PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

ESCUELA DE INGENIERIA

STUDY OF FURAN FORMATION STARCHY FOODS PROCESSED AT HIGH TEMPERATURE AND TECHNOLOGIES FOR ITS MITIGATION

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Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

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FRANCO PEDRESCHI

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A mis amados hijos

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PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
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STUDY OF THE FURAN FORMATION IN STRCHY FOODS PROCESSED AT HIGH TEMPERATURE AND TECHNOLOGIES FOR ITS MITIGATION

MARÍA SALOMÉ MARIOTTI CELIS

ABSTRACT

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences by

Furan is a potential human carcinogen that can be formed in a broad range of foods processed at high temperatures, such as coffee, baby foods, bread and snacks. Although it is still unclear what are the risks associated with the current intake levels of dietary furan, furan mitigation in foods may be considered as a challenge in the prevention of human diseases as cancer.

The hypothesis of this work is that it is possible to reduce the furan levels of low moisture starchy foods processed at high temperatures, by decreasing the content of its main precursors (reducing sugars and ascorbic acid) in the food raw materials. The overall objective of this thesis was to improve the understanding of furan formation in starchy foods processed at high temperature with the aim of developing technologies for its mitigation.

The available information about worldwide furan dietary exposure was exhaustively analyzed, concluding that it is strongly influenced by the geographical region, since both consuming habits and food preferences are distinctive characteristics of each country. Likewise, the proposed mechanisms responsible of furan formation in foods were deeply revised, suggesting that its generation is a specific process highly influenced by the intrinsic factors of the food matrix.

According to previous conclusions, the furan concentration of 14 types of Chilean commercial foods processed at high temperature was analyzed based on a modified Head Space-Gas Chromatography mass spectrometry method. In addition, a risk assessment was made with exposure estimates based on dietary data from national studies on different age groups. American coffee, potato chips and "Soda" type crackers presented the highest furan concentrations (936 ng g^{-1}, 259 ng g^{-1} and 91 ng g^{-1}, respectively). School children (9-13 years) and babies (9 months) represented the most exposed age sector to dietary furan, with margins of exposure lower than 10,000 (2,479 and 2,411; respectively), which points to a possible public health risk for a carcinogenic and genotoxic compound as furan. Thermally treated starchy foods represented more than the 50% of the total estimated daily intake of furan in Chile.

Based on these results, the furan formation of low moisture starchy foods (baked and fried) was studied. The role of ascorbic acid addition and heating conditions over the furan occurrence and its relation with the non-enzymatic browning in a wheat flour starchy food model system were investigated. Ascorbic acid addition significantly increased the furan generation after 7 min heating (P <0.05), having a strongest effect in baked products. Likewise, the furan content of fried products increased as oil uptake levels did. As for Maillard reactions in general, furan level in all samples linearly correlated with their degree of non-enzymatic browning, represented by L^* and a^* color parameters (*e.g.* wheat flour baked samples showed a R^2 of 0.88 and 0.87 for L^* and a^*, respectively) when the sample moisture content decreased during heating.

Finally, since potato chips were found to be a critical contributor to furan intake of Chilean population, a furan mitigation technology for this food matrix, which demonstrates the hypothesis of this thesis, was performed. In order to that, a central composite design was used to optimize the effect of blanching temperature and time over: (i) the extraction of the precursors of furan and acrylamide (reducing sugars and ascorbic acid) in potato slices and (ii) the reduction of furan, acrylamide and oil content in blanched chips. The content of both heat toxic compound significantly ($p<0.05$) decreased (> 50%) after blanching when the blanching temperature and time increased,

showing the same tendency obtained for the precursor levels. The optimum blanching conditions were 64°C and 17 min in which reductions of 91%, 53%, and 19% of furan, acrylamide, oil respectively, were obtained.

From a scientific perspective; it is concluded that the reduction of precursor concentration in the raw materials, specifically reducing sugars and ascorbic acid; produces a decreasing in the final furan content of thermally treated starchy foods such as potato chips, suggesting the Maillard reaction; as the responsible route of furan formation in this kind of matrix.

Thus, blanching of potato slices can be proposed as a feasible alternative to produce healthier chips, with lower amounts of food processing contaminants and a controlled level of oil.

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Santiago, April 2014

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
ESCUELA DE INGENIERÍA

ESTUDIO DE LA FORMACION DE FURANO EN ALIMENTOS AMILÁCEOS PROCESADOS A ALTA TEMPERATURA Y TECNOLOGÍAS PARA SU MITIGACION

Tesis enviada a la Dirección de Investigación y Postgrado en cumplimiento parcial de los requisitos para el grado de Doctor en Ciencias de la Ingeniería.

MARÍA SALOMÉ MARIOTTI CELIS

RESUMEN

El furano es un compuesto potencialmente cancerígeno en humanos que puede formarse en una amplia gama de alimentos procesados a altas temperaturas, tales como café, alimentos para bebé, pan y *snacks*. Si bien, aún son inciertos los riesgos asociados a los niveles actuales de ingesta de furano dietario; la mitigación de furano en alimentos podría ser considerada como un desafío en la prevención de enfermedades como el cáncer.

La hipótesis de este trabajo es que es posible reducir el nivel de furano presente en alimentos amiláceos procesados a altas temperaturas, mediante la disminución del contenido de sus principales precursores (azúcares reductores y ácido ascórbico) en las materias primas alimentarias. El objetivo de esta tesis fue mejorar el entendimiento de la formación de furano en alimentos amiláceos procesados a altas temperaturas con el fin de desarrollar tecnologías para su mitigación.

De acuerdo a las conclusiones anteriores, la concentración de furano de 14 tipos de alimentos comerciales chilenos procesados a altas temperaturas fue analizada en base a un método modificado de cromatografía gaseosa de espacio de cabeza con espectrometría de masas. Además, una evaluación de riesgos fue realizada con

estimadores de exposición basados en la data dietaria de estudios nacionales de diferentes grupos etarios. El café tipo "Americano", las hojuelas de papa frita y las galletas de "Soda" presentaron las concentraciones más altas de furano (936 ng g^{-1}, 259 ng g^{-1} y 91 ng g^{-1} respectivamente). Los escolares (9-13 años) y los bebés (9 meses) representaron las mayores ingestas de furano, con márgenes de exposición menores a 10.000 (2.479 y 2.411, respectivamente) lo cual apunta a un posible riesgo público para la salud, considerando que el furano es un compuesto posiblemente cancerígeno y genotóxico.

En base a estos resultados, la formación de furano en alimentos amiláceos de bajo contenido de humedad (horneados y fritos) fue estudiada. El rol de la adición de ácido ascórbico y las condiciones de calentamiento sobre la ocurrencia de furano y su relación con el pardeamiento no enzimático en un sistema modelo alimenticio a base de harina de trigo fueron investigados. La adición de ácido ascórbico aumentó significativamente la generación de furano después de los 7 min de calentamiento, teniendo un efecto mayor en los productos horneados. Asimismo, el contenido de furano en alimentos aumentó de manera paralela a los niveles de aceite penetrado. Como para las reacciones de Maillard en general, en todas las muestras el nivel de furano correlacionó linealmente con su grado de pardeamiento no enzimático, representado por los parámetros de color L^* y a^* (*ej*. las muestras de harina de trigo horneadas mostraron un R^2 of 0.88 y 0.87 para L^* y a^*, respectivamente) a medida que el contenido de humedad de la muestra disminuyó durante su calentamiento.

Finalmente, debido a que se encontró que las hojuelas fritas de papas eran un contribuyente crítico a la ingesta de furano de la población chilena, una tecnología de mitigación de furano para este tipo de matriz alimentaria fue propuesta. De acuerdo a lo anterior, un diseño central compuesto fue usado para optimizar el efecto de la temperatura y tiempo de blanqueado sobre: (i) la extracción de los precursores de furano y acrilamida (azúcares reductores y ácido ascórbico) en láminas de papa y (ii) el nivel de furano, acrilamida y aceite en hojuelas de papa blanqueadas y fritas. El contenido de furano y acrilamida disminuyó (> 50%) significativamente ($p<0.05$) al aumentar la

temperatura y tiempo de banqueado, mostrando la misma tendencia obtenida para los niveles de precursores. Las condiciones óptimas de blanqueado fueron 64°C y 17 min en las cuales se obtuvieron reducciones del 91%, 54% y 19% de furano, acrilamida y aceite, respectivamente.

Desde una perspectiva científica, se concluye que la reducción de la concentración de precursores en las materias primas, específicamente azúcares reductores y ácido ascórbico; produce una disminución del contenido final de furano en los alimentos térmicamente tratados, tales como las hojuelas de papas frita, sugiriendo la reacción de Maillard como la ruta responsable de la generación de furano en este tipo de matriz.

Por lo tanto, el blanqueado de láminas de papa puede ser propuesto como una alternativa factible para producir hojuelas de papa fritas más saludables, con menores contenidos de contaminantes alimentarios de proceso y un nivel de aceite controlado.

Miembros del Comité de Tesis Doctoral

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LIST OF PAPERS

This thesis is based on the following papers, referred in the text by their respective chapters:

CHAPTER 2: Mariotti, M.; Granby, K.; Rozowski, J.; Pedreschi, F. (2013). **Furan a critical heat induced contaminant.** *Food and Function,* 4: 1001-1015.

CHAPTER 3: Mariotti, M.; Toledo, C.; Hevia, K.; Gomez, J.P.; Fromberg, A.; Granby, K.; Rozowski, J.; Castillo, O.; Pedreschi, F. (2013) **Are Chilean exposed to dietary furan?** *Food Additives and Contaminants, Part A*: 30 (10): 1715-1721.

CHAPTER 4: Mariotti, M.; Granby, K.; Fromberg, A.; Risum, J; Agosín, E.; Pedreschi, F. (2012). **Furan occurrence in low moisture starchy foods processed at high temperatures: effect of ascorbic acid addition and heating conditions.** *Journal of Agricultural and Food Chemistry*, 60: 10162-10169.

CHAPTER 5: Mariotti, M.; Granby, K.; Fromberg, A.; Bysted, A; Pedreschi, F. (2014). **Heat toxic compound mitigation in potato chips.** *LWT- Food Science and Technology*. Submitted.

Proceedings

Parts of the work have also been presented at four international congresses and one national congress under the following references:

Mariotti, M.; Granby, K.; Fromberg, A.; Risum, J.; Pedreschi, F. Influence of heating conditions and ascorbic acid concentration over Furan Formation in Starchy Food Model Systems. In: Proceedings of the Institute of Food Technologist Annual Meeting (IFT 2012), June 25-28, Las Vegas, United States of America (2012).

Mariotti, M.; Granby, K.; Fromberg, A.; Risum, J.; Pedreschi, F. The role of ascorbic acid and oil uptake over furan content in fried starchy food model systems. In: Proceedings of the 16th IUFoST World Congress of Food Science and Technology, August 5-9, Foz de Iguazu, Paraná, Brazil. (2012).

Mariotti, M.; Granby, K,, Fromberg, A.; Risum, J.; Pedreschi, F. Furan content and non-enzymatic browning in starchy food model systems, In: Proceedings of the 11th International Symposium on the Maillard reaction (IMARS 2012), September16-20, Nancy, France. (2012).

Mariotti, M.; Granby, K.; Arvid, F. Rozowski, J.;Castillo, O.; Pedreschi, F. Are Chilean exposed to dietary furan? In: Proceedings of the XVII Conference of the Food Chemistry Division of the European Association of Chemical and Molecular Sciences (EUROFOOD CHEM XVII), May 7-10, Istambul, Turkey. (2013).

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1. INTRODUCTION

Food matrices can be considered as highly complex systems formed mainly by water, proteins, carbohydrates, lipids, vitamins and minerals. Due to the reactivity of these components, substantial interactions and changes can occur during the thermal processing of foods.

Thermal processes such as baking and frying are widely used in food manufacturing and have a strong impact on the final quality of foods. Desired and undesired results are produced in foods, as a consequence of various chemical reactions being Maillard reaction, caramelisation and lipid oxidation the most prominent (Capuano & Fogliano, 2011).

From a positive point of view, these high temperature processes improve the sensory properties of foods(Martins, Jongen, & van Boekel, 2000) because unique textures (Diamante, Savage, Vanhanen, & Ihns, 2012; Dueik, Robert, & Bouchon, 2010; Flander, Salmenkallio-Marttila, Suortti, & Autio, 2007; Jackson et al., 2009; Yang et al., 2012), aromas and flavors (Van Boekel et al., 2010) are generated during the heating of foods. Additionally the shelf life of products is extended as a consequence of the decreasing of water activity (Pittia, Furlanetto, Maifreni, Mangina, & Rosa, 2008). Moreover many antioxidants with positive effects on human health are generated during the heating of foods (Borrelli & Fogliano, 2005; Morales, Somoza, & Fogliano, 2012).

On the other hand, although the thermal processing of food has many advantages; some of its detrimental consequences should be carefully evaluated (Capuano & Fogliano, 2011). The heating of foods produce the loss of thermo labile compounds such as vitamins and essential amino acids decreasing the nutritional value the heated foods (Anjum, Ahmad, Butt, Sheikh, & Pasha, 2005; Fillion & Henry, 1998; Han, Kozukue, Young, Lee, & Friedman, 2004; Rickman, Barrett, & Bruhn, 2007; Rogers, Malouf, Langemeier, Gelroth, & Ranhotra, 1993). Furthermore, some undesired tastes and off-flavors can be formed in foods processed at high temperatures, affecting their sensorial

quality (Dresow & Bohm, 2009; Osawa, Goncalves, & Da Silva, 2013; Zhang, Saleh, Chen, & Shen, 2012).

In this sense, the major concern arising from the thermal processes comes from the formation of potential human carcinogens that are not naturally present in foods, but that may develop during heating processes (Capuano & Fogliano, 2011).

A well-known example of these food processing contaminants is furan, which is characterized by its wide occurrence in foods processed at high temperatures (EFSA, 2010).

Furan is classify as a possible carcinogenic compound to humans (2B) (IARC, 1995) and it has been detected in highly consumed foods such as baby foods, coffee, tomato sauces, chips and several starchy based products among other (Vranova & Ciesarova, 2009).The official methodology for furan determination in foods was established in 2004 by the US-FDA, who considering its volatility, developed a method based on automated headspace sampling (HS) followed by gas chromatography/mass spectrometry (GC/MS) analysis (FDA, 2004). In this sense, solid phase micro extraction (SPME) coupled to GC/MS has been proposed as an alternative technique for furan quantification in foods, however no comparative advantages in terms of sensibility has been observed compared to HS-GC/MS (Altaki, Santos, & Galceran, 2007; Goldmann, Perisset, Scanlan, & Stadler, 2005; Jestoi et al., 2009; Kim, Lee, Kim, Park, & Lee, 2009; Liu & Tsai, 2010). Additionally in terms of costs the use of fibres for SPME would increase the cost of analysis (Altaki, Santos, & Galceran, 2009).

On the other hand, although the presence of furan in food is not a recent issue, (IARC, 1995) to the best of our knowledge, to date there are no concluding remarks about the main pathway responsible of its formation in foods. Furan formation has shown to be strongly and specifically dependent on the food matrix, thus to extrapolate results from pure model systems to all foods may cause erroneous conclusions (Limacher, Kerler, Conde-Petit, & Blank, 2007; Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008; Owczarek-Fendor et al., 2012). To get deep into each food matrix considering factors

such as the heating medium, pH and water activity is necessary to understand the mechanism of generation and retention of this volatile food contaminant.

Furthermore the data base information of dietary exposure to furan is still incomplete (Schutte et al., 2012) and technologies for its reduction in all foods found to contain it, have not been developed yet (EFSA, 2010).

3.1. Scope and objectives of the thesis

From the above presentation it is clear that to reduce the level of food processing contaminants such as furan can be considered as a challenge in the prevention of diseases associated with their current intake. More research is needed to estimate the worldwide exposure to dietary furan and its main food contributors. Based on this information and elucidating the furan formation in foods it will be possible to develop furan mitigation technologies with the aim of improving the safety of population. This thesis covers the untapped areas previously commented and applies this prevention approach to the Chilean population, justifying a doctoral project in this field. The fundamental hypothesis of this thesis is that it is possible to develop starchy foods processed at high temperatures with lower levels of furan, by decreasing the content of its main precursors (reducing sugars, amino acids and ascorbic) in the food raw materials.

Therefore, the overall objective of this thesis was to improve the understanding of furan formation in starchy foods processed at high temperature with the aim of developing technologies for its mitigation.

To achieve this goal, the thesis was divided into the following specific objectives:

- Review the state of the art on the main issues of furan in foods (considering furan toxicity, human dietary exposure to furan and the mechanism found as responsible of its formation in thermally treated foods) and analyze those aspects of science and technology that need further development to produce foods with lower content of furan (Chapter 2).

- Determine the furan concentration of highly consumed Chilean foods and based upon this monitored data to perform an exposure assessment of Chilean population to this food processing contaminant (Chapter 3).
- Explore the effect of ascorbic acid addition and heating conditions (frying and baking) over furan occurrence and its relation with the non-enzymatic browning in a starchy food model system formulated from wheat flour (Chapter 4).
- To reduce the level of food processing contaminants (furan and acrylamide) in chips without increasing their oil uptake (Chapter 5).

3.2. Outline of the thesis

The central theme of this thesis is to develop low moisture starchy foods processed at high temperature with lower levels of furan.

Chapter 2 summarizes and discusses the most important aspects of dietary furan. The dietary exposure to furan is compared between different countries concluding that the geographical area is a critical factor on the furan exposure. On the other hand, furan formation in foods is presented as a pending issue, emphasizing the need of further research which clarifies both the main furan precursor and mechanism in real foods. Furthermore, different techniques to reduce the furan content are compared concluding that to date there is no methodologies to reduce the furan level in low moisture starchy foods such as bread and chips.

From these conclusions, two questions were generated. The first one which points to the furan exposure in Chile, was answered Chapter 3. The second one, related to elucidation of furan formation in foods, was discussed in Chapter 4.

In Chapter 3 of this thesis an exposure assessment to dietary furan in Chile was developed. The obtained results allowed identifying the most exposed age group and the main contributor food to furan dietary intake.

Chapter 4 of this thesis is focused into improve the understanding of furan generation in low moisture starchy foods, considering the role of ascorbic acid addition and heating

conditions over the furan occurrence (formation and retention) in wheat flour based model system.

In Chapter 5 the development of an effective furan and acrylamide mitigation technology is proposed, considering chips as the food matrix to study. This selection was based on the results obtained in Chapter 3, in which this food was the major contributor to furan intake in the most exposed age group (school children). In order to demonstrate the hypothesis of this thesis, this work studied the effect of blanching conditions over: (i) the extraction of furan and acrylamide precursors (reducing sugars and ascorbic acid) in potato slices and (ii) the level of furan, acrylamide and oil in blanched chips.

Finally in Chapter 6, most important findings of previous chapters and future perspectives are discussed.

A summary of the contents of Chapters 2 to 5 is schematically presented in Fig. 1.1, showing the relationship between the different Chapters of this thesis.

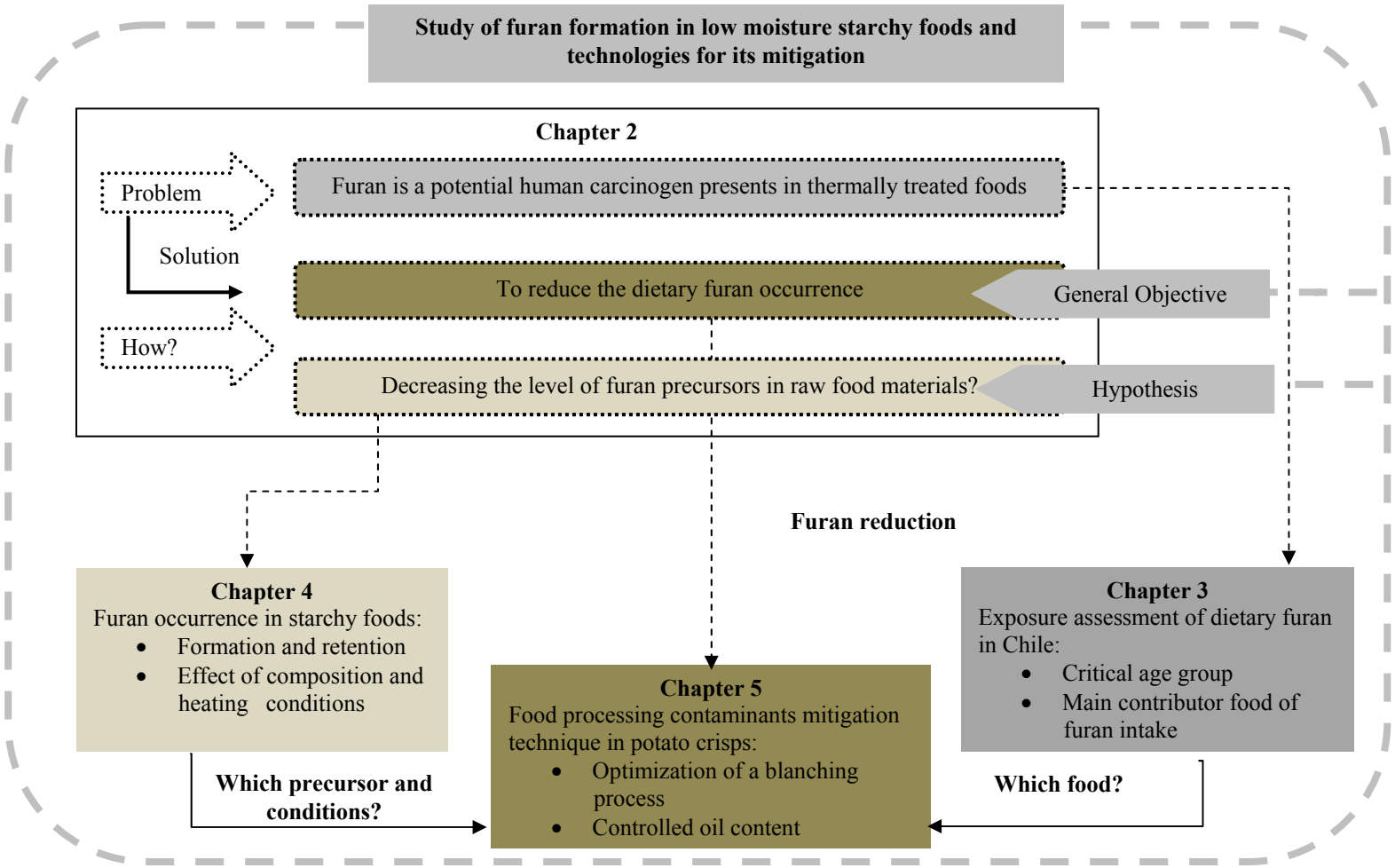

Figure 1.1: Overview of the studies comprising this thesis.

3.3. References

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2. FURAN: A CRITICAL HEAT INDUCED DIETARY CONTAMINANT

Abstract:

The presence of furan in a broad range of heat processed foods (0-6000 ppb) has received considerable attention due to the fact that this heat induced contaminant is considered as a "*possible carcinogenic compound to humans".* Since a genotoxic mode of action could be associated to furan-induced tumour formation; current human exposures levels to this contaminant may indicate a risk to human health and the necessity of its mitigation. This review summarizes and focuses on the main issues of furan toxicity, human dietary exposure to furan and mechanisms of furan formation. Additionally, the role of some critical factors such as heating conditions, pH and matrix microstructure are discussed in order to propose some potential methodologies for furan mitigation in a wide range of heated foods.

Key words: furan, heated foods, carcinogenicity, exposure assessment, mechanism of formation.

2.1. Introduction

Chemical food safety has become an area of concern throughout the past decades. In this sense, the presence of acrylamide, a probable human carcinogen (2A)(IARC, 1994) in starchy foods processed at high temperatures has shocked the food safety world provoking an international health alarm (Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). Since that time, most of the studies regarding to food processing contaminants generated by Maillard reaction have been focused on acrylamide (Mottram, Wedzicha, & Dodson, 2002). However, there are other contaminants generated during the heating of foods such as furan which could also have harmful effects on the health of the population (C. Crews & Castle, 2007).

