A SIMPLE AND SENSITIVE METHOD FOR DETERMINATION OF SUGAR CONTENT IN FRUITS AND CULTURE MEDIA DURING FERMENTATION

Djagal Wiseso Marseno and Retno Indrati

Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology,
Gadjah Mada University, Jl. Flora, Bulaksumur, Yogyakarta 55281
E-mail: djagal@ugm.ac.id

ABSTRACT

A colorimetric for sugar determination using cuprammonium reagent containing strong base has been improved with a heating process after a reaction started. The reaction was started after the sugar or sample containing sugar was added to the cuprammonium reagent consisted of 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH). This heating process could enhance the formation of sugar-cupper ammonium complex, so that the intensity of absorbance increased at wavelength of 280 nm. This simple and sensitive method was applicable for various sugars detection such as sugar fraction after HPLC separation, sugar content in biological extract or in culture medium during fermentation. Interestingly, different from previous report, this method was specific for non polyol sugars.

Keywords: *Sugar, determination, fruits, fermentation*

INTRODUCTION

Rapid and sensitive method for detection of sugars content is important and needed in research working with biological extract or culture media during fermentation. Some non-destructive methods to determine sugars in the tissue of fruits has been developed such as in apples (Yan-de *et al*., 2007), cantaloupe (Dull *et al*., 1989), mandarin (Kawano *et al*., 1993), and peach (Carlomagno *et al*., 2004). All of the above researches were based on Near-Infrared Reflectance (NIR) spectroscopic instruments which needs a high technology instrument, well trained technicians, and expensive.

Moreover, some destructive methods to determine sugar content in biological tissues have also been developed. The method of Nelson and Somogyi (1945) needs many chemical reagents. Grimble *et al*. (1983) proposed a new method to determine sugar in the post-column fraction after HPLC based on the hyperchromic shift in the ultraviolet due to the formation of a coordination complex between sugars and cuprammonium ion. This method has been improved by McKay *et al*. (1987) by the addition of strong base in the reaction mixture. They found that the addition of excess strong base produced a greatly improved response for not only sugars but also polyols and gave lower blank absorbance.

In this research, the method of McKay *et al*. (1987) was improved by a heating process so that the formation of sugar-cupper ammonium complex increased. This would resulted in the increased of absorbance, so that the sensitivity of the method could be improved. The aim of the research was to develop a simple and rapid method for sugar content determination in fruits tissue and culture medium of fermentation.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade quality. Some chemicals were obtained from Wako (D-sorbitol, maltose, fructose) and the others from Nacalai (D-glycerol, D-glucitol, D-mannitol, D-glucose, Lactose, Sucrose and soluble starch). Extra pure of Ammonium sulfate ($(NH_4)_2.SO_4$) was purchased from ICB (USA).

Reagents

The working reagents of McKay *et al.* (1987) were prepared as described by the authors. Stock reagent was prepared as follows: Reagent A: 2 M of ammonium sulfate $(NH_4)_2.SO_4$; Reagent B: 10 mM Cupric sulfate $CuSO_4.5\ H_2O$ and Reagent C: 6 M NaOH. Working reagent was prepared directly from stock reagent at the day of analysis at a final volume of 5 ml containing (final concentration) 0.4 M $(NH_4)_2.SO_4$, 1 mM $CuSO_4.5\ H_2O$, 0,6 M NaOH and various concentration of sugars

or polyols examined. The distilled water was added to give a final volume of 5 ml.

Spectrophotometer Assay Procedure

Standard curve of sugars was prepared by varying their concentrations in a 5 ml of working reagent. Working reagent was placed in screw cap tubes and heated for 10 min in boiling water then cooled for 5 min in tap water (25 - 27 ^{o}C). The absorbance of standard sugars in working reagent was read at 280 nm. Sugar content in the sample (extract of fruits or results of hydrolyzed soluble starch) was determined by comparing the absorbance of sample with the absorbance of standard curve.

Determination of Sugar Content in Fruits

Total and reducing sugars content in fruit were determined. In this research, peach has been used as a model of study. Peach was peeled and homogenized (ratio of fruit and cold water was 1 : 3) using homogenizer for 2 min. The resulting homogenate was centrifuge at 3,000 rpm for 10 min, the total and reducing sugar content in supernatant was determined. Total sugar in the supernatant was measured by the addition of 1 ml of supernatant into screw cap tube containing of 1 ml of HCl 6%, closed and mixed for 5 sec using vortex mixer, heated in boiling water for 10 min and cooled in ice for 5 min. The solution was centrifuged at 3,000 rpm for 10 min and a small volume of supernatant (20 µl – 40 µl) was added into working reagent (final volume 5 ml) followed by heating and cooling as described previously for standard curve preparation and the absorbance was measured at 280 nm. The sugar content was determined using standard curve of glucose.

