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Aconitic acid from sugarcane: production and industrial application

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ACONITIC ACID FROM SUGARCANE: PRODUCTION AND INDUSTRIAL APPLICATION

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LIST OF ABBREVIATIONS AND ACRONYMS

LATIN

AU:	Absorbance unit at 420 nm
ASI:	Audubon Sugar Institute
ASCL:	American Sugar Cane League
ASTM	American Society for Testing and Materials
ATBC:	Acetyl tributyl citrate
Ca:	Calcium
CLM:	Cane leaf matter
DEHP:	Di(2-ethylhelyl) phthalate
DINP:	Di-isononyl phthalate
DMA:	Dynamic Mechanical Analysis
DS:	Dry substance
DSC:	Differential Scanning Calorimetry
EPA:	Environmental Protection Agency
F:	Free energy of solution
FDA	Food and Drug Administration
GC:	Gas chromatography
GC-MS:	Gas Chromatography- Mass Spectrometry
H:	Enthalpy
HPIC:	High performance ionic chromatographic
HPLC:	High performance liquid chromatographic
ICUMSA:	International Commission for Uniform Methods

IU: ICUMSA units

IV: Index value

K: Potassium

LOC: Limiting organic concentration

LLE: Liquid-liquid extraction

Mg: Magnesium

MIBK: Methyl isobutyl ketone

MIK: Methyl ethyl ketone

M_w: Molecular weight,

NaOH: Sodium hydroxide

NIST: National Institute of Standards and Technology

NSW: New South Wales

OA: Organic/aqueous ratio

P: Distribution coefficient

Phr: Part per hundred parts of resin

pK_a: Acid dissociation constant

PVC: Poly(vinyl chloride)

S: Entropy

SBA: Strong base anion

SLM: Supported liquid membranes

Soly: Solubility

T: Temperature, °C

TAA: Trans-aconitic acid

TBA: Tributyl aconitate

TBC: Tributyl citrate

TBP: Tributyl phosphate

TGA: Thermogravimetry analysis

Tg: Glass transition temperature, °C

TFS: Total fermentable sugars

TRS: Theoretical recovery sugar

VR: Raffinate volume after phase separation, mL

VRo: Initial raffinate volume, mL

WBA: Weak base anion

WiA: Mass of component i in the aqueous phase (feed), g

WiO: Mass of component i in the organic phase, g

xiA: Mass fraction of component i in the aqueous phase (feed)

xiO: Mass fraction of component i in the organic phase.

GREEK

ϕ_s: Volume fraction of the solvent

ϕ_p: Volume fraction of the polymer

δ_s: Solubility parameter of the solvent

δ_p: Solubility parameter of the polymer

μ: Viscosity, mPa.s

ABSTRACT

Trans aconitic acid (TAA) is the predominant organic acid in cane leaf matter (CLM) juice. Its concentration is three to six times higher than the level found in sugarcane stalks. The variation of composition in terms of total fermentable sugars (TFS), anions, and cations of the LCP85-384 sugarcane variety during 2003-2006 seasons, as well as the ripener (Polado-L®) effect were analyzed.

TAA content ranged between 2.1-3.1 kg / t CLM. The TFS in CLM juice yielded a fermentation efficiency of 92%, four points lower than with sugar molasses.

Liquid-liquid extraction (LLE), solid-liquid extraction and ion exchange were evaluated for the recovery of TAA from CLM stillage. Tributyl phosphate -dodecane, ethyl acetate and butanol were evaluated for LLE. Maximum TAA extraction yield (92 g/100 g) was observed on acidification of CLM stillage to pH 2.0 with 50 % (v/v) sulfuric acid at an organic/aqueous phase ratio (OA) of 3.5 with butanol as extractant. Tributyl phosphate-dodecane had a similar extraction yield as butanol; however, in this mixed solvent formation of third phase was observed. Ethyl acetate had the lowest extraction yield. The purity of TAA extract in butanol was 32 g/100 g DS. Butanol extract was esterified with either sulfuric acid or a cation exchange resin as catalyst to yield tributyl aconitate (TBA). Resin efficiency was affected by the impurities. The overall yield using sulfuric acid was 84 %. TBA was decolorized with powder activated carbon. The attempts to esterify TAA from spray dried CLM stillage were unsuccessful. The highest conversion yield was only 2.5 %. Dowex Optipore SD-2, a non-ionic adsorbent, showed the best results among the resins and adsorbents evaluated.

TBA and two citrate esters were compared to di-isononyl phthalate (DINP) as plasticizers of polyvinyl chloride (PVC). Thermal and mechanical properties were similar to those observed with DINP.

CLM and leaves together represent up to 50% of the total fiber of cane. An economic model estimated that processing whole sugarcane can be a profitable business if it is transported at distance of no more than 20 miles from the field to the mill.

CHAPTER 1 INTRODUCTION

Research Problem Approach

The sugar industry around the world frequently faces fluctuations in sugar price that makes this industry vulnerable. The possibility of increasing profitability through diversification from traditional products obtained from sugarcane is without a doubt the best pathway to assure its existence in the future. The boom of ethanol around the world has brought a new air to the sugar industry, and it has been reflected in the positive trend of the international sugar price in recent years (Figure 1.1).

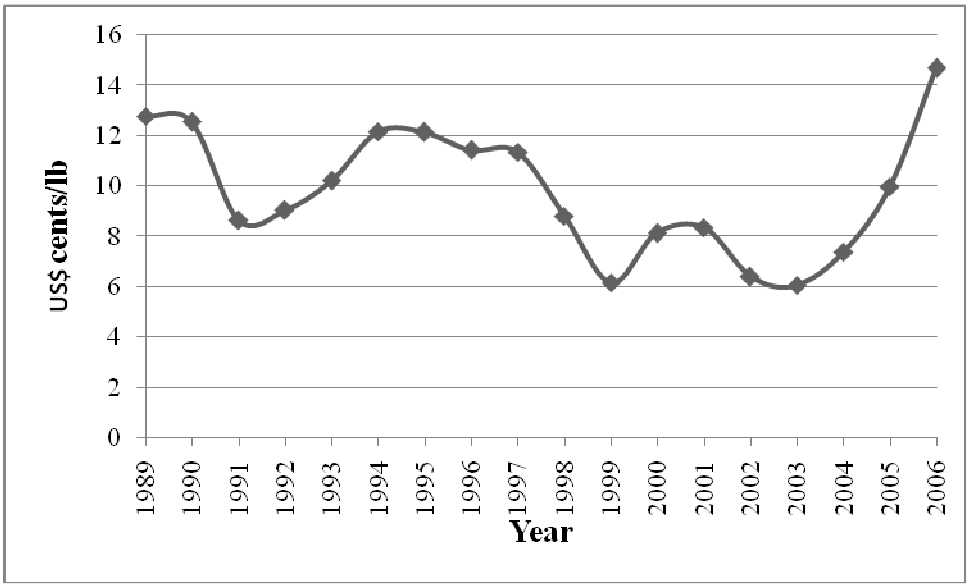

Figure 1.1 Annual average international sugar price 1989-2006 (Source: New York Stock Exchange)

The new environmental laws which prohibit the burning of cane before harvesting (Hassuani, 2001; Briceno et al. 2001), have given the sugar industry the incentive to re-evaluate potential uses for tops and leaves. Tops and leaves were considered in the past to be

trash matter because of their negative impact on the sugar processing, but due to their high fiber content, they can become a source for electric power, chemicals and liquid fuels.

This material referred to in this dissertation as cane leaf matter (CLM) is not only high in fiber but also in trans-aconitic acid (TAA) which is the main organic acid in sugarcane. Its concentration in CLM is between three to six times higher than in stalks. TAA was first recovered from sugar molasses in Louisiana about 60 years ago and used as a plasticizer for poly vinyl chloride (PVC) (Robert and Martin, 1954; Haines and Joyner, 1955). The high price of this product in comparison with plasticizers obtained from phthalic acid made it economically non competitive. The plasticizers from phthalic acid used in medical devices and toys have been recently found to be toxic. Their use has already been prohibited in Europe and others countries (Tullo, 2005). The market for these phthalic esters is around 500.000 tons/year (Di Gangi, 1999). These plasticizers can be replaced by bio-based plasticizers such as those obtained from TAA.

This research focused on three areas: 1) the characterization and extraction of TAA from CLM, 2) the separation of TAA from CLM juice using different separation techniques and 3) the evaluation of tributyl aconitate (TBA) as plasticizer of PVC.

Research Outline

The literature review presented in chapter 2 focuses on sugarcane composition, extraction techniques for organic acids and an overview of plasticizer use for PVC.

Most research has been done on the composition of sugarcane stalk in terms of its sugar content. However, relatively little information is available on cations and anions. The composition of stalk, CLM and leaves in terms of sugars, anions and cations of the sugarcane variety LCP85-384 (the most planted in Louisiana) (Legendre and Gravois, 2007), during

2003-2006 crushing seasons, the effect of a ripener (Polado-L®) on CLM composition, as well as the comparison between sugarcane bagasse and CLM are presented in chapter 3. No data have been reported in the literature on the preparation and extraction of the juice from CLM. A methodology to extract TAA in a pilot mill was developed and it is also presented in chapter 3. Included are two economic models: 1) the extra potential revenue generated from the utilization of bagasse, final molasses and CLM for non traditional uses such as ethanol production and cogeneration; and, 2) a model to estimate the potential revenue for harvesting whole cane using the extra fiber delivered to the mill for cogeneration.

In the early fifties, TAA was recovered as its calcium magnesium salt from sugarcane molasses by crystallization and sedimentation (Raceland mill, LA) (Godchaux, 1949; Haines and Joyner, 1955). However, the low yields and high cost of this process prevented its continued commercialization. More recently, the growing concerns about environmental issues as well as the demand of our society for natural products, prompted by the necessity to find alternatives to crude oil products created the conditions to re-evaluate the feasibility of extracting TAA from CLM. Three alternatives for the recovery of TAA were evaluated: liquid- liquid extraction, solid-liquid extraction and ion exchange. These methodologies as well as a decolorization method and esterification of TAA are described in detail in chapters 4 and 5.

PVC is, after polyethylene, the second most used polymeric resin in the world. Although this resin in its natural state is rigid, the addition of a plasticizer can convert this material into a soft and workable one. The plasticizers used to modify PVC are mostly derivatives of phthalic acid. These plasticizers have been found to migrate from PVC during the life time of the plasticized PVC product and have been found to be soluble in blood and

saliva. For this reason, plasticizers produced from phthalic acid have been identified as toxic at a certain level of exposure (Swan et al. 2005; Ema et al. 2003; Latini, 2000) and have been prohibited in toys and medical devices. A safe replacement for these plasticizers is bio-based plasticizers, which are not only non-toxic but also bio-degradable. In this category are the esters derived from citric acid such as tributyl citrate (TBC) and acetyl tributyl citrate (ATBC). Both are currently used at a commercial level in small quantities. These two, in addition to TBA, produced in this research, were compared to di-isononyl phthalate (DINP), one of the most used phthalate plasticizers. Thermal and mechanical properties of plasticized PVC were compared and summarized in chapter 6.

Finally, chapter 7 summarizes the conclusions of each chapter and presents recommendations for future work.

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CHAPTER 2 LITERATURE REVIEW

Sugarcane

Sugarcane, a type of giant grass, is cultivated in tropical and subtropical countries. It is complex hybrid constituted from at least two of the five species of the genus *Saccharum* (Irvine, 1993). Its composition and yield change from one place to another depending, among other factors, on 1) cane variety, 2) plant cane or stubble and 3) growing conditions such as fertilization, weather, and soil, diseases and uses of plant growth regulator (Rein, 2007). The vegetative period (growing season) can vary from 6 to 8 months in Louisiana to nearly two years in Hawaii (Rein, 2007). Cane is generally harvested during the fall and winter. The length of the season depends of the weather conditions (Rein, 2007). In tropical countries such as Colombia and Peru, cane is processed more than 300 days per year.

For the purpose of this dissertation, sugarcane will be considered as composed of three tissues or materials: 1) clean stalk which is constituted by the cane stalk from ground level to the natural breakpoint with leaves stripped off; 2) top is the part of cane above the natural break point without including the top leaves, and; 3) the dry and green leaves adhering to cane stalk. A term cane leaf matter (CLM) (Figure 2.1) which represents the top with the attached leaves, will be used through out this dissertation. For industrial purposes CLM may include also the green and dry leaves adhered to cane stalk because their separation from the tops may be not practical on the industrial scale.

Sugarcane Composition

Stalk

Stalk represents the traditional millable part of the sugarcane. The total solid content in clean stalk can vary from 24 to 27 g/100 g cane (Clarke, 1993). These solids are divided

into soluble solids, referred later as Brix, whose concentration can vary between 10 to 16 g/100 g cane, and fiber, which can vary from 11 to 16 g/100 g cane (Clarke, 1993). The soluble solids are divided into sucrose and non sucrose components. The sucrose content in cane fluctuates from 7.5 to 15g/100 g cane. The ratio sucrose/soluble solids, known as purity, is a parameter of cane quality. The purity of a good quality fresh clean cane is around 90% (Rein, 2007). The non-sucrose components are mainly glucose and fructose, simple monosaccharides that form sucrose, organic and inorganic ash, and in lower proportion among others by protein, starch, gums, and waxes (Clarke, 1993).

Figure 2.1 Cane Leaf Matter, CLM

Tops and Leaves

Tops and leaves have in common high fiber and ash content with respect to the stalk. However, the sugar content in tops is higher than in leaves due to the higher content of glucose and fructose found in this growing part of the stalk. The composition of these materials is reported only in few studies (Table 2.1) which were done in Louisiana (Birkett, 1965), South Africa (Scott et al, 1978), Australia (Ivin and Doyle, 1989) and Brazil (Finguerut, 2005). Significant differences can be observed among materials, especially in the accumulation of brix and in sucrose % brix (purity). The latter is lower in tops and leaves as

consequence of their non- sucrose compounds. Fiber is higher in leaves independent of its moisture content. The wide range of variability of the composition of tops may be related with the inherent differences in the sugarcane varieties, and with the criteria used to differentiate between top and stalk.

Table 2.1 Composition of clean stalk, tops and leaves g/100g sample

Material	Reference	Brix	Sucrose g/100 g Brix	Fiber	Moisture
Clean stalk	Birkett (1965)	15.3	84.3	13.0	71.7
Clean stalk	Scott et al. (1978)	16.7	88.6	12.8	70.5
Clean stalk	Ivin and Doyle (1989)	16.6	90.8	12.5	70.9
Clean stalk	Finguerut (2005)	19.1	87.4	12.8	68.1
Tops	Birkett (1965)	9.7	56.7	11.2	79.1
Tops	Scott et al. (1978)	6.7	20.9	16.6	76.7
Tops	Finguerut (2005)	9.0	42.7	10.7	80.3
Dry leaves	Scott et al. (1978)	7.8	19.2	58.6	33.6
Green leaves	Finguerut (2005)	7.5	34.4	23.8	68.7

Fiber

Fiber is composed of cellulose, hemicellulose and lignin (Manohar, 1997). Cellulose is a polysaccharide formed by units of glucose; hemicellulose by units of xylose (5 carbons) and in lower proportion by arabinose. Lignin is formed by aromatic phenolic compounds; its main function in the plant is provides the rigidity and hardness (Rein, 2007). Lignin forms a protective association with cellulose; thereby its removal is needed to have cellulose available for further processes.

The residue after juice has been extracted from shredded sugarcane is named bagasse. The average composition of a sample of bagasse from Hawaii is presented in Table 2.2 (NIST, 2001). Its high content in carbon makes bagasse useful for fuel in the boilers, for manufacture of pulp and paper, boards and furfural (Manohar, 1997). Attempts are being made to produce xylitol and carboxy methyl cellulose, at laboratory scale (Manohar, 1997).

Recently, the production of ethanol from the enzymatic hydrolysis of the cellulose and hemicellulose has been studied (Saska and Martin, 2006).

Table.2.2 Sugarcane bagasse composition

Material	g/100 g DS
Cellulose	40.2 ± 3.2
Hemicellulose	21.5 ± 3.1
Lignin	24.2 ± 3.6
Ash	4.0 ± 0.5

Sugarcane in Louisiana

The sugarcane industry in Louisiana is composed of 12 mills, one syrup mill and two refineries (Salassi and Legendre, 2007). In 2006, an estimated 403,402 acres were harvested to produce 12,434,452 short tons of sugarcane. The average yield of cane for each acre harvested was 31.1 short tons of cane per acre (69.7 metric ton/ha). The total production of the 12 raw sugar factories in 2006 was 1, 260,986 short tons of sugar (96 % pol). The average sugar recovery yield at the 12 factories was 10.14 kg sugar (96 % pol)/ 100 kg of cane (Salassi and Legendre, 2007).

Varieties

LCP85-384 was released in 1993(Legendre, 2001); and became a leading variety in Louisiana. It was planted on about 89% of the total area in 2003 (Legendre and Gravois, 2004), increasing the sugar per acre by 30 % or more. In addition with the higher yield, LCP 85-384 is a good stubbing variety (Legendre and Gravois, 2004). Due to the susceptibility of this variety to common rust and vigor reduction, the percentage of the acreage in plant cane crop has decreased, from 91 % in 2004 (Legendre and Gravois, 2005) to only 46 % in 2006 (Legendre and Gravois, 2007), According to 2006 survey on sugarcane distribution by variety and crop, which includes plant cane and stubbles, LCP85-384 is still the leading variety with

73% of the total acreage, followed by HoCP96-540 (14%), HoCP91-555 (5%), L97-128 (4%), and Ho95-988(2%)(Legendre and Gravois, 2007).

Sugarcane Weight Distribution

Eggleston et al. (2007) determined the weight distribution of the sugarcane in terms on clean stalk, tops, green and dry leaves on first stubble crop in the following varieties LCP 85-384, HoCP95-540, L97-128, L99-226 and L99 -233. The sugarcane weight distribution (Table 2.3) matched well with the data reported by Ivin and Doyle (1989) in Australian sugarcane, where clean stalk correspond to 81.2%, tops 6.1% and dry and green leaves to 12.7 %. Significant differences were observed in theoretical recovery, TRS, between the first four varieties and the L99-233(Table 2.4). In terms of tops and green leaves, a higher weight percentage and TRS were observed in LCP85-384, L97-128 and L99-226. Although TRS in these tissues represent only around 12% of the value reached in clean stalk for sugar production (Table 2.4), this value in terms of total sugars recovery,the main parameter for ethanol production, will change dramatically due to a higher percentage of glucose than sucrose in tops and green leaves (please see changes in CLM through the season in results section, Chapter 3).

Inorganic Constituents in Sugarcane

The inorganic constituents in sugarcane are present as soluble ions, salts or constituents of complex organic molecules, or as insoluble compounds. These constituents tend to increase with the age of the cane; their composition and percentage depend among other factors on the variety and soil types. Fine textured and poorly drained soils as Louisiana soils produce cane with higher potassium than well drained soils. The average content of potassium in Louisiana is 1.31 % DS (Clarke, 1993). Phosphate, magnesium and silica are the

most important inorganic compounds from a clarification point of view as these are partially removed. The other ions will remain in solution and become concentrated with processing (Walford, 1996).

Table 2.3 Average weight distribution of sugarcane plant, g/100 g cane

Tissue	LCP85-384	HoCP 95-540	L97-128	L99-226	L99-233
Clean stalk	80.2	83.6	81.4	82.6	83.3
Tops	5.1	4.6	6.9	4.6	5.8
Green leaves	11.0	8.4	8.2	10.2	9.0
Dry leaves	3.8	3.3	3.5	2.7	1.9
Tops and green leaves	16.1	13.0	15.1	14.8	14.8

Potassium

Potassium is the most abundant inorganic compound in the juice, up to 60% of the ash content. Its concentration is higher in the younger parts of the plant and decreases in the older parts of the stalk as can be seen in conductivity ash values in the cane variety CP65-357 (Table 2.5) (Clarke, 1993). Along the stalk its content decreases from 5.7 g/100 g DS in the top to 0.75 g/100 g DS in the basal internodes (Dillevijn, 1952).