Furan (C_4H_4O) is a small organic compound (MW: 68 $gmol^{-1}$) with high volatility (boiling point: 31°C) and lipophility used in various chemical-manufacturing industries

(C. Crews, Hasnip, Roberts, & Castle, 2007). Despite the fact that early reports of furan in heat treated foods has long been recorded (Maga, 1979), the study of this contaminant really became a potential concern in middle nineties, when based on research made in laboratories with animals exposed at high furan doses, this contaminant was considered as possible carcinogenic to humans (2B) by the International Agency for Research on Cancer (IARC). (IARC, 1995) Afterwards, there have been published several scientific articles of furan in foods regarding to its metabolism and toxicity, exposure assessment, and mechanisms of formation (C. Crews et al., 2007).

Toxicology and carcinogenesis studies of furan indicated that it is clearly carcinogenic to rats and mice, showing a dose-dependent increase in hepatocellullar adenomas and carcinomas in both sexes (EFSA, 2004; NTP, 1993). Several studies have been conducted on furan toxicity, however; the mechanism(s) of tumour induction by furan induction in laboratory animals have not been elucidated. Nowadays, there are still no reports about epidemiological studies which show possible associations between furan exposure and human cancer.

The monitoring of furan levels in foods has been conducted by world health and food authorities since 2004 until now in order to determine the real exposure assessment to furan in different populations around the world. In this respect, The Food and Drug Administration (FDA) published the first report on furan measurement in food in May 2004. Then, The European Food Safety Authority (EFSA) also analysed the available information about this issue and investigated the furan occurrence in European samples (EFSA, 2004; FDA, 2004). These reports outlined the occurrence of furan from non-detectable levels to approximately 5000 $\mu g\ kg^{-1}$ in a broad variety of foods, such as coffee, baked products and baby foods, among others.

Considering that population could be highly exposed to furan since it is present in several foodstuffs, EFSA has continued with its monitoring. In its last update on furan levels in foods and exposure assessment, a total of 5,050 analytical results of furan content in foods were submitted by 20 countries, with similar trends of occurrence, being the roasted coffee, the food which the highest furan concentration (6,407 $\mu g\ kg^{-1}$).

Additionally, mean exposure was estimated to range between 0.03 kg $_{bw}^{-1}$day^{-1} to 0.059 kg $_{bw}^{-1}$day^{-1}; 0.02 µg kg $_{bw}^{-1}$day^{-1} to 0.13 µg kg $_{bw}^{-1}$day^{-1}; 0.04 µg kg $_{bw}^{-1}$day^{-1} to 0.22 µg kg $_{bw}^{-1}$day^{-1}; 0.05 µg kg $_{bw}^{-1}$day^{-1} to 0.31 µg kg $_{bw}^{-1}$day^{-1} and 0.09 µg kg $_{bw}^{-1}$day^{-1} to 0.22 µg kg $_{bw}^{-1}$day^{-1} for adults, adolescents, other children, toddlers and infants respectively (EFSA, 2011).

Studies performed by JECFA (2010) have revealed the comparatively small margin of exposure (MoEs of 960 and 480 for average -0.001 mg kg $_{bw}^{-1}$ day and high -0.002 mg kg $_{bw}^{-1}$ per day dietary furan exposures, respectively) between human exposure and the furan doses which induce liver tumour in experimental animals. Similar results for infant exposure to dietary furan have been reported in Finland (Jestoi et al., 2009), Germany (M. Chen, Hsu, Lin, & J., 2012; Lachenmeier, Reusch, & Kuballa, 2009) and Brazil (Olivares C, Bustos Z, Lera M, & Zelada, 2007). Previous information clearly shows that furan exposure could represent a critical public health risk, specifically in infants since furan is a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite.

Regarding to the furan formation in foods, different authors have suggested that this food process contaminant could be produced by the thermal degradation and rearrangement of sugars, amino acids, ascorbic acid and also by the oxidation of polyunsaturated fatty acids (A. Becalski & Seaman, 2005; C. Crews & Castle, 2007; C. Crews et al., 2007; C. Crews, Roberts, Lauryssen, & Kramer, 2009; Maga, 1979; Perez Locas & Yaylayan, 2004; Roberts, Crews, Grundy, Mills, & Matthews, 2008). However, up to now, no conclusive remarks have been established regarding to the main mechanism and major precursor of furan formation in different kinds of food processed at high temperatures. Although there is still no clarity about the risks associated with the current furan dietary intake, the necessity to develop technologies to diminish its contents in foods could be considered as a challenge in the prevention of human diseases such as cancer. The aim of this review was to discuss the current available information about furan in foods considering the most relevant aspects of its

toxicology, exposure and formation. Finally, this review suggests some alternatives for developing new technologies for furan mitigation.

2.2. Furan Toxicology

The critical toxicological effect of furan is carcinogenicity, and liver is the main target organ of furan-induced toxicity in rats and mice, with a clear dose-dependency and probably acting by a genotoxic mechanism (EFSA, 2004; JECFA, 2010; NTP, 1993; Wilson, Goldsworthy, Popp, & Butterworth, 1992).

Furan has a low polarity and thus can easily pass through biological membranes. As shown in rats, orally administrated [2,5 -14 C]-labelled furan (8 mg kg_{bw}^{-1} in corn oil) was rapidly and extensively absorbed from the intestines. From the radioactivity remaining in the tissues 24 h post-dosing (19% of the dose), the main portion was found in the liver (13 % of the dose) followed by kidney and gastrointestinal tract (together < 1%). The tissue-bound radioactivity in the liver was found to be associated with protein, but not with DNA (Burka, Washburn, & Irwin, 1991; Parmar & Burka, 1993).

Considering that an important percentage of the total supplied furan is retained in liver tissues and it also presents hepatocarcinogenic effects, some authors have investigated about its toxicokinetics. Results have showed that furan is metabolized rapidly by cytochrome P-450 enzymes (especially CYP2E1) via ring opening to form carbon dioxide and cis -2-butene-1,4-dialdehyde (BDA) (L.J. Chen, Hecht, & Peterson, 1995). BDA has been identified as a key reactive genotoxic intermediate of furan which binds to proteins and nucleosides. BDA also has been found as a mono glutathione conjugate in the urine of rats exposed to radio labelled furan (Peterson, Cummings, Chan, Vu, & Matter, 2006).

On the other hand, first data on furan effect in humans showed a correlation of urinary furan with plasma γ-glutamyltranspeptidasa (γ- GT), a marker of liver damage in healthy men and women (Jun et al., 2008).

Although, this research represents important information, no epidemiological studies on furan in humans are available. Moreover, the mechanism of furan induced

carcinogenicity in rodents as well as the levels and effect of furan on humans, have not been clarified yet.

2.2.1 Biotransformation of furan

The liver possesses high capacity to eliminate furan from the bloodstream by first-pass metabolism. Some findings have supported the role of cytochrome P-450 in the activation of furan to a reactive metabolite. Studies in rats treated with furan, have shown that reduction of cell viability in freshly isolated hepatocytes caused by this toxic compound, can be inhibited by 1-phenylimidazole and enhanced by acetone pre-treatment of animals. Both the inhibition and also the induction agents have the same effect over cytochrome P-450 (Carfagna, Held, & Kedderis, 1993; L.J. Chen et al., 1995; Kedderis & Held, 1996; Parmar & Burka, 1993).

In the same way, the depletion of cellular glutathione levels and kinetics analyses of furan metabolism in gas uptake studies have indicated that there is a single saturable uptake process that can be blocked by pyrazole, a cytochrome P-450 inhibitor (Kedderis & Held, 1996). Considering previous studies, L.J. Chen et al. (1995) characterized the microsomal metabolites of furan in rat liver microsomal fractions. They identified BDA (a reactive aldehyde) as the major metabolic product of furan. During their investigation, these authors observed that BDA formation required NADPH, microsomes and semicarbazide and it was also concentration dependent. Conclusively, these investigators indicated that furan was oxidized by rat liver microsomes to BDA. They also investigate whether furan was directly activated to BDA by cythochrome P-450 suggesting that this enzyme system is a catalyst of furan oxidation. The above information suggests BDA as a major intermediate in furan treated animals.

Recently, some researchers have been focused into evaluating what happens with the in vivo biotransformation of BDA. For this purpose the urine of furan treated rats have been analysed in order to determine final furan metabolites. Different compounds which come from BDA have been identified as biomarkers of furan exposure in treated rats (Kellert, Wagner, Luiz, & Lutz, 2008; Peterson et al., 2006).

In order to improve the understanding of BDA carcinogen action, some researchers have evaluated different mechanism in which BDA reacts to form final urinary furan metabolites. They have found that there are multiple pathways by which furan can modify nucleophiles. In one pathway, BDA reacts directly with proteins to form cysteine-lysine products. BDA reacts also in another pathway with GSH to form GSH-BDA conjugates, which then react with cellular nucleophiles like free lysine or lysine moieties in proteins. Both pathways appear to be major pathways of furan biotransformation in vivo (Lu & Peterson, 2010; Lu, Sullivan, Philips, & Peterson, 2009).

2.2.2 Mechanism aspects of furan carcinogenicity

The mechanisms of furan and its metabolite BDA induced carcinogenesis are not well understood yet. Particular uncertainty exists concerning the possible effects in the low dose range of both tested items, which is of major importance for the risk assessment (Heppner & Schlatter, 2007). In this respect, studies have suggested that furan could act by both genotoxic (A. Becalski et al., 2004; F. Beland et al., 2013; Burka et al., 1991; Cordelli et al., 2010; EFSA, 2004; Leopardi et al., 2010; NTP, 1993; Peterson et al., 2006) and non-genotoxic mechanisms (F. A. Beland, 2010; Durling, Svensson, & Abramsson-Zetterberg, 2007; FranssonSteen, Goldsworthy, Kedderis, & Maronpot, 1997; Moser, Foley, Burnett, Goldsworthy, & Maronpot, 2009). On the other hand BDA has been mainly proposed as a genotoxic carcinogen. Next sections will summarize and discuss the available scientific evidence of furan and BDA modes of action.

2.2.2.1 Genotoxic evidence of furan

The available evidence of genotoxicity of furan mainly relies on *in vitro* studies in which furan was generally negative in bacteria, but showed mutagenic and clastogenic activity in mammalian cells, with or without metabolic activation (McGregor et al., 1988; NTP, 1993). Inconsistent results of mutagenic effect of furan in bacteria have been published. In this sense, some authors have concluded that furan is not mutagenic

in the *Ames Salmonella typhimurium* assay with and without metabolic activation in TA98, TA100, TA1535 and TA1537 (NTP, 1993), but other authors concluded that furan induced gene mutation in *Salmonella typhimurium* TA100 but not in TA98 (Lee, Bian, & Chen, 1994).

When analysing *in vitro* mammalian systems, furan induced gene mutations in mouse lymphoma cells (McGregor et al., 1988), DNA damage in Chinese hamster ovary (CHO) cells (NTP, 1993) and chromosomal damage in CHO cells with an exogenous metabolic activation system (IARC, 1995); but it did not induce DNA damage in mouse and rat hepatocytes.(NTP, 1993) Recently studies performed in splentocytes of mice cultured *in vitro* have shown statistically significant ($P<0.001$) increases of micronuclei in bi nucleated splentocytes (Leopardi et al., 2010). These controversial data could be due the high volatility of the compound.

Similarly, data on furan genotoxicity *in vivo* are also debatable, with no clear positive results following oral exposure. This is the case of the NTP (1993) study in which the obtained results showed that furan induced chromosomal aberrations, but not sister chromatid exchanges in bone marrow cells of mice treated by intra peritoneal injection and did not induce unscheduled DNA synthesis in liver of rats and mice treated with single oral dose. Furthermore, the positive results were observed only at the highest dose tested (250 mg kg_{bw}^{-1}) and no information on toxicity in the experimental animals was reported in this study. Additionally, Wilson et al. (1992) also obtained positive results of genotoxicity (extensive liver necrosis), when administrated a single oral dose of 250 mg kg_{bw}^{-1} to male mice. However, these positive *in vivo* genotoxic effects in bone marrow assays may not to be of special relevance, since they were obtained at dose levels associated with high toxicity.

Recently studies have shown the genotoxic effects elicited by furan when it is administered under the same treatment conditions that in NTP long-term studies: furan was given for 28 days by daily gavage at doses of 2, 8 and 15 mg kg_{bw}^{-1} (NTP, 1993). For instance, the genotoxic potential of furan *in vivo* was studied under NTP long term bioassays conditions by Leopardi et al. (2010). These authors evaluated the DNA

damage and the induction of micronuclei in mouse splentocytes. Their results showed a significant ($P<0.01$) increase of in foci of phosphorilated histone γ – H2AX, a marker for DNA double-strand breaks generation in the cells, in mitogen-stimulated splentocytes of animals treated with the two highest doses (8 and 15 mg kg_{bw}^{-1}). On the other hand, no effect of the *in vivo* furan was observed when γ – H2AX foci were scored in freshly isolated quiescent splentocytes. The analysis for induction of cross-links in freshly isolated splentocytes by alkaline comet assay yielded negative results, both with standard and radiation modified protocols. Authors mentioned that this situation has been observed in others studies since the sensitive of both standard and modified protocols of comet assay could be considered as low for detection of certain kinds of cross links (Speit & Hartmann, 2005). Consequently, these authors concluded that even though the underlying mechanism is not fully elucidated, this data support the formation of cross links by the BDA.

Cordelli et al. (2010) studied the genotoxic effects of oral administration of furan in mouse liver, in order to get more conclusive information about furan mode of action. In a long-term bioassay (under NTP conditions), these authors evaluated: (i) the polyploidy of DNA by flow cytometric analysis and (ii) the overall DNA methylation, gene expression and DNA damage by the analysis of immunofluorescence detection of foci of γ – H2AX and by alkaline comet assays. The researchers also determined the liver DNA damage by comet assays in mice receiving furan as a single acute oral dose (15, 100 or 250 mg kg_{bw}^{-1}).

Respect to polyploidy of DNA, authors observed that single cells suspensions of liver cells only showed a statistically significant increase at the highest furan dose (8N). On the other hand, authors did not recognize any direct evidence of genotoxicity in the liver of mice treated with lower doses. Evaluated liver sections did not present any changes in overall DNA methylation, γ – H2AX foci, DNA strand breaks and cross link at the end of the 4 week exposure period. However, several genes involved in DNA damage were over expressed in mice treated with the highest dose (15 mg/kg). Finally, acute administration of furan induced evident liver toxicity at the highest dose (250 mg kg_{bw}^{-1})

which was associated with a significant increase of DNA damage. Overall, the above information suggests that the contribution of genotoxicity to the mechanism of furan carcinogenicity in mouse liver should not be dismissed.

In this sense, recent studies performed in rats have suggested that furan may operate by a genotoxic mode of action. For instance F. Beland et al. (2013)assessed the potential of furan to covalently bind to DNA and its genotoxicity in F344rats treated with [3,4-^{14}C]-furan (0.1 and 2.0 mg $kg_{bwt}^{-1}day^{-1}$) during 28 days. Results showed that the ^{14}C-content in liver DNA was significantly increased in a dose-dependent manner. There was no evidence for genotoxicity of furan in peripheral blood and bone marrow cells. However, a dose-related increase in the incidence of chromosomal aberrations in rat splenocytes and some indication of DNA damage in liver were observed. Collectively, results from this study indicate that furan may operate at least in part by a genotoxic mode of action.

In the same way, A. Becalski et al. (2004) applied the *in vivo* Comet and micronucleus assays, combined with analysis of histopathological and gene expression changes to investigate the mechanisms for furan carcinogenicity in male F344 rats treated by gavage with 2, 4, 8, 12 and 16 mgkgbwt-1of furan doses during four consecutive days. In addition, these authors monitored the formamidopyrimidine DNA glycosylase (Fpg) and endonuclease III (EndoIII)-sensitive DNA damage as a measure of oxidative DNA damage. Liver Comet assays indicated that both DNA strand breaks and oxidized purines and pyrimidines increased in a near-linear dose-responsive fashion, with statistically significant increases detected at cancer bioassay doses. No DNA damage was detected in bone marrow, a non-target tissue for cancer, and peripheral blood micronucleus assays were negative. Histopathological evaluation of liver from furan-exposed animals produced evidence of inflammation, single-cell necrosis, apoptosis, and cell proliferation. In addition, genes related to apoptosis, cell-cycle checkpoints, and DNA-repair were expressed at a slightly lower level in the furan-treated livers. Although a mixed mode of action involving direct DNA binding cannot be ruled out, the data suggest that furan induces cancer in rat livers mainly through a secondary

genotoxic mechanism involving oxidative stress, accompanied by inflammation, cell proliferation, and toxicity.

2.2.2.2 Not genotoxic evidence of furan

Some authors have proposed that furan is an epigenetic or non- genotoxic carcinogenic compound, since furan would not directly modify the DNA. Concerning the non-genotoxic mechanism behind carcinogenicity it has been proposed that furan stimulates cell proliferation, which in turn increases the likelihood of tumour formation (FranssonSteen et al., 1997; Wilson et al., 1992). Reparative cell proliferation has been demonstrated in a variety of in vivo studies in mice and rats which single and multiple doses of furan, inducing liver cell necrosis and/or release of liver-associated enzymes (FranssonSteen et al., 1997; Wilson et al., 1992). Currently, some authors have tried to clarify whether furan carcinogenicity is caused by genotoxic effect or solely by an increased cell proliferation. In this regard, Durling et al. (2007) investigated the furan effect over micronucleus frequency both *in vivo* and *in vitro* experiments by using the flow cytometer- micronucleus assay in mice and the cytokinesis-block micronucleuos assay in human lymphocytes, respectively. These authors concluded that furan is a not genotoxic compound in the micronucleus assay *in vivo* or *in vitro*, since no increased level of micronucleus frequency was observed neither in mice erythrocytes nor in human lymphocytes treated with furan. However, researchers also suggested that for getting a better understanding of the mechanism behind the carcinogenic effect of furan, it would be necessary to apply a micronucleus assay restricted to liver cells, since liver is the target organ of furan carcinogenicity.

Regarding to this issue, Cordelli et al. (2010) have done studies in order to determinate the toxic effects of oral administration of furan in mouse liver. For this purpose these authors evaluated in a long-term bioassay under NTP conditions:

(i) The histological alterations, apoptosis and cell proliferation by using microscopic analysis among other parameters commented in the previous section (Genotoxic scientific evidence).

(ii) The epigenetic carcinogens whose proposed mode of action involves cytolethality.

Their results suggested that daily administration with hepatocarcinogenic furan doses elicited mild toxicity in mouse liver, producing slight histological alterations with necrotic figures (8-5 mg kg_{bw}^{-1}), apoptosis (15 mg kg_{bw}^{-1}) and limited compensatory cell proliferation (2 mg kg_{bw}^{-1}). It is hypothesized that dose response curve tumour development would closely parallel the dose-response curve for cell death with compensatory proliferation in the target organ.

Regarding to this, Moser et al. (2009) tested prospectively this hypothesis by evaluating furan induced hepatocyte toxicity and liver cell proliferation in a multi-dose 3 week study and liver neoplasia in a 2 year carcinogenicity study in female mice. Their studies were conducted under conditions similar to those used in NTP bioassay but utilizing dose levels which were not anticipated to be overtly cytotoxic. The obtained results demonstrated an association among furan-induced hepatic cytoxicity, compensatory cell replication, and liver tumour formation in mice; at high doses ($\geq$ 4mg kg_{bw}^{-1}) furan induced hepatotoxicity, compensatory cell replication and tumorigenesis in dose-related manner, while furan did not produce tumours at cytotoxic doses of 1.0 and 2 mg kg_{bw}^{-1}.

More recently, F. A. Beland (2010) tried to clarify the furan cancer mode of action, performing: (i) micronucleus (MN) frequency in normochromatic erythrocytes (NCEs) and reticulocytes (RETs), (ii) Pig-a gene mutation in total red blood cells (RBCs), (iii) liver cell transgene mutation assay and (iv) liver Comet assay in transgenic Big Blue rats treated by gavage five times a week for 8 weeks with 2, 8, 16 and 30 mg kg_{bw}^{-1}. Authors observed that, the responses in the MN assays conducted at both sampling times, and all the gene mutation assays, were uniformly negative; however, the Comet assay was positive for the induction of liver DNA damage at 30 mg kg_{bw}^{-1}. Since toxicity in the liver was only produced with doses in excess of the cancer bioassay doses, author concluded that furan has a predominantly nongenotoxic mode of action for cancer.

The above information suggests that furan induced hepatocarcinogenicity may be primarily the consequence of strong cytotoxic effects of furan. On the other hand, a

number of changes in gene expression take place following furan exposure, leading to altered cell proliferation. Thus, more studies will be necessary in order to draw more precise conclusions concerning this issue.

2.2.2.3 Genotoxicity of BDA

The proposed mechanism of furan-induced carcinogenesis involves reaction of BDA with proteins. BDA can bind covalently to cellular nucleophiles including proteins and nucleosides (Burka et al., 1991; Parmar & Burka, 1993). Additionally, some researchers have postulated that this metabolite would alkylate proteins leading to an acute cytotoxic response. This cytoxicity is believed to stimulate cell replication, increasing the likelihood of tumour production (L. W. Elmore & Sirica, 1993; Wilson et al., 1992).

On the other hand, studies accomplished by L. J. Chen, Hecht, and Peterson (1997) showed that BDA can cross link some amino acids such as lysine (amino group) and cysteine (thiol group), suggesting that it is a good candidate for the protein reactive microsomal metabolite of furan. When BDA reacts with thiols, the resulting product still retains reactivity towards nucleophiles. The subsequent reaction with an amino group yields stable pyrrole adducts. According to this, the characterization of products formed by BDA and nucleophiles, and the capability of these compounds to react with DNA is an important issue for a better understanding the mechanism by which furan induced carcinogenesis.

BDA is a highly reactive compound which has been proposed as a genotoxic carcinogen. In contrast to furan, it has shown to be mutagenic in the Ames Assay at not-toxic concentrations in *Salmonella typhimurium* TA104, a strain sensitive to aldehydes. On the other hand, this reactive compound tested negative in strains TA97, TA98, TA100, and TA102. Furthermore, the incubation of BDA with glutathione prior to addition of bacteria inhibited both genotoxic and cytotoxic activity of this metabolite (Peterson, Naraku, & D.P., 2000). Therefore, it is likely that BDA contributes to the carcinogen activity of furan by reacting with DNA to form mutagenic adducts.

Consistent with this hypothesis, M.C. Byrns, Predecki, and Peterson (2002) observed that in cell-free systems BDA reacted with 2´-deoxycytidine (dCyd); 2´-deoxyguanosine (dGuo) and deoxyadenosine (dAdo) -but not with thiamidine- to form adducts. Furthermore, the obtained reaction products presented different stability under similar physiological conditions. Thus, the dGuo and dAdo primary reaction products were substantially unstable and decomposed to secondary ones. On the other hand, dCyd-BDA adducts were relatively stable. Similar results were obtained in previous dCyd-BDA experiments realized by Gingipalli and Dedon (2001). Regarding to the relative reactivity of BDA with deoxyribonucleosides, these authors mentioned that it was paralleled to those observed for formaldehyde. Considering that dGuo and dAdo adducts rearrange to form secondary products, M. C. Byrns, Vu, and Peterson (2004) studied the structure of the rearrangement products. On the basis of UV absorbance, fluorescence, HNMR, and mass spectral data these authors concluded that primary dAdo and dGuo adducts undergo dehydratation to form substituted etheno products. Based on previous researches realized in CHO cells (Marinari et al., 1984), these authors concluded that since these secondary adducts retain a reactive aldehyde with the potential to form DNA and or DNA-protein cross-links, these product are likely to contribute significantly to furan's carcinogenic effects.

In order to determine the importance of DNA alkylation in furan induced carcinogenesis, M.C. Byrns et al. (2006) developed a sensitive liquid chromatography electro-spray tandem mass spectrometry assay for the detection of BDA derived adducts and its application to study their formation in DNA from BDA treated bacteria. Using this assay, the dCyd and dAdo adducts were detected in DNA isolated from *S.typhymurium* TA104 bacteria treated with mutagenic concentrations of BDA. Despite of the obtained results, authors mentioned that the efficiency of BDA reaction with DNA was much lower in bacteria than with isolated calf thymus. They also commented that this behavior could be explained due to not only differences in reaction conditions (time and temperature) but also to the protective role of plenty cellular nucleophiles in the bacteria cell which compete with DNA the reaction with BDA. Previous results

suggest that the reactive metabolite BDA could be a genotoxic compound and may also be involved in the carcinogenic mechanism of furan. Finally, although a sufficient dose of furan or its cytochrome P-450 induced metabolite BDA is clearly cytotoxic, genotoxic assays provide a mixed positive and negative response. Since carcinogenesis is a high complex process, it is possible that furan induced hepatocarcinogenecity is the result of not only a genotoxic but also a chronic cytotoxity mode of action, and the relative role of the two modes of action would vary with dose, treatment duration, species, sex, strain and environmental conditions.

