Correlation between acrylamide contents and antioxidant capacities of spice extracts in a model potato matrix

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Summary

In this study, the effect of methanol-water (4:1, v/v) extracts of spices on the acrylamide formation as well as correlation between acrylamide contents and antioxidant capacities of these extracts was investigated. The extracts of spices (pimento, black pepper, marjoram, oregano) were applied before the heat treatment to a model mixture simulating a fresh potato matrix. Antioxidant capacities of the extracts were determined by four assays, namely, determination of DPPH-scavenging capacity, thiobarbituric acid number, reducing power, and total phenolic compounds. Addition of the extracts resulted in a different reduction of acrylamide contents – up to 75% with pimento extract and 50% with black pepper extract in comparison with the control. Also, eugenol as the main component of pimento essential oil resulted in mitigation of acrylamide content at approx. 50%. The effect of the extracts on the reduction of acrylamide contents was in a correlation with antioxidant capacities of the applied spices, in particular with their DPPH-scavenging capacities (r = –0.996), reducing power (r = –0.978), and contents of oxidative products expressed as thiobarbituric acid number (r = –0.919).

Keywords

acrylamide; antioxidant capacity; natural antioxidant; spice; DPPH; potato

Acrylamide belongs to Group 2A as a probable human carcinogen according to the classification of International Agency for Research on Cancer (IARC) [1]. This compound arises from naturally occurring components in the process of Maillard reactions in thermally treated foods. Acrylamide has been found in a wide variety of foods including those prepared industrially, in catering and at home. It is found in staple foods such as bread, potatoes as well as in some specialty products such as crisps, biscuits, and coffee. Acrylamide is formed via the reaction of non-essential amino acid L-asparagine with reducing saccharides such as fructose and glucose at temperatures higher than 120 °C [2, 3]. The pathway of acrylamide formation has been extensively studied and key parameters of mitigation have been established. These include temperature and time of heat treatment, contents of amino acids and saccharides in raw materials, moisture, pH during the reaction, presence of additives such as inorganic salts, enzymes, amino acids, proteins etc. [4-7]. Based on this knowledge, many methods for acrylamide reduction or elimination have been proposed which are summarized in "Acrylamide Toolbox" published by the Confederation of the Food and Drink Industries of the EU (CIAA) [8]. However, owing to the vast range of different recipes, ingredients and processes used in the manufacture of high-acrylamide products, there is no simple way to reduce acrylamide formation.

Among the number of parameters affecting the level of acrylamide in foods, the effect of antioxidants has not yet been described satisfactorily. Studies conducted so far indicate that the addition of antioxidants and radical-scavenging compounds resulted in an ambiguous effect on acrylamide reduction [9-11]. Rare application of spices showed a 25% reduction of acrylamide in potato slices with the addition of rosemary herb into the olive and corn oil used for frying, but the mechanism of this action was not described [12]. VATTEM and SHETTY [13] used fried potato slices

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previously treated either with phenolic antioxidants from cranberry and oregano or coated with chickpea batter. They showed that formation of acrylamide in fried potato chips is not an oxidative phenomenon and can be reduced by the protective effects of chickpea proteins. Phenolic antioxidants stimulated the formation of acrylamide with oregano or did not change with cranberry extracts. Oregano has also been reported to possess strong antioxidant capacity [14]. FERNANDEZ and co-workers [15] studied the possibility of reducing the formation of acrylamide in potato chips by adding a flavonoid-containing spice. The addition of flavonoids caused a decrease in acrylamide contents by about 50%. It is possible that flavonoids can react with acrylamide precursors both in lipid oxidation and in Maillard reaction routes and thus significantly reduce acrylamide formation [15]. As a pioneer contribution to the reduction of acrylamide in various foods by natural antioxidants, the addition of antioxidants of bamboo leaves (AOB) to potato crisps and French fries can be considered. It has been reported that approximately 75% reduction in acrylamide was achieved by the addition of 0.1% and 0.01% (w/w) AOB, respectively [16]. However, even this case was ambiguous since results of acrylamide contents in both potato crisps and French fries showed opposite concentration-dependent relationships in different ranges of AOB treatments. Such reverse tendencies may relate to the inherent property of antioxidants and the antioxidant capacity of food matrices, which is called "antioxidative paradox" [16]. Recently, the suppressing effect of rosemary extract on the acrylamide contents was confirmed [17] as well as that of green tea flavonoids epicatechin or epigallocatechin gallate in an aqueous food model. A different point of view was applied by SUMMA et al. [18] who found a direct correlation between the concentration of acrylamide and the antioxidant capacity in cookies and demonstrated that combined long baking time, high protein content in the samples and low moisture could simultaneously increase the acrylamide level and the antioxidant capacity in the cookies.