Table.2.4 Average theoretical recovery of sugar, Kg

Tissue	LCP85-384	HoCP95-540	L97-128	L99-226	L99-233
Clean stalk	123.6	119.9	121.0	121.7	104.1
Tops	10.4	5.1	15.0	10.7	5.9
Green leaves	4.3	0.7	0.3	6.2	0.9
Tops and green leaves	14.7	5.7	15.3	16.8	6.8

Potassium aids in carbon assimilation, in the transformation and translocation of sugars, synthesis and transformation of proteins and starch formation (Dillevijn, 1952). Potassium is also related to the formation and neutralization of organic acids. It promotes the flavor and quality of many fruits and vegetables (Humbert, 1963).

Table 2.5 Conductivity of sugarcane parts, sugarcane variety CP65-357

Plant part	Juice extraction, %	Conductivity of juice, mhos*10^{-5}
Leaf blade	40.0	1378
Leaf sheath	38.6	1289
Leaf roll	48.2	1525
Stem top	47.6	927
Millable cane		
Top 60 cm	69.3	480
2^{nd} 60 cm	71.3	408
3^{rd} 60 cm	73.6	311
4^{th} 60 cm	71.1	249
Stubble	65.3	238
Dead leaves	37.1	229

Most of all the potassium present in plants is soluble in water, which explains its ability to migrate. The constant changes in potassium distribution in the plant led researchers to concluding that potassium migrates from leaves back to stalk before the leaves become physiologically inactive. The growing top takes the potassium supply from the older leaves and stalk (Humbert, 1963). The water-solubility of potassium plays an important role in its migration; Hart (1934) found the greatest migration of potassium in blades with the lowest moisture content.

Organic Acids

Organic acids represent a significant percentage of the total soluble non-sugars of sugarcane; they are responsible for most of the titratable acidity of the juice, which measures the total concentration of hydrogen ions. The organic acids have an important role in the fundamental life processes of plants (Honig, 1963). TAA, oxalic acid and citric acid are side products in the Krebs cycle.

Organic acids are frequently present in plants in the form of their salts; the actual concentration of free acid is usually low (Benett-Clark, 1933), with the exception of plants

such as lemon where its concentration increase with the maturity while the pH of the juice decrease (Sinclair and Eny, 1945). TAA is the main non- volatile organic acid of sugarcane, with an average of 1.54 % DS in juice (Martin et al. 1960). Acetic acid and lactic acid are not naturally found in cane, their presence is product of microbial infection. These acids increase their concentration in frozen cane (Clarke, 1993).

Some organic acids and their alkali earth salts are barely soluble in water. Reece (2003) found that CaTAA trihydrate was soluble to about 1.5- 1.7 g/100 g water at 25 °C and the solubility of CaMgTAA hexahydrate was 1.3-15 g/100 g water at 25 °C. The solubility of organic acids and their alkali salts increases with the temperature, and it is higher in sucrose solutions than in water (Figure 2.2); however, this changes at higher sucrose concentrations (Bubnik et al. 1995).

Organic acids affect the clarification process due to the sensitivity of this process to variation in hydrogen concentrations. These acids compete for the lime with phosphoric acid, and due to their buffer capacity (the ability to absorb large quantities of lime or other base with a small change in pH) increase the amount of lime required (Honig, 1963) These acids are able to participate in complex reactions with sugars and other organic constituents of the juice; they are associated with the formation of mellasigenic compounds during sugar processing. Oxalic and aconitic acid have been associated with the formation of scale in heaters and evaporators.

Aconitic Acid

Aconitic acid, ($C_6H_6O_6$), M_w 174g/gmol, an unsaturated tribasic aliphatic acid exists in two geometric forms, the trans- isomer, TAA, and the cis- isomer (Figure 2.3). TAA is a white to yellowish crystalline solid, with melting point 195 °C. It is soluble in water and

alcohol. Its solubility in water increases from 18.6 g/100 mL at 13 °C to 110.7 g/100 mL at 90 °C (Patarau, 1989).

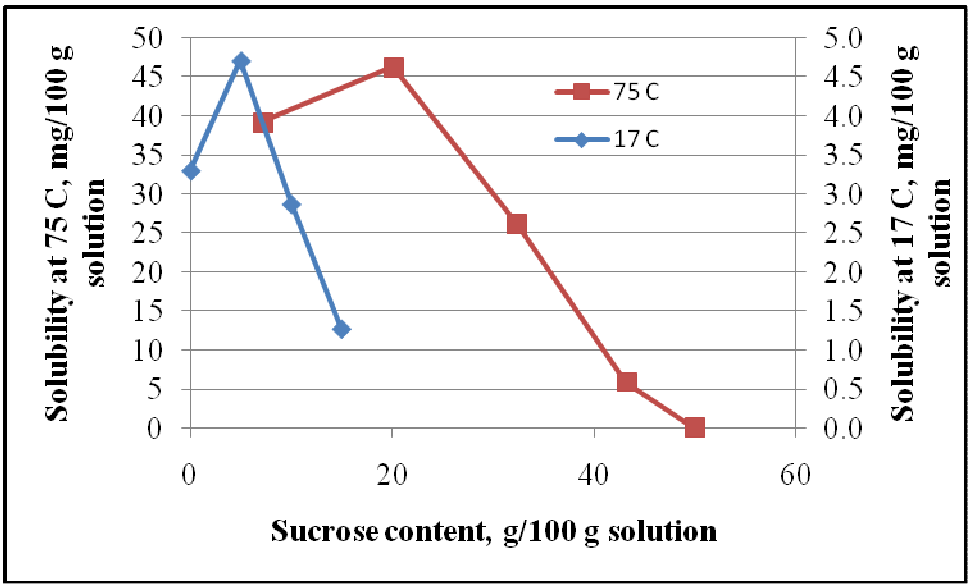

Figure 2.2 Solubility of calcium oxalate in pure sucrose solutions at two temperatures

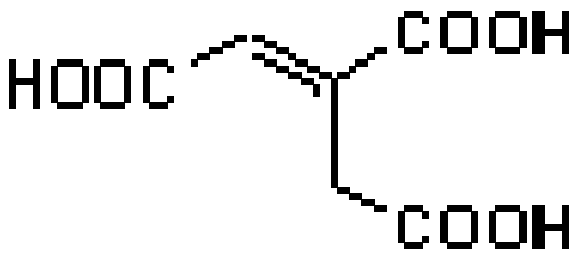

Figure 2.3 Trans -aconitic acid

The amount of cis-aconitic in growing cane is low because it is used in the Krebs cycle and not stored in the plant (Walford, 1998). Some cis acid will be isomerized to TAA while stored in the cell. Any TAA that may be isomerized to the cis isomer will be used in the Krebs cycle, preventing accumulation of the cis isomer in the plant (Walford, 1998). The kinetic of isomerization of trans to cis acid was studied by Walford (1998). He concluded that the isomerization rate increased at high temperatures, 70 – 90°C, and low pH. The presence of

divalent cations such as calcium and magnesium decreased the isomerization rate and the decarboxylation of TAA isomer into itaconic acid.

Fundaments of the Separation Techniques

Liquid – Liquid Extraction or Solvent Extraction

This separation technique is based on the different distribution of the components to be separated between liquid phases. In this process, the component to be extracted is transferred from first liquid phase to second one. In the liquid- liquid extraction process, it is common to use the terms feed or aqueous phase for the solution that contains the component to be separated, in this case the fermentation broths called A; the transferred component (the organic acid) called C, and the solvent or organic phase named B which is the liquid used to extract the component C, from the stream feed. The out streams of this process are the extract stream which mainly contains solvent and the solute and raffinate stream which is the liquid remaining from the feed after the solute is removed (Figure 2.4). The component extracted dissolves in the solvent (organic phase) until its concentration in water and organic phase are in equilibrium. Liquid- liquid extraction (LLE) is used in the separation of components with low volatility such as di and tri-carboxylic acids (Muller, 2002). Two additional steps of liquid-liquid extraction are solvent recovery and raffinate cleanup.

Aqueous Phase Conditions

Among the characteristics of carboxylic acids which affect their extractability are: the number of carboxylic groups and their acid strength, the kind and number of additional functional groups on the molecules and the size and hydration of the anion (Kertes and King, 1986).

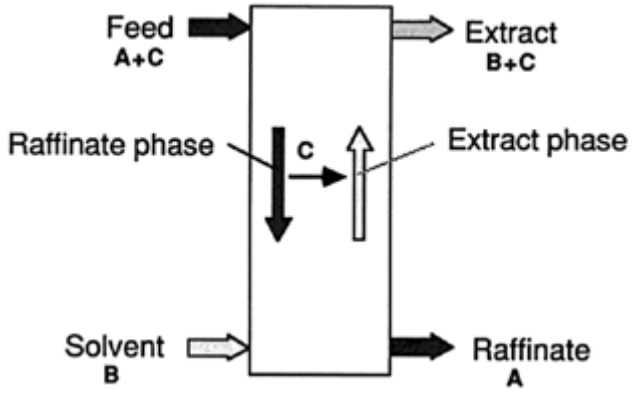

Figure 2.4 Liquid –liquid Extraction (Muller, 2002)

Acid strength is very important due to the fact that only un-dissociated acids are extractable. For di or polyprotic acids the acid strength is determined by the first dissociation constant, the contributions of others being marginal. Table 2.6 shows the dissociation constants K_a and the pK_a for citric, iso-citric, cis-aconitic and TAA as defined by the following equation:

$$pK_A = -\log(K_A) \quad \text{(Eq. 2.1)}$$

At a pH higher than the pK_a organic acids will be dissociated. Monocarboxylic acids are more extractable than di and polyprotic acids with equal number of carbons due to the higher affinity for the aqueous solution of acids with two or more carboxylic groups. The presence of additional groups such as ketones and hydroxyl also increase the affinity for the aqueous phase. On the other hand, the grade of the acid hydration and the energy bond of the water molecules decrease the carboxylic acid extraction (Kertes and King, 1986).

Organic Phase Conditions

The distribution coefficient (P) and the extraction yield (see below) are used to evaluate LLE efficiency. P is the mass fraction ratio of a compound i, in the two phases of a

mixture of two immiscible solvents at equilibrium. The extraction yield, also named partition coefficient, represents the percentage of component i, extracted for the organic phase.

Table 2.6 Dissociation constants and pK_a values for citric, iso-citric, cis-aconitic (Kertes and King, 1986) and TAA (http://www.zirchrom.com/organic.htm)

Organic acid	Molecular formula	Step	pK_a
Citric	$C_6H_8O_7$	1	3.14
		2	4.77
		3	6.39
Iso-citric	$C_6H_8O_7$	1	3.29
		2	4.71
		3	6.40
Cis-aconitic	$C_6H_6O_6$	1	1.95
TAA	$C_6H_6O_6$	1	2.80
		2	4.46
		3	6.30

The distribution coefficient P and extraction yield depend very strongly of the type of solvent used, the size of the molecule being extracted, pH and temperature (Harrison. et al. 2003). P values close to 1 mean that large volumes of solvent would be required and several serial extractions needed for full recovery, so it is desirable to have P as large as possible (Harrison et al. 2003).

$$P = {xiO}/{xiA}$$

Where:

P = Distribution coefficient

xiO= Mass fraction of component i in organic phase.

xiA= Mass fraction of component i in aqueous phase (feed).

$$\text{Extraction yield} = (WiO)/WiA) \times 100$$

Where:

WiO= Mass of component i in organic phase, g

WiA= Mass of component i in the aqueous phase (feed), g

The distribution coefficient of citric and succinic acids in different solvents is shown in Table 2.7. P is low in the aliphatic and aromatic solvents such as diethyl ether and methyl isobutyl ketone (Table 2.7) but higher in alcohols due to their capacity to be acceptors and donors of electrons as acids (Kertes and King, 1986). Using the same solvent, the distribution coefficient is higher for succinic acid than citric acid probably due to the hydroxyl group in citric acid (Table 2.7). However, on average P values are too low for both acids for LLE to be practical.

Ion Exchange

"In ion exchange, ions of a given charge (either cations or anions) in a solution are adsorbed on a solid material (*the ion exchanger*) and are replaced by equivalent quantities of other ions of the same charge that are released by the solid" (De Dardel and Arder, 2002). The solids are the ion exchange resin; they may be a salt, acid or base that is insoluble in water but hydrated. Among some of the applications of ion exchange resins are water purification and separation of antibiotics and organic acids from fermentation broths (De Dardel and Arder, 2002).

Ion exchangers are made of resins; polymers with 0.5 to 15 % of cross-linking (connections between long carbon chains in a polymer by adding i.e. divinyl benzene). The resin has active groups in the form of electrically charged sites. Ions of opposite charge are attracted but may be replaced by other ions depending on their relative concentrations and affinities for the sites. Cation exchange resins are classified according to their active groups as strongly acidic (sulfonic groups) and weakly acidic (carboxylic groups). Anion exchange

resins can also be strongly and weakly basic, i.e. anion exchange resins with quaternary ammonium groups are strongly basic (De Dardel and Arder, 2002).

Table 2.7 Distribution coefficient P of citric and succinic acid at 25^{o}C (Kertes and King, 1986)

Acid/Solvent	P
Citric acid	
Diethyl ether	0.009
Methyl isobutyl ketone	0.09
n-Butanol	0.29
Isobutanol	0.30
Succinic acid	
Diethyl ether	0.15
Metyl isobutyl ketone	0.19
n- Butanol	1.20
Isobutanol	0.96
n-Pentanol	0.66
n-Octanol	0.26

Cross-linking, usually on the order of 0.5 to 15 %, comes from adding divinyl benzene to the reaction mixture during the production of the resin. The size of the particles also plays a role in the utility of the resin. Smaller particles usually are more effective due to an increased surface area but cause large head losses that drive up pump equipment and energy costs. Temperature and pH also affect the effectiveness of ion exchange, since pH is inherently tied to the number of ions available for exchange, and temperature governs the kinetics of the process. The rate-limiting step is not always the same, and temperature's role is still not thoroughly understood (Dudas and Wierzchowski, 1995).

Extraction of Aconitic Acid from Sugarcane By-Products

TAA has been extracted from different sugarcane co- products at a pilot and commercial level. Also, its extraction from synthetic solutions has also been investigated. A summary of the techniques used and results obtained is discussed below.

Precipitation

As of now precipitation has been the only commercial process used to recover TAA. A pioneer in producing commercial quantities of calcium aconitate from sugar molasses was Iberia Sugar Cooperative, Inc in New Iberia, LA (International Sugar Journal, 1945). In 1946, Godchaux Sugars, Inc, at Raceland, LA (Godchaux, 1949; Haines and Joyner, 1955), designed a plant to produce 450 ton of dicalcium magnesium aconitate per year from B and final molasses based in the technology developed by Ambler, Turer and Keenan (1945) and Ventre (1949) of the U. S. Department of Agriculture. The process consisted on diluting the molasses to 55 g/100 g solids in a holding tank where lime and calcium chloride were added. The precipitation of dicalcium magnesium aconitate was helped by heating. The precipitate was separated by double centrifugation and the salt was rinsed with water, finally the salt was dried. The TAA content in the salt was 55 g/100 g of salt; only 38 g/100 g of TAA entering the plant was recovered as its salt, which was the main disadvantage of this process. As a result of the low yield, aconitate esters were sold at prices 25 – 30% higher than dioctyl phthalate (Haines and Joyner 1955), which was the main reason the factory closed sometime after 1955. Regna and Bruins (1956) increased the precipitation of calcium aconitate adding methanol to modify the solubility. They recovered between 70- 75 g TAA/100 g TAA from molasses, at laboratory scale; their modified process was not implemented at commercial level.

Liquid-Liquid Extraction

Several and diverse solvents, such as tributyl phosphate, ethyl acetate, alcohols, ketones, and amines, have been studied to extract TAA from B and final molasses, and from synthetic aqueous solutions. In all cases, the experiments have been conducted at laboratory

level; no experiences from larger scale experiments have been reported. A brief description of the methodology follows and the main results obtained are shown.

Tributyl Phosphate

This solvent has been the most studied to extract organic acids from synthetic aqueous solutions and from intermediate and final molasses. In all the cases, TBP is blended with a diluent to decrease its viscosity.

Malmary et al. (1995) contacted synthetic aqueous solution composed by TAA, citric and malic acid in different proportions, with a mixture of 70% vol. TBP and 30 % vol. dodecane saturated with water. The organic/solvent ratio (OA) varied from 0.5 to 5. The compositions of the simulated aqueous phase are presented in Table 2.8.

They found a direct relationship between the OA and the percentage of TAA extracted (Figure 2.5). At higher extraction, the TAA selectivity decreased, which was reflected in the lower TAA purity. High initial solid concentration help TAA extraction mainly at low OA(see samples with 68% and 70 % of TAA in the feed); However, the TAA selectivity was lower.

Table 2.8 Composition of simulated aqueous solutions A, B, C and D g/100g

Constituents	Solution A	Solution B	Solution C	Solution D
TAA	1.70	1.70	0.30	0.30
Malic acid	0.50	0.09	0.50	0.09
Citric acid	0.30	0.04	0.30	0.04
pH	1.1	1.3	1.7	1.9
TAA purity g/100 g DS	68	88	27	70
DS, g/100 g	2.50	1.83	1.10	0.43

A back extraction of TAA from organic phase was made in two steps, in the first one, the organic phase was stripped with pure water (ratio organic phase: water: 1:4); in this step all citric acid was recovered. In the second one, the remaining solution was stripped with

sodium hydroxide (0.1 N) in a ratio 2:1. The purity of the TAA in the basic aqueous solution was close to 100%. Although is not clearly established by the information reported, the salt, rather than the free form of the acid, could be obtained after back extraction process.

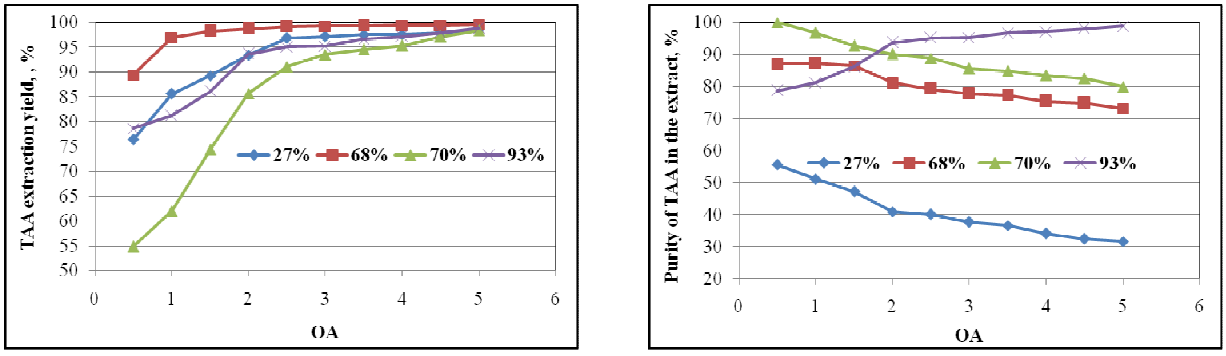

Figure 2.5 TAA extraction yield and purity at different OA and TAA feed purity, Malmary et al. (1995)

Blinco (2000) in her Ph.D. dissertation recovered TAA from diluted final molasses using as an organic phase both TBP and Alamine 336 diluted in Shellsol. In shake flask trials, she found that the mixture TBP and Shellsol 2046 (a non-polar organic liquid composed of paraffins, aromatics and napthenes), showed higher selectivity for TAA, recovering 95 % of TAA in organic phase. For back extraction process, she used as stripping agents different bases such NaOH, Na_2CO_3 and K_2 HPO_4. Blinco (2000) found that TAA recovery is independent of stripping agent and volume ratio (organic phase-stripping agent) as long as the pH of the receiving phase is about 6. TAA% organic acid content in the receiving phase fluctuated between 77 and 82 %. Again, for this process the salt is the final product instead of the free acid. No information was given on the purity of the final product and its color.