2.3. What foods commonly contain furan?

The presence of furan is common in food processed at high temperature, particularly in products packed on sealed containers (*e.g*. baby food). Due to its low boiling point, furan formed during thermal processes is easily vaporized and contained in the headspace of canned or jarred foods (Goldmann et al., 2005; Hasnip, Crews, & Castle, 2006). Despite the most important physicochemical property of furan is its high volatility, furan has also been found in low moisture foods not cooked in close containers such as potato chips, crackers, crisp breads and toasted breads (C. Crews & Castle, 2007; Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010; Nyman, Morehouse, Perfetti, Diachenko, & Holcomb, 2008; F. Van Lancker, Adams, Owczarek, De Meulenaer, & De Kimpe, 2009; Zoller, Sager, & Reinhard, 2007).

Besides the heating processing during manufacturing, it has been shown that consumer cooking also affects the amounts of furan in food products (Kim, Kim, & Lee, 2010; Roberts et al., 2008). As expected from its low boiling point, furan concentrations significantly decrease when the food product is reheated at moderate temperatures, especially in a saucepan or when it is stirred during reheating. However, it has been reported that furan volatilization during heating strongly depends on the food matrix. Especially lipophilic compounds, such as oils, caused significant retention of furan may be by their relatively polar affinity (Mariotti et al., 2012; F. Van Lancker, Adams, Owczarek-Fendor, De Meulenaer, & De Kimpe, 2010; F. Van Lancker et al., 2009).

Thus, it could explain the occurrence of furan not only in sealed container products but also in low moisture fried starchy products because in those last, the oil penetrates during frying (Bouchon, Aguilera, & Pyle, 2003; Bouchon, Hollins, Pearson, Pyle, & Tobin, 2001; Bouchon & Pyle, 2004).

Furan has also been known such a normal component of coffee flavour volatiles. High levels of furan have also been found in roasted coffee beans (~6000 μg kg^{-1}), probably due to the high temperatures required for roasting. Automatic coffee machines produced coffee brews with the highest levels of furan since a higher ratio of coffee powder to water is used (giving a lower dilution factor and because the closed system favours retention of furan) (Altaki, Santos, & Galceran, 2011).

Considering the presence of furan in foods is an issue of concern, since 2004 EFSA has continually monitored the furan levels in a wide range of foods. The last EFSA update published in 2011 summarized the data reported by the Member States from EU of furan content in food for a total of 5050 analysed samples of different food categories. Likewise, several studies around the world have been focused into evaluating the levels of furan present in their highly consumed foods.

Figure 2.1 represents the levels of dietary furan currently reported by EFSA (2011), per four defined main food categories: (i) coffee (instant and roasted); (ii) food packed in close containers (jarred baby food, canned foods, fruit juices and jams); (iii) low moisture starchy foods (breakfast cereals, bread, biscuits and snacks) and (iv) others (beer, soups, soy sauce and milk products).

In this sense, a more detailed information, can be observed in Table 2.1, which summarizes the mean furan values for different food samples reported by EFSA in 2010 (EFSA, 2010) and 2011(EFSA, 2011) as well as two Asian studies (Kim, Lee, Kim, et al., 2009; Liu & Tsai, 2010). Additionally some results of furan levels present in coffee and baby foods obtained in Brazil (A. P. Arisseto et al., 2011; Olivares C et al., 2007), Spain (Altaki et al., 2011), Germany (Lachenmeier et al., 2009) and Finland (Claeys, De Vleeschouwer, & Hendrickx, 2005; Jestoi et al., 2009) are reported in Table 2.2

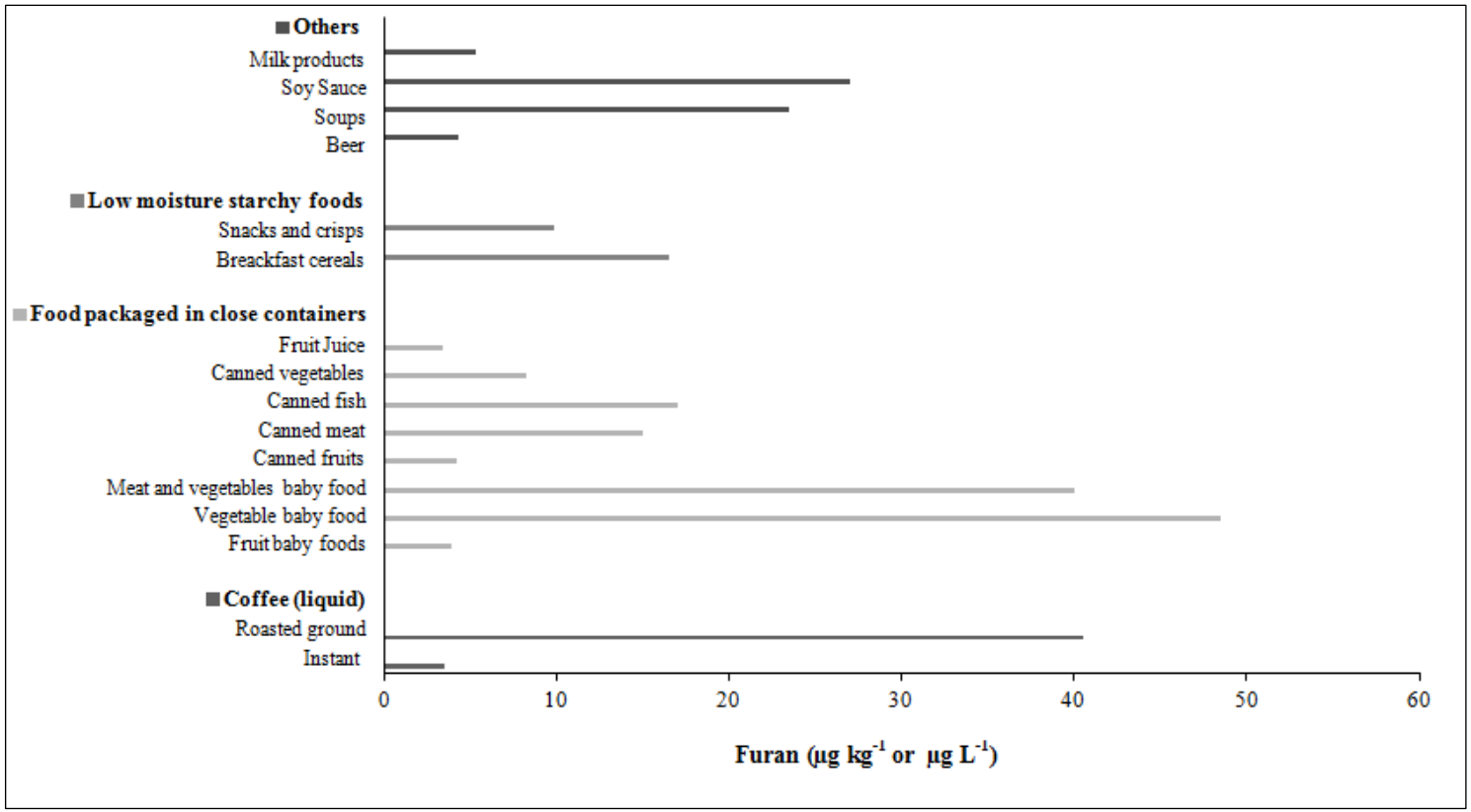

Figure 2.1: Furan levels in food processed at high temperatures.

Source: Adapted from EFSA (2011)

Table 2.1: Comparison of furan levels in foods of different geographical areas.

Sample description	**EFSA 2010 (EFSA, 2010)**	**EFSA 2011** (EFSA, 2011)	**Korea (Kim, Lee, Kim, et al., 2009)**	**Taiwan** (Liu & Tsai, 2010)
Instant coffee (liquid)*	0.0 - 9.0	0.0 - 7.0	3.5	58.0
Roasted ground coffee (liquid)*	27.0 - 30.0	39.0 - 42.0	48.5	70.3
Fruit baby foods	2.5 - 5.0	2.5 - 5.3	-	14.1
Vegetable baby food	39.0 - 40.0	48.0 - 49.0	22.5	58.5
Meat and vegetables baby food	39.0 - 40.0	40	-	124.1
Canned fruits	2.0 - 5.0	2.0 - 6.4	1.3 - 4.0	3.4 - 15.2
Canned meat	17.0 - 19.0	13.0 - 17.0	9.2 - 63.3	76.2
Canned fish	17.0 - 18.0	17	13.65 - 60.62	4.2 - 75.2
Canned vegetables	7.0 - 9.0	6.9 - 9.6	2.9 - 44.1	33.9 - 99.5
Fruit Juice	2.5 - 5.0	2.2 - 4.6	1.7 - 5.7	2.8 - 46.7
Jams	-.	-	2.3 - 4.8	9.2 - 15.2
Breakfast cereals	15.0 - 18.0	15.0 - 18.0	-	12.7 - 65.3
Bread	-	-	1.9	-
Biscuits	-	-	7.6	-
Snacks and chips	10	9.6 - 10	6.8	-
Beer	3.3 - 5.2	3.3 - 5.2	2.3 - 4.8	3.0
Soups	23.0 -24.0	23.0 - 24.0	17.6 - 18.5	-
Soy Sauce	23.0 -24.0	27	16.3	71.2
Milk products	5.0 - 6.0	5.0 - 5.6	3.8	2.4 - 28.7

*ng ml^{-1}

Table 2.2: Furan levels in coffee and baby foods of different countries.

Sample description	**Brazil** (A. P. Arisseto et al., 2011)	**Spain** (Altaki et al., 2011)	**Germany** (Lachenmeier et al., 2009)	**Finland** (Jestoi et al., 2009)
Instant coffee (liquid)*	-	12.1 - 35.2	-	-
Roasted ground coffee (liquid)*	10.0 – 288.0	32.3 - 35.3	-	-
Fruit baby foods	n.d. - 5.7	5.0	4.9 - 11.9	4.7 - 14.1
Vegetable baby food	23.8 - 77.4	37.8	13.9 - 48.5	22.6 -73.4
Meat and vegetables baby food	10.7 - 95.5	25.2	13.2 - 43.2	30.3 - 90.3

*ng ml^{-}

For all studies, the coffee category presented the highest furan concentration (figure 2.1), however some differences can be observed between the reports (Table 2.1 and Table 2.2), and for instance Brazilian roasted preparations reached furan levels of 288 μg kg^{-1}. This fact, could be explained since furan levels were quantified in liquid coffee, thus the handling of this beverages could heavily influence the final furan content in food samples.

On the other hand, in Table 2.1 it is possible to observe that Asian foods products, specially canned meat and canned fish, presented the highest furan levels (~ 76.2 μg kg^{-1} and 75.2 μg kg^{-1}, respectively). For the case of meat products, it could be attributed to the fact that Asian preparations include pork seasoned meat which is commonly fried, thus additionally to the canned processing the previous frying of foods may contribute to the furan generation. Likewise, the differences between canned fish products could be explained since those products are also seasoned and roasted before the canning.

Regarding to baby food, different values of furan have been reported (Table 2.2). For instance Spanish foodstuffs showed to have lower furan concentration than values reported by EFSA. On the other hand Taiwanese vegetable baby food presented the highest furan concentration in this food type (58 μg kg^{-1}). This situation suggests that more than processing conditions, the raw material composition could be the key factor over furan generation, since it is considered that all different baby foods are processed at similar conditions independently of their geographical region.

On the other hand, an interesting study performed by Colin Crews and Agency (2009) for EFSA in 2009 reported the levels of furan in a range of foods after cooking using normal domestic techniques. Additionally this study reports furan levels in the air surrounding kitchens during cooking. Results confirmed that *cafetiere* coffee is the major dietary furan source for adults (~30 μg kg^{-1} of powder coffee) since this beverage is prepared in hermetic automatic machines which reach elevated processing temperature.

Interestingly, high levels of furan were also measured in toasted bread (~200 to 400 μg kg^{-1}) while white bread did not contain more than 3 μg kg^{-1} furan before toasting.

Above information suggests that furan content in foods may be influenced by the habits of preparation and seasoning of the different countries. Finally, the raw material composition of foods, which is also affected by geographical region, could be considered as an important factor in furan occurrence.

In the following section we will summarize and discuss critical information about dietary exposure to furan and its risk characterization.

2.4. Exposure assessment for dietary furan

In 2004, EFSA presented its first estimated of furan exposure (Fromberg, Sisse, & Granby, 2009). Dietary furan exposures of 33.5 μg day^{-1} and 1.1 μg day^{-1} were reported for adults(15-75 years old) and children (4-6 years old), respectively. The 95% of dietary furan intake of adults came from the consumption of coffee. Additionally, children were the group which contributes the most to the intake of furan through breakfast cereals. Previous exposures revealed a high risk for population and the need of continuing dietary furan surveys in order to have a better estimation.

Thus, subsequent monitoring has been focused into evaluate the mean furan exposure, considering the average body weight of each age sector in order to obtain a more realistic estimation of dietary furan exposures.

Table 2.3 summarizes the current information, including not only the last update reported by EFSA in 2011 but also other studies performed in Asia and South America. Interestingly, for exposure estimated in infants, some difference can be observed, since values reported by EFSA are higher (< 50% for the worst exposure scenario) than those presented for Spanish, Finnish, German, Brazilian and Asiatic population. This fact could be attributed to the differences in food costumes and consume habits between geographical areas.

Besides the infant situation, for adult exposure to furan a similar trend can be observed. In this respect, it is worth noting that coffee continues being the major contributor to furan dietary exposition for this age sector (<95%).

Table 2.3: Furan exposure (µg kgbw-1day-1) per age sector in different geographical regions of the world.

Geographical region	Country	Infants	Adults	Reference
Europe	Germany	0.500 (6 months)	-	(Lachenmeier et al., 2009)
	Spain	0.140 (6 months)	0.380 (female)** 0.250 (male)**	(Altaki et al., 2011)
	UE(2011)	0.270 - 1.010 (3 - 12 months)	0.290 - 1.170	(EFSA, 2011)
North America	USA (2004)	0.410 (0 - 12 months)	0.250-0.230	(FDA, 2004)
	Canada	1.120 (1-4 years)	0.37	(Claeys et al., 2010)
South America	Brazil	0.460 (0 - 12 months)	-	(Olivares C et al., 2007)
Asia	Korea	0.017 (6 months)	0.011	(Kim, Lee, Kim, et al., 2009)
	Taiwan	0.470 (6 months)	0.177 (female)** 0.299 (male)**	(Liu & Tsai, 2010)

* Exposure estimate was calculated considering a daily intake of 234 g d-1 and a average weight of 8.5 kg for a baby of 6 months(Claus, Carle, & Schieber, 2008).

** Exposure values correspond only to coffee consumption.

On the other hand, Colin Crews and Agency (2009) studied furan intake from different foods considering the volatility of the contaminant. In this study, not only the final furan content of food after its handling but also the furan presents on the air surrounding the kitchen were considered for exposure estimations. Table 2.4 summarizes the changes of furan levels caused by food cooking as well the levels of volatilized furan on air surrounding the kitchen and consumer exposure to furan from cooked food and air surrounding the kitchen.

As was expected for a volatile compound, a change in furan levels was caused by food preparation (compared to values presented in Table 2.1 and Table 2.2). In this sense, not only a reduction (*e.g.* baby food after microwave heating) but also an increase (*e.g.* bread after toasted) in furan levels was observed for the different food samples analyzed. This contradictory response after the re-heating could suggest an important retention effect of the food matrix over the final furan content.

Interestingly, a decrease of furan level in the food was associated to an increase in the furan occurrence on the air surrounding the kitchen.

Additionally, the highest degree of furan inhalation resulted from: (i) addition of hot water to coffee in a *cafetiere* (5 ng L^{-1}), (ii) frying of chipped potatoes in an open chip pan (between 5 and 35 ng L^{-1}), and (iii) baking some foods in an oven (55-128 ng L^{-1}).

It is important to note that although this report indicates that coffee is the major contributor to furan exposure in adults, the reported furan levels are lower than those reported in the last scientific inform of EFSA presented in Table 2.1(ng v/s μg, respectively). It could be due to differences between the (i) varieties and (ii) roasting conditions of coffees analyzed in both studies.

Table 2.4: Consumer exposure to furan from food and air during food cooking.

Sample	Cooking method	Furan (ng kg^{-1})	Portion (g)	Furan intake (µg g^{-1})	Air furan (ng kg $-^{1}$)	Exposure time (min)	Air inhaled (L)	Furan inhaled (ng L^{-1})
Coffee instant	Cup brewed	2.0	220.0	0.4	0.6			
Coffee powder	*Cafetiere* brewed	28.0	220.0	6.1	8.0	10.0	50.0	400.0
White bread	Toasted	299.0	130.0	38.9	5.0	6.0	30.0	150.0
White bread	Toasted home	3.0	130.0	0.4	0.8	6.0	30.0	24.0
Wholemeal bread	Toasted	150.0	150.0	22.5	1.6	6.0	30.0	48.0
Wholemeal bread	Toasted home	5.0	150.0	0.8	0.5	6.0	30.0	15.0
Cookies	Baked from Mixture	7.0	150.0	1.1	0.3	15.0	75.0	23.0
Bean convenience meal	Microwaved	2.0	400.0	0.8	0.3	7.0	35.0	11.0
Meal convenience meal	Microwaved	5.0	400.0	2.0	0.4	8.0	40.0	16.0
Soup convenience meal	Microwaved	2.0	340.0	0.7	2.9	4.0	20.0	58.0
Infant food vegetable	Microwaved	15.0	180.0	2.7	2.5	4.0	20.0	50.0
Infant food meat	Microwaved	17.0	120.0	2.0	1.7	4.0	20.0	34.0
Tomato soup	Saucepan heated	26.0	400.0	10.4	2.1	5.0	25.0	53.0
Tomato soup	Microwaved	32.0	400.0	12.8	1.6	6.0	30.0	48.0
Pizza chilled	Baked	3.0	300.0	0.9	0.8	12.0	60.0	48.0
Pizza frozen	Baked	19.0	300.0	5.7	0.5	30.0	150.0	75.0
Breaded Chicken	Oven Cooked	7.0	300.0	2.1	0.7	20.0	100.0	70.0
Breaded vegetables	Oven Cooked	6.0	150.0	0.9	3.1	20.0	100.0	310.0
Breaded fish	Oven Cooked	5.0	200.0	1.0	0.9	22.0	110.0	99.0
Chips part cooked, frozen	Microwaved	2.0	100.0	0.2	0.9	3.0	15.0	14.0
Chips frozen	Oven Cooked	2.0	500.0	1.0	2.1	17.0	85.0	179.0
Chips from fresh potatoes	Fried	12.0	500.0	6.0	9.5	11.0	55.0	523.0

Source: Adapted from Colin Crews and Agency (2009)

Finally, the presence of furan in a wide range of foods confirms the fact, that it could be generated by several mechanisms in foods processed at high temperatures. Next section will be focused in describing the pathways and some factors responsible of furan formation.

2.5. Mechanistic pathways of furan formation

The primary source of furan in food was thought to be from thermal degradation and rearrangement of organic compounds, particularly carbohydrates (Maga, 1979). Furan and its derivatives have been associated with flavour of many foods and some studies have shown that there are several distinct pathways responsible for its formation (Morehouse, Nyman, McNeal, Dinovi, & Perfetti, 2008; Zoller et al., 2007). Literature information suggests multiple sources of furan formation originating from: (i) thermal degradation/Maillard reaction reducing sugars, alone or in the presence of amino acids, (ii) thermal degradation of certain amino acids, and thermal oxidation of (iii) ascorbic acid, (iv) poly-unsaturated fatty acids and (v) carotenoids (A. Becalski & Seaman, 2005; Perez Locas & Yaylayan, 2004).

According to the FDA (2004), a variety of carbohydrate/amino acid mixtures or protein model systems (*e.g.* alanine, cysteine, and casein) and vitamins (ascorbic acid, dehydroascorbic acid, and thiamin) have been used to generate furan in food. In this respect, some factors such as heating temperature, pH of and moisture content have shown to have a considerable effect over furan generation, since most of the mechanisms insights the formation of furan depend on them (Fan, Huang, & Sokorai, 2008; A. Owczarek-Fendor et al., 2010). Figure 2.2 summarizes the general pathways leading to the furan formation from these sources.

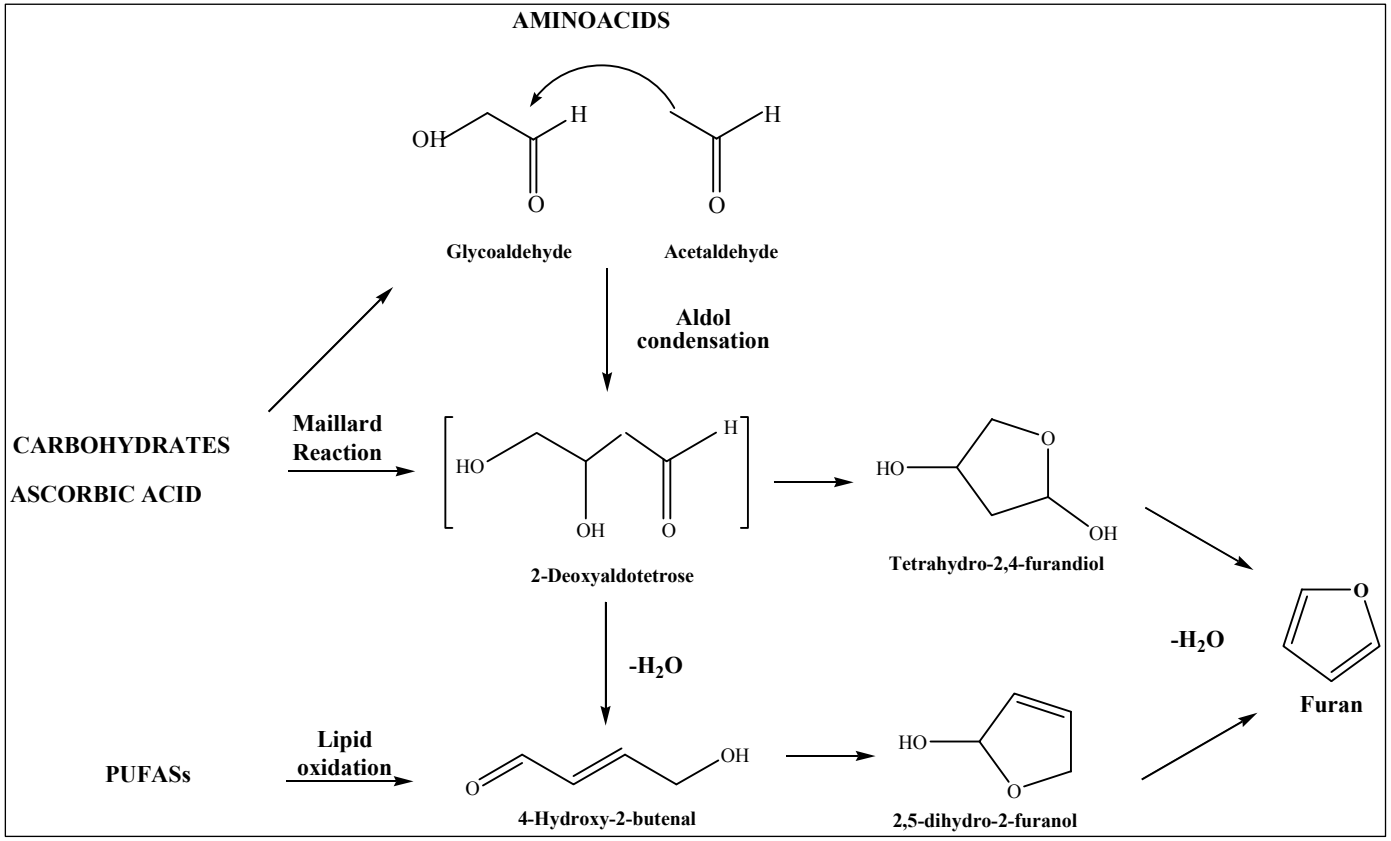

Figure 2.2: Different origins of furan formation.

