

Minireview

β -Glucosidase in cellular and acellular form for winemaking application

Rosa Palmeri, Giovanni Spagna*

Department of Horticulture, Floriculture, Arboriculture and Agroindustrial Technology (DOFATA), University of Catania,
Via S. Sofia 98, 95123 Catania, Italy

Abstract

β -Glucosidase (BG, EC 3.2.1.21) is one of the most interesting glycosidases, especially for hydrolysis of glycoconjugated precursors, in musts and wines, and the release of active aromatic compounds. We summarize the research on the use of commercial preparations selected yeasts and malolactic bacteria, as source of glycosidase activities, β G in particular. We illustrate our results on a new simple purification process and the physicochemical characteristics of the β -D-glucosidase (β G) from acellular and cellular origin. The β G from a commercial enzyme preparation chosen for its high activity and three yeast strains with the highest β G activity have been selected and characterized: Y8 (*Hanseniaspora uvarum*); AL 41 (*Saccharomyces cerevisiae*); AL 112 (*Pichia anomala*). An *Oenococcus oeni* strains (DSTVR 10) has been chosen among 10, it was isolated from wines of the Valpolicella.

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1. Introduction

Winemaking is a biotechnological process in which the use of exogenous enzyme preparations helps to overcome the problem of the insufficient activity of endogenous activity in the grapes. Some biotechnological techniques have been of fundamental importance in oenology, among these technological innovations, enzymatic treatments by commercial preparation in free or immobilized form, selected yeasts, improvement of microbial starters and enzyme immobilization.

But the use of the enzymes in the wine industry remains limited for several reasons that can be summarized as follows: traditionalism of winemakers, influence on enzymatic activities related to physicochemical characteristics of musts and wines (pH, temperature, ethanol, sugars, polyphenols, etc.) on enzymatic activities [1].

Several researchers have shown that in contrast to *Saccharomyces* species, the non-*Saccharomyces* yeasts produce endogenous and exogenous enzymatic activities (esterases, glycosidases, oxidases, etc.) into microbial cells able to develop odourous compounds, to interact also with grape precursor compounds to produce aroma active compounds, and play an important role in varietal aroma [2]. The activity of these enzymes, in

must and wine has not been studied extensively and therefore the effect on wine flavour is still unclear [3,4]. The contribution of yeast to the flavour of wine has always been controversial, even if some studies investigating starter cultures and indigenous yeasts have discovered that there are significant differences in the composition of the resultant wines.

The aroma of grapes includes volatile free odours substances, especially terpenes (linalool, geraniol, nerol, citronellol, α -terpineol linalool oxide, etc.), which play a fundamental role in giving character to certain cultivars. In addition, grapes also include compounds called “aroma precursors”, of which the more active are glycosides mainly of linalool, nerol and geraniol. From a technological point of view, the existence of such bound forms is interesting because these glycosides can give flavouring aglycons when hydrolytic reactions take place. Aromatic aglycon may be released through the sequential action of glycosidases (Fig. 1). Several studies have shown that enzymatic hydrolysis occurs in two stages. In the first stage, the intersugar bond is cleaved by rabinofuranosidase (Ara, EC 3.2.1.55), α -L-rhamnopyranosidase (Rha, EC 2.2.1.40) or β -D-apiofuranosidase (Api) and the corresponding monoterpenyl β -D-glucosides are released. In the second stage, the liberation of monoterpenols takes place after the action of a β G on the previous monoterpenyl β -D-glucosides. The selectivity of β G is prevalently for the sugar and it is related to the presence of glucose, and was found to be dependent on the structure of the aglycon. In fact, the β G is more selective towards tertiary alco-

* Corresponding author.

E-mail address: gspagna@unict.it (G. Spagna).

