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Verification of presence of caprolactam in sprouted achenes of *Fagopyrum esculentum* Moench and its influence on plant phenolic compound content

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ABSTRACT

The presence of caprolactam, a precursor of Nylon-6, among those synthetic polymers which are widely-spread throughout the environment, could be the reason for its being found in plants. The aim of this work was to confirm the previously described presence of caprolactam in dry and sprouted achenes, as well as in achene exudates of common buckwheat (*Fagopyrum esculentum* Moench). When the lyophilized sprouted and dry buckwheat achenes, along with exudates from growth experiments, with caprolactam-free medium were analysed by HPLC, no caprolactam was found. After addition of caprolactam into the growth medium, we confirmed the uptake of caprolactam in the lyophilized sprouted buckwheat achenes. The uptake of caprolactam is also a function of light conditions during the growth experiments. Caprolactam also inhibits the content of phenolic compounds; especially rutin, vitexin, isovitexin, orientin, and homoorientin in buckwheat plants.

1. Introduction

Caprolactam (ε-caprolactam; hexahydro-2-H-azepin-2-one; 2-oxohexamethylenimine; aminocaproic lactam) is a precursor of Nylon 6, is one of several widespread synthetic polymers used in the textile industry (about 53%), such as silon, perlon etc., for the production of industrial yarns (26%) and for engineering plastics/films (17%) e.g. for food and beverage package production (sausage, cooked meals, etc.) (Fisher, 2003).

Caprolactam is only mildly toxic (oral LD_{50} in the rat 1.1 g/kg). According to the International Agency for Research on Cancer, it is placed in Group 4 – lacking carcinogenicity in humans and experimental animals (INCHEM, 2013). The EPA (2013) includes ε-caprolactam among non-carcinogenic compounds. Irritation to mucosa, neurological, gastrointestinal, dermatological, immunological, and cardiovascular effects have only been reported in workers with chronic exposures to caprolactam. The OEHHA (2013) set the concentration of 7 μg/m^3 (1 ppb) of ε-caprolactam as 8 h inhalation REL and the concentration of 2 μg/m^3 (0.5 ppb) of ε-caprolactam as chronic exposure inhalation REL. According to Sheldon (1989) and Vogel (1989), caprolactam has been shown to be mutagenic. This compound also acts as a plant growth inhibitor (Hasegawa, Knegt, & Bruinsma, 1983).

Caprolactam is produced by Beckmann rearrangement of cyclohexanone oxime via acidic catalysis. About 2 billion kg are produced and consumed annually in Asia alone. Total World demand is estimated at 4 billion kg (EPA, 2013).

During the polymerisation reaction of caprolactam to Nylon-6, there is always a small percentage of caprolactam (up to 10%) that remains in the polymer (Sanches, Sendon, Cooper, Franz, & Losada, 2006). Mainly textile fibers and bags made from Nylon-6 are contaminated by this non-polymerized caprolactam (Stewart-Jones & Poppy, 2006). The residual caprolactam is easily released by heating, boiling, and microwaving (Bradley, Speck, Read, & Kastle, 2004), by immersion in a preheated diluted solution of acetic acid, or in the presence of ethanol (Bustos, Sendon, Sanchez, Perfecto, & Cirugeda, 2009). Caprolactam content in foods heated in their nylon packaging usually ranges from 2 to 13 mg/kg (Bradley et al., 2004). European Union regulations establish a specific migration limit of ε-caprolactam at 15 mg/kg for foodstuffs (EC Commission, 2002). The release of caprolactam from nylon films also supports gamma radiation (Young et al., 2006). Nylon cord and other nylon products may contribute contaminants into ground waters (Canova & Muthig, 1991).

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Environmental compartments are also directly contaminated by caprolactam during synthetic polymer production, e.g. the wastewater from caprolactam production by the Juhua Group Company of China contained approximately 5–10% caprolactam (He, Gao, Yang, & Edwards, 2004).

The presence of caprolactam in the environment could be the reason for the presence of this compound in plants. Hasegawa et al. (1983) identified caprolactam in sunflower achenes in 1983. However, Tin et al. (2009a) suggested that caprolactam is a naturally present compound in buckwheat and sunflower achenes.

The aim of this work was to confirm the presence of caprolactam in dry and sprouted achenes; and eventually in achene exudates of common buckwheat during growth experiments with caprolactam-free medium, as well as with medium supplemented with specific concentrations of caprolactam.