Source: Adapted from C. Crews and Castle (2007); Limacher et al. (2007); Limacher et al. (2008); Perez Locas and Yaylayan (2004); Vranova and Ciesarova (2009)

2.5.1 Furan formation from carbohydrates and Maillard reaction.

Reducing sugars are known to undergo Maillard reaction in the presence or absence of aminoacids and generate furan (Perez Locas & Yaylayan, 2004). Regarding to this issue, several pathways and reactive intermediates of furan have been proposed considering the fact that Maillard reaction is highly complex so the possibility of the existence of all of these pathways are probable. Mechanistic pathways of formation of furan from hexoses and pentoses can be observed in Figure 2.3.

Furan generation in model systems based on sugars and selected amino acids simulating food process conditions such as roasting and pressure cooking was recently studied by Limacher et al. (2008). These authors found that furan was preferably formed under roasting conditions (330 μmol/mol *vs* 20 μmol/mol for roasting and pressure cooking, respectively). In the absence of amino acids and under dry heating conditions, furan was mainly formed from the intact sugar skeleton. Nevertheless, the presence of alanine, threonine, or serine promoted furan formation by the recombination of C2 fragments, such as acetaldehyde and glycolaldehyde, which may originate from both sugars and amino acids. These results are consistent with the already proposed pathway of furan formation from Maillard reaction model systems proposed by Perez Locas and Yaylayan (2004). However, Limacher et al. (2008) just observed the mentioned pathway in aqueous solutions.

On the other hand, in pumpkin puree (which is a more realistic model system) they found that only 20% of furan was formed from sugars, preferably from the intact carbon skeleton.

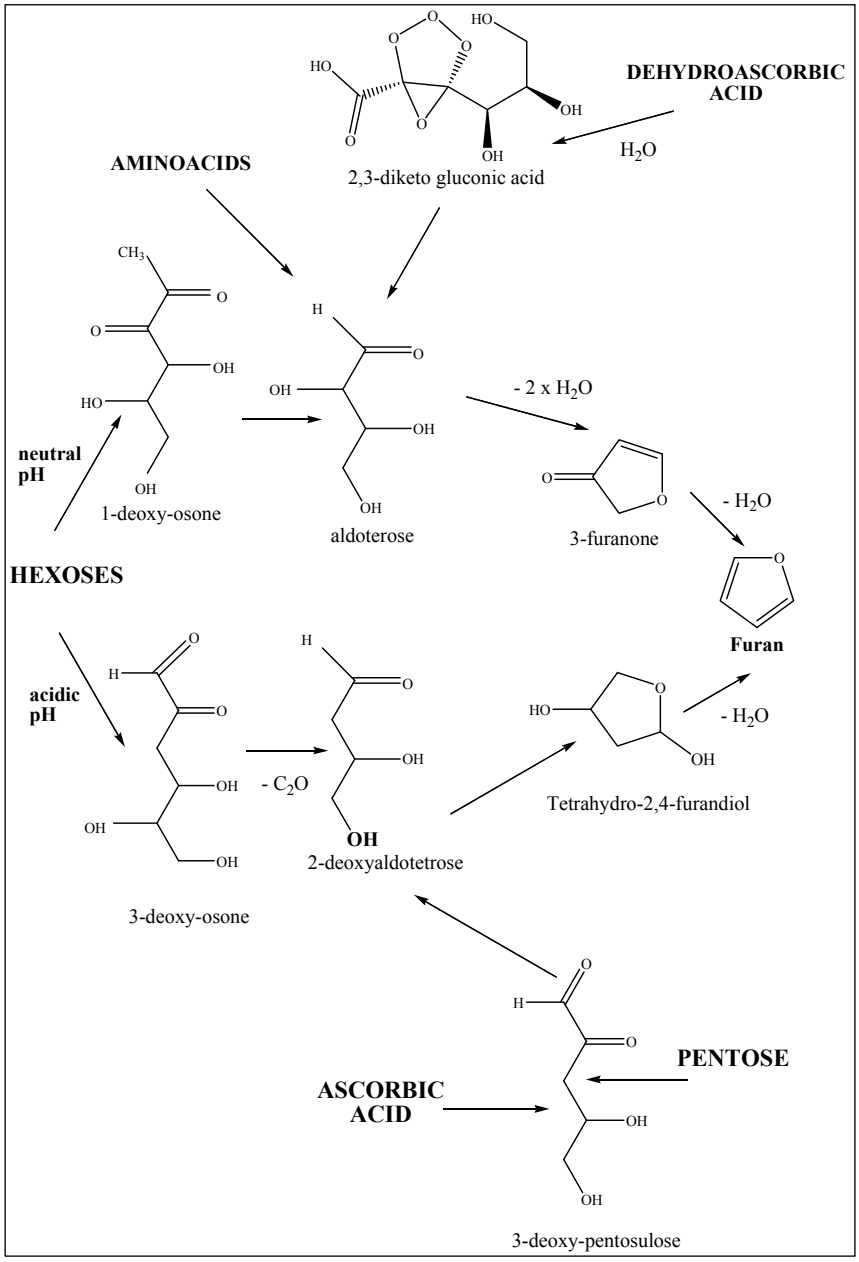

Figure 2.3: Furan formation from Maillard types reactions.

Source: Adapted from Vranova and Ciesarova (2009); Yaylayan (2006), Limacher et al. (2007); Limacher et al. (2008); Perez Locas and Yaylayan (2004).

Additionally, recent studies in simple Maillard reaction systems performed by F. A. Van Lancker, An Owczarek-Fendor, Agnieszka De Meulenaer, Bruno De Kimpe, Norbert (2011) revealed that under both dry-roasting and pressure cooking conditions, glucose-derived furan was formed from the intact sugar skeleton and not from fragmentation and recombination mechanisms. These authors also found that the presence of amino acids did not influence the formation pathways of furan from glucose. Interestingly some amino acids (especially alanine and serine) could provide an additional formation pathway as was proposed before by Perez Locas and Yaylayan (2004) since those could form not only acetaldehydes but also glycoaldehyes. Finally, evidence is given for furan formation by two principle pathways: (i) sugar degradation keeping the intact carbon skeleton; (ii) recombination of some fragments that may originate from both sugars and specific amino acids.

Pentoses have shown to be more efficient than hexoses in furan formation in simple model systems (Limacher et al., 2008). When amino acids were added to the reaction systems, contradictory results were found regarding to furan formation. Either with sugars model systems or with those with amino acids and sugars, heating condition is the most relevant factor in the molar yield of furan.

2.5.2 Furan formation from ascorbic acid

Ascorbic acid has been proposed as one of the major precursors of furan. In this respect, Perez Locas and Yaylayan (2004) studied the furan formation in model systems under pyrolysis conditions and proposed that furan generation from ascorbic acid can occur either under oxidative or non-oxidative conditions. Firstly, when the conditions are oxidative, the ascorbic acid is oxidized to dehydroascorbic acid which can then be converted to furan. However, this pathway is more improbable since only low furan amounts could be generated (Limacher et al., 2007; Mark, Pollien, Lindinger, Blank, & Mark, 2006). Additionally, when the ascorbic acid is heated in the absence of water, it can form a cyclic hemiketal which prevents furan formation. Secondly, when the conditions were non-oxidative, the ascorbic acid could also form furan and it is a more

efficient source of furan than dehydroascorbic acid. Proposed mechanisms of furan formation from both ascorbic and dehydroascorbic acid are shown in figure 2.3.

Likewise, Fan (2005) investigated the formation of furan from ascorbic acid as affected by ionizing radiation and thermal treatments, concluding that both thermal treatments and irradiation induced formation of furan from ascorbic acid. On the other hand, studies performed in pure model systems concluded that ascorbic acid was the most efficient precursor in furan generation. Then, additional research showed clearly that ascorbic acid efficiency in furan generation relied markedly on the heating conditions. For instance, ascorbic acid is less effective in furan generation than polyunsaturated fatty acid under cooking-pressure conditions (A. Becalski & Seaman, 2005).

The influence of other furan precursors such as sugars and amino acids over ascorbic acid efficiency has been studied by some researchers using carbon module labelling (CAMOLA). By using this technique, Limacher et al. (2007) observed that furan formation from this precursor was significantly slowed down in binary mixtures (*e.g*. the presence of erythrose led to 80% less furan) heated under roasting conditions. This result could be attributed to competing reactions in complex systems which disfavor furan formation. This mitigation effect could be explained since furan is formed without recombination of ascorbic acid fragments leading to foods with much lower furan levels than expected from those generated with pure ascorbic acid. Similar results in dry heating conditions (200 °C) were previously obtained by Mark et al. (2006) who found that simple binary mixtures of ascorbic acid and amino acids, sugars, or lipids reduced furan generation by 50-95%. These authors also observed that furan yields from ascorbic acid were lowered not only by an oxygen-free atmosphere (30%) but also by the presence of reducing agents (*e.g.,* sulfite, 60%) suggesting the important role of oxidation steps in the furan formation pathway. Contradictory to these results, A. Owczarek-Fendor et al. (2010) observed that the presence of starch enhanced drastically the furan formation from ascorbic acid during pressure cooking of starch-based model baby food simulated systems. Interestingly, Mariotti et al. (2012) obtained similar results in fried and baked starchy food model systems based on wheat flour where ascorbic acid

was added leading to a significant increasing in furan generation. These authors concluded that there is a synergism between ascorbic acid and the other furan precursors naturally present in the flour based model systems. Finally, Lachenmeier et al. (2009) found that the formation of furan in potato based baby foods was increased by addition of ascorbic acid when the heating temperature was above 50 °C and the heating time ~ 1h. .

2.5.3 Furan formation from poly unsaturated fatty acids

Since furan formation was detected during lipid oxidation, some authors have hypothesized that furan might be formed due to the radical reaction mechanism which is not particularly selective as it is shown in figure 2.4.

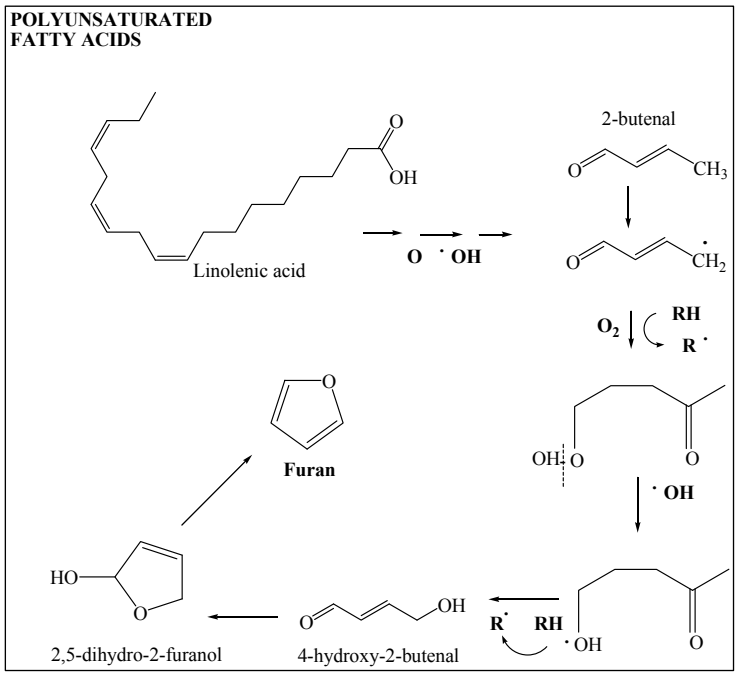

Figure 2.4: Furan formation from poly unsaturated fatty acids.

Source Adapted from Perez Locas and Yaylayan (2004) and A. Becalski and Seaman (2005).

A. Becalski and Seaman (2005) studied the oxidation of unsaturated fatty acids at elevated temperatures (130 °C, 30 min) as possible furan pathway finding that from 3 unsaturated acids tested (oleic, linoleic, and linolenic), only polyunsaturated acids generated furan upon heating as expected. These results shown that linolenic acid generates ~4 times more furan than linoleic acid does. Besides, catalytic amounts of ferric chloride increase furan production for these acids by factors of 2 and 4, respectively. The corresponding triglycerides of linoleic and linolenic acids formed furan at levels comparable to those of the parent acids; however, the triglycerides generated less furan than the acids did in the presence of ferric ions. Conclusively, these authors stated that the formation of furan is very likely to proceed via a radical hydroxyperoxide route, a reaction known to be catalyzed by iron ions. These findings are in agreement with those published by Perez Locas and Yaylayan (2004), suggesting a crucial role of 4-hydroxy-2-butenal, a typical 4-hydroxy- α,β-unsaturated aldehyde generated during the oxidation of ω-3 fatty acids such as R-linolenic acid, as a potent furan precursor. Recent research made by Agnieszka Owczarek-Fendor et al. (2010) shows the clear role of fat oxidation in furan generation of starch-based emulsions heated at 120 °C for 20 min. Their results shown that both the oil type (fatty acid composition), and the oil oxidation degree determined the susceptibility of the oils to generate furan upon heating. Thus, oils containing α-linolenic acid proved to be able to generate significant amounts of furan if the oils were oxidized. Surprisingly, no clear relationship between p-anisidine values of various oils and the amount of generated furan could be observed. Soybean oil has shown the tendency to increase significantly furan formation when its oxidation degree increases dramatically (extremely high levels which are not allowed and sensorial tolerable for the consumer). From all the above studies regarding to furan formation, it is possible to conclude that the efficiency of furan generation from different precursors in models systems depends not only on the heating conditions but also on other intrinsic factors of each model system leading sometimes to contradictory results and difficulties for data interpretation. Thus,

extrapolation of the results obtained from simple model systems to real foods could lead to mistaken conclusions.

2.5.4 Furan formation

"A function of heating conditions and some intrinsic factors"

2.5.4.1 Heating conditions

Previously, the formation of furan, typically produced by heat treatment in closed recipients, was discussed in model systems under conditions representing pasteurization and sterilization (A. Becalski & Seaman, 2005; Fan, 2005; Limacher et al., 2007; Limacher et al., 2008; A. Owczarek-Fendor et al., 2010; Agnieszka Owczarek-Fendor et al., 2010), at very high temperatures during pyrolysis (Perez Locas & Yaylayan, 2004) and under roasting conditions (Limacher et al., 2007; Limacher et al., 2008; F. A. Van Lancker, An Owczarek-Fendor, Agnieszka De Meulenaer, Bruno De Kimpe, Norbert, 2011). Moreover, the impact of exposure of model systems to ionizing radiation (Fan, 2005), frying and baking (Mariotti et al., 2012) on furan formation was also reviewed. In all of these studies the efficiency of furan formed from different precursors presented a high dependency on the heating conditions. For instance, under pressure-cooking conditions at 130°C, the potential of furan formation from ascorbic acid has shown to be lower compared to that exhibited by polyunsaturated fatty acids (A. Becalski & Seaman, 2005), while under roasting conditions (180°C) an opposite trend was observed (Limacher et al., 2007; Mark et al., 2006). Likewise, respecting to the efficiency of sugars in furan formation, under dry-roasting conditions glucose have shown to produce 25-100 times higher amounts of furan as compared to pressure cooking conditions. This important difference is coincident with the results obtained by Limacher et al. (2008). Additionally, F. A. Van Lancker, An Owczarek-Fendor, Agnieszka De Meulenaer, Bruno De Kimpe, Norbert (2011) observed in glucose/alanine model systems that the mechanism and kinetics of furan formation as well as its quantitative production were also different under dry-roasting conditions as compared to pressure-cooking conditions.

These ambiguous results could be attributed to the differences in processing temperatures and also to the heat transfer phenomena. Finally, considering the fact that furan is a very volatile compound, the effect of sample container should be considered as relevant factors over the final furan content since it could migrate easily from the food matrix to its surface, and then, from food surface to the surrounding air in open containers.

2.5.4.2 Intrinsic factors

The effect of some intrinsic factors such as precursor concentration, pH and head space volume has also been considered as a relevant issue in furan formation by several authors. For instance, Fan (2005) studied the effect of precursor concentration and pH over furan formation from major components in fruit juices (sugars and ascorbic acid) under ionizing radiation and thermal treatments, founding that both mentioned factors had an strong effect on furan formation. Furan generation increased with increasing concentration of ascorbic acid and carbohydrates. The fastest increase in furan generation was observed when concentrations of ascorbic acid and sugars ranged from 0 to 5 mg/ml. No significant increase in furan formation was observed when higher concentrations of the precursors were used in the model systems. Besides, when the pH of the system decreased, the furan formation rate increased significantly. Similar results were observed by Limacher et al. (2007) in ascorbic acid model systems heated under pressure cooking and roasting conditions, in which the furan content increased at the lowest pH tested (pH= 4.0). This fact could be explained since at acidic conditions sugar-amine reactions are unlikely (Fan, 2005; Fan et al., 2008; Limacher et al., 2007; Limacher et al., 2008; A. Owczarek-Fendor et al., 2010) and Maillard reaction occurs mainly from ascorbic acid degradation to highly reactive carbonyls compounds (*e.g.* 3-deoxypentonose and 3,4-dideoxypentosulos-3-ene) which then interact with amines (Clegg & Morton, 1965). Additionally, Fan et al. (2008) observed that heat-induced furan formation from free sugars, ascorbic acid, and linoleic acid was profoundly affected by pH and the presence of phosphate, an important food additive used as

emulsion stabilizer and pro-antioxidant in many processed foods. These authors observed that in linoleic acid emulsions, more furan was generated at pH 6 than at pH 3. On the other hand, the presence of phosphate increased furan formation for all substrates studied under the pH mentioned conditions. A. Owczarek-Fendor et al. (2010) observed that both the pH and the head space volume could affect the furan formation from ascorbic acid in heated starchy-gel model systems. pH related effects over furan formation were similar to those previously reported (Fan, 2005; Fan et al., 2008; Limacher et al., 2007; Limacher et al., 2008). Finally, when the head space volume was diminished, the furan formation was drastically reduced. Interestingly, these authors also found that changes in ascorbic acid concentrations in starchy model systems from 0.1 to 4.5 mg/g did not influence furan concentration. These results are not in agreement which those previously reported by Fan (2005), and this issue could be attributed to the effect of starch matrix over furan retention (which is a target remained to study deeply), since in Fan (2005) all experiments were performed in aqueous solutions. Finally, F. Van Lancker et al. (2009) studied the effect of heated model systems with different ingredients over furan retention. These authors spiked various samples (oils, starch gels, coffee, baby food and spinach) with D_4-furan and compared D_4-furan evaporation from these samples with comparable aqueous solutions. Additionally, they quantified furan in all the samples tested, finding that oils provoked higher furan retention than starchy gels. However, since furan retention was also found in defatted coffee and coffee grounds, other coffee constituents also have the ability to retain furan. The retention of furan is an important factor since it is directly correlated with the amount of furan that remains in the food matrix and consequently with the actual human intake. It is interesting to note that Mariotti et al. (2012) observed that furan content increased in fried starchy food model systems as oil uptake did. In addition the heating conditions, some important factors such as pH and matrix microstructure should be considered as critical parameters to improve the understanding of furan formation in different food matrixes. The proper management of this knowledge will contribute to the development of new processes and technologies for diminishing the furan content in foods.

2.6. How to mitigate furan in foods?

Heating foods has many advantages since it adds taste, colour, texture and minimizes harmful germs, among others. On the other hand, various hazardous compounds are produced by high temperature reactions, such as furan (Van Boekel et al., 2010). In this sense, a variety of technologies have been developed to reduce several heat processed food contaminants (*e.g*. acrylamide) based either on: (i) changing process parameters (*e.g.* time and temperature of cooking) which inhibits reactions of formation and (ii) reducing contaminant precursor levels in raw materials to be cooked at high temperatures (*e.g.* by using microorganisms, asparaginase, amino acids and saccharides, blanching, etc.)(Mariotti, Pedreschi, Carrasco, & Granby, 2011). More research is needed for developing previously mentioned technologies since before developing technologies to remove furan precursors it is necessary to identify their behaviour during processing in real foods. Considering that furan is a volatile compound, several researchers have evaluated the possibility of mitigating this contaminant by intervening traditional food preparation techniques (Al-Taher, Didsatha-Amnarj, Jackson, & Varelis, 2008; Bianchi, Careri, Mangia, & Musci, 2006; C. Crews et al., 2009; Guenther et al., 2010; Kim, Lee, Park, & Lee, 2009; Roberts et al., 2008; Zoller et al., 2007). For example, EFSA has evaluated the effect of food handling and of types of re-heating in products such as coffee, baby food, toasted bread and different soups in order to understand better furan generation mechanisms. They concluded that it is possible to reduce the furan content in some foods simply by volatilization through heating and stirring of canned or jarred foods in an open saucepan (Fromberg et al., 2009). Current research on furan has not been successful in identifying practical and consistently effective solutions for diminishing its content in foods. The interventions in the scientific literature on furan are mostly targeted at the level of consumer as is shown in Table 2.5.

Table 2.5: Potential interventions for furan reduction in food.

Area	Food category	Proposed Solutions	Furan reduction	Reference
Food handling	Jarred and canned foods	Heating (saucepan, conventional oven and microwave). Regular stirring increased furan reduction	≥ 40 % (higher reduction with saucepan heating)	(Lachenmeier et al., 2009)
		Heating (saucepan and microwave) Higher temperatures increase furan reduction	≥ 46 % (no differences between heating) methods)	(Bianchi et al., 2006)
		Opening , Heating (microwave), Refrigerating	≥ 22 %, 30% and 50% respectively	(Fromberg et al., 2009)
		Heating open jars (microwave) Stirring after heating increased furan reduction	≥ 29 %	(Guenther et al., 2010)
		Atypical home heating open jars (5.5 h) Opening jars (5.5 h)	85% and 50% respectively	(Kim, Lee, Kim, et al., 2009)
	Instant coffee	Transferring and letting stand for 4 hours	~ 50 %	(Guenther et al., 2010)
		Resting at room temperature for 1hour	~ 50 %	(Hasnip et al., 2006)
		Resting at room temperature for up to 20 min	~ 40 %	(Kim, Lee, Park, et al., 2009)
	Brewed coffee	Pouring in cups and letting stand up 30 min Keeping warm in a hotplate increase furan reduction	≥ 25 %	(Hasnip et al., 2006)
		Resting at room temperature for up to 20 min	~ 30 %	(Kim, Lee, Kim, et al., 2009)
		Heating in an open carafe for 1 hour	Significantly	(A. P. Arisseto et al., 2011)
	Bread	Diminishing the toasting level	Significantly	(Fromberg et al., 2009)
Ingredients and processing	Baby foods	Controlling the pH of food products Higher pH produce higher furan formation	Significantly	(A. Owczarek-Fendor et al., 2010)
		Controlling the processing temperature Higher T° produce higher furan formation	Significantly	(A. Owczarek-Fendor et al., 2010)
		No adding ascorbic acid	Significantly	(FDA, 2004)

Although formation and mitigation research of furan in foods suggest the potential for interventions in the areas of ingredient addition and thermal processing, such actions could have serious microbiological or nutritional effects (*e.i*. the changing of thermal profiles could decrease shelf-life of food and also the digestibility and bioavailability of its nutrients) and cannot be taken lightly.

2.7. Conclusions

Furan is considered a hazardous chemical for humans and its critical toxicological effect is carcinogenicity (Group: 2B). Although there is current available information about furan reproductive toxicity, the mechanism of furan induced carcinogenicity in rodents as well as its effect on humans, have not been clarified yet. Given that carcinogenesis is a highly complex process, furan could induce hepatocarcinogenicity as result of both genotoxic and chronic cytotoxic modes of action, and the relative role of each one would vary with dose, treatment duration, species, sex, strain, and environmental conditions.

Monitoring furan levels in foods have outlined its occurrence in a broad variety of foods (*e.g*. coffee, baked products and baby foods, etc) from non-detectable levels to ~6000 $\mu g\ kg^{-1}$ suggesting that furan could be formed from different precursors.

Despite ascorbic acid has shown the highest potential to form furan, furan levels present in foods are much lower than expected from trials with pure ascorbic acid. Then, conclusions about prior pathway for furan formation should be drawn with much caution from model reactions, avoiding extrapolation from oversimplified model systems to food products. The effect of different factors such as heating conditions, pH and matrix should be consider during the design of model systems, since they clearly affect the final furan content.

Considering that children can be exposed to particular high doses of furan via diet (since almost all of the baby foods contained furan) and that the knowledge about the mechanism (s) of tumour induction by furan remains uncertain, it is crucial to develop

some mitigation technologies of furan formation in foods in order to prevent some human diseases as cancer.

