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Short communication

Reduction of acrylamide formation in fried potato chips by *Aureobasidum pullulans* L1 strain

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ABSTRACT

Acrylamide is a potential carcinogenic molecule formed during food heat processing at high temperature (Maillard reaction). In the present study, the ability of the yeast *Aureobasidium pullulans* to deplete the acrylamide precursor free asparagine in fresh potatoes was investigated. *A. pullulans* applied before final frying changes the free amino acid composition of potatoes, decreasing the content of free asparagine by 16% and reducing acrylamide by 83% in fried potatoes. Potato browning was also reduced by yeast treatment without negative drawbacks on chip taste. This yeast, commonly used in fruit postharvest disease control, can therefore also be applied in potato and bakery industries to reduce food acrylamide content.

1. Introduction

Food products such as potato chips, French fries, bread or processed cereals, which are cooked at over 120 °C, accumulate acrylamide 'probably carcinogenic to humans' (Medeiros Vinci et al., 2012). Acrylamide reduction is at present considered one of the major challenges in the food industry. In order to decrease the acrylamide content, the EU commission has recently restricted the acrylamide level suggested for food commercialization (EU, 2017/2158) to 500 and 750 μg/kg (parts per billion) for French fries and potato chips, respectively. As is well known, the Maillard reaction is responsible for the formation of acrylamide in heated food (Halford et al., 2011). In potato, the relationship between precursor concentration and acrylamide formation is complex (Muttucumaru et al., 2017); reducing sugars and free asparagine are reported as the principal determinants of acrylamide formation but other free amino acids and food processing parameters and cooking conditions can also contribute to the reaction and increase the acrylamide level (Muttucumaru et al., 2013, 2014; Shepherd et al., 2010).

In potatoes, asparagine is usually the most abundant free amino acid (Muttucumaru et al., 2017) with a fairly narrow content range among different cultivars (Bethke and Bussan, 2013). Silencing of asparaginase genes in transgenic potatoes allowed a significant reduction in tuber acrylamide-formation, but led to small cracked potato tubers in open field (Chawla et al., 2012).

The total amount of free amino acids can also vary according to field management, water and nutrient availability, nitrogen fertilization and harvest timing (Muttucumaru et al., 2014, 2017). In fact, at present, in order to diminish acrylamide formation in potato chips, agronomic and storage practices are applied to reduce sugars and asparagine in tubers, such as the use of different cultivars and different storage temperatures, together with technical strategies such as dipping in additive solutions, reduction of thermal input or use of the asparaginase enzyme or yeast/lactic bacteria (Anese et al., 2009; Elmore et al., 2015; Halford et al., 2012). Processing methods are also used to moderate acrylamide formation: blanching, pH reduction and the use of the asparaginase enzyme are the methods most used. Blanching is used to leach acrylamide precursors and to obtain a uniform colour after frying by means of a layer of gelatinized starch that limits oil absorption and improves texture (Haase et al., 2003; Samir et al., 2013).

Lowering the pH of the foodstuff would block the nucleophilic addition of asparagine with a carbonyl compound, preventing the formation of the corresponding Schiff base, a key intermediate in the Maillard reaction and formation of acrylamide (Mestdagh et al., 2008). Finally, the use of asparaginase enzyme can be considered, and industrial production of this enzyme is nowadays carried out mostly using

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bacteria or fungi (Aiswarya and Baskar, 2018; Hendriksen et al., 2009). However, the enzymes produced by prokaryotes have some problems of hypersensitivity and immune inactivation (Narta et al., 2007), so eukaryotic microorganisms such as fungi (Krishnakumar and Visvanathan, 2014) and yeasts (Soler et al., 2015) have also been considered for L-asparaginase production (Muso Cachumba et al., 2016).

Aureobasidium pullulans L1 strain is a potential biocontrol agent active against different fruit postharvest pathogens, representing a promising alternative to common fungicides in the control of postharvest diseases (Di Francesco et al., 2016). Recently, we reported that the strain L1 has the ability to successfully assimilate peach juice free amino acids such as asparagine, making this nutrient unavailable to pathogens and allowing them to rapidly proliferate (Di Francesco et al., 2017). In light of these considerations, the present study was undertaken to explore the ability of the L1 strain to diminish the potato acrylamide content. The consumption of free amino acids, particularly asparagine, displayed by L1 yeast treatment was thus evaluated in potato homogenate and in potato slices before frying and acrylamide and quality parameters were then evaluated in the final fried potato chips.

2. Materials and methods

2.1. Aureobasidium pullulans strain L1

Aureobasidium pullulans strain L1, previously identified by Mari et al. (2012) and molecularly characterized by Di Francesco et al. (2018a), was maintained on nutrient yeast dextrose agar (NYDA) consisting of nutrient broth 8 g, yeast extract 5 g, dextrose 10 g and technical agar 15 g (all from Oxoid, Basingstoke, UK) in 1 L of distilled water, and stored at 4 °C until use. Two days before the experiments, the yeast was cultured in 250 mL conical flasks containing 50 mL of nutrient yeast dextrose broth (NYDB: NYDA without agar) on a rotary shaker at 200 rpm for 48 h at 25 °C. Washed cell suspension was prepared by centrifugation of the liquid culture samples at 6000*g* for 10 min at 4 °C, and then washed twice with sterile distilled water to remove the growth medium. The yeast was suspended in sterile distilled water and adjusted to an initial concentration of 1×10^8 cells/mL.

