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Modification of Cell Wall Degrading Enzymes from Soursop (*Annona muricata*) Fruit Deterioration for Improved Commercial Development of Clarified Soursop Juice (A Review)

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Abstract

The soursop fruit and the entire soursop plant is associated with a lot of health benefits which includes the killing of cancer cells in cancer patients. The treatment of cancer with soursop has been reported to be accompanied with the risk of Parkinson's disease while soursop juice is considered safe for consumption. The fruit had been associated with preharvest and postharvest deterioration caused by microorganisms and this reduces the total production of soursop fruit. The deterioration process is being accompanied with the production of cell wall degrading enzymes which has the advantage of being used for industrial purposes. One of the important uses of these enzymes is in the clarification of fruit juices. This review proposes the genetic modification of the genes coding for important microbial enzymes for the clarification of soursop juice for improved yield, taste, and colour.

Keywords: Soursop fruit; Health benefits; Soursop tree; Cancer; Cell wall degrading enzymes

Introduction

Soursop fruit as it is commonly called is derived from the *Annona muricata* plant. It has different names depending on different countries such as Graviola, Guanabana, Sauersak and Guayabano [1] (Badrie and Schauss). The tree is found in rain forests throughout Africa, South America and Southeast Asia. In Nigeria, it is commonly found in the Southern part of the country [2] (Abbo et al.). It is a sweet fruit with lots of health benefits associated with not only the fruit but other parts of the plant such as the seeds, the stem, roots and leaves [3] (Degnon et al.). The soursop fruit is very rich in nutrients with high moisture content [4,5] (Rice et al. and Vwioko et al.). Mexico is the largest producer of Soursop in the world and the state of Nayarit is the main producer with 13,022 ton per year [6] (Moreno-Hernandez et al.) (Figure 1).

Source: <http://ronellbridgemohan.files.wordpress.com/2013/04/soursopcancerfruit.jpg>

Important Benefits Associated with Soursop

There are important benefits associated with the soursop fruit (Table 1). The soursop fruit is known for its medicinal value [7] (Paul et al.). Soursop is loaded with nutrients such as amino acids, ascorbic acid, calcium, carbohydrates, iron, phosphorus, thiamine, fibres and riboflavin which can inhibit parasites inside the body. These are vital for the overall development of the body [4] (Rice et al.). The fruit is usually eaten raw but it has been processed into different forms such as the puree, juice, jam and into jellies.

Pre-harvest and postharvest losses of soursop caused by microorganisms

Despite all the benefits derived from the Soursop fruit, preharvest and post-harvest losses due to the activities of various microorganisms has been reported. Postharvest losses of Soursop of up to 60% have been reported [6,8] (Pareek et al. and Moreno-Hernandez et al.) and preharvest of soursop [9] (Amusa et al.). Therefore, different researchers are continually interested in investigating the postharvest treatments that may increase the shelf life and at the same time maintain the

nutritional quality of Soursop. A combination of 1-Methylcyclopropene and wax emulsions was utilized in postharvest handling of Soursop because the combination can preserve the nutritional composition and antioxidant activity of the soursop fruit [6] (Moreno-Hernandez et al.). Microorganisms associated with preharvest and postharvest losses of soursop and other fruits are aided by the actions of cell wall degrading enzymes.



Figure 1: The soursop fruit.

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Health Benefits from Soursop Plant	Part of Soursop Plant
1. The treatment of diabetes	Graviola Fruit
2. The fight against the herpes simplex virus-1 (The virus that causes cold sores)	Graviola Fruit
3. It is used in several Latin American countries as fresh fruits, sweetened juice and in yogurt.	Graviola Fruit
4. It is used externally against neuralgias, rheumatism and arthritic pains.	Graviola oil from unripe fruit
5. It is used as vermifuge and anti-helminthic, against internal and external parasites such as head lice and worms and to combat pea lice. Also, to kill moths and cockroaches	Ground Seeds of Soursop plant
6. The disappearance of the swelling around the cut on incisions after surgery without producing a scar.	Chewed leaves mixed with saliva
7. Selective Growth Inhibitor of human Breast Cancer cells	Graviola Fruit
8. Inhibition of tumorigenicity and metastasis of pancreatic cancer cells	Graviola Drug
9. To sober a drunk person	The leaves in water
10. Drunk on an empty stomach it alleviates liver diseases and leprosy.	Soursop Juice

Table 1: Health Benefits associated with consumption of Soursop Fruit.