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3. ARE CHILEAN EXPOSED TO DIETARY FURAN?

Abstract

Chilean consumer preferences include foods which may contain considerable amounts of furan, a potential human carcinogen. However, there is no information regarding to dietary exposure to furan in Chile. Thus, the objective of this work was to determine the Chilean exposure to dietary furan. To accomplish this objective, the furan concentration of 14 types of commercial foods processed at high temperature was analyzed based on a modified Head Space-Gas Chromatography mass spectrometry (HS-GC/MS) method in which the limits of detection for different food matrixes ranged from 0.01 ng g^{-1} to 0.6 ng g^{-1}. In addition, a risk assessment was made with exposure estimates based on dietary data from national studies on different age groups (9 month old babies, school children, adults and elderly people). Of the food items surveyed *"American"* type coffee (espresso coffee plus hot water) obtained from automatic coffee machine (936 ng g^{-1}) and low moisture starchy products like chips and "*Soda"* type crackers showed the highest furan concentrations (259 ng g^{-1} and 91 ng g^{-1} respectively). Furthermore, furan was also found in samples of breakfast cereals (~20 ng g^{-1}), jarred fruit baby foods (8.5 ng g^{-1}) and orange juice (7.0 ng g^{-1}). School children (9-13 years) represented the highest intake of furan (~ 500 ng kg^{-1}_{bw} day^{-1}), with margins of exposure of 2479 and 2411 respectively, which points to a possible public health risk.

Key words: furan, food, head space gas chromatography – mass spectrometry, dietary exposure.

3.1. Introduction

The occurrence of furan, a potential carcinogen for humans in several thermally treated foods has caused concern by several health organizations, which have emphasized the need to increase the information on the human dietary exposure to this compound.(EFSA, 2004, 2010; Heppner & Schlatter, 2007).

Furan (C_4H_4O) is a small lipophilic organic compound (MW: 68 $gmol^{-1}$) with high volatility (BP: 31°C). It is commonly detected in food processed at high temperature, particularly in products packed in sealed containers (*e.g*. baby food)(Hasnip et al., 2006). However, despite the most important physicochemical property of furan is its high volatility, furan has also been found in low moisture starchy foods not cooked in closed containers such as crackers, crisp, and toasted breads(C. Crews & Castle, 2007; C. Crews et al., 2007; C. Crews et al., 2009; Hasnip et al., 2006). Likewise, furan has been known as a normal component of coffee flavor volatiles. High levels of furan have been found in brewed coffee probably due to the high temperature required for its roasting (EFSA, 2004, 2010).

The diversity of foods found to contain furan suggests that multiple routes of formation are likely to form furan in foods(Morehouse et al., 2008). These mechanisms are based on decomposition of ascorbic acid and related compounds, oxidation of polyunsaturated fatty acids, Maillard reaction, and pyrolysis of sugars (A. Becalski & Seaman, 2005; Perez Locas & Yaylayan, 2004).

The monitoring of furan levels in foods initiated by the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) showedthat furan concentration range from non-detectable to approximately 6000 ng $g^{-1}$for different tested products (EFSA, 2010; FDA, 2004). Additionally, Margins of exposure (MoE) found in Germany (Lachenmeier et al., 2009) and Brazil (A. Arisseto, Vicente, & Toledo, 2010) have suggested that furan exposure in infants could be considered as a possible public health risk.

In this sense, although Chilean consumer preferences include foods which may contain considerable amounts of furan, currently there is no information regarding the dietary exposure to furan in Chile.

Food consumption in Chile has changed markedly in the last decades, showing an increase in ready to eat products (*e.g*. baby foods, chips) as well as an increase in the coffee consumption. The Chilean diet is principally based on cereal and potato products, e.g. bread and fried potato products are highly consumed by school children of 9-13

years old (Rozowski & Castillo, 2004). For instance, Chile is the world's second largest consumer of bread, only surpassed by Germany, reaching a Chilean annual per capita consumption of 98 kilos. Besides, Chilean consumers demand high amounts of fried products (59% of the population consume fried foods three times per week). Annual per capita consumption of coffee in Chile is 0.8 kg which corresponds to 180 cups of liquid coffee (Estrategia, 2012).

Considering that children can be exposed to particular high doses of furan via diet, it is crucial to evaluate the levels of exposure to this compound in this segment of Chilean population.

In the present study, the furan concentration in 14 types of processed foods including fruit baby foods, fruit juices, chips, breakfast cereals, Chilean bread, "*Soda*" type crackers, canned foods, meat and coffee were analyzed based on a modified gas chromatography – mass spectrometry method (GC-MS). In addition, exposure assessment was performed based upon the monitored data obtained.

3.2. Materials and Methods

3.2.1. Chemicals and Reagents

The standard solution of furan and d_4-furan (100 ug ml^{-1}) were provided by the Dr. Ehrenstorfer Company (Augsburg, Germany). Working standards of furan and d_4-furan (1.5 ug ml^{-1}) were prepared with 10 ml of HPLC grade water and 150 µl of standard solution of furan and D_4-furan. Methanol (HPLC grade) and sodium chloride were provided by Merck (Darmstadt, Germany).

3.2.2. Sample collection

14 types of foods were analyzed as follow: 8 brands of tomato sauce, 4 brands of orange juice, 2 brands of vegetable bay foods, three brands of fruit baby foods, three brands of canned corn, three brands of canned peas and five brands of peach marmalade; four brands of asparagus soap, four brand of powder milk and six brands of

powder milk; seven brands of chips, six brands of "Soda" type biscuits, six brands of toasted bread, four brands of whole breakfast cereal and four brands of breakfast cereal. For each type of food products all available brands in Chilean market were considered. The products were obtained from establishments selected randomly in seven different sectors (Center, North West, North, Northeast, South, South East and South west) of Santiago, Chile in order to reflect the diversity of the community. From each geographical sector, 2 samples of each brand were collected.

3.2.3. Sample preparation and spiking procedure

Sample preparation and spiking procedure were performed according to the methodology of furan determination in food of FDA (2004) with some modifications. All food products were stored at 4ºC for 4 hours prior to furan analysis. Liquid samples (5 g) were transferred to a headspace vial and spiked with the internal standard d_4-furan. For biscuits, cookies, and cereals 0.75 g sample was diluted with 5.5 ml sodium chlorine solution 5M. For low moisture samples with high fat content (*e.g*. chips), only 0.5 g of sample were used. For quantification of furan seven vials were used of which four vials were fortified with furan as follows: two vials at approx. half of the expected concentration of furan in the sample, one vial at approx. the expected concentration of furan in the sample, and one vial at approx. twice of the expected concentration of furan in the sample.

3.2.4. Gas chromatography – mass spectrometer (GC-MS) analysis

The samples were analyzed on a Model 7890A/7050 Gas chromatograph – mass spectrometer equipped with a CTC CombiPAL static headspace autosampler. (Agilent Technologies, Santa Clara, California, USA). A capillary column HP-PLOT/Q, 30 m × 0.32 mm ID × 0.20 μm film (Agilent Technologies), was used, with He (99.995%) as carrier gas at the flow-rate of 1 ml min^{-1}. The column temperature was programmed as follows: 50°C holding 1 min to 180 °C at 10 °C min^{-1}. The total analysis time was 14 min. The injector port was kept at 200°C and 1 μl sample was injected in split mode

(4:1). The MS source temperature was 230°C and the MS quad temperature 150°C. Dwell time 50 msec. The mass spectrometer was operated in electron ionization mode. Furan was detected using single ion monitoring of the fragments *m/z* 68 and *m/z* 39. The internal standard d_4-furan was detected by monitoring the *m/z* 72 and *m/z* 42 (FDA, 2004; Zoller et al., 2007).

3.2.5. Headspace operating conditions

The headspace operating conditions were as follow: incubation temperature: 60ºC, incubation time: 20 min; syringe temperature: 70 ºC; agitator speed: 50 ºC, fill speed: 500 mL s^{-1}; pull-up delay: 500 ms; injection speed: 500 µl s^{-1}; injection volume: 1 ml; pre injection delay: 500 ms; post injection delay: 500ms; flush hours: 60 s.

3.2.6. Determination of analytical quality parameters

The limit of detection (*LOD)* and the limit of quantification (*LOQ)* were established for all analyzed food categories. Both *LOD* and *LOQ* were determined by preparing the matrixes as described in the spiking procedure. The standard addition data were subjected to linear regression analysis. The *LOD* and *LOQ* values were defined at a signal to noise ratio (*S/N*) of 3 and 10 respectively. Besides, the recoveries were calculated from the slope of standard addition curve in all the analyzed food matrixes. Since blank material was not available, food samples were fortified with approx. twice the level of the incurred furan concentration found in each ones. For all calculations, each point of the standard addition curve was made in triplicate (Valcárcel, 1999). The recoveries were determined by using the following equation:

3.1 Recovery %= (ng furan found/ng furan added) x 100

Where, ng furan corresponded to the furan amount calculated with the standard addition curve (the background level was subtracted) and ng furan added, corresponded to the fortification level. Finally, analytical method precision was calculated by reference to the relative standard deviation (n =6)(Valcárcel, 1999).

3.2.7. Exposure Assessment of Furan

An exposure assessment of furan in analyzed food products was estimated based on the furan concentration of each sample and the dietary data of individual Chileans obtained from several national studies applied to different age sectors (Liberona, Castillo, Engler, Villarroel, & Rozowski, 2011; Rozowski & Castillo, 2004).

The estimation was carried out using the following equation:

$$\textbf{Estimated daily intake (EDI)} = \sum_{i=1}^{n} \frac{Ci \times Cri}{BW}$$

Where *Ci* is the mean concentration level of furan *i* food (ng g^{-1}); *Cri* is the daily consumption of *i* food (g d^{-1}); *BW* is the average body weight (kg); and *n* is the number of tested foods (Kim, Lee, Park, et al., 2009; Leighton et al., 2009).

Additionally, for the risk assessment the MoE method was used. The MoE of each age sector was determined considering the total furan intakes estimated here per each age sector and the lower confidence limit on the benchmark dose associated with a 10% response ($BMDL_{10)}$ dose of 1.23 mg $kg^{-1}{}_{bw}$ day^{-1} (Carthew, Dinovi, & Setzer, 2010). MoE calculation was carried out using the following equation:

$$\textbf{MoE} = \frac{\textbf{BMLD}_{10}}{\textbf{EDI}}$$

3.5. Results and discussion

3.3.1 Analytical performance

To evaluate the performance of the modified HS-GC-MS method the *LOD* and the *LOQ* were established for all analyzed food categories. Likewise both the accuracy of the analytical method as well as its precision was calculated in terms of recovery and relative standard deviation respectively (Table 4.1).As shown in Table 4.1 the HS-GC-

MS procedure in current research not only provides acceptable linearity (r=0.996-0.999) but also good sensitivities (*LOD* values ranged from 0.01 ng g^{-1} to 0.6 ng g^{-1} in orange juice and in blended coffee respectively) compared with other traditional headspace studies (Morehouse et al., 2008; Nyman et al., 2008; Zoller et al., 2007). On the other hand, both the accuracy as well as the precision of this method varied quite a lot between the different foods. The recoveries and relative standards deviations ranged from 88% to 120% and 0.6% to 25% respectively, suggesting a considerable effect of the food matrix over the furan analysis (C. Crews et al., 2007).

Table 3.1: Analytical features of a HS-GC/MS method for furan quantification in foods.

Food type	**r^2**	**LOD (ng g^{-1})**	**LOQ (ng g-1)**	**RSD (%)**	**Recovery (%)**
Tomato sauce	0.9999	0.01	0.02	4	98
Orange juice	0.9959	0.01	0.03	11	108
Canned peaches	0.9982	0.03	0.09	4	112
Jarred fruit baby food	0.9989	0.14	0.48	6	102
Peas	0.9998	0.06	0.21	10	117
Peach marmalade	0.9968	0.06	0.19	25	98
Brewed coffee	0.9924	0.02	0.07	11	99
Automatic coffee machine	0.9966	0.60	1.99	1	96
Chips	0.9976	0.07	0.25	3	88
" *Soda*"crackers	0.9939	0.01	0.03	1	96
Toasted bread	0.9924	0.03	0.10	9	96
Whole breakfast cereal	0.9905	0.01	0.04	9	92
Breakfast cereal	0.9988	0.02	0.06	3	97
Fried meat	0.9056	0.02	0.06	3	99

3.3.2 Determination of furan in Chilean foods

In this study the furan levels of a total of 69 types of Chilean commercial food products (which included all brands currently present on the Chilean market) were determined (Table 4.2). As expected, furan occurred in most analyzed samples. Furan levels varied considerably among the different food products from non-detectable levels in fried meat, to high concentrations both in chips (259 ng g^{-1}) and in coffee (936 ng g^{-1}) suggesting a clear effect of the food matrix not only over furan generation but also over its retention (F. Van Lancker et al., 2009).

Table 3.2: Furan content in Chilean foods.

Food type	**Number of tested brand**		**Furan(ng g^{-1})**		
	Total	**Positive**	**Minimum**	**Maximum**	**Average of positives**
Tomato sauce	8	6	3.9	4.9	4.0
Orange juice	4	4	4.3	7.0	6.5
Canned peaches	2	2	3.6	8.2	6.9
Jarred fruit baby food	3	3	6.1	12.3	8.5
Peas	3	3	3.3	3.9	3.5
Peach marmalade	5	5	2.9	3.1	3.0
Brewed coffee	4	4	6.1	7.0	6.5
Automatic machine coffee	6	5	928	944	936
Chips	7	6	254	268	259
" *Soda*" crackers	6	6	91	91	91
Toasted bread	6	3	1.7	4.0	3.95
Whole breakfast cereal	4	4	14.6	23.0	20.0
Breakfast cereal	6	2	21.8	23.0	22.2
Fried meat	5	0	n.q.	n.q.	n.q.

n.q.: no quantifiable

Obtained results are in accordance with those previously reported in other studies specifically for the group of food products packaged in close containers such as canned peaches (C. Crews et al., 2009; Kim et al., 2010; Liu & Tsai, 2010; Morehouse et al., 2008) and jarred fruit baby foods (A. Arisseto et al., 2010; Jestoi et al., 2009; Lachenmeier et al., 2009). Interestingly, although food products prepared by autoclaving were expected to represent the highest furan levels, since this technology has been suggested to favor furan formation over other processing (Roberts et al., 2008; Zoller et al., 2007); higher furan levels were found in low moisture starchy foods (chips and "*Soda*" type crackers) than those previously reported (Nyman et al., 2008). For the case of potato based products, this fact could be explained because Chilean potatoes varieties used in food industry (*Desiree*) have been shown to have higher ascorbic acid levels. Additionally, the frying oil commonly used by Chilean food industry is a vegetal oil mix, which mainly contains soy oil. Thus, considering that soy oil has elevated levels of linolenic acid, the most efficient poly unsaturated fatty acid in furan generation, the furan formation could be increased by its thermo oxidation (A. Becalski & Seaman, 2005; Agnieszka Owczarek-Fendor et al., 2010; Perez Locas & Yaylayan, 2004).

With regard to wheat crackers it was observed that Chilean products were darker than those from European countries. This color difference could explain the higher concentration of furan in the Chilean product, considering that color development has shown to increase with increased furan concentration (Mariotti et al., 2012).

On the other hand, as was expected automatic machine coffee contained the highest levels of furan in comparison to the other food categories. This result could be explained because automatic coffee machines produce more concentrated brews and because of the closed system favoring retention of furan (A. P. Arisseto et al., 2011).

Obtained results confirm that the levels of furan for different foods are remarkably different, suggesting that food composition as well as its processing and packaging conditions could be considered as key factors not only over the furan formation but also over its retention (Jestoi et al., 2009; Lachenmeier et al., 2009; Liu & Tsai, 2010).

3.3.3 Exposure assessment of dietary furan in the Chilean population

In terms of studies of food consumption in Chile, the first nutrition survey performed at the national level was the Interdepartmental Committee on Nutrition for National Defense (ICNND) survey in 1960. After that, a national survey was performed in 1974 but it was never published in its entirety. Currently, most information on food consumption comes from studies that have been focused in different sectors of the population. In this sense, Olivares C et al. (2007) performed a cross sectional study in groups of 200 and 358 girls from 10 to 13 years in schools of medium-high and low socioeconomic level (SEL) respectively from Santiago, Chile. The same type of study was also realized by Liberona et al. (2011) in school children from 9 to 12 years, but in this opportunity the evaluated group were more representative of the population (n=1732). The consumption data of both studies were used to estimate the dietary exposure to furan corresponding to this age sector (9-13 years) (Table 4.3). Obtained results, showed no considerable differences between the two social economical sectors in terms of furan exposure (furan intake of Medium high-SEL and Low-SEL reached 496 ng day^{-1} kg_{bw}^{-1} and 510 ng day^{-1} kg_{bw}^{-1} respectively). Interestingly, for both socio-economical levels, the major contributor to dietary furan in Chilean school children was chips representing ~80 % of its total intake. In this sense, no surveys have been conducted in this age sector, since most of researches have been focused on dietary exposure to furan in adults and babies. Considering the fact that the Chilean snacks market is in dramatically increase (projecting a growth higher than 10 % per year in terms of consumption)(Bull et al., 1984), development of furan mitigation technologies may be considered a relevant challenge in the prevention of human disease as cancer.

On the other hand, considering elderly people as immune vulnerable population, it was decided to estimate their exposure to dietary furan. For accomplishing this purpose, an interesting stud

y conducted by Bagdonaite, Derler, and Murkovic (2008) in a group of elderly people (64 years) (n= 41) from a middle-income community from Santiago, Chile, was used (Table 4.4). Obtained results indicated a lower dietary furan exposure for this age sector compared with those of school children (94 ng day^{-1} kg_{bw}^{-1} and 109 ng day^{-1} kg_{bw}^{-1} for men and women respectively). Interestingly, for this age sector cereal-based foods more specifically "*Soda*" type crackers (a typical Chilean product characterized by its high consume); were the major contributor of the furan intake (~90 %). In terms of adult's exposure to dietary furan, some data about foods which are commonly consumed by the Chilean adult population (39 years)(A. Arisseto et al., 2009) were considered in order to estimate their furan intake (Table 4.5). For this case, lower values than those in elderly people for dietary furan exposure were found (63 ng day^{-1} kg_{bw}^{-1}). However, for this age sector it is also necessary to consider the contribution from coffee. In this sense, considering a daily coffee (brewed) intake of 99 ml day^{-1}, for men of 75.5 kg the furan exposure provided by this would be 8.5 ng $day^{-1}kg_{bw}^{-1}$ (Bråthen, Kita, Knutsen, & Wicklund, 2005). On the other hand, the furan exposure contribution from jarred fruit baby food was estimated to be appr. 250 ng $day^{-1}kg_{bw}^{-1}$ (considering a baby of 9 month with an average weight of 8.5 kg and a daily intake of 200 g /day). These results are in agreement with those previously reported in other countries such as Brazil (A. Arisseto et al., 2010)Spain(Altaki et al., 2011), Finland(Jestoi et al., 2009) and Germany (Lachenmeier et al., 2009). Finally regarding the risk characterization, dietary furan exposure in Chile could be considered as a public health concern only for school children and babies, since MoE values obtained for the other age sectors were higher than 10,000 as can be observed in table 4.6 (Chapin et al., 1995).

Although, previous results reveal that the Chilean population might be exposed to dietary furan; it is necessary to access a national survey in order to obtain more representative information of the current Chilean food consumption.

Table3.3: Furan exposure (µg kg-1 bw day-1) in school children (10-13 years) according to socio-economic level (SEL), in metropolitan region of Santiago, Chile**.**

Food category	**Furan content (ng g^{-1})**	**Edible portion (g day^{-1}) Mean Medium-high SEL**	**Furan Intake (ng day^{-1} Kg_{bw}^{-1})**	**Edible portion (g day^{-1}) Mean Low SEL**	**Furan Intake (ng day^{-1} Kg_{bw}^{-1})**
Cereals	22.2	127	62.7	200	98.7
Bread	4.0	136	11.9	214	18.8
Legumes	3.5	10.7	0.83	10.6	0.82
Red meat	0	67	0	56	0
Salty snacks	259	73	421	68	392
Total intake (ng day^{-1} Kg_{bw}^{-1})			496		510

*Average body weight (bw): 45 kg

Table 3.4: Furan exposure (ng kg-1 bw day-1) in older adults in metropolitan region of Santiago, Chile.

Food category	**Furan content (ng g^{-1})**	**Men edible portion (g day^{-1})**	**Furan Intake (ng $Kg_{bw}^{-1}day^{-1}$)**	**Women edible portion (g day^{-1})**	**Furan Intake (ng $Kg_{bw}^{-1}day^{-1}$)**
Red meat	0	74.6	0	57.6	0
Cereals ("*Soda*" crackers)	91.1	67.6	81.5	74.2	99
Legumes	3.5	11.3	0.52	16.6	0.86
Bread	4.0	232	12.1	142	8.3
Total intake (ng day^{-1} Kg_{bw}^{-1})			94		109

*Average body weight (bw): men: 75.5 kg

*Average body weight (bw): women: 67.9 kg

Table 3.5: Furan exposure (ng kg -1bwday -1) in adults from food of lunch time from a Chilean metal mechanic company.

Food	**Furan content (ng g^{-1})**	**Men Edible portion (g day^{-1})**	**Furan Intake ng $kg^{-1}_{bw}day^{-1}$**
Fruits (canned peaches)	6.9	47.0	4.3
Red meat	0	104	0
Legumes	3.5	13.0	0.60
White bread	4.0	80.0	4.2
Cereals	91.1	45.0	54.3
Total intake (ng $kg^{-1}_{bw}day^{-1}$)			63.4

*Average body weight (bw): men: 75.5 kg

Table 3.6: Margins of exposure to furan of different age sector of Chilean population.

.Age sector	**Total intake (ng $kg_{bw}^{-1}day^{-1}$)**	**MoE**
School children Medium-high SEL	496	2479
School children Low SEL	510	2411
Men elderly people	94	13056
Women elderly people	109	11321
Adults*	72	17119
9 month babies	250	4922

*: total intake includes coffee

3.4. Conclusion

The results presented in this research provide the first data on furan occurrence in foods purchased and consumed in Chile. Good analytical performance was obtained for the analytical furan quantification HS-GC-MS method in foods.

The levels of furan in analyzed food samples varied from below the limit of detection in fried meat to 936 ng g^{-1} in automatic machine brewed coffee.

When furan exposures from different food types were estimates for different age sectors, the Chilean school children were exposed to the highest levels of furan (496 ng day^{-1} kg_{bw}^{-1} for Medium high-SEL and 510 ng day^{-1} kg_{bw}^{-1} for Low-SEL).

For all age sectors studied low moisture starchy food such as chips and wheat flour biscuits, were the major source of dietary furan. Additionally, considering MOE values obtained both for school children and babies, dietary exposure to furan in Chile may be considered as of potential public health concern.

Finally, considering the fact that the exposure estimate reported in this study only expressed an approximation of Chilean exposure to dietary furan, it is necessary to improve the database of food consumption in Chile in order to have more representative furan exposure estimation.

3.5. References

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4. FURAN OCCURRENCE IN STARCHY FOOD MODEL SYSTEMS PROCESSED AT HIGH TEMPERATURES: EFFECT OF ASCORBIC ACID AND HEATING CONDITIONS.

Abstract:

Furan, a potential carcinogen, has been detected in highly consumed starchy foods such as bread and snacks; however, research on furan generation in these food matrixes has not been undertaken, so far. The present study explored the effect of ascorbic acid addition and cooking methods (frying and baking) over furan occurrence and its relation with the non-enzymatic browning in a wheat flour starchy food model system. Results showed that furan generation significantly increased in the presence of ascorbic acid after 7 min heating (P <0.05). The strongest effect was observed for baked products. Additionally, furan content in fried products increased as oil uptake levels did. As for Maillard reactions in general, furan level in all samples linearly correlated with their degree of non-enzymatic browning, represented by L^* and a^* color parameters (*e.g.* wheat flour baked samples showed a R^2 of 0.88 and 0.87 for L^* and a^*, respectively) when the sample moisture content decreased during heating.