2.2. Potato and analysis of reducing sugars

Potato tubers (*Solanum tuberosum* cv "Primura") were obtained from the CREA experimental field located in Budrio (Bologna, Italy) and were harvested from plants at the senescence growth stage BBCH 97907 (leaves and stem dead, stems bleached and dry). At least 10 U.S. N.1 tubers (5 cm or 112 g minimum) of uniform shape were stored at 7 °C with 90% RH (relative humidity). For the experiment, potatoes were manually washed and peeled. The two ends of the potato were eliminated and slices (2.0 ± 0.3 mm thickness) were cut using an electric slicing machine (model HR7776/90, Philips, Amsterdam, the Netherlands). The reducing sugar content of the potatoes used in the described experiments was determined on freeze-dried samples by extracting with distilled water 1:50 (w/v). The aqueous suspension was first mixed by vortex for 8 min and further subjected to ultrasound-assisted extraction for 20 min. After centrifugation (30,500*g*, 30 min, 10 °C), the supernatant was filtered on paper filter and diluted 1:2 with water. Sugar analysis was performed by HPLC using a Hewlett-Packard Model series 1100 system, coupled with an evaporative light scattering detector (SEDEX LT-ELSD Model 85LT SEDERE), a Shodex Sugar SP-G column and the corresponding guard column. Isocratic elution was performed at 80 °C by water at a flow rate of 0.5 mL/min. 20 µL of sample solution spiked with arabinose as internal standard were injected and sugar quantification was calculated from a previous determined calibration curve using pure standards.

2.3. Qualitative method to detect L1 strain L-asparaginase activity

Asparaginase enzyme activity was tested through a rapid plate assay (Saxena and Sinha, 1981) made by suspending glucose 2 g, L-asparagine (Sigma Aldrich, Saint Louis, USA) 10 g, KH_2PO_4 1.52 g, KCl 0.52 g and traces of $CuNO_3 \cdot 3H_2O$ (trace), $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, agar technical 15 g in 1000 mL of distilled water. The medium was supplemented with 3 mL of a phenol red stock solution (2.5%) dissolved in ethanol (pH 6.2). Plates were inoculated with a yeast plug (5 mm diameter) cut from a 48 h colony and incubated at 25 °C. Control plates consisted of the medium without yeast plug. After 7 days of incubation, the agar shading to pink colour was verified. At least 30 plates of treated or control samples were analyzed. The assay was repeated once.

2.4. Pre-frying treatments

Treatments with L1 strain were performed on raw sliced potatoes in order to evaluate their effect on free asparagine reduction and acrylamide formation after frying. Sliced potatoes, two replicates of 40 slices each (from at least 20 different tubers) homogeneous in size, were immediately rinsed in distilled water for a few seconds to eliminate starch material on the surface and dipped in 250 mL (1:2 w/v) of L1 suspension (1×10^8 cells/mL) for 30 min at 25 °C. As a control, potato slices were rinsed and immersed in distilled water under the same conditions. At the end of the treatments, potatoes were quickly rinsed again in distilled water, dried with absorbent paper, and deep-fried in 2 L of vegetable oil (high oleic sunflower oil) at 180 °C ± 3 °C for 150 s, by using a thermo stable electrical fryer (Deep Fryer, De Longhi, Italy) with a static basket. The frying was repeated once.

After frying, potato slices were drained to remove oil excess and subjected to pH, colour and acrylamide analyses. Before frying, samples of raw potatoes, treated and control, were collected for HPLC free amino acid analysis.

2.5. Preliminary evaluation of yeast free asparagine consumption in potato homogenate and potato slices by free amino acid HPLC analysis

Raw potatoes were homogenized by using a mixer (Imetec Ch4, Italy) and samples of the obtained potato homogenate (1 g) were incubated for 30 min at 25 °C with 1 or 2 mL (1:1 or 1:2 w/v) of L1 suspension (1 × 108 cells/mL) or distilled water (control). Control and yeast-treated suspension after incubation were extracted for free amino acid analysis by first diluting up to 1:5 (p/v) with water and then by 30 s of vortex agitation followed by 10 s of sonication. The final extracts were centrifuged at 16,000*g* for 20 min at 4 °C, micro-filtered (Millex sterile syringe filter 0.22 µm, Merck, Germany) for yeast elimination, and analyzed by HPLC for free amino acid detection as described below. Three independent biological replicates of each treated and control sample were prepared, pulled together and analyzed. The analysis was repeated once.

For free amino acid determination, 5 g of sliced potatoes were extracted in 50 mL of H_2O for 4 min by using a lab blender at room temperature; the suspension was then sonicated for 10 min and finally centrifuged (31,500*g* for 30 min), micro-filtered (0.22 µm) and diluted with 0.1 M hydrochloric acid (HCl) if necessary before HPLC analysis. Free amino acids were determined by reversed phase HPLC analysis and automated pre-column derivatisation with o-phtalaldehyde-3-mercaptopropionic acid (OPA) for primary and 9-fluorenylmethyl-chloroformate (FMOC) for secondary amino acids, according to Agilent procedures (Application note 5990-4547EN 2010), with some modifications. A Hewlett-Packard Model series 1100 system, coupled with a diode array detector (UV wavelength set at 338 and 262 nm), a Gemini C18 column (110 Å–4.6 × 250 mm, 5 µm Phenomenex) and the corresponding guard column were used. Chromatographic conditions are described in Di Francesco et al. (2018b). Quantitative analysis was performed by using norvaline and sarcosine as internal standards for

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