The work reported here focuses on the reduction of acrylamide in a heated potato model matrix by addition of methanol-water extracts of four spices (pimento, black pepper, marjoram, and oregano). Antioxidant capacity of these extracts was determined by four assays (determination of DPPH-scavenging capacity, thiobarbituric acid number, reducing power, and total phenolic compounds). Finally, correlation between the antioxidant activities of spice extracts and their impact on acrylamide reduction was demonstrated.

MATERIAL AND METHODS

List of spices

Pimento *(Pimenta dioica)*, black pepper *(Piper nigrum)*, marjoram (*Origanum majorana)* and oregano *(Origanum vulgare)* were purchased as dried spices from a local market in consumer packings.

List of chemicals

Acrylamide analytical grade (Serva Feinbiochemica, Heidelberg, Germany), starch from potatoes (Fluka Chemie, Buchs, Switzerland), D-glucose monohydrate for biochemistry (Merck, Darmstadt, Germany), L-asparagine monohydrate 99% (Sigma Aldrich Chemie, Buchs, Switzerland), 2,3,3-D_3-labelled acrylamide 98% (Cambridge Isotope Laboratories, Andover, USA), eugenol for synthesis (Merck Schuchardt, Hohenbrunn, Germany), methanol HPLC grade (Sigma Aldrich Chemie), deionized water (HP 340 Deionizer, Purite, Thame, United Kingdom).

Design of the experiment

Spice extracts were prepared in the following way: 2 g of dried ground homogenized spices were extracted with 50 ml of a methanol-water mixture (4:1; v/v) for 1 h at laboratory temperature using a laboratory shaker (Innova 2000, New Brunswick Scientific, Edison, New Jersey, USA) at 3.3 Hz and then all extracts were filtered. Antioxidant capacity of spice extracts was determined before heat treatment by the assay described in detail previously [19] and expressed as antiradical DPPH-scavenging capacity, thiobarbituric acid number, reducing power, and total phenolic compounds. The extracts were added to the model potato matrix, thoroughly stirred, and the solutions in sample tubes sealed with Teflon caps underwent heat intake at 180 °C for 20 min (Thermochem Metal-block, Thermostat Liebisch Labortechnik, Bielefeld, Germany). Model potato matrix consisted of the equimolar mixture of L-asparagine monohydrate and D-glucose monohydrate (0.2 g), potato starch powder (1.0 g), and the extracts themselves (4.0 ml). In case of eugenol testing, solution of eugenol of different concentrations were added to the model matrix. After the heat treatment, the sample was cooled to laboratory temperature and acrylamide was extracted with 2 × 5 ml of methanol in an ultrasonic bath (Teson 1, Tesla, Banská Bystrica, Slovakia) at 60 °C for 20 min and centrifuged at 500 × *g* for 5 min (MLW T52.1, MLW Zentrifugenbau, Engelsdorf, Germany). All samples were prepared in triplicates. Acrylamide was analysed by GC-MS (Agilent 6890/MSD 5973 inert, Agilent Technologies, Santa Clara, California, USA) in NCI mode at conditions established previously [20]. Analyses were done running in duplicates.

