High Fructose Rectified Concentrate Must

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Rectified Concentrate Must (RCM) from grapes is a mix of glucose and fructose in almost equal proportions. A substantial increase in the proportion of fructose with respect to glucose by using enzymes is proposed in this work. Glucose is converted enzymatically into gluconic acid. Soluble glucose-oxidase catalase is used. A 97% fructose sirup is obtained. Sweetening power of High Fructose Rectified Concentrate Must (HFRCM) is 30% higher than regular RCM. Relationship between glucose concentration, enzyme concentration, H_2O_2 supply and air supply are studied.

Fructosereicher rektifizierter Konzentrat-Most. Rektifizier-Konzentrat-Most (RCM) aus Trauben ist eine Mischung aus Glucose und Fructose in fast gleichem Verhältnis. Eine beträchtliche Erhöhung des Fructoseanteils gegenüber der Glucose durch Verwendung von Enzymen wird in dieser Arbeit vorgeschlagen. Glucose wird enzymatisch zu Gluconsäure umgewandelt. Dazu wird lösliche Glucoseoxidase-Katalase benutzt. Ein 97%iger Fructosesirup wird erhalten. Die Süßkraft des fructosereichen rektifizierten Konzentrat-Mostes (HFRCM) ist 30% höher als die von RCM. Die Beziehung zwischen Glucosekonzentration, H_2O_2- und Luftversorgung wird untersucht.

1 Introduction

RCM is used mainly as sweetener. It is produced from juice grape at a final concentration of 60 to 70° Brix. An enzymatic step of oxidase glucose into gluconic acid is proposed in this work as a way to increase the amount of fructose with respect to glucose to enhance the sweetening power of the sirup. Reaction of oxidation of glucose to gluconic acid is:

$$C_6H_{12}O_6 + H_2O + O_2 \xrightarrow{\text{glucose oxidase}} C_6H_{12}O_7 + H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

H_2O_2 and air are added as a oxigen source. The enzymatic step does not modified esencially the production scheme of RCM.

2 Materials and Methods

2.1 Substrate

Commercial RCM from grapes produced in Mendoza, Agentina, was used. It contained 445g/l of glucose and 460g/l of fructose.

2.2 Enzyme

MKC-Glucose oxidase p-1500 (Solvay Enzimas S.A., Buenos Aires, Argentina) obtained by a controlled fermentation of *Aspergillus niger* var, was used. It was supplied as a powder standardized on 2100GOU/g for colorimetric assay.

2.3 Methods

All the trials were carried out with diluted RCM with deionized water. PH was adjusted with 1M NaOH for the first assay and $CaCO_3$ (powder) for all other trials. Preliminary experiments were carried out in a 250ml-reactor. Final assays were carried out in a 5 L-reactor.

2.4 Analytical methods

Glucose was determined enzymatically (glucose oxidase-catalase, Trinder, 1969). Reducing sugars were determined by dinitrosalicilic acid method (Miller, 1959). Both analysis were carried out in a UV spectrofotometer (UV 160 Shimadsu Corporation, Kioto, Japan).

2.5 Equipment

Preliminary experiments were conducted in a 250ml-reactor as show of Fig.1. Final experiments were conducted in a Bio Flow IIC Bioreactor (New Brunswick Scientific Co., Inc; Edison, NJ, USA), equipped with pH, temperature, nutrient feed, DO, agitation and electronic foam controls.

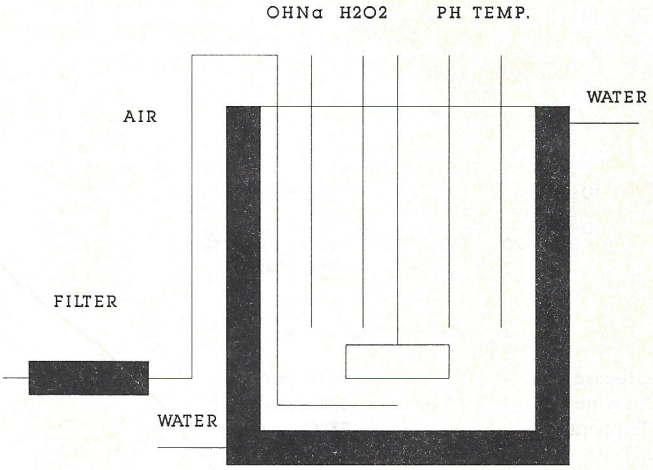

Figure 1. Laboratory equipment for HFRCM production.

3 Results

Basic parameters were determined under two different glucose concentrations (1%w/v, Graphic 1 and 10%w/v, Graphic 2). Conversion of 99% in the first case and 97% in the second are reached.

