ELSEVIER

Carbohydrate Polymers 38 (1999) 283–286

Carbohydrate Polymers

Carbohydrate-flavour conjugates in wine

Katrina M. Hemingway*, Mark J. Alston, Colin G. Chappell[1], Andrew J. Taylor

Samworth Flavour Laboratory, Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

Received 15 January 1998; revised 6 April 1998; accepted 20 April 1998

Abstract

Although flavour glycosides have been reported in wine, the possibility that they might contribute to perceived flavour by being hydrolysed in the mouth during consumption has not been previously documented. The release of hexanol from hexyl β-D-glucoside was studied in-mouth by measuring hexanol in expired air with atmospheric pressure ionisation–mass spectrometry. About 10% (0.2 mg) of the glucoside was hydrolysed over a 2 min period due to the activity of glucosidase enzymes from the oral microflora. Since wine contains ethanol, the inhibitory effects on alpha and beta glucosidases from different sources were ascertained using *p*-nitrophenyl glucosides and spectrophotometric determination. Enzymes from yeast and almonds were inhibited to a greater extent (70–80%) than the *Aspergillus niger* enzyme (35%). Overall, the results show that there is potential for flavour glycosides to be hydrolysed in-mouth and therefore contribute to perceived flavour but further work in real food systems is needed to confirm this hypothesis.

Keywords: β-D-glucoside; Flavour glycosides; Glucoside hydrolysis

1. Introduction

There are many reports of flavour glycosides occurring in the roots, fruits or bark of plants (McIlroy, 1951). Flavour molecules possessing an alcohol group (the aglycone) are glycosidically linked with sugars to produce a stable, flavourless glycoside, which can be converted back to the flavour alcohol and sugar by an appropriate enzyme or by acid hydrolysis of the glycosidic link. The potential of flavour glycosides to act as a source of flavour was recognised by Hewitt et al. (1956), but most published work since then has been concerned with the identification of different glycosides in a variety of plant tissues (Williams et al., 1993).

In our laboratory, techniques to follow the release of aroma during eating have been developed (Linforth et al., 1996) and it has been clearly demonstrated that volatile flavours are released at different rates. The purpose of this preliminary investigation was to determine whether glycosides could contribute to the flavour of wine during consumption. Grapes contain a number of flavour glycosides as listed in Table 1 (Günata et al., 1990) and there have been suggestions that some of the subtle changes in wine flavour during maturation are due to changes in the flavour glycosides. Fig. 1 shows the hydrolysis of one of the grape glycosides (neryl β-D-glucoside) and the formation of the flavour active molecule, nerol. Glucose is also formed but has no flavour activity as the amounts formed by hydrolysis (typically μg/kg) are well below the taste threshold of this compound (typically g/kg).

Williams et al. (1992) proposed that slow hydrolysis of glycosides occurred during maturation due, either to the residual enzyme activity from the grapes (e.g. β-glucosidase, β-arabinosidase and β-rhamnosidase) or due to fungal enzymes which are found on the surface of grapes. However, the possibility of glycosides contributing to flavour during wine consumption has not received attention despite the fact that trained wine tasters generate a complex and changing verbal description of wine as it is consumed, part of which might be caused by the release of some flavours from glycosides in-mouth.

Initially, the hypothesis seems far-fetched, since, although there are glycosides present in wine, a source of enzymes capable of hydrolysing the glycosides rapidly enough for them to be sensed during the short time in-mouth (30–60 s) is not obvious. The glycosidase activity in wine is too low to be effective and saliva contains amylase, which, while capable of hydrolysing glycosidic linkages between glucose molecules, does not hydrolyse the flavour glycosides. However, a range of glycosidases are found in saliva, which are derived from the microflora of the mouth (Nakamura et al., 1983).

* Corresponding author

[1] Dalgety plc., DFI Food Ingredient Development Centre, Station Road, Cambridge CB1 2JN, UK

0144–8617/98/$ - see front matter
PII: S0144-8617(98)00103-9

Table 1
Glycosides present in grapes and wine

Glycoside	Aglycone	Glycone
Geranyl β-D-glucoside	Geraniol	β-D-glucoside
Neryl β-D-glucoside	Nerol	β-D-glucoside
Citronellyl β-D-glucoside	Citronellol	β-D-glucoside
Linalyl β-D-glucoside	Linalool	β-D-glucoside
α-Terpinyl β-D-glucoside	α-Terpineol	β-D-glucoside

Source: Günata et al., 1990.

