UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

ACRILAMIDA EM ALIMENTOS: OCORRÊNCIA, MÉTODOS ANALÍTICOS E ESTIMATIVAS DE INGESTÃO

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SUMÁRIO

Índice de tabelas

Índice de figuras

Resumo Geral

Uma importante descoberta de pesquisadores suecos em abril de 2002 mostrou que acrilamida, uma substância provavelmente carcinogênica a seres humanos, pode ser formada em determinados alimentos que são submetidos a tratamento térmico em altas temperaturas.

No presente estudo, 111 amostras de diferentes categorias de alimentos foram coletadas em supermercados, lojas de "fast-foods" e restaurantes da cidade de Campinas-SP, entre os meses de setembro de 2004 e abril de 2006, e analisadas para verificar a presença de acrilamida. As amostras foram selecionadas em função dos resultados divulgados por outros países e incluíram, além de produtos à base de batata, trigo e café, alimentos tipicamente brasileiros à base de mandioca e milho, processados em altas temperaturas. Os níveis de acrilamida foram determinados por cromatografia líquida de alta eficiência e espectrometria de massas em série (LC-MS/MS), conforme método previamente desenvolvido. Como a aplicação deste método em matrizes de cacau não apresentou resultados satisfatórios, o mesmo foi modificado através da inclusão de uma etapa de precipitação de proteínas e de alterações no procedimento de limpeza, o que resultou em melhor desempenho nesta matriz. As concentrações de acrilamida determinadas nas amostras analisadas confirmam que produtos à base de batata, tais como batatas fritas e batatas chips, biscoitos e café são os alimentos que apresentam os maiores níveis de acrilamida.

Em etapa posterior, a ingestão potencial diária deste contaminante foi estimada combinando-se os dados analíticos de ocorrência de acrilamida obtidos no presente estudo com dados de consumo dos alimentos analisados, disponíveis para a população em geral e para uma população de adolescentes da cidade de Piracicaba-SP. Os valores médios de ingestão estimados para ambas as populações (0,14 e 0,12 µg/kg de peso corpóreo/dia, respectivamente) são inferiores aos valores relatados para populações de países da América do Norte e Europa (0,3 a 2 µg/kg de peso corpóreo/dia), o que pode ser parcialmente

atribuído ao fato de que as estimativas de ingestão nestes países levaram em conta a contribuição de um maior número de alimentos. Dessa forma, é importante que mais amostras e grupos de alimentos sejam investigados para que, futuramente, possa ser calculada a contribuição da dieta total como fonte de acrilamida para a população brasileira e avaliados os possíveis riscos à saúde relacionados à exposição a este contaminante.

Palavras-chaves: acrilamida, reação de Maillard, batata, ingestão, LC-MS/MS.

Summary

ACRYLAMIDE IN FOODS: OCCURRENCE, ANALYTICAL METHODS AND INTAKE ESTIMATES.

An important discovery of Swedish researchers in April 2002 showed that acrylamide, a probable carcinogen to humans, can be formed in certain foods which are submitted to thermal treatment at high temperatures.

In the present study, 111 samples of different food categories were collected at supermarkets, fast-food restaurants and restaurants, in Campinas-SP, between September 2004 and April 2006, and analysed to verify the presence of acrylamide. The samples were selected according to results reported in other countries and included, beyond potato- and wheat-based products and coffee, typical Brazilian foods made from cassava and maize, and processed at high temperatures. The levels of acrylamide were determined by high performance liquid chromatography *tandem* mass spectrometry (LC-MS/MS), according to a method previously developed. As the application of this method in cocoa matrices did not presented satisfactory results, there was a need to modify it by the inclusion of a protein precipitation step and changes in the clean-up procedure, which improved its performance in this matrix. The concentrations of acrylamide determined in analysed samples confirm that potato-based products, such as French fries and potato chips, biscuits and coffee are the foods containing the highest levels of acrylamide.

In a next step, the potential daily intake of this contaminant was estimated by combining analytical data on the occurrence of acrylamide obtained in the present study with data on food consumption for the analysed foods, available for the general population and for a population of adolescents from the city of Piracicaba-SP. The mean intakes estimated for both populations (0.14 e 0.12 µg/kg body weight/day, respectively) are below the values reported for populations from North American and European countries (0.3 to 2 µg/kg body weight/day),

which may be partially attributed to the fact that intake estimates conducted in these countries took into account the contribution of a greater number of foods. In this way, it is important that more samples and food groups be investigated so that, in the future, it may be possible to calculate the contribution of the total diet as source of acrylamide for the Brazilian population, and to assess the possible risks to health with regard to the exposure to this contaminant.

Keywords: acrylamide, Maillard reaction, potato, intake, LC-MS/MS.

Introdução geral

Acrilamida (C_3H_5NO, peso molecular 71), substância produzida industrialmente através da hidratação de acrilonitrila e utilizada principalmente na síntese de poliacrilamida, foi encontrada em uma grande variedade de alimentos processados em altas temperaturas por um grupo de pesquisadores suecos em abril de 2002 (Tareke et al., 2002; SNFA, 2002).

Esta descoberta motivou uma série de ações em nível mundial devido aos possíveis riscos que a ingestão de acrilamida através de alimentos poderia representar à saúde humana. De acordo com a Agência Internacional de Pesquisa sobre o Câncer (IARC), a acrilamida pode provocar danos ao sistema nervoso em humanos, além de apresentar propriedades genotóxicas e carcinogênicas, confirmadas em estudos experimentais com animais (IARC, 1994). A partir de então, muitos pesquisadores têm se dedicado ao estudo da acrilamida em alimentos, sendo que importantes questões já se encontram elucidadas na literatura.

Sabe-se hoje que a formação de acrilamida em alimentos ocorre a partir da reação de Maillard entre aminoácidos e açúcares redutores, em temperaturas acima de 120ºC (Mottram et al. 2002; Stadler et al. 2002; Becalski et al. 2003). Foi observado que a presença de asparagina aumentava significativamente o nível de acrilamida formada e foi confirmado, através de experimentos com ^{15}N e ^{13}C marcados, que átomos de carbono e nitrogênio da molécula de acrilamida eram provenientes dos locais correspondentes da molécula de asparagina. Sendo assim, asparagina foi identificada como o principal precursor da acrilamida, embora o mecanismo exato da reação ainda não esteja completamente esclarecido.

Os primeiros dados sobre a ocorrência de acrilamida em alimentos indicaram que produtos à base de batata, como batatas fritas e chips, e produtos à base de cereais, como pães, torradas, biscoitos e cereais matinais, apresentavam

os maiores teores de acrilamida, podendo atingir até 3500 µg/kg, dependendo do tipo de produto (FDA, 2002; NFCA, 2002; SOPH, 2002; UK FSA, 2002). Níveis moderados de acrilamida (5-50 µg/kg) foram encontrados em produtos à base em proteínas processados termicamente, enquanto que em alimentos crus ou cozidos em água a presença deste contaminante não foi detectada (<5 µg/kg) (Tareke et al., 2002).

Embora muitos países já tenham reportado os níveis de acrilamida em seus produtos de mercado, nenhum dado sobre sua ocorrência em países da África e América Latina foi submetido ao Comitê de Especialistas em Aditivos Alimentares da FAO/OMS (JECFA) quando da avaliação deste contaminante em 2005 (FAO/WHO, 2005). Desta forma, esta pesquisa teve como objetivo principal investigar os níveis de acrilamida em alguns alimentos que fazem parte da dieta da população brasileira, utilizando-se método analítico desenvolvido e validado previamente por colaboradores deste projeto. Observando-se que este método apresentava limitações para produtos à base de cacau, buscou-se também, no presente estudo, testar algumas técnicas para melhorar o seu desempenho nestas matrizes. A partir dos níveis de ocorrência de acrilamida obtidos no presente estudo e de dados de consumo dos alimentos analisados, a ingestão potencial diária deste contaminante foi estimada para a população em geral e para uma população de adolescentes da cidade de Piracicaba-SP. Espera-se que as informações geradas nesta pesquisa possam contribuir para avaliações de risco realizadas mundialmente e permitir uma melhor compreensão da distribuição de acrilamida em alimentos de diferentes países.

O capítulo 1 deste trabalho, já publicado na revista *Brazilian Journal of Food Technology, v. 9, n. 2, p. 123-134, abr./jun. 2006*, apresenta uma revisão bibliográfica sobre os principais tópicos abordados e investigados desde a descoberta da formação de acrilamida em alimentos. O artigo apresentado no capítulo 2, publicado na revista *Food Additives and Contaminants, v.24, n. 3, p. 236-241, March 2007*, discute os resultados da ocorrência de acrilamida em alimentos no Brasil. No capítulo 3, redigido de acordo com as normas da revista

Analytica Chimica Acta, são discutidos os resultados do desenvolvimento e validação do método de análise de acrilamida em matrizes à base de cacau. Os capítulos 4 e 5 referem-se aos estudos de estimativa de ingestão e foram redigidos de acordo com as normas das revistas *Food and Chemical Toxicology* e *Food Additives and Contaminants*, respectivamente.

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CAPÍTULO 1: Revisão bibliográfica

Artigo publicado na revista

Brazilian Journal of Food Technology, v. 9, n. 2, p. 123-134, abr./jun. 2006

Acrilamida em alimentos: uma revisão

Resumo

Em abril de 2002, pesquisadores suecos comunicaram a formação de altos níveis de acrilamida durante o processamento térmico de diversos alimentos como batata frita, batata chips, cereais matinais e pão. Esta descoberta motivou uma série de ações em nível mundial, justificadas pelo conhecimento de que a acrilamida pode provocar danos ao sistema nervoso em humanos, além de apresentar propriedades genotóxicas e carcinogênicas, confirmadas em estudos experimentais com animais. De acordo com as informações disponíveis até o presente, o principal caminho para a formação da acrilamida envolve a reação de Maillard entre aminoácidos e açúcares redutores, sendo o aminoácido asparagina identificado como principal precursor. Entretanto, o mecanismo exato da formação de acrilamida em alimentos ainda não está completamente esclarecido. Muitos estudos relativos à otimização de parâmetros de processo para minimizar a formação de acrilamida nos alimentos, métodos analíticos, metabolismo e efeitos biológicos deste contaminante têm sido publicados. Embora progressos significativos já tenham sido obtidos, ainda existe a necessidade de um melhor entendimento de seu papel na saúde humana. O presente artigo apresenta uma revisão de literatura sobre acrilamida, incluindo aspectos toxicológicos, ocorrência, mecanismos de formação, estimativas de exposição e métodos analíticos para sua determinação em alimentos.

***Palavras-chaves**: carcinógeno, reação de Maillard, carboidratos, asparagina.*

Summary

Acrylamide in foods: a review*. In April 2002, Swedish researchers announced the formation of high levels of acrylamide during the thermal treatment of many foods such as French fries, potato chips, breakfast cereals and bread. The discovery attained worldwide concern, justified by the knowledge that acrylamide can cause damage to the nervous system in humans and present genotoxic and carcinogenic properties confirmed in experimental studies with animals. According to the information available at this moment, the main pathway for acrylamide formation is the Maillard reaction between amino acids and reducing sugars, with asparagine identified as main precursor. However, the exact mechanism of acrylamide formation in foods is not completely understood. Many studies concerning to the optimization of process parameters to minimize the acrylamide formation in foods, analytical methodologies, metabolism and biological effects have been published. Although significant progress have been obtained, a need exist of a better understanding of its role in human health. This paper presents a literature review on acrylamide including toxicological aspects, occurrence in food, mechanisms of formation, exposure assessments and analytical methodologies for its determination in food.*

Keywords: *carcinogen, Maillard reaction, carbohydrates, asparagine.*

1 – INTRODUÇÃO

Durante a construção de um túnel na Suécia em 1997, a utilização de material selante contendo altos níveis do monômero acrilamida foi responsável pela contaminação ambiental de lençóis freáticos localizados na região da construção. Este acidente, que resultou na morte de peixes e paralisia de vacas, foi atribuído a uma reação de polimerização incompleta do material, com liberação de acrilamida no ambiente. Altos níveis de adutos de acrilamida com hemoglobina

foram encontrados no sangue dos animais que apresentaram paralisia, comprovando a origem da contaminação.

Adicionalmente, trabalhadores envolvidos na construção do túnel relataram o aparecimento de sintomas como paralisia e formigamento nas mãos e nos pés. Ao se avaliar quantitativamente a exposição destes trabalhadores à acrilamida, através da medida de adutos com hemoglobina, observou-se que não somente o grupo de indivíduos ocupacionalmente expostos à substância, mas também o grupo controle, com exceção de fumantes, apresentava níveis significativos de adutos no sangue (TÖRNQVIST *et al*., 2000).

Os níveis de adutos de acrilamida encontrados no sangue de indivíduos não expostos se tornou uma questão preocupante, já que as fontes de exposição até então conhecidas, como a água, cosméticos e a fumaça de cigarro, foram consideradas não significativas para explicá-los, sugerindo a existência de uma nova fonte de exposição. A ocorrência de acrilamida na fumaça de cigarro poderia indicar que esta substância era formada durante combustão incompleta ou aquecimento de matéria orgânica (BERGMARK, 1997).

A formação de acrilamida durante o aquecimento foi posteriormente confirmada em estudos experimentais através da identificação de adutos de acrilamida com a hemoglobina em animais alimentados com ração frita a 200ºC, sendo que a presença deste aduto não era observada no sangue de animais alimentados com ração não frita (TAREKE *et al*., 2000). Em abril de 2002, os mesmos pesquisadores comunicaram a descoberta de altos níveis de acrilamida em diversos alimentos submetidos a tratamento térmico em altas temperaturas (TAREKE *et al*., 2002).

Devido às preocupações sobre os possíveis efeitos tóxicos à saúde humana relacionados à ingestão de acrilamida através da dieta, a Agência das Nações Unidas para Agricultura e Alimentação (FAO) e a Organização Mundial da Saúde (OMS) realizaram um encontro, em junho de 2002, para discutir as conseqüências sanitárias da presença deste contaminante em alimentos. Diversas recomendações foram estabelecidas com a finalidade de se obter mais

informações e de se proceder a novos estudos para um melhor entendimento dos riscos que a ingestão de acrilamida através de alimentos representava para a saúde humana (FAO/WHO, 2002).

Desde então, muitos pesquisadores têm se dedicado ao estudo da acrilamida em alimentos e significativos progressos já foram obtidos com relação ao metabolismo e efeitos biológicos desta substância. Diferentes métodos analíticos foram desenvolvidos e validados, viabilizando a determinação do teor de acrilamida em vários tipos de alimentos, em níveis de concentração cada vez menores. Indústrias e institutos de pesquisa têm trabalhado juntos na otimização de parâmetros de processos para minimizar a formação de acrilamida em alimentos, sendo que diferentes estratégias potencialmente promissoras já foram identificadas e apresentadas.

2 – ASPECTOS TOXICOLÓGICOS DA ACRILAMIDA

Acrilamida (C_3H_5NO, peso molecular 71) é um sólido branco cristalino, estável à temperatura ambiente, solúvel em água, etanol, metanol, dimetil éter e acetona, e insolúvel em benzeno. Fazem parte de sua estrutura química uma função amida polar, que confere a característica de alta solubilidade em água, e uma função vinil, que permite a polimerização. A acrilamida também é conhecida como 2-propenamida (US EPA, 1994).

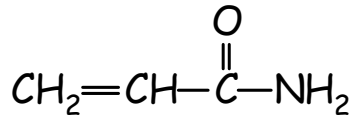

Figura 1 – Estrutura molecular da acrilamida

Acrilamida tem sido produzida industrialmente desde a década de 50 através da hidratação de acrilonitrila e é utilizada, principalmente, para produzir

poliacrilamida, que é empregada, por exemplo, no tratamento de clarificação da água e do esgoto e na produção de géis para eletroforese. Além de produzir poliacrilamida, acrilamida é utilizada em fundações para a construção de túneis e barragens (WHO, 2002).

Os riscos associados à acrilamida não são recentes e, provavelmente, a população tem sido exposta a esta substância por algumas gerações. Segundo a Agência Internacional de Pesquisa sobre o Câncer (IARC), acrilamida é classificada como uma substância provavelmente carcinogênica em humanos (grupo 2A) e, além disso, pode ser tóxica ao sistema nervoso e reprodutivo de homens e animais em determinadas doses (IARC, 1994).

Devido ao potencial tóxico deste contaminante e, conseqüentemente, aos riscos que a sua ingestão através de alimentos poderia representar para a saúde humana, o Comitê do Codex sobre Aditivos e Contaminantes em Alimentos (CCFAC), em 2004, recomendou ao Comitê de Especialistas em Aditivos Alimentares da FAO/OMS (JECFA) que realizasse uma avaliação do risco da acrilamida e, eventualmente, estabelecesse limites para sua ingestão, já que esta substância nunca tinha sido avaliada anteriormente.

O JECFA solicitou dados relacionados a todas as áreas de pesquisa sobre acrilamida e conduziu a primeira avaliação toxicológica deste contaminante durante sua 64ª Reunião, em fevereiro de 2005. Os resultados da avaliação confirmaram o potencial tóxico da acrilamida, tendo o Comitê concluído que a substância deveria ser reavaliada assim que os resultados de pesquisas em andamento sobre carcinogenicidade e neurotoxicidade estivessem disponíveis. Foi recomendado também que esforços deveriam ser direcionados para a redução do teor de acrilamida formada durante o processamento e era desejável que os países em desenvolvimento apresentassem dados sobre a ocorrência de acrilamida nos alimentos representativos das dietas nacionais (FAO/WHO, 2005).

2.1 - Absorção, distribuição, metabolismo e excreção

Em animais experimentais, acrilamida é rapidamente absorvida pelo trato gastrintestinal e largamente distribuída nos tecidos. É metabolizada ao epóxido glicidamida através de uma reação catalisada pela enzima citocromo P2E1. A conjugação da acrilamida com glutationa é um caminho alternativo para o seu metabolismo. A biodisponibilidade absoluta da acrilamida varia entre 23 e 48% em roedores para uma dose de 0,1 mg/kg de peso corpóreo (pc), e tanto a acrilamida quanto a glicidamida são rapidamente eliminadas na urina.

A glicidamida é muito mais reativa com o DNA do que a acrilamida e vários adutos da base purina têm sido identificados *in vitro*. Já com a hemoglobina, acrilamida e glicidamida podem se ligar covalentemente aos aminoácidos da proteína do sangue para formar adutos (FAO/WHO, 2005).

2.2 - Carcinogenicidade, genotoxicidade e neurotoxicidade

Os possíveis efeitos carcinogênicos da acrilamida foram testados em dois experimentos de longa duração com ratos Fischer 344 através da administração de doses de até 3 mg de acrilamida/kg pc/dia na água, durante até dois anos de estudo. Verificou-se um aumento significativo na incidência de vários tipos de tumores nos animais experimentais de ambos os sexos quando comparados ao grupo controle (JOHNSON *et al*., 1986; FRIEDMAN *et al*., 1995). Estudos de curta duração com camundongos que receberam de 0 até 60 mg/kg pc de acrilamida na água, três vezes por semana, durante oito semanas, também mostraram um aumento significativo do número de adenomas no pulmão, proporcional à dose administrada (BULL *et al*., 1984).

Além de demonstrar efeitos carcinogênicos em animais experimentais, acrilamida e glicidamida possuem propriedades mutagênicas e clastogênicas em células de mamíferos. Embora a acrilamida não tenha apresentado mutagenicidade no teste de Ames para *Salmonella*, a administração desta

substância induziu mutações genéticas e aberrações cromossômicas em células de roedores *in vivo* e *in vitro* (IARC, 1994; RICE, 2005). A genotoxicidade da acrilamida parece estar relacionada com a sua biotransformação em glicidamida, que mostrou maior potencial mutagênico que a própria acrilamida em uma determinada dose (FAO/WHO, 2005).

Estudos de toxicidade reprodutiva com roedores demonstraram redução da fertilidade, efeitos letais dominantes e efeitos adversos na morfologia e no número de esperma em machos em doses de acrilamida maiores que 7 mg/kg pc/dia. Em fêmeas, nenhum efeito adverso na fertilidade foi observado. Acrilamida mostrou-se não teratogênica em ratos ou camundongos (FAO/WHO, 2005).