Glucose concentration. Trials were carried out at three different glucose concentration levels (1%w/v, 10%w/v, 13,3w/v). Graphic 3 shows that conversion rate decreases when glucose concentration increases. 10%w/v glucose concentration was selected on the base of a conversion of 97% in a period of time of 170min. Such a concentration is compatible with industrial scheme processes. Higher glucose concentration showed a lower conversion. 1%w/v glucose concentration showed a higher conversion (99%), but the volume was ten time higher. All trials for optimization of other process parameters were based on a substrate concentration of 10%w/v in glucose.

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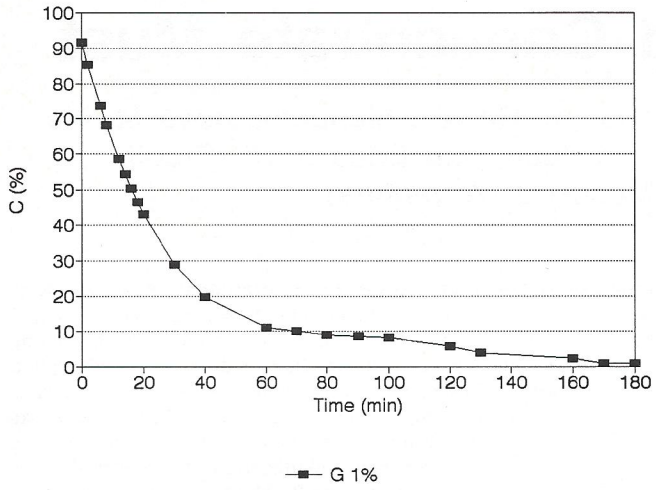
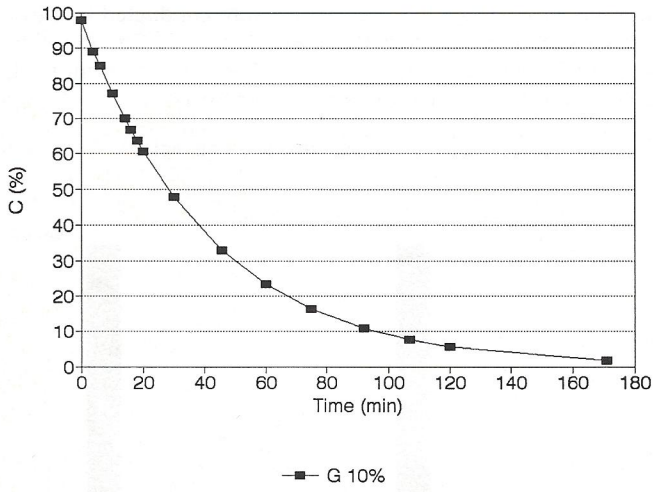


Glucose	1.0%(w/v)
Enzyme	0,4%(w/v)
Temperature	25°C
pH	6.0
Agitation	200rpm
Air supply	1 l/l·min

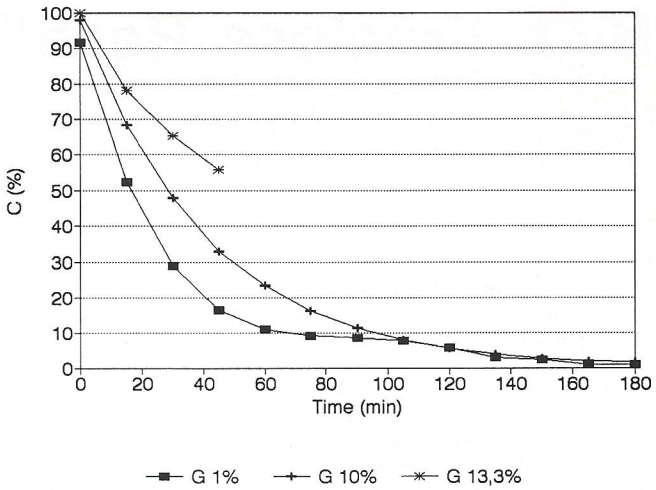
Graphic 1. Basic reaction for HFRCM production.



Glucose	10.0%(w/v)
Enzyme	1,0%(w/v)
Temperature	25°C
pH	6.0
Agitation	200rpm
Air supply	1 l/l·min

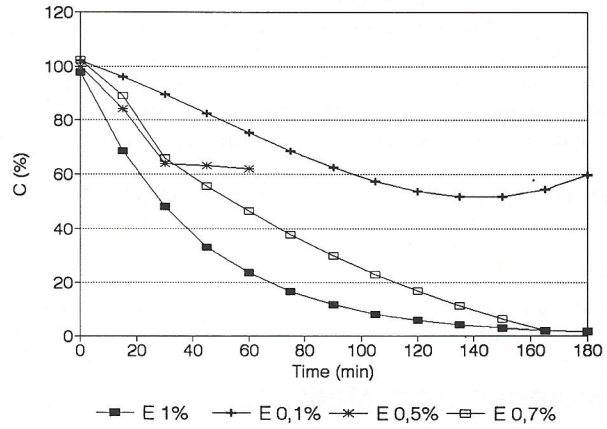
Graphic 2. Basic reaction for HFRCM production.

