SOLUBLE COMPLEXES OF COPPER AND ZINC IN WHISKEY DISTILLERY SPENT WASH

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Received 24 September 1981

The capacity of samples of malt whiskey distillery spent wash to complex added ionic copper was examined, and copper binding capacities of up to 3250 mg litre^{-1} found. A correlation between the copper binding capacity and the total hexose content of spent wash was demonstrated. Gel filtration studies indicated that the copper was probably bound to an organic fraction containing both carbohydrate and ninhydrin positive material, whereas zinc appeared to be complexed by a lower molecular weight fraction containing both hexose and phenolic moieties.

Key words: *copper, zinc, distillation.*

Introduction

Quinn *et al*[12] have investigated the distribution of copper in malt whiskey distillery spent wash ('pot ale'), in which it is found as a result of the prolonged heating of the acidic spent wash during distillation in traditional 'copper pot stills'. Total soluble copper levels of 8·5 ppm were found (in addition to a large insoluble fraction associated with the spent wash solids) with no more than 6% of the total soluble copper present as the free ion.

These findings are important in view of considerable current interest in the utilisation and treatment of distillery effluent through microbial biomass production, which has recently been reviewed by Sheehan and Greenfield.[15] In the case of malt whiskey distillery spent wash, which in most plants is presently disposed of by an energy intensive evaporation process,[7] the economics of an alternative biological treatment process would seem to be extremely favourable provided the resultant biomass were to gain acceptance as an animal feedstuff. However, Quinn *et al*[13] have demonstrated that three of the organisms which would be most suitable for such a treatment process may actively accumulate dissolved copper from spent wash, although subsequent compounding would reduce feedstuff copper concentrations to an acceptable level. Mackel[7] has also reported the presence of 200 ppm copper similarly accumulated in the sludge produced during biological treatment of 'foul condensate' from malt whiskey distillery spent wash evaporation plants.

This report considers the distribution of dissolved copper among the potential ligands present in spent wash as part of a survey of the mechanisms involved in copper accumulation by micro-organisms. Some results of this study have already been published.[13] The distribution of zinc complexes is also investigated since unpublished results in our laboratory have shown that malt whiskey spent wash consistently contains about 5 ppm dissolved zinc, much of it presumably also derived from distillery pipework and fittings. The dezincification of brass and corrosion of copper materials has recently been reviewed by Matson,[8] and several instances of corrosion of copper from food and drinks processing equipment are quoted by McMullen.[9]

Materials and methods

Samples were collected over the period 1979–81, from an Irish malt whiskey distillery at Bushmills, Co. Antrim, immediately after distillation and were stored deep frozen. Prior to analysis samples were filtered through a membrane filter (pore size 0·45 μm). Total levels of chelating agents in these samples were determined by the method of Kunkel and Manahan[6] which is based on the fact that copper added (as $CuSo_4$) will be kept in solution by the chelating agents present in the sample, and remain so when the pH is raised to 10·0, with the equilibrium concentrations of the free copper ion being negligible. Following removal of the excess unchelated copper as a $Cu(OH)_2$ precipitate the soluble copper remaining in the filtrate was determined by atomic absorption spectrophotometry. Levels of protein in the spent wash samples were determined by the biuret method[5] using bovine serum albumin as a standard, and total free amino acids as glycine by reaction with ninhydrin according to the method of Yem and Cocking.[16] Hexoses were measured by reaction with the anthrone reagent[5] using glucose as a standard, and polyphenolic components as tannic acid by the Folin Denis method.[1]

To separate the components of spent wash on the basis of their molecular sizes, and determine the fractions responsible for the binding of copper and zinc, a sample of spent wash (collected in February 1981) was fractionated by gel permeation chromatography using a 1·6 × 60 cm column of Sephadex G50–80 (Pharmacia Fine Chemicals, Uppsala, Sweden). As a preliminary step it was necessary to increase the copper and zinc concentrations of the samples to levels higher than those normally encountered under distillery conditions. 100 ml samples of spent wash were acidified to pH 3·0 with lactic acid, heated at 80°C for 30 min with 5 g of either copper of zinc granules, cooled and filtered through 0·45 μm membrane filters. The filtrates were concentrated eight fold by rotary evaporation and 2·0 ml samples were applied to the Sephadex column. The column was eluted with distilled water at the rate of 0·5 ml min^{-1}, and the eluate collected in 5 ml fractions. The bed volume of the column was 120 ml and the void volume (determined using Blue Dextran 2000; Pharmacia Fine Chemicals) was 50 ml. Copper and zinc in the fractions were determined by atomic absorption spectrophotometry and the fractions were also analysed for protein, total free amino acids, total hexose and polyphenols as described above.