Reducing sugar was determined by the addition of 1 ml of supernatant into screw cap tube containing 1 ml of TCA 10 %, closed the tube and mixed for 5 seconds using vortex mixer, heated in boiling water for 10 min and cooled in ice for 5 min. The solution was centrifuged at 3,000 rpm for 10 min and a small volume of supernatant (20 µl – 40 µl) was used for sugar determination as described previously.

Determination of Sugar as a Result of Hydrolysis of Soluble Starch

Soluble starch (3 g) was dissolved in total volume of 100 ml of hot distilled water (80 ^{o}C), 3 ml of this solution was added into screw cap tube containing 3 ml of HCl 6 %, closed and heated in boiling water for 0 – 40 min, cooled in ice for 5 min. The resulting solution (100 µl) was added into working reagent (final volume 5 ml, included the samples). The next procedure was same as for standard curve preparation.

Determination of Sugar Content During Fermentation

Change of sugar content in the culture medium of *Mortierella* sp. during incubation was examined. *Mortierella* sp. was used to produce some fatty acids (Indrati *et al*., 2003). At defined interval time, a small volume of the culture media was taken and its total and reducing sugar content were determined using the method mentioned above.

RESULT AND DISCUSSION

Effect of Heating on the Absorbance

The improvement of the method of McKay *et al.* (1987) for sugar determination by the treatment of heating process was very interesting. As shown in Figure 1, heating process could significantly increased the absorbance, compared to that of original procedure without heating. The increase of absorbance was concomitant with that of glucose concentration. The data suggested that heating process would increase the interaction between cupper ammonium and sugars, so this finally will increase the intensity of the color formed.

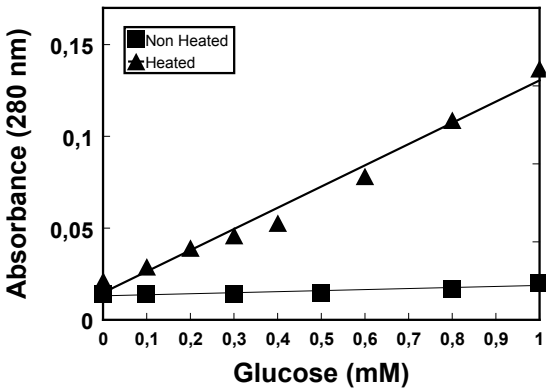

Figure 1. Effect of heating process on the absorbance of glucose-cupper ammonium complex at various glucose concentrations. The final concentration of the components are 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH and various concentration of glucose

The increasing of absorbance of the sugar – cupper ammonium complex were depending on the temperature and period of heating process (Figure 2 and 3). The higher temperature or the prolonged heating time will give the higher absorbance. Spectra pattern in various wavelength indicated that the highest absorbance for samples or blank was at 270 – 280 nm (Figure 4). Based on these data, the wavelength of 280 nm and heating at 100 ^{o}C for 10 min were decided as the best conditions for sugar determination as used for further experiments.

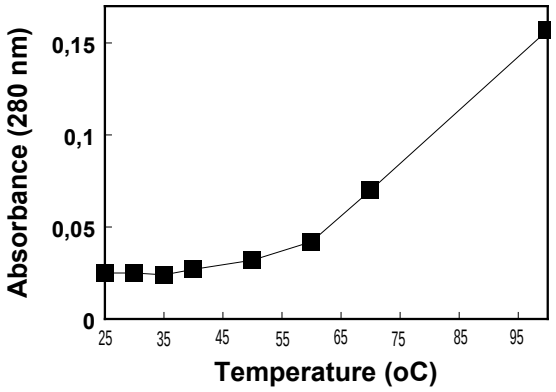

Figure 2. Effect of temperature on absorbance at 280 nm. The final concentration of the components are 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH and 1 mM glucose. Heating time is 10 min

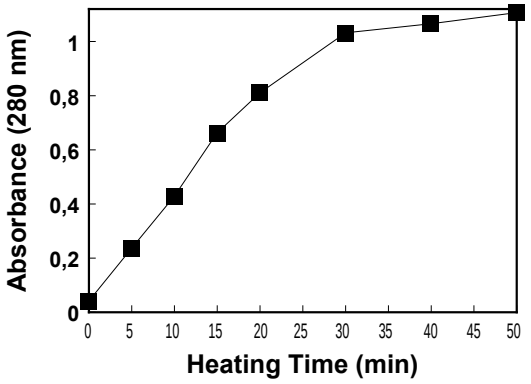

Figure 3. Effect of heating time on absorbance at 280 nm. The final concentration of the components are 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH and 3 mM glucose. The mixture was boiled for various time of heating as shown on the figure