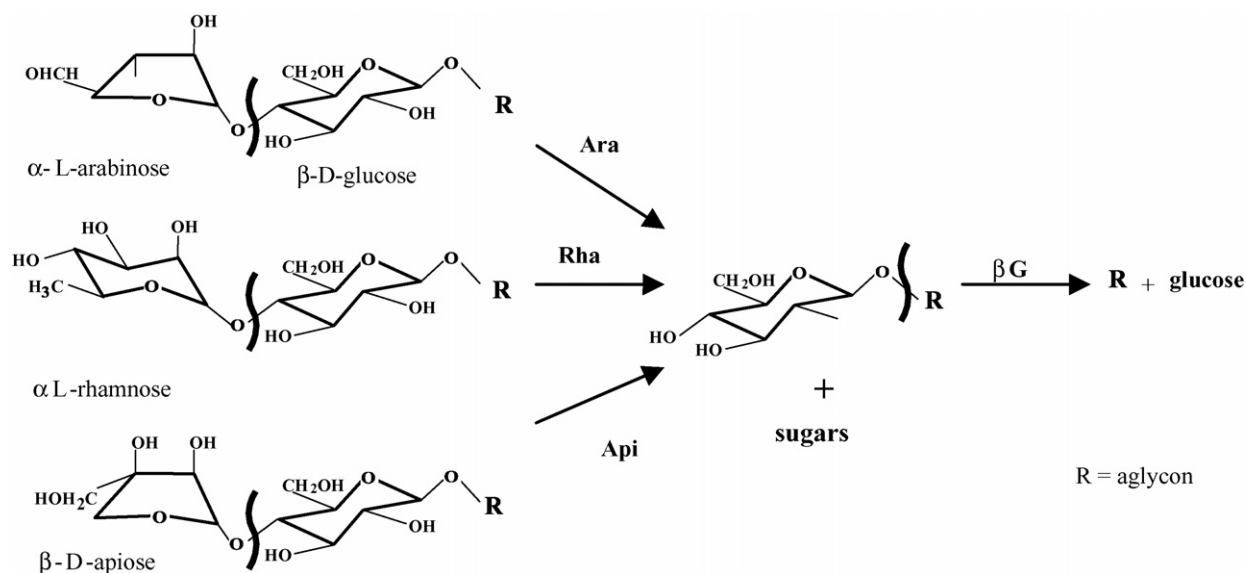


Fig. 1. Sequential mechanism of glycosidases [1]. Ara: α -L-furanosidase; Rha: α -L-pyranosidase; Api: β -D-apiofuranosidase; β G: β -D-glucopyranosidase. R = monoterpenes, sesquiterpenes, norisoprenoids, benzene derivatives, aliphatic alcohols.

hols than to primary alcohols (steric hindrance), and to the origin of the enzyme [1].

The aglycon moieties of glycosides include monoterpenes, and also C_{13} -norisoprenoids, benzene derivatives, and aliphatic alcohols, the sugar moiety is represented by glucose or disaccharides. This glycosidically bound aroma fraction, upon hydrolysis, can give rise to odourous volatiles or to volatiles able to generate odour-active compounds during wine storage by acid–base mechanism. The volatile compounds from glycosides can be released by acid or enzyme hydrolyses. The acid hydrolysis occurs quite slowly in winemaking conditions. Due to the limited effect of glycosidases from grape and *Saccharomyces cerevisiae* in winemaking a large part of glycosides is still present in young wines. Therefore, attention has been focused on the case of exogenous glycosidases from filamentous fungi and from yeast to enhance wine aroma, among these the β G is the most studied [5]. More recently a great interest has been also focused on lactic acid bacteria (LAB) strains involved in malolactic fermentation (MLF) [6].

In red fruit juice and in wines some β G activities can induce a loss of colour. Anthocyanins, responsible for the colour of red fruits, are formed by the addition of a mono or disaccharide to anthocyanidins. The main sugar residue of anthocyanins is glucose, the decolourization is due to the breaking of the bond between the sugar residue and the anthocyanidin which degrades spontaneously into colourless compounds. In grapes juices and wines, since the main anthocyanins are monoglucosides, β G could induce a loss of colour. Le Traon-Masson et al. [7] studied the specificity of two β G purified from an *Aspergillus niger* preparation substrate specificities and kinetic studies (specific activities, affinity, catalytic and inhibition constants) confirmed that the isolated β -D-glucosidases had totally different activity spectra. β G A was highly active on cellobiose and genanyi- β -D-glucoside and very slowly degraded malvidin-3-glucoside. Its effect on the colour of a whole grape anthocyanin extract was

negligible; thus β G A seemed to present an excellent potential to be used for both cellulolysis and aroma enhancing. On the contrary, β G B can be defined as a specific anthocyanase since its activity is directly related to the release of glucose from anthocyanidin-3-glucosides [7].

An action on wine colour of a *Candida molischiana* anthocyanin- β -glucosidase has been reported, and more recently was conducted a screening for anthocyanin- β -glucosidase activity, at wine pH and in the presence of ethanol, in 53 yeasts belonging to various genera *Candida*, *Dekkera*, *Hanseniaspora*, *Metschnikowia*, *Pichia*, *Rhodotula*, *Schizosaccharomyces*, *Zygosaccharomyces*. But nine yeast strains demonstrating greatest β G activity was checked using an anthocyanin extract, and all degraded the anthocyanin extract.

Moreover, the efficiency of β G is influenced by the concentration of the glucose and gluconolactone, and in sweet wines, the hydrolysis is interrupted at the stage of the monoglucoside without freeing of the odours monomers. In the case of dry wines there is a considerable increase in most of the free terpenes [8].

2. β -Glucosidase from commercial preparations

The typical flavour of wines is mainly due to volatile compounds that come from the grapes. It is now well established that apart from free flavour compounds, a significant part of several flavour compounds is accumulated in grape berry and several fruits as odourless non-volatile glycosides. Actually, researchers and tools to study the presence of terpenes and their evolution in grape but it is not yet possible to translate all of the acquired knowledge to the wineries because an efficient methodology to improve the terpene content of all the wines present in the market has not been found [9].

Filamentous fungi originated pectinolytic enzyme preparations were good candidates to use as exogenous glycosidases source. These preparations, obtained from generally recognised

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