RESULTS AND DISCUSSION

Impact of spice extracts on acrylamide contents in a model potato matrix

Analysis of acrylamide contents showed that addition of each spice extract resulted in a significant reduction of the acrylamide contents. The most pronounced decrease (75%) was observed with the application of pimento extract. Oregano and marjoram extracts caused a decrease of approx. 60% and the black pepper extract caused a decrease of approx. 50% (Fig. 1). These results suggest that there is a real possibility of minimizing the acrylamide contents by the addition of natural antioxidants, which was observed in some cases in previous studies [15, 16]. Although the mechanism of the effect of antioxidants on acrylamide level has not been explained yet, one hypothetical mechanism contributing to the acrylamide formation is a pathway via acrolein, which is formed by the degradation of lipids, mainly oxidized fatty acids or glycerol. Acrolein may react by oxidation to generate acrylic acid or by formation of an intermediate acrylic radical [12]. Both intermediates could then induce acrylamide formation in the presence of nitrogen source under favourable reaction conditions. Therefore, the addition of antioxidants seems to block the oxidation of acrolein to a certain extent and further reduce the generation of acrylamide. However, in the absence of lipids, as in the case of this study, the mentioned blocking mechanism cannot be considered. An alternative to the proposed mechanism involving interaction of reactive saccharide fragments with the conjugate system of polyphenols, is a mechanism in which the polyphenols scavenge free radical fragments of saccharides otherwise being intermediates in a reaction sequence leading to acrylamide

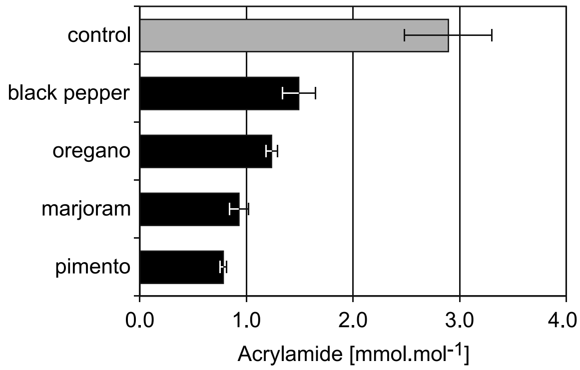

Fig. 1. Effect of the spice extracts on acrylamide contents expressed as mmol acrylamide per mol asparagine in model potato matrix after heat treatment (180 °C, 20 min).

[21]. Different elimination reactions are also possible since the polymerization of acrylamide can be initiated by heat or peroxide radicals [17] or by inorganic salts [6, 7].

Antioxidant capacity of spice extracts

For the clarification of differences in the effect of particular spices on acrylamide contents, antioxidative properties of all extracts before its use in model mixtures were determined. These properties were expressed as DPPH radical-scavenging capacity, thiobarbituric acid number, a measurement of reducing power (ability of the extract to reduce Fe^{3+} to Fe^{2+}), and the determination of total phenolic compounds expressed as the amount of gallic acid. By the above mentioned methods, pimento extract was found to show the highest values in all parameters except for total phenolics contents, while black pepper extract displayed the lowest values in all parameters except for the reducing power (Tab. 1).

Finally, the relationship between particular antioxidant capacity of spice extracts and the acrylamide level was attempted to be defined (Fig. 2).

Tab. 1. Antioxidant activity of the spice extracts before heat treatment.

	Thiobarbituric number*	DPPH [%]	Reducing power**	Total phenolic compounds***
Pimento	0.3068 ± 0.0072	77.11 ± 1.34	1.926 ± 0.021	1931 ± 71
Marjoram	0.2410 ± 0.0038	65.48 ± 0.12	1.797 ± 0.039	2251 ± 21
Oregano	0.2005 ± 0.0040	58.79 ± 1.25	1.536 ± 0.005	1918 ± 46
Black pepper	0.0828 ± 0.0043	46.67 ± 0.74	1.657 ± 0.037	377 ± 9

* - thiobarbituric acid number expressed in absorbance units at 532 nm, ** - reducing power expressed in absorbance units at 700 nm, *** - total phenolic compounds expressed as mg equivalent of gallic acid per 1 l of the extract.