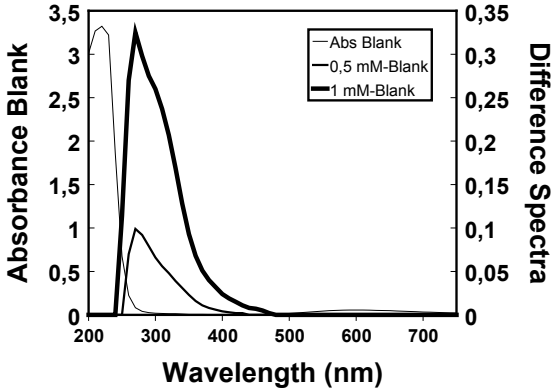

Figure 4. Difference spectra of the sugar-cupper ammonium complex. The final concentration of the components are 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH and glucose 0,5 mM and 1 mM. The absorbance was detected at wavelength as shown on the figure

Stability of the Sugar-Cuprammonium Complex

One of the important factors in chemical substances determinations using spectroscopy method is the stability of the mixture before absorbance reading. Figure 5 shows that the absorbance of sugar-cuprammonium complex was stable at least for 60 min. This result was useful when working with a large number of samples.

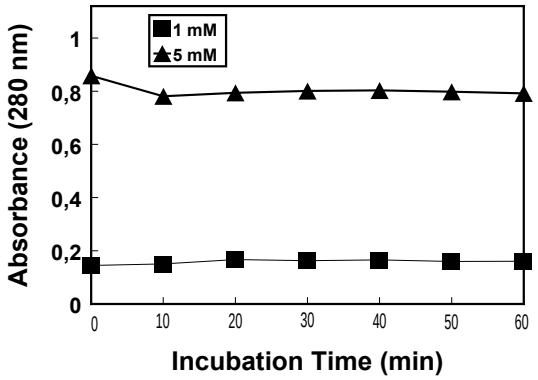

Figure 5. Stability of the sugar-cupper ammonium complex. The final concentration of the components are 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH and glucose 1 mM and 5 mM

Hydrolysis of Soluble Starch

Hydrolysis of soluble starch during incubation under acid condition could be detected easily. Product of hydrolysis (sugar) increased linearly with the increase of hydrolysis time (Figure 6). These results suggested that the method is sensitive to detect any change of sugar concentration during starch hydrolysis. Repetition of the experiment at least for 3 times gave the same results, indicated that the method has a high reproducibility.

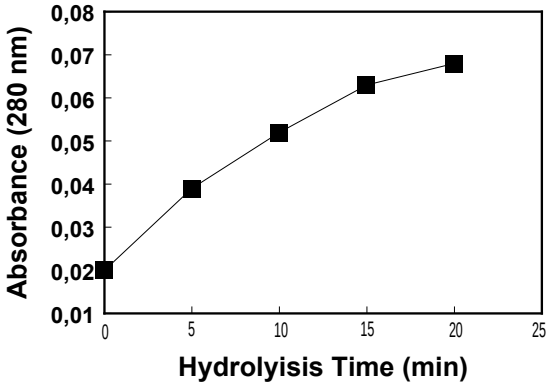

Figure 6. Change of sugar content during hydrolysis of soluble starch under acid condition. The final concentration of the working reagent is 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH and 100 µl of sample was taken at interval time and determined its sugar content using working reagent

Determination of Total and Reducing Sugar in Fruit

Sensitivity of the method to determine total sugar (sample was treated with HCl) and reducing sugars (sample was treated with trichloroacetic acid, TCA) in peach fruits was examined (Figure 7). The results showed that the absorbance increased linearly with the increased of extract added. These data suggested that the method was very sensitive so that it is applicable for determination the change of both total and reducing sugar in fruits during storage or ripening.

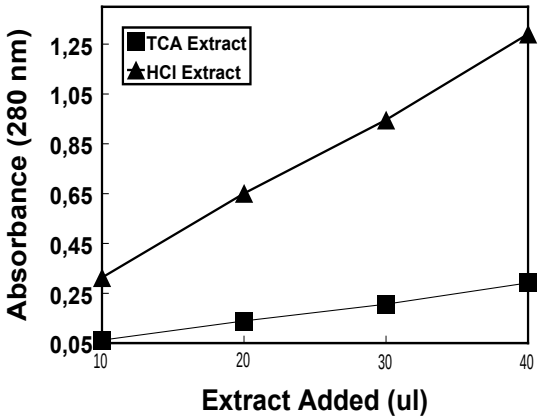

Figure 7. Determination of total (treated with HCl) and reducing (treated with TCA) sugars content in peach extract. The final concentration of the component are 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH and small volume of peach extract after acid hydrolysis

Determination of Reducing Sugar in Culture Media During Fermentation

Changes of sugar content in culture media during fermentation process could be determined effectively (Figure 8). Hydrolysis of soluble starch to total and reducing sugars in culture media during fermentation could be detected easily. Both total sugars (treated by HCl) and reducing sugars (treated by TCA) content in culture media decreased during fermentation.