There is therefore sufficient certitude to investigate the hypothesis further and factors that may affect the production of flavour from glycosides in-mouth have been investigated. Initially, the rate of hydrolysis of a readily available glycoside (hexyl β-D-glucopyranoside) was measured in-mouth by monitoring expired air from the nose for the presence of hexanol. Atmospheric pressure ionisation–mass spectrometry (API-MS) was used for this analysis. Secondly, since wine contains ethanol, its effect on the activity of a range of glycosidases was measured spectrophotometrically in model systems using the conventional substrates for glycosidases (*p*-nitrophenyl glycosides; Halvorson, 1966).

2. Materials and methods

2.1. API–MS in-mouth analysis

The hydrolysis of hexyl β-D-glucopyranoside (Sigma, Poole) to hexanol (Firmenich, Geneva) in the mouth was determined by API–MS. A sample (2 ml; 5 mM) was placed in the mouth, and circulated around the mouth in a drinking action; the sample was held in the mouth for the full time duration without swallowing. During this time, air was sampled from the nose of the subject, through a custom built interface (Linforth and Taylor, 1996) and analysed by API–MS. The MS was set in selected ion mode to follow the $[M + H - H_2O]^+$ ion of hexanol (m/z 84.9).

The API–MS was calibrated using standards of hexanol in water, at concentrations of 0.01 mM to 0.5 mM. These were placed in the mouth and circulated as described above. The hexanol signal from air sampled from the nose was measured for each of the concentrations and this calibration curve used to estimate the amount of hexanol released in mouth from the hexyl β-D-glucopyranoside during eating.

2.2. P-nitrophenol assay

p-Nitrophenyl β-D-glucopyranoside, *p*-nitrophenyl β-D-glucopyranoside, β-glucosidase (source Brewers yeast), β-glucosidase (source almonds) were supplied by Sigma, Poole. β-Glucosidase (source *Aspergillus niger*) supplied by Fluka, Poole, UK. Sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate were supplied by Fisher Scientific, Loughborough, UK.

Samples consisted of 100 μM *p*-nitrophenyl β-D-glucopyranoside or *p*-nitrophenyl β-D-glucopyranoside; β-glucosidase or β-glucosidase; and ethanol (0–15% v/v) in 0.2 M sodium phosphate buffer, pH 6.8. Assays were performed using a Perkin Elmer Spectrophotometer at a temperature of 37°C and a wavelength of 400 nm.

3. Results

3.1. In-mouth analysis of glycoside hydrolysis

The hydrolysis of hexyl β-D-glucopyranoside in mouth was investigated by placing a solution of the glucoside in mouth (2 ml; 5 mM) and monitoring expired air from the nose for hexanol. The conditions used were artificial in that the solution was held in-mouth for 2 mins without swallowing and regular mouth movements were made to mix the solution in the oral cavity. This approach was adopted to maximise the chances of hydrolysis occurring. The trace obtained (Fig. 2) clearly shows hydrolysis of hexyl β-D-glucoside in-mouth and the subject reported sensory detection of the flavour at about 5 s. The trace shows the effects of the mouth movements, which pump small quantities of air from the mouth into the pharynx where they are conveyed to the nose. The regular small spikes on the trace are the individual mouth movements, which are superimposed on the regular breathing pattern of the subject with a periodicity of about 5 s. An estimation of the amounts of hexanol formed was obtained by placing solutions of hexanol in mouth and measuring peak height of hexanol in the expired air collected from the nose (data not shown). From these experiments, the degree of hydrolysis over 2 mins was estimated at 10%.

This result is interesting as it shows that hydrolysis of glycosides can occur in-mouth due to the natural glycosidase activity. However, the extent of hydrolysis will depend

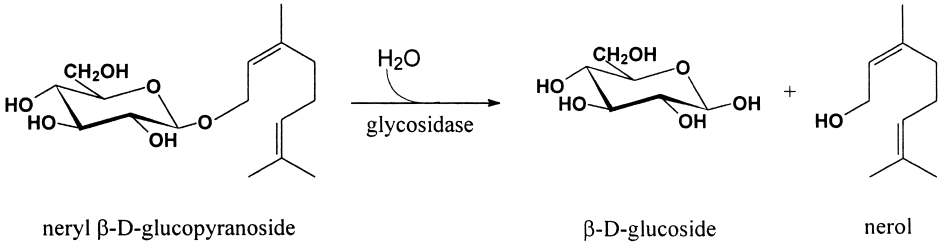

Fig. 1. Hydrolysis of Neryl β-D-glucoside to nerol and β-D-glucose.