Mc Murray and Griffin (2002) extracted TAA from simulated organic acid mixtures and final molasses using supported liquid membranes SLM. Simulated organic acid mixtures

were prepared using TAA, citric acid, oxalic acid, malic acid and glucose in double-distilled water. Equal mass concentrations (25- 40 g/dm^3) were used.

In the SLM process, a membrane, e.g. Acurrel PP flat membrane made from polypropylene with 92.5 ± 17.5 μm of thickness, is immersed in an extract-diluent mixture (TBP and Shellsol) under vacuum conditions between 30 - 60 min. The impregnated membrane is placed between the cells, one called departure phase and the other receiving phase; each one containing a Perspex stirrer. The temperature of the cells is kept constant by using a jacket with water around the cell. The economic advantages of SLM compared with bulk extraction are to combine solvent extraction and stripping process, low energy demand, low capital and cost operations.

After 25 minutes of contact, the extraction yield for TAA and oxalic acid was around 40 g/100 g in the feed, while for citric and malic acids were lower than 10g/100g in the feed. The low values for citric and malic acid were explained in terms of their hydroxyl functional groups that reduces the lipophilicity of these compounds.

No effects were shown on the recovery of TAA when the stirring rate was changed. This means that the transport phenomenon was controlled by the diffusion resistance across the membrane and/or the chemical kinetics and the membrane interfaces.

Mc Murray and Griffin (2002) found that it was better to perform the separation at room temperature due to the high temperature decreases TAA selectivity and the stability of the SLM diminished by decreasing of capillarity and surface forces.

To extract TAA from final molasses, these were diluted with distilled water to 27 and 40 mg/mL of TAA, and then acidified to pH 1.0 with sulfuric acid. The purity of TAA in receiving phase ranked between 40 to 60 g/ 100g total soluble solids, indicating the transport

of some impurities through the membrane. The extraction yield was only 10% after 25 minutes of contact and increased up to 30% after 3 hr. The decrease in TAA transportation rate, compared with synthetic organic solutions rate, was explained by the large number of impurities that fouled the membrane.

SLM technique showed good results when used in simulated organic acid solutions; however, in final molasses, recovery and purity of TAA decreased. This means that additional effort is required to use this technology to extract TAA from final molasses. In addition, the paper did not mention anything about the life time of this membrane.

Ethyl Acetate

Azzan and Radwan (1986) extracted TAA from final molasses, acidified with sulfuric acid up to pH 1.3. After acidification, they neutralized the molasses with calcium hydroxide and then centrifuged. The purified molasses was mixed with ethyl acetate. After heating, the vapors containing TAA and ethyl acetate were collected in a flask. Ethyl acetate was separated in a second distillation by boiling point difference. Finally, the TAA was re-crystallized from glacial acetic acid. The authors reported a 90% TAA recovery efficiency.

Saska and Gil (2006) evaluated the volatility of TAA at different conditions. Only traces of TAA were found the condensates. In addition, with the neutralization of acidified final molasses the organic acids would not be present in their un-dissociated form; the extraction yield was very low.

Hill and Kancharla (1999) recovered TAA from B molasses diluted at 60 ^{o}Brix, acidified with concentrated sulfuric acid at pH 1.3, followed by centrifugation. The supernatant was placed in a liquid- liquid extractor and extracted with sequential charges of ethyl acetate. The solvent was removed from the extract by evaporation and the residue

diluted in hot glacial acetic acid. Finally, TAA crystals were obtained when the solution cooled. TAA extraction yield was around 81% at OA = 2.5.

Hill and Kancharla (1999) also compared the feasibility of extracting TAA from solutions of pure TAA, mixtures of TAA, glucose and fructose and final molasses (Table 2.9). No significant differences were observed in the TAA extraction yield between pure TAA and TAA, glucose and fructose solutions. In contrast, with final molasses, the yield decreased around 11 points at OA=1.5. They observed the formation of a third phase in the final molasses-ethyl acetate system. The volume of the emulsion formed was higher at low OA, TAA losses in the third phase were 34 % at OA =1.0 and, only 2.2% at OA= 4.

Other Solvents

Regna and Bruins (1956) extracted TAA from final molasses diluted to 60 g/100 g and acidified with sulfuric acid. The amount of acid was related to the percent of ash. After 30 minutes of reaction period, calcium sulphate and other impurities were removed. The acidified molasses was contacted in counter current with a solution containing 88% methyl ethyl ketone and 12 % water. The extracted phase was evaporated and the gases containing methyl ethyl ketone and water were condensed and re-used. The mother liquid/solids was centrifuged, the solids containing raw TAA were rinsed with water, decolorized with carbon and then crystallized in a vacuum pan. The TAA obtained had a melting point of 179-182^{o}C. It assays 96.8% and is not ash. However, the paper did not report much data to verify the yield of the process. The author compared the costs to produce TAA by ion exchange and solvent extraction. The results showed that solvent extraction is 24% cheaper than ion exchange.

Vasantdada Sugar Institute (2003) reported on a study on extraction of TAA from a 2% pure TAA aqueous solution. They studied six solvents: ethyl acetate, methyl ethyl ketone

(MIK), methyl isobutyl ketone (MIBK), 1-butanol, n-amyl alcohol and isoamyl alcohol. In all cases, at OA =4. MIBK had the lowest TAA extraction yield, less than 65g/100 g, while ethyl acetate just 65 g/100 g. For the other solvents, the extraction yield was higher than 65 g/100 g. Solvents with moderate miscibility in water (~8%) gave better TAA extraction, while MIBK and ethyl acetate with less miscibility in water (~3%) gave the lower TAA extraction. They also studied the pH effect, with ethyl acetate as solvent, at pH 2.4, the TAA extraction was 65 g/100 g, after decreasing the pH at 1.3, with chloridic acid, the extraction was 75 g/100 g, and after increase the pH up to 6.5 the extraction yield decreased to l7.5 g/100 g, which corroborates that only undissociated acids are extracted.

Table 2.9 TAA extraction yield, g TAA/100 g TAA in the feed, for acidified solutions of pure TAA, and a mixture of TAA and reducing sugars, and final molasses

OA	Pure TAA	TAA + glucose+fructose	Final molasses
0.25	20.4	18.5	-
0.67	39.3	36.8	-
1.00	49.7	49.6	32.2
1.50	61.2	62.9	54.0
2.33	-	-	71.6
4	-	-	76.3

Extraction by Ion Exchange

Regna and Bruins (1956) extracted TAA by passing diluted final molasses, 50 g/100 g solids, through columns containing Amberlite 1R-4B, a weak exchanger in chloride form, which removes aconitic ions. The exhausted resin was eluted with sulfuric acid giving three fractions; the first one was a color fraction containing little TAA, the second one an acid-rich fraction, and the last one a TAA poor fraction. The fraction rich in TAA was concentrated under vacuum conditions and then centrifuged. The raw TAA was rinsed and diluted to 20% solution and then treated with bentonite to remove some color bodies. The clarified solution

was pumped through a decolorizing resin bed and concentrated under vacuum conditions. Finally, the slurry produced was centrifuged, and the TAA obtained was dried. About 64 Kg TAA were taken up per cubic meter of resin. The weakness of the process is that the authors estimated but did not measure the extraction yield, assuming a value of 97%.

Hanine et al. (1992) combined ion exchange and precipitation technology to recovery TAA from sugar cane juice concentrated at 30 g/100 g solids and enriched with pure TAA at 0.7 g/100 mL. The juice was demineralized passing successively through polystyrene absorbent resin, gel-type styrene- DVD copolymer cation strongly acidic resin, and finally by polystyrene hydroxyl form anionic resin. They found that sodium aconitate was eluted at the same time from the anionic resin as polyphenols and colored substances. The regeneration eluates from anionic resins were contacted with an excess on calcium chloride; finally the precipitates were separated under vacuum conditions and dried. The pH effect showed the recovery in the precipitation reaches a maximum at pH 7. At this pH, TAA is recovered with an efficiency of 90 % while at pH 13 where, despite the fact that the TAA is almost 100 ionized, the recovery efficiency was only 68%, due to probable formation of lime and the presence of crystallization inhibitors.

Nothing is mentioned about ion exchange efficiency and purity and final color of TAA. This becomes a long process to recover TAA with effort and generation of side products. The main point here is their simultaneous elution of TAA and phenol compounds and other color bodies from the anionic resin.

Process for the Recovery of Organic Acids

Moore and Sanborn (2004) described in their patent a process to recover organic acids from fermentation broths. Fermentation broths are dried up to 0.1- 10% of moisture content,

by means of a spray dryer, a spin flash dryer or a fluidized bed dryer. The partial removal of insoluble materials is recommended before the drying process. The dried fermentation broth is contacted with lower alcohol such as methanol, propanol or butanol in the presence of an acid such as sulfuric, hydrochloric or nitric. The dehydration and acid reaction process must be carried out between 25 – 60^{o}C. After acid reactions, the insoluble materials can be easily removed by filtration or centrifugation. Further purification processes are not presented in detail. The inventors claim a total recovery yield about 96% of the organic acid and its ester.

Polyvinyl Chloride

Polyvinyl chloride (PVC) is classified as a linear polymer with a slightly syndiotactic structure where chloride atoms are added in an alternating position with respect to the plane of carbon atoms. PVC is formed by the free- radical polymerization of vinyl chloride monomer (Figure 2.6) (Summers, 2005). The slightly syndiotactic structure causes PVC to have less than 10 % crystallinity. This low crystallinity provides PVC with the ability to be plasticized and work as a thermoplastic elastomer with the crystallites acting as the pseudo-cross-links (Summers, 2005). PVC is the most versatile of all thermoplastics. It can be converted either into rigid products of considerable strength and hardness such as pipe and fittings, fencing and packaging sheet or into flexible articles as film and sheet, wire and cable insulation and floor covering among others (Linak and Yagi, 2003). Contrasting with crystalline materials characterized in terms of melting and crystallization temperatures, PVC is characterized in terms of glass transition temperature Tg. Below this temperature PVC it is brittle and elastic and above is like a rubber with a plastic behavior.

PVC is available in different molecular weights, depending on its applications: from MW 39000 (inherent viscosity= 0.51, weight average degree of polymerization, n, = 625),

used for injection molding of thin-walled parts (Summers et al. 1996, and Garcia et al. 2000), to MW = 168000 (inherent viscosity=1.60, n= 2700) used for plasticized PVC having exceptional compression set (Shah et al. 2002). PVC molecular weight is controlled by manipulating the polymerization temperature; the higher the polymerization temperature the lower the molecular weight. Normal polymerization temperatures vary from 50 °C to approx. 70 °C.

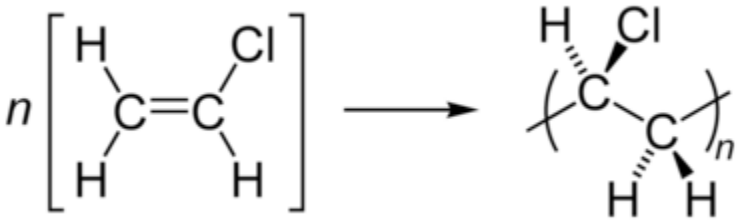

Figure.2.6 PVC polymerization

PVC is more resistant to the combustion and less dependent of prices of natural gas and oil than other polymers due to its high content in chlorine, 57%, (Summers, 2005). PVC is highly insoluble in vinyl chloride. This insolubility makes it possible to produce PVC as porous granules (Witenhafer and Poledna, 2005).

PVC degrades thermally to liberate acetic acid. The colored residue after degradation is presumed to have a polyacetylene type structure. The loss of hydrogen chloride is assumed to start at the chain ends and then to be propagated by a free-radical process along the chain from these terminal sites (Allcock and Lampe, 1990).

PVC Global Market

Worldwide installed capacity production of PVC resins in June 2003 was approximately 32 million metric tones, with 85% of this capacity concentrated in USA,

Asia/Pacific and Western Europe (Smalley, 2005). The worldwide demand of PVC in 2002 was approximately 26.5 millions metric tons. Asia/Pacific was the region with the highest consumption (34%). However, an important portion of this volume was converted in goods and exported around the world (Smalley, 2005). Some 65 -70% of PVC production is used in its rigid state for pipes and construction profile market: flexible formulation is used in cable insulation, packing material and medical devices.

Plasticizers

A plasticizer is usually a clear and colorless liquid of very high boiling point with an oil-like appearance. Chemically, it is an ester made from an anhydride or acid and a suitable alcohol that usually has between 6 to 13 carbon atoms (Titow, 1990). It is inert and very stable over extended periods of time. The main function of the plasticizers is to improve the flexibility and process ability of polymers by lowering the second order transition temperature, named as glass transition temperature (Tg), which is defined as the threshold temperature from a completely rigid state of a material to a state where movement of large segments of composing polymeric chains are allowed to move (Saska and Negulescu, 2004). Plasticizers low molecular weight, resins or liquids, form secondary bonds to polymer chains and spread them apart. Therefore, plasticizers reduce polymer-polymer chain secondary bonding and provide mobility for the macromolecules (Rahman and Brazel, 2004). Plasticizers work by incorporating in the amorphous parts of the polymers while the structure and size of any crystalline part continues unaffected (Krauskopf and Godwin, 2005). Plasticizers reduce the modulus, tensile strength, hardness, density, glass transition temperature, viscosity, electrostatic chargeability and volume resistivity of a polymer, while

simultaneously increasing its flexibility, elongation at break, toughness, dielectric constant and power factor (Rahman and Brazel, 2004; and Krauskopf and Godwin, 2005).

The properties of ideal plasticizers include their high compatibility with polymers, stability in both high and low temperature environments, sufficient lubrication over a wide temperature range, insensibility to solar ultraviolet (UV) radiation, leaching and migration resistant, low cost and finally they should fulfill health and safety regulations. In addition it is required that they have little to no migration tendencies. The interaction plasticizer-polymer generally decreases Tg. The decrease in Tg with the increase in plasticizer concentration is a reference of plasticizer effectiveness. Below Tg, the polymer exists in a glassy state that is characterized by a substructure in which there is minimal chain movement; above Tg, a polymer is in a rubbery state, which is usually characterized by regions with increased polymer chain movement and polymer elasticity (Krauskopf and Godwin, 2005).

Plasticizer Requirements

The permanence of a polymeric plasticizer in a flexible PVC compound depends on its structure, molecular weight/ viscosity and polarity. Polymers composed of branched structures are more permanent than those based upon linear structures. Branching tends to hinder movement or entangle the plasticizer within the polymer matrix making it more difficult to migrate or be removed by volatilization or extraction. Although linear structures provide less permanency, they do yield better low temperature properties (Rahman and Brazel, 2004).

The greater the viscosity/molecular weight of a plasticizer, the greater will its permanence be. Polarity which is roughly defined as the ratio of oxygen to carbon atoms in a plasticizer; it should be properly matched to that of the polymer, in this case PVC. If the

polarity of the plasticizer is not sufficiently similar to that of PVC, varying degrees of plasticizer incompatibility may result. Plasticizers that are somewhat incompatible are more prone to migration, volatilization, and extraction (Krauskopf and Godwin, 2005).

Types of Plasticizers

Plasticizers can be either internal or external. In external plasticizers the molecules are not joined to polymer chain by primary bonds and can be separated by evaporation, migration or extraction. In contrast, internal plasticizers are intrinsically part of the polymer and remain part of the product. Internal plasticizers have problems with maintaining dimensional stability at elevated temperatures (Rahman and Brazel, 2004). Plasticizers may be divided into primary and secondary types. Primary plasticizers are used as the sole plasticizer or as the major component of plasticizer, whereas secondary plasticizers are mixed with primary ones to improve performance properties and/or to lower cost, if used as sole plasticizer, they will be exuded from the blend.

Plasticizers for Phthalic Esters

Phthalic acid esters have been used as plasticizers since 1920 and until now still the most sold. The esters are produced by esterification of two molecules of a monohydric alcohol with one mole of phthalic acid. The polarizable benzene nucleus in phthalates makes these esters highly compatible with PVC. Di (2-ethylheyl) phthalate, DEHP, was introduced in 1930 and since then it has been the most widely used plasticizer of the more than 30 different phthalates esters on the market. Phthalates account for 92% of plasticizer production worldwide, whose market was estimated in 4.6 million metric tons by 2003 (Smalley, 2005). DEHP production represents 51% of the phthalates market (Rahman and Brazel, 2004). PVC accounts for 80 – 90 % of world plasticizer consumption (Bizarri et al. 2003).

Health and Environmental Concerns of Plasticizers

Several investigations have found that phthalates slowly migrate out of many plastic products; because they are not at all bound to the PVC molecule (Krauskopf and Godwin, 2005). The mechanism controlling the plasticizer migration will be the lowest rate between the loss occurring at the surface and the rate which plasticizer diffuses to the surface (Krauskopf and Godwin, 2005). Plasticizer losses in oily media, in which plasticizers are highly soluble, are controlled by diffusivity rates. Leaching of DEHP from PVC medical devices into medical solutions and deposition in the tissues was reported approximately 40 years ago among others by Jaeger and Rubin (1972) and Hillman et al. (1975). DEHP has been found to leach out of PVC as function of temperature, amount of plasticizer present, storage time while in contact with medical solutions, kind of medium storage and agitation of the device (Tickner, et al. 2001). The market for phthalate esters in medical devices represents around 10%. Blood bag and tubing in blood bag contain among 65 to 80 % wt DEHP while intravenous storage and catheter among 30 – 40 % wt DEHP (Di Gangi, 1999).

Phthalate esters have been studied in detail in the last two decades for environmental groups and related to potential carcinogenicity and possible endocrine modulating effects (Latini, 2000; Sheftel, 2000; NIH, 2001). They found carcinogen effects of DEHP on experimental animals. DINP is the plasticizer most widely used in teething rings. The results of an experiment administrating this plasticizer to laboratory animals at high doses showed DINP caused cancer and damaged the liver and other organs.

Swan et al. (2005), in a study that included 85 male babies, found that those babies whose mothers were exposed to high levels of four phthalates, DEHP, dibutyl phthalate, benzyl phthalate and isbotuyl phthalate had shorter anogenital indexes (the anogenital

distance adjusted by weight) and were more likely to have small genitalia and partially un-descended testicles. These results are in agreement with those found in rodent studies after high dose phthalate exposure (Ema et al. 2003, Gray et al. 2000, and Mylchreest et al. 2000). The higher dose phthalate exposure in rodents, compared to humans, is explained by the fact that rodents have a higher metabolic rate and more rapid inactivation of toxicants. Factors found to be inversely correlated to body and size were higher than in humans (White and Seymour, 2005).

Regarding phthalic anhydride, the U.S. Environmental Protection Agency (EPA) determined that: "Exposure to phthalic anhydride may occur during its use as a chemical intermediate in the plastic industry. The acute (short-term) effect from exposure to phthalic anhydride in humans consists of irritation to the eyes, respiratory tract, and skin, but no permanent injury is observed. Chronic (long-term) effects observed in workers exposed to phthalic anhydride included conjunctivitis, rhinitis, rhino conjunctivitis, bronchitis and irritation of the skin and mucous membranes of the respiratory tract. Animal studies indicate that chronic exposure to phthalic anhydride vapor causes congestion, irritation, and injury to lung cells. No studies are available on the reproductive, developmental or carcinogenic effects of phthalic anhydride in humans. EPA has not classified phthalic anhydride for carcinogenicity."

On sources of potential exposures, the EPA report says: "Exposure to phthalic anhydride may occur during the manufacture of phthalate-derived products. It has been suggested that exposure to phthalic anhydride may occur from the use of plastics from which phthalate plasticizers are leached, specifically certain medical plastics such as blood bags,

plastic syringes and plastic tubing. Phthalate esters have been identified as environmental pollutants."

As a result of the studies indicating the adverse health effects in humans of phthalates esters, their use has begun to be prohibited. In Europe, starting in 2006, three phthalate esters including DEHP were permanently banned for use in toys, and another three including di-isononyl phthalate (DINP) were prohibited in toys that can be mouthed. Japan also has prohibited DINP and DEHP in toys as well as DEHP in food-handling gloves. In USA, California has proposed similar laws of phthalate toys (Tullo, 2005).

