UNIVERSITI PUTRA MALAYSIA

PROPERTIES OF POLYPHENOLOXIDASE OF SUGARCANE AND DEVELOPMENT OF METHODS TO PREVENT DISCOLOURATION OF JUICE

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PROPERTIES OF POLYPHENOLOXIDASE OF SUGARCANE AND DEVELOPMENT OF METHODS TO PREVENT DISCOLOURATION OF JUICE

By

MOHAMMAD SHAMSUL HOQUE

Thesis Submitted in Fulfilment of the
Requirements for the Degree of Master of Science
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DEDICATION

This piece of research work is dedicated as a token of respect and compliment to the happy soul of my deceased father ***Mohammed Mokbul Hossain.***

`O may ***Allah****! Have mercy on him as he did care for me when I was very little, forgive him and protect him from the torment of the grave and the torment of the hell fire and receive him with honour and admit him to the happy place '****Jannatul Ferdous****' ..... **Ameen.***

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Chairman : Assoc. Prof. Dr. Salmah Yusof
Faculty : Food Science and Biotechnology.

The objective of the research was to develop a method to extend the shelf life of freshly extracted sugarcane juice. The work focused on understanding the properties of sugarcane juice as well as determining methods of preserving the colour. Polyphenoloxidase (PPO) from sugarcane juice was extracted, partially purified and characterised. Results indicated that the temperature for optimum PPO enzyme activity was 30°C at pH 7.6. Heat inactivation studies showed that enzyme lost 50% activity by exposure to 80, 75, 70 and 65°C for 1.2, 2.8, 3.6 and 7.8 min, respectively. The use of ascorbic acid (0.5mM concentration), erythorbic acid (0.5mM concentration) and sodium metabisulphite (0.5mM concentration) inhibited the browning reaction 80%, 74% and 92%, respectively.

The effect of different blanching conditions on the quality of juice was also investigated. Sugacane was blanched at various temperatures and time intervals using

both steam (100 °C) and hot water (75 °C, 80 °C and 85 °C). After blanching the juice was analysed for chlorophyll content, colour, PPO activity and tannin content and sensory evaluated for colour and taste. Unpeeled sugarcane stems which were steam blanched for 13±1 min yield the highest quality juice.

A method to prepare an acceptable ready-to-drink bottled cane juice was developed. The process consisted of steam blanching of unpeeled uncut cane followed by the addition of 25 ppm. of ascorbic acid. Shelf life of cane juice with and without preservative (ascorbic acid) packed in HDPE bottles stored at 28°C , 5°C and -18°C for up to 16 days were assessed in terms of physico-chemical characteristics, microbiological quality, colour, viscosity and sensory properties. Results indicated that samples stored at 5 and -18°C retained acceptable colour, flavour and taste for 10-12 days. The physico-chemical parameters, such as pH, TSS, TA, sugars (sucrose glucose and fructose), L, a, b values, and viscosity varied little during 10-12 days storage at 5°C while they remained completely unchanged during 16 day-storage at -18°C.

Abstrak Tesis Yang Dikemukakan Kepada Senat Universiti Putra Malaysia Sebagai Memenuhi Keperluan Ijazah Master Sains.

CIRI-CIRI POLIPHONOLOKSIDASE TEBU DAN PENGEMBANGAN KAEDAH-KAEDAH PENGAWETAN TANPA PEWARNA JUS TEBU

Oleh

MOHAMMAD SHAMSUL HOQUE

Mei, 1998

Pengerusi : Prof. Madya Dr. Salmah Yusof
Fakulti : Sains Makanan dan Bioteknologi

Objektif kajian ini adalah untuk menghasilkan kaedah penstabilan jus tebu bagi penyimpanan jangkamasa panjang. Kajian yang terperinci telah dijalankan ke atas ciri-ciri jus tebu dan kaedah pengawetan warna jus tebu. Enzim poliphenoloksidase daripada ekstrak jus tebu, telah ditulinkan dan diciri. Keputusan yang didapati menunjukkan aktiviti enzim adalah maksimum pada suhu 30°C dan pada pH optimum 7.6. Kajian ke atas penyahaktifan pemanasan pula menunjukkan bahawa enzim dinyahaktifkan apabila terdedah kepada suhu 80, 75, 70 dan 65°C selama 1.2, 2.8, 3.6 dan 7.8 minit masing-masingnya, kehadiran asid askorbik, asid erythorbik dan sodium metabisulfite di dalam jus tebu telah merencatkan tindakbalas keperangan dengan begitu ketara sekali.

