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MONOGRAPH

First report on the presence of Alloxan in Bleached Flour by LC-MS/MS Method

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ABSTRACTS Alloxan is a ketone with a low molecular weight and neutral functional groups. In this work the presence of Alloxan in bread, pastry and cake bleached flour was investigate in order to verify possible risk for consumers related to the use of chemicals for flour bleaching.

KEY WORDS Alloxan; UHPLC MS/MS; toxicology.

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INTRODUCTION

Alloxan is an oxygenated pyrimidine synthesized by uric acid oxidation that can be found in hydrated form. It was found to have toxicological effects on pancreatic b-cells leading to diabetogenic action, therefore (Pincus, 2013) is commonly employee for the development of Type-I Diabetes Mellitus in animal models (Carvalho et al., 2003). Alloxan also demonstrated to have a carcinogenic action in rats and fishes; furthermore, it can induce adenohypophysis cancer in mices (Suganuma et al., 1993). The aim of this work is to prove the presence of Alloxan in bleached flour, given the absence of data in literature on the relative toxicity of this molecule in bakery products. A selective UHPLC MS/MS method using precolumn derivatization was developed for the determination of Alloxan in flours.

MATERIAL AND METHODS

A total of 175 bleached flour samples were collected from manufacturers and local market of Sicily (Southern Italy). All the samples considered in this study were randomly collected by choosing different texture and size of granulation: 62 bread flour (slightly coarse), 55 pastry flour and 58 cake flour (smooth and fine), respectively. 2 g of the homogenized flour samples were weighed in a polypropylene centrifuge tube and spiked with 200 ml of Alloxan working solution at 10 mg mL 1 in Hydrochloridric acid 0.1 M to obtain a concen- tration of 1 mg kg 1. Every sample was mixed and allowed to rest for 15 min. Subsequently, 10 mL of hydrochloride acid 0.1M were added in the tube and then mixed for 1 min. The tube was vigorously centrifuged for 10 min at 3500 rpm; the supernatant was collected in a 50 mL polypropylene centrifuge tube that was filtered with filters of 0.45 mm. A 0.5 mL aliquot was added to 1.5 mL of 0.1% aqueous formic acid solution. This solution was spiked with 2 mL of o-phenylenedi- amine at 1 mg mL 1. After a gentle stirring, an aliquot of 1 mL was transferred into vials and set at the appropriate temperature of 25 C for 24 h, prior to LC-MS/MS analysis.

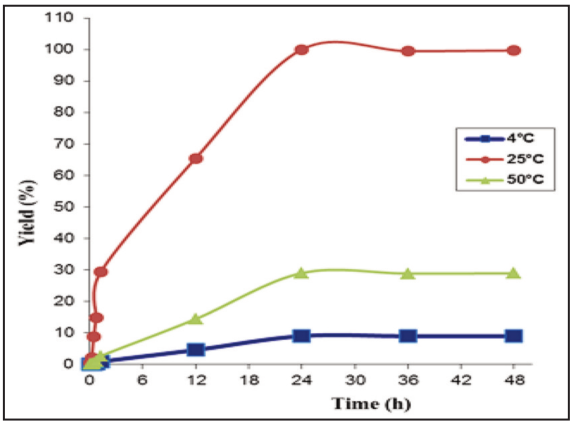

Figure 1. Graphic representation of the yield percentage of the derivatization reaction: kinetic of reaction studied as a function of the time at three different temperatures.

RESULTS AND DISCUSSION

The Alloxan fragmentation is difficult due to unstable transitions, so the ionization efficiency in ESI is low. The pre-column derivatization with o-phenylenediamine, which produces Alloxazine, has provided a better way to detect the analyte in question, due to a greater stability. Alloxazine is the result of reaction between a primary amine and carbonyl groups with formation of a product containing carbon nitrogen double bonds. The reaction is acid catalyzed by hydrochloric acid with elimination of two molecules of water. An alloxan solution was admixed with an excess of derivatizing agent (o-phenylenedi- amine), considering the stoichiometry as known and the yield as unknown. The instrumental results was collected at different time intervals: 5 min, 15 min, 30 min, 45 min, 90 min, 12 h, 24 h, 30 h and 48 h, in order to evaluating the reaction times. It was found an increasing trend of the yield percentage, up to a maximum value in the 24 h, following by a plateau in the next hours. The effect of the temperature on the yield was 4% at 4 C, 100% at 25 C and 29% at 50 C, respectively. Alloxan trace levels were found in 42 (24%) of the analyzed samples, with mean values of 0.95 ± 0.04 mg kg 1 and a range between 0.88 and 1.02 mg kg 1. Therefore, most of the cake flour are bleached in order to improve their baking per- formance and responding to a wide request for production. The use of chemical oxidizing agents and bleaches were developed to produce quick aging of wheat flour (48 h), instead the natural conditions that require several months. Chlorine gas used as bleaching agent may reacts with some proteins in the flour (including the gluten) producing Alloxan as a by-produc. High-gluten flours, such as the cake flour.

CONCLUSIONS

According to our knowledge (Vadlamudi et al., 1982; Galland & Senger, 1988; de Oliveira et al., 2005), this work represents the first report on the presence of Alloxan in cake bleached fl our, suggesting a potential risk for consumers due to the application of chlorine gas and other chemicals for baking cakes. The reported UHPLC e MS/MS method was found very sensitive and accurate for the determina tion of Alloxan in wheat fl our starting from 0.85 mg kg. The results obtained show that the fl our bleached with chlorine dioxide and chlorine gas may contain Alloxan as a minor product of a series of oxidation reactions. As a pilot stu dy, further studies are needed with a larger number of fl our samples, in order to understand the real risk for the consumers.

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