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This is the accepted version of:

Joseph H.Y. Galani, Nilesh J. Patel, Jayant G. Talati,

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Food and Chemical Toxicology,

Volume 108, Part A,

2017,

Pages 244-248,

ISSN 0278-6915,

https://doi.org/10.1016/j.fct.2017.08.011.

Acrylamide-Forming Potential of Cereals, Legumes and Roots and Tubers Analyzed by UPLC-UV

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Abstract

For directing scientists, consumers, industry and stakeholders on mitigation strategies, there is a need to understand the acrylamide-forming potential of important Indian foods. Flour obtained from total 16 varieties of 9 Indian cereals, legumes and roots and tubers was heated at 160 °C for 20 min, acrylamide was extracted and quantified by UPLC-UV. Acrylamide level was above the European Commission indicative value in potato- and cereal-based food products, it ranged from 3436.13 to 5562.56 µg/kg in roots and tubers (potato and sweet potato). Among the cereals, maize (2195.31 µg/kg) and wheat (161.12 µg/kg) had the highest and lowest contents, respectively, whereas rice, sorghum and pearl millet showed intermediate values. Among the 2 legumes, soybean contained higher acrylamide (337.08 to 717.52 µg/kg) than chickpea (377.83 to 480.49 µg/kg). Analysis of variance revealed that roots and tubers acrylamide was highly significantly greater than the content in cereals (*p*<0.0001) and in legumes (*p*<0.0001) while there was no significant difference between cereals and legumes (*p*=0.443). These results support the combination of pulses and minor cereals (chickpea, soybean, millets and sorghum) in cereal-based foods for improving the nutritional value and reducing acrylamide formation.

Highlights

- Acrylamide content in roots and tubers heated flour was higher than cereals and legumes flour.
- Acrylamide levels were above the European Commission indicative value in potato- and cereal-based food products.
- Pulses and minor cereals (chickpea, soybean, millets and sorghum) should be combined in cereal-based foods for improving the nutritional value and reducing acrylamide formation

Keywords: Food safety; Acrylamide formation; Heating; UPLC-UV; India.

1. Introduction

Next to agrochemical residues, processing contaminants in foods have become a major concern for consumers, industry and stakeholders. A processing contaminant is a substance that is produced in a food when it is cooked or processed, but which is not present, or is present at much lower concentrations, in the raw, unprocessed, food and is undesirable either because it has an adverse effect on product quality or because it is potentially harmful (Curtis et al., 2014). Acrylamide, a processing contaminant formed during the cooking or high-temperature processing of mainly plant-derived foods and present in popular foods is now one of the most pressing problems facing the food industry (Muttucumaru et al., 2017). Acrylamide is formed essentially during the Maillard reaction when carbohydrate-rich foods are fried, baked, roasted or processed at high temperatures above 120 °C (Pedreschi, 2007), in low moisture condition, especially in foods containing free amino acids (asparagine) and reducing sugars (glucose and fructose). The major contributors of acrylamide estimated intake in the adult population of Europe are potato, coffee and cereal products like bread, crisp bread, breakfast cereals, cookies, crackers and biscuits (EFSA, 2015).

In fact, glycidamide, a metabolite of acrylamide has been reported to bind to DNA and cause genetic damage in animals and cultured animal cells, leading to skin and lung cancer in mice (Hashimoto and Tanii, 1985). Moreover, the International Agency for Research on Cancer (IARC) classified acrylamide as a probable human carcinogen (Group 2a) with reproductive and neurotoxicological effects at high doses (Friedman, 2003; IARC, 1994), and European Commission together with WHO have listed acrylamide as Category 2 of mutagens (WHO, 2002). Therefore, food safety measures are being taken to monitor and lower the levels of acrylamide in food in Europe by the European Food Safety Authority (EFSA, 2015), in USA by the Food and Drug Administration (FDA, 2016) and worldwide by FAO and WHO. FAO/WHO Expert Committee on Food Additives recommended food manufacturers to develop and implement methods for reducing acrylamide in foods of major importance for dietary exposure (WHO, 2011). The European Commission indicative value of acrylamide levels is between 600-1000 µg/kg for potato-based products and 50-500 µg/kg in cereal-based foods (European Commission, 2013).