Levels of individual free amino acids in a sample of spent wash (concentrated five fold by rotary evaporation and suspended in citrate buffer pH 2·2) were determined using an LKB 4101 Amino Acid Analyser.

Results

Table I illustrates the considerable variation found over a two year period in the capacity of samples of filtered malt

TABLE I. Levels of Copper Chelating Agents in Malt Whiskey Distillery Spent Wash.

Date of sample	Chelating agent concentration (mg litre^{-1} copper chelating capacity)
June 1979	3250
October 1979	1687
November 1979	1375
February 1980	3187
March 1980	1250
June 1980	2187
September 1980	2125
February 1981	2350

TABLE II. Free Amino Acids in Malt Whiskey Distillery Spent Wash.

Amino acid	Concentration in spent wash (mg litre^{-1})
Aspartic acid	50·9
Threonine	42·1
Serine	34·1
Proline	130·2
Glutamic acid	64·0
Glycine	81·5
Alanine	96·5
Cysteine	24·4
Valine	48·7
Methionine	17·5
Isoleucine	27·4
Leucine	42·7
Tyrosine	27·4
Phenylalanine	27·0
Histidine	23·0
Lysine	58·0
Arginine	20·7
	Total 816·1

whiskey distillery spent wash to form stable soluble chelates with added ionic copper. When equal amounts of ionic zinc and copper were added to replicate samples the copper binding capacity decreased by 14·8%. The method of Kunkel and Manahan[6] which was employed does not permit an assessment of the relative importance of the different potential ligands present in such a complex effluent. However, analysis of the eight samples showed that their total hexose levels ranged from 5·2 to 20·0 g litre^{-1}, protein from 8·7 to 13·2 g litre^{-1}, free amino acids (as glycine equivalents) from 1·5 to 3·7 g litre^{-1} and polyphenols (as tannic acid equivalents) from 1·5 to 2·0 g litre^{-1}. When correlation coefficients between these and the copper chelating capacities of the samples were calculated the relationship was only significant at the 5% level ($r=0·71$) in the case of total hexose. A significant relationship between the copper chelating capacity and free amino acid content had been anticipated.[12] Accordingly, a sample containing 3·5 g litre^{-1} total free amino acids as determined by the method of Yemm and Cocking[16] was analysed for individual free amino acids. The results, shown in Table II, indicate that the sum of the individual free amino acids is much lower than the value for ninhydrin positive material. It can be inferred from this that approximately 76% of this material is present as ninhydrin positive peptides and not as the free amino acids.

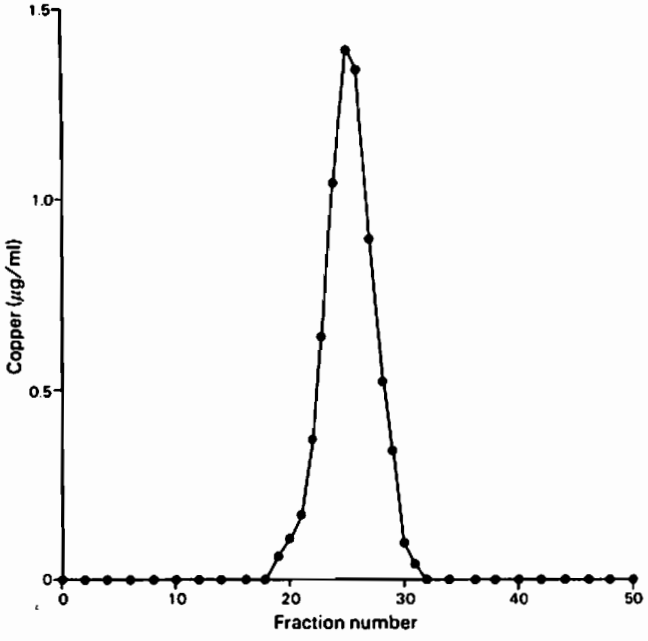

Fig. 1. Sephadex G 50–80 elution pattern of malt whiskey distillery spent wash heated with copper turnings. Fractions were assayed for copper.

