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Effects of variety, agronomic factors, and drying on the amount of free asparagine and crude protein in chicory. Correlation with the acrylamide formation during roasting

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ABSTRACT

Most thermally processed foods are affected by the Maillard reaction which leads to the formation of not only desired flavor and aroma compounds but also neoformed contaminants with probable unwanted biological effects. Among the latter, acrylamide was found to be formed from free asparagine in plant foods such as potatoes, cereals and coffee. Roasted chicory which is used as a common coffee substitute is also known to be high in acrylamide and indicative values have been proposed for this food category by the European Commission. The current study is aimed at understanding the impact of varietal selection and agronomic practices on the concentration of free asparagine, a major acrylamide precursor, in chicory roots, and the effect of these parameters on the amount of acrylamide in roasted chicory. The study showed that the concentration of free asparagine in raw chicory roots was variety- and year-dependent (from 444 to 2786 mg/kg). All five varieties tested showed an increase in free asparagine level in response to increased nitrogen application. The duration of chicory cultivation and the date of harvest had limited effects which need to be further understood. The drying process reduced free asparagine concentration in chicory roots without generating acrylamide. Overall the reduction of free asparagine in the raw chicory roots tested in this study led to a significant decrease in acrylamide formation in the roasted chicory (from 11097 to 2249 μg/kg). This positive correlation does not exclude the possibility that other factors such as those related to roasting conditions could be involved in the acrylamide formation.

1. Introduction

In 2002, the discovery by the Swedish National Food Administration of the presence of acrylamide at various levels in cooked foods led to an important study to investigate the origin of this contaminant and its possible adverse health effects (Swedish National Food Administration, 2002; Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002). Despite an ongoing debate about a possible association between the acrylamide intake from food and the risk of pathologies (Lipworth, Sonderman, Tarone, & McLaughlin, 2012) the safety of the food products in question is still a matter of concern. In the absence of a firm conclusion different committees of experts have recommended a reduction in the dietary exposure to acrylamide, and encouraged the food producers to lower acrylamide levels as low as reasonably achievable (JECFA, 2005). The European Food Safety Authority (EFSA) has also published indicative levels for acrylamide in the most relevant food categories (EFSA, 2012). These levels which are neither regulatory limits nor safety thresholds are values which can act only as a guide to investigating methods for the mitigation of acrylamide levels. They have been revised recently (EFSA, 2012) and, in addition to the 10 food categories already listed, new indicative values have been set for the category called "coffee substitutes" (CSs) (2000 μg/kg for coffee substitutes mainly based on cereals and 4000 μg/kg for other substitutes such as roasted chicory). Based on a European monitoring report the higher middle bound mean

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acrylamide values were found in the CS category and an increase was observed in this food group from 2007 to 2010 (EFSA, 2012). Our recent survey on CSs on sale in France indicates that the acrylamide level varies from 474 to 4937 μg/kg of CS (Loaëc, Jacolot, Helou, Niquet-Léridon, & Tessier, in press). Although the consumption of coffee and CSs can vary widely among European countries it has been estimated that the coffee category alone could contribute up to 40% to the dietary exposure to acrylamide for the adult population in the so-called 'high coffee consuming countries' (EFSA, 2012). More data on food consumption are needed to assess the relative contribution of CSs to the overall dietary intake of acrylamide. Pending the outcome of these data it is essential to set up strategies for reducing acrylamide formation in the roasting of CSs.

Soon after the discovery of acrylamide in cooked foodstuffs it was found that the Maillard reaction between free asparagine (Asn) and reducing sugar was the main chemical pathway for the formation of this neoformed compound (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002). In pursuit of an improvement, aside from the revision of food processing which could limit the formation of acrylamide, the food industry has adopted another strategy, namely a better selection of raw materials which have low contents of the two acrylamide precursors, asparagine and reducing sugars (FDE, 2014). An important study on the strategies for mitigating acrylamide formation in fried potato and cereal based products is now available (Claus, Carle, & Schieber, 2008; Claus et al., 2006; De Wilde et al., 2006; Elmore et al., 2010; Knutsen et al., 2009; Muttucumaru, Powers, Elmore, Mottram, & Halford, 2013; Postles, Powers, Elmore, Mottram, & Halford, 2013; Weber et al., 2008; Wicklund et al., 2006; Williams, 2005). In both food categories pre-harvest parameters such as cultivar selection and agronomy have been studied to control the formation of the precursors and subsequently to limit the formation of acrylamide.

A very limited number of studies have been conducted to improve the CS raw materials despite this major concern (CIAA, 2004). Among the crops used for the production of CSs, chicory root is one of the main plant materials. In chicory roots the concentrations of inulin and sucrose are in large excess (15.3 to 18.1 and 2.57 to 2.98 g/100 g, respectively) (Clarke & Macrae, 1987) compared to the amount of free asparagine (40 to 230 mg/100 g) (FDE, 2014) and vary little among varieties. Therefore we assume that the latter will be a major determinant of the formation of acrylamide in roasted chicory. Here we report on the effect of genotypic and environmental factors on the free asparagine concentration in post-harvest chicory roots and after drying. The present study compares the asparagine accumulation and the crude protein content from five cultivars with varying levels of nitrogen fertilization in the course of two years of cultivation, and different harvesting dates. The relationship between chicory root asparagine and acrylamide formation during roasting is also reported.