Alternative Plasticizers

Potential substitutes for phthalate esters are among others trimellitates, aliphatic dibasic esters, phosphates, benzoates, citrate esters, polymeric plasticizers, sulfonic acid, chloroparaffins and sorbitol (Tullo, 2005). However, none of these substitutes offer the broad application that phthalate esters do. In addition, adipates, trimellitates and citrates are up to 3 times more expensive than DEHP. The plasticizer may form up to a third of compounded PVC. Therefore, an alternative plasticizer can increase the cost from about US$ 1.80 to about US$ 2.1/kg. Tullo (2005) mentioned that alternatives to replace phthalate esters will not be researched in depth; until prohibition on phthalates will be imposed on industry.

Aconitate Esters

The presence of the three carboxylic acid groups in TAA gives it a high compatibility with resins (Magne and Mod, 1946); this was the reason why aconitate esters with high molecular weight were produced from calcium magnesium aconitate (Hanson and Coggin, 1942; Cox, 1947) salt recovered from sugar cane molasses at Godchaux Sugars, Inc., Raceland, Louisiana (Haines and Joyner, 1955). Among the alcohols used for the

esterification were n-propyl, n-butyl, n-amyl, iso-amyl, and 4-methyl-2-pentyl. These esters were evaluated as plasticizers of PVC. The results showed that they could replace DEHP, due to their high performance and unique light stability. However, the high cost of aconitate esters, 25 - 50 % higher than phthalates ones, due to low efficiencies and high cost recovery from sugarcane bio-products (Haines and Joyner, 1955), caused the aconitate esters to be withdrawn from the market.

Citrate Esters

Citrate plasticizers esters such as triethyl citrate, acetyl triethyl citrate, tributyl citrate (TBC), acetyl tributyl citrate (ATBC) and tri-(2-ethylhexyl)-citrate are used to plasticize vinyl resins in applications including medical equipment and packaging films due to its poor oil extraction properties compared with DEHP. The FDA approves both the acid and its ester as additives in food.

Of the approximately 230,000 tons of citric acid used annually in Western Europe, 58% is used in food and beverages, 24% in household detergents and cleaners, 9% in pharmaceuticals and 9% for industrial uses such as plasticizers.

TBC is used in PVC, polyvinyl chloride/vinylidene chloride copolymers or polyvinyl chloride/vinyl acetate resins, which are subsequently used for items such as food-wrapping film. Some advantages of TBC are its heat-stability and unchanging color when being processed in compounded resins. ATBC is used in electrical coatings and casings because of its solvating characteristics. It is also used in the production of inks. Acetyl tributyl citrate and acetyl triethyl citrate are also used in hair sprays and aerosol bandages. Triethyl citrate has applications in the food industry as flavor and flavor emulsion.

Production and Evaluation of PVC- Plasticizer Blends

The formulation for the production of PVC- plasticizer blends includes, in addition to PVC powder and the plasticizer, other reagents with specific functions such as fillers, stabilizers and some miscellaneous compounding ingredients. The main function of fillers is to reduce cost. However some of them confer desirable characteristics to the product such as improving the insulation resistance. The principal requirement of fillers is a degree of uniformity. It should be as white as possible, particle size, no more than 3- 5 μm, with low plasticizer absorption. Filler to be used is calcium carbonate. In addition, calcium stearate is used as lubricant and stabilizer of heat and light. Paraloid K-120 is used as processing aids, contributing to improve the mixture and to reduce or eliminate the melt fracture (Titow, 1990).

The physical/mechanical properties of polymers depend on both environmental factors and the chemical composition of the polymer. Structural properties of the polymer include molecular weight, cross linking and branching, crystallinity and crystal morphology, type and amount of plasticizes and the use of additives or fillers. Environmental factors included are: temperature, time, rate of polymer stress, pressure, stress and strain (some change in shape), and amplitude (Sears, 1982).

The degree of plasticization of the blend is evaluated by determining Tg. Some polymers are used above their Tg, in the rubbery state, where they are soft and flexible; others like hard plastics are used below their Tg; this is in their glassy state, the higher the percentage of plasticizer, the lower Tg value.

The properties of PVC-plasticizer blends can be evaluated by thermal and physical/mechanical analysis. Thermal analysis consists of a group of methods where the

chemical and physical properties of a blend are measured in function of temperature or time while the sample is subject to a controlled temperature program. Among these methods are differential scanning calorimetry (DSC), dynamic mechanical analysis (DMA) and thermogravimetry analysis (TGA).

DSC characterizes melting and softening behavior of a blend, by measuring the heat absorbed or given off as it is heated or cooled under a controlled temperature and atmosphere (Cassel, 2002). DSC is used to measure the Tg in rigid or un-plasticized materials, which have high Tg values, i.e. 75 – 85°C for un-plasticized PVC. When plasticizer is added the Tg value will decrease until room temperature values or lower. Low Tg values are more difficult to measure by DSC, because this method measures the heat capacity difference between the two states of the material, i.e., before and after the Tg threshold, read on the curve as the half temperature of inflexion. Just in this case it is better to use DMA.

Polymers are viscoelastic materials; they have the properties of both solid and liquid states (Allcock and Lampe, 1990). In these materials, the relationship between stress and strain depends on time. The deformation (strain) lags behind the applied stress. DMA determines the storage modulus E′ that quantifies the elastic component; loss modulus E″ that reflects the viscous character of the sample (Figure 2.7). The change of the viscoelastic properties of the material will correspond to its Tg. The loss factor or loss tangent calculated as the tangent of the phase angle δ or as the tangent of the ratio between E''/E' it is known as Young's Modulus. This constant is characteristic of each polymer blend; its value gives an idea of the stiffness or rigidity. High modulus means high rigidity and therefore a considerable stress will be required to cause a deformation (Allcock and Lampe, 1990). The phase angle is a function of temperature, time or frequency (at which the dynamic force is

applied). For purely elastic material the phase angle will be zero while for purely viscous material this angle will be 90^{o} (Figure 2.8).

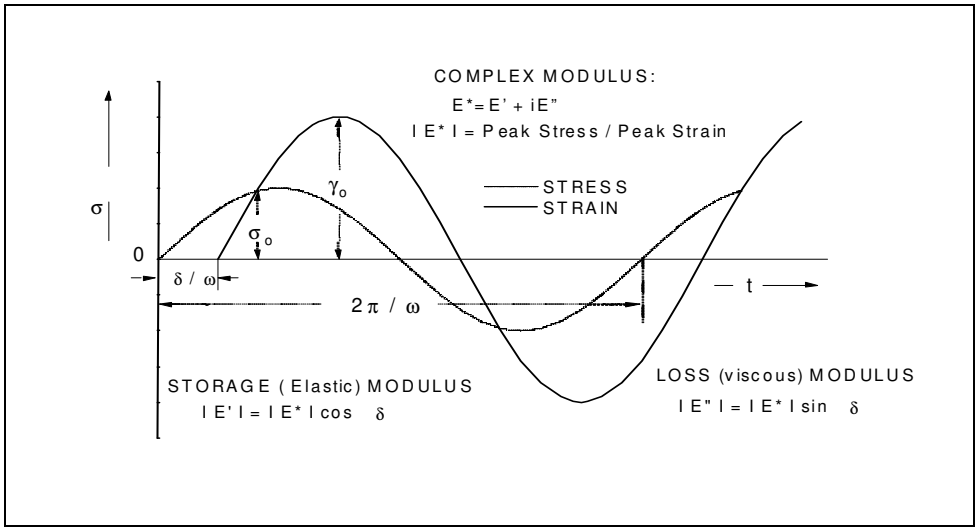

Figure 2.7 Schematic representation of the stress (σ) as a function of time (t) with dynamic (sinusoidal) loading (strain)

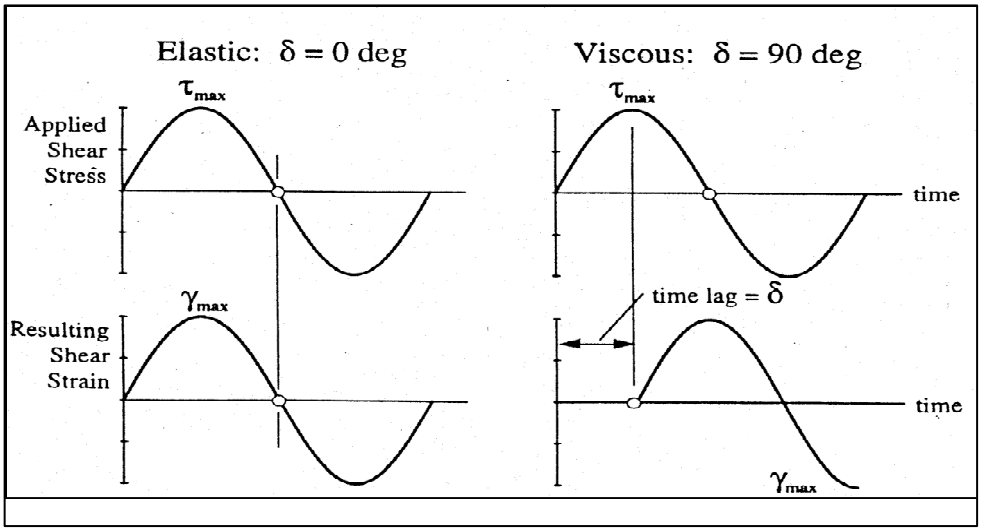

Figure 2.8 Definition of elastic and viscous materials under shear.

Finally, TGA determines the percentage of weight loss that occurs when a material is heated in a chosen atmosphere (generally nitrogen) as a function of temperature. This analysis indicates the temperature range on which materials such as polymers can be safely used.

The effect of physical aging which will represent a stiffening of the material can be evaluated by a stress- strain profile (tensile equipment); from where it is possible to obtain for a standard sample the modulus of elasticity, tensile strength and film elongation at breakpoint.

Conclusions

The literature review showed that there is a lack of information regarding the composition of stalk, CLM, and leaves of sugarcane in terms of cations and anions. It is recognized that TAA is the main non- sucrose component in the sugarcane juice. Several studies have been reported on the extraction of TAA, by precipitation, liquid-liquid extraction and ion exchange. Precipitation has been the only one used at commercial level. The literature review showed the negative effect of molasses impurities on TAA extraction yield, when compared with TAA extraction from synthetic organic solutions. An inverse relationship was found between TAA extraction yield and selectivity. At higher extraction yield, the selectivity of TAA is reduced; therefore the TAA purity will be lower. The color of the extracted TAA was neither evaluated nor reported in most of the studies; however, in a study using ion exchange resin to extract TAA, it was shown that color compounds and TAA eluted at the same time from the resin. Therefore color will be an issue especially when extracting TAA from CLM juice, which is a material with high content of color compounds such as phenols. TAA was used to produce plasticizers fifty years ago in Louisiana; however, the low yield and the high price of aconitate esters, when compared with phthalate esters, made this business unprofitable. Now, the awareness of migration and toxicity of some phthalates esters and the improved extraction technologies, present another opportunity to extract TAA from CLM juice, material with the highest content of TAA in the sugarcane.

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CHAPTER 3 CANE LEAF MATTER (CLM) COMPOSITION, EXTRACTION AND UTILIZATION

Introduction

CLM

In many sugarcane producing countries, it was a common practice to burn off the leaves before harvesting. Nowadays, this practice is becoming less common due to environmental laws (Hassuani, 2001; Briceno et al. 2001). The tops and top leaves called here cane leaf matter (CLM) together with other leaves attached along the stalk (Figure 3.1) represent a potential new feedstock to produce chemicals and energy.

Figure 3.1 Sugarcane distribution

The yield of CLM and leaves depends mainly on the variety, weather and growing conditions. Their amount on wet basis was reported between 200 to 350 kg/t of whole cane by Schembri et al. (2002) in Australia, and 83 to 300 kg/t of whole cane by Victoria et al. (2003) in Colombia.

The negative impact of processing CLM and leaves with clean stalk on the sugar mill factories has been studied in detail by many researchers and this will not be discussed in this

dissertation. Instead, the focus will be on the potential use of CLM to produce chemicals, such as trans-aconitic acid (TAA), and energy due the similarities in the composition between the CLM fiber and clean cane fiber as shown later. TAA in the juice obtained from CLM and leaves was reported to be 3 to 5 times higher than the TAA in juice from mature cane stalks (Balch et al. 1945).

Trans-aconitic Acid

TAA (Figure 3.2) is a white crystalline solid, melting at about 195°C with decomposition. It is soluble in water (18.6 g/100 mL at 13 °C) and alcohol (Paturau, 1989). TAA can be obtained as a byproduct in sugar manufacture or by dehydration of citric acid (Umbdenstock and Bruins, 1945). During sugar manufacture, this acid is partially removed in the clarification stage (Reece, 2003). However, a high percentage remains and forms part of the final molasses composition. This acid contributes to the formation of scale in juice heaters and evaporators. TAA also forms complex melasigenic compounds with sugars, affecting the formation of the sugar crystal (Mane, et al. 2002).

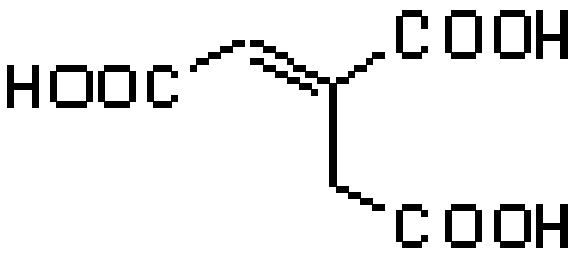

Figure 3.2 Trans-aconitic acid

TAA was used to produce plasticizers during early fifties (Roberts and Martin, 1954; Haines and Joyner, 1955) but by reason of the high cost associated and low recovery efficiency from sugar molasses, it was replaced by phthalate, a less expensive plasticizer.

TAA is also used as a flavoring agent when added with sclareolide (a natural byciclic terpenoid) increasing the organoleptic properties of the food, especially mouthfeel (initial perception on palate to the first bite through mastication to swallowing) and fullness in absence of amino acids (Buckholz et al. 1994).

Possible Reason for Aconitate Accumulation

Organic acids accumulate in plants when the cation uptake is higher than anion uptake (Beevers et al. 1966). In addition to maintaining a charge balance, the accumulation of organic acids increases the carbon dioxide fixation (Hiatt and Hendricks, 1967). A study (Orioli and Thompson, 1990) concluded that in wheat seeds TAA is accumulated in higher proportion by culturing seedlings on a potassium sulfate solution than in a calcium sulfate solution. A higher accumulation was observed in leaves, where the rapid uptake of potassium ions in comparison with sulfate ions leads to an imbalanced charge with an accelerated organic acid synthesis. The study also showed that TAA is less metabolically active than other organic acids such as malic, succinic and citric acid. This low activity of TAA was explained by its accumulation in a vacuole, possibly helped by potassium (Orioli and Thompson, 1990).

Comparison between Sugarcane and CLM Bagasse Composition

Until now, the traditional use for bagasse is combustion in the boilers to produce steam and electric energy. The ultimate analysis of sugarcane bagasse, whole CLM, CLM without juice extraction, and leaves is shown in Table 3.1. On a dry ash free basis, which excludes water and inorganic compounds, the ultimate analysis shows no significant differences between bagasse and whole CLM. Leaves have the highest percentage of ash and the lowest of carbon (ECN, 2003; Jorapur and Rajvanshi, 1995; Ohman, 1997). Even though whole CLM has higher ash content than sugarcane bagasse, a part of ash will eventually be

extracted with the CLM juice. No significant differences were observed in carbon between bagasse and whole CLM.

Table 3.1 Ultimate analysis (g/100 g) and calorific values of bagasse, whole CLM and leaves (ECN, 2003; Jorapur and Rajvanshi, 1995; Ohman, 1997

	Bagasse		Whole CLM		Leaves	
	Dry	Daf*	Dry	Daf*	Dry	Daf*
Carbon	48.6	49.9	47.0	49.7	39.8	43.1
Hydrogen	5.87	6.0	5.9	6.2	5.5	6.0
Oxygen	42.8	43.9	40.8	43.2	46.8	50.8
Nitrogen	0.16	0.16	0.70	0.74	0.19	0.20
Sulphur	0.04	0.04	0.11	0.12		
Low heating value, Mj/kg	17.71	18.96			14.80	16.04

* Dry, ash free

Plant Growth Regulators

In Louisiana the cane harvested early in the season is immature due to the growing period being only seven months, from March to September (Legendre et al. 2005). Moreover the reduction in temperature and daylight as the fall advances, affect negatively the maturation (natural ripening) process of the cane harvested through the season that ends in early January (Legendre et al. 2005). For this reason, Louisiana sugarcane industry, as many others around the world, use artificial plant growth regulators, chemical ripeners which accelerate the sugar cane maturation and increase the sugar yield.

One of the most effective chemical ripeners is glyphosate [isopropylamine salt of N-(phosphomethyl) glycine], whose commercial name is Polado-L®, manufactured by Monsanto (St. Louis, MO). The Polado -L® label for sucrose enhancement in Louisiana, Florida, Texas, Hawaii and Puerto Rico stipulates its use only in stubble crops, in a dose from 283 to 993 g/ha with a treatment period before harvest of 35 - 49 days (Legendre et al. 2005). Polado-L® is

not labeled in plant- cane crops to avoid a possible phytotoxicity to crown buds, which could decrease re-growth.

To increase maturity, that is the sucrose- soluble solids ratio in the stalk, it is necessary to reduce the size of green top and force the drying and dying of the lower leaves (Humbert, 1963), therefore the effect of chemical ripener on CLM composition and weight was studied.

Alternatives to Collect and Transport CLM

Due to environmental concerns due to cane burning, un-burnt or green cane harvesting has been adopted in many sugar countries around the world. This system has been used in Louisiana since 1994. Contrary to other tropical countries where the green material is left on the field to help maintain temperature and moisture of the soil control the weeds and increase biological activities, in Louisiana where the season occurs during humid winters and on soils deficient in internal drainage, the residue layer keeps the soil saturated and cool. This material must be removed to avoid delay in cane ratooning and decreasing sugar cane yield (Viator et al. 2005). The material should be removed seven or ten days after harvest.

One of the big challenges to face in order to use the CLM for chemical and energy purposes is finding a feasible collection and transportation system. The bulk density of the CLM is considerable lower than sugarcane. The amount of material transported by wagon, when CLM is transported together with the sugarcane to the mill without compacting, is reduced by more than 50% (see below). A review of some of the studies on this subject around the world identified the following options:

1. Combine harvesting, as done at present, then collect CLM off the ground, and transport it to the mill. In Brazil (Macedo, et al. 2001) found that on average 68% of the CLM and dry leaves are blown out of the harvester and 32% are taken with the cane to the mill. The

trash is left on the field for around 5 days until the average moisture content is below 20%. Finally trash is baled and transported to the mill. This procedure is applicable for CLM and dry leaves used as a fuel and also where the hot temperature helps to dry this material; however, when the purpose is to recover the sugars and TAA present in CLM, this material must be collected green.

2. Top off cane, collect and transport whole cane to mill, separate green and dry leaves in the mill The New South Wales (NSW) Sugar milling co-operative Ltd, (Broadwater, NSW Australia), harvests and transports complete cane to the Condong and Broadwater mills in large road bins (to compensate the low density of the green and dry leaves) provided with a tarp system to avoid loss of leaves during transportation (University of Ballarat, 2004). In the mill, green and dry leaves are separated from the billets in a dry clean system with double air chamber (Schembri et al. 2002). The size of green and dry leaves is reduced in a trash shredder prior their combustion in the boilers. The power required is 12 Kw /t trash per hour, value around 50% higher of the traditional cane shredder (Schembri et al. 2002). Complete cane will be processed in Condong and Broadwater sugar mills in Australia during 2007 season, the mills have a capacity to generate 60 MW of electricity (Lamb, 2007 personal communication).