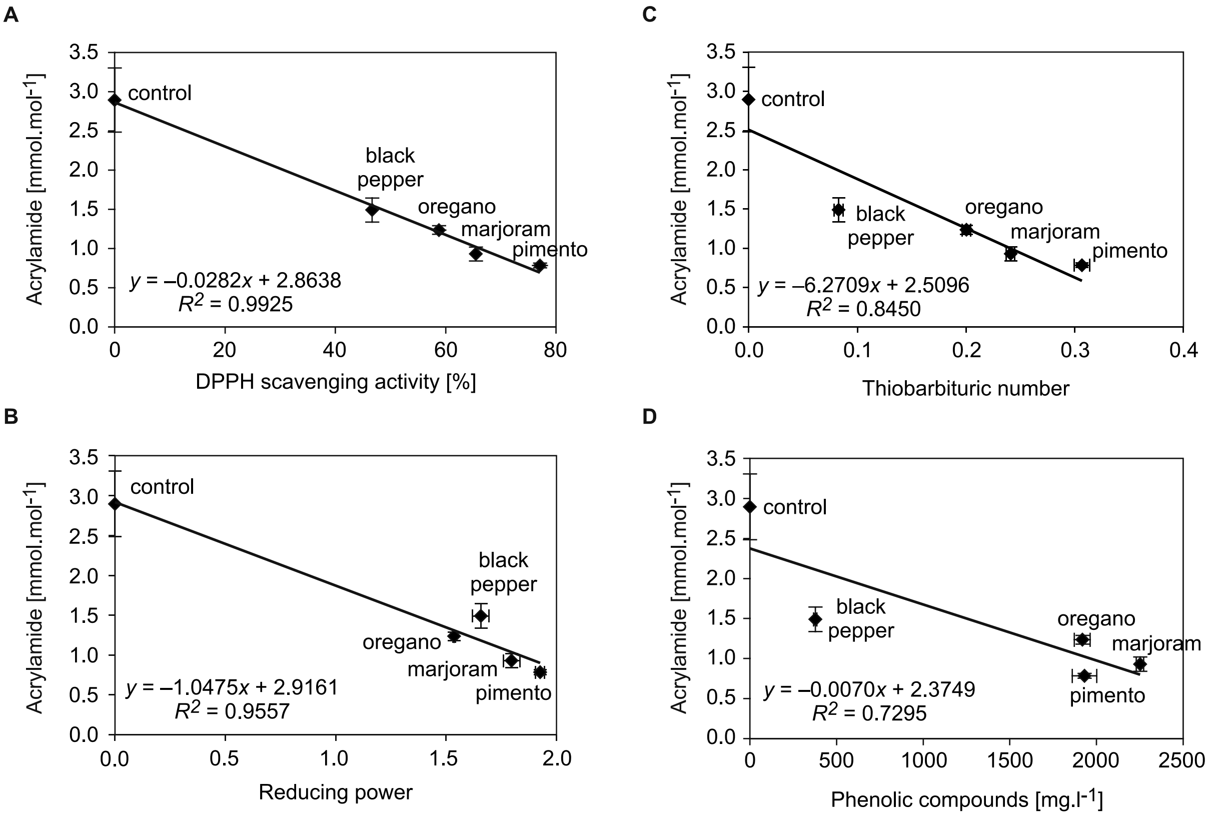

Fig. 2. Correlation between acrylamide contents (as mmol acrylamide per mol asparagine) after heat treatment (180 °C, 20 min) and DPPH-scavenging activity (A), reducing power expressed in absorbance units at 700 nm (B), thiobarbituric acid number expressed in absorbance units at 532 nm (C), and total phenolic compounds expressed as mg equivalent of gallic acid per 1 l of the extracts (D) added to the model potato matrix.

