Macedonian - Chinese Scientific and Technological Cooperation

New Project Proposal for 2018-2019

Title of project proposal: **"Dynamic monitoring of ethyl carbamate and its precursors synthesis during wine production and developing a control strategy"**	**Project number**

Macedonian organization:

Faculty of Agriculture, University "Goce Delčev" - Štip

Chinese organization:

China Agricultural University, College of Food Science and Nutritional Engineering

Project period: 01.01.2018 – 31.12.2019

Expected period of stay in China for the Macedonian Researchers:
4 persons, 10 days
From 15.05.2018 to 24.05.2018

Expected period of stay in Macedonia for the Chinese Researchers:
__4_ persons, _10__ days
From 15.07.2018 to 24.07.2018

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Project description:

INTRODUCTION

Ethyl carbamate (EC) ($NH_2COOCH_2CH_3$), also known as urethane, is the ester of carbamic acid, which presents potential toxicity for human health. This compound was re-classified in 2007 as probable human carcinogen compound (Group 2A) by the International Agency for Research on Cancer (IARC) (IARC, 2010). EC occurs in many fermented foods (e.g. yoghurt, bread) and alcoholic beverages (e.g. wine, beer, whiskey) (Weber & Sharypov, 2009). It is obtained from the reaction between ethanol and nitrogenous compounds like urea, citruline, hydrocyanic acid and cyanogenic glycosides and other N-carbamyl compounds.

One of the most common pathways proposed to explain the development of EC in acid media is the reaction of urea with ethanol (Weber & Sharypov, 2009). It has been demonstrated that EC formation from urea (which results from the degradation of arginine by yeasts) is greatly enhanced by the temperature increase. Urea and citruline can be detected in wine and are both derived from the arginine metabolism during the fermentative processes. Another precursor of EC is hydrogen cyanide derived from cyanogenic glycosides, produced by several plant species, including Vitis vinifera L. The formation of EC via cyanide is mostly originated through procedures that include thermal treatments like distillation or baking. Moreover, arginine content in grape must influence the ethyl carbamate content in wine, increasing the EC concentration when higher amounts of arginine in the juice are available. In addition, the wine yeasts also play an important role in influencing the ethyl carbamate content in wine. During the fermentation, urea is produced as a by-product of arginine metabolism. The yeast strains differ in their ability to produce urea, excrete urea into must and even reabsorb the urea from the must back into the cells.

Maximum level of EC was firstly established in Canada in 1985, for alcoholic beverages: 30 μg/L for table wine, 100 μg/L for fortified wines, 150 μg/L for distilled spirits, and 400 μg/L for fruit brandies and liquors. In Europe, only Czech Republic follows the Canadian legislation for fortified wine (EFSA, 2007).

Several extraction and chromatographic techniques have been used for EC analysis in wine, such as continuous liquid–liquid extraction, solid phase extraction, derivatization with 9-xanthydrol, combined with one-dimensional gas chromatography coupled with mass spectrometry detection (GC–MS) or high performance liquid chromatography (HPLC) with fluorescence detection (Canas et al. 1994: Herbert et al. 2002). The reference method set by the International Organization of Vine and Wine (OIV) uses solid phase extraction (SPE) preceding GC–MS quantification (OIV).

In addition, determination of the elemental composition of wines is very important from toxicological point of view, since it could contain harmful elements, such as Pb, As and Cd. From nutritional point of view, wine contains essential elements for the human organism, such as Ca, Cr, Co, K, Se and Zn (Ivanova-Petropulos et al. 2013). Therefore, their concentration has to be controlled. For their accurate determination, sensitive techniques are required, such as flame atomic absorption spectrometry, atomic fluorescence spectrometry, inductively coupled plasma mass spectrometry, inductively coupled plasma optical emission spectrometry, electroanalysis or neutron activation analysis.

Wine production has a long tradition in Republic of Macedonia since the ancient Roman times and now it is the second most important export agro-food product after the tobacco, thus representing an economic opportunities for the new generation. Chemical composition of Macedonian wines has already been studied (Ivanova et al. 2013; Ivanova Petropulos et al. 2014; Ivanova-Petropulos et al. 2015, 2016; Tašev et al. 2016), but to the best of our knowledge, there has been no report on the identification and quantification of EC and its precursors in the wines. Moreover, in Macedonia there is no a legislation that controls the concentration of this toxic compound. Therefore, its concentration has to be monitored during wine production. Thus, the aim of this project is to develop an efficient, fast simple and sensitive methodology to study the EC and its precursor's concentration in wine using SPE combined with GC-MS method. In addition, the precursor synthesis during the wine production (alcohol fermentation, malolactic fermentation, aging stage) will be monitored and a dynamic change model will be established. Moreover, toxic elements, such as Pb, As, Cd, Cu will be determined in wines applying ICP-MS technique.

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