Role of spices on acrylamide formation in buckwheat ginger cakes

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ABSTRACT

Acrylamide is an undesirable carcinogenic component of thermally processed foods arising from reducing saccharides and asparagine at the temperature more than 120 °C. The acrylamide content in food could be influenced by many factors such as temperature, time, water content, pH and matrix constituents. Recently, antioxidants such as phenolic compounds, flavonoids, vitamins and phenolic extracts from various spices have been reported to inhibit acrylamide formation. According to our previous model study, the antioxidant capacity of some spice extracts highly correlated with acrylamide formation in simplified simulated food matrices [1].

The aim of this study was to assess the impact of selected spices on acrylamide occurrence in ginger cakes with the addition of buckwheat flour. Buckwheat flour is frequently used as an additive in functional foods due to its high level of antioxidants [2]. Spices as a part of recipe of ginger cakes (clove, cinnamon, allspice, white pepper, coriander, star anise, anise, fennel, nutmeg, cardamom, and vanilla) were evaluated as promoters or suppressors of acrylamide formation.

Antioxidant capacity of extracts from spices with different polarity was measured by the analysis of free radical scavenging - TEAC (Trolox Equivalent Antioxidant Capacity) using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH\textsuperscript{•}). Final acrylamide content was analyzed by GC/MS using negative chemical ionization.

Keywords: acrylamide; antioxidant capacity; ginger cakes; spices; buckwheat

INTRODUCTION

Acrylamide is a genotoxic, mutagenic, neurotoxic substance. According to the International Agency for Research on Cancer (IARC), acrylamide is classified into Group of substances probably carcinogenic to humans [3, 4]. Acrylamide is formed in foods during heat treatment at temperatures higher than 120 °C and especially during Maillard reaction. In particular, foods rich in asparagine and reducing sugars are the source of acrylamide. Asparagine provides the backbone of the molecule acrylamide [5, 6].

An evaluation of health risks has led to recommendations that should reduce acrylamide levels in food in order to protect health [7]. For application of possibilities of acrylamide elimination is necessary to take into account a wide range of recipes, processing and techniques. The content of acrylamide in foods can be reduced by elimination of its precursors, modification of recipes, processing or final finish. The options are also the use of substances that allow effective elimination of acrylamide in foods such as inorganic salts, enzymes and antioxidants [8].

Some recent studies suggest that antioxidants such as phenolic compounds, flavonoids, vitamins and phenolic extracts from various spices inhibit acrylamide formation [9, 10, 11, 12, 13]. According to our previous model study, the antioxidant capacity of some spice extracts highly correlated with acrylamide formation in simplified simulated food matrices [1]. The aim of this study was to assess the impact of selected spices on acrylamide occurrence in ginger cakes with the addition of buckwheat flour which it is frequently used as an additive in functional foods due to its high level of antioxidants [2].
MATERIALS & METHODS

Chemicals
2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma Chemical Co., St. Louis, MO, USA); acrylamide (Serva, New York, USA); d3–acrylamide (98 %) (Cambridge Isotope Laboratories, Andover, USA); Carrez II. solution (zinc sulfate p.a., 300 g/l), glacial acetic acid 100 %, anhydrous p.a., ethanol (Merck, Darmstadt, Germany); Carrez I. solution (Potassium ferrocyanide p.a., 150 g/l) (Slavus, Bratislava, Slovak Republic); deionized water (device: Purite LTD, Thame Oxon., United Kingdom).

Spice extracts preparation and DPPH• Radical Scavenging Assay
Dried and pulverized spice samples were separately extracted with mixture ethanol/water (1:1, v/v) by sonication. Final extracts were kept at -20ºC prior to further determination of the antioxidant capacity DPPH assay.
The DPPH• radicals scavenging activity assay was based on a modified method of Brand-Williams at al. (1995) [14]. In this assay antioxidants present in the sample reduce the DPPH• radicals, which have an absorption maximum at 515 nm. The DPPH• solution was prepared by dissolving 10 mg DPPH in 25 mL ethanol. The decrease in absorbance of the resulting solution was monitored at 517 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Kyoto, Japan). The Trolox standard solution (concentration 0.1 – 2.5 mM) in ethanol was prepared and assayed under the same conditions. The DPPH• scavenging activity was expressed as Trolox equivalents, on the basis of percentage inhibition of absorbance at 515 nm of standards and samples.