3. Combine harvesting without topping and fan off (whole cane), transporting the whole cane to the mill. A trial to harvest whole cane was carried out at Lake Charles Cane – Lacassine Mill, LLC (Lacassine, LA) during the end of 2006 season, by reducing the speed of the harvester machine 50% and turning the fan off. Complete cane, with most of the tops and leaves dried as a result of the subfreezing temperatures to which the cane was exposed three weeks prior to the harvesting, was cut without topping and transported to the mill in

conventional bins. The cane load per bin was reduced around 50% by the lower bulk density of dry leaves and tops. An advantage of this process was that the sucrose losses for the billets left on the field and the stalk adhered to the top during harvesting were reduced. No impact was observed on juice extraction in the diffuser. The amount of fiber was increased 25% with respect to the sugarcane harvested with the fan on and topping (Saska and Gil, 2007). This alternative increases the amount of fiber and also recovers fermentable sugars from the tops. Complete cane can be processed in factories producing bio-ethanol and/or raw sugar but there is a considerable increasing in the juice color. TAA is also potentially extractable.

The objectives of this chapter are: 1) to contrast the composition in terms of sugars, anions and cations between the main parts of the sugarcane, clean stalk, CLM and dry leaves; 2) to analyze its variation before and during the season; 3) to analyze the differences among varieties as well as the effect of ripener; 4) to discuss the results reached during the extraction of CLM juice at pilot plant level; and, 5) finally, to present an economic model about the profit/loss of harvesting and processing complete cane.

Materials and Methods

Changes in CLM through the Season

Cane samples of the LCP85-384 variety, the most widely grown variety in Louisiana, were sampled prior (July to middle of September) and during the processing season of 2002. The samples were taken from plant-cane materials at LSU Agcenter in Sugar Research Station. Six whole plants sampled and manually separated into stalk, CLM and leaves which were then weighed at Audubon Sugar Institute. Each material was shredded in a 15x8 model shredder (Jeffrey Specialty Equipment, Woodruff, SC) to reduce particle size and homogenized. Shredded samples of each material were blended with deionized water using

the following water/ sample ratio, 3 for stalk, 5 for CLM and either 5 or 7 for leaves. The ratio was changed to assure a complete disintegration of the material. Samples were disintegrated in the Reitz disintegrator, cooled with a tap water jacket, for 30 minutes.

After disintegration, the liquid and solid components were separated by filtration under vacuum. °Brix, pH, and conductivity were determined in the filtered extract; the samples were kept frozen until determination of sugars, anions and cations by HPLC. The solids, after being washed with water, were dried under vacuum and used to determine fiber content.

Effect of Sugarcane Ripener on CLM Composition

The effect of ripener application on weight and composition of CLM was quantified in an experiment conducted by Legendre (2004) at the Sugar Research Station (St. Gabriel, LA) on LCP85-384 plant-sugarcane. In the experiment, a small acreage of sugarcane was divided in two. One half was treated with Polado-L® with 425 g/ha diluted in 30 l of water. The other half was kept without ripener application. Following the above described methodology, six whole plants were sampled and analyzed, at zero, two, five, and seven weeks after ripener application. The last sampling was simultaneous with the harvest of the trial field.

CLM Extraction at Pilot Level

CLM collected in commercial fields, either manually or after mechanical harvesting, was processed at Audubon Sugar Institute (ASI). CLM was first shredded in a 15x8 model shredder (Jeffrey Specialty Equipment, Woodruff, SC) and then crushed in a 3- roller pilot mill 0.3x0.3 m (Farrell Ansonia, CN). CLM was passed through the mill three times, the first pass without water addition, followed by two passes with 36 kg/100 kg CLM water as imbitition. The amount of water added was 10% higher than the usually amount added at the

commercial sugarcane mills to compensate for the higher fiber content in CLM compared with clean stalks. Before crushing, a sample of shredded CLM was kept for disintegration analysis; the same was done with a sample of the final bagasse after extraction process. The shredded CLM, CLM juice extracted and CLM bagasse were weighed.

Determination of TAA Extraction

The fraction of TAA extracted from the CLM was determined using the following equation:

$$\text{Extraction} = \frac{\text{Weight of TAA in CLM juice}}{\text{Weight of TAA in CLM}} x100$$

Where weight of TAA in CLM juice or CLM is determined as:

$$\text{TAA in material (kg)} = \text{weight of material (kg)} \times \text{TAA in material (kg/100kg)}$$

Economic Model to Estimate the Cost of Harvesting, Transporting and Extracting Complete Cane

During 2006 season, two tests were conducted in order to determine the feasibility of delivering CLM to the mill for its use in cogeneration and/or conversion to liquid fuels. The first test was conducted at the Lacasssine Mill where complete cane, harvested with the fan off, was processed for a period of 4.5 h. Then, normal cane harvested with the fan on, was evaluated for a period of 7 h. In this test, information was collected regarding weight per wagon; cane and bagasse samples were analyzed following the previously described methodology. In a second test, organized by Frank Martins Farms, Inc. (Franklin, LA) and American Sugar Cane League (ASCL, Thibodaux, LA), cane, of the variety HoCP96-540, was harvested both with the fan on and off. For both the complete and normal cane, the weights per wagon and sugarcane quality were determined. A sample of the residues left in

the field for both systems was taken from a determined area. The load densities, for both cane qualities, were determined in a wagon with a capacity of 3 tons.

Analytical Methodologies

Anions

The determination of TAA, cis-aconitic, citric, and iso-citric acids and 10 others anions were done with Dionex (Sunnyvale, CA) HPIC systems provided with an IonPac AS11 column and suppressed-conductivity detector. Sample volumes of 50µl were injected into a mobile phase with a Spectra System AS 3000 auto sampler. The mobile phase was run in a gradient composed by three eluents; dionized water and sodium hydroxide at two concentrations, 4mM and 100 mM. The time of analysis was 60 minutes. The concentrations were determined from the peak areas calibrated against three standards run before and after samples, using the software Dionex Peaknet System (Version 4).

Cations

Sodium, potassium, calcium and magnesium were quantified by HPLC using an Ionpac CS 12 column (Dionex, Sunnyvale, CA) and conductivity detector. A Dionex AS 40 automated sampler was used to inject 10µL of each sample into a mobile phase of 0.22 mM methanesulfonic acid (MSA) solution eluted isocratically at 0.7 mL/min. Concentrations of the cations were calculated from peak areas of HPLC calibrated against external standards of 2, 5 and 10 ppm using the Dionex Peaknet System (Version 4) software. The time analysis was 15 minutes.

Sugars

Sucrose, glucose and fructose were determined by HPLC using HPX-87 K Bio-Rad laboratories (Richmond, CA), column kept at 85 °C and a refraction index detector. The

mobile phase was a solution of 0.01 M of K_2SO_4 eluted isocratically at 0.6mL/min. The time analysis was 16 minutes. Concentrations of the sugars were calculated from peak areas of HPLC against external standards at three concentrations levels.

Results and Discussion

Changes in CLM through the Season

The weight, the fiber and the solids distribution of the three components of the cane are showed in Tables 3.2 and 3.3. A wide variation is observed in fiber and soluble solids, associated with the changes in cane composition when growing, and weather conditions during the sampling.

Table 3.2 Weight distribution (%) of cane variety LCP85-384

Test date	Stalk	Leaves	CLM
25-Jul-02	72	12	16
8-Aug-02	79	11	10
21-Aug-02	76	13	11
4-Sep-02	74	16	10
18-Sep-02	73	14	13
21-Oct-02	75	10	15
2-Dec-02	78	4	18

The December 2 sample presented the biggest difference in the leaves content; this cane had only a few leaves, and the majority of them were dry. Noticeable is the increase in fiber and soluble solids in the stalk as consequence of the maturation process. CLM represents 10 to 18 g/100 g of whole cane (Table 3.2). The amount of fiber in millable sugarcane varies between 13 – 18 g/100 g cane. After crushing, the cane is separated into juice and bagasse. Bagasse, containing mostly of the fiber present in sugarcane; equals 26- 36 g/100 g cane usually with a 50 % of moisture. CLM represents between 20-25 % of the total sugarcane fiber (Table 3.3); counting the leaves, the fiber availability can be duplicated (Table 3.3). In

immature cane, leaves and CLM have 15 g/100 g DS; however with the maturation process and aging of the leaves, this content is reduced to half.

Table 3.3 Fiber and soluble solids distribution of cane variety LCP85-384

	Fiber, g/100 g total			Soluble solids, g/100 g total		
Test date	Stalk	Leaves	CLM	Stalk	Leaves	CLM
25-Jul-02	52	23	25	84	7	9
8-Aug-02	59	22	19	87	7	6
21-Aug-02	62	24	14	92	5	4
4-Sep-02	47	40	13	89	7	5
18-Sep-02	53	30	16	92	4	4
21-Oct-02	59	20	21	91	4	5
2-Dec-02	73	9	18	89	1	10

Sugar Content

As can be seen in Table 3.4, Brix and sucrose content tends to increase with the maturity of the cane in the stalk, and reducing sugars content to decrease. The sugar contents in tops (Table 3.5) varied acrosss a small range during the sampling time with the exception of the sample of December 2; apparently a part of stalk was considered as top, because the sucrose content was very high. The soluble solids in leaves and tops are very similar; however, tops have more sugars (Table 3.5)

Table 3.4 Brix and sugar content g/100 g DS in stalk juice

Test date	Brix	Sucrose	Glucose	Fructose
25-Jul-02	6.71	42.61	21.02	17.61
8-Aug-02	5.87	38.56	21.57	19.61
21-Aug-02	11.56	77.21	3.74	3.74
4-Sep-02	8.60	58.74	9.42	6.73
18-Sep-02	13.78	80.32	6.02	5.22
21-Oct-02	13.06	83.84	1.35	1.68
2-Dec-02	15.79	86.62	1.01	1.26

In contrast with stalk, sugars in CLM are composed mainly for glucose and fructose. The total sugars in CLM represents 25g/100 g DS, this value can be increased up to 60 g/100

g DS when a part of stalk is added to CLM; as it happened on December 2 sample. This situation occurs frequently at the commercial level (See CLM extraction at pilot level).

Table 3.5 Brix and sugar content g/100 g DS in CLM and leaves juice

	CLM				Leaves			
Test date	Brix	Sucrose	Glucose	Fructose	Brix	Sucrose	Glucose	Fructose
25-Jul-02	3.5	8.1	12.8	12.8	3.7	6.0	8.3	8.3
8-Aug-02	3.6	13.1	8.3	8.3	3.8	11.7	7.4	7.4
21-Aug-02	3.6	12.8	7.0	8.1	4.2	8.7	3.8	3.8
4-Sep-02	3.5	6.8	6.8	8.0	3.8	1.1	6.4	6.4
18-Sep-02	3.7	10.0	6.7	6.7	3.5	3.4	5.2	5.2
21-Oct-02	4.1	12.3	7.0	7.0	4.6	3.9	5.3	5.3
2-Dec-02	7.6	50.2	6.3	7.5	4.7	0.7	16.0	15.3

Anions and Cations Content

Potassium and sodium have melasigenic effects; they increase the sucrose solubility and decrease the exhaustion of the final molasses. Tables 3.6 and 3.7 and 3.8 reveal that the content of these components in stalk is 4 or 5 times lower than leaves and tops. The average content for potassium during the middle and the end of the season (September- December) is 2 to 4 times higher than the value reported for South Africa by Walford (1996).

Table 3.6 Ion concentrations in stalk juice in g/100 g DS

Test date	K^{+}	Mg^{2+}	Ca^{2+}	$PO4^{2-}$	$Citrate^{3-}$	TAA^{3-}
25-Jul-02	3.31	0.27	0.35	0.34	0.09	4.55
8-Aug-02	4.56	0.24	0.33	0.45	0.35	5.83
21-Aug-02	2.58	0.16	0.18	0.30	0.15	3.40
4-Sep-02	4.63	0.24	0.37	0.39	0.12	7.36
18-Sep-02	1.85	0.11	0.15	0.29		2.50
21-Oct-02	2.85	0.20	0.31	0.31	0.10	3.74
2-Dec-02	0.97	0.06	0.24	0.46	0.24	1.06

TAA also has melasigenic effect (Mane et al. 2002); it decreases in the stalk with the age of the cane. TAA content in tops and leaves is on average 8 or 10 times higher than

mature stalk; which is higher than reported by Balch et al. (1945). Potassium and TAA decreased in CLM and leaves through the season, this behavior appears to agree with the reported by Hart (1934) and Humbert (1963) on the migration of potassium to dry leaves.

Table 3.7 Ion concentrations in CLM juice in g/100 g DS

Test date	K^+	Mg^{2+}	Ca^{2+}	$PO4^{2-}$	$Citrate^{3-}$	TAA^{3-}
25-Jul-02	9.75	0.53	0.86	1.53	0.28	12.78
8-Aug-02	11.13	0.58	1.00	1.44	0.48	12.20
21-Aug-02	12.51	0.60	0.85	2.02	0.55	14.36
4-Sep-02	12.43	0.63	1.07	1.72	0.58	15.79
18-Sep-02	10.88	0.45	1.40	1.25	0.35	14.43
21-Oct-02	11.54	0.72	1.98	1.74	0.45	12.19
2-Dec-02	4.53	0.52	1.08	1.14	0.88	3.47

Table 3.8 Ion concentrations in leaves juice in g/100 g DS

Test date	K^+	Mg^{2+}	Ca^{2+}	$PO4^{2-}$	$Citrate^{3-}$	TAA^{3-}
25-Jul-02	8.89	0.96	1.75	0.95	0.73	10.00
8-Aug-02	10.20	0.93	1.59	0.88	0.62	10.56
21-Aug-02	10.23	0.76	1.69	0.88	1.08	10.71
4-Sep-02	10.59	0.66	1.59	0.70	1.10	9.89
18-Sep-02	9.50	0.62	1.88	055	1.14	8.79
21-Oct-02	7.36	0.64	2.14	0.66	0.95	6.23
2-Dec-02	2.96	1.10	2.32	0.85	2.04	0.99

A study on scale composition in Louisiana (Godshall and Wartelle, 2002) indicated that calcium, phosphorus, silica and magnesium are some of the main constituents of the scaling in the heaters and evaporators. This scaling reduces the heat transfer, thus increasing the cleaning frequency of these equipments. Calcium, phosphate and magnesium content in CLM and leaves are between to 2 and 5 five times higher than in stalk; this corroborates the potentially large effect of CLM and leaves in the formation of scaling.

CLM Extraction at Pilot Level

An average of 100 Kg of CLM was collected from the field after mechanical harvesting in the 2003 and 2005 seasons and cut by hand in the 2004 and 2006 seasons. The mass balance of CLM juice extracted in 2003 following the described methodology is shown in Table 3.9.

Table 3.9 Mass balance of TAA extraction from CLM juice

Material	21-Nov	22-Nov	26-Nov	3-Dec
CLM (kg)	47.2	44.7	31.8	40.6
First juice extracted (kg)	13.7	12.7	7.3	14.4
First juice % CLM	29.0	28.4	23.0	35.4
Imbibition water (kg)	15.0	17.5	12.5	13.3
Imbibition water % CLM	31.8	39.1	39.3	32.8
Total juice (kg)	25.7	27.4	19.4	28.0
Total juice % CLM	54.4	61.3	61.0	68.9
Brix juice, %	6.4	7.8	7.1	9.4
TAA (mg/L)	4,627	5,483	5,340	3,304
TAA (g/100 g DS)	7.23	7.03	7.52	3.52
TAA (g/kg CLM)	2.5	3.4	3.3	2.3
Bagasse final (kg)	25.5	29.7	24.5	25.5
Bagasse final % CLM	54.1	66.4	77.0	62.9

The amount of juice extracted is slightly less than the amount of bagasse. Significant differences were observed in TAA concentration in CLM which ranged between 2.5 to 3.3 g/kg.

The average composition of CLM for each season is provided in Tables 3.10 and 3.11. The high value of Brix and sugars during the CLM 2005 season, clearly illustrate the sugar losses during mechanical harvesting as a result of the differences in the height of the cane; but at the same time, showed the potential use of this juice to produce ethanol. Differences also were observed in cation and anion composition related to sugarcane varieties and growing conditions.

Table 3.10 Brix, sugars and cations in CLM juice

		Sugars, g/100g DS			Cations, g/100 g DS			
Year	Brix, %	Sucrose	Glucose	Fructose	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}
2003					0.45	5.66	0.99	1.53
2004	3.52	7.51	15.79	15.14	0.09	9.00	0.78	1.69
2005	11.50	53.81	8.35	9.01	0.01	4.40	0.51	0.85
2006	4.24	7.55	12.73	14.38				

Table 3.11Anions in CLM juice, g/100g DS

Year	$Malate^{2-}$	$SO4^{2-}$	$Oxalate^{2-}$	$PO4^{3-}$	$Citrate^{3-}$	Iso-$citrate^{3-}$	Cis-$Aconitate^{3-}$	TAA^{3-}
2003	1.32	1.39	0.33	0.71		1.30		6.32
2004	0.34	3.89	0.32	1.37	0.48	0.38	0.23	7.63
2005		2.70	0.22	0.95	0.31	0.85	0.11	5.05
2006		2.16	0.50	1.74	0.56	0.55	1.38	8.55

The amount of TAA, expressed as TAA in kg/ton CLM, remained relatively stable through the seasons, except for 2005, with a 33 % decrease with respect to the average, due to the portion of stalk adhered to tops that enriched the amount of sugars but decreased TAA content (Table 3.12). In CLM collected after mechanical harvesting, sugars, cations and anions represent nearly 90% of the CLM composition. In contrast, in samples cut by hand (2004 and 2006) these components only correspond to roughly 70%, indicating that other components, probably of high molecular weight, form part of CLM composition. Taking an average of 2.6 kg TAA/ton CLM (Table 3.12), the potential production of TAA in Louisiana with a crushing rate of 12 million tons/year should be 5600 tons.

Table 3.12 Total amount of sugars, cations, anions and aconitic acid, g/100 g DS in CLM juice

Year	Sugars	Cations	Anions	TAA	TAA, g/ 100 g CLM
2003	59.9	8.7	14.4	6.3	0.29
2004	34.6	14.9	19.0	8.7	0.31
2005	71.2	5.8	12.5	5.1	0.22
2006	32.7		17.5	8.3	0.23

Effect of Sugarcane Ripener on CLM and Stalk Composition

Chemical ripener effects not only the composition but also the weight of the three main components of sugar cane: stalk, CLM and leaves. Tables 3.13 and 3.14 illustrate the composition in terms of sugars, anions and cations of stalk and CLM juice samples with and without ripener.

Table 3.13 Ripener effect on the composition of stalk juice expressed in g/100 g DS

Treatment	Week	Brix	Sucrose	Glucose	Fructose	K^+	Mg^{2+}	Ca^{2+}
Initial sample (control)	0	12.30	70.29	11.94	7.81	1.18	0.18	0.17
Initial sample (ripener)	0	14.00	75.51	8.70	5.43	1.26	0.16	0.14
Ripener	2	15.26	79.80	8.23	5.32	1.24	0.13	0.12
Control	2	14.11	77.91	8.44	5.44	1.34	0.17	0.15
Ripener	5	18.80	84.40	5.28	4.00	0.95	0.12	0.10
Control	5	14.70	82.65	5.62	4.57	1.17	0.18	0.19
Ripener	7	20.30	85.48	4.17	3.23	0.87	0.12	0.10
Control	7	16.80	84.49	3.76	2.30	0.79	0.13	0.12

The variation in soluble solids and sugars in stalk show an increasing trend through the seven weeks; however, the rate of increment in terms of soluble solids and sucrose is faster in the samples treated with ripener (Figure 3.3).

Table 3.14 Ripener effect on stalk juice. Composition of anions expressed in g/100 g DS

Treatment	Week	$SO4^{2-}$	$PO4^{3-}$	$Citrate^{3-}$	Iso-$citrate^{3-}$	Cis-$Aconitate^{3-}$	TAA^{3-}
Initial-control	0	0.63	0.19	0.07	0.01	0.01	1.16
Initial-ripener	0	0.46	0.15	0.08	0.01	0.00	1.13
Ripener	2	0.46	0.17	0.07	0.02	0.01	1.00
Control	2	0.56	0.18	0.08	0.02	0.00	1.00
Ripener	5	0.33	0.19	0.11	0.02	0.01	0.81
Control	5	0.56	0.21	0.09	0.02	0.02	1.07
Ripener	7	0.40	0.18	0.12	0.02	0.01	0.82
Control	7	0.46	0.19	0.10	0.02	0.01	0.61

The drying process, after spraying with ripener, leads to a loss in the weight of stalk. This reduction on weight is less than the increase in sucrose (Figure 3.3). The weight fluctuation between 2 and 5 week samples, in the control test, reflects the inherent variations among sugarcane plants. The maturation process reduces not only the monosaccharide concentrations in the stalk but also the anions and cations as well (Tables 3.13 and 3.14).