Embora os estudos realizados com animais experimentais tenham considerado a carcinogenicidade e a genotoxicidade como possíveis conseqüências da exposição à acrilamida, a neurotoxicidade é o único efeito adverso identificado através de estudos epidemiológicos envolvendo populações humanas ocupacionalmente expostas (LO PACHIN, 2005). A exposição ocupacional de trabalhadores suecos durante a construção de um túnel resultou em leves e reversíveis sintomas do sistema nervoso periférico (TÖRNQVIST *et al*., 2000; HAGMAR *et al*., 2001).

Alguns estudos epidemiológicos sobre a incidência de câncer em populações humanas, envolvendo operários ocupacionalmente expostos à acrilamida, começaram a ser publicados na década de 80. Os resultados indicaram que nenhuma tendência era encontrada com relação ao aparecimento de casos de câncer ao se comparar grupos de operários com alta e baixa exposição à acrilamida e que havia pouca evidência para se estabelecer uma relação entre exposição à acrilamida e mortalidade por câncer em humanos (SOBEL *et al*., 1986; COLLINS *et al*., 1989; MARSH *et al*., 1999). As únicas informações disponíveis que consideram os riscos à saúde humana relacionados à ingestão de acrilamida através dos alimentos estão baseadas em estudos de casos-controle (MUCCI *et al*., 2003; PELUCCHI *et al*., 2004). Entretanto, o JECFA considera que estes estudos apresentam poder limitado para detectar um

aumento significativo no risco de câncer provocado pela ingestão de acrilamida através da dieta (FAO/WHO, 2005).

Baseado em dados de estudos experimentais com roedores, o JECFA (FAO/WHO, 2005) estabeleceu o nível sem efeito observado (NOEL) para acrilamida como sendo:

- 0,2 mg/kg pc/dia para indução de alterações morfológicas em nervos
- 10 mg/kg pc/dia para neurotoxicidade durante desenvolvimento
- 2 mg/kg pc/dia para efeitos reprodutivos e no desenvolvimento

2.3 – Cálculos do risco associado à exposição à acrilamida

Alguns pesquisadores têm estimado o número de casos de câncer em humanos, em órgãos não específicos, como resultado da ingestão de acrilamida através de alimentos e bebidas, baseados em extrapolações de resultados obtidos em experimentos laboratoriais com roedores (NFCA, 2002; KONINGS *et al*., 2003).

Os resultados das avaliações de risco podem não ser os mesmos, já que são baseados em modelos matemáticos diferentes. Por exemplo, o consumo de 1 µg de acrilamida/kg pc/dia ao longo de toda a vida, considerando-se 10.000 indivíduos expostos, poderia representar o surgimento de:

- 45 casos de câncer, segundo a Agência Americana de Proteção Ambiental (US EPA, 1993)
- 13 casos de câncer, segundo a Agência de Controle de Alimentos da Noruega (NFCA, 2002)
- 7 casos de câncer, segundo a OMS (WHO, 1996)

O risco de surgimento de câncer devido à exposição à acrilamida através do café foi estimado em 2 casos por 10.000 indivíduos noruegueses expostos,

considerando-se homens e mulheres. Em relação à ingestão de acrilamida através de alimentos e bebidas, este valor subiu para 7 casos de câncer por 10.000 indivíduos expostos durante 53 anos (NFCA, 2002).

Com base na média de exposição da população holandesa à acrilamida, KONINGS *et al.* (2003) calcularam uma incidência anual de 75 casos de câncer, quando utilizada a estimativa de risco da OMS (WHO, 1996). Este valor aumentou para 130 casos de câncer quando o cálculo foi baseado na estimativa de risco da NFCA (2002).

É importante ressaltar que, embora muitos órgãos de regulamentação de alimentos concordem com a identificação qualitativa da acrilamida como um perigo carcinogênico, a FAO e a OMS consideram que modelos teóricos que calculam um risco quantitativo ao homem não são suficientemente seguros para se tirar conclusões, principalmente na ausência de resultados epidemiológicos positivos (RICE, 2005).

3 – ACRILAMIDA EM ALIMENTOS

3.1 – Ocorrência

Após a descoberta da formação de acrilamida em alimentos pelos pesquisadores da Universidade de Estocolmo, a Agência Nacional de Alimentos da Suécia foi o primeiro órgão que realizou um estudo sobre a determinação de acrilamida em produtos disponíveis no mercado, confirmando sua presença em diferentes níveis, em muitos alimentos processados termicamente (SNFA, 2002). Em seguida, outros países como Estados Unidos, Noruega, Suíça e Reino Unido iniciaram suas pesquisas, e também confirmaram que acrilamida estava presente em diversos alimentos (FDA, 2002; NFCA, 2002; SOPH, 2002; UK FSA, 2002).

De modo geral, os resultados destas avaliações indicaram que alimentos ricos em carboidratos submetidos a altas temperaturas apresentavam altos níveis de acrilamida. Alimentos que não eram fritos ou assados durante seu

processamento ou preparação e alimentos ricos em proteínas apresentavam baixos e moderados níveis do contaminante, enquanto que em alimentos crus ou cozidos em água, a sua presença não foi detectada. Produtos à base de batata, como batatas fritas e batatas chips, torradas, biscoitos, cereais matinais e café apresentaram os maiores teores de acrilamida.

Os dados destas primeiras pesquisas foram compilados e discutidos durante o encontro da FAO/OMS sobre as conseqüências sanitárias da presença de acrilamida em alimentos. Em 240 amostras avaliadas, o nível de acrilamida encontrado estava entre menos de 30 µg/kg e 3500 µg/kg, dependendo do tipo do produto. Observou-se ainda que estes níveis variavam significativamente dentro de uma mesma categoria de alimento, sendo sugerido que o modo de preparo dos alimentos poderia interferir no teor de acrilamida formada. Esta avaliação tornou-se uma orientação para outros países em relação à escolha das amostras a serem analisadas e ao intervalo dos resultados obtidos (FAO/WHO, 2002).

Entre os anos de 2002 e 2005, muitos países apresentaram dados sobre a ocorrência de acrilamida em diversos alimentos, incluindo aqueles onde a presença do contaminante já tinha sido confirmada e outros produtos característicos de cada região. A *Tabela 1* apresenta um resumo dos resultados obtidos em alguns países, mostrando que eles estão de acordo com os dados apresentados pela FAO/OMS em 2002. Somente na Turquia o biscoito apresentou o maior teor de acrilamida. Na França e Canadá, altos níveis de acrilamida foram encontrados em café.

Tabela 1. Ocorrência de acrilamida em alimentos de diferentes países.

País	*n*	Mín.-Máx. (µg/kg)	Alimentos com maior teor de acrilamida	Referência
Bélgica	63	<100-1210	batata chips, pão de ervas, biscoito infantil	AFSCA, 2002
França	206	<10-1300	café, batata chips e batata frita	AFSSA, 2003
Austrália	112	<25-1270	batata chips, biscoitos	CROFT *et al.* (2004)
EUA	490	<20-1970	batata chips, batata frita e biscoitos	FDA, 2005
Canadá	96	<2-4300	café e substitutos de café, batata chips e batata frita	HC, 2003
Holanda	344	<30-3100	batata chips, batata frita, snacks, pão de gengibre	KONINGS *et al.* (2003)
China	425	<3-1700	batata chips, torrada de centeio	LEUNG *et al.* (2003)
Áustria	158	<30-2410	batata chips, biscoitos, café	MURKOVIC (2004)
Japão	63	5-3540	batata chips, snacks à base de batata, grãos de cevada torrados	ONO *et al.* (2003)
Turquia	120	<20-3789	biscoitos, batata chips	SENUYVA & GÖKMEN (2005b)

(*n*) Número de amostra

No relatório final da avaliação toxicológica da acrilamida, o JECFA apresentou uma compilação dos resultados das análises do teor de acrilamida em alimentos de 24 países da Europa, América do Norte, Ásia e Pacífico, durante o período de 2002 a 2004 (FAO/WHO, 2005). O número total de resultados analíticos foi significativamente maior (6752) que o número avaliado pela FAO/OMS em 2002, o que permitiu um melhor entendimento da distribuição deste contaminante nos alimentos. A *Tabela 2* mostra os grupos de alimentos analisados e os resultados obtidos segundo a avaliação da FAO/OMS em 2002 (FAO/WHO, 2002) e do JECFA em 2005 (FAO/WHO, 2005).

Tabela 2. Grupos de alimentos e teores de acrilamida avaliados pela FAO/OMS e pelo JECFA.

Grupo de alimento	FAO/OMS			JECFA		
	n	**média (µg/kg)**	**máximo (µg/kg)**	***n***	**média (µg/kg)**	**máximo (µg/kg)**
batata chips	38	1312	2287	874	752	4080
batata frita	39	537	3500	1097	334	5312
biscoitos e torradas	58	423	3200	1270	350	7834
pão	41	50	162	1294	446	3436
cereais matinais	29	298	1346	369	96	1346
café	3	200	230	205	288	1291
produtos à base de cacau	2	75	100	23	220	909
bebidas alcoólicas	1*	<30	<30	66**	6,6	46
peixes e frutos do mar	4	35	39	52	25	233
carnes	2	52	64	138	19	313
laticínios	-	-	-	62	5,8	36
frutas processadas	-	-	-	37	131	770

(*n*) Número de amostra
*cerveja
**vinho, cerveja, gim

Embora muitos países já tenham reportado os níveis de acrilamida em seus produtos de mercado, nenhum dado sobre sua ocorrência em países da África e América Latina foi submetido ao JECFA quando da avaliação deste contaminante. Um estudo sobre a ocorrência de acrilamida em alimentos brasileiros foi realizado entre 2004 e 2006 através da análise de 111 amostras, representando 19 categorias diferentes de alimentos. Os níveis de acrilamida variaram entre menos de 20 µg/kg e 2528 µg/kg, dependendo do tipo do produto. Os resultados mostraram que os maiores níveis eram encontrados em produtos à base de batata, como batata frita, chips e batata palha (n=26), variando entre 144 e 2528 µg/kg. Outros alimentos que apresentaram elevados teores de acrilamida foram

café, bolacha água e sal, biscoito cream cracker e torrada (ARISSETO *et al*., 2007).

3.2 – Mecanismos de formação

Logo após a descoberta da presença de acrilamida em alguns alimentos processados, muitos pesquisadores se dedicaram ao estudo dos mecanismos envolvidos na sua formação, já que havia poucas evidências de como a molécula era formada nos alimentos. Foi então observado que a acrilamida era produzida em alguns alimentos processados em altas temperaturas e que seus níveis pareciam variar de acordo com o tempo e o modo de aquecimento, mostrando uma correlação com o escurecimento do produto (AHN *et al*., 2002).

Diversos autores têm demonstrado que o principal caminho para a formação de acrilamida em alimentos envolve a reação de Maillard entre aminoácidos e açúcares redutores (MOTTRAM *et al*., 2002; STADLER *et al*., 2002; BECALSKI *et al*., 2003; YAYLAYAN *et al*., 2003; ZYZAK *et al*., 2003; ROBERT *et al*., 2004; STADLER *et al*., 2004). Portanto, de acordo com as evidências existentes até o presente, este é o principal mecanismo que tem sido aceito pelos pesquisadores para explicar a formação de acrilamida em alimentos, embora ainda não esteja completamente elucidado (YAYLAYAN & STADLER, 2005).

Os primeiros estudos envolvendo misturas de aminoácidos e açúcares redutores submetidas a altas temperaturas mostraram que a presença de asparagina aumentava significativamente o nível de acrilamida formada, principalmente acima de 120ºC. O aminoácido asparagina foi identificado como o principal precursor da acrilamida e, além disso, foi confirmado, através de experimentos com ^{15}N e ^{13}C marcados, que átomos de carbono e nitrogênio da molécula de acrilamida eram provenientes dos locais correspondentes da molécula de asparagina (MOTTRAM *et al*., 2002; STADLER *et al*., 2002; BECALSKI *et al*., 2003). A formação de acrilamida a partir de asparagina poderia

explicar as altas concentrações encontradas em produtos à base de batatas e cereais, que são ricos neste aminoácido (MOTTRAM *et al*., 2002).

Em relação aos açúcares redutores, não foi observada formação de acrilamida quando os mesmos eram aquecidos na ausência de aminoácidos (STADLER *et al*., 2002). Na presença de asparagina, alguns autores verificaram que a frutose era mais eficiente que a glicose na formação de acrilamida. Embora aldoses sejam mais reativas que cetoses, a eficiência da frutose na reação poderia estar relacionada à liberação de água de cristalização e aumento da mobilidade molecular dos precursores, já que o ponto de fusão da frutose é inferior ao da glicose (ROBERT *et al*., 2004; STADLER *et al*., 2004).

Mesmo com a identificação dos principais precursores e mecanismos envolvidos na formação de acrilamida em alimentos, ainda faltam informações, principalmente em relação aos compostos intermediários da reação que atuam como precursores diretos da acrilamida. Alguns autores sugeriram o aldeído de Strecker como composto intermediário, que necessitaria de etapas posteriores de redução e desidratação para ser convertido em acrilamida (MOTTRAM *et al*., 2002). Outros autores demonstraram que o N-glicosídeo da asparagina ou da base de Schiff, produto da reação inicial entre glicose e asparagina, era o precursor direto de acrilamida (STADLER *et al*., 2002). Outros compostos intermediários foram sugeridos baseados no mecanismo que envolvia a formação da base de Schiff. Um deles era o composto oxazolidina-5-ona, formado a partir de iminas (YAYLAYAN *et al*., 2003), e o outro era a 3-aminopropionamida, formada pela hidrólise da base de Schiff descarboxilada por aquecimento (ZYZAK *et al*., 2003). Apesar de vários compostos intermediários já terem sido sugeridos, ainda não existem evidências concretas sobre o verdadeiro envolvimento destas moléculas na formação da acrilamida.

Embora a reação de Maillard seja reconhecida como o principal caminho para a formação de acrilamida em alimentos, outros possíveis mecanismos foram propostos e testados, como a hipótese de sua formação a partir de acroleína ou ácido acrílico (BECALSKI *et al*., 2003; VATTEM & SHETTY, 2003; YASUHARA *et*

al., 2003). Entretanto, foi verificado que batatas fritas em óleo de milho e em óleo de parafina (destituído de triglicerídeos e, portanto, de acroleína) apresentavam níveis de acrilamida muito próximos, sugerindo que a formação de acrilamida a partir de acroleína não era relevante (BECALSKI *et al*., 2003). Por outro lado, observou-se a formação de acrilamida a partir de ácido acrílico, porém em quantidades muito inferiores às geradas a partir de asparagina (aproximadamente 5%). Devido à limitação de amônia livre e necessidade de altas temperaturas para a reação proceder eficientemente, é provável que este mecanismo tenha pouca importância em relação à formação de acrilamida em alimentos (YASUHARA *et al*., 2003).

3.3 – Variáveis envolvidas na formação de acrilamida em alimentos

Muitos grupos de pesquisas têm se dedicado ao estudo das variáveis que estão envolvidas no processo de formação de acrilamida em alimentos, sendo a presença de seus precursores na matéria-prima e as condições de processamento as mais discutidas. O estudo destas variáveis tem sido útil no desenvolvimento de estratégias para a diminuição do potencial de formação deste contaminante em alimentos. Devido à presença de altos níveis de acrilamida em produtos à base de batata e cereais, estas categorias de alimentos têm sido as mais estudadas.

3.3.1 – Precursores

O potencial para a formação de acrilamida em alimentos está relacionado não somente com a presença de seus precursores (asparagina e açúcares redutores), mas também com as concentrações desses compostos na matéria-prima, que podem variar significativamente entre diferentes espécies e práticas de cultivo, e serem fortemente influenciadas pelas condições de estocagem. Em produtos à base de batata, a concentração de açúcares redutores é o fator determinante para a formação de acrilamida (BIEDERMANN *et al*., 2002b; AMREIN *et al*., 2003; NOTI *et al*., 2003; BECALSKI *et al*., 2004; OLSSON *et al*.,

2004; DE WILDE *et al*., 2005), enquanto que em cereais o teor de asparagina associado ao método de cozimento parece ser mais relevante (FREDRIKSSON *et al*., 2004; BRATHEN *et al*., 2005).

Um estudo realizado com 17 variedades de batatas suíças mostrou que o conteúdo de glicose variava entre 0,04 e 2,7 mg/g (em base úmida) e o conteúdo de asparagina entre 1,4 e 5,2 mg/g (AMREIN *et al*., 2003). Outros autores mostraram uma variação de 5,5 a 13,9 mg/g (em base seca) para o teor de glicose e de 3,9 a 10,2 mg/g para o de asparagina entre 8 variedades de batatas da Suécia (OLSSON *et al*., 2004). Selecionando-se matérias-primas adequadas, com um menor conteúdo de açúcares redutores, foi possível reduzir a formação de acrilamida em 28 vezes utilizando-se o mesmo tratamento térmico (AMREIN *et al*., 2003).

Em relação às práticas de cultivo, nenhuma diferença significativa foi observada na concentração dos precursores de acrilamida quando batatas eram cultivadas em sistemas orgânico, convencional e integrado (AMREIN *et al*., 2003). Verificou-se um aumento significativo no teor de asparagina relacionado à adição de nitrogênio durante a fertilização de batatas (EPPENDORFER, 1996; DE WILDE *et al*., 2005). Entretanto, para outros autores, a fertilização do solo com nitrogênio mostrou ser um fator não relevante para os conteúdos de asparagina e açúcares redutores e, conseqüentemente, para a formação de acrilamida (BIEDERMANN *et al*., 2002b; AMREIN *et al*., 2003).

O armazenamento de batatas a temperaturas abaixo de 8ºC resulta em aumento das concentrações de glicose e frutose, fenômeno conhecido como adoçamento por baixas temperaturas (COFFIN *et al*., 1987). BIEDERMANN *et al.* (2002b) observaram um aumento de até 15 vezes no conteúdo de frutose e de aproximadamente 40 vezes no de glicose durante a estocagem de diferentes variedades de batatas a 4ºC. O recondicionamento em maiores temperaturas pode reverter parcialmente o aumento da concentração de açúcares redutores. De acordo com os mesmos autores, foi observada uma redução de 53 e 41% nos

conteúdos de frutose e glicose, respectivamente, durante o recondicionamento a 25ºC.

3.3.2 - Condições de processamento

A influência da temperatura, do tempo e do modo de cozimento na formação de acrilamida em alimentos tem sido demonstrada desde a sua descoberta (BIEDERMANN *et al*., 2002a; MOTTRAM *et al*., 2002; STADLER *et al*., 2002; TAREKE *et al*., 2002; BECALSKI *et al*., 2003). O cozimento de batata em água a 100ºC ou em panela de pressão a 120ºC produziu baixos níveis de acrilamida, em torno de 20 µg/kg, sugerindo que sua formação estava relacionada a métodos de cozimento que resultassem primeiramente em uma secagem da superfície do produto (BIEDERMANN *et al*., 2002a).

Quando batatas foram assadas a 100ºC, não foi possível detectar a formação de acrilamida. A 120ºC observou-se um pequeno aumento no teor de acrilamida formada, de aproximadamente 30 µg/kg, indicando que a temperatura necessária para a sua formação era maior que 100ºC (TAREKE *et al*., 2002). Entretanto, a combinação de temperaturas maiores ou iguais a 200ºC com longos tempos de aquecimento resultou em uma diminuição do conteúdo de acrilamida formada em batatas (RYDBERG *et al*., 2003; TAUBERT *et al*., 2004) e em café (SENYUVA & GÖKMEN, 2005a), após um aumento ocorrido no início do tratamento térmico.

A influência do tipo de óleo utilizado na fritura de batatas também tem sido estudada em alguns trabalhos. Batatas fritas em óleo de oliva apresentaram maior concentração de acrilamida comparativamente às batatas fritas em óleo de milho (BECALSKI *et al*., 2003). Por outro lado, não foram observadas diferenças significativas na concentração de acrilamida formada em batatas fritas em óleo de palma, de soja e parafina (MESTDAGH *et al*., 2005). A influência do óleo de fritura na formação de acrilamida deve estar relacionada com a taxa de transferência de calor e não com a presença de acroleína (BECALSKI *et al*., 2003).