Preparing ginger cakes
Dough for the production of ginger cakes consisted of light buckwheat flour, light rye flour, buckwheat honey, raising agent, sugar, butter and spices (2 % content of flour). Light rye flour and light buckwheat flour was in a ratio 0.7 : 0.3. Spices as a part of recipe of ginger cakes were clove, cinnamon, allspice, white pepper, coriander, star anise, anise, fennel, nutmeg, cardamom (Mäspoma spol. s r.o., Zvolen, Slovak Republic) and vanilla (Johann Kotányi spol. s r.o., Praha, Czech Republic). The dough was cut into 0.5 cm thick discs of 5.5 cm diameter that were baked at 180 °C for 18 min in a DC-32E electric oven (Sveba-Dahlen, Fristad, Sweden). Samples without additional spices and without additional buckwheat flour were used as controls.

Sample preparation and determination of acrylamide by LC/ESI-MS-MS
Extraction of acrylamide from the samples was carried out according to the procedure Ciesarová et al. (2009) [15] with modifications. As an internal standard d3–acrylamide (20 mg/ml) was used. The extraction step (CH3COOH, 0.2 mmol/l) was implemented and the clarifying step with Carrez solutions was used in the procedure. The samples were re-extracted into ethyl acetate to remove negative influence of salts on the chromatographic system. Triplicate samples were run for each set.
The LC/ESI-MS-MS analyses were performed by HPLC system 1200 series (Agilent Technologies, USA) coupled with an Agilent 6410 Triple Quad detector equipped with ESI interface. The analytical separation was performed on a Purospher ® STAR RP-8ec (150 mm x 4.6 mm, 3 µm) using isocratic mixture of 100 ml of acetonitrile and 900 ml of aqueous solution of perfluorooctanoic acid (0.05 mmol/g) at a flow rate 0.5 ml/min and room temperature. All parameters of LC/ESI-MS-MS system were set up identically to Ciesarová et al. (2009) [15].
Calibration samples were prepared from the stock solution of acrylamide (0.02 g/100 ml methanol) in the range 50 to 2000 ng/10 ml with 50 µl of the internal standard (d3–acrylamide, 20 mg/ml).

RESULTS & DISCUSSION
Antioxidant capacity of spices was determined in EtOH/water (1:1 v/v) extracts by DPPH• Radical Scavenging Assay (Figure 1). The results show that spice with the highest antioxidant capacity is cinnamon. Also, star anise has a relatively high antioxidant capacity compared to the rest of the spices. Lower antioxidant capacity was observed in nutmeg and allspice. Other spices have antioxidant capacity of less than 50 µmol Trolox/g.
Furthermore, the influence of spices on the acrylamide content in buckwheat ginger cakes was investigated. Effects of selected spices on the content of acrylamide in ginger cakes were compared with the ginger cake control without the added spice (Figure 2). Significant decrease of acrylamide content was observed in samples of ginger cake with nutmeg (approximately 23%) and fennel addition (approximately 21%).
decrease of acrylamide content by approximately 17% was determined in samples of ginger cakes with anise or clove. Only a very small decrease of acrylamide content was found out in ginger cakes with white pepper, cardamom and vanilla. No change of acrylamide content was observed in samples of ginger cakes with star anise and allspice. On the contrary, the increase of acrylamide content was in samples of ginger cakes with coriander and cinnamon. Acrylamide content increased in ginger cakes with coriander by approximately 18% and in ginger cakes with cinnamon even by 29%.

Content of acrylamide in ginger cakes could be influenced by chemical constituents which are present in spices [16, 17, 18, 19]. The main components of spices are summarized in Table 1. Nevertheless, only weak correlation between results of antioxidant capacity of spices extracts and acrylamide content of ginger cakes with correlation coefficient of 0.68 was observed.