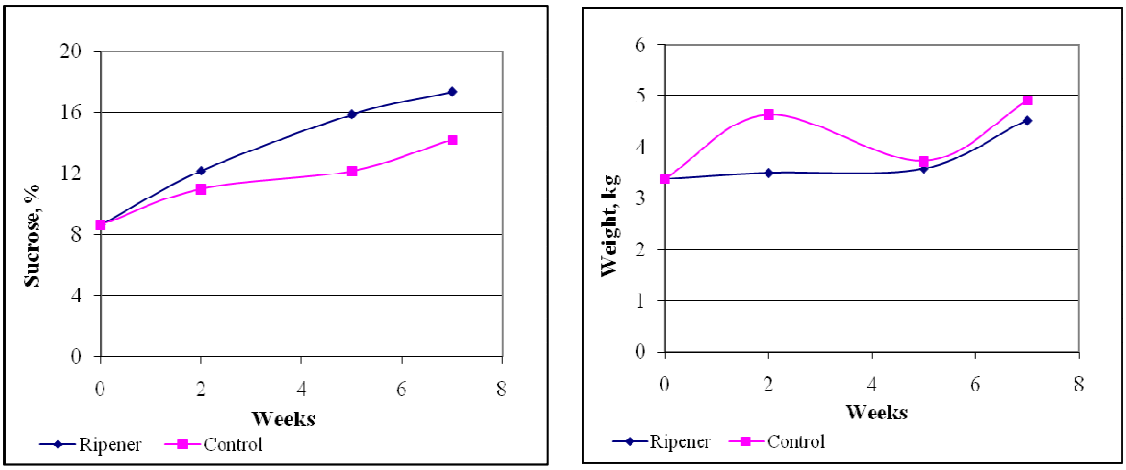

Figure 3.3.Effect of ripener on sucrose content and weight of sugarcane stalk

The effect of ripener on CLM and leaves at the end of the seven week treatment is illustrated in Figure 3.4; the growing process slowed, and a high percentage of the leaves dried out. Although Brix in CLM juice with ripener is higher than without ripener, at the end of seven weeks, the total amount of sugars and TAA in g/100 g DS was significantly lower (Tables 3.15 and 3.16). In addition, a considerable difference in the weight in CLM, between ripener and without ripener, made CLM ripening economically un-feasible for recovering sugars and/or TAA.

As mentioned by Humbert (1963) the natural ripening process is also helped by low temperatures, therefore for purposes of TAA recovery it would better to harvest the cane early in the season when the temperature is relatively high, without ripener.

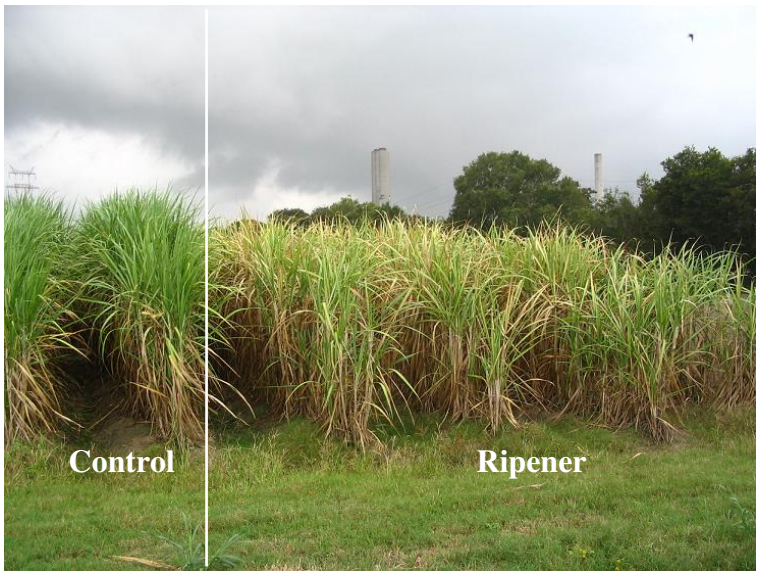

Figure 3.4 Ripener test. Right side sugarcane treated with ripener, left side sugarcane control. Sugarcane variety LCP85-384, Sugar Research Station, St. Gabriel, LA

Table 3.15 Ripener effect on Brix, %, sugars and cations, g/100 g DS of CLM juice

Treatment	Week	Sucrose	Glucose	Fructose	K^+	Mg^{2+}	Ca^{2+}
	0	17.6	11.2	11.8	8.2	0.7	1.0
	0	10.8	16.2	16.6	8.2	0.5	0.5
Ripener	2	33.5	13.8	12.9	6.5	0.6	0.8
Control	2	23.8	12.7	12.6	8.0	0.6	0.9
Ripener	5	49.2	8.1	7.3	3.7	0.4	0.5
Control	5	30.4	9.3	8.9	6.7	0.5	0.8
Ripener	7	19.1	13.3	12.0	5.2	0.6	0.9
Control	7	28.1	12.0	10.8	6.1	0.6	1.0

Moreover, frequently at the end of the season, Louisiana sugar industry faces freezing temperatures that damage the cane and dry almost completely the tops and leaves. An analysis of TAA in CLM that was harvested from canes submitted two and three weeks after freezing

temperatures gave concentrations of only 0.01 g/100 g DS (Saska and Gil, 2007). This is up to 800 times lower than the typical value found in CLM juices in samples analyzed between 2002 and 2006 seasons.

Table 3.16 Ripener effect on CLM juice, composition of anions expressed in g/100 g DS

Treatment	Week	$SO4^{2-}$	$PO4^{3-}$	$Citrate^{3-}$	Iso-$citrate^{3-}$	Cis-$Aconitate^{3-}$	TAA^{3-}
	0	1.74	1.06	0.33	1.04	0.15	9.14
	0	1.57	1.11	0.36	0.87	0.14	9.92
Ripener	2	1.11	0.76	0.34	0.85	0.13	6.99
Control	2	1.35	1.05	0.37	0.80	0.17	8.43
Ripener	5	0.90	0.52	0.27	0.42	0.08	3.92
Control	5	1.30	1.28	0.47	1.05	0.16	7.09
Ripener	7	1.13	0.71	0.42	1.11	0.16	4.70
Control	7	1.84	0.711	0.39	1.00	0.16	6.60

Differences in CLM among Varieties

Tables 3.17 and 3.18 present the changes in CLM juice composition between early season (October) and middle season (November) of LCP85-384 and two other commercial varieties, HoCP91-555 and L97-128. L97-128 had the highest amount of sugars on both sampling dates and HoCP91-555 the lowest. This is consistent with the report by Eggleston et al. (2007). On average, cations remained constant between sampling. TAA, as observed before, decreased with the maturation of the cane, L97-128 showed the lowest TAA concentration (Table 3.18), which can be related with the highest amount of sugar found.

Table 3.17 Variety effects on CLM juice, composition of sugars and cations expressed in /100 g DS

Variety	Date	Sucrose	Glucose	Fructose	K^{+}	Mg^{2+}	Ca^{2+}
LCP85-384	Oct- 8-04	22.61	14.50	13.83	4.74	0.59	0.98
HoCP 91-555		30.71	8.08	8.18	6.49	0.71	1.55
L97-128		37.24	8.53	8.71	4.84	0.40	1.21
LCP85-384	Nov- 2-04	16.98	17.19	16.26	4.61	0.69	1.01
HoCP 91-555		22.28	12.07	11.05	3.63	0.82	1.69
L97-128		26.55	14.93	13.67	4.47	0.50	1.49

Table 3.18 Variety effects on CLM juice, composition of anions expressed in g/100 g DS

Variety	Date	$SO4^{2-}$	$PO4^{3-}$	$Citrate^{3-}$	Iso-$citrate^{3-}$	Cis-$Aconitate^{3-}$	TAA^{3-}
LCP85-384	Oct-04	0.78	0.86	0.61	0.82	0.12	4.20
HoCP 91-555		1.07	1.03	0.83	2.26	0.19	4.87
L97-128		1.16	0.87	0.59	1.06	0.08	1.96
LCP85-384	Nov-04	0.79	0.76	0.54	0.55	0.09	2.76
HoCP 91-555		1.06	0.64	0.86	2.18	0.26	2.10
L97-128		1.25	0.78	0.69	1.18	0.11	0.99

Comparison between Sugarcane and CLM Bagasse Composition

The compositional analysis of bagasse and CLM are illustrated in Table 3.19 (Rao, 1997; Lasser et al. 2002; Tiedje, 2004; Saska, and Gray, 2006). On average, bagasse has a higher content of glucan while CLM has a lower lignin but higher ash content. Having less lignin is an advantage since this material needs to be removed in order to hydrolyze the glucan and xylan. Table 3.19 also illustrates a wide variation in CLM composition which can be related with the constant changes in this part of the sugarcane.

A relatively new use for these lignocellulosic materials, bagasse and CLM, is their hydrolysis after pre-treatment to produce bio-ethanol. The technology to hydrolyze the bagasse to monosaccharides of 5 (xylose) and 6 (glucose) carbons and their fermentation is up till now in development (Gray, 2007). In a lime pre-treatment followed by enzymatic hydrolysis Saska and Gray (2006) obtained 350 kg of a mixture of glucose and xylose per ton of dry leaves. Rein (2007) estimated the potential for ethanol production in Louisiana, from bagasse and CLM, at the actual crushing of 14 million tons cane/year, after discounting the amount required to generate the steam and power in the mills, to be close to 946.3 millions l / year.

Table 3.19 Compositional analysis of sugarcane and CLM bagasse, in g/100 g DS

Analysis	Sugarcane bagasse	CLM bagasse
Carbohydrates		
Glucose (cellulose)	34.5 - 38.4	26.0 - 39.4
Xylan (hemicellulose)	22.2 - 24.1	22.8 – 24.5
Lignin		
Acid insoluble	19.7 - 20.1	15.3 – 21.5
Acid soluble	5.3	7.4
Ash	5.2 – 8.8	7.0 – 13.4
Others	7.3 – 10.6	6.4 – 8.1

Assessment of Gross Income from Harvesting and Processing Complete Sugarcane

An estimate of the extra revenue for harvesting whole or complete cane is illustrated in Table 3.20. In the calculations, it was considered that all bagasse from the stalk is used as fuel in the boilers. Two possibilities were evaluated for molasses: 1) Sale for animal feed and other use, which is quite profitable at the moment because the molasses price has increased from US$ 60 a few years ago to US$ 120 per ton since the expansion of the Brazil ethanol program; and, 2) the production of ethanol and TAA. CLM was considered for production of ethanol and TAA from the juice and use of the fiber as boiler fuels for co-generation (steam and electricity) it has same calorific values as bagasse. The increment in the revenue from selling the molasses, even at this high price, or to use it to produce ethanol is around 80 %. The revenue obtained by using CLM fiber as fuel is nearly equal to the revenue obtained by selling the molasses. Another possibility for bagasse and CLM fiber is to burn in the boilers to produce steam but also to cogenerate electricity, this alternative will depend of the Kw-h price and it is evaluated in the next section. The data showed the advantages the sugar industry would have by giving added value to its traditional (bagasse and final molasses) and non-traditional (CLM) sub-products.

Table 3.20 Estimated gross income per 1 MT of complete sugarcane

Material	Product	Unit	Amount	$/unit	$
Stalk	Sugar[1]	MT	0.101	440	44.44
	Molasses	MT	0.028		
	Animal feed, etc[1]	MT	0.028	120	3.36
	Ethanol[2]	L	9.9	0.57	5.64
	TAA[3]	Kg	0.4	1.58	0.63
CLM	Juice (5 % dry solids)	MT	0.224		
	Ethanol[2]	L	3.8	0.57	2.16
	TAA[3]	Kg	0.4	1.58	0.63
	Fiber (50 % moisture)	MT	0.185		
	Co-generation[4]	KWh	43.5	0.065	2.83

[1] http://www.amscl.org/SugarIndustry.pdf, predicted prices 2007
[2] http://www.ethanolmarket.com/fuelethanol.htmL,prices at September, 2007
[3] http://www.purchasing.com/article/CA6458587.htmL, TAA price was fixed as 20% higher than citric acid price. Prices at July, 2007
[4] 1 MT (50 % moisture) CLM fiber = 235 KWh

Economic Model to Estimate the Cost for Harvesting, Transporting and Extracting Complete Sugarcane

In the test conducted in L.C. Cane- Lacassine Mill, LLC (Lacassine, LA), an average load per wagon of 25.3 t for conventional cane and 12.6 t for complete cane was observed. This reduction is higher than reported by Eiland et al. (2003) in Florida, with an average load per wagon of 25 t and 15.3 t for conventional and complete cane, respectively. The reason for the greatest change in LA test can be related to the lower moisture of the CLM due to the freezing temperatures that affected this zone early December 2006. The CLM and leaves' content, in a Florida test, increased from 8.4 % in cane harvested with extractor fans on to 25.0 % without. The wagons per ha were increased from 3.4 wagons /ha to 7.4 wagons /ha. No changes were found in juice quality.

A model was built in Excel to predict the feasibility to harvest, transport and process CLM using as reference: a) the information collected at Lacassine mill; b) in the test organized by Frank Martins Farms and ASCL and c) as reported by Eiland et al. (2003) in

Florida. The following costs were considered: the increased cost of transportation which depends on the load density and the distance from the field to the mill; the increased sucrose losses in bagasse; the extra power required for cane preparation and crushing, and the cost for the separation of CLM in the factory by a pneumatic system. The extra revenue includes the reduction of sucrose losses in the field, the electric power cogenerated from the excess of fiber and the difference in the fuel/acre required by the combine. The economic and environmental benefits from not burning the CLM in the field were not included. The model does not include any capital investment.

The cane load densities, with fan on and fan off, and the CLM content % cane, were taken from the Frank Martins Farms /ASCL test and are shown in Table 3.21. The data was fitted with a linear correlation.

Cane density, $kg/m^3 = 650.5 - 9.155x$ % CLM

The transportation cost of complete cane was estimated by taking as a reference the cost to transport conventional cane, which varies between US$ 1 to US$ 6/t, depending on the distance between the field and the mill. This cost was multiplied by the ratio between the cane load density of conventional and complete cane. The extra transportation cost and the sucrose lost in bagasse were chosen to illustrate different cases. The cane losses in the field are substantially reduced while harvesting with the fan off. These cane losses were estimated to be between 2 to 10 % by Eiland et al. (2003).

Table 3.21 Load density and CLM % cane

Parameter	Fan on (1050 rpm)	Fan off
Density, kg/m^3	469	285
CLM % cane	10	30

The extra power required in preparation and diffusion was quantified by taking as a reference the values reported by the Lacassine mill (Lacassine, LA). The fuel saved by harvesting with the fan off was estimated as 0.06 gal/t of cane as reported by Eiland et al. (2003). The power required in the pneumatic cleaning system was estimated as 3.7 Kwh/ton CLM consistent with the values reported by Rein et al. (2003). The efficiency of the pneumatic system depends on the weather conditions. In dry weather, it is around 80% while in wet weather it decreases to about 50 %.

The input data for the model are the following:

Milling rate of clean cane: 4500 t/d
Conventional: clean cane ratio: 1.11
Complete: clean cane ratio: between 1.11 and 1.43
Clean cane fiber: 13 %
Clean cane sucrose: 12.5 %
CLM moisture: 50 %
Bagasse moisture: 50%
Extra cane from field: 8%
Sugar sale price: 400 US$/t
Electrical power generation: 111 Kwh /t of bagasse (50% moisture):
Electrical power sale price: 0.07 US$/kWh
Power required, cane preparation and milling: 98 kWh/t fiber
Power required, diffuser and dewatering: 127 kWh
Power required, CLM separation: 3.7 kWh/t CLM
CLM separation efficiency: 50%
Fuel consumption harvester with fans on: 1.00 l/t cane
Fuel consumption harvester with fans off: 0.80 l/t cane
Fuel cost: 0.64 US$/l

Three cases were studied using the model. Case 1 used the values reported above, with out a dry clean system. Case 2 a dry clean system was included with a separation efficiency of 80%. In Case 3, the electrical power generation index, Kwh generated /t of bagasse , was changed from 111, a common value in Louisiana mills to 235, a value expected for modern turbines; this case did not include dry clean system.

The profit / loss per day for case 1 is presented in Table 3.22. From the results, it is evident that processing complete cane is profitable the closer the field is to the factory, as well as if the mill/diffuser is able to handle the excess fiber without increasing the sucrose loss in bagasse. Under the conditions used, it seems to be economically attractive if the transportation cost of the conventional cane is not higher than US$3/t with sucrose content in bagasse up to 1.5%.

In Case 2, the profit increased, due to the reduction of the pol losses in bagasse as a result of less amount of fiber processed (Table 3.23). The revenue increased appreciably in comparison with the previous case, and the model predicts a cost-effective operation for almost all the conditions considered in Table 3.23.

Table 3.22 Estimated additional profit/loss, US$/day for harvesting, transporting and processing complete cane. Case 1

	Transportation cost of conventional cane, TCCC, US$/t					
Pol in bagasse, %	1	2	3	4	5	6
0.5	18375	12792	7209	1626	-3957	-9540
0.7	17226	11644	6061	478	-5105	-10688
0.9	16078	10495	4912	-671	-6254	-11837
1.1	14930	9347	3764	-1819	-7402	-12985
1.3	13871	8198	2615	-2968	-8551	-14134
1.5	12633	7050	1467	-4116	-9699	-15282
2.0	9762	4179	-1404	-6987	-12570	-18153

Table 3.23 Estimated additional profit/loss, US$/day, for harvesting, transporting and processing complete cane in a factory with dry clean system, Case 2

	Transportation cost of conventional cane, TCCC, US$/t					
Pol in bagasse, %	1	2	3	4	5	6
0.5	20300	14717	9134	3551	-2032	-7615
0.7	20070	14487	8904	3321	-2262	-7845
0.9	19841	14258	8675	3092	-2491	-8074
1.1	19611	14028	8445	2862	-2721	-8304
1.3	19381	13798	8215	2632	-2451	-8534
1.5	19152	13569	7986	2403	-3180	-8763
2.0	18577	12994	7411	1828	-3755	-9338

The major changes, in the profitability among the three cases presented, occurred when the index that measures the cogeneration efficiency was increased from 111kWh/t bagasse to 235 kWh/t bagasse. The revenue not only increased significantly in comparison with the previous conditions but also, the model prediction was profitable for almost all the conditions studied (Table 3.24).

Table 3.24 Estimated additional profit/loss, US$/day, for harvesting, transporting and processing complete cane in a factory with dry clean system, Case 3

	Transportation cost of conventional cane, TCCC, US$/t					
Pol in bagasse, %	1	2	3	4	5	6
0.5	30835	25252	19669	14086	8503	2920
0.7	29687	24104	18521	12938	7355	1772
0.9	28538	22955	17372	11789	6206	623
1.1	27390	21807	16224	10641	5058	525
1.3	26241	20658	15076	9493	3910	-1673
1.5	25093	19510	13927	8344	2761	-2822
2.0	22222	16639	11056	5473	-110	-5693

Conclusions

The variations in CLM through the season, differences among varieties, effect of ripener on CLM composition, the extraction of CLM juice at pilot level, as well as an economic model to predict the economic viability to harvest complete cane were addressed in this chapter. The main findings found are presented as follows:

CLM represents 10 – 18 % of the total weight of cane. CLM and leaves are a potential feedstocks for cogeneration and lignocellulosic ethanol and together can represent up to 50% of the total fiber of cane. The content of TAA in immature sugarcane is higher than in mature one, which is reflected in the relative high contents of TAA in sugarcane stalk before the season. In mature canes, TAA on dry basis is 3 to 6 times higher in CLM than in clean stalk. The effect of maturation on TAA content was also clearly observed during the study of

ripener effect on CLM composition. In immature canes, TAA content was almost twice as high as in cane matured either by ripener and age or only by age. In addition to the negative effect of maturation on TAA content, it was found that cane exposure to freezing temperatures will produce significant reduction in TAA content in both stalk and CLM.