3.4 - Estratégias para a redução do teor de acrilamida formada em alimentos

Os experimentos realizados em alimentos e sistemas modelos têm indicado um grande número de possíveis alternativas para a redução da formação de acrilamida. Algumas estratégias para diminuir a concentração de seus precursores na matéria-prima podem ser empregadas em diferentes estágios da cadeia produtiva como, por exemplo, através da seleção de cultivares de batatas com um menor conteúdo de açúcares redutores (BIEDERMANN *et al*., 2002b; AMREIN *et al*., 2003), do armazenamento de batatas a temperaturas acima de 8ºC, para evitar o aumento da concentração de açúcares redutores (NOTI *et al*., 2003; DE WILDE *et al*., 2005), de técnicas simples como o branqueamento (KITA *et al*., 2004; PEDRESCHI *et al*., 2004; BRATHEN *et al*., 2005) e da utilização da enzima asparaginase, que remove seletivamente a asparagina antes do tratamento térmico (HENDRIKSEN *et al*., 2005).

Outras possíveis alternativas para a diminuição do teor de acrilamida formada em alimentos são: o controle do tempo e da temperatura de processamento (BIEDERMANN *et al*., 2002a; BIEDERMANN *et al*., 2002b; TAUBERT *et al*., 2004), a diminuição do pH, através da imersão de batatas em soluções de ácido clorídrico, cítrico ou acético (RYDBERG *et al*., 2003; KITA *et al*., 2004; PEDRESCHI *et al*., 2004), o aumento do tempo de fermentação durante o processamento de pães (FREDRIKSSON *et al*., 2004), a alteração da composição do produto através da adição de aminoácidos e ingredientes protéicos (RYDBERG *et al*., 2003; VATTEM & SHETTY, 2003; BRATHEN *et al*., 2005), a adição de compostos com propriedades antioxidantes como alecrim (BECALSKI *et al*., 2003) e flavonóides (FERNANDEZ *et al*., 2003) em batatas e a utilização de bicarbonato de sódio na formulação de pão de gengibre (AMREIN *et al*., 2004).

No âmbito do CCFAC, foi recomendada, em sua 38ª reunião, a elaboração de um Código de Práticas para a redução de acrilamida em alimentos, visando à proteção do consumidor pela diminuição da ingestão de acrilamida através da dieta. Este Código apresentará os principais aspectos da produção comercial de alimentos, incluindo práticas agrícolas, estocagem, matérias-primas,

processamento e preparação de alimentos, e métodos potenciais para a redução de acrilamida nas áreas de agronomia, formulação de produtos, condições de processamento e preparação final (CCFAC, 2006).

4 – ESTIMATIVA DE INGESTÃO

Valores médios da ocorrência de determinado contaminante podem ser combinados com informações disponíveis sobre o consumo médio de alimentos ou grupos de alimentos para se calcular a estimativa de exposição a este contaminante, em uma dada região (WHO, 2000). Muitos autores têm estimado valores de ingestão potencial diária de acrilamida para diferentes populações e investigado os alimentos ou grupos de alimentos que mais contribuem para a sua ingestão, de acordo com os hábitos alimentares de cada país. A *Tabela 3* mostra os resultados obtidos em diferentes países.

Tabela 3. Ingestão potencial diária de acrilamida em diferentes países.

País	**Ingestão de acrilamida (µg/kg pc/dia)**		**Referência**
	consumidores médios	**grandes consumidores**	
França	0,5	2,9	AFSSA (2003)
Alemanha	1,1	3,4	BfR (2003)
Austrália	0,4	1,5	CROFT *et al.* (2004)
Noruega	0,32	1,35	DYBING & SANNER (2003)
EUA	0,43	2,31	JIFSAN (2004)
Holanda	0,48	1,1	KONINGS *et al.* (2003)
Bélgica	0,51	1,09	MATTHYS *et al.* (2005)
Suíça	0,28	-	SOPH (2002)
Suécia	0,45	1,03	SVENSSON *et al.* (2003)
Reino Unido	0,3	1,8	UK FSA (2005)

O JECFA estimou uma ingestão de acrilamida entre 0,3 e 2,0 µg/kg pc/dia para consumidores médios, considerando dados submetidos por 17 países de todas as regiões com exceção da América Latina e África. Para os grandes consumidores, ou seja, aqueles cujos hábitos diferem amplamente da média, as estimativas de ingestão variaram de 0,6 a 3,5 µg/kg pc/dia, podendo chegar a até 5,1 µg/kg pc/dia. Os dados disponíveis indicaram que crianças apresentavam valores de ingestão de acrilamida de 2 a 3 vezes maiores que os de consumidores adultos, quando estes eram expressos em base de peso corpóreo. Foi concluído que poderia ser definido um valor de ingestão de 1 µg/kg pc/dia de acrilamida (ou 60 µg, assumindo-se um peso corpóreo de 60 kg) para representar consumidores médios e de 4 µg/kg pc/dia de acrilamida (ou 240 µg) para representar grandes consumidores (FAO/WHO, 2005).

Na ausência de níveis de ingestão toleráveis para a acrilamida, o JECFA relatou a contribuição relativa dos alimentos à ingestão total. Os produtos que mais contribuíram para a exposição na maioria dos países avaliados foram batatas fritas (16-30%), batatas chips (6-46%), café (13-39%), produtos de panificação e bolachas doces (10-20%), e pães e torradas (10-30%). Outros alimentos contribuíam menos que 10% da exposição total à acrilamida (FAO/WHO, 2005).

Uma maneira alternativa de se estimar a exposição à acrilamida é através da medida do aduto formado com a hemoglobina do sangue. Neste caso, o valor estimado corresponde à acrilamida proveniente de diversas fontes de exposição, como alimentos, bebidas, água, fumaça de cigarro e cosméticos, por exemplo. Para a população sueca de não fumantes, o nível de adutos determinado correspondeu a uma ingestão diária média de, aproximadamente, 100 µg de acrilamida por dia, ou 1,4 µg/kg pc/dia, assumindo-se um peso corpóreo de 70 kg (TÖRNQVIST *et al*., 1998).

5 – MÉTODOS ANALÍTICOS PARA A DETERMINAÇÃO DE ACRILAMIDA EM ALIMENTOS

Progressos significativos têm sido obtidos com relação ao desenvolvimento e validação de métodos analíticos para a determinação de acrilamida em alimentos. Atualmente, muitos métodos já estão publicados e este tópico foi revisado por diversos autores (WENZL *et al*., 2003; CASTLE & ERIKSSON, 2005; ZHANG *et al*., 2005).

5.1 – Extração

A extração de acrilamida do alimento ocorre na presença de água e/ou solventes orgânicos, dependendo da natureza da matriz. Devido à alta solubilidade da acrilamida em água, a extração em meio aquoso é geralmente suficiente (ROSÉN & HELLENÄS, 2002; TATEO & BONONI, 2003). A Agência Americana de Alimentos e Medicamentos (FDA) relatou que o aquecimento durante a etapa de extração deve ser evitado, já que este procedimento poderia gerar uma grande quantidade de partículas finas que, por sua vez, poderiam saturar as colunas de extração em fase sólida utilizadas nas etapas posteriores de limpeza (FDA, 2003). Entretanto, AHN *et al*. (2002) utilizaram água a 80ºC durante a extração de acrilamida presente em pão, batata frita e batata chips, sem ter relatado qualquer problema durante a limpeza do extrato.

Para amostras ricas em gordura, a extração com solventes orgânicos polares é mais efetiva. Alguns métodos têm sugerido a utilização de uma mistura de água e acetona para extração de acrilamida (FAUHL *et al*., 2002; TAKATSUKI *et al*., 2003). Metanol também tem sido utilizado, com taxas de recuperação variando de 68 a 75,4% (TATEO & BONONI, 2003). Uma outra alternativa é a inclusão de uma etapa de desengorduramento, adicionando-se hexano, éter de petróleo ou ciclohexano, antes ou em combinação com a etapa de extração (WENZL *et al*., 2003; ZHANG *et al*., 2005).

A adição de um padrão interno ao extrato tem sido recomendada por muitos autores para controlar as recuperações alcançadas e acompanhar as possíveis perdas que ocorrem durante as etapas de extração e limpeza da amostra. Os padrões internos mais utilizados são: [$^{13}C_3$]-acrilamida (TAREKE *et al*., 2002; BECALSKI *et al*., 2003), [$^{13}C_1$]-acrilamida (TAKATSUKI *et al*., 2003) e [$^{2}H_3$]-acrilamida (AHN *et al*., 2002; BECALSKI *et al*., 2003; ROSÉN & HELLENÄS, 2002).

A maioria dos procedimentos de limpeza da amostra tem utilizado a extração em fase sólida (SPE). Uma das técnicas empregada com sucesso foi a combinação de cartuchos Oasis® HLB (Waters, Milford, MS, USA) e Bond Elut-Accucat® (Varian, Palo Alto, CA, USA) (FDA, 2003; GOVAERT *et al*., 2006). A combinação de três cartuchos, Oasis® MAX (Waters), Oasis® MCX (Waters) e ENVI-Carb® (Supelco, Bellefonte, PA, USA), foi utilizada por BECALSKI *et al.* (2003). Outros pesquisadores optaram pelo uso de cartuchos Isolute Multimode® (International Sorbent Technology, Hengoed, UK) combinado com filtração (ROSÉN & HELLENÄS, 2002).

Os resultados de dois estudos interlaboratoriais conduzidos pelo Instituto Federal Alemão de Avaliação de Risco (BfR) e pelo Centro de Pesquisa Conjunto da União Européia (JRC) revelaram desempenho satisfatório dos métodos empregados na análise de acrilamida para a maioria das matrizes avaliadas. Entretanto, muitos laboratórios tiveram problemas na determinação de acrilamida em alimentos à base de cacau e café, matrizes consideradas complexas ou difíceis (KLAFFKE *et al*., 2005). Um potencial inconveniente é a existência de interferentes co-extrativos, com tempos de retenção muito próximos ao da acrilamida (WENZL *et al*., 2003). DELATOUR *et al*. (2004) obtiveram resultados satisfatórios na análise de acrilamida em cacau e café incluindo uma etapa de desproteinização durante a extração, realizada através da adição de soluções salinas Carrez I e Carrez II.

5.2 - Determinação cromatográfica

A cromatografia gasosa acoplada ao espectrômetro de massas (GC-MS), com a etapa de derivatização, tem sido utilizada por diversos autores (AHN *et al*., 2002; TAREKE *et al*., 2002). O procedimento é geralmente realizado através da adição de uma solução de brominação pré-preparada, contendo brometo de potássio, brometo de hidrogênio e bromo. A acrilamida brominada é menos polar comparativamente ao composto original sendo, portanto, mais solúvel em solventes orgânicos apolares. Em muitos casos, acetato de etila ou misturas de acetato de etila e ciclohexano são usados para a extração do analito da fase aquosa. A separação das fases é, na maioria das vezes, realizada por centrifugação.

Com a finalidade de se evitar uma longa preparação da amostra e obter métodos rápidos destinados ao maior número de matrizes diferentes, a cromatografia líquida acoplada ao espectrômetro de massas em série (LC-MS/MS) foi alternativamente investigada. Muitos procedimentos baseados nesta técnica analítica foram então desenvolvidos, com resultados comparáveis aos obtidos por cromatografia gasosa (WENZL *et al*., 2003). A cromatografia em fase reversa tem sido a técnica mais utilizada para a separação cromatográfica de acrilamida e diversas colunas têm sido sugeridas, como Hypercarb (ROSÉN & HELLENÄS, 2002; TAREKE *et al*., 2002; BECALSKI *et al*., 2003), Atlantis d-C_{18} (ONO *et al*., 2003) e µ-Bondapak C_{18} (FDA, 2003; GOVAERT *et al*., 2006).

Muitos pesquisadores desencorajam o uso da cromatografia líquida com detecção por ultravioleta ou arranjo de diodos (LC-UV ou LC-DAD) pelo fato de a acrilamida não possuir um forte espectro no UV, gerando falta de seletividade, e pela inviabilidade da utilização de acrilamida isotopicamente marcada como padrão interno (CASTLE & ERIKSSON, 2005). Entretanto, alguns trabalhos têm incentivado a utilização destas técnicas. VATTEM & SHETTY (2003) utilizaram detector de arranjo de diodos e obtiveram resultados considerados maiores que os esperados, embora os mesmos não tenham sido confirmados por outra técnica. Outros autores também testaram um novo método empregando LC-DAD e

obtiveram resultados precisos e reprodutíveis, confirmados por espectrometria de massas (GÖKMEN *et al*., 2005). Outro método utilizando cromatografia líquida de alta eficiência em fase normal com detecção por UV mostrou aplicabilidade em diversos tipos de alimentos, fornecendo resultados similares aos encontrados na literatura (PALEOLOGOS & KONTOMINAS, 2005). Segundo os autores, estas técnicas podem ser uma alternativa simples, rápida e barata para análises de rotina de acrilamida em alimentos quando comparadas ao detector de massas.

5.3 - Detecção e confirmação

Para a detecção de acrilamida, a espectrometria de massas tem sido o método mais freqüentemente escolhido. LC-MS/MS, operando no modo de monitoramento de reações múltiplas (MRM) apresenta alta seletividade. O modo MRM significa que a transição a partir de um íon precursor, que é separado no primeiro quadrupolo, a um íon produto, gerado pela colisão com argônio no segundo quadrupolo, é monitorada no terceiro quadrupolo. A transição 72>55 foi selecionada para quantificar acrilamida devido a sua intensidade relativamente alta (AHN *et al*., 2002; ROSÉN & HELLENÄS, 2002; TAREKE *et al*., 2002; BECALSKI *et al*., 2003). Outras transições, como 72>54, 72>44 e 72>27, têm sido utilizadas em alguns casos para confirmação. Para a detecção de acrilamida marcada isotopicamente, usada como padrão interno, as transições monitoradas são 75>58 para [$^{2}H_{3}$] e [$^{13}C_{3}$]-acrilamida e 73>56 para [$^{13}C_{1}$]-acrilamida.

6 – CONCLUSÕES

Desde a descoberta da formação de acrilamida em alimentos pelos pesquisadores suecos em 2002, grupos de pesquisas e indústrias de alimentos, principalmente na Europa e nos Estados Unidos, têm se dedicado ao estudo desta substância, sendo que importantes progressos já foram conseguidos em várias áreas relevantes. Os principais precursores e mecanismos envolvidos na sua formação foram identificados, o que têm contribuído para o entendimento dos

níveis de acrilamida encontrados em alimentos e do impacto das condições de processamento. Métodos analíticos têm sido desenvolvidas para a determinação de acrilamida em diversas matrizes, em concentrações cada vez menores. Entretanto, a principal questão ainda se refere à maneira como o teor de acrilamida em alimentos pode afetar a saúde humana. Embora efeitos neurotóxicos tenham sido documentados em humanos, os riscos associados à carcinogenicidade e genotoxicidade são baseados somente em estudos com animais. Desta forma, pesquisas ainda são necessárias antes de se tirar qualquer conclusão relativa ao potencial genotóxico e carcinogênico da acrilamida em seres humanos.

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CAPÍTULO 2

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Determination of acrylamide levels in selected foods in Brazil

Abstract

Selected carbohydrate-rich foods available on the Brazilian market (111 samples representing 19 product categories) were analysed for acrylamide content using a liquid chromatography – tandem mass spectrometry method. A limit of detection of 10 µg kg^{-1}, a limit of quantitation of 20 µg kg^{-1} and mean recoveries ranging from 100 to 115% were obtained during a laboratory validation procedure. The concentration of acrylamide in the samples ranged from <LOQ to 2528 µg kg^{-1}, with a considerable variation between individual foodstuffs within the same food product class. The highest levels were found in potato products processed at high temperature and in instant coffee, while the lowest concentrations were detected in cassava- and maize-based products, bread and beer. These results are comparable with those reported in other countries.

Keywords: *Acrylamide, carcinogen, cooked foods, Brazilian foods, LC-MS/MS.*

Introduction

Acrylamide (CH_2=CH-CO-NH_2) is an important industrial chemical used since the 1950s in the production of polyacrylamides, which are used as flocculants for clarifying drinking water and other industrial applications. The neurotoxicity of acrylamide in humans is well established from occupational and accidental exposures, and experimental studies have shown reproductive, genotoxic and carcinogenic effects in animals. Acrylamide has been classified as probably carcinogenic to humans by the International Agency for Research on Cancer (IARC 1994).

In April 2002, the Swedish National Food Administration (SNFA 2002) and researchers from Stockholm University reported the presence of high levels of acrylamide in a variety of common consumed foods. Acrylamide, which is formed during normal cooking practices, such as roasting, baking or frying, was found primarily in carbohydrate-rich foods prepared or cooked at high temperatures. The highest acrylamide levels (150-4000 μg kg^{-1}) were measured in heated potatoes and in some heated commercial potato products, while moderate levels (5-50 μg kg^{-1}) were found in heated protein-rich foods. So far no acrylamide has been detected in foods that are boiled or in unheated control samples (<5 μg kg^{-1}) (Tareke et al. 2002). This discovery caused worldwide concern about the possible public health risks from dietary exposure to acrylamide and a consultation was held jointly by the Food and Agriculture Organization and the World Health Organization in 2002 (FAO/WHO 2002).

The major pathway involved in the acrylamide formation is the Maillard reaction between amino acids and carbonyl compounds, such as reducing sugars, at temperatures above 120ºC (Mottram et al. 2002; Stadler et al. 2002; Becalski et al. 2003). Initial investigations have led to the unambiguous identification of asparagine as the main amino acid precursor of acrylamide by confirmation of the three-carbon backbone of acrylamide and the amide nitrogen originating from the corresponding locations in asparagine. These studies also confirmed that the presence of sugars was necessary to effect the conversion of asparagine into acrylamide. However, the mechanism for the decarboxylation of asparagine in the presence of sugars is not well understood and concrete evidence is still lacking, in particular on formation of the key intermediates in food products (Yaylayan and Stadler 2005).

There have been numerous reviews on different analytical techniques for determination of acrylamide in foods (Wenzl et al. 2003; Castle and Eriksson 2005; Zhang et al. 2005). GC/MS and LC-MS/MS are the most common analytical techniques. Water is usually used as the extraction solvent and solid-phase extraction (SPE) is routinely used for sample clean-up. A variety of SPE phases

have been employed, including graphitized carbon black, mixed mode anion and cation exchange, and polymeric materials.

Various studies on the occurrence of acrylamide in foods have been carried out, confirming the Swedish findings and providing additional information on the levels of acrylamide in different products (NFCA 2002; SOPH 2002; UK FSA 2002; BVL 2003). Online public databases have been set up in Europe by the European Commission's Joint Research Center (JRC 2005) and in the USA by the Food and Drug Administration (FDA 2005), showing that data on acrylamide levels from North America and European countries have been well established. Moreover, some reports on the level of acrylamide in foods from other countries have been published. In Hong Kong, a large number of samples of Asian foods were analysed by Leung et al. (2003). In Japan, a study on various food categories was carried out by Ono et al. (2003). Croft et al. (2004) have conducted a survey on the occurrence of acrylamide in several carbohydrate-based foods available on the Australian market. In Jordan, Al-Dmoor et al. (2004) have investigated acrylamide levels in selected fried and baked foods. A survey of retail Turkish foods was conducted for acrylamide by Senyuva and Gökmen (2005).

However, during the first toxicological evaluation of acrylamide carried out by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in February 2005, it was noted that no data from Latin America and Africa were submitted. The Committee recommended that it would be useful to have occurrence data on acrylamide in foods consumed in developing countries, since this information will be used in conducting intake assessment, as well as considering mitigation approaches to reduce human exposure (FAO/WHO 2005). The aim of this study was to evaluate the levels of acrylamide in a variety of processed foods consumed in the Brazilian diet, including cassava-based products, using a laboratory-validated liquid chromatography – tandem mass spectrometry (LC-MS/MS) method.

Experimental

Chemicals and Consumables

Acrylamide was obtained from Sigma (St. Louis, MO, USA) and acrylamide-D_3 was purchased from Polymer Source Inc. (Dorval, Quebec, Canada). Ultra pure water was used throughout the experiments (MilliQ system; Millipore Corp., Bedford, MA, USA). All organic solvents were of HPLC grade. Glacial acetic acid (purity of 99%) was supplied by Merck (Darmstadt, Germany). SPE cartridges Oasis® HLB (200 mg, 6 ml) were from Waters Corp. (Milford, MA, USA) and Bond Elut-Accucat® (200 mg, 3 ml) from Varian Inc. (Harbor City, CA, USA). Syringe filters (0.45 µm pore size, 17 mm diameter) with nylon membranes were supplied by Euroscientific (Lint, Belgium).

