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Effects of selected amino acids and water-soluble vitamins on acrylamide formation in a ripe olive model system

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ABSTRACT

A ripe olive model system was used to evaluate the potential inhibiting effects on acrylamide formation from a set of amino acids and water-soluble vitamins. The system was based on ripe olive juice heated at 121 °C for 30 min in a stainless steel tubular reactor. The most potent acrylamide inhibitors were proline and sarcosine, both with inhibition rates of ~75% at a 100 mM level. In addition, glycine, ornithine, taurine, and γ-amino butyric acid were effective (50–65% inhibition) while the rest of the compounds demonstrated weak or non-significant effects. Acrylamide contents in the model system were found to be highly correlated with the corresponding contents in the real product. The kinetic pattern for the formation of acrylamide in the absence and presence of two selected amino acids, added separately or together, was well fitted using a simple logistic function.

1. Introduction

Acrylamide (AA) has been a focus of attention by the scientific community in recent years. This compound has been classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen (IARC, 1994). In early 2002, high AA levels were reported in potato products and cereals, such as fried potatoes, baked potatoes, bread, breakfast cereals, and biscuits (Tareke et al., 2002). Subsequent studies confirmed this finding (FDA, 2006) and also reported high AA levels in other products such as ripe olives (Casado and Montaño, 2008). Previous surveys also showed that AA was not detected in the olives of other processing types (e.g. Spanish-style green olives, directly brined olives, etc.), or in other low acid canned vegetables such as asparagus, green beans, and spinach (FDA, 2006).

Ripe olives (also called "Californian-style table olives") are one of the most important classes of table olive commercialized in the world. In this type of processing the olives are treated with a series of dilute NaOH solutions (lye) to remove their natural bitterness, which is caused by glucoside oleuropein. Between lye treatments, the fruits are aerated. During this operation, the fruits are progressively darkened due to polyphenol oxidation. After the lye treatments and oxidation, the olives are washed several times with water to remove most of the residual lye, reaching a final pH of around 7, and placed in 3–5% brine with ferrous gluconate or ferrous lactate to maintain their color (Sánchez et al., 2006). Finally, the olives are canned in mild salt brine, and heat sterilized (generally at 121–126 °C).

The use of additives for the potential inhibition of AA formation is a strategy that has been investigated in different foods, including ripe olives. Of the additives tested in this product, only sodium bisulfite was able to totally eliminate AA with a negligible repercussion on sensory quality (Casado et al., 2010). However, sodium bisulfite is currently not permitted as an additive in table olives in accordance with European regulation (Commission Regulation (EU) No 1129/2011). Therefore, studies to find other additives to be used by the table olive industry with significant AA-reducing effects and without any negative effects are necessary. Amino acids or vitamins are especially attractive because their addition to food may also improve its nutritional value. However, the results previously found with a few amino acids in ripe olives were generally not satisfactory (Casado et al., 2010). Cysteine at 50 mM was demonstrated to be a strong inhibitor of AA formation in ripe olives, but did generate unpleasant off-flavors. In contrast, arginine and methionine at 50 mM had no negative impact on the sensory quality of ripe olives, but their AA-reducing effects were little or negligible. Protein amino acids such as tryptophan, proline, and histidine have demonstrated significant AA-reducing effects in asparagine-glucose model systems (Koutsidis et al., 2009). It has

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been suggested that these amino acids could form amino acid-AA adducts through Michael addition type reactions thereby reducing the AA content (Friedman and Levin, 2008; Koutsidis et al., 2009). Taurine, a non-protein amino acid, has been reported to be an inhibitor of AA formation in aqueous and potato chip model systems (Shin et al., 2010). Several water-soluble vitamins were reported to significantly inhibit the formation of AA in model systems and in fried potatoes (Zeng et al., 2009; Yuan et al., 2011). Although the action mechanism has not been characterized, the presence of nucleophilic groups, in particular, an amino group in some vitamins, might contribute to their inhibitory activity against AA formation.

The reduction of AA levels by using additives may significantly affect the kinetic behavior of acrylamide formation. The kinetics of AA formation have been previously studied in model systems of asparagine and glucose (Claeys et al., 2005; Knol et al., 2005; Zhang and Zhang, 2008), potato chips (Granda and Moreira, 2005), potato crisps (Knol et al., 2009), and potato powder (Franke et al., 2009). Empirical models have been proposed for modeling the formation of AA in foods and model systems (Corradini and Peleg, 2006), In fact, the "Logistic-Fermi" and "Logistic-Exponential" models have been used to fit the formation of AA in fried potato crisps (Knol et al., 2009) and in an asparagine-glucose model system (Zhang and Zhang, 2008). Using these models that only give a mathematical description of the formation or degradation of AA in food bypasses the problem of considering all the mechanisms that occur during the processing of foods. Besides, AA precursors in olives are still unknown. In the case of ripe olives the AA precursors appear to be different from those in other heated foods. Thus, it is well-demonstrated that the Maillard reaction from amino acids, mainly asparagine, along with reducing sugars, represents the main formation route of AA in potatoes products (Amrein et al., 2004; Taubert et al., 2004), roasted almonds (Amrein et al., 2005) and roasted tea (Mizukami et al., 2006). However, previous studies in olives showed no correlation between their AA content and any of the sugars or amino acids determined before sterilization, which appears to indicate that these compounds are irrelevant as AA precursors in olives (Amrein et al., 2007; Casado and Montaño, 2008). The aim of the present work was twofold. The first aim was to assess the efficiency of selected amino acids and water-soluble vitamins to eliminate or reduce AA in a ripe olive model system, which mimicked the chemical composition and the heat treatment of ripe olives. For comparison purposes, a strong inhibitor of AA formation in ripe olives, namely sodium sulfite, was also tested. To evaluate the reliability of the model system, some results obtained from the model system were compared with those obtained with the real product. The second aim was to investigate the kinetic profile of AA formation in the ripe olive model both in the absence and presence of selected additives.