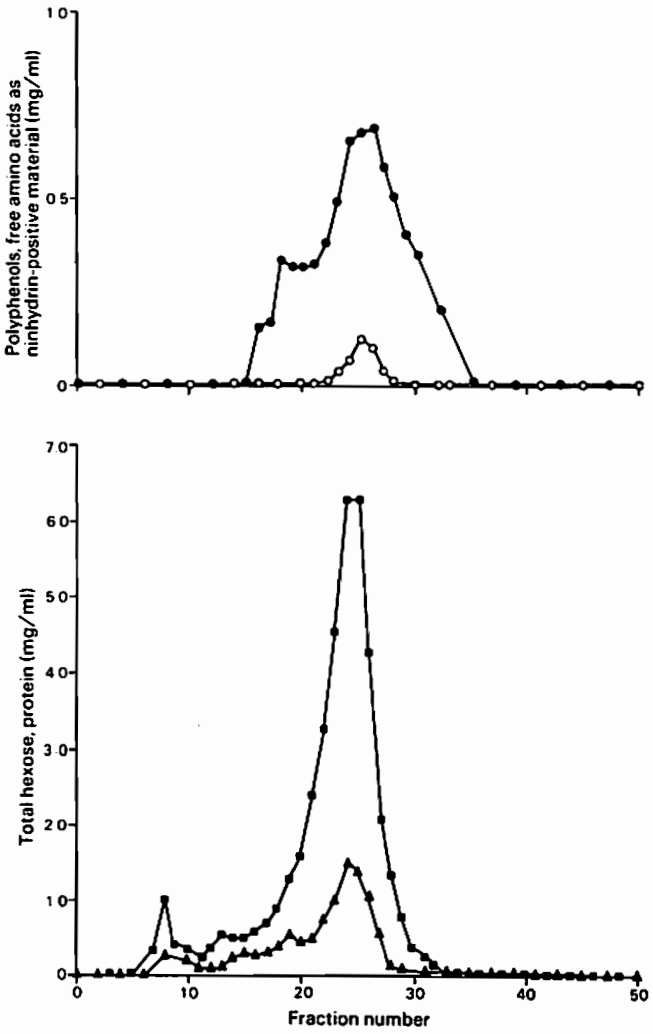

Fig. 2. Sephadex G 50–80 elution pattern of malt whiskey distillery spent wash heated with copper turnings. Fractions were assayed for polyphenols (●—●), free amino acids (○—○), total hexose (■—■) and protein (▲—▲).

Various attempts have been made[2,3,10,14] to characterise copper chelates in plant materials by differential extraction and/or paper chromatography but they have proved largely inconclusive. However, polyacrylamide gel filtration has recently been successfully applied to the characterisation of copper complexes in human alimentary secretions.[4] We therefore decided to attempt to characterise the copper and zinc containing fractions of spent wash which had been previously heated with copper turnings or with zinc granules by gel filtration on a cross-linked dextran gel (Sephadex G50–80). A single well defined copper elution peak was observed (Fig. 1) in fractions 18–32 indicating that all the copper was present in a complexed form. After calibration of the column with a mixture of standard proteins (cyanocobalamin MW 1353; insulin MW 6000; cytochrome c MW 12,384; lysozyme MW 14,600; chymotrypsinogen MW 24,000) the apparent molecular weight of this copper binding component was found to be approximately 2000. It was not possible to obtain on the column clear resolutions of the major organic compounds likely to be involved in copper complex formation (Fig. 2) since all co-eluted with copper to some degree. There was, however, a precise coincidence in the elution patterns of ninhydrin positive and the lower molecular weight anthrone positive materials (Fig. 2).