In India, cereals, especially rice, wheat, maize, sorghum and millets, are integral part of the diet; they are used for preparing many house made traditional foods and are also used in various cereals products from the food industry. Various combinations of legumes or pulses are generally added when making the cereal-based staples. Pulses play a key-role in the food composition of these foods, as they supply proteins which are essential in the diet of the mostly vegetarian Indian population. Flat bread such as *chapatti, paratha, phulka* and *tandoori roti*, and deep fried products such as *pooris* and *bhaturas* are examples of daily cereal staples consumed in India. These foods have a desirable palatability and digestibility, and they owe their characteristic flavor, aroma and color to Maillard reaction which proceeds rapidly on heat processing of foods above 120 °C (Mulla et al., 2010). *Papad*, a popular food item in India and other Southeast countries, is prepared from various combinations of cereals and legumes and is regularly consume as a meal accompaniment, after roasting or frying (Shaikh et al., 2009). Considerable levels of acrylamide were found in many of these foods (Mulla, 2012; Mulla et al., 2010; Shaikh et al., 2009), because of their intrinsic composition rich in proteins (amino acids) and carbohydrates (reducing sugars) which make them susceptible to form acrylamide during cooking at high temperatures.

The potato is an essential basic vegetable worldwide as well as in the Indian subcontinent, and makes up a considerable proportion of the Indian diet (Galani et al., 2017). Because potatoes contain high levels of asparagine and reducing sugars, and are commonly prepared for consumption by frying or baking, high levels of acrylamide are often found in fried or heated potato products, which are major contributors to dietary acrylamide intake (EFSA, 2015; Muttucumaru et al., 2017; Quayson and Ayernor, 2007). A considerable part of potato production in India is used by industries for processing into chips (Mulla et al., 2011; Paul et al., 2016). Besides, sweetpotato, which has a relatively close composition to potato, is also prone to acrylamide formation during high-temperature cooking and processing. As there is an increasing demand for good-quality fries from carotene-rich sweetpotatoes, acrylamide formation in sweetpotato French fries is likely a potential health concern (Truong et al., 2014). Acrylamide presence in roots and tubers foods have been intensively studied in potato (Elmore et al., 2015; Khoshnam et al., 2010; Mekawi, 2015; Mesias and Morales, 2015;

Powers et al., 2013) and in sweetpotato (Lim et al. 2014; Truong et al. 2014). Likewise, disturbing levels of acrylamide were found in potato products found on Indian market (Paul et al., 2016; Shamla and Nisha, 2014).

While variety, crop management and storage are the pre-processing factors affecting acrylamide content in food product (Curtis et al., 2016; Elmore et al., 2015; Granvogl and Schieberle, 2006; Halford et al., 2012; Muttucumaru et al., 2006; Postles et al., 2013), the pH, temperature, reaction time, moisture content, reactant concentration, and type of reactants are important determinants of the acrylamide content in the food during processing (Shaikh et al., 2009). One of the most effective methods of acrylamide mitigation would be to reduce the accumulation of acrylamide precursors in plant material used for food production (Postles et al., 2013). Considering the important place of cereals, legumes and roots and tubers in Indian diet, added to the worrisome levels of acrylamide found in these food products, there is a need to investigate their acrylamide-forming potential. This will provide useful information to breeders for cultivar selection and to the food industry to comply with indicative levels or regulatory limits and deliver good-quality processed products with low acrylamide levels to the consumers. The present study investigates the acrylamide formation in different varieties of cereals, legumes and roots and tubers mostly consumed in India, when they are subjected to high temperatures.

2. Materials and Methods

2.1. Chemicals

Acrylamide standard (≥99.9%) was obtained from Sigma–Aldrich (St. Louis, Mo., U.S.A.). HPLC grade water was procured from Merck Specialties (Mumbai, India) and purified water from Milli-Q-system (Millipore, Bangalore, India) was used for acrylamide extraction throughout the work.