Di dalam kajian ini, kesan penceluran pada keadaan yang berbeza-beza ke atas kualiti jus tebu telah dilakukan. Perlakuan penceluran dengan menggunakan stim ataupun air panas (75, 80 dan 85°C) telah dilakukan ke atas tebu. Selepas dicelur jus tebu yang diekstrak dianalisa bagi klorofil, PPO, tannin, warna dan penilaian deria telah

dilakukan ke atas jus tebu yang diekstrak daripada tebu yang dicelurkan. Perlakuan penceluran selama 13 minit telah dilakukan ke atas tebu yang tidak dibuang kulitnya. Adalah didapati hasilan jus tebu yang mengalami perlakuan penceluran mempunyai nilai kualiti yang lebih tinggi daripada hasilan jus tebu tanpa mengalami perlakuan penceluran.

Di dalam kajian ini, satu proses penyediaan jus tebu yang sedia diminum yang telah dibotolkan di mana perlakuan penceluran tanpa pembuangan kulit diikuti dengan penambahan agen perencat enzim telah diperkembangkan. Untuk menentukan hayat penyimpanan jus tebu yang dicelurkan (13minit) dan dibotolkan di dalam botol HDPE samada dengan penambahan bahan pengawet atau tanpa bahan pengawet dan disimpan pada suhu 5 dan -18°C dan pada suhu bilik selama 16 hari, ujian seperti penentuan ciri-ciri pisiko-kimia, kualiti mikrobiologi, warna, kelikatan dan penilaian deria telah dilakukan. Adalah didapati sampel yang disimpan pada suhu 5 °C dan -18°C telah dapat mengekalkan ciri-ciri penerimaan warna, rasa dan aroma selama 12 hari. Daripada analisis yang dilakukan ke atas jus tebu yang disimpan pada suhu 5 °C dan -18°C selama 12 hari didapati tiada perubahan yang ketara pada nilai TSS, pH, TA, L, a, b, gula dan kelikatan jus tebu. Sebaliknya jus tebu yang disimpan pada suhu bilik didapati tidak dapat mengekalkan ciri warna, rasa dan aroma selepas penyimpanan selama 2 hari. Daripada kajian ini, didapati bahawa tempoh hayat penyimpanan selama 12 hari adalah sesuai bagi jus tebu yang disimpan pada suhu 5 dan -18°C. Walau bagaimanapun, didapati jus tebu yang disimpan pada suhu -18°C dapat mengekalkan kualiti penyimpanan selama 16 hari.

CHAPTER I

INTRODUCTION

There are many varieties of sugarcane being cultivated in Malaysia, some are grown for sugar production while others are for fresh juice consumption. Fresh sugarcane juice is very popular as a refreshing drink throughout Malaysia because of the tropical heat, high humidity and temperature.

Sugarcane juice has good organoleptic characteristics like flavour, colour and taste. It has also calorific and medicinal values (Khamar *et al.,* 1965). Sugarcane juice has a great demand in Malaysia throughout the year for its thirst-quenching taste and flavour; if these characteristics are retained in their original form, it can be a very good bottled drink.

Proper methodology is yet to be developed to preserve the fresh cane juice. After a certain period, the extracted juice undergoes marked deterioration in quality in terms of taste, colour and flavour, thus affecting its sensory characteristics. Previous works have suggested that enzymatic browning was mainly responsible for discolouration of sugarcane juice (Smith, 1978) and polyophenoloxidase, copper containing catalyses the ortho- hydroxylation of monophenols and the oxidation of o-diphenols to o-quinones (Mayer and Harel, 1979), is the major enzyme involved

