THE DETECTION OF PROTEIN IN REFINED SUGAR

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Abstract

The sporadic appearance of floc, and more particularly acid beverage floc (ABF), in soft drinks is a source of concern to cane sugar refiners. Although floc is unsightly, it is a quality parameter that is unlikely to impact on the health and nutritional status of sugar. The refiner is often hastily blamed and compensation sought despite the fact that claims may not always be valid.

There is agreement among researchers that ABF in soft drinks arises from aggregation of macromolecules in the solution after acidification. This is believed to be initiated by a protein component that complexes with polysaccharides, giving rise to observable floc. Furthermore, it has been shown that a relationship exists between the protein concentration and the initiation of floc. Two rapid dye-binding techniques were therefore investigated for the detection and quantification of protein in refined sugar.

The Amido Black dye method involves staining a membrane after filtering the sugar solution to give a visual indication of floc positive sugar.

The Coomassie Blue protein assay method is a modification of the colorimetric Bradford protein dye-binding method in which absorbance is measured at a specific wavelength. A protein standard was used for the calibration graph.

Results to date indicate that the protein content of floc positive sugars ranges from 2 to18 mg/kg. There are indications that the protein concentration correlates with the 10-day low Brix Coca-Cola test (Anon, 1998).

Keywords: sugar, floc, protein

Introduction

The appearance of ABF in soft drinks is a quality problem that is difficult to manage. ABF usually only manifests in a soft drink at the point of retail. The standard ABF test is the 10-day Coca-Cola test, but the delay in this result is a disadvantage as it causes distribution problems with refined sugar producers. There is thus the need for a rapid and reliable method to predict the propensity for ABF formation. Cohen *et al.* (1970) reported that trace amounts of protein were a major contributor to floc formation in granulated cane sugar.

The aims of the current investigation were two-fold:

(i) To implement a rapid test to indicate the possible presence of protein in sugars, as an initial screening for further testing.

(ii) To confirm the suitability of a protein dye-binding technique for quantifying protein in sugar.

The Amido Black dye method (Liuzzo and Wong, 1982; Anon, 2002) was considered for the first application. The Coomassie protein assay method (a modification of the colorimetric Bradford protein dye-binding method (Bradford, 1976) was evaluated for the second application.

Materials and method

Amido Black dye membrane staining method

A visual indication of floc positive sugars can be obtained by using Amido Black to stain a membrane through which a sugar solution has been filtered. A dilute sugar solution was filtered through a membrane filter. The membrane was stained drop-wise with an acetic acid solution of Amido Black dye for 10 minutes. The membrane was de-stained for15 minutes. Cane sugars (10-day Coca-Cola floc positive and floc negative), a floc negative first boiling refined sugar as control, and a protein spiked sugar solution (standard), were tested.

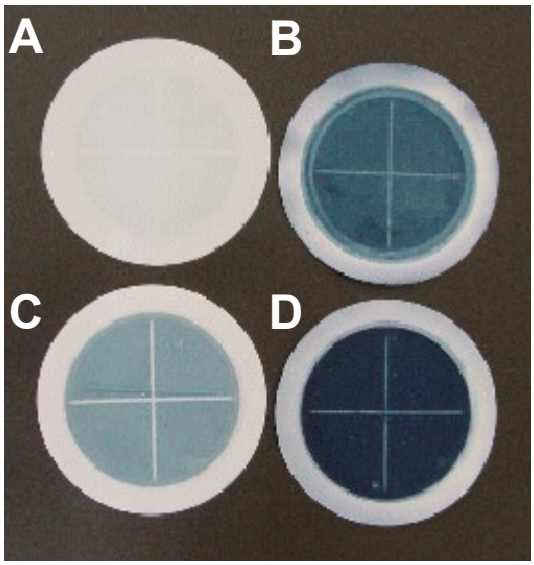
Coomassie protein dye-binding method

The Coomassie Blue dye and the standard protein are commercially available. Six dilutions of standard protein were prepared in 50°Brix sugar solutions covering the range 2 to 12 mg/kg. The standards and the sugar sample were mixed with an equal volume of dye. The solution was allowed to stand at room temperature for at least five minutes. The absorbance was measured and the protein concentration of the sample was estimated from the calibration curve. Three 50°Bx sugar solutions were spiked with 2.0, 4.0 and 6.0 mg/kg protein on Brix. These solutions were treated in exactly the same way as the samples. A total of 140 sugars were tested.