Standard stock solutions of both 1 mg ml^{-1} acrylamide and acrylamide-D_3 in water were prepared and stored at 4°C for 3 months. Working standard solutions of 10, 1 µg ml^{-1}, 500 and 60 ng ml^{-1} for acrylamide, and 10, 1 µg ml^{-1}, 500 and 150 ng ml^{-1} for acrylamide-D_3 were obtained by diluting the stock solution in water. For acrylamide only, additional working standard solutions diluted in water at concentrations of 600, 300, 150, 60, 30 and 15 ng ml^{-1} were also prepared for the calibration curve. All these solutions were stored at 4°C for 1 month.

Food Samples

A total of 111 samples of different brands and batches, representing 19 food categories, were purchased at supermarkets, fast-food restaurants and restaurants, in Campinas, SP, Brazil, between September 2004 and April 2006. The analytical survey included French fries, potato chips, bread, crispbread, crackers, breakfast cereals, coffee, beer and other typical Brazilian foods rich in carbohydrates and processed at high temperature. The samples were transported to the Scientific Institute of Public Health (Brussels, Belgium), where the analyses were carried out. When necessary, frozen foods were kept at low temperatures and prepared according to the instructions on the package before homogenization.

The samples were minced in a Moulinex apparatus and sub-samples of the homogenate were stored at –20ºC in polypropylene tubes with plastic screw-capped lids until analysis.

Apparatus

The system consisted of a HPLC quaternary pump Alliance 2695 incorporating an autosampler (Waters) coupled to a Quattro Micro triple quadrupole mass spectrometer (Micromass, Manchester, UK). The analytical column was a *µ*-Bondapak C_{18} (Waters), 10 µm particle size, 300 x 3.9 mm I.D., equipped with a guard column of the same phase.

Sample extraction

A sample of 1.0 $\pm$ 0.1 g was weighed in a 50 ml polypropylene tube and 500 µl of 500 ng ml^{-1} acrylamide-D_3 solution and 9.5 ml of water added. After 10 min, the sample was first vortexed and then mixed on an orbital shaker. The mixture was centrifuged (10 min, 2500*g*, 5ºC) and a 3 ml aliquot of the supernatant filtered through a 0.45 µm nylon filter. The Oasis® HLB SPE cartridges (200 mg, 6 ml), preconditioned first with methanol (5 ml) and then water (5 ml), were loaded with 2 ml of extract. The elution was performed with 2 ml of water and the eluate collected. The eluate was further cleaned-up on Bond Elut-Accucat® SPE cartridges (200 mg, 3 ml) after preconditioning with 3 ml of methanol and 3 ml of water. A 1.5 ml aliquot of the eluate was concentrated to 500 µl by evaporation under vacuum at 45ºC before injection.

LC-MS/MS analysis

An isocratic elution was performed with water containing 0.1% of acetic acid at a flow-rate of 0.6 ml min^{-1}. The mobile phase was diverted post-column to achieve a flow-rate of 0.3 ml min^{-1} in the MS interface. The injection volume was 100 µl, typical retention time of acrylamide was approximately 8 min and total run time was

15 min. The LC-MS/MS was operated in positive electrospray mode. Nitrogen was used as desolvation gas at a flow of 600 l h^{-1} at 300ºC and argon was used as collision gas at a pressure ~3 x 10^{-3} mbar. The source temperature was maintained at 120ºC, the capillary voltage was set at 4 kV and the cone voltage at 20 V. For the transitions *m/z* 72>72 and 72>55 of acrylamide, the collision energies were set at 1 and 10 eV, respectively. For the transition *m/z* 75>58 of acrylamide-D_3, the collision energy was set at 10 eV.

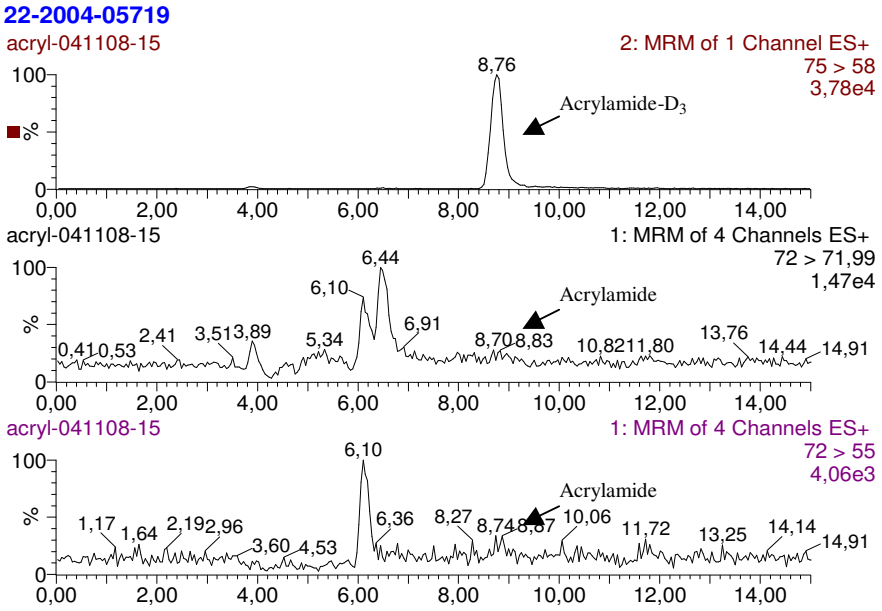
Results and discussion

Validation procedure

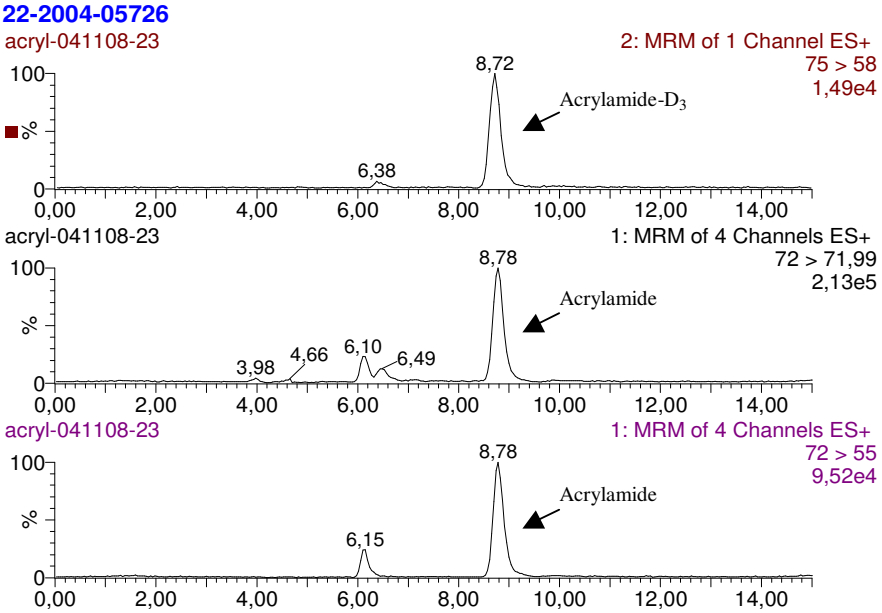
The method was validated according to a laboratory validation protocol. As no blank matrix samples were available, most calculations were carried out on blank aqueous solutions. Identification of acrylamide is made from the relative retention time (RRT) and diagnostic ions, consisting mainly of the precursor ion (protonated molecule) at *m/z* 72, and one daughter ion at *m/z* 55 originated from the fragmentation of the precursor ion. For confirmatory purposes, a comparison with quality control samples was made using acceptable deviations of ± 2.5% for RRT and ± 20% for ionic relative abundance, as described in the European Commission Decision 2002/657/EC laying down performance criteria of analytical methods for residues in products of animal origin (EC 2002). The determination of acrylamide concentration in samples proceeded by extrapolation from a linear analytical curve (0-1000 µg kg^{-1}) by monitoring the most intense MS/MS transition at *m/z* 72>72.

The acrylamide response was linear over a concentration range of 0-1000 µg kg^{-1}, as shown by the Mandel's fitting test, with correlation coefficients >0.995. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated considering a signal-to-noise ratio of three and six, respectively. A LOD of 10 µg kg^{-1} and a LOQ of 20 µg kg^{-1} were set, taking into account the identification criteria (relative retention time, presence of the most abundant ion and with respect to relative ion ratios). The recovery of acrylamide, determined by analysing extracts in

blank aqueous solutions spiked with 30 and 250 µg kg^{-1}, was 115 and 100% respectively. For repeatability, extracts in blank aqueous solutions spiked with 30 and 250 µg kg^{-1}, as well as in different food samples, were considered and the resulting coefficients of variation (CVs) ranged between 1.36 and 8.06%. For within-laboratory reproducibility, the CVs ranged between 3.3 and 10.9%, and all calculated CVs were within the tolerances set by European Decision 2002/657/EC, which are based on the Horwitz equation. More details on results regarding the development of the method and the validation procedure are given by Govaert et al. (2006). Figure 1 shows SRM chromatograms of a deep-fried cassava sample containing a level of acrylamide below the LOQ and a potato chips sample containing 1999 µg kg^{-1} acrylamide.



(a)



(b)

Figure 1. SRM chromatograms of (a) a deep-fried cassava sample and (b) a potato chips sample.

Acrylamide content in foods

The determination of acrylamide in food samples was carried out using an ISO 17025 accredited method. In this study, foods were only sampled in the Campinas area and, therefore, may not be representative of national food supply. Concentrations refer, in some cases, only to a single randomly selected sample of each specific product. Variations among production batches and among brands within a product type due to differences in the processing conditions are presumed. However, the results are a general guide to acrylamide concentrations in a selected segment of Brazilian food resources. The median, and minimum and maximum acrylamide levels found in each food group are listed in Table I.

The acrylamide concentrations observed in this study were within those reported in data compiled from several countries by the FAO/WHO (2005). The levels of acrylamide varied considerably between samples in each food group, as shown by the range of minimum and maximum values. Heating temperature, types and amounts of carbohydrates and amino acids, and other factors partly still unknown, cause this large variation in the amount of acrylamide formed in a particular food product (Tayemans et al. 2004). The highest concentrations were found in a sample of French fries (2528 µg kg^{-1}) and in a sample of potato chips (1999 µg kg^{-1}), the only samples in which the acrylamide level exceeded 1000 µg kg^{-1}. On the other hand, acrylamide levels were below the LOQ in all 14 brands of beer, in deep-fried cassava, in cheese bread (which is made from cassava starch), in three brands of cassava starch biscuit, in four kinds of bread, in two brands of toasted bread flour, in one brand of toasted cassava flour, in one brand of breakfast cereal, in “curau” powder and in two samples of deep-fried “polenta” (which are made from maize flour).

Table I. Acrylamide levels in food samples from the Brazilian market analysed by LC-MS/MS (LOQ = 20 µg/kg).

Food	Number of samples (n)	Median (µg kg^{-1})	Minimum/Maximum (µg kg^{-1})
Potato products			
French fries	7	264	146/2528
Potato chips	12	591	144/1999
Potato "palha"	7	649	198/803
Cassava products			
Toasted cassava flour	3	30	<LOQ/81
Cassava starch biscuit	11	22	<LOQ/62
Deep-fried cassava	3	<20	<LOQ/<LOQ
Cheese bread	3	<20	<LOQ/<LOQ
Maize products			
Breakfast cereal	8	30	<LOQ/49
Deep-fried "polenta"	3	<20	<LOQ/33
"Curau" powder	1	-	-
Wheat flour products			
Water and salt biscuit	4	187	154/361
"Grissini"	2	128	125/131
Cracker	3	116	107/131
Crispbread	7	71	25/231
Bread	14	21	<LOQ/124
Toasted bread flour	3	<20	<LOQ/67
Coffee products			
Instant coffee	3	582	333/683
Roasted coffee	3	174	128/202
Beverages			
Beer	14	<20	<LOQ/<LOQ

As shown in Table I, potato products contained the highest levels of acrylamide in comparison to other foods. Potato "palha" is a common product in Brazil, manufactured with grated potato, deep-fried in oil at high temperatures. The results confirm early data on the occurrence of high levels of acrylamide in potato products treated at high temperatures (SNFA 2002).

Typical Brazilian foods, such as cassava-based products, were investigated in this study to evaluate their potential to form acrylamide during traditional cooking procedures. Cassava is considered an excellent dietary source of energy in tropical countries (Redhead 1989) and, in Brazil, is mainly consumed as deep-fried cassava snack or in meals. Deep-fried cassava is commonly prepared by first cooking the strips of cassava in boiled water and then frying in oil at high temperatures. Alternatively, the cassava starch is used in the manufacture of several typical Brazilian products, such as biscuit and cheese bread, which are baked in oven at 200-220ºC for approximately 20 min. As presented in Table I, the highest acrylamide level found in cassava products was detected in toasted cassava flour, at a concentration of 81 µg kg^{-1}. This may suggest that cassava is poor in acrylamide precursors, such as free asparagine and reducing sugars. A study by Ngudi et al. (2002) showed that the concentration of free asparagine in processed cassava is, in general, very low, ranging from undetectable to 0.096 mg g^{-1} dry weight (DW), whereas Vitrac et al. (2000) reported reducing sugars levels in cassava in the range of 0.05-1.02 mg g^{-1} DW. The results of the present study demonstrate that Brazilian cassava-based meals contain very low acrylamide levels and may not be a concern, as raised by the FAO/WHO (2002, 2005), in spite of their high consumption in this country.

Typical maize-based products, such as deep-fried “polenta” and “curau”, were also investigated. Deep-fried “polenta”, which is made by cooking maize flour with water followed by frying in oil at high temperature for a few minutes, showed levels ranging from <LOQ to 33 µg kg^{-1}. “Curau”, another typical Brazilian food also manufactured from maize flour, showed levels of acrylamide below the LOQ. All samples of breakfast cereals analysed were manufactured with maize flour and the acrylamide levels ranged from <LOQ to 49 µg kg^{-1}. Levels of acrylamide below 50 µg kg^{-1} were also reported in maize extrudates (Kretschmer 2004). The low levels of acrylamide observed in these products may be attributed to the low concentration of precursors in maize. According to literature data, the average levels of free asparagine and reducing sugars reported in maize are 0.09 and 0.39 mg g^{-1} DW, respectively (ILU-EV 2004).

Within the wheat flour products category, water and salt biscuit, “grissini”, crackers and crispbread showed the highest levels of acrylamide. In breads, crust and crumb were analysed jointly and, since acrylamide formation occurs mostly by surface phenomena (Senyuva and Gökmen 2005), the acrylamide content in the whole bread (crust + crumb) was, in general, low. The highest level of acrylamide (124 µg kg^{-1}) was found in a brand of Swedish-type bread. Variations on the acrylamide content in bakery products may be attributed to differences in either the ingredients, leavening agents, dough pH before baking of products or heat treatment intensity (Al-Dmoor et al. 2004).

High contents of acrylamide were found in coffee, confirming data from other countries (FAO/WHO, 2005). On the other hand, no detectable amounts of acrylamide were found in beer, suggesting that barley is poor in acrylamide precursors and/or the processing conditions are not conducive for the formation of acrylamide.

Conclusions

The occurrence of acrylamide in selected foods in Brazil is reported. The levels of acrylamide were within those reported from other countries and varied considerably between samples in each food group. It was demonstrated that typical cassava- and maize-based products consumed in Brazil contain negligible levels of acrylamide and may not represent an important source of this contaminant in the Brazilian diet. Although the data from the survey are useful for a preliminary estimate of the dietary intake of acrylamide by Brazilians, additional research is needed on a wider range of foodstuffs and a larger number of samples, including cocoa products, snacks, baby foods, infant biscuits and home-cooked foods. It is expected that these results will contribute to data accumulation for worldwide health risk assessment and be helpful in establishing approaches to lower acrylamide formation during processing.

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CAPÍTULO 3

A modified sample preparation for acrylamide determination in complex matrices such as cocoa and coffee

Abstract

A modified sample preparation for acrylamide determination in cocoa and coffee products by a liquid chromatography – tandem mass spectrometry method is presented in this work. For the sample extraction, different solvents were evaluated. The performance of the method was increased by introducing a protein precipitation step and a liquid-liquid extraction during the sample clean-up. The analyses were carried out on a µ-Bondapak C_{18} column using acrylamide-D_3 as internal standard. For identification, relative retention time and two diagnostic ions were monitored. A limit of detection of 10 µg kg^{-1}, a limit of quantitation of 20 µg kg^{-1}, mean recoveries ranging from 93 to 99%, coefficients of variation of 3.42% for repeatability and from 1.68 to 10.75% for within-laboratory reproducibility were obtained during a laboratory validation procedure.

Keywords: *Acrylamide, cocoa, complex matrices, LC-MS/MS.*

1. Introduction

In April 2002, the Swedish National Food Administration (SNFA) and researchers from Stockholm University reported the findings of elevated levels of acrylamide in heat-treated carbohydrate-rich foods such as French fries, chips, breads and biscuits [1, 2]. The Swedish discovery has attracted worldwide attention since acrylamide has been classified as probably carcinogenic to humans by the International Agency for Research on Cancer (IARC) [3].

Acrylamide is formed in foods during common cooking practices, i. e. roasting, baking or frying. The major pathway involved is the Maillard reaction between amino acids and carbonyl compounds like reducing sugars at temperatures above 120ºC [4-6]. Asparagine was identified as the main amino acid precursor, but the presence of reducing sugars is also necessary to form acrylamide. However, the mechanism of the decarboxylation of asparagine in presence of sugars is not completely elucidated and concrete evidence is still lacking, in particular with regard to the formation of key intermediates in food products [7].

The initial analytical method employed by Swedish researchers for acrylamide analysis in feed relied on derivatisation of acrylamide by bromine, including solvent partitioning and detection by gas chromatography - mass spectrometry (GC-MS) [8]. This technique has been adapted and commonly used by many authors to determine acrylamide in foods, but its conversion to dibromopropionamide or 2-bromopropenamide derivatives prior to GC-MS is often incomplete and time-consuming [9-11]. In order to avoid these inconveniences and obtain rapid methods destined for a larger variety of samples, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was alternatively investigated and many procedures based on this analytical approach were developed [6, 12-14]. More details on analytical methodologies are given by Wenzl et al. [15], Castle and Eriksson [16] and Zhang et al. [17] in their review manuscripts.

The assessment of results from six rounds of proficiency tests organized by Food Analysis Performance Assessment Scheme (FAPAS®) [18] showed that the performance of the methods employed for acrylamide analysis for some relevant food matrices, such as potato chips, crispbread and breakfast cereal, is generally acceptable, with percentage of satisfactory results between 71 and 87%. However, many laboratories showed problems in determining acrylamide in complex food matrices, such as cocoa and coffee [19]. The problems with these samples have been associated to the loss of the analyte throughout the sample preparation and the presence of undesirable co-extractives.

As indicated by the results of an interlaboratory study conducted by the Federal Institute Risk Assessment (BfR) [20] on the determination of acrylamide in cocoa, the criterion for performance acceptability of analytical methods was not fulfilled. Moreover, the proficiency test organized by FAPAS® on the determination of acrylamide in coffee revealed that 24% of the laboratories which required the test material did not return the results [18]. In this context, the European Commission's Directorate General Joint Research Center (JRC) organized in 2004 in collaboration with BfR an interlaboratory study on the determination of acrylamide in difficult matrices [21].

Therefore, laboratories have worked in adapting their methods to achieve the required precision and sensitivity for cocoa and coffee products and some reports have been published on determination of acrylamide in complex matrices mainly by LC-MS/MS [22-27]. Water is most commonly used as extraction solvent [22, 23, 25-27], but methanol can also be employed successful [24]. Some authors have carried out a deffating step with petroleum ether [22] while others have used dichloromethane [23, 25]. A precipitation step by adding Carrez I and II solutions have been included in some procedures [25, 27]. Solid-phase extraction (SPE) has been routinely used and a variety of SPE phases have been suggested, such as Oasis® HLB [22, 24], Isolute Multimode® [25-27] and aminopropyl-bonded silica [23]. Some authors have also recommended a liquid-liquid extraction performed with ethyl acetate prior to the SPE step [22, 25].