Keyword: furan, starchy food model system, ascorbic acid, oil uptake, moisture, non-enzymatic browning

4.1. Introduction

Furan is a potential human carcinogen that can be formed in a broad range of foods processed at high temperatures, such as coffee, baby foods, bread and snacks (C. Crews & Castle, 2007). Although it is still unclear what are the risks associated with the current intake levels of dietary furan, furan mitigation in foods may be considered as a challenge in the prevention of human diseases as cancer (IARC, 1995). The presence of furan is common in foods processed at high temperatures, particularly in products packed in sealed containers (*e.g.* baby foods). Due to its low boiling point, the furan generated

during thermal processes easily evaporable, accumulates in the headspace of canned or jarred foods (Hasnip et al., 2006). However, despite its high volatility, furan has also been found in low moisture foods processed in open containers, such as potato chips, crackers, crisp breads and toasted breads (Guenther et al., 2010; Hasnip et al., 2006; F. Van Lancker et al., 2009; Zoller et al., 2007).

The broad number of foods that have been shown to contain furan suggests that multiple pathways might be involved in its formation in foods (Morehouse et al., 2008). Thermal degradation and rearrangement of sugars was suggested as the primary source of furan in food (8); more recently, amino acids, polyunsaturated fatty acids (PUFAS) and ascorbic acid have also been implicated (C. Crews & Castle, 2007; Fan, 2005; Hasnip et al., 2006; Maga, 1979; F. A. Van Lancker, An Owczarek-Fendor, Agnieszka De Meulenaer, Bruno De Kimpe, Norbert, 2011; Vranova & Ciesarova, 2009; Zoller et al., 2007). The latter formed the highest amount of furan in aqueous model systems heated at high temperature.

It is worthy to note that furan content determined in foods was much lower than predicted from trials with pure ascorbic acid. Therefore, caution must be drawn about the plausibility of the proposed pathways for furan formation determined in model systems, and their direct extrapolation to the more complex food products (Roberts et al., 2008).

Few authors have evaluated furan generation in more real systems that considered the interaction between potential precursors. Limacher et al. (2008) and F. Van Lancker et al. (2010) determined furan formation from Maillard reaction in Carbon Module Labeling (CAMOLA) model systems under both, dry-roasting and pressure cooking conditions. They concluded that glucose-derived furan was formed from the intact sugar skeleton and not from fragmentation and recombination mechanisms. However, some amino acids (especially alanine and serine) could provide an additional formation pathway, as previously proposed by Perez Locas and Yaylayan (2004).

The role of ascorbic acid and PUFAS on furan occurrence has recently been investigated by Limacher et al. (2007) in starchy model systems that mimic baby foods. The authors

showed that for CAMOLA model systems heated under roasting conditions, furan formation from ascorbic acid was significantly reduced in binary mixtures (*e.g*. the presence of erythrose led to 80% less furan). These results agreed with previous findings in which simple binary mixtures of ascorbic acid and amino acids, sugars, or lipids could reduce furan by 50-95% (Mark et al., 2006). Thus, more complex reaction systems result in lower furan generation as compared to the individual precursors, most likely due to competing reaction pathways. A. Owczarek-Fendor et al. (2010) observed, however, that the presence of starch drastically enhanced furan formation from ascorbic acid. They hypothesized that furan synthesis was stimulated when ascorbic acid was incorporated in the starchy gel (inclusion complex); thus, its degradation was favored over the condensation with other compounds present in the reaction medium.

Furan formation from lipid oxidation was influenced not only by the fatty acid composition but also by the interactions with other matrix ingredients (Agnieszka Owczarek-Fendor et al., 2011). For example, while linolenic acid has been identified as responsible of furan generation in most research studies (A. Becalski & Seaman, 2005; Agnieszka Owczarek-Fendor et al., 2011; A. Owczarek-Fendor et al., 2011; Agnieszka Owczarek-Fendor et al., 2010; Perez Locas & Yaylayan, 2004; Şenyuva & Gökmen, 2007), the importance of the degree of fat oxidation is still unclear. Finally, the effect of different intrinsic and extrinsic factors such as pH, matrix and heating temperatures also has considerably impact on both, furan generation and its retention (Ames, Guy, & Kipping, 2001; C. Crews & Castle, 2007; Hasnip et al., 2006; Huang, Duan, & Barringer, 2011; Roberts et al., 2008; F. Van Lancker et al., 2009).

Since high levels of furan were found in baby foods, most model systems focused in replicating as reliably as possible the physicochemical features of these matrices. To the best of our knowledge, research on furan generation in other food matrixes, such as bread, crackers, or potato chips, where its presence was demonstrated, has not been carried out yet. Considering the significant worldwide consumption of thermally-processed starchy foods, in this work we investigated the mechanisms involved in furan generation in these matrixes. The present study explored the effect of ascorbic acid and

heating conditions (frying and baking) over furan occurrence, as well as the relationships between non-enzymatic browning and furan content in a starchy food model system.

Finally, as these low moisture starchy food products are characterized by the development of non-enzymatic browning during high temperature processing (Nursten, 2005), we explored if color development could be a good predictor of furan generation.

4.2. Materials and methods

Two different dough formulations with the same moisture content of 40 % in wet basis (wb) were prepared: (i) WF: wheat flour; (ii) WF-AA: wheat flour and ascorbic acid. Then, both formulations were laminated and cut in circle slices in order to be either baked or fried. Furan concentration of the fried or baked slices on dry de-fatted weight basis (ddb) was quantified by gas chromatography coupled to mass spectrometry (GC/MS). Finally, color development of the cooked samples was quantified in *L**, *a** and *b** units by using a colorimeter.

4.2.1. Materials

Dough formulations were prepared with the following materials: (i) Wheat flour (moisture content of 15% in wb); (ii) Anhydrous ascorbic acid (<99%, Sigma-Aldrich, Steinheim, Germany); (iii) Milli-Q water. For frying experiments, sunflower oil (Solsikkeolie Copenhagen, Denmark) was used as heating medium.

Chemical reagents for furan analyses were: (i) Furan (>99%, Sigma-Aldrich, Steinheim, Germany); (ii) d_4-furan (98 atom% D, Isotec, Ohio, USA); (iii) Methanol (HPLC grade, Rathburn, Walkerburn, Scotland); (iv) NaCl (>99%, Merck, Darmstadt, Germany). Finally, petroleum ether (>99%, Sigma-Aldrich, Steinheim, Germany) was used as extraction solvent for oil determination by Soxhlet.

4.2.2. Dough preparation

Dough formulations were prepared based on the criteria that both formulations (with and without ascorbic acid) would have the same moisture content of 40 ± 0.6% wb before being fried or baked. To calculate the amount of water that had to be added to the solid materials, the exact dry solid content of wheat flour was determined experimentally by drying it until constant weight. For WF-AA samples, anhydrous ascorbic acid was added in a concentration of 300 mg/kg of wheat flour. Then, the amount of wheat flour, ascorbic acid and water necessary to prepare 500 g of each dough formulation was calculated in dry basis (db). For WF and WF-AA formulations, a 100 % and 99.5 % of wheat flour (db) was added. In WF-AA formulation near to 0.5% (db) corresponded to the ascorbic acid necessary to reach the required concentration.

WF and WF-AA dough formulations were prepared by using a food mixer (Teddy Bear Varimixer, Copenhagen, Denmark) and water was added according to the protocol described by Gazmuri and Bouchon (2009). Half of the water was gradually added at 15 °C while mixing for 1 min. After mixing for one extra minute, the remaining water previously heated at 90 °C was added to the dough, and then all the ingredients were homogenized for 2 min. The resultant dough was then wrapped in a plastic bag and left for 1 h at room temperature (20 °C). Then, the dough was kneaded to ensure homogeneity, laminated to get the required thickness using a dough sheeter (Rollmatic, Vicenza, Italy), and cut into 40 mm diameter circles. The exact thickness of the resultant dough slices ranged from 2 to 2.3 mm. Approximately 500 g of dough was prepared for each batch of experiment.

4.2.3. Thermal processing of dough

The resulting samples were fried and baked at 170 and 200 °C for 5, 7 and 9 min respectively.

4.2.1.1. Frying conditions

The samples were fried in a 20 L capacity deep-fryer (FKI, Copenhagen, Denmark). The fryer was filled with 15 L of oil that was preheated for 2 h prior to frying (Blumenthal, 1991) and was discarded after 90 min of frying time. Chip-to-oil mass ratio was maintained as low as possible in order to keep a constant temperature of frying. Throughout the frying process, 10 chips of 3.7 ± 0.03 g were placed in a basket and held in position with a wire grid to prevent them from floating. The fried chips were drained over a wire screen for 5 min (Moyano & Pedreschi, 2006). Then, fried samples were homogenized and refrigerated for 30 min. Then, chemical and color analyses were carried out.

4.2.1.2. Baking conditions

The samples were baked in a forced-air oven (drying rate: 1m/s; Heraeus, Copenhagen, Denmark) preheated for 1 h prior to baking. Throughout the baking process, 10 chips were cooked, and then the baked samples were homogenized and refrigerated for 30 min (J. S. Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005). Chemical and color analyses were then carried out.

4.2.4. Analytical Methods

4.2.1.1. Solid content

Raw material (wheat flour) was placed in a Petri dish, dried in a forced air oven at 105 °C to constant weight, and cooled in a desiccator (A.O.A.C., 1995). The solid content of baked samples was determined using the same procedure. For fried products, the solid content was determined in extracted, oil-free sample. The solid content of samples was used to calculate their furan concentration on a de-fatted dry weight basis (ddb). Hence, changes in these concentrations with high temperature processing times were not influenced by changes in moisture and fat content.

4.2.1.2. Oil content

Total oil content of fried chips was determined gravimetrically by Soxhlet extraction with petroleum ether (A.O.A.C., 1995).

4.2.1.3. Furan quantification

Furan was quantified according to the methodology of the National Food Institute of Technical University of Denmark (DTU). This method is a revised version of the FDA (2004) methodology.

0.5 g of fried sample and 0.75 g of baked sample, both previously pulverized, were weighed into headspace vials, diluted with 5M NaCl solution. After adding the internal standard (d_4-furan), the vials were sealed. Automated headspace sampling followed by gas chromatography/mass spectrometry (GC/MS) analysis was used to detect furan and d_4-furan in the scan mode. Furan was quantified by using a standard addition curve, where the concentration of furan in the fortified test portions was plotted versus the furan/d_4-furan response factors. For constructing the calibration curve, seven vials were used of which four vials were fortified with furan, as follows: two vials at ca. half of the expected concentration of furan in the sample, one vial at ca. the expected concentration of furan in the sample, and one vial at ca. twice of the expected concentration of furan in the sample.

The analyses were performed on a gas chromatograph – mass spectrometer (GC-MS) (Agilent 6890N GC with Agilent 5973 N MSD, Palo Alto, California, USA) fitted with a CTC CombiPAL static headspace autosampler. The syringe was heated to 70°C and the sample vial was heated at 60°C for 30 min. 1 ml of the headspace from a 10 mL headspace vial was injected splitless on the gas chromatograph. A 15 m x 0.32 mm x 20 µm HP-Plot Q column was used. The following conditions were set: helium flow, 1.7 mL/min; injector temperature , 200°C; oven temperature, 50°C (1 min.), with a temperature ramp of 10 °C/min to 130°C, then a temperature ramp of 3°C/min. to 157°C and finally a temperature ramp of 20°C/min to 260°C, and held for 2.5 min. The MS

source temperature was 230°C and the MS quad temperature 150°C.; dwell time, 50 msec. The mass spectrometer was operated in electron ionization mode. Furan was detected using single ion monitoring of the fragments *m/z* 68 and *m/z* 39. The internal standard d_4-furan was detected by monitoring the *m/z* 72 and *m/z* 42. This method has a limit of quantification of 2.4 ng g^{-1}. All analytical determinations were carried out in three replicates.

4.2.1.4. pH estimation

The pH of the dough was calculated considering that pure water was used and also the pH of wheat flour was 7.0. For WF formulation, the pH corresponded to –log $[H_3O^+]$, where the molar concentration of H_3O^+ was 10^{-7} mol/l, thus pH_{WF} =7. For the case of WF-AA formulation the Henderson-Hasselbach equation was used considering that the ascorbic acid solution used has a molar concentration of 0.00365 mol/l (which corresponded to the added ascorbic acid amount 300 mg/kg of wheat flour) and a pK_a of 4.17, thus $pH_{WF\text{-}AA}$ = 3.3.

4.2.5. Color development

The color of baked and fried samples was measured using a colorimeter (Minolta Chromo Meter CR 200b, USA) attached to a data processor DP-100 using the CIE L*, a*, b* color scale. Triplicate readings were carried out at 20 °C on each three equidistant locations of each chip and the mean value was recorded. Color changes were followed by the lightness (*L**) and redness (*a**) parameters, since these color components presented the highest and significant variations during high temperature processing due to non-enzymatic browning reactions (Pedreschi, Leon, et al., 2007).

4.2.6. Statistical Analysis

The experiments were replicated three times. Statistical analysis was carried out using Statgraphics Centurion XV software (Manugistic Inc., USA). One–way variance analysis was performed to confirm that there were no significant differences between

measurements of a sample processed under same specific conditions. Differences between treatments were analyzed by least significant difference (LSD) test using the linear general model. All significant differences were determined with a confidence level of 95%.

4.3. Results and discussion

The occurrence of furan and its relation with some intrinsic factors (ascorbic acid content, oil uptake and moisture) and non enzymatic browning were explored in a starchy food model system simulating frying and baking conditions, in order to elucidate a main furan pathway for this kind of products.

4.3.1. Role of ascorbic acid in furan formation

The impact of ascorbic acid over furan generation in model systems mimicking complex foods has not been completely understood yet. Some results have suggested that furan formation from ascorbic acid is negatively affected by the presence of additional molecules such as carbohydrates, lipids and amino acids, which may increase the fragmentation rate or change the redox status of the reaction system (Mark et al., 2006; Perez Locas & Yaylayan, 2004). However, at our model conditions, ascorbic acid significantly increased furan generation (P <0.05) after 7 min of processing, having a stronger effect in baking, as shown in figure 3.1 (*e.g.* WF-AA samples baked and fried during 9 min contained ~74% and 33 % more furan than their WF counterparts). Interestingly, not only ascorbic acid concentration but also processing time were critical factors for furan formation, suggesting a synergistic effect between the ascorbic acid added and the reducing sugars and amino acids naturally present in wheat flour, at least under the dry heating conditions applied in this work. The latter could be explained since under similar dry heating conditions (180°C), ascorbic acid behaves like reducing sugars in the Maillard reaction (Vernin, Chakib, Rogacheva, Obretenov, & Párkányi, 1998).

On the other hand, despite ascorbic acid increased the final content of furan in WF-AA fried samples, this effect was weaker than in baked samples. Since furan formation strongly depends on the heating temperature, these results could be attributed to the fact that frying experiments were performed at lower temperatures (170°C) than the baking ones (200 °C). It is worthy to mention that processing temperatures for both unit operations were selected according to common requirements in food industry.

Furthermore, pH significantly affects the yield of furan generation in Maillard model systems (A. Becalski & Seaman, 2005; Fan, 2005; Mark et al., 2006; Perez Locas & Yaylayan, 2004); the higher the pH of the system, the higher the formation of 1-deoxysones, which is the most effective furan synthetic route in Maillard reaction. However, for our model conditions, the final furan content was similar both in WF-AA and WF samples despite their differences in the pH (WF-AA and WF samples presented a pH ~ 3.3 and ~ 7 respectively).

This is in accordance with a previous study in starch-based model systems with and without ascorbic acid of the relationship between pH (range 3.5-6.5) and furan formation, showing that the highest formation was found in model systems with ascorbic acid at pH 3.5 (A. Owczarek-Fendor et al., 2010). This fact may be explained since these experiments were performed under more drastic heating conditions (~ 190 °C) than previous research (~120 °C) performed in simple model systems of sugars (Fan et al., 2008).

For the present model system the addition of ascorbic decreased the pH but increased the furan occurrence under the low moisture conditions and higher processing temperatures applied.

4.3.2. Influence of oil uptake over the final furan content

Additionally to the role of ascorbic acid, and specifically for fried products, the influence of oil uptake could also affect the final furan content of the product, since at high temperatures commonly used during atmospheric frying, the penetrated oil (Moreira, Sun, & Chen, 1997) can easily be oxidized and form furan (Vranova &

Ciesarova, 2009). On the other hand, considering that furan is a non-polar compound, the penetrated oil could also have retention effect over the furan generated from the others precursors, originally present in these samples (sugars, amino acids and ascorbic acid). In this respect, our results showed that not only for WF but also for WF-AA fried products; the amount of furan generated increased during frying as oil uptake did (Figure 3.2).

Some authors have suggested that the overall role of lipids in furan formation was restricted in practice, since it is necessary to significantly oxidize the oil which is sensory unacceptable by the consumer (Owczarek-Fendor et al., 2012). Moreover, recent studies have shown that for PUFAS only α- linolenic fatty acid is a precursor for furan, because of its unique potential among fatty acids to produce 2-butenal upon oxidation (Agnieszka Owczarek-Fendor et al., 2010).

Since our frying experiments were performed with commercial sunflower oil which contains antioxidant (BHA) but lacks α- linolenic fatty acid, the retention effect of penetrated oil could be considered as the major contribution of oil in the final furan occurrence of the tested model system.

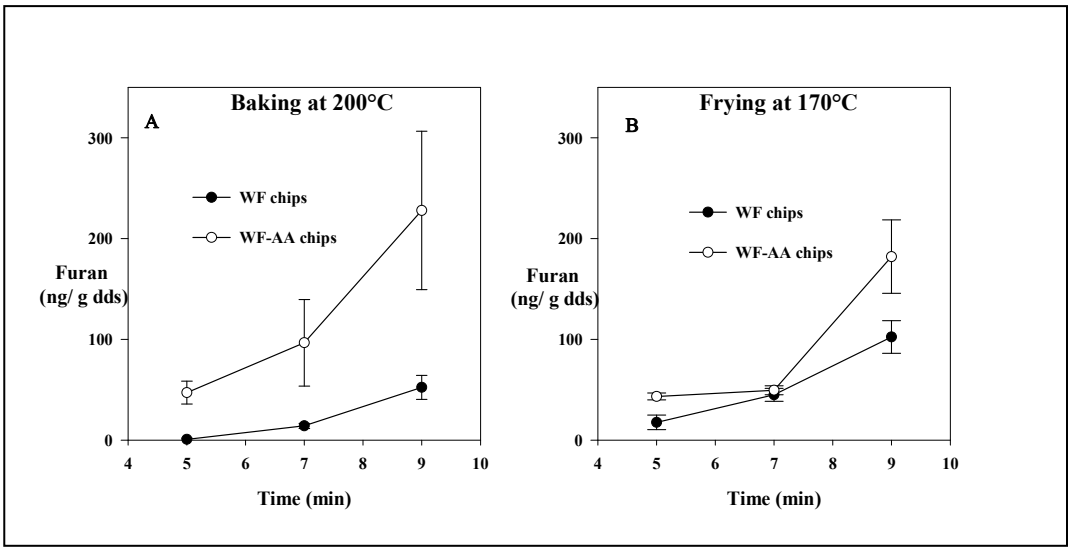

Figure 4.1: Role of ascorbic acid over furan formation in starchy food model systems processed at high temperatures. Error bars represent standard deviations (n = 3).

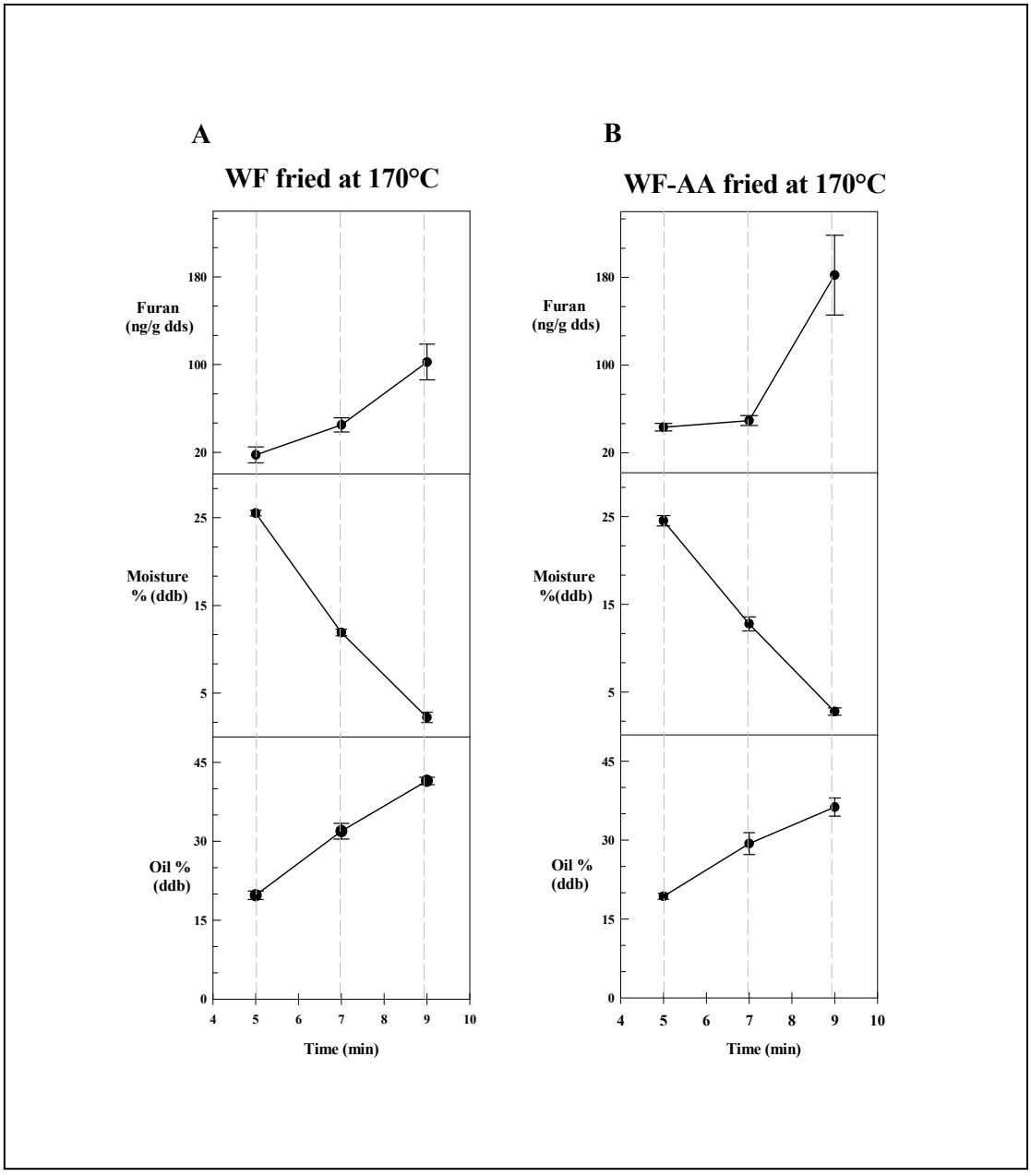

Figure 4.2: Influence of oil uptake and moisture over the final furan content in fried starchy food model systems. Error bars represent standard deviations (n = 3).

4.3.3. Effect of moisture content over furan generation

As for Maillard reaction in general, for all samples, an exponential increase in furan level was observed when the moisture content decreased (figures 3.2 and 3.3). The furan concentration of fried and baked samples (both WF as WF-AA) did not significantly change (P> 0.05) until the moisture content was below 12% (ddb), achieving the highest values at moisture levels of 2.23 % (ddb) and 2.77 % (ddb) for WF (102.32 ng g^{-1}dds) and WF-AA (182.04 ng g^{-1}dds) fried samples and 6.22 % (ddb) and 5.48 % (ddb) for WF (52.36 ng g^{-1}dds) and WF-AA (227.00 ng g^{-1}dds) baked samples respectively. Similar results were found in carrot slices (Duan & Barringer, 2012) and hazelnuts (Şenyuva & Gökmen, 2007) which were dried at temperature ranges of 113-133 °C and 50-150°C, respectively.