The composition of CLM varied depending on other factors: cane variety, growing and weather conditions. The preparation and extraction of CLM juice at pilot level were carried out without difficulties. The amount of TAA ranged from 2.2 kg/t CLM to 3.1 kg/t CLM, which for a crushing rate of 12 million tons of cane/year would give a potential production of 5600 t of TAA/year.

The similarity found in composition between sugarcane bagasse and CLM confirmed the possibility to use CLM as fuel in the boilers to increase the cogeneration capacity or in the near future as cellulosic feedstock to produce ethanol. More studies are needed to determine the effect of higher content of ash in CLM for the purposes mentioned above.

Finally, the economic feasibility to harvest complete cane was studied. Although transportation cost is increased more than twice in comparison with conventional cane as a result of the low load density of CLM, harvesting and processing whole cane can be profitable when the cane is transported a distance no more than 20 miles from the field to the mill. This profit is increased significantly if the mill has a turbo generator of high efficiency.

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CHAPTER 4 ACONITIC ACID EXTRACTION FROM CLM AND ITS ESTERIFICATION AND PURIFICATION

Introduction

The extraction of trans aconitic acid (TAA) from sugar cane products, sorghum and synthetic solutions of organic acids has been studied in detail in two different periods. The first one was 60 years ago (Ventre, 1949 and 1955, Godchaux II, 1949, Regna and Bruins, 1956), and the second one was mainly since early the nineties (Malmary et al. 1995, Malmary et al. 1998; Azzan and Radwan, 1986; Hill and Kancharla, 1999; Blinco, 2000; McMurray and Griffin, 2002). The first person to attempt recovery TAA from its natural sources was Ventre (1940), who in his patent described the separation of calcium aconitate after evaporation of sorghum juice. Nine years later, he also patented the process for sugarcane. On industrial scale, TAA was recovered from sugar molasses through precipitation and sedimentation with calcium and magnesium at Raceland mill, LA (Godchaux II, 1949). Calcium magnesium aconitate was produced in a plant with a capacity of 5 tons /day (Godchaux II, 1949). The low yield of 38 % in addition the need to convert the salt to acid and its consequent esterification, made this product too expensive in comparison with the phthalate esters (Haines and Joyner, 1955). The factory closed after a few years of commercial operation.

In addition to precipitation and sedimentation techniques, liquid-liquid extraction (LLE) has been studied. At laboratory level, Regna and Bruins (1956) recovered TAA from final molasses using as solvent a solution containing 88% methyl ethyl ketone and 12 % water. In absence of a technique to directly determine the TAA, decarboxylation, a technique

which measures the amount of carbon dioxide formed on decomposition of organic acid was used (Roberts, and Ambler, 1947). Because TAA is not the only organic acid in final molasses, the results cannot be considered. With this approximation in mind, Regna and Bruins (1956) claimed an extraction yield of 93% after six extraction cycles.

More recently, TAA has been recovered from synthetic solutions composed of citric, malic and TAA with a mixture of tributyl phosphate (TBP) and dodecane as solvent by LLE (Malmary et al. 1995). They found a direct relationship between higher organic/aqueous phase ratio (OA) and TAA recovery efficiency; however the selectivity (purity) of TAA decreased under these conditions. Hill and Kancharla (1999) recovered TAA from molasses, by extraction using ethyl acetate as solvent. They claimed an efficiency of 43 % when the residue, after ethyl acetate evaporation, was dissolved in hot acetic acid and allowed to crystallize, then diluted in ethyl acetate and decolorized with activated carbon, followed by a second crystallization. Later, Blinco (2000) recovered TAA from final molasses with different solvents. She found Alamine 336 and TBP, each diluted in Shellsol as the best. At laboratory level, she reported 95% extraction efficiency. For back extraction process she used, among others, sodium hydroxide and sodium carbonate as stripping agents, recovering between 77 to 82% of TAA as its salt.

McMurray and Griffin (2002) used supported liquid membranes. Membranes made of polypropylene (Accurel, TM) were immersed in the same solvents utilized as by Blinco (2002) and put between two cells. The TAA and other components migrated from the departure to the receiving phase. They found good results with synthetic solutions, but when using final molasses, the recovery and selectivity of TAA significantly decreased. Finally,

Moore and Sanborn (2004) patented a procedure to recover organic acids directly as their esters from fermentation broths previously dried either in a spray or fluidized bed dryer.

After a critical analysis of the methods used to recover organic acids, LLE, solid-liquid adsorption (see chapter 5) and the method of Moore and Sanborn (2004) were chosen as the techniques to be tested for recovery of TAA from cane leaf matter (CLM) and final molasses. CLM is the name given to the tops and green leaves of sugarcane. The TAA content in CLM juice is between 8 – 12 g/100 g DS, and in final molasses between 2- 4 % g/100 g DS. CLM juice and final molasses were fermented prior to TAA extraction to increase TAA concentration. For LLE, the solvents studied were TBP mixed with dodecane, ethyl acetate, and butanol. Butanol was chosen as an extractant due to the good results obtained with tributyl aconitate (TBA) as a plasticizer for PVC (Gil et al. 2006). The reason for choosing the Moore and Sanborn process was to obtain the aconitate ester directly from the dry acidified CLM stillage. The TAA solubility in each solvent was determined as well as the miscibility between CLM stillage and each solvent.

Materials and Methods

TAA Solubility in Organic Solvents and CLM - Solvent Miscibility

The solubility of pure TAA in water, TBP, ethyl acetate, dodecane, and 1-butanol was determined at 20^{o}C, 50^{o}C and 80^{o}C. In all cases, an excess of TAA crystals was mixed with the solvent and kept for 3 hours at constant temperature with intermittent agitation. Experiments at 50^{o}C and 80^{o}C were done in a water bath (Iso Temp 3016 H, Fisher Scientific with precision of 0.1 ^{o}C). Then, a sample of homogeneous liquid phase was taken and weighed and dry solids content determined. TBP samples were filtered prior to drying because the high viscosity prevented complete separation of the TAA crystals by settling.

To qualify the feasibility of phase separation and solvent-CLM extracts miscibility, CLM stillage at two concentrations, 35 g DS/100 g and 0.5 g DS/100 g was acidified to pH 2.0 with 50 % sulfuric acid. The acidified solution was centrifuged in an IEC Centra CL2 centrifuge, Thermo Electron Corporation (Waltham, MA) for 30 min at 3000 rpm. 3 g of the supernatant were collected for each OA: (0.25, 0.5, 1.0, 2.0 and 3.5) and transferred into 15 mL cone-bottom centrifuge tubes, Corning ® with screw-top caps and graduations. Three solvents were studied: butanol, ethyl acetate and the mixture of tri-butyl phosphate (70 %v/v) and dodecane (30 %v/v). The tubes were shaken in the wrist shaker for 5 minutes and then allowed to settle for up to 60 minutes in some cases. Photos were taken at every 1, 2, 3, 5, 10, 15, 20, 25 and 60 minutes until no more changes were noticeable. At the end, the tubes were centrifuged again in the same centrifuge for 10 min at 3000 rpm. Observations of the time required for the phases to separate and changes in the volume of each phase were recorded.

Reagents: TAA, (98%), TBP, (97%), and dodecane (99%), were purchased from Sigma Aldrich (St. Louis, MO). 1- butanol, (>99%), was obtained from Fisher Scientific (Fair Lawn, NJ) and ethyl acetate, (99.8%), was purchased from EMD (Darmstadt, Germany). All reagents were used as received.

CLM and Molasses Stillage Preparation

CLM collected in commercial fields, either manually or after mechanical harvesting, was reduced in size in a shredder, 15x8 (Jeffrey Specialty Equipment Woodruff, SC). The juice was extracted by crushing three times in a 3-roller pilot mill, 0.3x0.3m (Farrell, Ansonia, CN) at Audubon Sugar Institute. Preparation of the stillage involved four steps: the batch fermentation of CLM juice or final molasses, the separation of yeast by centrifugation, the separation of ethanol by distillation, and the concentration of stillage. The batch fermentation

was made in a glass reactor with capacity of 22 L. The equipment was provided with a thermostat to control the temperature between 34-36°C and a variable speed impeller (Figure 4.1). The initial total fermentable sugars (TFS) fluctuated between 60 and 80 g /L. RapidRise yeast (Fleischmann's), in a proportion of 25 g/100 g TFS, was used for the fermentation. The initial pH fluctuated between 5.1 and 5.3, and was not controlled as it was found not to vary during the fermentation. The fermentation was finished when the soluble solids content in two consecutive hourly samples did not change by more than 0.1 units. A sample of final molasses was fermented under the same conditions to compare the yields obtained between these two materials.

After fermentation, yeast was separated from the fermentation broth by centrifugation in an IEC-EXD centrifuge, (Damon/IEC division), for 20 min at 1800 rpm (*g*-force= 756 *g*). After that, the fermentation broths were concentrated in a 10 L rotary-evaporator RE-71 (Yamato Scientific America Inc, Santa Clara, CA), heated with water at 85°C. The evaporator was operated at 76.2 kPa of vacuum (Figure 4.2). After ethanol removal, the stillage was concentrated in the same equipment and conditions until 30 % DS.

Liquid- Liquid Extraction

Trials were conducted by contacting the aqueous phase, the concentrated CLM stillage acidified with diluted 50% vol. sulfuric acid, and the organic phase in a sealed plastic flask. The mixture was shaken either with a Wrist Shaker Action 75 (Burrell, Pittsburgh, PA) when the experiments were conducted at room temperature (22± 2°C), or a 360 Orbital Shaker Bath (Precision Scientific) provided with speed and temperature controller when the experiments were conducted at different temperatures. After a pre-determined period, the material was allowed to settle for one hour. To complete the separation, raffinate was centrifugated at 1800

rpm for 20 minutes, using the centrifuge described before. The weight of light phase, combined, and raffinate phase were recorded. Samples of CLM stillage, raffinate and in some trials, extracts were analyzed for anions. The TBP- dodecane extract was kept for back extraction and the butanol extract for esterification tests. No further treatments were done on the ethyl acetate extract.

Figure 4.1 Equipment used for CLM Fermentation

Three extractants were used in LLE trials: TBP, butanol, and ethyl acetate. Dodecane was chosen as inert diluent of TPB for its relative low viscosity $3.75x10^{-3}$ Pa.s in comparison $15.8x10^{-3}$ of TBP and its insolubility in water. The OA varied from1 to 5 depending on the extractans. Temperature effect was studied at three levels: room temperature (22± 2° C), 50 °C and 80 °C. The pH of CLM stillage was adjusted between 2.6 and 2.

Back Extraction of TBP-dodecane Extract

TBP-dodecane extract was blended with these stripping agents: water, at 1:1 ratio or 0.1 and 0.2 M NaOH, at 1:2 ratio. The mixture was shaken for 60 min at room temperature

(22± 2° C), then the phase separation was completed by centrifugation at 1800 rpm for 20 min. The TAA recovered in the receiving phase was quantified.

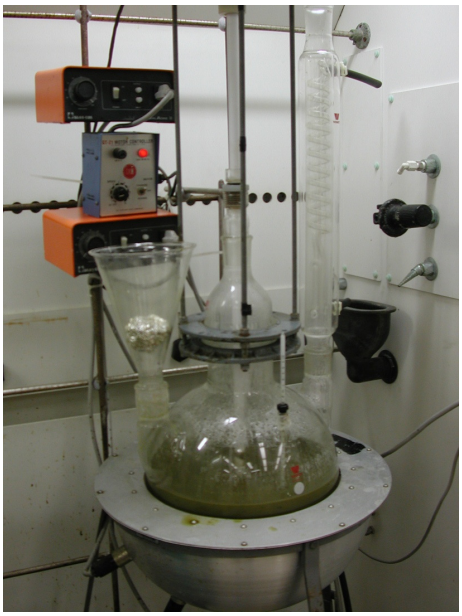

Figure 4.2 Rotary-evaporator used to concentrate the stillage

Solid-Liquid Extraction

Following the procedure described by Moore and Sanborn (2004), CLM stillage was dried in a spray dryer (Pulvis GB 22, Yamato Scientific; Tokyo, Japan) (Figure 4.3). For the recovery of organic acids (iso-citric, citric, cis-aconitic and TAA) as esters, 6 g of CLM powder stillage containing approximately 18% of the organic acids and 30 g of butanol were mixed with variable amounts of concentrated sulfuric acid (0.3, 0.5 and 0.8 g) and boiled in a 250 mL round bottom flask at atmospheric pressure under reflux, allowing for continuous removal of water. Samples for GC analysis were taken at different times.

Esterification of Butanol Extract

The TAA recovered in butanol extract was esterified following a Fisher esterification procedure with sulfuric acid or a cation exchange resin, AG 50W-X4, 100-200 mesh,

hydrogen form (Bio-Rad, Sunnyvale, CA) as a catalyst. A mixture of 50 g of butanol extract with either sulfuric acid, 0.5 – 1.2 g/100 g butanol extract, or a resin up to 16 g/100 g butanol extract was boiled in a 250 mL round bottom flask at atmospheric pressure under reflux in an arrangement similar to a Dean-Stark apparatus. This arrangement allowed a continuous removal of water formed in the reaction. After about one and half hour of boiling when temperature of about 120°C was reached and no more water was being produced, the reaction was considered complete. The resin, when used, was screened off from the liquid product and the amount of TBA formed was determined by GC.

Figure 4.3 CLM drying process 1) CLM stillage 30 g/100 g DS, 2) Detail of CLM powder

To compare the effect of the impurities on the esterification yield, butanol extract and pure TAA were esterified in parallel. To simulate the conditions of butanol extract, 0.4 g of pure TAA was blended with 50 g of butanol. Cation exchange resin and sulfuric acid were used as catalysts.

Color in CLM Stillage and Color Transfer from CLM Stillage to Butanol Extract

Color in CLM stillage was determined by adjusting the pH of CLM stillage to pH 7 and measuring the absorbance (AU) value at 420 nm (ICUMSA, 2005). To provide more information about color bodies, the index value, IV, which is the ratio of color at pH 9 and pH 4, was determined. In order to determine if the transference of color from aqueous to organic phase was associated with the contact time, butanol and CLM stillage, 35 g /100g DS , acidified at pH 2.6 with sulfuric acid diluted 50% v, were blended at OA= 3.5. Seven tests were carried out at the following contact times, 1 min, 2 min, 5 min, 10 min, 20 min, 40 min, and 60 min. The AU at 420 nm, using butanol as blank, and dry substance were measured for all samples, without adjusting the pH.

Decolorization

Decolorization tests were conducted prior to and after esterification process. Preliminary trials were carried out with granular and powder activated carbon, at 20 °C and 50° C. The best decolorization was reached using powdered activated carbon at 50 °C; these conditions were used during further tests.

Analytical Methodologies

Organic Acids Determination

The concentration of TAA, cis-aconitic, citric, iso-citric, and ten other acids were determined as their corresponding anions with a Dionex (Sunnyvale, CA) HPIC system provided with an IonPac AS11 column and suppressed-conductivity detector. Sample volumes of 50µl were injected into the mobile phase with a Spectra System AS 3000 auto sampler. The mobile phase was composed by three eluents; dionized water and sodium hydroxide at 4mM and 100 mM. Its composition changed according to the gradient program

shown in Table 4.1. The time of analysis was 60 minutes. Samples were diluted in deionized water and filtered through a Cole Palmer (Vernon Hills, IL) syringe filter SCFA membrane 0.45 µm pore size. The concentrations were determined with the Dionex Peaknet System (Version 4) software from the peak areas calibrated against three standards (Table 4.2 and Figure 4.4) run before and after the samples. The chromatogram for a No 2standard is illustrated in Figure 4.5. For determination of anions in the organic phase, a sample of the extract was put in a vacuum oven until solvent was completely removed. Then, the dry solids were diluted in deionized water.

Table 4.1 Gradient program for anion separation

		Eluent, %		
Time, min	Flow, mL/min	1	2	3
0.0	1.0	85	0	15
14.0	1.1	85	0	15
15.0	1.2	85	0	15
18.8	1.2	80	0	20
22.9	1.3	80	10	10
26.0	1.4	70	20	10
35.2	1.4	40	60	0
47.6	1.4	35	65	0
51.5	1.3	85	0	15
54	1.2	85	0	15

Eluent 1: de-ionized water
Eluent 2: 100 mM sodium hydroxide
Eluent 3: 4 mM sodium hydroxide

Determination of Sugars and Ethanol

Sucrose, glucose, fructose and ethanol were determined with a Bio-Rad Laboratories, (Richmond, CA) HPLC, using HPX-87K column kept at 85 °C and refractive index detector. The mobile phase was a solution of 0.01 M of K_2SO_4 eluted isocratically at 0.6mL/min. The time of analysis was 16 minutes. Samples were diluted in deionized water and filtered with a

Cole Palmer (Vernon Hills, IL) syringe filter SCFA membrane 0.45 µm pore size. Their concentrations were calculated from calibration standard curve for each component (Table 4.3 and Figure 4.6).

Table 4.2 Retention time and concentrations (mg/L) of the three standards for anion determination

Anions	Retention time, min	Standard 1	Standard 2	Standard 3
Lactate	13.85	1.000	5.001	10.285
Acetate	14.50	2.005	10.023	20.046
Shikimate	16.00	1.998	9.991	19.982
Chlorate	28.40	5.000	25.000	50.000
Malate	31.45	1.005	5.023	10.045
Sulfate	33.10	2.000	10.000	20.000
Oxalate	33.50	2.000	10.001	20.001
Phosphate	36.40	1.000	5.000	10.000
Citrate	38.00	2.005	10.026	20.052
Iso-citrate	38.90	2.001	10.007	20.014
Cis-aconitate	39.50	1.998	9.991	19.982
TAA	41.45	9.894	49.471	98.942

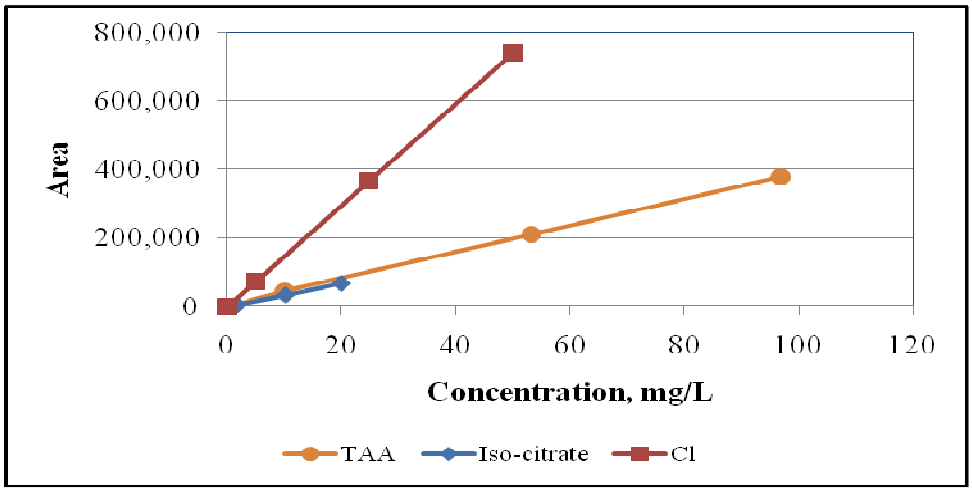

Figure 4.4 Calibration curves of three of the anion standards

Table 4.3 Retention time and standard concentrations (mg/L) for determination of sugars and ethanol

Sugar/alcohol	Retention time, min	Standard 1	Standard 2	Standard 3
Sucrose	8.40	1030.8	2003.2	3001.3
Glucose	11.52	91.2	300.3	600.7
Fructose	12.51	90.6	298.3	595.7
Ethanol	14.52	2500	5000	10000

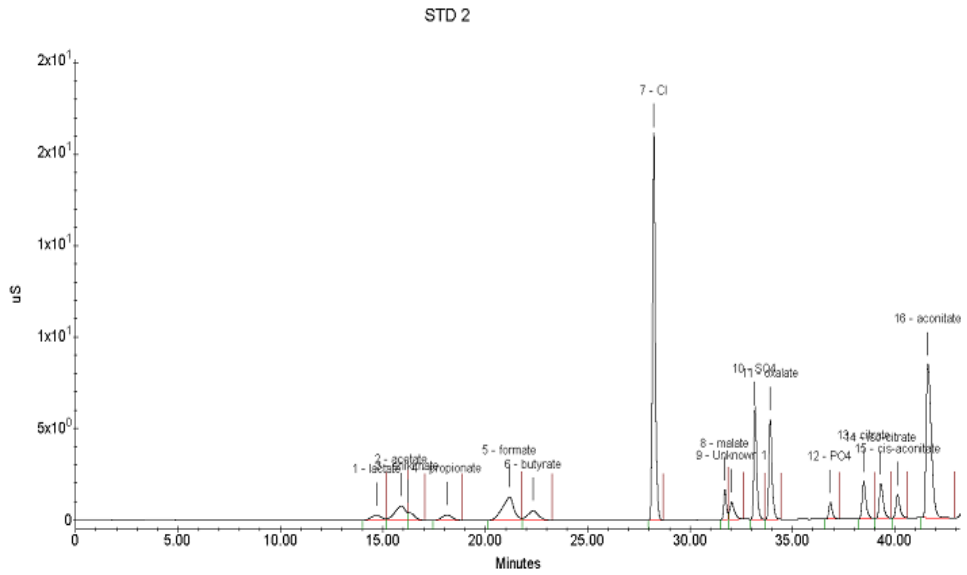

Figure 4.5 Chromatogram of the standard No 2 for anion determination

Characterization of Esterification Products

The amount of TBA was quantified with an Agilent Technologies (Foster City, CA) 6890 N GC instrument, with a flame ionization detector and a DB-5 capillary column (30 m, 0.25µ film) under the following conditions: oven temperature 250 °C, run time 32 min, Helium carrier gas at 30 mL/min, and a 2µL injection volume. The esterified butanol extracts were diluted in ethyl acetate. Concentrations were calculated from peak areas of GC against external standards at 3 concentrations levels of TBA (Figure 4.7) diluted in ethyl acetate.

