Food Chemistry 140 (2013) 245–254

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Different influences of β-glucosidases on volatile compounds and anthocyanins of Cabernet Gernischt and possible reason

Yuxia Wang [a], Chao Zhang [b], Jiming Li [c], Yan Xu [d,*]

[a] Key Laboratory of Fermentation Resources and Application of Institutes of Higher Learning in Sichuan, School of Life Science and Food Engineering, Institute for Bioengeering, Yibin University, 8 Jiusheng Road, Yibin, Sichuan 644000, China
[b] Key Laboratory of Fermentation Resources and Application of Institutes of Higher Learning in Sichuan, School of Life Science and Food Engineering, Yibin University, 8 Jiusheng Road, Yibin, Sichuan 644000, China
[c] Centre of Science and Technology, Changyu Group Company Ltd., Yantai 264001, China
[d] State Key Laboratory of Food Science and Technology, Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, 1800 Lihu Ave., Wuxi, Jiangsu 214122, China

ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form 4 February 2013
Accepted 11 February 2013
Available online 22 February 2013

Keywords:
β-Glucosidase
Wine aroma
Anthocyanidin
Secondary structure

ABSTRACT

The effects of three β-glucosidases (BG1, BG2 from *Trichosporon asahii*, AS from *Aspergillus Niger*) on the aroma profiles of Cabernet Gernischt (CG) were investigated, coupled with an exploration of the possible reasons for the different performances of β-glucosidases under the two different conditions (hydrolysis of grape glycoside extract and wine-making). The analysis of headspace solid-phase micro-extraction and gas chromatography–mass spectrometry revealed that volatile flavour compounds in the β-glucosidase-treated samples were significantly increased. Specially, the wines treated with β-glucosidase BG1 occupied the highest concentrations of 19 out of 23 volatile compounds that exhibited significant differences. The investigation of the effects of pH or glucose on β-glucosidases showed that low pH is the main factor that exerts a more critical and irreversible influence on the activities and structures of β-glucosidase proteins. The stronger resistances to pH and glucose provided β-glucosidase BG1 a better ability in hydrolysing aromatic precursors than other enzymes under winemaking conditions. With the HPLC analysis, eight anthocyanins were identified from CG wine. Among the three β-glucosidases, BG1 showed the lowest influence on the main anthocyanin glycosides. These results suggested that the β-glucosidase BG1 may have some potential values to complement wine quality during the winemaking process.

1. Introduction

Among the various factors that contribute to the enjoyment of wine, its flavour is possibly the most important one. The typical flavour of wines is mainly related to volatile compounds that derived from the grapes where they are in free volatile form or in bound form (Loscos, Hernández-Orte, Cacho, & Ferreira, 2010). Apart from free flavour components, a significant part of important flavour compounds is accumulated as non-volatile and flavourless glyco-conjugates, which are known as glycosidic precursors (Pogorzelski & Wilkowska, 2007).

The odourless non-volatile glycosides can be hydrolysed by heat-acid or enzymatic hydrolysis. Fast heat-acid hydrolysis may cause rearrangements in the aglycone structure together with the formation of undesirable flavours (Hernandez-Orte et al., 2009). However, enzymatic hydrolysis just cleaves the glycosidic linkage without altering the aglycone, which makes this procedure more favourable (McMahon, Zoechlein, Fugelsang, & Jasinski, 1999). Therefore, much attention has been attracted in flavour enhancement of juices or wines through the hydrolysis of the glycoside aroma precursors using glycosidic enzymes, particularly β-D-glucosidase. β-D-Glucosidase is the most important flavour enzyme in the enzymatic hydrolysis of non-volatile glycosidic precursors present in fruit juices, musts, and wines (Dignum, Kerler, & Verpoorte, 2001).