2.2. Plant samples and treatments

Plant materials were obtained from agricultural research stations of Gujarat, India. They included common Indian varieties of 5 cereals i.e. wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pear millet (*Pennisetum glaucum*); 2 legumes *viz*. soybean (*Glycine max*) and chickpea (*Cicer arietinum*); and 2 roots & tubers i.e. potato (*Solanum tuberosum*) and sweetpotato (*Ipomoea batatas*). Cereals and legumes flour was obtained by grinding approx. 20 g of grains in a tissue lyser (TissueLyser II, QIAGEN) for 1 min at 30 Hz. For potato and sweetpotato, 3 tubers were randomly taken and thoroughly washed with tap water, they were peeled and the flesh was immediately cut into small dice and pooled. The pooled sample was grinded to powder in liquid nitrogen using a mortar and pestle and freeze-dried to obtain a tuber flour. For inducing acrylamide formation, the different flours were heated for 20 min at 160 °C and then allowed to cool at room temperature for 1h (Halford et al., 2012).

2.3. Extraction and analysis of acrylamide by UPLC-UV

The protocol for acrylamide extraction was adapted from Muttucumaru et al. (2017). Precisely 0.5 g of the cooled flour was added to 20 mL water and shaken for 20 min at room temperature. The mixture was centrifuged for 15 min at 10 °C and 9000 rpm and 2 mL of the supernatant was filtered through a 0.2 µm Nylon membrane (EMD Millipore, Billerica, USA) into a 2 mL vial. The extracted acrylamide was analyzed using a UPLC protocol adapted from Khoshnam et al. (2010) on a Waters Acquity UPLC H Class System (Waters Corp., Milford, MA) coupled to a UV photodiode array detector (PDA). Analyses were performed on an

XBridge BEH C18 analytical column (3.5 µm, 4.6 mm x 50 mm, Waters Corp., Milford, MA) heated at 40°C while the Sample Manager FTN was maintained at 10°C. The mobile phase consisted of 0.1% formic acid in water run at a flow rate of 0.3 mL/min. Samples (2 µL) were run for 5 min, the PDA spectra were measured over the wavelength range of 200–800 nm and acrylamide occurring at 2.4 min was detected at 238 nm in steps of 2 nm, with a data acquisition rate of 20 points/sec with 1.2 resolution. Empower 3 software was used for chromatographic data gathering. Concentration of acrylamide was calculated by means of an external calibration curve built in a range between 1 and 10 ng/mL of standard, and expressed in µg/kg.

2.4. Statistical analysis

All experiments were performed in triplicate. Data obtained were analyzed using analysis of variance (ANOVA) (p<0.05) and the significance of difference in acrylamide content among the 3 crop groups was determined using the Fisher's least significant difference. The statistical analyses were performed using XLSTAT version 2017.2 software (Addinsoft, New York, NY).

3. Results and Discussion

Acrylamide occurred at 2.4 min as shown on the chromatograms depicted on Figure 1.