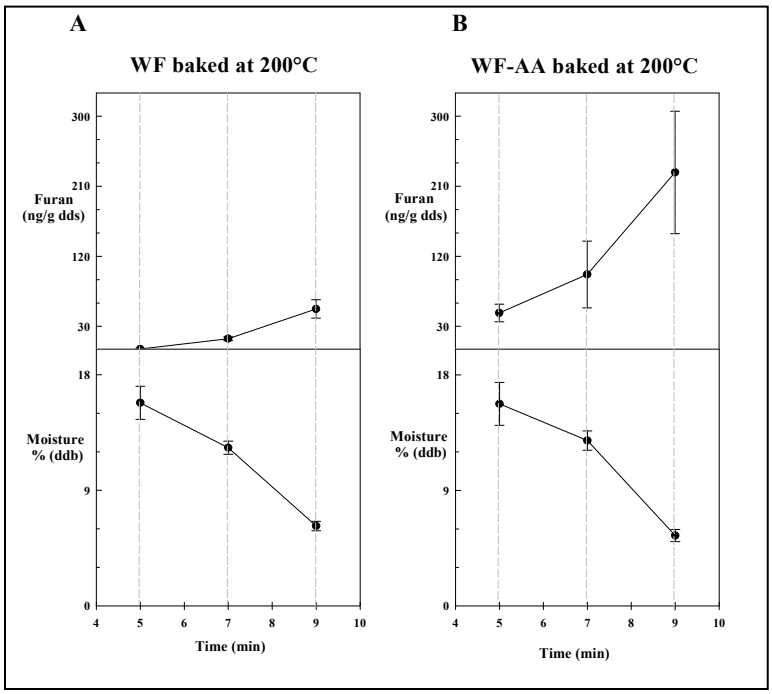

Figure 4.3: Effect of moisture content over furan generation in baked starchy food model systems. Error bars represent standard deviations (n = 3).

4.3.4. Correlation between furan content and non-enzymatic browning

The presence of furan in foods is related to the thermal degradation of carbohydrates, ascorbic acid, amino acid and PUFAS (Maga, 1979). Considering that non-enzymatic browning in low moisture starchy foods is also a consequence of these reactions (Zamora & Hidalgo, 2005), we determined the color development of WF and WF-AA formulations processed at high temperatures. Figures 3.4 and 3.5 show that color represented by the parameters L^* and a^* followed linear correlations with furan content, both for frying (WF formulation: R^2 of 0.72 and 0.82 for L^* and a^* values, respectively; WF-AA formulation: R^2 of 0.56 and 0.94 for L^* and a^* values, respectively) and baking (WF formulation: R^2 of 0.70 and 0.88 for L^* and a^* values, respectively; WF-AA formulation: R^2 of 0.72 and 0.87 for L^* and a^* values, respectively) experiments. These results suggest that preliminarily color development could be considered as a good predictor of furan formation in starchy matrixes. Lower correlation values obtained for lightness in fried products could be attributed to distortion effects caused by oil located in the fried sample surface.

L^* values tended to decrease with the processing time since the samples get darker in the surface due to non-enzymatic browning reactions. On the other hand, a^* value showed an increase during the tested cooking processes since the sample surface got more red coloration due to the reactions mentioned before. Interestingly, these results agreed with those obtained by other researchers when they studied acrylamide formation phenomena in real systems, such as potato chips and French fries (Pedreschi, Bustos, et al., 2007; Pedreschi, Kaack, & Granby, 2006; Pedreschi, Moyano, Kaack, & Granby, 2005). Maillard reaction might be the main route of formation of furan in low moisture starchy foods.

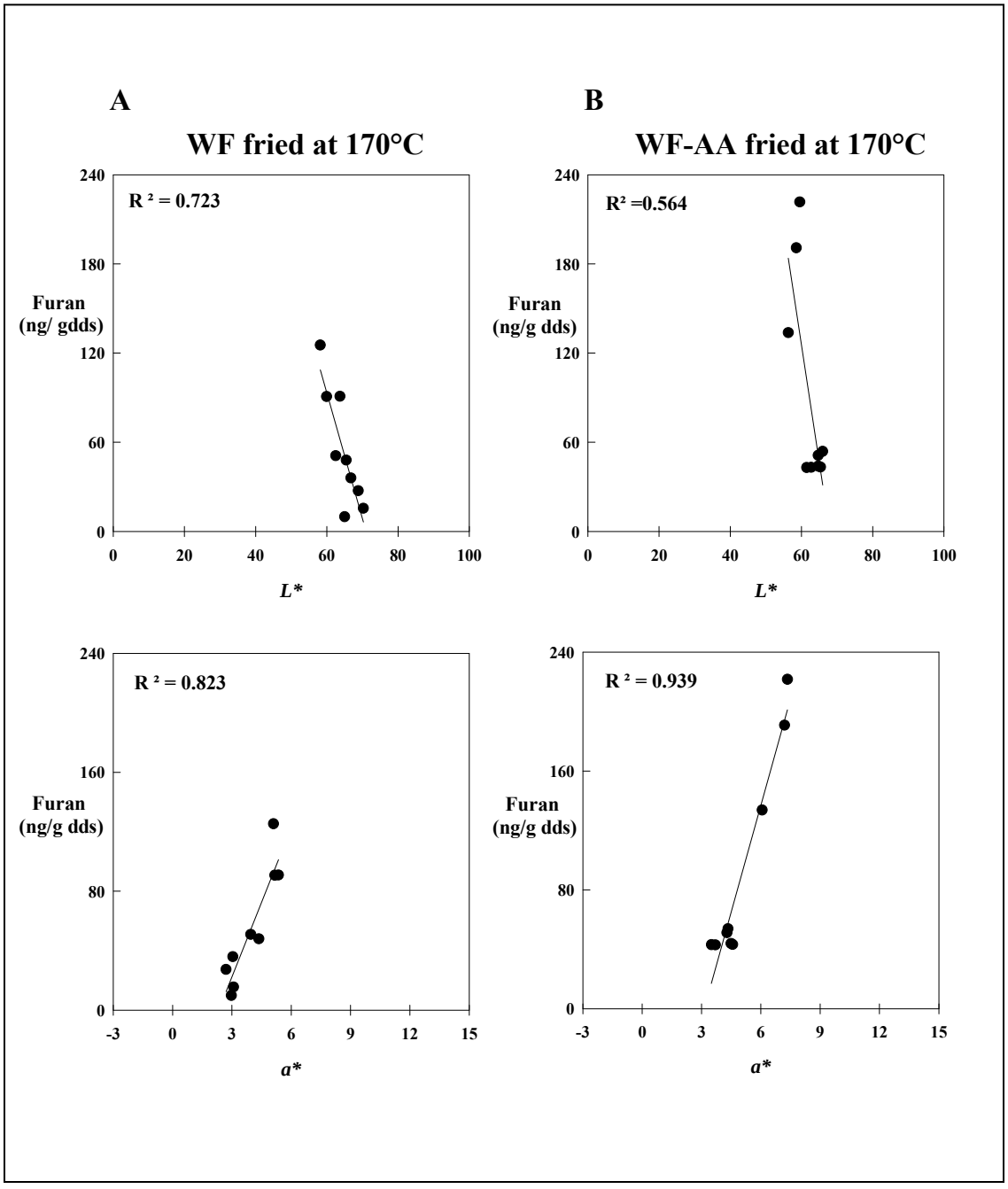

Figure 4.4: Relationship between color development and furan content in fried starchy food model systems.

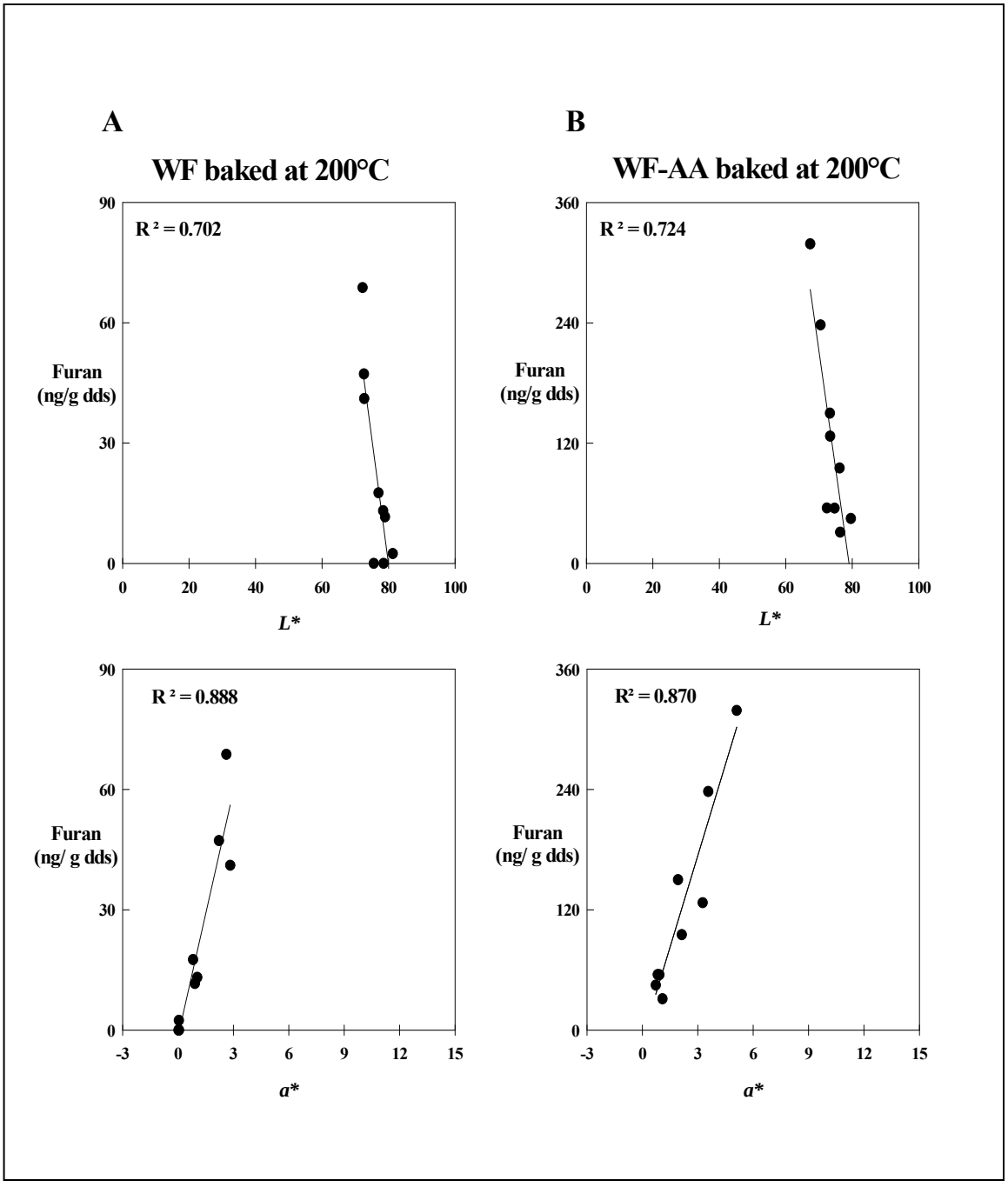

Figure 4.5: Relationship between color development and furan content in baked starchy food model systems.

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5. HEAT TOXIC CONTAMINANT MITIGATION IN POTATO CHIPS

Abstract

Heating foods immersed in oil during frying provides many attractive sensorial attributes including taste, flavor and color. However, some toxic compounds formed during frying of potatoes such as furan and acrylamide may constitute an increased cancer risk for consumers. The objective of this work was to mitigate the furan and acrylamide formation in potato chips without increasing their oil uptake by optimizing the blanching treatment before final frying. Potato slices were blanched in order to simultaneously leach out ascorbic acid and reducing sugars, the most important precursors of furan and acrylamide generation in thermally treated starchy foods. A central composite design was implemented to optimize the temperature-time blanching conditions under which furan, acrylamide and oil content in potato chips were minimized. The optimum blanching conditions were 64°C and 17 min in which significant reductions of furan, acrylamide and oil content (91%, 54% and 19% respectively) were reached.

Key words: Furan; Acrylamide; Precursors; Oil Uptake; Blanching.

5.1 Introduction

The processing of foods at high temperature has many advantages since it adds taste, color, texture and minimizes harmful germs. However, during the heating of foods various hazardous compounds are produced by high temperature reactions, such as furan and acrylamide (Van Boekel et al., 2010). Both furan and acrylamide are potential human carcinogens that can be formed in foods processed at high temperatures (IARC, 1994, 1995).

Multiple pathways have shown to be involved in furan formation in foods. The thermal degradation and rearrangement of sugars and amino acids, as well as the thermo oxidation of polyunsaturated fatty acids (PUFAS) and ascorbic acid have been proposed

as the mechanisms responsible for its formation in thermally treated foods (Hasnip et al., 2006; Vranova & Ciesarova, 2009). Furan is commonly detected in food packed in sealed containers such as baby foods (Hasnip et al., 2006). However, despite the most important physicochemical property of furan is its high volatility, furan has also been found in foods not cooked in closed containers such as crackers, chips, and toasted breads (C. Crews et al., 2007; C. Crews et al., 2009). Since high levels of furan were found in coffee (~50 μg L^{-1}) and baby foods (70 μg kg^{-1}), most mitigation efforts have been focused into reducing the furan content of these products by its evaporation (Mariotti, Granby, Rozowski, & Pedreschi, 2013). To the best of our knowledge, research on furan mitigation in other food matrixes, such as potato chips, has not yet been carried out.

On the other hand, acrylamide is formed through Maillard reaction, being their main sources of the dietary exposure fried potatoes (~272 - 570 μg kg^{-1}) (EFSA, 2012).

In this sense, a variety of techniques have been proposed to decrease the acrylamide level in potato products. Some of these methods are based on the reduction of acrylamide precursor levels such as reducing sugars in the food raw materials, for instance blanching (Mariotti et al., 2011). In this sense, blanching is a unit operation widely used in the potato-processing industry for leaching of accumulated sugars to control the Maillard reaction during subsequent frying (Gonzalez-Martinez, Ahrne, Gekas, & Sjoholm, 2004). The usual range of blanching water temperature is from 60-80°C and the blanching residence time vary between 5-20 min (Califano & Calvelo, 1988).

Studies performed in potato chips have shown that although blanching at high temperature and short time (85°C, 3min) improved their color it also increased their oil uptake (Pedreschi & Moyano, 2005b). It could be attributed to the fact that, under extreme blanching conditions; the vegetal tissue loss firmness as consequence of softening of cell walls and decreasing in the turgor pressure (Alvarez, Morillo, & Canet, 2000). Previous information suggests that an optimization of the blanching conditions is necessary to improve the chemical safety of chips, not only by diminishing the amounts

of heat contaminants such as furan and acrylamide, but also by avoiding an increase in the oil uptake of the fried products. The objective of this work was to reduce the level of furan and acrylamide in chips without increasing their oil uptake. To accomplish this purpose a central composite design was used to study the effect of blanching time and temperature on the reduction of furan and acrylamide precursors (reducing sugars and ascorbic acid) in potato slices. Additionally, the impact of both factors was evaluated over the furan and acrylamide formation as well as over oil absorption. Finally and a multiple optimization of blanching conditions was performed to obtain potato chips with lower content of furan, acrylamide and oil.

5.2 Materials and Methods:

5.2.1. Raw Materials

Potatoes (variety Ditta, 80% of moisture) and sunflower oil (Solsikkeolie, Copenhagen, Denmark). Potatoes (stored at 8°C and 95% relative humidity) were washed and cut in slices of 2.2 mm thickness from the pith of the parenchymatous region of tubers. A circular cutting mold was used to provide chips with a diameter of 40 mm.

5.2.2. Blanching and frying conditions

Potato slices were blanched between 5 and 15 min (Mestdagh et al., 2008) in combination with blanching temperature varying from 50°C to 80°C (potato/water ratio of 0.015). All blanching treatments were performed in distilled water (Pedreschi, Kaack, & Granby, 2004). After the pre-treaments, potatoes slices were fried at 170°C (±1°C) until reaching 2% of moisture. The samples were fried in a 20 l capacity deep-fryer (FKI, Copenhagen, Denmark). The fryer was filled with 15 l of oil that was preheated for 2 h prior to frying (Blumenthal, 1991). Throughout the frying process, 10 chips of 2 ± 0.03 g were placed in a basket and held in position with a wire grid to prevent them from floating. The fried chips were drained over a wire screen for 5 min. Then, fried

samples were refrigerated for 30 min in order to prevent the furan evaporation (Mariotti et al., 2012).

5.2.3. Reagents and chemicals

Chemical reagents for furan analyses were: furan (>99%, Sigma-Aldrich, Steinheim, Germany); d_4-furan (98 atom% D, Isotec, Ohio, USA); Methanol (HPLC grade, Rathburn, Walkerburn, Scotland); NaCl (>99%, Merck, Darmstadt, Germany).

For acrylamide analysis acrylamide (2-propene amide) (>99.5%, Sigma-Aldrich, St. Louis, MO, US); Labelled d_3-acrylamide (>98%, Polymer Source Inc., Dorval, Quebec Canada) and Acetonitril (HPLC grade, Rathburn, Walkerburn, Scotland) were used.

D-(+) Glucose (PM:180.16 g mol^{-1}, G-7528, Sigma-Aldrich, Steinheim, Germany); Fructose: (180.16 g mo^{-1}, F-0127 Sigma-Aldrich, Steinheim, Germany); L(+) Ascorbic acid (Sigma-Aldrich, Steinheim, Germany) were used to measure reducing sugar and ascorbic acid respectively. For both analyses the eluent was made from a 50% w/w solution of NaOH (J.T. Baker 7067, NJ, USA) and water.

Petroleum ether (>99%, Sigma-Aldrich, Steinheim, Germany) was used as extraction solvent for oil determination by Soxhlet.

5.2.4. Chemical analysis

5.2.4.1. Solid content

Raw material (potato slices) was placed in a Petri dish, dried in a forced air oven at 105 °C to constant weight, and cooled in a desiccator (A.O.A.C., 1995). The solid content of chips was determined in extracted, oil-free sample. The solid content of samples was used to calculate their acrylamide and furan concentration on a de-fatted dry weight basis (ddb). Hence, changes in these concentrations with high temperature processing times were not influenced by changes in moisture and fat content.

5.2.4.2. Oil content

Total oil content of chips was determined gravimetrically by Soxhlet extraction with petroleum ether (A.O.A.C., 1995).

5.2.4.3. Precursors: glucose, fructose and ascorbic acid

Both sugars and ascorbic acid analyses were performed by High Performance Anion Exchange Chromatography with Pulsed Amperometic Detection (HPAEC-PAD).

– Sample extraction:

All samples (blanched an control) were frozen at -18°C, for 12 hours in order to ensure a correct homogenization between the dry solids and the water content of potatoes. Furthermore the samples were homogenized using a mixer machine (model 4169/4297, Braun AG, Kronberg, Germany) and 0.3 grams of firmly homogenized sample were put in a ultrasonic bath (Branson 5510, Thermo Fisher Scientific, USA) with 30.0 ml of Milli-Q water (60°C) for 20 min. After that, the samples were centrifuged (Heraeaus Multifuge, Osterode, Germany) 10 min at 3500 rpm. Finally 1 ml of supernatant was transferred through a 0.45 µm filter (Whatman Inc., USA) into a HPLC vial. The samples were made in three replicates.

– Instrumentation and chromatographic conditions:

The precursors were detected by HPAEC-PAD with an AS-model autosampler (Dionex ICS-3000, Thermo Fisher Scientific, Sunnyvale, CA, USA) using a Carbopac PA20Guard precolumn (Dionex, 3 x 30 mm) and CarboPac PA20 column (Dionex, 3×150 mm) and a gradient of deionized water and 0.2 M NaOH.

The autosampler temperature, the run time and the flow were set at the same conditions for sugars and ascorbic acid analyses: 40 min, 0.45 ml min^{-1} and 10 °C. On the other hand, the injection volume was different: 5 µl for sugars and 15 µl for ascorbic acid,

since different concentration of these compounds are present in potatoes. The column temperature was set at 30°C (slightly above room temperature). The detection was made with PAD in Integrated Amperometry mode. Retention times for glucose, fructose and ascorbic acid were 5.78, 6.75 and 8.85 min respectively.
Finally, for the operating of the system and data collection Chromeleon Windows-based data system with full control and digital data acquisition was used.

5.2.4.4. Acrylamide

Acrylamide was quantified by chromatography–tandem mass spectrometry analytical methodology according to the methodology of (Granby & Fagt, 2004) .

– Sample extraction and clean up:

All the samples were before extraction milled to fine particles using a mixer machine (model 4169/4297, Braun AG, Kronberg, Germany). Three grams of milled sample were transferred to a 50 ml centrifuge tube. Furthermore, 150 µl of 10 µg ml^{-1} d_3-acrylamide were added as an internal standard to the centrifuge tube before 30 mL of Milli-Q water were added. The samples were placed on a homogenizer (model Ultra Turrax T25, Janke and Kunkel, Germany) at 10,000-12,000 rpm for 2 min to extract the acrylamide.
After that, the samples were centrifuged (Heraeaus Multifuge, Osterode, Germany) per 20 min at 3500 rpm and an aliquot of 4 ml was transferred to Eppendorf vials and frozen to -18°C for 12 hours and subsequently centrifuged in an Eppendorf centrifuge at 12,100 x *g* for 10 min (Minispin Centrifuge, Eppendorf Ag, Hamburg, Germany). The sample thawed during centrifugation and starch precipitated from the supernatant at this low temperature.
The SPE (Solid Phase Extraction) cleanup was performed by an automated sampler (Gilson Aspec Xl, Gilson Company Inc., Ohio, USA) using Isolute Multimode SPE-cartridges (300 mg) from (International Sorbent Technology Ltd., Hengoes UK). The eluate was transferred to Miniprep PTFE filter HPLC vials with a pore diameter of 0.45 µm (Whatman Inc., USA). The samples were made in duplicates.

– Instrumentation and chromatographic conditions:

The LC system consisted of a liquid chromatograph (model HP1100, Agilent Technologies, USA). Separation was performed with 0.1g/100g formic acid in water on a Hypercarb column (dimensions 2.1 mm x 100 mm, particle size 5 µm). The MS-MS detection was performed on a Quattro Ultima triple quadropole instrument from Micromass (UK). The source was kept at 120°C and the desolvation temperature was 400°C.

Nitrogen was used as cone and desolvation gas with flow rates of 150 and 500 l/h, respectively. Argon was used as collision gas and kept at a pressure 240 Pa. Detection was performed in positive ion mode by multiple reactions monitoring (MRM) using *m/z 72>55* of acrylamide relative to *m/z 75>58* of d_3-acrylamide for quantification.

Quantification of the fragmentations was done using MassLynx software version 4.1 including QuanLynx.

5.2.4.5. Furan quantification:

Furan was quantified according to the methodology of the National Food Institute of DTU which is a revised version of the FDA (2004) methodology.

– Sample preparation:

0.5 g of fried sample and 0.75 g of baked sample, both previously pulverized, was weighed into headspace vials, diluted with 5M NaCl solution. After adding the internal standard (d_4-furan), the vials were sealed.

– Instrumentation and chromatographic conditions:

Automated headspace sampling followed by gas chromatography/mass spectrometry (GC/MS) analysis was used to detect furan and d_4-furan in the scan mode. Furan was quantified by using a standard addition curve, where the concentration of furan in the

fortified test portions was plotted versus the furan/d_4-furan response factors. For constructing the calibration curve, seven vials were used of which four vials were fortified with furan, as follows: two vials at ca. half of the expected concentration of furan in the sample, one vial at ca. the expected concentration of furan in the sample, and one vial at ca. twice of the expected concentration of furan in the sample.

The analyses were performed on a gas chromatograph – mass spectrometer (GC-MS) (Agilent 6890N GC with Agilent 5973 N MSD, Palo Alto, California, USA) fitted with a CTC CombiPAL static headspace autosampler. The syringe was heated to 70°C and the sample vial was heated at 60°C for 30 min. 1 mL of the headspace from a 10 ml headspace vial was injected splitless on the gas chromatograph. A 15 m x 0.32 mm x 20 µm HP-Plot Q column was used.

The following conditions were set: helium flow, 1.7 ml min^{-1}; injector temperature , 200°C; oven temperature, 50°C (1 min.), with a temperature ramp of 10 °C min^{-1} to 130°C, then a temperature ramp of 3°C min^{-1} to 157°C and finally a temperature ramp of 20°C min^{-1} to 260°C, and held for 2.5 min.

The MS source temperature was 230°C and the MS quad temperature 150°C. dwell time, 50 msec. The mass spectrometer was operated in electron ionization mode. Furan was detected using single ion monitoring of the fragments *m/z* 68 and *m/z* 39. The internal standard d_4-furan was detected by monitoring the *m/z* 72 and *m/z* 42. This method has a limit of quantification of 2.4 ng g^{-1}. All analytical determinations were carried out in three replicates.