β-D-Glucosidases (β-D-glucopyranoside glucohydrolases, E.C. 3.2.1.21) are enzymes that hydrolyse glycosidic bonds to release nonreducing terminal glucosyl residues from glycosides and oligosaccharides (Ketudat Cairns & Esen, 2010). These enzymes are found universally in all domains of living organisms, including archaea, eubacteria, and eukaryotes (Ketudat Cairns & Esen, 2010). However, typical winemaking conditions such as high sugars, ethanol concentrations, low pH, and high concentrations of polyphenols, may inhibit the activity of grape and microbial β-glucosidases (Ugliano, 2009). For this reason, the hydrolysis of glycosylated precursors of volatile compounds by β-glucosidases from grape and *Saccharomyces* strains is often incomplete during the winemaking process (Barbagallo, Spagna, Palmeri, Restuccia,

* Corresponding author. Tel.: +86 0510 85964112.
E-mail address: yxu@jiangnan.edu.cn (Y. Xu).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.02.044

& Giudici, 2004; Rodríguez, Lopes, Valles, Giraudo, & Caballero, 2007). Furthermore, commercial enzyme preparations of fungal origin, mainly *Aspergillus* (Spagna, Barbagallo, Martino, & Pifferi, 2000), are a mixture of non-specific multiple forms other enzymes. These non-specific enzymes may cause collateral hydrolysis reactions detrimental to the final wine (Villena, Iranzo, & Perez, 2007), or the formation of undesirable flavours (Hernandez-Orte et al., 2009). Therefore, in order to modify or enhance wine aroma, especially for some typically regional wines, it is necessary to use β-glucosidases from indigenous yeasts. Because these strains have adapted to the ecological environment and formed part of the wine ecosystem, thus, β-glucosidases from indigenous yeasts could serve as better options to embody the regional characters of wines (Villena et al., 2007). The belief that native fermentations enhance aroma may be partly supported by the higher hydrolytic enzyme production in these genera (Rodríguez et al., 2007). Therefore, the use of β-glucosidases from indigenous microbial strains has a distinct advantage to improve wine aroma reflecting the distinguishing feature of district.

Wine flavour modification involves not only β-glucosidase but also glycoside precursors. Due to the limited effect of glycosidases from grape and *Saccharomyces cerevisiae* in winemaking, a large amount of glycosidic precursors is still present in young wines (González-Pombo, Fariña, Carrau, Batista-Viera, & Brena, 2011). So, during winemaking process, the addition of β-glucosidase with special properties, which derived from enological environment, has been considered as an industrial interest to ensure the occurrence of efficient enzymatic hydrolysis of aromatic precursors.

Besides aroma profiles, colour is one of the main distinguishing characteristics of red wines. Anthocyanins are responsible for the colour of red wines. These compounds are natural antioxidants, and possessing neuro-protective and potent cancer-preventive properties, which are considered beneficial and proved (Yoo, Al-Farsi, Lee, Yoon, & Lee, 2010). Anthocyanins are formed by the addition of a mono or disaccharide to anthocyanidins. Due to the breaking of the glucosidic bonds, anthocyanidin can degrade into colourless compounds which may result in off-colour in red wines. Since the main anthocyanins are monoglucosides, many β-glucosidases can induce a loss of colour of red grape juices or red wines (Barbagallo, Palmeri, Fabiano, Rapisarda, & Spagna, 2007). Therefore, more and more attentions have been focused on the case of glycosidases without or with a very low anthocyanin-β-glucosidase activity in the wine industry.

Cabernet Gernischt (*Vitis vinifera* L. cv.) is the most widely grown red grape variety in Jiaodong Peninsula, Shandong, which is one of the main raw grape and wine production bases in China. The quality of CG wines can be improved by exploiting the varietal characteristics, which may be associated with bound volatile compounds. However, the research about the bound aroma of this grape variety has rarely been reported.