Cell wall degrading enzymes and their roles in pathogenicity

The major prerequisite for phytopathogenicity is the ability of the causal agent to secrete enzymes necessary to degrade the components of the plant cell wall [10,11] (Wood and Lisker et al.). Many plant pathogenic fungi and bacteria are known to produce a variety of cell wall degrading enzymes [12,13] (Walton, Cervone and Sethuraman et al.). Cell wall degrading enzymes are involved in tissue maceration of the host tissue [14] (Collmer and Keen). Plant cell wall degrading enzymes play a major role in host-pathogen interaction by allowing the pathogen to gain entrance into the protoplasm of the plant cells where the food substances manufactured by the host plants are kept [15] (Agrios). These enzymes make the prevention of the pathogens through the cell wall possible by allowing them to penetrate the outer barrier formed by the cell walls [16] (Lehtinen).

Various cell wall degrading enzymes are produced by the white-rot fungus *Ceriporiopsis subvermispora* when monitored in shake-flask cultures and these included various cellulolytic, hemicellulolytic and ligninolytic enzymes [13] (Sethuraman et al.). Bruno et al. [17] reported that starvation from both ammonium and glucose resulted in very early expression and secretion of all the cell wall degrading enzymes examined by them. *Phaeosphaeria nodorum* produces a range of cell wall degrading enzymes that enables its penetration and infection of the host tissues [16] (Lehtinen).

Types of pectinases (cell wall degrading enzymes)

Pectic enzymes are among the cell wall degrading enzymes produced by many phytopathogens [18] (Hagar and McIntyre). They degrade the pectic substances of the middle lamella which is made up of galacturonic acid units joined by a α -1,4 glycosidic linkages [19] (Talboys and Busch). The ability of pathogens to produce pectic enzymes capable of degrading the host tissues is an important factor in virulence [10,20] (Wood, Alabi and Naqvi). Pectic enzymes can be divided into two major groups [21] (Dube and Bilgrami). The first group is the de-esterifying enzymes or the depolymerases [22-24] (Rexova-Benkova, Markovic, Chesson and Rose). The pectinesterases remove methoxyl groups hydrolytically from pectin or pectinic acid to give methyl alcohol and pectic acid [25] (Bateman and Miller). The chain splitting enzymes are further divided into two categories, the polygalacturonases (PG) and the pectin transeliminase (PTE) (Dube and Bilgrami). Polygalacturonases cleaves the bonds by hydrolytic action with the introduction of water across the oxygen bridge. Pectin transeliminase cleaves glycosidic bonds by transelimination reaction which leads to the formation of double bond between C4 and C5 in

one of the products. Goel and Mehvotva [26] reported that pectin methylesterase occurs in healthy tissues while polygalacturonase occurs only in infected tissues. Pectic enzymes have been implicated in tissue maceration and cell death [27,28] (Albersheim et al., Talboys and Busch). The pathogenicity of those organisms that cause infection is linked to the ability to elaborate pectic and cellulolytic enzymes [11,28] (Lisker et al., Ishii and Kihō) however, Urbanek and Zelewskia-Sobezak [29] reported that the initial disintegration of plant cell wall tissues result from the hydrolytic action of polygalacturonase.

Mahmood and Greenman [30] reported that a strain of *Bacillus subtilis* produced pectic enzymes from the crude peel of orange and potato. Igbasan and Guenter [31] reported that the addition of a combination of pectinase and β -galactosidase as supplement to the nutritive value of peas for broiler chickens improved the growth rate of broiler chickens. Purified pectic enzymes produced by *Rhizoctonia fragariae* and *Botrytis cinerea* were used as a selective tool for *in vitro* recovery of strawberry plant with fungal disease resistance [32] (Orlando et al.). Laats et al. [33] observed that pectic enzymes are present in green beans and they often influence the texture of the green beans after commercial sterilization. Cano et al. [34] studied pectic enzymes in strawberry and orange products and reported that they can be inactivated by high pressure and temperature effects. The synthesis of pectinase using *Aspergillus niger* can be enhanced by optimizing the carbon and nitrogen sources present in the medium [35] (Nair and Panda). Pectinases in combination with other enzymes are important in obtaining higher palm kernel oil yield during extraction [36] (Tano-Debrah and Yashiyuki). Pectinesterase obtained from soursop (*Annona muricata*) pulp was purified using the techniques of ammonium sulphate fractionation, ion-exchange chromatography and gel-filtration [37] (Arabaisah et al.).