A summary of the literature indicates that little work has been carried out in this area. Khamar *et al.* (1965) initiated work in this area and reported that conventional method of heat processing of the juice imparted a jaggery-like taste and flavour. Rao *et al.* (1936) was of the opinion that changes in colour was due to both enzyme action and metal contamination during juice extraction. According to Mann and Singh (1988), the fresh sugarcane juice when blended with equal quantity of whey/milk improve both the flavour and colour of the drink. They observed that the shelf life of product was lengthened to one week at refrigeration (5-10^{0}C) but beyond this the quality deteriorated rapidly. The microbes proliferated very fast at ambient temperature and fermented the sugars in juice within a few hours, making it sour and unfit for human consumption (Sharma *et al.*, 1989). Thus it is evident that preservation of fresh juice with its keeping qualities intact is still a major problem. Considerable research is needed to address the issue as to how it can be preserved for a longer time. Therefore, the following objectives were set out for this study. These are: -

1. To understand the characteristics of the browning enzyme by extraction, partial purification and characterisation of the enzyme polyphenoloxidise.
2. To determine the effects of blanching on the quality of sugarcane juice
3. To evaluate the stability of sugarcane juice.

If these objectives are achieved, the problem of preservation of sugarcane juice will be partially solved. Thus marketability will be easier and it will be a new economic product in Malaysia

CHAPTER II

LITERATURE REVIEW

Sugarcane

Origin of Sugarcane Cultivation

Sugarcane has been grown since the earliest time. However, nobody knows for certain where it was first cultivated. Researchers generally believe that sugarcane originated either in Northern India (*Saccharum barbara*, Jeswiet), Southeast China (*Saccharum sinense*, Roxb), or in the Malaya Archipelago (Rosenfeld, 1956).

According to Brandes (1956), New Guinea was the home of the *Saccharum* species where it is said to have been grown 8000 years ago as a garden plant, from where it probably spread about 3000 years ago through the Malaya Archipelago, and then to Indonesia and Bengal. Brandes (1956) described the migration of *Saccharum officinarum* that began approximately in the year 8000 BC from the Solomon Island to the New Hebrides, and the New Hebrides to New Caledonia. The second migration which began at about 6000 BC was by way of the Philippines, Borneo, Java, Malaya, Burma to India; and the third, between the years AD 6000 and AD 1100 was from Fiji

to Tonga, Tahiti, the Marquees, Hawaii, as well as others part of Ocean. In Chinese literature, sugarcane was cultivated in 475 BC in Southeast China. The Egyptians, who were skilled in agriculture and chemistry (Deerr, 1950) developed clarification, crystallisation and refining of sugarcane. Sugarcane later reached Morocco, South Africa (AD 755), Sicily (AD 950), Madeira (1420) and the Canaries. Then it spread in the 1500 from Santo Domingo to Mexico, Brazil, Peru and West Indian Island. In 1511, sugarcane was first planted in Cuba. Its cultivation was introduced in Mauritius, Reunia, and Hawaii in the 1700 and Australia, Fiji and South Africa in the 1800. The regions where sugarcane was produced before 1900's include Queensland, New South Wales, Fiji, Hawaii, South and Central America, Mexico, Java, Egypt, Philippines, South Africa, Mauritius, Caribbean's Island and India.

Since the beginning of the century, sugarcane has been successfully established as an important agriculture crop for sugar production. Sugar is an indispensable household commodity, which is widely used in food, beverage, drinks and many other uses. World sugarcane production is given in Table 1

Malaysia started to grow sugarcane on a commercial scale during the 19th century in Perak and Province Wellesley, mainly for sugar production (Tan, 1989). However, the importation of sugar beet from Europe and rapidly developing oil palm caused closure of many sugarcane plantations in 1913. Sugarcane industry was again revived in the 1960's when the Government of Malaysia introduced its Agriculture diversification program to overcome the country's dependence on imported sugar.

Table 1: World Sugarcane Production for Sugar

Region and Country	Area harvested (1000 ha)	Sugarcane Yield (Kg/ha)
World	17606	61108
Malaysia	23	67722
Bangladesh	181	39329
Pakistan	963	46144
Indonesia	405	77778
Asia	7873	58592
India	3578	64561
China	1058	52060
North America	2755	55212
South America	5222	68135
Brazil	4213	66041
Australia	361	90582
Central America	379	76251
Columbia	4213	90625
Thailand	945	39756
Philippines	380	73158

Source FAO (1994, Statistical Year Book)
Statistical Handbook, Malaysia (1993)

Survey teams from Taiwan (1964) and Australia (1965) reported the feasibility of viable sugarcane projects in northern part of Peninsular Malaysia (Tan, 1989) Tables 2 and 3 indicate the increasing sugarcane cultivation in Malaysia. Since 1967 to 1994, total area under sugarcane cultivation has increased manifold from 6000 acres to 18000 hectares (Malaysia Statistical Handbook, 1993)