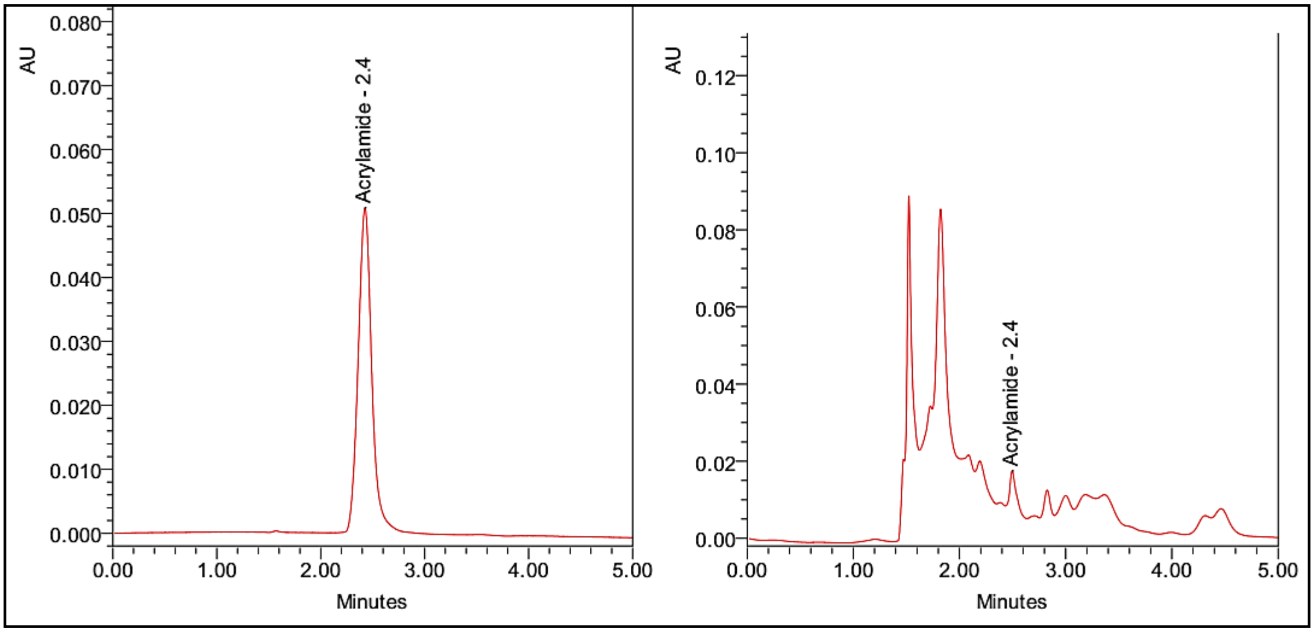

Figure 1. Examples of UPLC chromatogram of acrylamide standard (left) and sample (right) at 238 nm. Acrylamide was extracted in water and 2 µL of extract was separated through XBridge BEH C18 column with 0.1% formic acid mobile phase run at 0.3 mL/min.

Acrylamide content of cereals, legumes and roots and tubers flour after heating at 160 °C for 20 min is presented in Figure 2. It appears that the content of acrylamide differed among the crop groups. Acrylamide in roots and tubers ranged from 3436.13 µg/kg in potato (Kufri Badshah) to 5562.56 µg/kg in potato (Kufri Laukar), intermediate values were found in sweet potato. It was highly significantly greater than the content in cereals ($p<0.0001$) and in legumes ($p<0.0001$). There was no significant difference between cereals and legumes ($p=0.443$). Among the cereals, maize (GYH-0965) showed the highest acrylamide content (2195.31 µg/kg) while the lowest amount (161.12 µg/kg) was obtained in wheat (GAW-112). Regarding the legume crops, soybean (NRC-37) with 717.52 µg/kg and soybean (Veg variety) with 337.08 µg/kg contained the highest and the lowest acrylamide amount, respectively, the values of chickpea being in between.