Pectinases are important in host tissue maceration and cell death during pathogenesis [14] (Collmer and Keen). The enzyme polygalacturonase, cellulase, pectin methylesterase and pectin transeliminase play important roles in the deterioration of cocoa beans by *Penicillium steckii* [38] (Olutiola). Some environmental and chemical factors greatly influence the activity of pectic enzymes as shown by many researchers [11,20] (Lisker et al., Alabi and Naqvi). Temperature and pH are important factors influencing the activity of pectinases [39] (Dixon and Webb).

Cell wall degrading enzymes and soursop juice

The use of enzymes in the juice industry has contributed immensely to yield increase and the production of various types of juices while

the production of enzymes that are able to remove bitterness of citrus juice, extract pigments amongst other applications is gaining increased interest in the juice industry [40] (Ribeiro et al.). Different enzymes have been employed for the clarification of various fruit juices.

Are there disadvantages associated with the consumption of soursop?

There is the school of thought that when chemotherapy fails, soursop fruit can be tried. Previous researchers have observed that the smell of soursop fruit is very similar to that of chemotherapy drugs and there are several reports that it is a powerful weapon against cancer whereby it kills the damaging cells up to 10,000 times more efficiently than chemotherapy drugs because graviola is known to attack cancer cells without harm to the healthy cells [6,41] (Montroc, 2014; FDA, 2014). Despite all the benefits from soursop fruit, there are previous reports that alkaloids extracted from soursop fruit may alter the function and survival of nerve cells and therefore probably contribute to the development of movement disorders similar to Parkinson's disease. This is because the consumption of the fruit had been linked with harmful effects on the nervous system because of the alkaloids found in Graviola [42] (Lannuzel et al.). Alkaloids are a class of naturally occurring chemicals, mostly made up of nitrogen atoms.

Advantages of Soursop Juice

Soursop juice is highly encouraged [43] (Nwachukwu and Ezeigbo). This is because fruit juices are important sources of nutrients and they contain several important therapeutic properties that may reduce the risk of various diseases. Soursop juice has many therapeutic properties which includes diuretic, antiurethritis, antihaematuria, antibacterial, anticancerous, astringent, sedative, and anti-aging. They contain large amounts of antioxidants, vitamins C and E and the juice possess pleasant taste and aroma [2] (Abbo et al.). When taken on an empty stomach, soursop juice is believed to alleviate liver diseases and leprosy and the juice from the fruit is also used to increase milk secretion in breast-feeding mothers. Soursop fruit juice is rich in nutrients such as amino acids, vitamins, fibre, proteins, unsaturated fats and essential minerals [4,9] (Rice et al. and Amusa et al.).

Clarification of fruit juice

Clarification of fruit juice is a process whereby the juice from fruits is filtered to remove the pulp from the fruit. It can be carried out by centrifugation or by enzyme treatment. Centrifugation achieves a separation of particles in suspension in the juice and can be considered as a pre-clarifying step. This operation is carried out in centrifugal separators with a speed of 6000 to 6500 RPM [44] (FAO, 2008) while enzyme clarifying is based on pectic substance hydrolysis which decreases the juices' viscosity and facilitate the filtration. The treatment is the addition of pectolytic enzyme preparations in a quantity of 0.5 to 2 g/l and will last 2 to 6 hours at room temperature, or less than 2 hours at 50°C, a temperature that must not be exceeded. The control of this operation is done by checking the decrease in juice viscosity. Sometimes, the enzyme clarifying is completed with the step called "sticking" by the addition of 5-8 g/hl of food grade gelatine which generates a flocculation of particles in suspension by the action of tannins [45] (FAO, 2013). The juice from different fruits had been clarified with different enzymes with increased yield, decreased viscosity and turbidity [46-48] (Pal, Khanum, Kareem, Adebowale and Nakkeeran et al.). Available reports on the clarification of soursop juice with microbial enzymes are scanty. Khan et al. [48] reported that an understanding that enzymatic degradation of fruit