Since the LC-MS/MS method used by our laboratory for acrylamide analysis in potato and cereal products [28] did not present satisfactory results regarding peak identification and confirmation of acrylamide in cocoa-based products, the aim of this study was to modify the sample preparation of the existing method, in order to achieve good performance for this type of matrix. Results of tests and data from an in-house validation performed are presented.

2. Experimental

2.1. Standards

Acrylamide was obtained from Sigma (St. Louis, MO, USA) and acrylamide-D_3 was purchased from Polymer Source Inc. (Dorval, Quebec, Canada). Stock solutions of both 1 mg ml^{-1} acrylamide and acrylamide-D_3 in water were prepared and stored at 4°C for 3 months. Working solutions of 10, 1 µg ml^{-1}, 500 and 60 ng ml^{-1} for acrylamide, and 10, 1 µg ml^{-1}, 500 and 250 ng ml^{-1} for acrylamide-D_3 were obtained by diluting the stock solution in water. For acrylamide only, additional working solutions diluted in water at concentrations of 1000, 500, 250, 100, 50 and 25 ng ml^{-1} were also prepared for the analytical curve. All these solutions were stored at 4°C for 1 month.

Spiking of quality control samples was made by adding 500 µl of acrylamide solutions at 500 and 60 ng ml^{-1}. The analytical curve was obtained by mixing 100 µl of the acrylamide solutions at 25, 50, 100, 250, 500 and 1000 ng ml^{-1}, with 100 µl of the acrylamide-D_3 at 250 ng ml^{-1}.

2.2. Chemicals and Consumables

Water was of ultra pure quality (Milli-Q system; Millipore Corp., Bedford, MA, USA) and all organic solvents were HPLC grade. Glacial acetic acid (purity of 99%) was supplied by Merck (Darmstadt, Germany). Potassium hexacyanoferrate (II) trihydrate and zinc sulfate heptahydrate were obtained from Sigma. SPE cartridges Isolute Multimode® (300 mg, 3 ml) were from IST (Hengoed, Mid Glamorgan, UK), Oasis® HLB (200 mg, 6 ml) were from Waters Corp. (Milford, MA, USA) and Bond Elut-Accucat® (200 mg, 3 ml) from Varian Inc. (Harbor City, CA, USA).

2.3. Apparatus

A Reax 2 orbital rotative system from Heidolph (Germany) was used to perform the extraction of acrylamide. Centrifugation was carried out on a 5810R centrifuge from Eppendorf (Hamburg, Germany). Preconcentration of samples proceeded with a RC10-10 speed vacuum system from Jouan (St. Herblain, France).

The LC-MS/MS system consisted of a HPLC quaternary pump Alliance 2695 including an autosampler (Waters) coupled to a Quattro Micro triple quadrupole mass spectrometer from Waters/Micromass (Manchester, UK). The analytical column was a µ-Bondapak C_{18} (Waters), 10 µm particle size, 300 x 3.9 mm id, equipped with a guard column of the same phase.

2.4. Sample extraction

A portion of 1 ± 0.1 g was weighed in a 50 ml polypropylene tube. A volume of 500 µl of the internal standard solution, acrylamide-D_3 at 500 ng ml^{-1}, was added. After 10 min, acrylamide was extracted by adding 9.5 ml of water. The samples were homogenized during 1 min on a Vortex and 10 min on an orbital shaker. The extract was centrifuged (10 min at 3000 rpm, 5ºC) and the supernatant was collected in another 50 ml polypropylene tube. Aliquots of 1 ml of a 0.68 mol L^{-1} potassium hexacyanoferrate (II) trihydrate solution (Carrez I) and 1 ml of a 2 mol L^{-1} zinc sulfate heptahydrate solution (Carrez II) were added for protein precipitation. The samples were shaken during 10 min on an orbital shaker and then centrifuged (15 min at 10000 rpm, 5ºC). For liquid-liquid extraction, 13 ml of ethyl acetate were added to 6 ml of the supernatant (ethyl acetate seems to be a suitable solvent to “salt-out” acrylamide from the aqueous supernatant). The mixture was homogenized during 10 min on an orbital shaker and centrifuged (10 min at 3000 rpm, 5ºC). The organic phase was transferred into a glass tube containing 2 ml of water, and the mixture was stirred vigorously for 1 min on a Vortex. The ethyl acetate was evaporated under a stream of nitrogen at 40ºC (the

presence of 2 ml of water will prevent the loss of acrylamide during the evaporation and transfer the analyte back into the aqueous phase). One further extraction with ethyl acetate of the 6 ml supernatant portion was conducted. The volume of the final aqueous extract, after evaporation of ethyl acetate, was adjusted to 2 ml and cleaned-up on SPE cartridges. Isolute Multimode® cartridges, preconditioned firstly with methanol (3 ml) and then with water (2 x 3 ml), were loaded with the aqueous extract which was directly collected. An aliquot of 1 ml of water was loaded onto the cartridge, eluted, collected and mixed with the previous fraction. A 1.5 ml volume of the mixture was concentrated to 500 µl by evaporation under vacuum at 45ºC before injection.

2.5. LC-MS/MS analysis

An isocratic elution was performed with water containing 0.1% of acetic acid (v/v) at a flow rate of 0.6 ml min^{-1}. The mobile phase was diverted post-column (split 1:1) to achieve a 0.3 ml min^{-1} flow rate in the MS interface. The injection volume was 100 µl, typical retention time of acrylamide was approximately 8 min and the total run time was 15 min. The LC-MS/MS was operated in positive electrospray mode. Nitrogen was used as desolvation gas at a flow of 600 L/h at 300ºC and argon was used as collision gas at a pressure ~3 x 10^{-3} mbars. The source temperature was maintained at 120ºC, the cap illary voltage was set at 4 kV and the cone voltage at 20 V. For the transitions *m/z* 72>72 and *m/z* 72>55 of acrylamide, the collision energies were set at 1 and 10 eV, respectively. For the transition *m/z* 75>58 of acrylamide-D_3, the collision energy was set at 10 eV.

2.6. Identification and quantification

Identification of acrylamide was based on the relative retention time (RRT) and the presence of diagnostic ions, consisting mainly of the precursor ion (protonated molecule) at *m/z* 72, and one daughter ion at *m/z* 55 originated from the fragmentation of the precursor ion. For confirmatory purposes, a comparison

with a quality control sample was performed using an acceptable deviation of ± 2.5% for RRT and ± 20% for ionic relative abundance [29]. The quantification of acrylamide in samples proceeded by extrapolation from a linear analytical curve (0–1000 µg Kg^{-1}) by monitoring the transition *m/z* 72>72.

3. Results and discussion

3.1. Method development

A LC-MS/MS method for the determination of acrylamide in several foodstuffs, including mainly potato and cereal products, was previously reported by our laboratory [28]. Briefly, acrylamide was extracted with water and the aqueous extract was centrifuged, filtered and cleaned-up on Oasis® HLB and Bond Elut-Accucat® cartridges. The extract was then preconcentrated to a small volume before LC-MS/MS analysis on a µ-Bondapak C_{18} column using acrylamide-D_3 as internal standard. Z-scores between -0.44 and 2.27 obtained in proficiency tests organized by FAPAS® and JRC for potato and cereal products as well as coffee were generally within the acceptable range ($-2 < z < 2$). In only one of the eight tests of which the laboratory participated, the z-score was in the range of questionable performance.

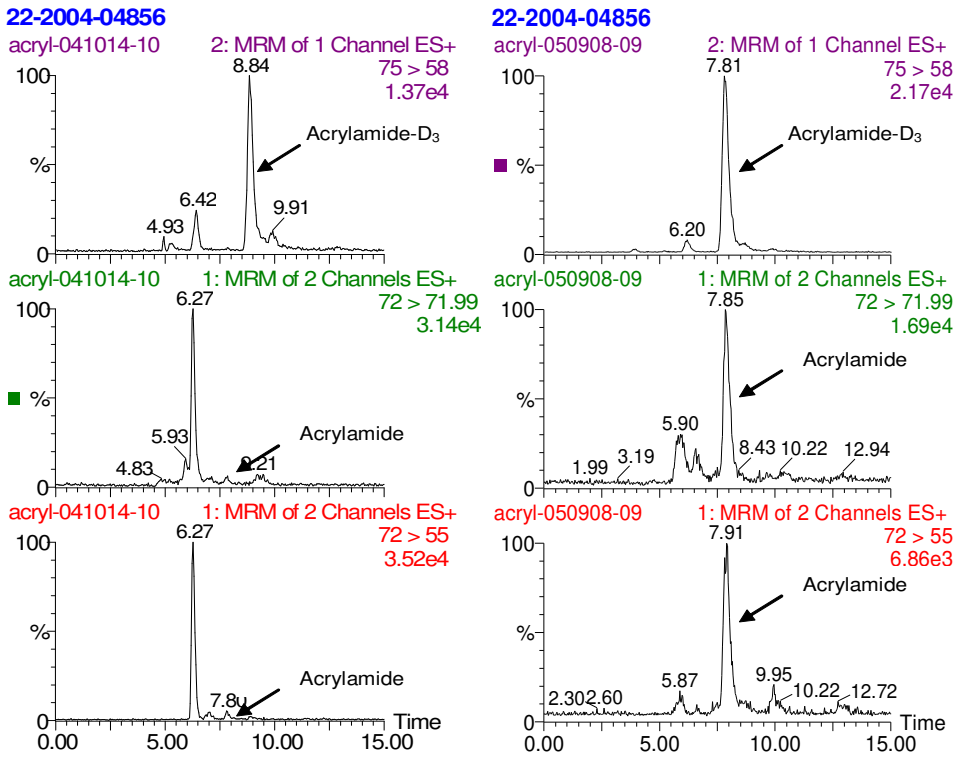
However, the proficiency test organized in 2004 by JRC and BfR on the determination of acrylamide in difficult matrices [21] revealed that this method presented strong limitations for cocoa powder in terms of identification and confirmation of the peak of acrylamide, making impossible the calculation of its concentration in these samples. The laboratory did not return the results and initiated the tests to modify the existing extraction procedure used for potato and cereal products in order to increase its performance for cocoa-based matrices.

In the study conducted by BfR in 2002, it was noted that the extraction of acrylamide from cocoa with non-aqueous solvents (e.g., methanol, acetone, acetonitrile) led to the assignment of almost double the amount of acrylamide

compared to extraction with water [20]. Therefore, different non-aqueous solvents were tested instead of water for the primary extraction of acrylamide including methanol, acetonitrile, ethyl acetate and dichloromethane. The extraction procedure was followed by centrifugation, filtration, clean-up on Oasis® HLB and Bond Elut-Accucat® cartridges and finally a preconcentration to a small volume before LC-MS/MS analysis as described previously for potato and cereal products. No satisfactory results were obtained and the problem of the method was attributed to the lack of preliminary separation of proteins from the initial sample.

To improve the sample preparation, a precipitation of matrix constituents with Carrez I and II solutions was tested before clean-up on Oasis® HLB and Bond Elut-Accucat® cartridges. The criteria for ionic relative abundance were not fulfilled and mean recoveries ranging from 141 to 146% showed that this procedure was not sufficient to solve the problem. The challenge was then focused into modifying the SPE step. For that, Isolute Multimode® cartridges were tested separately, as suggested by Granby and Fagt [26], and in combination with a liquid-liquid extraction with ethyl acetate, as suggested by Delatour et al. [25]. The best cleaned-up extract was univocally obtained with the last combination.

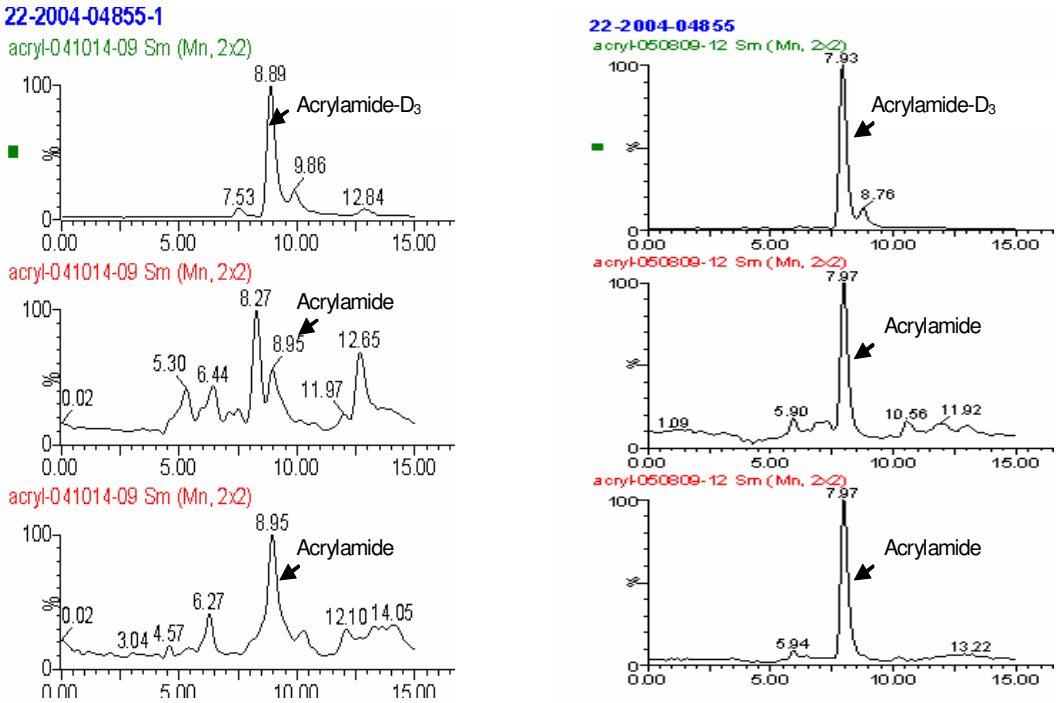
The existing procedure used for potato and cereal products was then modified by introducing a protein precipitation step with Carrez I and II solutions and by performing the SPE on Isolute Multimode® cartridges in combination with a liquid-liquid extraction with ethyl acetate. In order to demonstrate that the modified procedure performs better in difficult matrices than the procedure used for potato and cereal products, the sample of cocoa powder coming from the proficiency test conducted by JRC and BfR was extracted and analysed by both methods and the chromatograms are illustrated in Figure 1. When the extraction procedure for potato and cereal products was used (1a), the criteria for acrylamide identification were not fulfilled. Consequently, it was not possible to confirm the identity of the peak at the retention time of acrylamide. When the modified procedure was used, the chromatogram clearly showed a peak at the retention time of acrylamide and the criteria for identification and confirmation were fulfilled, thus allowing the quantification of the analyte in the sample (1b).



(1a) (1b)

Figure 1. Sample of cocoa powder extracted (1a) by the procedure used for potato and cereal products [28] and (1b) by the modified procedure for complex matrices. Conditions: column: µ-Bondapak C_{18} (300 x 3.9 mm id.; 10 µm); mobile phase: 0,1% acetic acid (v/v); flow: 0,6 ml min^{-1}; injection volume: 100 µl; ionization source: positive electrospray; capillary voltage: 4000 V; desolvation gas: nitrogen (600 L/h, 300ºC); collision gas: argon (~3 x 10^{-3} mbars); collision energy: 1 eV (72>72) and 10 eV (72>55).

As several authors consider that coffee is a difficult matrix to analyse for acrylamide [23, 25, 27], the modified procedure was also tested in samples of roasted and instant coffee. The results indicated that, although the extraction procedure used for potato and cereal products had performed well in these matrices, as shown by the z-score of 1.5 obtained in the FAPAS® proficiency test [28], the chromatograms generated by using the modified procedure had a higher resolution and less co-extractive peaks (Figure 2).



(2a) (2b)

Figure 2. Sample of instant coffee extracted (2a) by the procedure used for potato and cereal products [28] and (2b) by the modified procedure for complex matrices. Conditions: column: µ-Bondapak C_{18} (300 x 3.9 mm id.; 10 µm); mobile phase: 0,1% acetic acid (v/v); flow: 0,6 ml min^{-1}; injection volume: 100 µl; ionization source: positive electrospray; capillary voltage: 4000 V; desolvation gas: nitrogen (600 L/h, 300ºC); collision gas: argon (~3 x 10^{-3} mbars); collision energy: 1 eV (72>72) and 10 eV (72>55).

3.2. In-house validation

An in-house validation of the proposed method was performed. The acrylamide response was linear over a concentration range of 0-1000 µg kg^{-1} (R^2 = 0.9997), as shown by the Mandel's fitting test [30]. A slight, but significant matrix effect was observed by comparison of slopes between curves set on standard

solutions and on chocolate powder sample using a student *t*-test (p=0.02) (Figure 3).

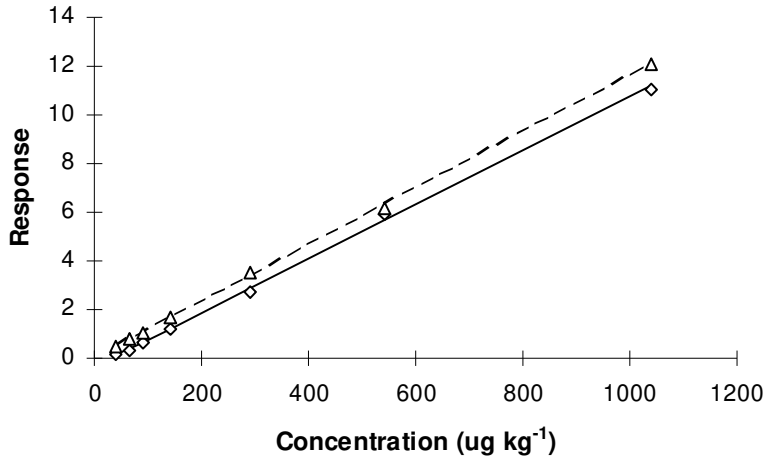


Figure 3. Comparison of slopes between curves set on standard solutions and on chocolate powder sample. (——— standard solutions; – – – – – – chocolate powder)

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated considering a signal-to-noise ratio of three and six, respectively, measured on 20 blank aqueous solutions since no blank matrix samples were available. Mean concentrations of 5.5 µg kg^{-1} for LOD and 9.6 µg kg^{-1} for LOQ were obtained. To verify these limits in practice, five replicates of blank aqueous solutions spiked at concentrations of 5, 10, 15 and 20 µg kg^{-1} were analysed. The identification criteria were fulfilled for all concentrations, however, at 5 µg kg^{-1} a signal-to-noise ratio below three was obtained. Thus, a LOD of 10 µg kg^{-1} and a LOQ of 20 µg kg^{-1} were finally set. It must be emphasized that the determination of the LOD and LOQ from aqueous solutions does not take matrix effects into account and, therefore, the values calculated here could be low. This might be verified further, if blank matrix or very low contaminated samples are found.

Recovery and bias were measured to evaluate the trueness of the method. Recovery was evaluated by fortifying three replicates of various complex food

samples with 150 µg kg^{-1} acrylamide and by comparing the measured concentration of acrylamide in fortified samples with the expected concentration (= incurred + supplemented concentration). Mean recoveries ranging from 93 to 99% were obtained (Table 1). Bias was determined on three replicates of samples of instant coffee and roasted coffee, for which an assigned value was known [21], and the results are summarized in Table 2.

Table 1. Recovery results from in-house validation trial.

Samples	Acrylamide mean concentrations (µg kg^{-1})			%
	Incurred	Expected[a]	Measured	
Chocolate powder	38	188	175	93
Cocoa powder	67	217	204	94
Chocolate	150	300	292	98
Chocolate pasta	40	190	181	95
Instant coffee	715	865	859	99
Roasted coffee	206	356	351	99

[a]Expected = incurred + 150 µg kg^{-1} acrylamide

Table 2. Bias results determined on instant and roasted coffee.

Samples	Acrylamide mean concentrations (µg kg^{-1})		%
	Assigned value	Measured	
Instant coffee	858	715	-17
Roasted coffee	258	206	-20

Precision was evaluated by calculating the coefficients of variation (CV) under repeatability and within-laboratory reproducibility conditions (Table 3). For repeatability, six replicates of chocolate powder were analysed on the same day and the CV was 3.42%. For within-laboratory reproducibility, minimum three

replicates per extract considering different matrix samples such as cocoa powder, chocolate and coffee, were analysed over different days. The CVs ranged between 1.68 and 10.75%, and all calculated CVs were below the maximal CV of the Horwitz equation [31].