**Table 1. Chemical constituents with antioxidant capacity in spices**

<table>
<thead>
<tr>
<th>Spice</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>anise</td>
<td><em>trans</em>-anethole, estragole, anise ketone, other components in minor concentrations: β-caryophyllene, anisaldehyde, anisic acid, linalool, limonene, α-pinene, acetaldehyde, p-cresol, creosol, hydroquinine, β-farnesene, γ-himachalene ar-curcumene [16]</td>
</tr>
<tr>
<td>star anise</td>
<td><em>trans</em>-anethole, α-pinene, camphene, β-pinene, linalool, safranal, anisaldehyde, acetoisole, estragole, limonene, p-allylanisole, anisyl methyl ketone, 1,8-cineole, p-cymene, terpinen-4-ol, α-terpineol, δ-3-carene, α-phellandrene, β-phellandrene [17, 19]</td>
</tr>
<tr>
<td>white pepper</td>
<td>α-pinene, linalool, β-damasconeone, eugenol, skatole, m-cresol, guaiacol, piperonal [18]</td>
</tr>
<tr>
<td>fennel</td>
<td><em>trans</em>-anethole, fenchone, estragol, limonene, α-pinene, fenchyl alcohol, anisaldehyde, myristicin, dillapiol [19]</td>
</tr>
<tr>
<td>cardamom</td>
<td>1,8-cineole, α-terpinyl acetate, limonene, borneol, methyl eugenol, α-pinene, β-pinene, sabine, α-myrcene, α-phellandrene, γ-terpine, p-cymene, terpinolene, linalool, linalyl acetate, α-terpineol, α-terpinyl acetate, citronellol, nerol, geraniol, <em>trans</em>-nerolidol [16]</td>
</tr>
<tr>
<td>clove</td>
<td>eugenol, eugenol acetate, β-caryophyllene, α-cubebene, α-coapene, isoeugenol, nerolidol, farnesol [16]</td>
</tr>
<tr>
<td>coriander</td>
<td>linalool, α-pinene, γ-terpine, geranyl acetate, camphor, geraniol, β-pinene, camphene, myrcene, limonene, p-cymol, dipentene, α-terpinene, α-decalaldehyde, borneol, acetic acid esters [17]</td>
</tr>
<tr>
<td>nutmeg</td>
<td>sabine, α-pinene, β-pinene, terpinen-4-ol, γ-terpine, myristicin, campfene, myrcene, α-hellandrene, α-terpine, limonene, 1,8-cineole, p-cymene, terpinolene, trans-sabinene hydrate, copaene, linalool, cis-sabinene hydrate, cis-p-meth-2-en-ol, cis-piperitol, safrole, methyl eugenol, eugenol, elemicin [16]</td>
</tr>
<tr>
<td>cinnamon</td>
<td>cinnamaldehyde, eugenol, eugenol acetate, cinnamyl acetate, cinnamyl alcohol, linalool, methyl eugenol, benzaldehyde, cinnamaldehyde, benzyl benzoate, monoterpane, hydrocarbon, caryophyllene, safrole, pinene, phyllandrene, cymene, cineol [16]</td>
</tr>
<tr>
<td>vanilla</td>
<td>vanillin, vanillic acid, p-hydroxybenzaldehyde, p-hydroxybenzoic acid, linoleic acid, acetic acid, hexadecanoic acid, p-hydroxybenzyl alcohol, vanillyl alcohol, acetovanillone, anisic acid [19]</td>
</tr>
</tbody>
</table>

Eugenol is a very potent antioxidant. The mechanism of its strong antioxidant effect is not fully known. Ciesarova et al. (2008) [1] described in their study a strong correlation between the content of eugenol added and the final content of acrylamide.

Eugenol is the main phenolic compound of clove, meanwhile clove decreasing acrylamide content in buckwheat ginger cakes. In a small content eugenol is also present in allspice. Allspice had no effect on the acrylamide content in ginger cakes. This could be explained by the presence of other volatiles in allspice. Methyl eugenol is a major component of allspice is methyl eugenol. The minor amount of eugenol is also found in cinnamon, white pepper and nutmeg. Cinnamon increases the acrylamide content in ginger cakes. A major compound in cinnamon is cinnamaldehyde. In contrast, nutmeg effectively reduced the acrylamide content in ginger cakes from selected spices. The main volatiles of nutmeg are sabinene, pinenes and terpinene. White pepper also contains α-pinene and linalool, in
approximately equal proportions. But white pepper reduced the acrylamide content only small. Linelool is also the dominant component in coriander, which conversely increased the acrylamide content. Acrylamide content in ginger cakes was also effectively reduced in the presence of fennel and anise. A major components of fennel and anise are mainly trans-anethole and also estragole. Trans-anethole is also main volatiles in star anise. Another important volatile of star anise is also α-pinene. But changes acrylamide content was observed in ginger cakes with star anise. Change of the acrylamide content was observed in the ginger cakes with vanilla, whose dominant component is vanillin, and ginger cakes with cardamom, which contains mainly 1,8-cineole, α-terpinyl acetate and limonene. Vanilla and cardamom have minimal effect on the acrylamide content in buckwheat ginger cakes.

CONCLUSION

The aim of this study was to determine the role of spices on acrylamide formation in buckwheat ginger cakes. Acrylamide content was significantly reduced in ginger cakes with nutmeg, fennel, anis and clove. Minimal decrease of acrylamide content was in ginger cakes with vanilla, cardamom and white pepper. In contrast, the highest antioxidant capacity was determined in extracts of cinnamon and star anise. Change of acrylamide content was not observed in ginger cakes with star anise. And acrylamide content in the ginger cakes with cinnamon increased.

The final acrylamide content was probably influenced by chemical constituents with antioxidant capacity in particular kinds of spices. The investigation of the effect of chemical constituents with antioxidant capacity of spices is proposed in further studies using model systems.

ACKNOWLEDGEMENTS

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REFERENCES