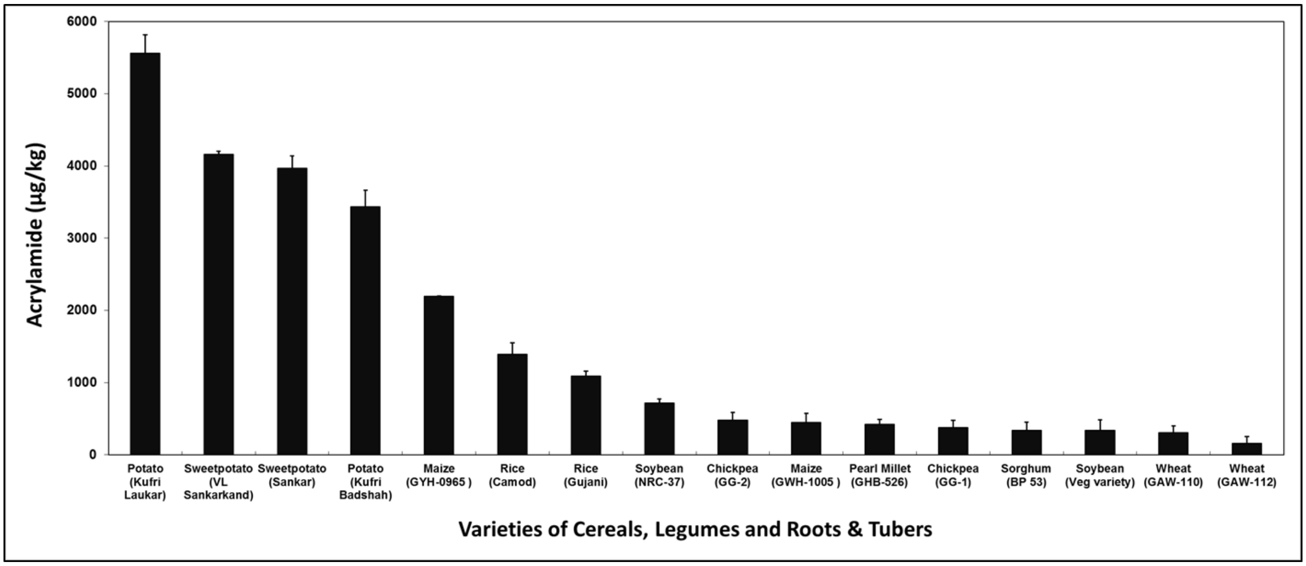

Figure 2: Acrylamide content of cereals, legumes and roots and tubers flour after heating at 160 °C for 20 min. Error bars represent standard error of mean of three replications.

Several authors have reported different levels of acrylamide in products made from cereals, legumes and roots and tubers. Among these crops, potato is one of the most studied food. As compared to our findings, lower acrylamide amounts (235.82 to 245.39 µg/kg) were found in potato chips (Khoshnam et al., 2010), 763 to 358 ng/g were measured in potato crisps in Europe in 2002 and 2011, respectively (Powers et al., 2013). Between 560 and 1226 µg/kg was detected in potato chips in Egypt (Mekawi, 2015). Acrylamide content in commercial potato crisps from Spanish market from 2004 to 2014 ranged between 108 and 2180 µg/kg, with an average value of 630 µg/kg (Mesias and Morales, 2015). However, our results are consistent with the studies of (Elmore et al., 2015) who found 131-5360 µg/kg acrylamide in UK cold stored potatoes, and (Shamla and Nisha, 2014) who reported 82.0 to 4245.5 µg/kg acrylamide, with an average mean of 1456.5 µg/kg in southern India potato chips.

With sweetpotato, acrylamide concentrations of 366 µg/kg were measured in sweetpotato chips in a Thai market and varied from 1443 to 2019 µg/kg depending on the saturation level of frying oil (Lim et al., 2014); in sweetpotato French fries from strips fried at 165 °C for 2, 3, and 5 min it was 124.9, 255.5, and 452.0 ng/g fresh weight, respectively (Truong et al., 2014). These values are smaller than the content measured in the present study.

In cereal-based foods, acrylamide content in heated flour of 3 wheat varieties was between 2600-5200 and 600−900 μg/kg for sulfate-deprived and normal sulfate crops, respectively (Muttucumaru et al., 2006), while Curtis et al. (2016) found values between 134 to 992 μg/kg. Similarly, various wheat cultivars contained from 94 to 3124 μg/kg acrylamide, depending on sulfur application on the crop (Granvogl and Schieberle, 2006). These reports are very high as compared to the contents we obtained in this work. The values of Mekawi (2015) who found that in Egyptian foods, acrylamide content in μg/kg was, 533 to 620 in corn snacks, 233 to 354 in breakfast cereals, 70 to 528 in bread, 170 in biscuits, and 20 to 56 in powdered baby food cereal and milk agree with our findings. Lower values of acrylamide levels of 22 to 84 μg/kg (Mariod et al., 2016) and 51.50 to 59.43 μg/kg (Omar et al., 2015) were found in Sudanese sorghum-based fermented foods. In Indian domestic staples prepared from damaged starch wheat flour, acrylamide ranged from 12.5 to 65.5 μg/kg in *chapatti*, and 25.5 to 130.5 μg/kg in *pooris* (Mulla et al., 2010). Also, 12.7 μg/kg acrylamide was found in roasted Indian *papad* (Shaikh et al., 2009).