Table 3. Precision results from within laboratory tests.

Samples	Mean concentrations (μg kg^{-1})	CV (%)	Horwitz CV (%)
Repeatability			
Chocolate powder	45 (*n* = 6)	3.42	26
Reproducibility			
Chocolate powder	40 (*n* = 9)	10.75	26
Cocoa powder	67 (*n* = 3)	6.86	24
Chocolate	150 (*n* = 3)	1.68	21
Chocolate pasta	40 (*n* = 3)	3.79	26
Instant coffee	715 (*n* = 3)	3.52	17
Roasted coffee	206 (*n* = 3)	1.68	20

In conclusion, this work reported a reliable and efficient method for acrylamide determination in complex food matrices such as cocoa powder, chocolate and coffee. The original extraction procedure of the laboratory applied for potato and cereal products was modified by including a protein precipitation step with Carrez I and II solutions, liquid-liquid extraction with ethyl acetate and solid-phase extraction on Isolute Multimode® cartridges. The overall performance of the method was improved by increasing the recovery and eliminating interferences arisen from complex food matrices.

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CAPÍTULO 4

Estimate of the dietary exposure to acrylamide in Brazil

Abstract

A preliminary estimate of the dietary exposure to acrylamide by the total population in Brazil is reported. The daily intake was assessed by combining measured levels of acrylamide in selected food categories with national food consumption data inferred from a household economic survey, using a deterministic approach for calculations. Acrylamide levels were determined analytically by an accredited liquid chromatography – tandem mass spectrometry method. Mean intakes of 0.14 and 0.86 µg/kg body weight/day were estimated for the total Brazilian population using median and maximum acrylamide levels, respectively. The lowest mean intakes were estimated in the Central West, Northeast and Northern regions (0.09, 0.11 and 0.12 µg/kg body weight/day, respectively) while the Southern region showed the highest mean intake (0.20 µg/kg body weight/day). The foods that most contributed to acrylamide exposure in Brazil were French fries (57%), roasted coffee (14%), toasted cassava flour (8%), crackers (7%) and French bread (4%). Risk characterization made by calculating margins of exposure and by estimating the number of human cancer cases based on extrapolations from results of bioassays indicated a concern for human health.

***Keywords**: acrylamide, dietary intake, carcinogen, risk assessment.*

1. Introduction

Findings of Swedish researchers in April 2002 showed that acrylamide could be formed at relatively high levels in certain fried, baked and roasted foods (SNFA, 2002; Tareke et al., 2002). It is now generally agreed that acrylamide is formed by

the Maillard reaction at temperatures above 120ºC and that the major determinants of acrylamide formation in foods are the presence of asparagine and reducing sugars (Mottram et al., 2002; Stadler et al., 2002; Becalski et al., 2003).

Since the discovery of acrylamide formation in foods, significant progress has been made in understanding how acrylamide can affect the human health. The neurotoxicity of acrylamide in humans is well established from occupational and accidental exposures and experimental studies with animals have shown reproductive, genotoxic and carcinogenic effects. Acrylamide has been classified as probably carcinogenic to humans by the International Agency for Research on Cancer (IARC, 1994).

To evaluate whether acrylamide contributes to a risk for a population, there is a need to perform an exposure assessment (Boon et al., 2005). Many authors and regulatory agencies have estimated the potential exposure to acrylamide from the diet and investigated the foods that most contribute to acrylamide intake (SOPH, 2002; BfR, 2003; Dybing and Sanner, 2003; Konings et al., 2003; Svensson et al., 2003; Croft et al., 2004; JIFSAN, 2004). Based on intake data of 17 countries from all regions excepted Latin America and Africa, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that acrylamide intakes ranged from 0.3 to 2.0 µg/kg body weight (bw)/day for average consumers and from 0.6 to 5.1 µg/kg bw/day for high consumers (90th to 99th percentiles). Generally, the most important sources of acrylamide in the diet are French fries, potato chips, coffee, biscuits and breads.

According to the JECFA, intakes of 1 and 4 µg/kg bw/day of acrylamide could be taken to represent the exposure of average and high consumers, respectively. Based on margins of exposure (MOEs) calculated at these intake levels, the Committee concluded that adverse neurotoxic and reproductive toxic effects are unlikely to occur, but morphological changes in nerves could not be excluded for some individuals with very high intake. For adverse carcinogenic effects, MOEs calculated by the Committee may indicate a human health concern (FAO/WHO, 2005).

The present paper describes a preliminary estimate of acrylamide intake from dietary sources in Brazil as well as the relative contribution of foodstuffs or food groups to acrylamide exposure. The potential risks of acrylamide to Brazilians were also assessed by comparing the estimated intakes with toxicological reference values.

2. Material and methods

2.1. Food consumption data

Food consumption data were inferred from a Household Budget Survey conducted by the Brazilian Institute of Geography and Statistics (IBGE – Instituto Brasileiro de Geografia e Estatística), from July 2002 to June 2003 (IBGE, 2005). The survey covered the urban and rural areas of all 27 federal states, which are grouped into 5 large regions: North, Northeast, Central West, Southeast and South (Figure 1).

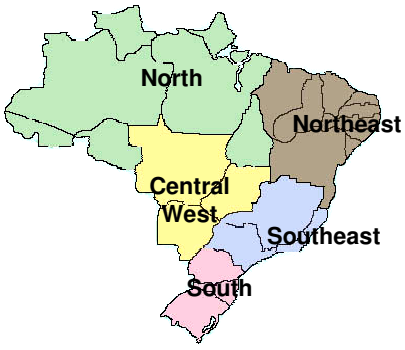

Figure 1. Brazilian geographic regions.

A total of 45,348 households (93.6% from the total surveyed) that reported data were considered, comprising 174,378 individuals. Information on the amount of food entering the household was recorded in a diary over 7 consecutive days.

For each household, the total week consumption of each food obtained from the Household Budget Survey data was divided by the household size to generate weekly consumption per individual. Finally, the food consumption data, expressed as kg per capita per year, were reported for selected food categories as mean national consumption and as mean regional consumption for each geographic region. A detailed description of the survey was reported by Caldas et al. (2006).

Assumptions regarding consumption data of certain food categories were made since household budget surveys do not provide information on how food is handled by the household. Thus, to illustrate a extreme situation of exposure, it was considered that all potato *in nature* acquired by the household was consumed as French fries, all cassava *in nature* was consumed as deep-fried cassava and all maize flour was consumed as deep-fried “polenta”, a typical Brazilian meal made by cooking maize flour with water followed by frying in oil at high temperature for a few minutes. Snacks were considered as potato chips and corn flakes as breakfast cereal since all samples of breakfast cereals analysed for acrylamide were maize extrudates products. The national and regional per capita daily intakes of foods considered in the calculations are shown in Table 1.

Table 1. Food consumption data (g/person/day) in Brazil (IBGE, 2005).

Food category	National	Regional[1]				
		N	NE	S	SE	CW
Potato	17.98	7.81	8.14	33.28	22.16	10.81
French fries	0.21	0.03	0.06	0.16	0.36	0.14
Snacks	0.46	0.24	0.09	0.83	0.66	0.20
Cassava	6.21	9.45	4.24	13.47	4.51	5.51
Toasted cassava flour	21.28	92.68	42.01	2.85	3.91	3.72
Cheese bread	0.43	0.31	0.09	0.35	0.61	1.05
Corn flakes	1.03	0.37	2.12	0.18	0.86	0.23
“Fubá” (maize flour)	8.73	4.69	16.46	7.47	5.87	2.46
Crackers	5.17	5.83	8.73	3.37	3.76	2.62
Crispbread	0.29	0.81	0.09	0.10	0.43	0.08
Toasted bread flour	0.16	0.06	0.08	0.19	0.23	0.07
French bread	33.79	29.36	33.37	26.23	39.16	23.43
Whole bread	0.20	0.15	0.21	0.42	0.15	0.11
Other breads	0.52	0.18	0.50	1.53	0.31	0.07
Roasted coffee	6.77	5.49	6.17	5.83	7.73	6.78
Instant coffee	0.35	0.18	0.41	0.90	0.20	0.14
Beer	12.53	5.18	4.92	16.93	17.06	14.16

[1]Regions: N = North; NE = Northeast; S = South; SE = Southeast; CW = Central West

2.2. Acrylamide analytical data

Acrylamide was determined in different brands and batches of selected foods purchased at supermarkets, fast-food restaurants and restaurants in the region of Campinas, SP, Brazil, from September 2004 to April 2006 (Arisseto et al., 2007). The analytical survey comprised French fries, chips, breads, crispbreads, crackers, breakfast cereals, coffee, beer and other typical Brazilian foods rich in carbohydrates and processed at high temperature such as deep-fried cassava, toasted cassava flour, cheese bread and deep-fried “polenta”.

The analyses were carried out using an accredited LC-MS/MS method, according to Govaert et al. (2006). Briefly, acrylamide was extracted with water and the aqueous extract was centrifuged, filtered and cleaned-up on Oasis® HLB and Bond Elut-Accucat® cartridges. The extract was then pre-concentrated to a small volume before injection into a system consisting of a HPLC quaternary pump Alliance 2695 with an autosampler from Waters coupled to a Quattro Micro triple quadrupole mass spectrometer from Waters/Micromass. The LC-MS/MS analysis was performed on a µ-Bondapak C_{18} column using acrylamide-D_3 as internal standard. The limit of detection (LOD) and limit of quantitation (LOQ) were 10 and 20 µg/kg, respectively.

2.3. Dietary exposure modeling

The exposure assessment was conducted using the deterministic approach. This modeling, also called point estimate, refers to a method whereby a fixed value for food consumption is multiplied by a fixed value for the residue/concentration, and the intakes from all sources are then summed (Kroes et al., 2002).

Calculations were made considering mean food consumption data and median acrylamide levels (scenario 1). Maximum acrylamide levels were also used in order to simulate a “worst case” scenario (scenario 2). Analytical results below the LOQ were replaced by half of the LOQ since the number of samples below the LOQ was lower than 60% of the total number of samples, as proposed by the World Health Organization (WHO, 2003).

To estimate the acrylamide exposure of high consumers, intakes of average consumers were multiplied by 3, as no data on extreme consumers were available from IBGE. This approach has been suggested by WHO in the Guidelines for the Study of Dietary Intake of Chemical Contaminants (WHO, 1985). A consumer average body weight of 60 kg was used in the calculations.

2.4. Risk characterization

To evaluate the potential risks of acrylamide to Brazilians, MOEs were calculated by comparing the estimated intakes with toxicological reference values, as proposed by the JECFA (FAO/WHO, 2005). For neurotoxic effects, the intakes were compared with the no-observed-effect level (NOEL) of 0.2 mg/kg bw/day for morphological changes in nerves detected in rats (Burek et al., 1980). For reproductive and developmental toxic effects, the NOEL of 2 mg/kg bw/day (Johnson et al., 1986, Friedman et al., 1995) was used for comparison with the acrylamide intakes. For carcinogenic effects, the benchmark dose lower confidence limit (BMDL) of 0.3 mg/kg bw/day for induction of mammary tumors in rats (Johnson et al., 1986) was used in the calculations.

3. Results

Table 2 shows the median and maximum acrylamide levels determined analytically and summarizes the estimates of the acrylamide daily intake, based on mean national food consumption data from Table 1. Intakes of 0.14 and 0.42 µg/kg bw/day were estimated for average and high consumers, respectively, when using median acrylamide levels (scenario 1). For maximum acrylamide levels (scenario 2), the corresponding estimated intakes were 0.87 and 2.60 µg/kg bw/day, respectively.

Table 2. Estimated acrylamide daily intake for average and high consumers based on mean national food consumption data from IBGE (2005).

Food category	Acrylamide levels (µg/kg)		Acrylamide intake (µg/kg bw/day)	
	Median	**Maximum**	**Scenario 1**	**Scenario 2**
French fries	264	2528	0.0800	0.7661
Potato chips	591	1999	0.0045	0.0153
Deep-fried cassava	<20	<20	0.0010	0.0010
Toasted cassava flour	30	81	0.0106	0.0287
Cheese bread	<20	<20	0.0001	0.0001
Breakfast cereal	30	49	0.0005	0.0008
Deep-fried “polenta”	<20	33	0.0015	0.0048
Crackers	116	361	0.0100	0.0113
Crispbread	71	231	0.0003	0.0011
Toasted bread flour	<20	67	0.0000	0.0002
French bread	<20	<20	0.0056	0.0056
Whole bread	<20	<20	0.0000	0.0000
Other breads	21	124	0.0002	0.0011
Roasted coffee	174	202	0.0196	0.0228
Instant coffee	582	683	0.0034	0.0040
Beer	<20	<20	0.0021	0.0021
Total average consumers			**0.1396**	**0.8652**
Total high consumers			**0.4188**	**2.5956**

Acrylamide daily intakes were also estimated for average consumers from different geographic regions, using median acrylamide levels. Although all analysed samples were purchased in the city of Campinas and, because of that, may not represent the national food supply, many of these foods are nationally distributed and may be taken as sources of exposure to acrylamide for consumers from other regions. The results indicate that the lowest intakes were found in the Central West (0.09 µg/kg bw/day), Northeast (0.11 µg/kg bw/day) and Northern

(0.12 µg/kg bw/day) regions while the highest intake was observed in the Southern region (0.20 µg/kg bw/day).

Figure 2 illustrates the foods that most contributed to the exposure of Brazilians to acrylamide. As can be seen, French fries were the most important source of exposure followed by roasted coffee, toasted cassava flour, crackers and French bread.

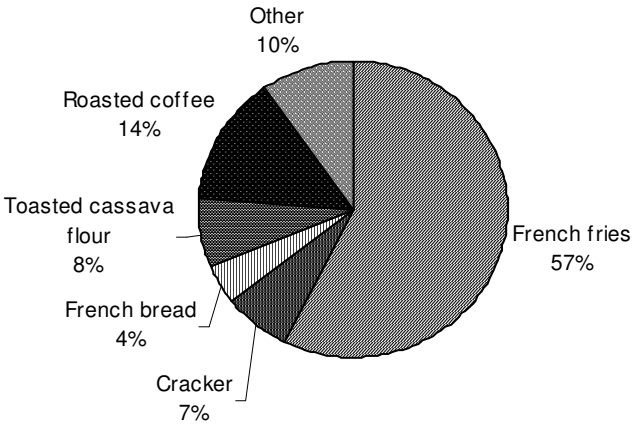

Figure 2. Contribution (%) of different foods to the dietary intake of acrylamide in Brazil.

MOEs were calculated at intakes of 0.14 µg/kg bw/day for average consumers and 0.42 µg/kg bw/day for high consumers. Comparison of these intakes with the NOEL of 0.2 mg/kg bw/day for morphological changes in nerves in rats provided MOEs of 1429 and 476, respectively. For reproductive and developmental effects, comparison of the selected intakes with the NOEL of 2.0 mg/kg bw/day provided MOEs of 14,286 and 4762, respectively. For carcinogenic effects, MOEs of 2143 and 714 were calculated for average and high consumers, respectively, by comparing the selected intakes with the BMDL of 0.3 mg/kg bw/day for induction of mammary tumors in rats.

4. Discussion

4.1. Dietary exposure to acrylamide

The deterministic approach was used in this work as a first step in order to provide an indication of the level of exposure of Brazilians to acrylamide. It is important to emphasize that in deterministic models the following assumptions may be considered: 1) all individuals consume the specified food(s) at the same level, 2) the chemical is always present in the food(s) and 3) the chemical is always present at a median/mean/high level (Kroes et al., 2002).

In scenario 1, the intake of 0.14 µg/kg bw/day estimated for average consumers is below the lowest acrylamide daily intake reported in the literature, i.e. 0.28 µg/kg bw/day in Switzerland (SOPH, 2002). For high consumers, the intake of 0.42 µg/kg bw/day is also below the lowest value reported, i.e. 0.6 µg/kg bw/day in The Netherlands (Konings et al., 2003). Acrylamide intakes estimated in scenario 1 are approximately 7- and 9-fold lower than the intakes of 1 and 4 µg/kg bw/day taken by the JECFA to represent average and high consumers, respectively (FAO/WHO, 2005).

In the “worst case” scenario, the intake of 0.87 µg/kg bw/day estimated for average consumers is compared to the results reported in other countries (0.3 to 2.0 µg/kg bw/day). For high consumers, the same trend is observed. Acrylamide intakes estimated in scenario 2 are approximately 1.2- and 1.5-fold lower than the intakes taken by the JECFA (FAO/WHO, 2005). According to Kroes et al. (2002), when high levels are used to represent either food consumption or chemical concentration values, the summing of the intake from multiples sources may lead to high and often implausible overestimates of intake. Therefore, daily intakes from scenario 1 were taken to represent the dietary exposure of Brazilians to acrylamide.

Differences observed on intakes estimated in Brazilian geographic regions may be attributed to different patterns of food consumption, as can be seen from

Table 1. The Southern region showed the highest consumption of potato and consequently the highest acrylamide intake. The Northern and Northeast regions presented the lowest consumption of potato and a very high consumption of toasted cassava flour, which contains a level of acrylamide much lower than French fries. The Southeast region, where the city of Campinas is located, showed the mean acrylamide intake most close to the mean national intake, with the highest consumption of roasted coffee and French bread.

It is important to note that the assumptions made in this work, due to the lack of information on cooking methods, may lead to overestimated intakes. By the other hand, household budget surveys do not account for outside household consumption, which could underestimate the results since the most significant consumption of French fries is outside home. Although the presented data may not indicate the real dietary intake of acrylamide by Brazilians due to several sources of uncertainty associated to food consumption data, food sampling and modeling, they can be considered as a preliminary estimate of the exposure to acrylamide via foods in Brazil.

4.2. Contribution of different foods to acrylamide dietary exposure

If assumed that all potato acquired by the household is consumed as French fries, the contribution of this food category to acrylamide exposure is 57%. Based on results from 17 countries, mainly from Europe and North America, the JECFA (FAO/WHO, 2005) considered that this product contributes from 16 to 30% of the total acrylamide dietary intake, which confirms that the assumptions made in this work could overestimate the results. As shown in Figure 2, the other food categories that most contributed to acrylamide exposure were roasted coffee (14%), toasted cassava flour (8%), crackers (7%) and French bread (4%). Other foods summed contributed less than 10%.

In a previous study on the occurrence of acrylamide in selected foods in Brazil (Arisseto et al., 2007), it was noted that cassava based meals contain very

low acrylamide levels and should not be a concern issue as raised in the consultation held jointly by the Food and Agriculture Organization (FAO) and WHO in 2002 (FAO/WHO, 2002). Nevertheless, due to the high consumption of toasted cassava flour in the Northern and Northeast regions, this food item was identified as the third major contributor to the overall intake of acrylamide.

4.3. Risk characterization

In order to discuss the risks that the estimated intakes of acrylamide represent to Brazilians, MOEs calculated in this study were compared with MOEs calculated by the JECFA at intakes of 1 and 4 µg/kg bw/day, for average and high consumers, respectively. The data are presented in Table 3.

Table 3. Margins of exposure (MOEs) for neurotoxic, reproductive and carcinogenic effects of acrylamide.

	MOEs			
	Average consumers		**High consumers**	
Effects	**Brazil**	**JECFA**	**Brazil**	**JECFA**
Neurotoxic	1428	200	476	50
Reproductive	14,286	2000	4762	500
Carcinogenic	2143	300	714	75

As the MOEs calculated for Brazilian population are higher than the MOEs calculated by the JECFA, it can be assumed that adverse neurotoxic and reproductive effects are also unlikely to occur at the intake levels estimated in this study. For carcinogenic effects, although MOEs calculated in Brazil are higher than MOEs calculated by the JECFA, they can still be considered low for a compound that is both genotoxic and carcinogenic, indicating thus a human health concern.

For comparison purposes, when intakes of benzo(a)pyrene at levels of 0.004 and 0.010 µg/kg bw/day (taken by the JECFA to represent average and high consumers, respectively) are compared with the BMDL of 100 µg/kg bw/day for carcinogenic effects, the calculated MOEs are 25,000 and 10,000, respectively, which was considered of low concern for human health by the Committee (FAO/WHO, 2005).