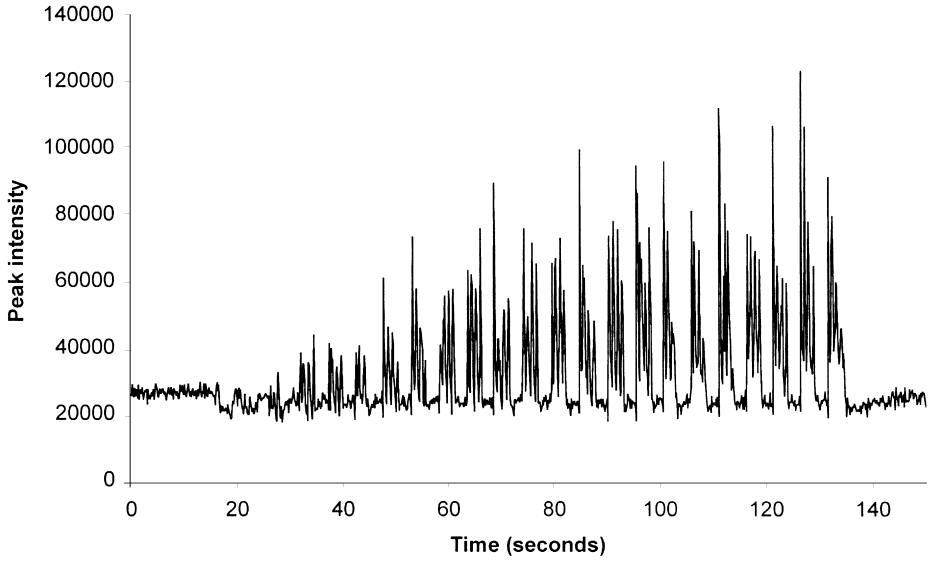

Fig. 2. APCI–MS of Hexanol release from Hexyl β-D-glucopyranoside in the mouth of the subject. The sample was introduced at 15 s.

both on the concentration (and type) of the substrate as well as the enzyme activity in-mouth. To determine the sensory significance of the results, it is convenient to compare the concentrations used with the concentrations necessary to detect hexanol sensorially. From Van Gemert and Nettenbreijer (1977), the mean odour threshold of hexanol in water is 1.9 mg/kg (19 μM). The hexyl β-D-glucopyranoside solution was placed in-mouth at a concentration of 5 mM. The solution was therefore 260 times the threshold, a situation that frequently occurs in food flavourings. Substantial variations in oral microflora have been reported both in one individual over a period of time and between individuals (Nakamura et al., 1983). This leads to speculation that flavour perception, in some instances, may depend more on the composition of the individual's mouth microflora rather than an innate ability to differentiate flavours.

However, of the factors that might limit glycoside hydrolysis from wine in-mouth, the most important is the potential inhibitory effect of ethanol on the activity of the glycosidases.

3.2. Effect of ethanol on glycosidase activity in model systems

Three glucosidases were commercially available and provided comparison between the rate of hydrolysis of α and β forms and between different sources of the enzyme, (yeast, almonds, *A. niger*). The activity of the glucosidases was