pectin can clarify juices and improve juice yields resulted in the search for microbial pectinase and their application in vegetable and fruit processing industries and the identified enzymes were classified on the basis of their catalytic activity to pectin or its derivatives and in terms of industrial use. Sandri et al. [49] analyzed the efficiency of fungal pectinolytic preparations produced in the laboratory and commercial products used for the clarification of apple, Butia palm fruit, blueberry and grape juices and they reported that the crude enzymatic extracts produced by *Aspergillus niger* and *Aspergillus oryzae* in comparison with commercial preparations of Pectinex[®] Clear and Pectinex[®] BE produced similar results. Their results indicated the potential of *A. niger* T0005007-2 enzymes for biotechnological application in fruit processing and for the juice production industry. A Protease (Enzeco) and a pectinase (Pectinex Smash[®]) for clarification of cherry juice [50,51] (Pinelo et al.), a novel acidic and low-temperature active endopolygalacturonase from *Penicillium* sp. CGMCC 1669 for clarification of apple juice (Yuan et al.), the clarification of orange juice by crude fungal pectinase from citrus peel by *Rhizopus oryzae* [47] (Kareem and Adebowale), Polygalacturonase from *Aspergillus carbonarius* for apple juice clarification [52] (Nakkeeran et al.), xylanases for enrichment of citrus fruit juice clarification [53] (Dhiman et al.) and a combination of xylanases from *Aspergillus niger* DFR-5 with pectinase and cellulase for clarification of pineapple juice [46] (Pal and Khanum). Joshi et al. [54] studied the isolation, purification and characterization of pectinase (Pectin methyl esterase) produced from apple pomace and they reported that the addition of pectinase significantly increased the colour, total soluble solids (TSS), titratable acidity and total sugars in the enzymatically extracted juices with desirable activity of the enzyme. Seifollah and Khodaverdi [55] reported the presence of pectinesterase in citrus fruit particularly in cloud loss of citrus juice which is one of the most intensively studied problems in food technology and they therefore carried out a study on soursop pulp pectinesterase and its effect on cloud stability of soursop juice because of paucity of information on the enzyme in soursop. The clarification of fruit juices or the different stages of fruit juice processing is not only limited to enzymes from fungi. Prathyusha and Suneetha [56] identified and reported the involvement of pectinolytic bacteria employed for fruit juice processing. Nagar et al. [57] reported the efficacy of absolutely purified xylanases obtained from a hyper-produced *Bacillus pumilus* SV-85S was studied on juice enrichment of apples (*Malus domestica*), Pineapples (*Ananas comosus* L.) and tomatoes (*Lycopersicon esculentum*). Also, pectinolytic yeast (*Saccharomyces* sp.) had been associated with the production of cold-active polygalacturonase at room temperature (25°C) at 5°C. This is recommended for fruit juice clarification at cold (mild) conditions because it is a cold active enzyme and because the enzyme is produced at room temperature by a yeast considered as a GRAS (Generally regarded as safe) organism [58] (Padma et al.). Yeasts are an attractive source of pectinases for industrial applications and they have attracted a great deal of attention from various researchers worldwide as an alternative to fungal pectinases [59] (Alimardani-Theuil et al.). There are other technologies used for fruit juice processing. Membrane technology is used mainly to clarify the juice by means of ultrafiltration and microfiltration and to concentrate it by means of nanofiltration and reverse osmosis [60] (Echavarria et al.). Laorko et al. [61] studied the effects of membrane property on the permeate flux, membrane fouling and quality of clarified pineapple using both microfiltration and ultrafiltration membranes. They reported that membrane filtration did not have significant effects on the pH, reducing sugar and acidity of the clarified juice while the suspended solids and microorganisms were completely removed. The use of non-thermal technologies such as UV irradiation is advantageous with low energy dosages which

are a valid alternative to thermal processing to eliminate pathogenic microorganisms while still maintaining quality in reconstituted apple juice [62] (Caminiti et al.).

Genetic modification of enzyme for juice clarification

Discovery of gene sequences that code for enzymes, protein engineering and molecular biology tools had also resulted in defined microbial strains that over-produced enzymes for cost effective technologies [48] (khan et al.).

Conclusion

This report reviewed literature and summarized the importance of the soursop tree, bark, its fruits, leaves and juice, the activities of pectin-degrading enzymes and their industrial applications in juice clarification and the role of soursop fruit in the treatment of cancerous cells. The genetic modification of microbial enzymes for the clarification of soursop juice for improved commercial development is therefore proposed.

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