The best correlation was ascertained at DPPH-scavenging capacity, reducing power and thiobarbituric acid number with correlation coefficients $r = -0.996$, -0.978, and -0.919, respectively. Slightly weaker correlation was found with total phenolic compounds ($r = -0.854$). It means that higher antioxidant capacity of the extracts before the heat treatment resulted in a larger suppression of the generated acrylamide. These results point out that spices have a good potential to reduce acrylamide during the heat treatment probably due to their high antioxidative properties. Actually, these most efficient spices include high levels of phenolic compounds such as eugenol in pimento, and carvacrol in marjoram and oregano, which is the reason of their high antioxidant capacities [22]. Eventually, a relationship was found also between concentration of added eugenol itself and the generated acrylamide (Fig. 3), which confirmed the liability of phenolic compounds in the suppression of acrylamide. Interestingly, the effect of flavonoids epicatechin and epigallocatechin gallate on the Maillard reactions, to form adducts with saccharide fragments due to a carbonyl-trapping capacity of the compounds, was reported recently [17]. The presented results suggest that polyphenolic compounds and radical-scavenging antioxidants may inhibit acrylamide formation in a similar way as they inhibit Maillard reactions.

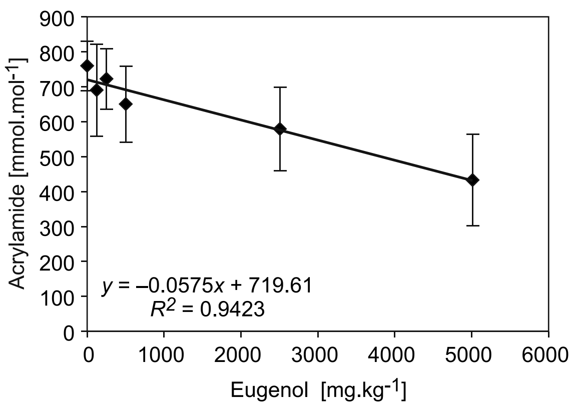

Fig. 3. Correlation between eugenol concentration and acrylamide contents after heat treatment (180 °C, 20 min) in the potato model matrix.

CONCLUSIONS

In this study, a positive effect of the addition of methanol-water extracts of spices on the suppression of acrylamide formation was observed, and a correlation between antioxidant capacity of these extracts and the acrylamide level was observed. The role of eugenol as an important phenolic compound frequently present in spices was also confirmed.