2. Materials and methods

2.1. Chemicals

Individual amino acids (L-arginine, glycine, L-tryptophan, L-proline, L-histidine, DL-ornithine hydrochloride, taurine, sarcosine, and γ-amino-*n*-butyric acid), vitamins (thiamine hydrochloride, VB1; nicotinic acid, VB3; pyridoxine, VB6; biotin, VB7; and sodium-L-ascorbate, VC), and sodium sulfite were supplied by Sigma–Aldrich (St. Louis, MO). Alliin (*S*-allyl-L-cysteine sulfoxide) was isolated from garlic powder by the method of Mochizuki et al. (1997). The garlic powder was prepared as follows: fresh garlic cloves were frozen in liquid nitrogen, immediately peeled, and lyophilized. Methiin (*S*-methyl-L-cysteine sulfoxide) was synthesized as described by Shen and Parkin (2000) using S-methyl-L-cysteine (Sigma) as the starting material. Deionized water (Milli-Q; Millipore Corp.) was used throughout. All reagents and chemicals used for the AA analysis were as described by Casado and Montaño (2008). All other chemical and solvents were of analytical grade from various suppliers.

2.2. Preparation of ripe olive model systems

Green olives (Hojiblanca cultivar) were stored in 2.4% acetic acid for about three months before processing, and were then subjected to darkening as follows: olives (≈22 kg) were treated in a horizontal stainless steel cylindrical container (0.4 m diameter × 0.7 m length) with a lye solution of 3%, which progressively penetrated the flesh until the alkali reached the pit. Next, the lye was removed and the olives were washed with water until the pH reached 8.0. During lye treatment and washing, air was injected through the bottom of the container. Pressurized air is introduced through 15 spigots (0.5 mm diameter) uniformly located in the bottom of the container so that the oxidation process is uniform. Then, a 0.1% ferrous gluconate solution with pH corrected to 4.5 was added to fix the black color (López-López et al., 2009). One portion of ripe olives (≈10 kg) was then subjected to the following operations: pitting, homogenization using a mixer, filtration through cheesecloth, and centrifugation at 20,000*g* for 20 min. After separation of the oil, the resulting juice was stored at −30 °C until the moment of performing the different tests. The selected compounds were added separately to juice in known concentrations. The addition levels of the amino acids were 50, 100, and 200 mM whereas vitamins were assayed at 25, 50, and 100 mM. In all cases, prior to heating, the pH of the mixture was adjusted to 7. Heat treatment was performed by placing olive juice (1 mL) in a custom-made cylindrical stainless steel tubular reactor (internal diameter 0.7 cm, length 3.0 cm) having one end closed. The reactor was sealed with a stainless steel tube plug, and then heated in an oil bath at 121 ± 1 °C for 30 min. The bath was equipped with a stirrer to ensure a homogeneous temperature in the oil. After heating, the sample was immediately cooled in ice water for 3 min to stop any further reaction and analyzed for its AA content. The temperature profile inside the reactor tube was obtained using a stainless steel temperature probe (Pt100 sensor) coupled to a Crison thermometer model 620/3 (Crison Instruments, Barcelona, Spain). The probe (3 mm diameter) was inserted through the hole of a rubber washer (14 mm external diameter, 1 mm internal diameter, 3 mm thickness) placed under a nut previously connected to the open end of the reactor. The temperature profile is shown in Fig. 1. The control was treated with the same experimental steps but without additives. All heating experiments were performed in triplicate.

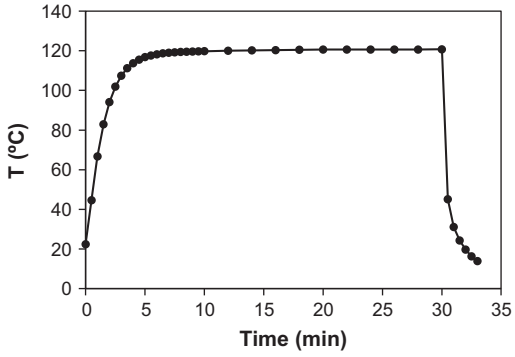

Fig. 1. Temperature–time profile of samples heated in a custom-made cylindrical stainless steel tubular reactor (internal diameter 0.7 cm, length 3.0 cm).