Although most international regulatory agencies agree on the qualitative identification of acrylamide as a carcinogenic hazard, theoretical models to calculate quantitative risk are not sufficiently reliable, especially in the absence of any positive epidemiological results (FAO/WHO, 2002). However, various estimates of the number of human cancer cases (sites not specified) resulting from the dietary intake of acrylamide have been made based on extrapolations from results of bioassays in rodents. The US Environmental Protection Agency (US EPA, 1993) calculated a lifetime cancer risk of 4.5×10^{-3} at an acrylamide intake level of 1 µg/kg bw/day. For the same acrylamide intake level, Dybing and Sanner (2003) and WHO (1996) calculated a lifetime cancer risk of 1.3×10^{-3} and 0.7×10^{-3}, respectively.

Correlating the hazard level of 0.7×10^{-3} from WHO (1996) with the acrylamide intake estimated in this study for the average population, a lifetime cancer risk of 0.1×10^{-3} was calculated, which implies that 1 out 10,000 individuals may develop cancer due to acrylamide. Using the hazard levels from Dybing and Sanner (2003) and US EPA (1993), the estimated mean acrylamide intake could represent a cancer incidence of 2 and 7 per 10,000 individuals, respectively.

In conclusion, a preliminary assessment of the exposure of Brazilians to acrylamide is reported in this work. Mean dietary intakes were estimated to be 0.14 and 0.42 µg/kg bw/day for average and high consumers, respectively, which are lower than intakes reported in other countries. As the food consumption data generated by the IBGE survey does not necessarily reflect the real dietary habit of the Brazilian population, it is important that data from individual food consumption

surveys be made available and that a larger number of food groups and samples be analysed in order to design a more realistic exposure scenario. Despite the many sources of uncertainty inherent in the results, these are the only data available in Brazil and the dietary exposure to acrylamide estimated in this work may be considered as a first assessment which should be refined in the future. Margins of exposure calculated for carcinogenic effects indicate a human health concern, however, for neurotoxic and reproductive toxic effects, the risks associated to acrylamide intake from foods are unlikely to occur.

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CAPÍTULO 5

Acrylamide dietary intake by adolescents from a selected region in Brazil

Abstract

Acrylamide dietary intakes by a selected population of Brazilian adolescents are estimated in this work. The exposure assessment was carried out by combining levels of acrylamide in foods, determined analytically by an accredited LC-MS/MS method, with individual food consumption data, using a deterministic approach. Data on food consumption were generated using 24-hour recall applied to 578 individuals aged 11 to 17 years, between July and August 2001. The mean and maximum acrylamide intakes were estimated to be 0.12 and 1.92 μg kg^{-1} bw day^{-1}, respectively. At 50th, 95th and 97.5th percentiles, the average intakes were 0.04, 0.55 and 0.77 μg kg^{-1} bw day^{-1}, respectively. Boys presented exposure levels lower than girls, while the acrylamide intake by younger adolescents (11-14 years) was higher as compared to the older group (15-17 years). The foods that most contributed to acrylamide exposure were French fries, French bread, water and salt biscuit and coffee.

Keywords: *acrylamide, exposure assessment, adolescents, carcinogen.*

Introduction

Acrylamide, a probable carcinogen to humans (IARC 1994), can be formed in some heated foods as a result of a reaction between asparagine and reducing sugars during treatment at high temperatures (Mottram et al. 2002; Stadler et al. 2002; Becalski et al. 2003). Acrylamide has shown reproductive, genotoxic and

carcinogenic properties in experimental studies with animals. In humans, only neurotoxic effects have been documented after occupational and accidental exposures (Friedman 2003).

Since the discovery of acrylamide formation in foods (SNFA 2002; Tareke et al. 2002), intensive efforts have provided a better knowledge of how this compound can affect the human health. To evaluate whether acrylamide represents a risk for a population, there is a need to perform an exposure assessment. According to Kroes et al. (2002), only intakes of toxicologically significant amounts can lead to adverse health effects.

There are two ways to combine data on food consumption and concentration of the chemical in food in order to conduct an exposure assessment. Deterministic modeling, also called point estimate, is a simplistic way consisting of multiplying a single level of consumption by a single level of contamination. This method is very useful as a screening tool. Probabilistic modeling is a more sophisticated approach which describes variables in terms of distributions to characterize their variability and uncertainty. It takes into account all the possible values that each variable could have and weight each possible model outcome by the probability of its occurrence (Kroes et al. 2002; Dybing et al. 2005).

In 2005, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated data on acrylamide dietary intake of 17 countries from all regions except Latin America and Africa, calculated mainly using deterministic approach. Acrylamide intakes ranged from 0.3 to 2.0 μg kg^{-1} body weight (bw) day^{-1} for average consumers and from 0.6 to 5.1 μg kg^{-1} bw day^{-1} for high consumers (90th to 99th percentiles). Based on the available data, the Committee noted that children may have intakes of acrylamide around two to three times those of adult consumers when expressed on a body weight basis (FAO/WHO 2005).

It is expected that children and adolescents have consumption patterns different from adults. Most of the types of foods in which acrylamide was detected are popular among children and adolescents, such as French fries, snacks, biscuits and breads. Moreover, they have a lower average body weight and,

consequently, a higher average food intake per kilogram body weight than adults. For that, acrylamide intake by these individuals is considered a concern.

Although several estimates of acrylamide dietary intake are available for general populations, few data on exposure of children and adolescents to acrylamide can be found in the literature. In The Netherlands, the mean exposure to acrylamide of individuals 7-18 years was estimated to be 0.71 μg kg^{-1} bw day^{-1}. For the total population (1-97 years), the estimated acrylamide intake was 0.48 μg kg^{-1} bw day^{-1} (Konings et al. 2003). The mean acrylamide intake by German individuals aged 7-19 years was estimated to be 0.30 μg kg^{-1} bw day^{-1} and the data do not confirm the presumption that the intake by children is two to three times higher than those by adults (Hilbig et al. 2004). For Norwegian 13 years old youngsters, the mean exposure to acrylamide varied from 0.49 to 0.52 μg kg^{-1} bw day^{-1}, respectively for girls and boys. Acrylamide intakes were 0.46 and 0.49 μg kg^{-1} bw day^{-1} for females and males, respectively, considering the total population aged 16-79 years (Dybing and Sanner 2003). The median intake by Belgian adolescents was estimated to be 0.51 μg kg^{-1} bw day^{-1}, and ranged from 0.19 to 1.09 μg kg^{-1} bw day^{-1} at the 5th and 95th percentiles, respectively (Matthys et al. 2005).

This work reports the results of a deterministic assessment of the dietary exposure of Brazilian adolescents to acrylamide, using individual food consumption data and levels of acrylamide determined in selected foods.

Experimental

Food consumption data

Individual food consumption data were originated from a survey carried out to quantify food and nutrient intakes, using a 24-hour diet recall (Caroba 2002). In this kind of survey, the subject is asked by a trained interviewer to recall and describe the type and amount of all foods and beverages ingested during the last 24 hours (Kroes et al. 2002). The survey was based on a representative sample of 578

adolescents (289 boys and 289 girls), aged from 11 to 17 years. All participants were from public schools of the city of Piracicaba-SP, which is located in the administrative region of Campinas-SP, where the food sampling for acrylamide analysis was made. Although the dietary survey was conducted from July to August 2001, it is assumed that the pattern of food consumption identified at that time has not substantially changed. The food groups cited in the questionnaires by the participants included all relevant acrylamide containing foods analysed. As 114 participants (19.7%) did not consume any of the foods of interest in the present assessment, there was a need to separate consumers from non-consumers. The weight and height of all participants were recorded. Table I summarizes the consumption data of the food groups used in the intake calculations.

Table I. Average food consumption of adolescents.

Food category	Food consumption (g day^{-1})				
	All (n=464)	**Boys (n=233)**	**Girls (n=231)**	**11-14 years (n=312)***	**15-17 years (n=152)***
French fries	12.53	8.26	17.16	13.82	10.38
Potato chips	0.17	0.00	0.35	0.26	0.00
Potato “palha”	0.19	0.00	0.38	0.28	0.00
Deep-fried cassava	0.12	0.24	0.00	0.18	0.00
Toasted cassava flour	0.84	0.55	1.15	0.88	0.80
Cassava starch biscuit	0.33	0.51	0.15	0.16	0.68
Breakfast cereal	0.53	0.90	0.17	0.69	0.20
Water and salt biscuit	3.31	3.18	3.52	2.67	4.74
Cream cracker biscuit	0.17	0.14	0.20	0.25	0.00
Crispbread	0.93	1.07	0.81	0.37	2.11
French bread	68.49	80.98	57.68	69.71	68.70
Hot dog bread	1.60	1.29	1.95	2.08	0.66
Italian bread	0.10	0.19	0.00	0.00	0.30
“Bisnaguinha” bread	0.26	0.00	6.52	0.19	0.39
Coffee (beverage)**	36.22	42.48	30.85	40.09	29.71

*Boys and girls.
**Consumption of coffee is given in ml/day.

Acrylamide analytical data

Different brands and batches of selected foods were purchased at supermarkets, fast-food restaurants and restaurants in the region of Campinas-SP, Brazil, between September 2004 and April 2006, and analysed for acrylamide using an accredited liquid chromatography–tandem mass spectrometric (LC-MS/MS) method (Arisseto et al. 2007). Briefly, acrylamide was extracted with water and the aqueous extract was centrifuged, filtered and cleaned-up on Oasis® HLB and Bond Elut-Accucat® cartridges. The extract was then pre-concentrated to a small volume before injection into the system consisting of a HPLC quaternary pump Alliance 2695 with an autosampler from Waters coupled to a Quattro Micro triple quadrupole mass spectrometer from Waters/Micromass. The LC-MS/MS analyses were performed on a µ-Bondapak C_{18} column using acrylamide-D_3 as internal standard. The limit of detection (LOD) and limit of quantitation (LOQ) were 10 and 20 µg kg^{-1}, respectively (Govaert et al. 2006). The median and maximum acrylamide levels found in the analysed foods are shown in Table II.

Table II. Acrylamide levels in selected food categories.

Food category	Number of samples	Acrylamide levels (µg kg^{-1})	
		Median	Maximum
French fries	7	264	2528
Potato chips	12	591	1999
Potato “palha”	7	649	803
Deep-fried cassava	3	<20	<20
Toasted cassava flour	3	30	81
Cassava starch biscuit	11	22	62
Breakfast cereal	8	30	49
Water and salt biscuit	4	187	361
Cream cracker biscuit	3	116	131
Crispbread	7	71	231
French bread	2	<20	<20
Hot dog bread	2	21	21
Italian bread	1	<20	<20
“Bisnaguinha” bread	1	39	39
Roasted coffee	3	174	202

Dietary exposure modeling

The deterministic approach was used to estimate the mean acrylamide exposure of the total population of adolescents and of sub-groups according to gender (boys and girls) and age (11-14 and 15-17 years). Dietary intakes were also reported at 50th, 95th and 97.5th percentiles. The subject's daily intake (*Y*) was computed according to Equation (1):

$$Yi = \sum_{v} (F_{v,i} \times C_v) \quad (1)$$

where *i* is the subject, *v* is the food item, $F_{v,i}$ is the amount (g) of the food item *v* consumed by the subject *i*, and C_v is the acrylamide content in the food item *v*, expressed in μg kg^{-1}.

To express the acrylamide daily intake (DI) in μg kg^{-1} bw day^{-1}, Equation (2) was used for each subject *i*:

$$DIi = Y_i / bw_i \quad (2)$$

where *Yi* is the subject's total daily intake of acrylamide and bw*i* is its body weight (kg).

Analytical results below the LOQ were replaced by half of the LOQ since the number of samples below the LOQ was lower than 60% of the total number of samples, as proposed by the World Health Organization (WHO 2003).

As the consumption of coffee in the dietary survey was recorded as coffee drink and the acrylamide content of coffee was determined on basis of coffee powder, there was a need to recalculate the levels of acrylamide in coffee drink. This was done by using a conversion factor of 0.08 (20 g ground coffee to 250 ml water), as proposed by Camargo et al. (1999).

Results and discussion

Estimated acrylamide intakes are shown in the distribution plot illustrated in Figure 1. A mean intake of 0.12 µg kg^{-1} bw day^{-1} was estimated for the total population of adolescents considered in this study. At 50th percentile, the dietary intake was estimated to be 0.04 µg kg^{-1} bw day^{-1}, which means that 50% of the total population had an intake of acrylamide at or below 0.04 µg kg^{-1} bw day^{-1}. At high percentiles, the acrylamide intake was approximately 4.5- (95th percentile) and 6.5-fold (97.5th percentile) higher than the mean. A total of 12 subjects had an exposure level above 97.5th percentile, and the maximum intake reported was 1.92 µg kg^{-1} bw day^{-1}.

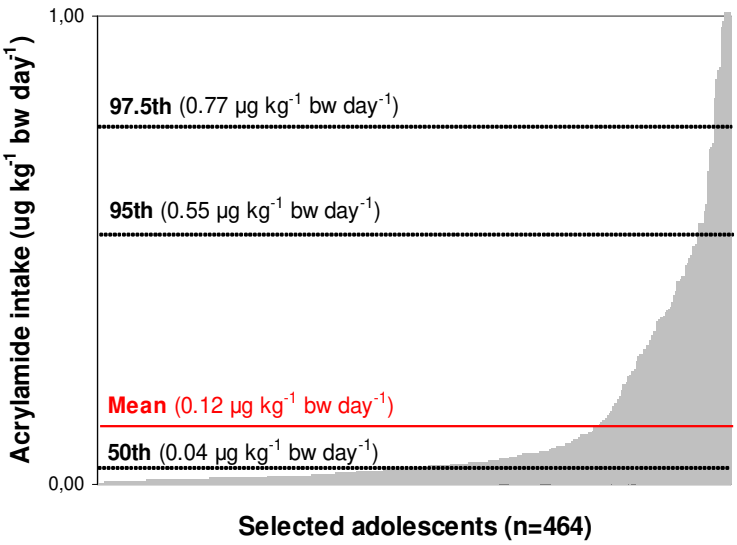

Figure 1. Distribution plot of acrylamide daily intake by Brazilian adolescents.

Figure 2 shows a frequency plot of acrylamide daily intake, based on acrylamide levels found in the groups of food included in the calculations and on the consumption of these products by the studied population. Most of the respondents consumed acrylamide from dietary sources up to 0.10 µg kg^{-1} bw day^{-1}, while relatively few consumers had intakes above 0.60 µg kg^{-1} bw day^{-1}.

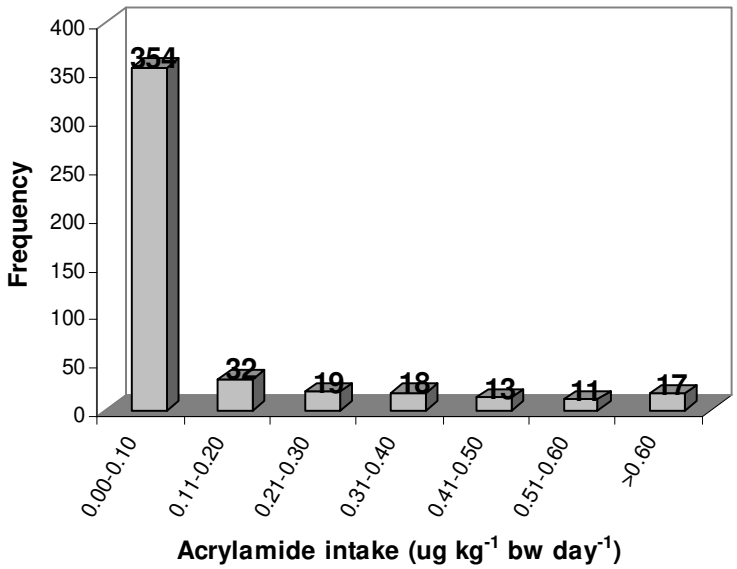

Figure 2. Frequency plot of estimated acrylamide daily intake by adolescents.

Acrylamide daily intakes estimated for sub-groups according to gender and age are summarized in Table III. The results indicated that boys have an exposure to acrylamide significantly lower than girls ($p<0.05$). To assess the intake according to age, sub-groups were divided in 11-14 and 15-17 years, as recommended by the European Commission (Kroes et al. 2002). As seen in Table III, the intake by the younger sub-group (11-14 years) was significantly higher ($p<0.05$) as compared to the older sub-group (15-17 years).

Table III. Acrylamide daily intake according to gender and age.

	Acrylamide intake (µg kg^{-1} bw day^{-1})			
	Boys	**Girls**	**11-14 years**	**15-17 years**
Mean	0.10	0.15	0.14	0.09
50th percentile	0.03	0.04	0.04	0.03
95th percentile	0.38	0.60	0.57	0.46
97.5th percentile	0.59	0.89	0.89	0.53

An assessment of the contribution of each food group to acrylamide exposure showed that French fries are the most important source of acrylamide in the diet, contributing to 60% of the total average intake. The highest contribution of French fries was observed among girls (66%), when considering sub-groups. French bread, water and salt biscuit, and coffee were also significant sources of acrylamide, contributing to 13, 11 and 9% of the total average intake, respectively. In other countries, potato products and bread are also considered the most important sources of acrylamide intake by adolescents (Dybing and Sanner 2003; Konings et al. 2003; Hilbig et al. 2004; Matthys et al. 2005).

The percentile distribution for each food group that most contributed to acrylamide exposure was estimated independently, and is shown in Table IV. The results indicated that French bread is the most important source of acrylamide from the 16th to 84th percentiles. From the 85th percentile, French fries are the main contributor to acrylamide intake. From the 92nd percentile on, water and salt biscuit becomes the second most important source of acrylamide. In spite of the low content of acrylamide in French bread, its large daily consumption in Brazil could explain its importance as source of acrylamide in the diet.

Table IV. Percentile distribution of acrylamide intake via the most important food groups.

Percentile	**Acrylamide intake (μg kg^{-1} bw day^{-1})**			
	via French bread	**via coffee**	**via French fries**	**via water and salt biscuit**
16th	0.005	0	0	0
50th	0.013	0	0	0
62nd	0.016	0.003	0	0
85th	0.028	0.027	0.175	0
90th	0.032	0.038	0.325	0
92nd	0.033	0.043	0.372	0.055
95th	0.041	0.049	0.467	0.107
97.5th	0.046	0.066	0.667	0.153

In order to compare the results of this study with similar information available in the literature (Dybing and Sanner 2003; Konings et al. 2003; Hilbig et al. 2004; Matthys et al. 2005), Table V and VI present data on daily intake of acrylamide by adolescents in different countries (total population and sub-groups, respectively).

Table V. Daily intake of acrylamide in different countries by total population.

Country (age of the studied population)	**Acrylamide intake (μg kg^{-1} bw day^{-1})**				
	Mean	**Median**	**90th**	**95th**	**97.5th**
Brazil (11-17)	0.12	na	na	0.55	0.77
Germany (7-18)	0.30	na	0.67	na	na
The Netherlands (7-18)	0.71	na	0.7	0.9	1.1
Belgium (13-18)	na	0.51	na	1.09	na

na – not available
References: Germany (Hilbig et al. 2004), The Netherlands (Konings et al. 2003), Belgium (Matthys et al. 2005).

As can be seen from Table V, the acrylamide intake by Brazilian adolescents is lower than that reported in European countries, even at high percentiles. These differences can be attributed to different patterns of food consumption, especially regarding the consumption of potato products in Brazil and Europe. According to Matthys et al. (2005), the mean consumption of French fries by Belgian adolescents is 39.88 g day^{-1} while Caroba (2002) determined a mean intake of 12.53 g day^{-1} of French fries by the selected individuals in Brazil, i. e. approximately three-fold lower than in Belgium. As the median acrylamide level detected in French fries is of the same order in Belgium and in Brazil (254 and 264 μg kg^{-1}, respectively), it can be assumed that the consumption of French fries alone accounts for an acrylamide intake by Brazilian adolescents approximately three-fold lower than in Belgium.

Table VI. Daily intake of acrylamide in different countries by sub-groups.