The present work reports the effects of β-glucosidases from non-*Saccharomyces* yeast (*Trichosporon asahii*) on the volatile compounds of both glycoside extraction from CG grape and CG wine, in comparison with a commercial enzyme under the same conditions. *T. asahii* was confirmed as a good source of β-glucosidase and produced two β-glucosidases (BG1 and BG2) in our previous study (Wang, Kang, Xu, & Li, 2011; Wang, Li, & Xu, 2011). Although the bound aromatic profiles treated with the partial purified β-glucosidase (a mixture of BG1 and BG2) or purified BG1 were studied, this is the first study of the effects of β-glucosidase BG1 and BG2 on the volatile compounds of CG variety (Wang, Kang et al., 2011; Wang, Xu, & Li, 2012). Furthermore, the possible explanation for the different performances of β-glucosidases was investigated under the conditions of extract-hydrolysing and wine-making by assaying enzymatic activities and second structures at different pH or under different glucose concentrations. The influences of β-glucosidases on the anthocyanins in CG wine were also investigated under winemaking conditions. This is also the first utilisation of β-glucosidase from *T. asahii* during winemaking process.

2. Materials and methods

2.1. Materials and reagents

CG grape (supplied by Changyu Group Company Ltd.) were used. A pectinase preparation AR2000 (DMS Food Specialties B.V., Delft, The Netherlands), containing β-glucosidase from *Aspergillus niger*, was used in the current study. All volatile standards were purchased from Aldrich (Milwaukee, Wis., USA) and Fluka (Buchs, Switzerland). Anthocyanins were purchased from Polyphenols Laboratories (Sandnes, Norway). All other chemicals used were of analytical or high performance liquid chromatography (HPLC) grade.

2.2. Extraction of the glycosides

Five-hundred grams CG grape were used for bound precursors extraction. After destemmed and homogenised, grape must was treated in an ultrasound bath (Elma E60H elmasonic 500 W, Germany) for 20 min at 4 °C. Then, grape juice was separated from skin by centrifugation (5000*g*, 15 min, 4 °C). Extraction of the bound volatile compounds was carried out using 500 mg Sep-pak C_{18} cartridges (Waters, Milford, MA, USA) according to the method proposed by Arévalo Villena and modified by Wang (Arévalo Villena, Díez Pérez, Úbeda, Navascués, & Briones, 2006; Wang, Kang et al., 2011).

2.3. Enzymatic hydrolysis of bound aroma compounds and winemaking

Two β-glucosidases (BG1 and BG2) from *T. asahii* were purified before used (Wang, Li et al., 2011). The *A. niger* β-glucosidase (AS) from pectinase AR2000 was also isolated according to the method of Todaro (Todaro, Palmeri, Barbagallo, Pifferi, & Spagna, 2008). In order to ensure the same enzymatic activity, 1 U/100 mL of each β-glucosidase was added to each glass container, which was used to contain CG grape must or glycosidic mixtures. A control experiment (CK) without any enzyme was performed.

Prior to enzymatic hydrolysis, the glycoside extract was recovered to the original concentration with 0.1 M citrate phosphate buffer (pH 5.0). The same enzymatic activity was used in the hydrolysis of grape glycoside extract. Then, the glycosidic mixtures were incubated at 40 °C for 72 h, and cooled to room temperature before analysis of volatile compounds. Treatments were carried out in triplicate. The condition of enzymatic hydrolysis of glycoside extract was named as condition one.

Before wine-making, CG grapes were hand-harvested at 20 °Brix. The grapes were destemmed and crushed and then transferred to glass containers. Five litres of each treatment were produced in three replications. Sixty mg/L of SO_2 were added to the CG grape must, and then the following were added: 5 g/h L of dried active yeast (*S. cerevisiae* BM45, Lallemand Company, Toulouse, France), 20 g/h L maceration enzyme preparation (Lallzyme EX, Lallemend Company, Toulouse, France) and different β-glucosidases. Then, fermentation, maceration, and bottling were carried out according to the method of Xi, Zhang, Cheng, & Li (2010). The condition of wine-making was defined as condition two.

2.4. HS-SPME and GC–MS conditions

A 50/30 μm DVB/CAR/PDMS fibre (Supelco, Inc., Bellefonte, PA, USA) was used for aroma extraction. A 8-mL aliquot of the sample,