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REFERENCES

1. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Vol. 60. Some Industrial Chemicals. Lyon : International Agency for Research on Cancer, 1994; 560 pp. ISBN 92 832 1260 6.
2. Mottram, D. S. - Wedzicha, B. L. - Dodson, A. T.: Food chemistry: Acrylamide is formed in the Maillard reaction. Nature, *419,* 2002, pp. 448-449.
3. Stadler, R. H. - Blank, I. - Varga, N. - Robert, F. - Hau, J. - Guy, P. A. - Robert, M. C. - Riediker, S.: Food chemistry: Acrylamide from Maillard reaction products. Nature, *419,* 2002, pp. 449-450.
4. Ciesarova, Z.: Minimization of acrylamide content in food. Chemicke Listy, *99,* 2005, pp. 483-491.
5. Ciesarova, Z. - Kiss, E. - Kolek, E.: Study of factors affecting acrylamide levels in model systems. Czech Journal of Food Sciences, *24,* 2006, pp. 133-137.
6. Kolek, E. - Šimko, P. - Šimon, P. - Gatial, A.: Confirmation of polymerization effects of sodium chloride and its additives on acrylamide by infrared spectrometry. Journal of Food and Nutrition Research, *46*, 2007, pp. 39-44.
7. Kolek, E. - Šimon, P. - Šimko, P.: Nonisothermal kinetics of acrylamide elimination and its acceleration by table salt - A model study. Journal of Food Science, *72*, 2007, pp. E341-E344.
8. CIAA Acrylamide Toolbox [on-line]. Brussels : Confederation of the Food and Drink Industries of the European Union, 29 September 2006 [cited 13 September 2007]. <http://www.ciaa.be/documents/brochures/CIAA_Acrylamide_Toolbox_Oct2006.pdf>
9. Rydberg, G. P. - Eriksson, S. - Tareke, E. - Karlsson, P. - Ehrenberg, L. - Tornqvist, M.: Investigations of factors that influence the acrylamide content of heated foodstuffs. Journal of Agricultural and Food Chemistry, *51,* 2003, pp. 7012-7018.
10. Biedermann, M. - Grob, K.: Model studies on acrylamide formation in potato, wheat flour and corn starch; ways to reduce acrylamide contents in bakery ware. Mitteilungen der Lebensmittel Hygiene, *94,* 2003, pp. 406-422.
11. Jung, M. Y. - Choi, D. S. - Ju, W. U.: A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. Journal of Food Science, *68,* 2003, pp. 1287-1290.
12. Becalski, A. - Lau, B. P. Y. - Lewis, D. - Seaman, S. W.: Acrylamide in foods: occurrence, sources, and modeling. Journal of Agricultural and Food Chemistry, *51,* 2003, pp. 802-808.
13. Vattem, D. A. - Shetty, K.: Acrylamide in food: a model for mechanism of formation and its reduction. Innovative Food Science and Emerging Technologies, *4,* 2003, pp. 331-338.
14. Shahidi, F.: Antioxidants in food and food antioxidants. Nahrung, *44,* 2000, pp. 158-163.
15. Fernandez, S. - Kurppa, L. - Hyvoenen, L.: Content of acrylamide decreased in potato chips with addition of a proprietary flavonoid spice mix (Flavomare®) in frying. Innovations in Food Technology, 2003, pp. 24-26.
16. Zhang, Y. - Chen, J. - Zhang, X. - Wu, X. - Zhang, Y.: Addition of antioxidant of bamboo leaves (AOB) effectively reduces acrylamide formation in potato crisps and French fries. Journal of Agricultural and Food Chemistry, *55,* 2007, pp. 523-528.
17. Hedegaard, R. V. - Granby, K. - Frandsen, H. - Thygesen, J. - Skibsted, L. H.: Acrylamide in bread. Effect of prooxidants and antioxidants. European Food Research and Technology. Online first. Published online 26 September 2007. DOI 10.1007/s00217-007-0750-5.
18. Summa, C. - Wenzl, T. - Brohee, M. - De La Calle, B. - Anklam, E.: Investigation of the correlation of the acrylamide content and the antioxidant activity of model cookies. Journal of Agricultural and Food Chemistry, *54,* 2006, pp. 853-859.
19. Horváthová, J. - Suhaj, M. - Šimko, P.: Effect of thermal treatment and storage on antioxidant activity of some spices. Journal of Food and Nutrition Research, *46,* 2007, pp. 20-27.
20. Ciesarova, Z. - Kiss, E. - Boegl, P.: Impact of L-asparaginase on acrylamide content in potato products. Journal of Food and Nutrition Research, *45,* 2006, pp. 141-146.
21. Schieberle, P. - Kohler, P. - Granvogl, M.: New aspects on the formation and analysis of acrylamide. In: Friedman, M. - Mottram, D. (Ed.): Chemistry and safety of acrylamide in food. Vol. 561. New York : Springer, 2005, pp. 205-222.
22. Suhaj, M.: Spice antioxidants isolation and their antiradical activity: a review. Journal of Food Composition and Analysis, *19,* 2006, pp. 531-537.

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