2. Experimental

2.1. Materials

Common buckwheat (*F. esculentum* Moench), variety Spacinska, was grown on plots (plot = 10 m^2) in Ceske Budejovice (48° 57′ 42″, 14° 28′ 05″, 380 m a.s.l., sandy-loam soil with pH 5.6, mean temperature, total precipitation) 2009. No chemical treatment was applied during the crop season. Achenes were sampled at harvest time (September). Dry achenes were stored in paper bags in dark dry 12 °C the experiment or analysis. The thirty dry buckwheat achenes were put on a stainless strainer inserted in a crystallizing dish, moistened with 25 ml preboiled ultrapure water (25 °C) and covered with a cover. The samples were cultivated in the light (9 h 30 min light/14 h 30 min dark) or in the dark (0 h light/24 h dark) for 1 and 3 days at temperature 22 ± 0.5 °C. Thirty dry achenes were used as control. All samples have been prepared triplicate. The whole sprouted achenes and control dry achenes were immediately frozen and then lyophilized to dryness. The residues of cultivation solution with achene exudates were transferred from the dish by 4 × 1 ml of 5% methanol in vials and stored in a refrigerator to the HPLC analysis. Two concentrations of caprolactam 1 a 5 g/l of medium were used for the next experiment. Buckwheat achenes were cultivated same way as above with addition of caprolactam in preboiled ultrapure water. Only 3 day sprouted achenes (the sprout with a coat) without roots were in contact with caprolactam, were used for analysis. Achenes cultivated in distilled water were used as the control.

2.2. Extraction of ε-caprolactam from seeds and exudates

The lyophilized sprouted achenes and dry achenes were milled by a mixer mill for 50 s (Retsch®; MM 200); the homogenized samples were then extracted by 5 ml of 80% acetone in an ultrasonic bath for 5 min. The capped tubes were vortexed before centrifugation at 1800*g* for 10 min. The extraction in two similar steps was performed. The upper layer was then filtered through filter paper (Whatman™; 55 mm; GF/A) and transferred to 15 mm vials, measured by HPLC, and then 5 ml of the extract was evaporated to dryness *in vacuo* at 40 °C. These concentrates were dissolved in methanol (3 × 1 ml) and analysed by HPLC.

2.3. Extraction of caprolactam from polymers fibers

The three types of polymer materials (latex gloves, bags, Parafilm), which could be a source of contamination by caprolactam within the experiments were tested. Additionally, two types of materials (polyamide technical sieves, non-woven textiles) were analysed for confirmation of caprolactam in polyamide materials, and for assessment of the trial concentration for the experiment. The samples of polymer materials were extracted by 7 ml of preboiled ultrapure water (25 °C) in an ultrasonic bath for 5 min, stored for 3 days with sunlight for 9 h and 30 min, then analysed by HPLC, before centrifugation at 3000 RPM for 10 min. All samples were analysed twice.

2.4. HPLC analysis

Caprolactam in the extracts was analysed using an HPLC apparatus (Hewlett Packard 1050) with a diode array detector (DAD) and Phenomenex Luna C18(2) (3 μm, 2 × 150 mm) column. The volume of the injected sample was 5 μl. Column temperature was 25 °C. The mobile phase consisted of acetonitrile and *o*-phosphoric acid. Mobile phase A: 5% acetonitrile + 0.1% *o*-phosphoric acid; mobile phase B: 80% acetonitrile + 0.1% *o*-phosphoric acid. Gradient for separation: gradient from 0% to 35% of mobile phase B within 55 min was used; thereafter, from 35% to 100% of B within 10 min. The flow rate was 0.25 ml/min. The spectra were recorded in the range from 190 to 600 nm. Data were quantified at 200 and 220 nm by using linear calibration curves of standards. Detection limits of caprolactam were calculated according to Graham (1993) ($X_D\alpha$ = 1.83 mg/l, $X_D\beta$ = 5.23 mg/l), Miller and Miller (2005) (X_m = 2.78 mg/l) and IUPAC (1980) (LOD = 1.1 mg/l), and the limit of quantification (LOQ = 3.67 mg/l) according to IUPAC (1980).

The content of phenolic compounds (rutin, orientin, homoorientin, vitexin, and isovitexin) with retention times from 21.5 to 28.5 min were determined in the buckwheat extracts as a group, using the above-mentioned chromatographic conditions and using the calibration curve for rutin.

2.5. Chemicals

Standards of caprolactam, rutin, orientin, homoorientin, vitexin, and isovitexin were purchased from Sigma–Aldrich. The methanol and acetone solvents were purchased from Merck (Czech Republic).

2.6. Statistical analysis

Statistical analyses were conducted using Statistica 8.0 (StatSoft) software. Analysis of variance with Tukey HSD test was used to determine significant differences among variants.

3. Results and discussion

All plastic materials that were in the contact with the buckwheat achenes, and could have contaminated the achenes with caprolactam by this means, were analysed (gloves, bags, Parafilm). No caprolactam was detected in them. To the contrary, there was

Table 1
Caprolactam content (mg/kg DM) in the analysed plastic materials (mean ± standard deviation).

Material	Caprolactam
Latex gloves	ND
PARAFILM® M	ND
Bags used for the harvest of buckwheat achenes	ND
Non-woven textiles – RIOBA	1048.3 ± 139.12
Polyamide technical sieves – UHELON 100 T	786.0 ± 11.30
Polyamide technical sieves – UHELON 20 T	5.5 ± 1.09

ND – not detected.