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Effect of Wine Inhibitors on Free Pineapple Stem Bromelain Activity in a Model Wine System

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ABSTRACT: The influence of potential inhibitors, naturally present in wine, on the activity of stem bromelain was investigated in order to evaluate the applicability of this enzyme for protein stabilization in white wine. Bromelain proteolytic activity was tested against a synthetic substrate (Bz-Phe-Val-Arg-pNA) in a model wine system after adding ethanol, sulfur dioxide (SO_2), skin, seed, and gallic and ellagic tannins at the average range of their concentration in wine. All the inhibitors of stem bromelain activity tested turned out to be reversible. Ethanol was a competitive inhibitor with a rather limited effect. Gallic and ellagic tannins have no inhibitory effect on stem bromelain activity, while both seed and skin tannins were uncompetitive inhibitors. The strongest inhibition effect was revealed for sulfur dioxide, which was a mixed-type inhibitor for the enzyme activity. This study provides useful information relative to a future biotechnological application of stem bromelain in winemaking.

KEYWORDS: pineapple stem bromelain, wine inhibitors, model wine system

1. INTRODUCTION

Stem bromelain is a cysteine proteinase extracted from the stem of the pineapple plant, *Ananas comosus*.[1] It has multiple uses in food processing, including meat tenderization, applications in the baking industry[2] and enhancement of protein stability in beverages, especially fruit juices and beers.[3,4] To our knowledge, no one apart from us has proposed the use of pineapple stem bromelain for biotechnological application in wine. However, in a recent study there was a preliminary characterization of this protease under wine-like conditions, showing that stem bromelain might find productive biotechnological applications in winemaking.[5]

Since wine contains various compounds (such as ethanol, polyphenols and sulfur dioxide (SO_2)) that could have inhibitory effects on the enzymatic activity of stem bromelain, further studies are necessary in order to evaluate its applicability for protein stabilization in white wine. Among these potentially inhibitory compounds, ethanol is derived from the alcoholic fermentation carried out by yeasts, which convert grape sugars to carbon dioxide and alcohol. Ethanol concentration, depending on the wine style and degree of maturity of the grapes, can range from 8% to 18% v/v in dry white and red wines.[6]

Wine is also an excellent source of various classes of polyphenols, most of which originate in the grape berry; white wines contain significantly lower amounts of total phenols compared with red wines.[7] Wine tannins are phenolic compounds classically divided into condensed and hydrolyzable forms. While the first involve flavan-3-ol units with various degrees of substitution and polymerization and constitute the largest group of proanthocyanidins, the latter are composed of gallic acid and ellagic acid esters formed with glucose or related sugars.[8]

The biological activity of phenolic compounds can be summarized as comprising three main mechanisms: metal chelation, antioxidant activity and enzyme inhibition.[9] In particular, tannins have the ability to act as protein-complexing agents, inhibiting the activity of proteolytic enzymes such as trypsin.[7] Liang et al.[10] have studied the effect of polyphenols extracted from Chinese green tea on the activity of bromelain from pineapple juice, proving that tea polyphenols act as competitive inhibitors of bromelain.

Sulfur dioxide is industrially used as an antioxidant, an inhibitor of oxidizing enzymes and an antiseptic. Sulfur dioxide can be present in two different forms in wine, namely, free (as HSO_3^- or SO_2) or bound to carbonyl or unsaturated compounds and/or phenols.[11] Only free SO_2 possesses reducing and antiseptic properties. Excessive levels of SO_2 must be avoided during the winemaking process as they result in poor wine aroma and flavor. Finally, the content of this toxic substance in the end-product must comply with existing legal limits (EC regulations 1493/1999 and EC 753/2002).

Inhibition studies can tell us something about the specificity of an enzyme, the physical and chemical architecture of the active site, and the kinetic mechanism of the reaction. An "inhibitor" can be defined as any substance that reduces the velocity of an enzyme-catalyzed reaction. In order to supply a complete characterization of stem bromelain to evaluate its suitability for protein stabilization of white wine, the influence on its protease activity of potential inhibitors naturally present in wine must be determined. Ethanol, tannins and sulfur dioxide have never been tested as inhibitors of stem bromelain activity under wine like conditions. In this work the inhibitory effects of ethanol, sulfur dioxide (SO_2), grape skin, seed, gallic and ellagic tannins were investigated over the average range of their respective concentrations of wine.

2. MATERIALS AND METHODS

2.1. The Enzyme and Chemicals. Stem bromelain (EC 3.4.22.32) was obtained from Sigma-Aldrich (Milan, Italy). The synthetic peptide substrate Bz-Phe-Val-Arg-p-nitroaniline (pNA) was

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