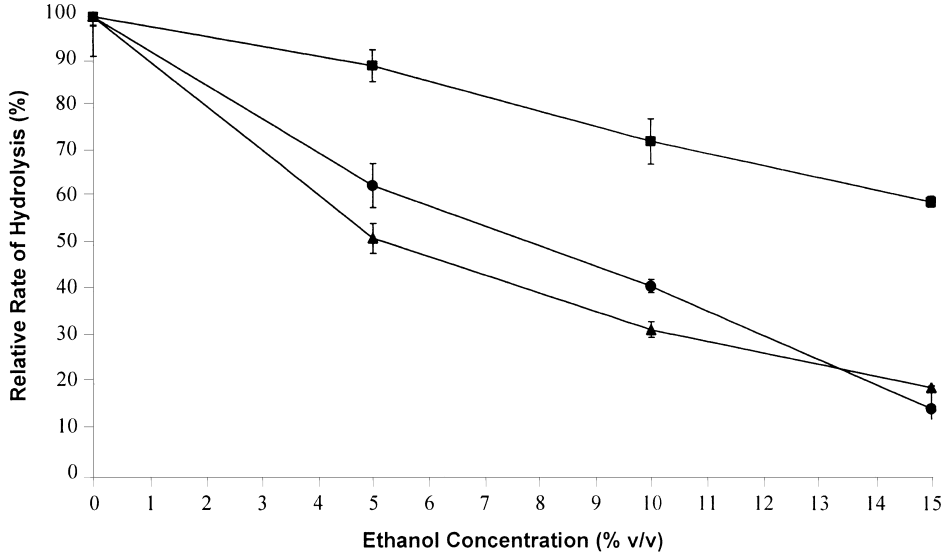

Fig. 3. Inhibition of glycosidases by ethanol. –●– α-Glucosidase (Brewers Yeast), –▲– β-glucosidase (almonds), –■– β-glucosidase (*A. niger*). Values are the means of three replicates, error bars are ±1 S.D.

tested at ethanol concentrations of 0, 5, 10 and 15% ethanol to represent the initial ethanol content of wine and a range of values that might be attained in the oral cavity as wine becomes diluted with saliva. Fig. 3 shows the inhibition of the enzymes on a relative scale for easy comparison. The α- and β- glucosidases from yeast and almonds respectively, were inhibited to a similar degree with 50–70% inhibition at 5% ethanol, rising to around 80% inhibition at 15% ethanol. In contrast, the *A. niger* (glucosidase was inhibited to a lesser degree with only 35% inhibition at 15% ethanol. The differences in inhibition follow the trend of bacterial enzymes being more stable than their plant counterparts. The enzymes in mouth are bacterial in origin and therefore more likely to follow the *A. niger* pattern of inhibition. However, it is impossible to extrapolate from the activities measured in model systems using *p*-nitrophenol substrates, as the activity of glucosidases varies significantly with the type of aglycone present.

4. Summary

The preliminary experiments reported here demonstrate that in-mouth hydrolysis of flavour glycosides is potentially feasible and may contribute to the flavour of foods but further experiments are required to determine whether this actually occurs in real food systems.

Acknowledgements

KMH acknowledges support from the BBSRC and Dalgety plc. through an Industrial CASE studentship, while MJA is supported through a BBSRC ROPA award.

References

Günata, Y.Z., Bayonove, C.L., Cordonnier, R.E., Arnaud, A., & Galzy, P. (1990). *J. Sci. Food Agric., 50*, 499–506.

Halvorson, H. (1966). A glucosidase from Yeast. In: *Methods in enzymology*, Vol. VIII Complex Carbohydrates (pp. 559–562). London: Academic Press.

Hewitt, E.J., Mackay, D.A.M., Konigsbacher, K., & Hasselstrom, T. (1956). *Food Tech*., Oct., 487–489.

Linforth, R.S.T., Ingham, K.E., & Taylor, A.J. (1996). Time course profiling of volatile release from foods during the eating process. In: A.J. Taylor, & D.S. Mottram (Eds.), *Flavour science: recent developments* (pp. 361–369). London: Royal Society of Chemistry.

Linforth, R.S.T., & Taylor, A.J. (1996), Apparatus and Method for the Analysis of Trace Constituents in Gases, UK Patent Application 9615303.6.

McIlroy, R.J. (1951). *The plant glycosides* (pp. 1–13). London: Edward Arnold.

Nakamura, M., & Slots, J. (1983). *J. Periodontal Res., 18*, 559–569.

Van Gemert, L.J., & Nettenbreijer, A.H. (1977). *Compilation of odour threshold values in air and water*. Zeist, Netherlands: National Institute for Water Supply, Voorburg and Central Institute for Nutrition and Food Research TNO.

Williams, P.J., Sefton, M.A., & Leigh Frances, I., (1992). Glycosidic Precursors of Varietal Grape and Wine Flavour. In: *Flavour precursors—thermal and enzymic conversions* (pp. 74–85). Washington, DC: ACS.

Williams, P.J., Sefton, M.A., & Marinos, V.A., (1993). Hydrolytic flavor release from non-volatile precursors in fruits, wines and some other plant-derived foods. In: R. Hopp & K. Mori (Eds.), *Recent developments in flavor and fragrance chemistry* (1st ed, pp. 283–289). Weinheim: VCH.