Country	Acrylamide intake (μg kg^{-1} bw day^{-1})*			
	Gender		Age	
	Boys	Girls	Younger	Older
Brazil	0.10	0.15	0.14 (11-14 years)	0.09 (15-17 years)
Norway	0.36-0.52	0.32-0.49	0.32-0.36 (9 years)	0.49-0.52 (13 years)
Belgium	0.64	0.46	na	na

na – not available
References: Norway (Dybing and Sanner 2003), Belgium (Matthys et al. 2005).
* The acrylamide intake is reported as mean in Brazil and Norway, and as median in Belgium.

With regard to gender differences in the exposure to acrylamide, no pattern of intake could be identified (Table VI). The results from Norway and Belgium showed that a higher intake of acrylamide was estimated for boys. However, boys had a lower intake as compared to girls in Brazil. This may be due to the higher consumption of French fries by girls observed in the studied population. In relation to age, the available data indicate that it is likely that younger people have higher intakes as compared to older people when expressed in kg of body weight (Dybing et al. 2005). According to data reported in Table VI, this pattern was observed in Brazil, but it was not verified in Norway, where the acrylamide intake estimated for younger people was lower than the intake by older people.

It must be emphasized that there are several sources of uncertainty in this exposure assessment, which could under or overestimate the results. As for example, the limited number of analytical measurements in a limited number of food samples. In addition, there is a considerable variation in the detected levels of acrylamide within the same food category. The use of half of the LOQ in calculations may also add some uncertainty. On the other hand, in spite of the fact that not all foodstuffs consumed in the diet have been included in the calculations due to the lack of analytical results, this assessment was based on the main food categories in which acrylamide occurs. Moreover, the survey on food consumption

was carried out in the region where the sampling for acrylamide analysis was done. Another important consideration is that 24-hour diet recalls seem very appropriate for investigating acrylamide exposures by providing greater levels of detail about the processing and preparation procedures of the consumed foods (Dybing et al. 2005). Other countries, such as Australia (Croft et al. 2004) and USA (JIFSAN 2004, USDA 2004), have also used 24-hour diet recalls to perform the exposure assessment to acrylamide.

To assess whether a toxic compound contributes to a risk for a population, calculated exposure levels are compared with relevant toxicological reference values (Boon et al. 2005). However, the data presented in this work are not appropriate for risk assessment purposes since the length of time over which dietary samples are to be collected should be several consecutive days at multiple intervals of months, seasons and even years (Kroes et al. 2002). Until now, epidemiological studies found no association between consumption of foods containing acrylamide and cancer risk (Mucci et al. 2003, 2004; Pelluchi et al. 2003). However, these studies have limited power to detect small increases in tumor incidence (FAO/WHO 2005), and the lack of positive epidemiological results is not proof that no such effects exist. For a compound that is carcinogenic and, presumably acts via a genotoxic mechanism, no safe levels of intake can be derived, meaning that the level in foods should be as low as reasonably achievable (Tritscher 2004).

Conclusions

This work reports the results of an exposure assessment to acrylamide via foods of adolescents from a selected region in Brazil. The mean dietary intake was estimated to be 0.12 μg kg^{-1} bw day^{-1}, which is lower than the intakes reported for adolescents from other countries. The foods that most contributed to acrylamide exposure of the studied Brazilian adolescents were French fries, French bread, water and salt biscuits, and coffee. French fries were the most important contributor at higher percentiles while French bread was more important at lower

and median percentiles. Although individual food consumption data were used in this assessment, only the food groups which were analysed for acrylamide were included in the calculations. As a consequence, the contribution of other foods, in some of which the presence of acrylamide has been reported in the literature, were excluded, thus underestimating the intake of the contaminant. Therefore, it is highly desirable that a larger number of foods and samples of the same product be analysed in the future, in order to allow an estimate of the real contribution of the diet as a source of acrylamide in Brazil.

As it remains difficult to predict the total impact of all sources of uncertainty on intake evaluations and because of undefined, although quite low, risks for neurotoxic and carcinogenic effects, people should be advised to avoid excessive frying or baking, especially with regard to potato products, and have a balanced diet by increasing the consumption of fruits and vegetables and decreasing the consumption of fried foods.

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Conclusão Geral

Este trabalho inicialmente revisou os principais tópicos abordados em cinco anos de pesquisas desde a descoberta da formação de acrilamida em alimentos. Muitos pesquisadores, principalmente de países da Europa e América do Norte, têm se dedicado ao estudo deste contaminante e elucidado importantes questões referentes à formação, metabolismo e técnicas analíticas, entre outras. Entretanto, nota-se que poucos dados de países de outras regiões encontram-se disponíveis na literatura, o que evidencia a necessidade da participação de países em desenvolvimento na geração de dados analíticos e estudos de estimativa de ingestão.

Os resultados experimentais obtidos mostram que os níveis de acrilamida encontrados nos alimentos selecionados para este estudo variaram de <20 a 2528 µg/kg e estão de acordo com os dados reportados em outros países. Os maiores teores foram encontrados em produtos à base de batata, café e biscoitos. Produtos à base de mandioca, para os quais ainda não havia dados disponíveis, apresentaram baixos níveis de acrilamida. O método proposto para determinação de acrilamida em produtos à base de cacau pode ser utilizado para a obtenção de resultados confiáveis em matrizes complexas, como mostraram os resultados do processo de validação. Estimativas preliminares de ingestão indicaram que a exposição de brasileiros à acrilamida é inferior àquelas reportadas em outros países. Para a população geral, valores médios de 0,14 e 0,42 µg/kg pc/dia foram estimados para médios e grandes consumidores, respectivamente. Para adolescentes, valores de ingestão de até 1,92 µg/kg pc/dia foram observados.

Esta primeira obtenção de dados em alimentos brasileiros foi muito importante para ajudar a suprir a demanda por informações sobre a ocorrência de acrilamida em outras regiões, como América Latina, por exemplo, e permitir um melhor entendimento de sua distribuição nos alimentos. Apesar de significativos progressos já terem sido obtidos, pesquisas estão sendo realizadas para que se

possam avaliar os verdadeiros riscos associados à exposição à acrilamida através da dieta. Por enquanto, é aconselhável evitar o cozimento excessivo de alimentos, principalmente de produtos ricos em carboidratos, e manter uma alimentação equilibrada e variada, aumentando-se o consumo de frutas e legumes e diminuindo-se a ingestão de alimentos fritos e gordurosos.

ANEXOS

Anexo 1. Lista das amostras analisadas e teor médio de acrilamida encontrado. (limite de quantificação = 20 µg/kg)

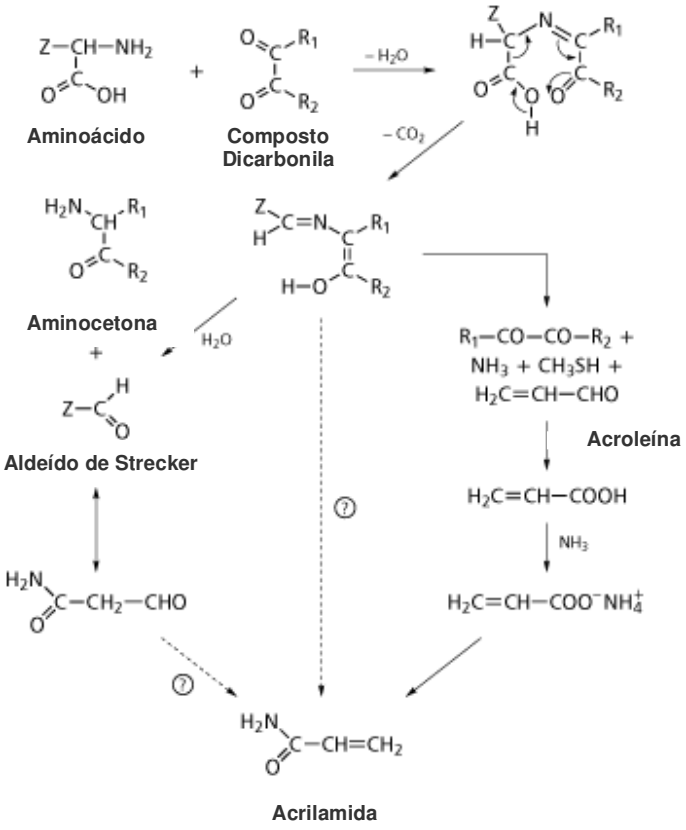
Amostras	Acrilamida (µg/kg)
Batata frita, fast-food 1	171
Batata frita, fast-food 2	264
Batata frita, fast-food 3	351
Batata frita, restaurante 1	181
Batata frita, restaurante 2	873
Batata frita congelada, marca 1, frita por 5 minutos	146
Batata frita congelada, marca 1, frita por 8 minutos	2528
Batata chips, marca 1, sabor tradicional	851
Batata chips, marca 1, sabor ervas finas e azeite	649
Batata chips, marca 1, sabor cebola e salsa	653
Batata chips, marca 2, sabor tradicional, lote 1	255
Batata chips, marca 2, sabor tradicional, lote 2	144
Batata chips, marca 2, sabor tomate e azeitona	168
Batata chips, marca 2, sabor azeite de oliva	267
Batata chips, marca 2, sabor cebola e salsa	275
Batata chips, marca 3, sabor tomate e manjericão	533
Batata chips, marca 4, sabor tradicional	769
Batata chips, marca 5, sabor tradicional	889
Batata chips, marca 6, sabor tradicional	1999
Batata palha, marca 1, lote 1	803
Batata palha, marca 1, lote 2	744
Batata palha, marca 2, lote 1	733
Batata palha, marca 2, lote 2	388
Batata palha, marca 3, lote 1	198
Batata palha, marca 3, lote 2	307
Batata palha, marca 4	649
Farinha de mandioca torrada, marca 1	81
Farinha de mandioca torrada, marca 2	30

Farinha de mandioca torrada, marca 3	<20
Biscoito de polvilho, marca 1, natural, lote 1	22
Biscoito de polvilho, marca 1, natural, lote 2	<20
Biscoito de polvilho, marca 1, doce	34
Biscoito de polvilho, marca 2, salgado, lote 1	<20
Biscoito de polvilho, marca 2, salgado, lote 2	<20
Biscoito de polvilho, marca 2, doce, lote 1	26
Biscoito de polvilho, marca 2, doce, lote 2	<20
Biscoito de polvilho, marca 3, salgado, lote 1	35
Biscoito de polvilho, marca 3, salgado, lote 2	62
Biscoito de polvilho, marca 4, salgado	<20
Biscoito de polvilho, marca 5, doce	30
Mandioca palito congelada, marca 1, frita por 5 minutos	<20
Mandioca palito congelada, marca 2, frita por 5 minutos	<20
Mandioca palito congelada, marca 2, frita por 8 minutos	<20
Pão de queijo, estabelecimento 1	<20
Pão de queijo, estabelecimento 2	<20
Pão de queijo congelado, marca 1, assado por 40 minutos a 180ºC	<20
Cereal matinal, marca 1, sabor original, lote 1	49
Cereal matinal, marca 1, sabor original, lote 2	24
Cereal matinal, marca 1, sabor chocolate, lote 1	27
Cereal matinal, marca 1, sabor chocolate, lote 2	38
Cereal matinal, marca 1, sabor banana, lote 1	35
Cereal matinal, marca 1, sabor banana, lote 2	32
Cereal matinal, marca 1, sabor frutas, lote 1	<20
Cereal matinal, marca 1, sabor frutas, lote 2	22
Polenta palito congelada, marca 1, frita por 5 minutos	<20
Polenta palito congelada, marca 1, frita por 8 minutos	33
Polenta frita, restaurante 1	<20
Curau de milho em pó, marca 1	<20
Biscoito água e sal, marca 1	174
Biscoito água e sal, marca 2	199

Biscoito água e sal, marca 3	154
Biscoito água e sal, marca 4	361
Grissini, marca 1	131
Grissini, marca 2	125
Biscoito cream cracker, marca 1	107
Biscoito cream cracker, marca 2	131
Biscoito cream cracker, marca 3	116
Torrada salgada, marca 1	25
Torrada salgada, marca 2, lote 1	71
Torrada salgada, marca 2, lote 2	58
Torrada salgada, marca 3, lote 1	79
Torrada salgada, marca 3, lote 2	54
Torrada salgada, marca 4, lote 1	184
Torrada salgada, marca 4, lote 2	231
Pão francês, estabelecimento 1, lote 1	<20
Pão francês, estabelecimento 1, lote 2	<20
Pão tipo mini baguete, estabelecimento 1, lote 1	<20
Pão tipo mini baguete, estabelecimento 1, lote 2	25
Pão italiano, marca 1, lote 1	<20
Pão italiano, marca 1, lote 2	<20
Pão integral, marca 1	<20
Pão de hot dog, estabelecimento 1, lote 1	21
Pão de hot dog, estabelecimento 1, lote 2	21
Pão de hambúrguer, estabelecimento 1, lote 1	21
Pão de hambúrguer, estabelecimento 1, lote 2	25
Pão tipo bisnaguinha, marca 1	39
Pão tipo sueco, marca 1, com gergelim	124
Pão tipo sueco, marca 2, com alho	53
Farinha de rosca, marca 1	<20
Farinha de rosca, marca 2	<20
Farinha de rosca, marca 3	67
Café instantâneo, marca 1	333
Café instantâneo, marca 2	582

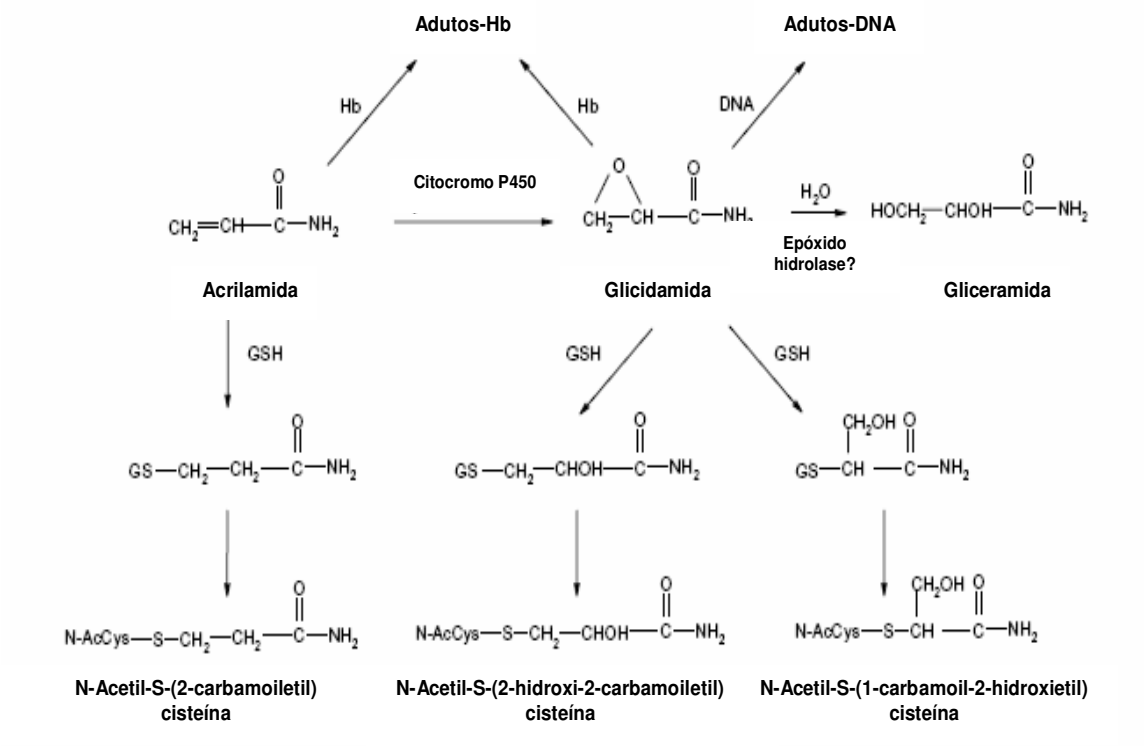
Café instantâneo, marca 3	683
Café torrado e moído, marca 1	128
Café torrado e moído, marca 2	174
Café torrado e moído, marca 3	202
Cerveja escura, marca 1	<20
Cerveja escura, marca 2	<20
Cerveja escura, marca 3	<20
Cerveja clara, marca 1	<20
Cerveja clara, marca 2	<20
Cerveja clara, marca 3	<20
Cerveja clara, marca 4	<20
Cerveja clara, marca 5	<20
Cerveja clara, marca 6	<20
Cerveja clara, marca 7	<20
Cerveja clara, marca 8	<20
Cerveja clara, marca 9	<20
Cerveja clara, marca 10	<20
Cerveja clara, marca 11	<20

Anexo 2. Possíveis mecanismos de formação de acrilamida em alimentos.



Fonte: Stadler et al. (2002). Acrylamide from Maillard reaction products, *Nature* 419: 449-450.

Anexo 3. Principais rotas metabólicas da acrilamida.



Fonte: Dybing et al. (2005). Human exposure and internal dose assessments of acrylamide in food, *Food and Chemical Toxicology* 43: 365-410.

Anexo 4. Influência do tempo de fritura na formação de acrilamida em batata frita. (limite de quantificação = 20 µg/kg)

5 minutos
(146 µg/kg)

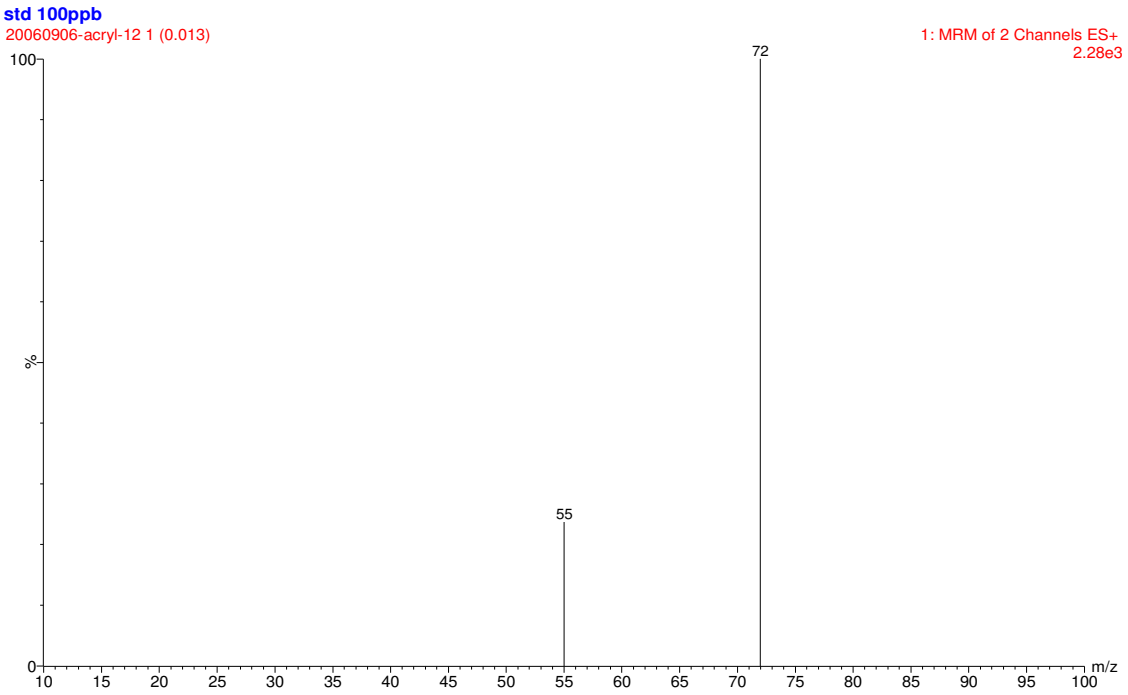
8 minutos
(2528 µg/kg)

Anexo 5. Influência do tempo de fritura na formação de acrilamida em mandioca frita. (limite de quantificação = 20 µg/kg)

5 minutos
(<20 µg/kg)

8 minutos
(<20 µg/kg)

Anexo 6: Espectro de massas da acrilamida (padrão).



Condições: coluna: µ-Bondapak C_{18} (300 x 3.9 mm d.i.; 10 µm); fase móvel: 0,1% de ácido acético em água; vazão: 0,6 ml/min; volume de injeção: 100 µl; fonte de ionização: electrospray positivo; voltagem do capilar: 4000 V; gás de dessolvatação: nitrogênio (600 L/h, 300ºC); gás de colisão: argônio (~3 x 10^{-3} mbars); energia de colisão: 1 eV (72>72) e 10 eV (72>55).