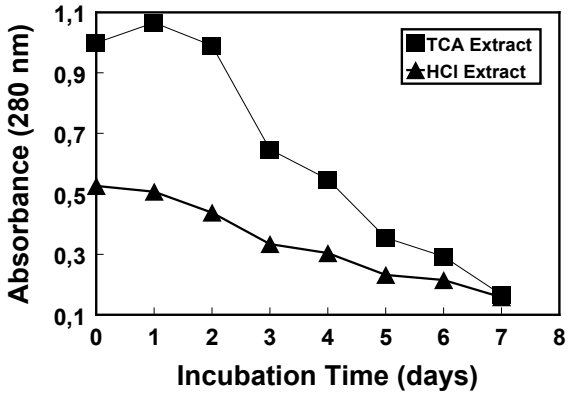

Figure 8. Change of total (treated with HCl) and reducing (treated with TCA) sugars in the culture media during fermentation. The final concentration of the component are 1 mM $CuSO_4$ – 0,4 M $(NH_4)_2SO_4$ – 0,6 M NaOH and small volume of acid hydrolised-culture media during fermentation

Detection of Some Polyols

Ability of the method to detect some polyols and sugars were examined and the data was presented on Table 1. The data show that the method was suitable to detect sugars but not polyols, such as D-Glucytol, D-Mannitol, and D-Sorbitol. This was differed with that of the original method of McKay *et al.* (1987) which is preferable for the measurements of sugar and polyols.

Table 1. Reaction of cuprammonium reagents with sugars and polyols

Concentration (mM)	Sugars					Polyols		
	D-Glucose	Sucrose	D-Fructose	Lactose	Maltose	D-Glucytol	D-Mannitol	D-Sorbitol
Blank	0.025							
0.1	0.034	0.022	0.035	0.051	0.039	0.016	0.014	0.018
0.3	0.054	0.022	0.089	0.122	0.085	0.017	0.017	0.019
0.5	-	0.021	0.200	0.202	0.186	0.017	0.016	0.018
0.8	0.128	0.021	0.404	0.354	0.367	0.016	0.018	0.017
1.0	0.161	0.021	0.449	0.456	0.489	0.017	0.017	0.023

CONCLUSION

Sugar determination method using formation sugar-cuprammonium complex could be intensify its sensitivity by the addition of heating process to increase the complex formation. The suggested heating condition was at 100 ^{o}C for 10 min and absorbance at 280 nm. This method was sensitive for sugars detection but not for polyols.

ACKNOLEDGEMENT

Financial assistance from the Rotary Yoneyama Memorial Foundation of Japan and The Habibie Center of Indonesia is gratefully acknowledged. Special thank to Professor Yoshiyuki Ohta of the Laboratory of Applied Microbiology, Faculty of Applied Biological Science, Hiroshima University, Hiroshima, Japan for giving a permission to work in his laboratory during post doctoral program.

REFERENCES

Carlomagno, G. and Capozzo, L. (2004). Non-destructive grading of peaches by near-infrared spectrometry. *Infrared Physics and Technology* **46**: 23-29.

Dull, G.G., Birth, G.S. and Leffler, R.G. (1989). Near infrared analysis of soluble solids in intake of cantaloupe. *Journal of Food Science* **54**: 393-395.

Grimble, G.K., Barker, M.H. and Taylor, R.H. (1983). Chromatographic analysis of sugars in physiological fluids by post-column reaction with cuprammonium: A new and highly sensitive method. *Analytical Biochemistry* **128**: 422-428.

Indrati, R., Sardjono and Marseno, D.W. (2003). Pengaruh pengaturan kadar glukosa terhadap produksi asam arakhidonat dari *Mortierella sp*. *Prosiding Seminar Nasional, PATPI*, 22 – 23 Juli 2003, Yogyakarta.

Kawano, S., Fujiwara, T. and Iwamoto, M. (1993). Nondestructive determination of sugar content in Satsum mandarin using near infrared transmittance. *Journal of Japan Society of Horticulture Science* **62**: 465-470.

McKay, D.B., Tanner, G.P., MacClean, D.J. and Scott, K.J. (1987). Detection of polyols and sugars by cuprammonium ion in the presence of strong base. *Analytical Biochemistry* **165**: 392-398.

Nelson, N. (1945). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry* **153**: 375-380.

Somogyi, M. (1945). A new reagent for the determination of sugars. *Journal of Biological Chemistry* **155**: 61-68.

Yan-de, L., Yi-bin, Y., Xiaping, F. and Huishan, L. (2007). Experiments on predicting sugar content in apples by FT-NIR Technique. *Journal of Food Enginering* **80**: 986-989.