5.2.5. Experimental design and statistical analysis

In order to investigate the effect and the optimization of two factors: blanching time (at 2 levels: 5 and 15 min) and temperature (at 2 levels 50 and 80°C) over the reduction of five responses: reducing sugars and ascorbic acid contents and subsequent furan and acrylamide content as well as oil uptake, a central composite experimental design was applied using Statgraphics Centurion XV software (Manugistic Inc., USA).

The design was developed using thirteen combinations of the independent variables (blanching time and temperature) performed in random order, including five replicates of the central region (called central points) and four star points (situated outside of the experimental region defined by the level factors) , allowing the estimation of quadratic response surface curvatures which show the maximum and minimum of each response (Canet, Alvarez, & Fernandez, 2005). The first step of the statistical analysis consists into determine if the studied factors and their interactions produce a significant change in the studied responses by using an analysis of variance (ANOVA). Then, considering the factors with a significant effect over the response; it is possible to build a model (quadratic surface response equation) which represents the relationship between the factors and the response. In this way, it is obtained a model for each studied response and the optimal level is found for each response separately.

Accordingly, quadratic response surface equations were able to be calculated of the type:

$$\text{Response} = c_0 + c_1 T + c_2 t + c_3 T^2 + c_4 T t + c_5 t^2$$

Where c_0 is a constant, T and t are the blanching temperature and time, and c_1, c_2, c_3, c_4 and c_5 are the coefficients.

Finally, a multifactorial optimization is performed by using the model obtained previously per each response. That is, the factor combination that achieves the best overall response which is expressed as the desirability function.

5.3 Results and discussion

One of the approaches to mitigate the food processing contaminants is the reduction of their precursors in the food raw material (Mariotti et al., 2011). In this sense, for the case of furan formation, not only carbohydrates but also ascorbic acid have been proposed as main precursors (Perez Locas & Yaylayan, 2004) with a synergistic effect in low moisture starchy foods (Mariotti et al., 2012).

On the other hand, and specifically for the case of acrylamide formation in potato products, reducing sugars have shown to be its limiting substrate (Amrein et al., 2003).

Thus, we decided to evaluate the influence of blanching time and temperature as well as their interactions over the extraction of reducing sugars and ascorbic acid in potato slices. Subsequently, the impact of these blanching conditions over the formation of food processing contaminants (furan and acrylamide) and the oil uptake were analyzed in chips. The mentioned experiments were performed following a central composite design and the response surface equations obtained for furan, acrylamide and oil content as function of blanching time and temperature were used to optimize the blanching process considering the minimum level of food processing contaminants and oil uptake as the best response. The results obtained from these analyses are presented ad discussed in the following sections.

5.3.1. Effect of blanching conditions over the precursor´s levels

Firstly, it was tested if the studied factors (blanching time and temperature) produce a significant effect over precursor's levels (reducing sugars and ascorbic acid). Blanching time produced a significant effect over the reducing sugars and ascorbic acid content showing P-values of: 0.0012 and 0.0128 respectively; using a significance level of 5%. Since P-values obtained in both responses were lower than 0.05, blanching time produces a significant effect over the reducing sugar and ascorbic acid content. Similarly, blanching temperature is a significant factor over the reducing sugar and ascorbic acid content (P-values of: 0.0015 and 0.0022 respectively) -Table 5.2-.

Then, the coefficients of each model and their corresponding estimated effect were calculated. For both reducing sugar and ascorbic acid the blanching time showed the highest effect over their extraction (Table 5.2). These results are contradictory to those previously reported by Mestdagh et al. (2008) in which blanching temperature presented the highest effect over the reducing sugar extraction. This difference could be attributed to the fact that they performed their experiment in a different experimental region to that we used in this investigation. Interestingly, kinetics studies of sugar lixiviation performed in potato slices blanched between 60-90 °C clearly showed that even though the constant effective reducing sugar diffusivity increased with blanching temperature

during the first 40 min of blanching; there were no considerable differences between the reducing sugars levels extracted during the first stages of the lixiviation (first 20 min) (Pedreschi, Travisany, Reyes, Troncoso, & Pedreschi, 2009). Besides, similar results were reported by Arroqui, Rumsey, Lopez, and Virseda (2002) who studied the losses of ascorbic acid by diffusion in blanched potato tissue at 65°C, 80°C and 93°C. These authors observed an Arrhenius-type relationship between the ascorbic acid apparent diffusivity and temperature, during the first 60 min of blanching; however, a separation in the ascorbic acid content between the different temperatures applied is possible to be observed at longer times of extraction as it was mentioned before. Previous information could explain that for the experimental blanching region selected in the present research, blanching time was the most important factor over the extraction of the two studied precursors (ascorbic acid and reducing sugars), since the highest level of blanching time studied was 17 min.

Table 5.1: Analysis of quadratic response surface equation for precursors (reducing sugars and ascorbic acid).

	Reducing sugars			**Ascorbic acid**		
Terms	Coefficient	P-value	Estimated effect	Coefficient	P-value	Estimated effect
Constant	131,9640	-	-	815.3930	-	-
T	-0.9029	0.0015	-27.0856	-6.53801	0.0022	-126.1400
t	-2.8077	0.0012	-28.0766	-14.0789	0.0128	-140.7890

The second step was to build the surface responses by using the factors previously found as significant (blanching time and temperature). The obtained surface response graphics show the relationship between the blanching time and temperature conditions and the reducing sugars and ascorbic acid content (Figure 5.1) as well as the maximum and minimum values obtained for these responses. From Figure 5.1, it is possible to observe that the level of both precursors decreased after blanching upon increasing blanching

time and temperature. Finally, reducing sugar quadratic surface response shows an R^2 (adjusted for degree of freedom) of 0.82 which indicates that the model as fitted explains 82% of the variability in reducing sugar content. For ascorbic acid content a response of R^2 (adjusted for degree of freedom) of 0.73 was obtained. Consequently, industrial blanching process should be performed at higher temperatures in order to decrease the costs of processing, since shorter blanching times would be necessary to obtain significant reduction in precursor levels, and subsequently in the lower formation of furan and acrylamide. Although this approach is correctly, it is also necessary to analyze some secondary effects produced by the high temperature over the microstructure of potato slices. The diffusion of the precursors is associated with a breaking in vegetal tissues. This loss of firmness will affect the texture of potato slices after the frying process, increasing the oil uptake of potato chips.

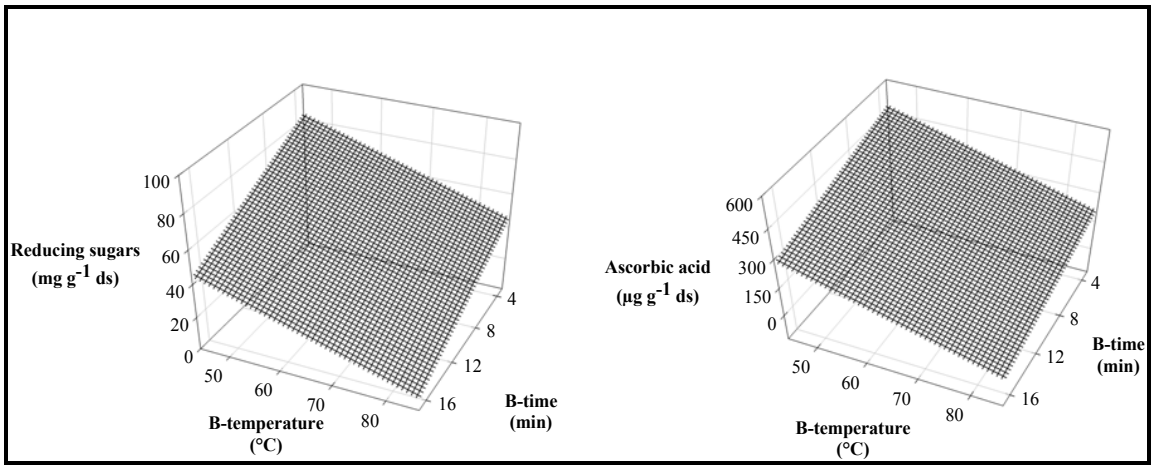

Figure 5.1: Response surface plot of the sugars and ascorbic acid content of potato slices as function of blanching conditions.

5.3.2. Impact of blanching conditions over the level of food processing contaminants and oil uptake.

Both blanching time, temperature and the quadratic interaction of temperature were significant factors (*P≤0.05)* over the occurrence of furan (*P:* 0.0004, *P*: 0.0030 and *P*: 0.0200 for time, temperature and quadratic interaction of temperature, respectively).

On the other hand, for acrylamide only blanching time and temperature were significant factors (*P:* 0.0194 and *P*: 0.0018 for time and temperature, respectively). Blanching temperature presented the highest effect over the minimization of both contaminants under the experimental conditions tested as it can be observed in Table 5.2. After analysing the response surfaces statistically build for furan and acrylamide, the obtained quadratic equations presented R^2 (adjusted for degree of freedom) of 0.87 and 0.73 for furan and acrylamide, respectively. Both furan and acrylamide content significantly decreased ($P \leq 0.05$) in blanched potato chips when the blanching temperature and time increased (Figures 5. 2).

Interestingly, a linear relationship was observed between the ascorbic acid content and furan concentration (R^2: 0.80). Likewise, a similar behaviour was found between the reducing sugars level and furan and acrylamide concentration (R^2: 0.84 and $R^{2:}$ 0.81 respectively) (Figures 5.3).

Finally, for the case of oil uptake most of the factors were significant ($P \leq 0.05$), except the quadratic term of time (*txt*). Oil uptake increased as blanching temperature did (Figure 5.2) as it is reflected in the constructed quadratic response (R^2: 0.95). These results confirm previous findings which suggest that low-temperature blanching improves the textural quality of fried potatoes, since the limpness and oil content of fried product are reduced (Pedreschi & Moyano, 2005a, 2005b).

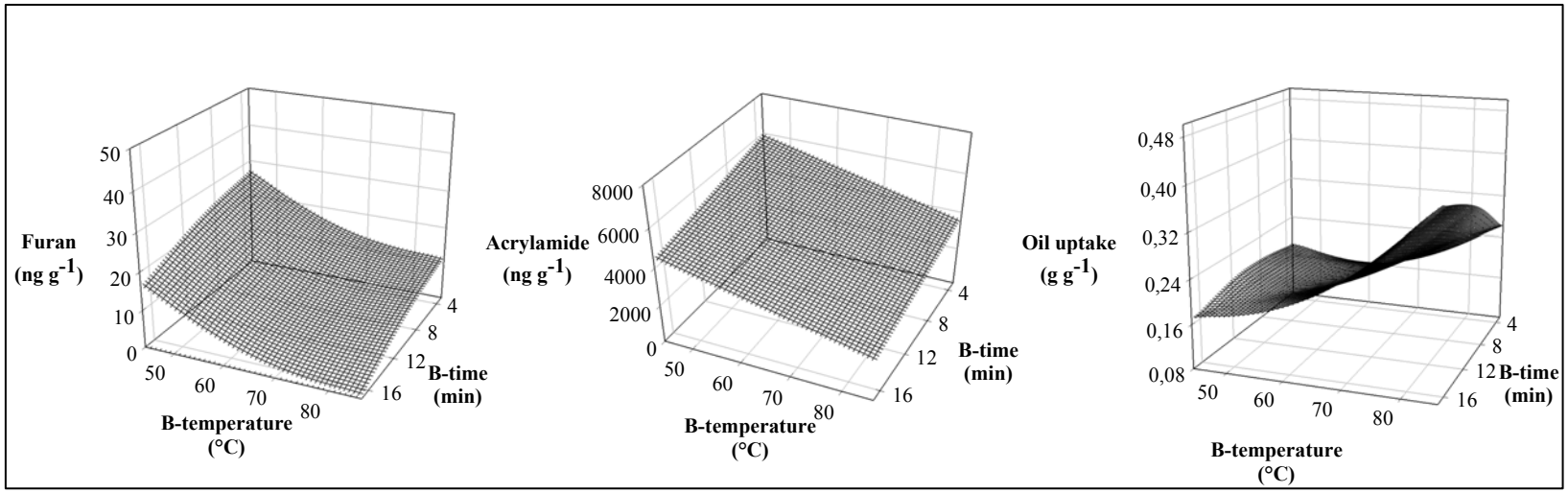

Figure 5.2: Response surface plot of the acrylamide, furan and oil content in crisps as function of blanching conditions.

Table 5.2: Analysis of quadratic response surface equation for food processing contaminants and oil uptake.

	Acrylamide			**Furan**			**Oil**		
Terms	Coefficient	P- value	Estimated effect	Coefficient	P-value	Estimated effect	Coefficient	P -value	Estimated effect
Constant	8776.75	-	-	98.9556	-	-	0.8064	-	-
T	-56.0889	0.0018	-1682.6700	-2.1294	0.0004	-11.3416	-0.02092	0	0.1101
t	-106.697	0.0194	-1066.9700	-0.7878	0.0030	-7.87841	-0.01366	0.0033	0.04544
TxT	-	>0.05	-	0.01347	0.0200	6.0624	0.0002	0.0005	0.07545
Txt	-	>0.05	-	-	>0.05	-	0.0003	0.0219	0.04201
txt	-	>0.05	-	-	>0.05	-	-	>0.05	-

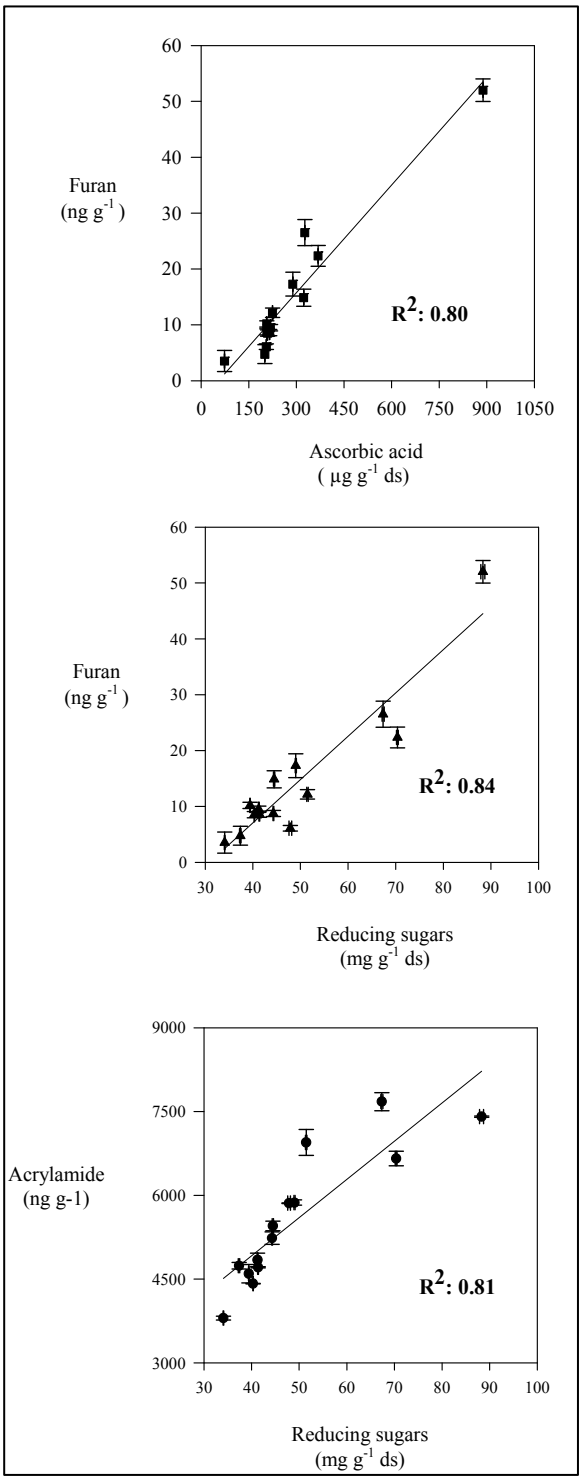

Figure 5.3: Relationship between the levels of food processing contaminants and their precursors.

5.3.3. Optimization of Blanching by desirability function

Since consumer´s preference for low oil content and fat –free products has been the driving force of snack industry, to optimize the blanching minimizing not only the level of food processing contaminants but also the oil uptake was the objective of this research. In this sense, the desirability function approach is one of the most widely used methods for the optimization of multiple response processes. It is based on the idea that the "quality" of a product or process that has multiple quality characteristics, with one of them outside of some "desired" limits, is completely unacceptable. The method finds operating conditions that provide the "most desirable" response values (Myers, Montgomery, & Anderson-Cook, 2009). In the present investigation, blanching variables such as time and temperature were optimized by a desirability function in order to calculate the minimum value of furan content; acrylamide content and oil content (4.49 ng g^{-1}, 3377 ng g^{-1} and 0.23 g g^{-1}, respectively) in potato chips after being fried at 170°C. The estimated optimum values for blanching conditions were 64°C and 17 min. These values were within the range of the selected parametric values indicating proper adequacy of the range selection (Burande, Kumbhar, Ghosh, & Jayas, 2008; Verma, Agrawal, Sharma, Sarkar, & Sharma, 2005). Under these conditions, an optimum desirability (maximum desirable response) of 0.81 was obtained as it can be observed in figure 4. This optimum desirability corresponded to reductions of 91%, 54% and 19% in the contents of furan, acrylamide, and oil, respectively, in comparison with the control samples.

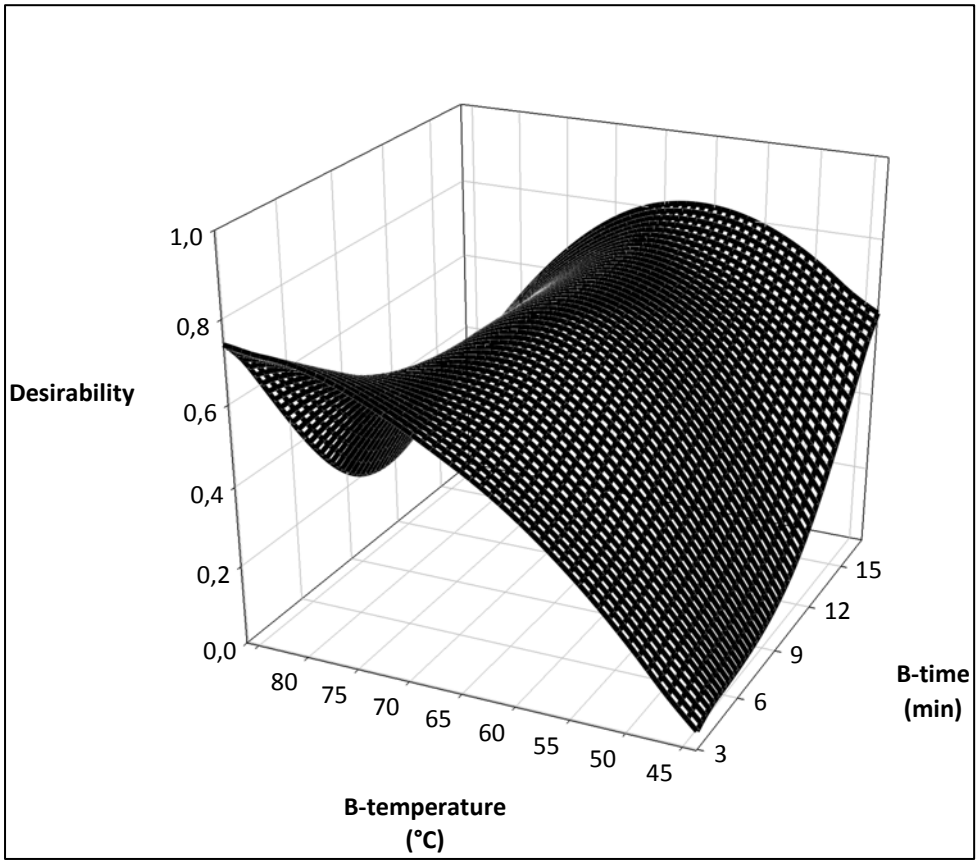

Figure 5.4: Desirability function of potato slices blanching

5.4 Conclusions

Blanching process may be considered as an alternative to produce healthier chips with lower levels of furan, acrylamide and oil. The blanching time was the main factor related to the leaching out of the precursors. On the other hand, blanching temperature presented the highest effect over the reduction of the furan, acrylamide and oil content. The optimum blanching conditions estimated in the experimental region studied were 64°C and 17 min.

5.5 References

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6. GENERAL CONCLUSIONS AND FUTURE PROSPECTS

6.1. General conclusions

The furan generation in foods is highly complex process and depends on the specific features of food matrix and heating conditions. In this sense, our results showed that furan formation in starchy foods processed at high temperatures such as snacks and bread was significantly affected by the formulation (ascorbic acid addition) and cooking methods (frying and baking). Additionally, furan content in fried products increased as oil uptake levels did. As for Maillard reactions in general, furan level in all samples linearly correlated with their degree of non-enzymatic browning when the sample moisture content decreased during heating. Previous results would indicate that Maillard reaction might be the main route of formation of furan in low moisture starchy foods.

On the other hand, the first data on furan occurrence in foods purchased and consumed in Chile was successfully determined. The levels of furan in Chilean foods presented considerable differences, varying from below the limit of detection in fried meat to 936 ng g^{-1} in automatic machine brewed coffee. Furthermore, the dietary furan exposure assessment showed that low moisture starchy food such as chips and wheat flour biscuits were the major source of dietary furan for all the age sectors studied.

Based on the MOE values obtained in this doctoral research, dietary exposure to furan in Chile may be considered as of potential public health concern both for school children and babies.

Finally, considering the fact that chips were found to be a critical food in the dietary furan exposure of Chilean population, a furan mitigation technique for this food matrix was developed. The optimization of a blanching process to minimize the acrylamide and furan content of chips without increasing their oil uptake was carried out. According to our results, blanching process can be proposed as an alternative to produce healthier chips with lower levels of food processing contaminants and a controlled oil uptake.

6.2. Future prospects

The present thesis got deep into the formation of furan in highly consumed starchy foods processed at high temperatures, and proposed the blanching of potato slices as a feasible alternative to improve the chemical safety of chips. Likewise, during the development of this dissertation research new channels of study were opened, indicating that the study of food processing contaminants can be considered as an emerging area not only from a scientific perspective but also from a technological point of view.

In this sense, it is proposed that future work can be conduct in two directions. The first one points to explore deeper some of the results obtained in this thesis. The second one points to consider the sensorial quality of furan reduced-foods as critical response for the developing of food processing mitigation techniques.

The relation of non-enzymatic browning and furan content in low moisture starchy food can be a possibility of further research. Computer vision techniques may be used to build calibration models for predicting the furan content of foods as function of their browning ratio.

It would be quite interesting to study the effect of a_w and temperature over rate of furan generation with the aim of limiting its occurrence in different food matrices. Through the following of the kinetics of furan formation at different a_w values and temperatures it would be possible to determine the activation energy involved in the rate-determining steps leading to furan.

Also, to determine the influence of lipid oxidation over the furan generation in fried foods is a topic that could be explored, in order to elucidate the real contribution of this pathway to the final furan content of these products. The formation of polar compound in the penetrated oil of fried products could be considered as a good parameter of lipid oxidation. To explore the existence of some correlations between this one and the furan content of the fried samples would clarify the commented pending issue.

Furthermore, although, our results reveal that the Chilean population might be exposed to dietary furan; it is necessary to access a national survey in order to obtain more representative information of the current Chilean food consumption.

Finally, a crucial challenge is generated from this thesis, which is related to the realistic application of the furan mitigation technologies to starchy foods.

To develop healthier foods which improve the life quality of the consumers is one of the most important objectives of food scientists. In this sense, the reduction of food processing contaminants of products can be considered as an important contribution. However, the improvement of life quality of people not only considers the safety and nutritional aspects of the products. The pleasure caused by the consuming of a food is also a relevant component of this objective. It is here, where the sensory evaluation of furan-reduced foods would allow accomplishing this purpose. Sensory evaluation is a powerful tool that businesses use to understand their target market, define product concepts, streamline formulation development, substitute ingredients, resolve quality issues, and ensure long shelf life. Characterization of the appearance, aroma, flavor, and texture of the furan-reduced foods product is a necessary knowledge for setting performance standards and evaluating the progress of the furan mitigation technologies.