Zinc was more readily solubilised than copper by acidified

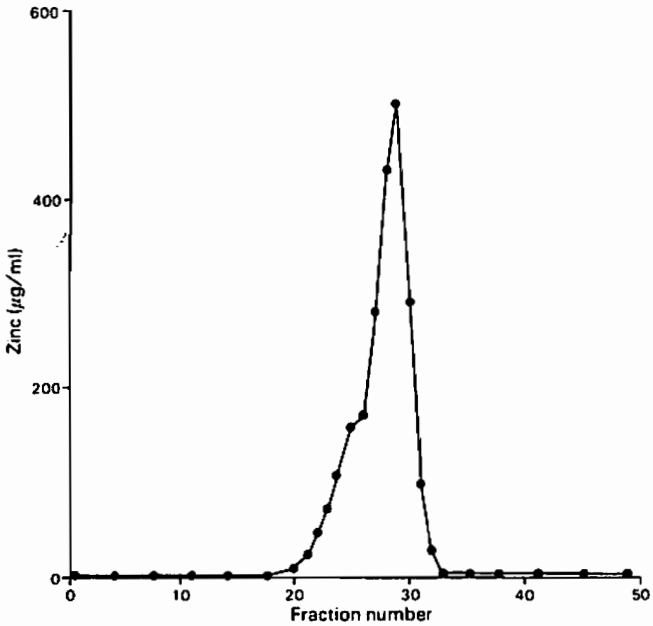

Fig. 3. Sephadex G 50–80 elution pattern of malt whiskey distillery spent wash heated with zinc granules. Fractions were assayed for zinc.

spent wash and, as with copper, all the zinc was found in a complexed state (Fig. 3). Two types of zinc complex appeared to be present; one eluting in fraction 18 onwards, with a peak in fraction 25, coincided with the copper binding peak (Fig. 1) and was possibly identical to it, while a second major component was observed (Fig. 3) with a peak in fraction 29. This larger peak would appear to be a zinc complexing agent of lower molecular weight, and at this elution volume a considerable proportion of the organic material present is polyphenolic in nature (Fig. 2).

Discussion

It is apparent from Table 1 and Figs. 1 and 3 that malt whiskey distillery spent wash is able to complex copper and zinc in concentrations far in excess of those likely to be leached from pipework in normal distillery practice. These results confirm our earlier findings[12] that over a four year sampling period the free ionic copper concentration only exceeded 2% of the total soluble copper on one occasion, despite the low pH of the spent wash which was frequently below pH 3·5.

As an intermediate between the 'hard' and 'soft' electron acceptors, cupric copper forms strong complexes with many organic chelons with nitrogen and/or oxygen donor atoms being typically involved. The complexity and multiplicity of naturally occurring organic compounds precludes indentification of Cu (II) compounds in most natural environments and, although formation constant data exist for simple chelates, the formation of mixed ligand complexes is a common phenomenon.[11] Such complexes are almost certainly present in malt whiskey distillery spent wash, and may be associated with the anthrone and ninhydrin positive fractions which we believe to be Maillard reaction products formed during distillation. The elution volume of much of the complexed zinc in spent wash suggests that this ion is bound by a smaller macromolecule containing both hexose and phenolic moieties, which we believe to be a condensed polyphenol. The extent to which these two ions are accumulated by micro-organisms during a biological treatment process should depend on the ability of the organisms to degrade and assimilate the macromolecular species by which they are complexed. If these copper complexing agents are, as we believe, Maillard reaction products, which are generally weakly biodegradable, it would explain our previous findings[13] that there is partial active uptake of copper by the micro-organisms employed in a treatment process. It would also explain why, despite a proven[12] strong negative correlation between the apparent free amino acid content of spent wash measured by the ninhydrin method and the 'free' copper content, the uptake of copper by the microorganisms employed in a treatment process was not antagonised by the addition of casamino acids.[13]

Acknowledgements.—Our thanks are due to the directors and staff of the Old Bushmills Distillery for their co-operation, and to Dr Alastair Flinn, of the New University of Ulster, for assistance with the amino acid analysis. This work was supported by a grant from the Wolfson Foundation.

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