Table 2: Total Land under Sugarcane Production (1967-1979)

Name	Location	Approx. Area (ac.)	Plantation start
Johor Plantation & Indus	Kulai	6000	1967
Perak Sugars	Dinding	8000	1970
Perlis Sugars	Chuping	20000	1971
Negeri Sembilan Sugars	Bahau	10000	1972
Padang Terap Sugars	Kedah	8000	1973

Source Tan, 1989

Table 3: The Present position of Land Area under Sugarcane Cultivation in Malaysia

Year	Hectare
1980	12705
1986	23318
1987	20489
1988	23970
1989	23000
1990	23000
1991	23000
1992	20000+
1993	18000+
1994	18000+

Source: FAO (1994 Statistical Yearbook)
Malaysia (1993, Statistical Handbook)

Sugarcane Species and Varieties

Five species of sugarcane are cultivated in different parts of the world. These are: -

1. *Saccharum officinarum*
2. *Saccharum robustum*
3. *Saccharum barberi*
4. *Saccharum sinense*
5. *Saccharum spontaneum*

Each of the species can be sub-divided into varieties with different genotype and phenotypic characteristics. *Saccharum officinarum* known as `Noble Cane' is rich in sucrose and low in fibre content. The stems are vigorous and long and are used for

commercial sugar production all over the world. *Saccharum robustum*, found in New Guinea, is similar to the Noble Cane in its stem, leaf, and blossom characters. It is higher in fibre and lower in sugar content than that in *S. officinarum* and hence suitable for agricultural production. The stems are longer and vigorous. *S. robustum* when crossed with *S. officinarum,* produces a popular hybrid which is fertile. *S. barberi* called 'Indian Species' is sturdier than *S. officinarum* and is disease resistant. These qualities, together with higher sugar and ample fibre content are used for breeding purposes to enhance sugarcane production. *S. barberi* has few varieties namely Sunnabile, Mango, Nargori and Saretha. Since they are less important their production and chemical characteristic are not known (Jeswiet, 1927).

S. sinense, known as 'Chinese cane' has few varieties such as Uba, Oshima, Cayania and Zwinga. The colour of the stem is green to greenbronze, and leaves are long and narrow. The species is vigorous and resistant to infection. They have a low sucrose, high fibre content unsuitable for manufacturing purposes. *S. spontaneum* also known as 'wildcane', this variety is short and thin (less than 2-cm thickness), the leaves are narrow and hard, and the plant is very sturdy and resistant to most diseases. It is also known as 'wildcane'. *S. officinarum* has the following varieties: -

1. Otaheik - produced in Hawaii and Peru for many years
2. Cheribon - this comes from Indonesia (Java); the best known and widely distributed is 'black cheribon'. In Latin America it is known as 'Morada' or 'Regencia', in Louisiana purple; variation; light cheribon and striped cheribon.
3. Preanger - this cane originated from Java; the noblest known in Latin

America is Lacristalina (Cuba) and has many synonyms.

4. Tanna-Caledonia - light, dark and striped Caledonia; the best known were white and yellow Caledonia, Australia, Fiji, Mauritius, and Hawaii.
5. Beadle - this comes from New Guinea and from thence to Australia.
6. Black Borneo, Borneo
7. Creole - this is called 'Criolla' in Latin America and is identical with the Indian pure variety.

None of these groups are cultivated commercially at present in the world, although genetically they are resourceful. Old varieties deteriorate in their production capacity and will have to be constantly replaced by more production, newer varieties of sugarcane. According to Buzacott (1965), the reasons for deteriorative change in sugarcane varieties include: -

1. Declining fertility of the sugar producing soil
2. The development of an unfavourable physical condition in soil
3. The cumulative effect of disease and pest, and
4. The existence of symptom less or unidentified disease.

In recent years there have been few varieties developed in many parts of the world. These hybrid varieties grow vigorously with increased yield from previously 20 - 35 tone/ha which are resistant to diseases and pest infestation.

The promising and outstanding varieties of sugarcane grown all over the world include the following: Co740, Co775, Co62174, Co851, Co853, Co951, Co957,