Our results on higher acrylamide content in root and tubers crops as compared to cereals agree with those of Boroushaki et al. (2010) who found that in different Iranian brands products acrylamide was higher in potato (244–1688 μg/kg) than in corn (<30–410 μg/kg), and Leung et al. (2003) who reported that highest acrylamide levels were detected in potato crisps (1500–1700 μg/kg), lower levels were found in rye flour-based crisps (440 μg/kg), followed by corn-based (65 to 230 μg/kg) and wheat flour-based crisps (61–200 μg/kg), and then rice flour-based crisps (15–42 μg/kg).

The multiple factors affecting the acrylamide formation in foods before and during processing can explain the different results we obtained. Variety, crop management and storage determine the content of reactants involved in acrylamide formation, with subsequent consequence on acrylamide content in food products. Crop management practices like lack of fungicide treatment during cropping of wheat resulted in increases in acrylamide ranging from 2.7 to 370% (Curtis et al., 2016). Moreover, Granvogl and Schieberle, (2006) and Muttucumaru et al. (2006) demonstrated that acrylamide content was higher in products from sulfate-deprived wheat as compared to wheat grown with normal levels of sulfate fertilization. In potato and sweet potato, storage conditions greatly influence acrylamide concentration. When potatoes were cold-stored, the acrylamide content increased (Elmore et al., 2015) because of increased reducing sugars resulting from starch hydrolysis induced by low temperature in a process known as cold-induced sweetening (Galani et al., 2016). The potatoes and sweetpotatoes we used in this study were coming from cold storage (4 °C) because the research was conducted the month of December, the potato sowing season in Gujarat, it was the only possible option.

During processing, pH, temperature, reaction time, moisture content, reactant concentration, and type of reactants are important determinants of the acrylamide content in the food (Shaikh et al., 2009). In sweetpotato acrylamide content increased with increased frying duration (Truong et al., 2014), or with the degree of unsaturation of frying oil (Lim et al., 2014). Additionally, lower values of acrylamide levels found in Sudanese sorghum-based fermented foods can be due to the added ingredients and long fermentation period, which converted their reducing sugars to ethanol and changed the pH of the foods, thus influencing their proteins and free amino acids (asparagine) content (Mariod et al., 2016; Omar et al., 2015). Likewise, lower acrylamide levels previously obtained in *chapatti*, *pooris* and *papad* can be justified by the added ingredients like alkaline salt (*papad khar*) during the making of the dough, which influence on the acrylamide formation during cooking (Mulla et al., 2010; Shaikh et al., 2009).

Our results suggest that low acrylamide-forming flours like chickpea and soybean, millets and sorghum should be incorporated in widely used flours such as wheat and maize. These flours will contribute not only as a strategy of acrylamide mitigation in baked foods, but might also improve their nutritional value (protein content), which will be a great additional benefit for Indian vegetarians. Our assertion is supported by the findings of (Miśkiewicz et al., 2012) who reported that cookies obtained from the blend of wheat and chickpea flours (1:1, w/w) contained less acrylamide (5.7 μg/kg) than those derived from wheat flour only (41.9 μg/kg). Moreover, addition of chickpea flour to a foodstuff affects the sensory and textural properties, increases its nutritional value and reduces the acrylamide content (Rachwa-Rosiak et al., 2015).

4. Conclusion

This study has demonstrated that when submitted to high temperatures, selected Indian cereals, legumes and roots and tubers generate acrylamide levels above the European Commission indicative value in potato- and cereal-based food products. It appears that potatoes and sweetpotatoes have a higher acrylamide-forming potential than cereals and legumes, suggesting that high sugars content conditioned acrylamide formation more than high proteins / amino acids (asparagine) content. The data obtained here could be helpful to support the adding of more pulses and minor cereals in cereal-based foods for improving the nutritional value and reducing acrylamide formation without the need for additives or potentially costly changes to processes. Further studies on the effects of variety and storage conditions on acrylamide content, the effect of flour blending on sensorial parameters and nutritional quality, will bring more evidences to support these suggestions.

6. Acknowledgement

We are thankful to TWAS Fellowships for Research and Advanced Training who supported the principal investigator, the officials of Anand Agricultural University for their incredible support, and all the staff of Department of Biochemistry, B.A. College of Agriculture, for their valuable help and assistance provided during this work.

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