2. Materials and methods

2.1. Chemicals

Acrylamide, formic acid and Carrez solutions were provided by Sigma-Aldrich (Saint Quentin Fallavier, France). Internal standard of ($^{13}C_3$)-acrylamide was from CortecNet (Voisins-le-Bretonneux, France) whereas ($^{15}N_2$)-asparagine was from Euriso-top (Saint-Aubin, France). Stock solutions of calibrators were prepared in UHQ water. Acetonitrile HPLC Grade, water HPLC Grade, *n*-hexane and methanol were purchased from Sodipro (Echirolles, France). Perfluoropentanoic acid 97% (NFPA) was provided by VWR International (Fontenay sous bois, France).

2.2. Varieties, growth conditions and processing of the chicory samples

Field trials were conducted by Florimond Desprez (Cappelle-en-Pévèle, France) near Coutiches in 2011 and near Nomain in 2012, both in France. The residual nitrogen at the end of winter (measured by colorimetry assay) were 144.5 and 116 kg/ha respectively. The experimental designs were randomized blocks. Plots were three 45 cm rows wide by 6.75 m long (9.1 m^2). Plot populations were adjusted to 155,000 plants/ha by thinning.

In 2011 a genetic study comparing 24 genotypes (2 replications/entry) was realized under advised solid nitrogen input (90 kg/ha of ammonitrate 27). The response of five commercial varieties (Malachite, Silex, Chrysolite, Calcite and Orchies named A to E in the figures, respectively) grown under 5 levels of nitrogen input (0, 30, 60, 90 and 120 kg/ha) was evaluated in a 4 replicated 2-factor design. A last experiment was conducted to evaluate the effect of sulfur fertilization on the asparagine content. Three levels of liquid sulfur were applied (0, 15 and 30 kg/ha) and repeated 3 times on the variety Orchies.

In 2012 the effect of the harvesting date was studied. The same set of commercial varieties were evaluated in 4 replication designs harvested at 7 different dates, every other week, from early September to early December.

Chicory roots of the entire plots were harvested and washed to determine the agronomic traits. Ten kilograms of roots randomly selected from each plot was delivered at the chicory processing plant (Leroux, Orchies, France) to provide dried and roasted samples and a homogenous fresh sample of the remaining roots was immediately freezed, lyophilized and stored in a desiccator until analysis.

At the chicory processing plant the roots were dried on a Variable Circulation Lab Dryer (VCLD) (CPM Wolverine Proctor LLC, Glasgow, UK). Basically the roots were placed on perforated trays in the VCLD and dried for 125 min at 110–130 °C with an alternative upward and downward air flow. Representative samples were taken for analysis and the remainders of the dried roots were roasted for 20 min at 180 °C with the same VCLD. In these conditions a light roasting was obtained across all roasted samples. It was estimated by reflectance measurement and an arbitrary unit of 180 was the target for all samples. All dried and roasted root samples were finely ground and stored at room temperature protected from light before analysis.

2.3. Methods

2.3.1. Analysis of free asparagine in raw and dried chicory roots

Asparagine content was measured with a validated method used routinely in our laboratory. One hundred milligrams of ground sample was weighed and diluted in 7 mL of pure water. The internal standard ($^{15}N_2$-asparagine) was added to an adequate amount. The sample was vortexed for 10 min for homogenisation and then centrifugated for 10 min at 5000 rpm. Supernatant was collected in a 50 mL centrifuge tube and the previous steps were repeated twice. The pool of supernatant was clarified by addition of 500 μL of each Carrez solution. After centrifugation at 5000 rpm for 10 min, the supernatant was transferred into a 25 mL volumetric flask and diluted to the mark with UHQ water. Samples were filtered through a 0.45 μm filter before LC–MS/MS analysis.

Chromatographic separation was operated on an Accela HPLC system (ThermoFisher Scientific, Courtaboeuf, France). Twenty microliters of sample was injected on a Hypercarb column (100 × 2.1 mm, 5 μm; ThermoFisher Scientific, Courtaboeuf, France) with a Hypercarb guard column (10 × 2.1 mm, 5 μm). The mobile phase was composed of NFPA 20 mM (solvent A) and acetonitrile (solvent B). The flow rate was set at 200 μL/min and column oven temperature was at 25 °C. The percentage of solvent B increased from 0 to 10% at 10 min and reached 50% at 12 min. After 5 min at 50% solvent B, the column was equilibrated with 100% solvent A for 5 min.

Tandem MS analyses were performed on a TSQ Quantum Discovery Max triple stage quadrupole mass spectrometer (ThermoFisher Scientific, Courtaboeuf, France). The LC–MS/MS system was controlled by an Xcalibur™ MS software, version 2.0 (ThermoFisher Scientific, Courtaboeuf, France). Tandem MS analyses were performed in selected reaction monitoring mode (SRM). The specific transitions