Enzyme concentration. Trials were carried out at three different enzyme concentration levels (0,1%w/v; 0,7%w/v and 1%w/v). Graphic 4 shows an increase in the conversion rate with the increase of the enzyme concentration. 0.7%w/v enzyme concentration was selected on the base of the results. A conversion of 98% was reached in a period of 100min.
Trials for optimization of the all others process parameters were carried out at an enzyme concentration of 0,7%.
H_2O_2 supply. Assays were carried out at three different values of H_2O_2 supply (20ml/l·h; 40ml/l·h and 62ml/l·h). Graphic 5 shows a conversion of 57% at an addition rate of 20ml/l·h, 91% at 40ml/l·h and 89% at 62ml/l·h. It can be seen that conversion increases when hydrogen peroxide supply increase. 40ml/l·h of H_2O_2 supply was selected on the base of the conversion reached. By the other hand such a value involve a diminution of



Ratio glucose/enzyme	10
Ratio glucose/H_2O_2 supply	1,61(w/v)
Temperature	25°C
pH	6.0
Agitation	400rpm
Air supply	1 l/l·min

Graphic 3. Influence of glucose concentration.



Glucose	10.0%(w/v)
Temperature	25°C
pH	6.0
H_2O_2	62ml/l·h
Agitation	400rpm
Air supply	1 l/l·min

Graphic 4. Influence of enzyme concentration.

35% of H_2O_2 comsuption with respect to the maximum value of H_2O_2 supply.
Air supply: one trial was carried out at H_2O_2 supply of 20ml/l·h, air supply at 2 l/l·min and agitation at 500rpm (Graphic 6).

4 Conclusions

A sirup with a fructose concentration of 98% is obtained. The enzymatic step is fully compatible with the industrial production scheme of rectified concentrate must. Basic variables of enzymatic process have been optimized.

Acknowledgements

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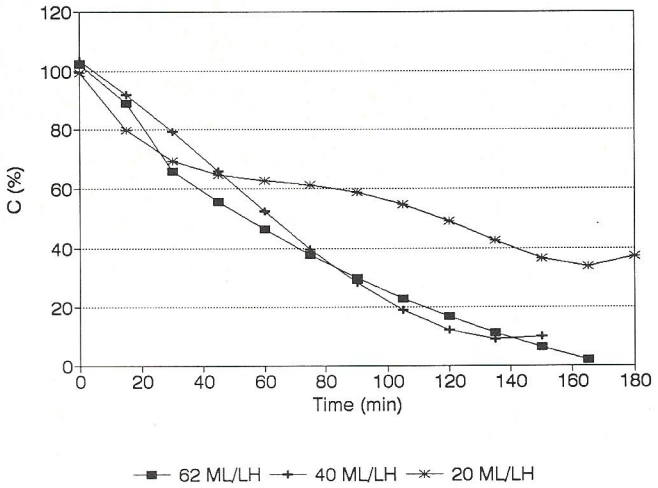
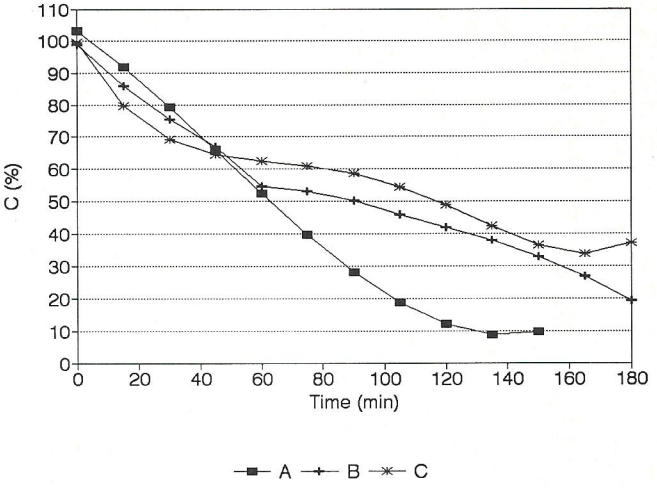


Glucose	10.0%(w/v)
Temperature	25°C
pH	6.0
Enzyme	0,7%(w/v)
Agitation	400rpm
Air supply	1 l/l·min

Graphic 5. Influence of H_2O_2 supply.



Glucose	10.0%(w/v)		
Enzyme	0,7%(w/v)		
Temperature	25°C		
pH	6.0		
	Curve A	Curve B	Curve C
air supply	1 l/l·min	2 l/l·min	1 l/l·min
agitation	400rpm	500rpm	400rpm
H_2O_2 supply	40ml/l·h	20ml/l·h	20ml/l·h

Graphic 6. Influence of air supply.

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