Results and discussion

Amido Black dye membrane staining method

In Figure 1, the membrane filters from some of the samples show an increase in blue colour as the protein level increases. The protein standard, membrane B, stained a deep blue. The sugar control, Membrane A, de-stained well and was floc negative. Membranes C and D stained with varying degrees of blue, depicting different concentrations of protein. This procedure has potential as a screening test to be confirmed using the Coomassie Blue test and the 10-day Coca-Cola test.



a = control; B = protein standard;
C and D = floc positive refined cane sugar by the 10-day Coca-Cola test

Figure 1. Membranes stained with Amido Black after filtration of sample.

Coomassie protein dye-binding method

The criteria for choice of a protein assay are usually based on convenience, availability of protein for calibration, presence or absence of interference agents and the need for accuracy. Table 1 gives concentrations and corresponding absorbances of the standard protein solutions. A good correlation between the protein concentration and absorbance was obtained (r^2=0.993).

From Table 2 it is clear that the recovery of protein in spiked samples was adequate, effectively validating the Coomassie protein dye-binding method. The Coomassie protein dye-binding method is sensitive and results can be obtained within five minutes.

Table 1. Protein calibration using 500 µl Coomassie Blue reagent.

Protein standard (0.1 mg/cm^3) (µl)	Diluent 50°Bx sugar solution (µl)	Final concentration (mg/kg)	Absorbance
0	500	0	0.00
5	495	1.00	0.056
12.5	487.5	2.50	0.099
25	475	5.00	0.163
50	450	10.00	0.255
60	440	12.00	0.294

Table 2. Validation of the Coomassie Blue protein dye-binding method (conditions as for Table 1).

Protein standard (mg/ml) (µl)	Diluent 50°Bx sugar solution (µl)	Absorbance (595 nm)	Expected concentration on Bx (mg/kg)	Final concentration on Bx (mg/kg)
20	490	0.086	2.00	2.02
40	480	0.137	4.00	3.98
60	470	0.186	6.00	6.18

Figure 2 shows protein levels in both floc positive and floc negative sugars using the Coomassie Blue test. Protein in most floc positive samples ranged from 2 to 18 mg/kg. About 20% of the floc positive samples had protein levels of less than 2 mg/kg. It is possible that this floc is not true ABF, but is caused by carry-over at the filter station. Such floc would include calcium and silicates, for example, and will be investigated further.

About 18% of the floc negative sugar samples contained approximately 2 mg/kg protein. The other floc negative sugar samples had less than 2 mg/kg. Hence, protein levels greater than 2 mg/kg are probably indicative of floc positive sugars.

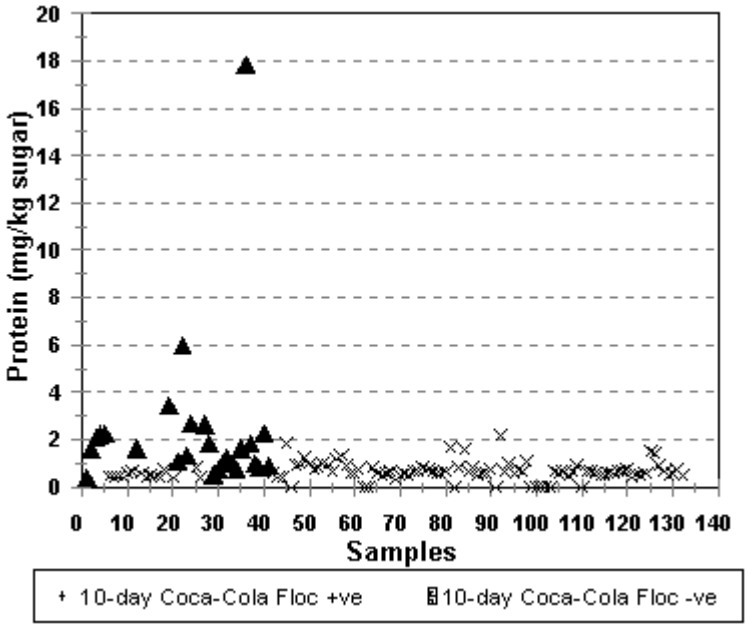


Figure 2. Scatter plot of protein levels in floc positive and floc negative (10-day Coca-Cola test) refined cane sugar samples.

Conclusion

- The Amido Black staining procedure is a useful screening test that gives a qualitative indication of protein and therefore floc potential.
- The Coomassie Blue method is simple, sensitive, rapid and readily quantified.
- Floc positive sugars (10-day Coca-Cola test) generally contain more than 2 mg/kg protein when tested with the Coomassie Blue method.
- Both procedures will be useful to flag sugars with the potential to floc.

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