A TBA standard was prepared in the laboratory, because a commercial product could not be obtained. The standard was prepared by mixing 30 g of TAA was mixed with 57.4 g of butanol (a 50 % excess) and 4 g of pre-dried cation exchange resin AG 50W-X4, 100-200 mesh, hydrogen form (Bio-Rad, Sunnyvale, CA) as catalyst. The esterification was performed following the procedure described before. After esterification reaction has finished, the excess alcohol was removed by heating to about 80 °C for an hour under vacuum. The resulting chromatogram for a standard 2, 1013 mg/L of TBA diluted in ethyl acetate, is shown in

Figure 4.8. The peak at five minutes corresponds to traces of butanol in the sample and the main peak at 7.9 minutes to ethyl acetate used to dilute TBA. TBA had a retention time of 29.6 min.

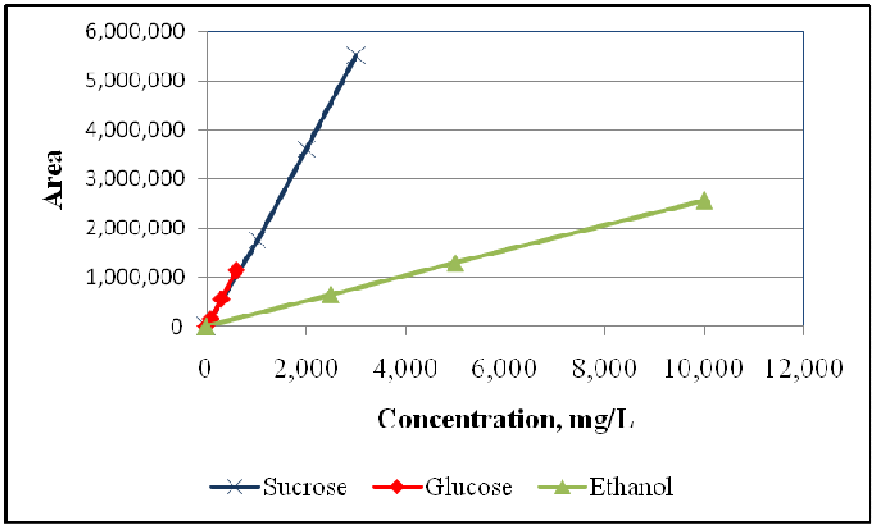

Figure 4.6 Calibration curve for sucrose, glucose and ethanol determination

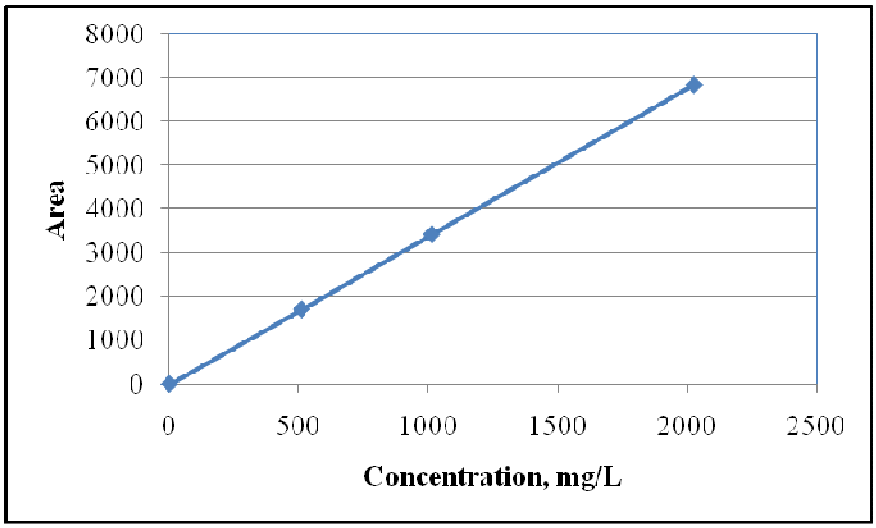

Figure 4.7 Calibration curve for TBA determination

Determination of Dry Solids

Dry solids were determined by drying in a vacuum oven at 76.2 kPa and 65°C. Approximately 2 g of sample were weighted in an aluminum dish and kept in the oven for 18

h or until constant weight. For the samples of TBP - dodecane extract the temperature was increased up to 120°C, at the same pressure

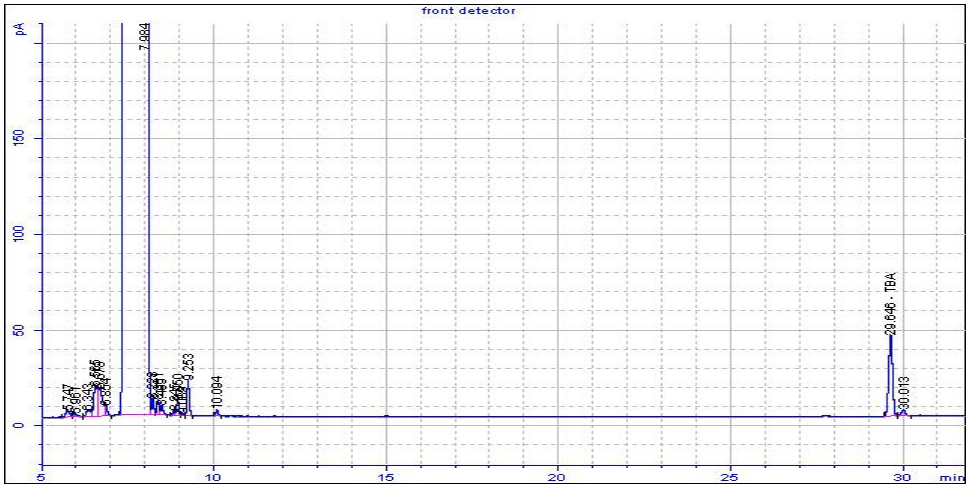

Figure 4.8 Chromatogram for standard No 2 for TBA determination

Results and Discussion

Aconitic Acid Solubility in Different Solvents

The solubility was calculated as grams of TAA dissolved in 100 g of solvent. The results (Table 4.4) indicated that TAA is almost insoluble in dodecane and slightly soluble in TBP at 20°C.

Table 4.4 TAA solubility in different solvents at three temperatures

Solvent	TAA, g/100 g of solvent		
	20 °C	50 °C	80 °C
Water	24.25	52.15	97.29
Butanol	11.78	20.54	33.68
Ethyl acetate	2.29	4.61	7.48
TBP	5.15	3.37	3.08
Dodecane	0.00	0.00	0.10

Water is the best solvent for TAA followed by butanol and, ethyl acetate. The solubility of TAA in water was reported by Patarau (1989), at 13 °C, it was 18.6 g/100 mL

and it increased to 110.7 g/100 mL at 90 °C. These values combined with the found in this study (Table 4.4) fit in a first lineal equation. For all solvents, except for TBP, the solubility increased as temperature increased (Figure 4.9).

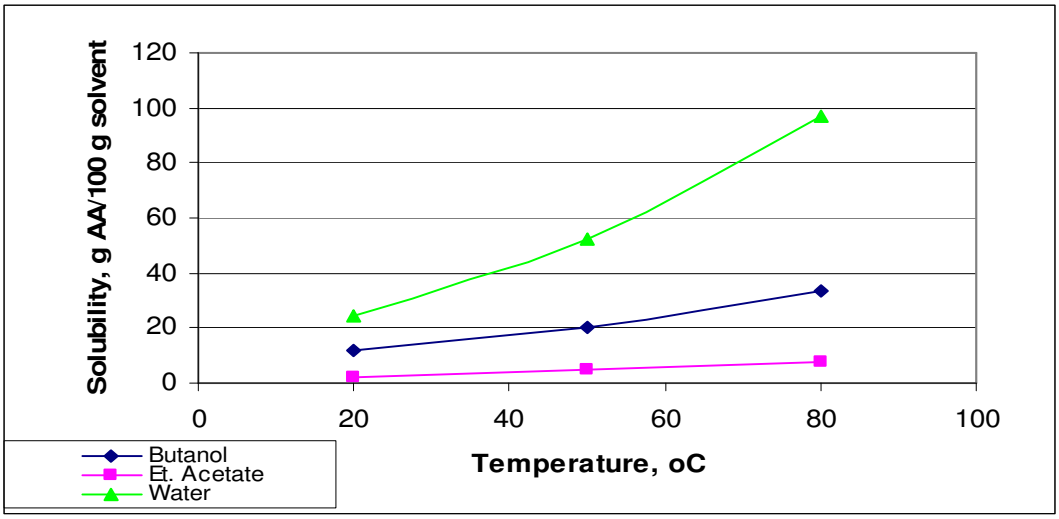

Figure 4.9 TAA solubility in different solvents and at different temperatures

CLM Juice and Diluted Final Molasses Fermentation

CLM juice and diluted final molasses with TFS of 7.4 g/100 g and 6.8 g/100 g respectively, were fermented under the same conditions. In both cases, the fermentation time was 5 h. The rate of TFS decrease with time was similar for both materials (Figure 4.10). The sugar remaining was mainly fructose. An apparent sampling error, one hour after the fermentation started, caused a small increment in TFS of final molasses between two consecutive samples. The yield expressed as percent of theoretical (0.51 g ethanol/ g TFS), was 92 and 96 for CLM and final molasses respectively. The effect of TAA on yield is apparently lower than reported by Mane et al. (2005). They found that for each percent of TAA, the ethanol yield decreased by 1.6%. In our work, the difference in TAA content

between CLM juice and final molasses was 3.2 %, therefore the reduction of yield by each percent of TAA was 1.25%.

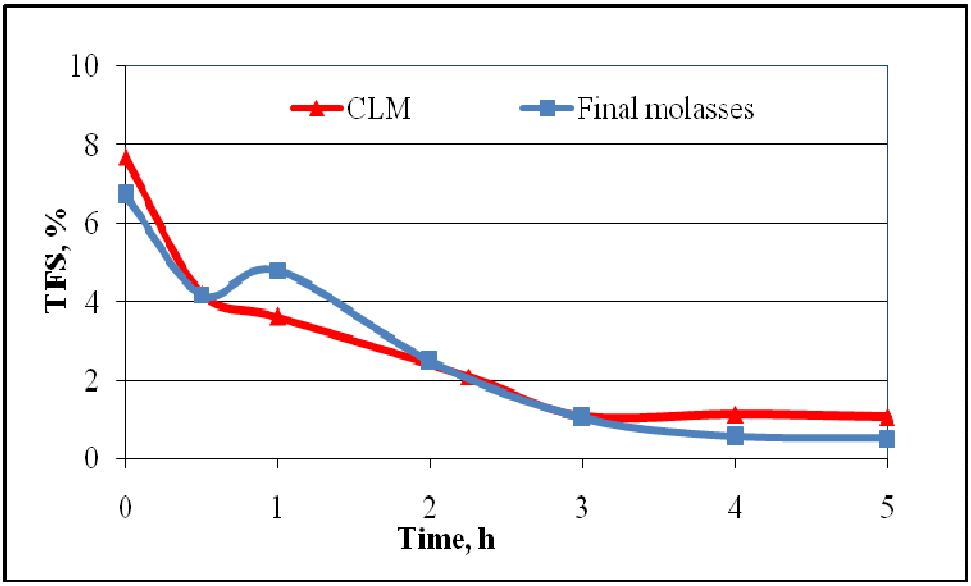

Figure 4.10 TFS profile during fermentation of CLM and final molasses

Physical Properties of the Solvents

The viscosity and solubility (soly) of solvent in water and water in solvent, for each of the solvents are presented in Table 4.5 (Flick, 1985; Caudwell et al. 2004).

Solvent – CLM Stillage Miscibility

Aqueous phase: CLM Stillage 30 g DS/100 g Solution

For all experiments, two photos are presented. The first one shows the final phase separation reached by settling alone. The second photo shows the system after the phase separation has been completed by additional centrifugation.

Butanol: Figure 4.11-a shows the two phases after 1.5 minutes of settling, and Figure 4.11-b after centrifugation. The high solubility of water in butanol causes two effects with increasing OA, the first one is the reduction in the volume of raffinate (bottom phase), and the

second one is the formation of a third heavy phase. At the highest OA, the DS in raffinate is increased from 35g DS/100g up to 65 g DS/100 g; around 70 % of water, initially in aqueous phase, was co-extracted. As the water content decreases, precipitation in the aqueous phase occurs and forms the third heavy phase that can be observed in Figure 4.11.

Table 4.5 Viscosity and solubility of the four solvents with water at specific temperature (°C)

Solvent	μ, cp (T,°C)	soly in water (T,°C)	soly of water in solvent (T,°C)
n- butanol	2.948 (20)	7.8 g/100g (20)	20.1 g/100 g (20)
Ethyl acetate	0.455 (20)	9.7 g/100 mL (25)	9.8 g/100mL (25)
TBP	15.80 (25)	0.1 g/100mL (25)	6.4 g/100 mL (25)
Dodecane	3.75 (25)	Insoluble	0.0065 g/100g (25)

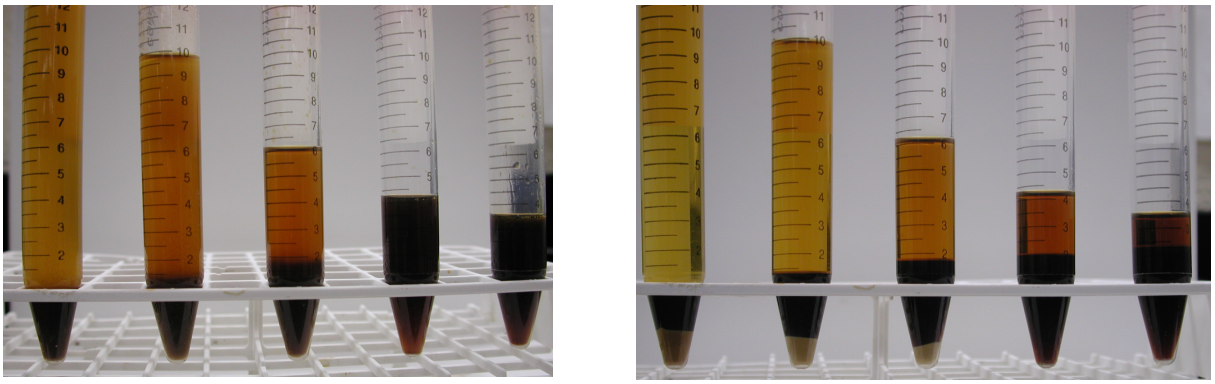

Figure 4.11 Phase separation for the butanol- CLM stillage system. OA: 3.5, 2, 1, 0.5, 0.25 (left to right). a) after settling for 1.5 min. b) after centrifugation

Ethyl acetate: Figures 4.12-a and 4.12-b show the phase separation after 40 sec of sedimentation, and after centrifugation. The separation by sedimentation of the two phases was faster than with the other two solvents, which is related to the low viscosity of ethyl acetate. After centrifugation (Figure 4.12-b) a third phase is observed at higher OA. No

changes in the amount of raffinate were observed, which does not means that an interchange of water and ethyl acetate could not have occurred. This is corroborated by the noticeable reduction of viscosity and the strong odor of ethyl acetate in the raffinate after separation of the two phases. The DS in raffinate decreased slightly from 37.5 g DS/100g in CLM stillage to about 33- 34 g DS/100 g in the raffinate as consequence of the solute extraction to ethyl acetate phase

Tributyl phosphate - dodecane: This solvent required 10 minutes for separation by sedimentation at the highest OA (Figure 4.13- a). After centrifugation, formation of a third (middle) phase was observed, at all OA. The system TBP-dodecane- CLM stillage has an almost ideal behavior in terms of volume change for all O/A ratios evaluated.

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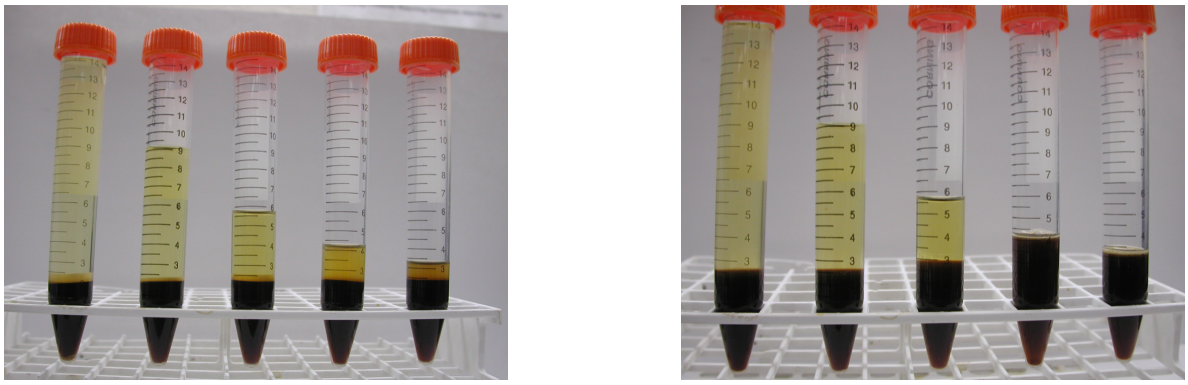

Figure 4.12 Phase separation for the ethyl acetate- CLM stillage system. OA: 3.5, 2, 1, 0.5, 0.25 (left to right). a) after sedimentation for 40 sec. b) after centrifugation

Since in all experiments, the only variable was the relation between the organic and the aqueous phases, the increase in the volume of the organic phase, when butanol was used as solvent, was directly related to the high solubility of water in butanol.

The water co-extraction, that is water that enters the organic phase with the solute, depends on the solubility of the water in the solvent. Water co-extraction also depends on the carboxylic acid extracted. Mono-carboxylic acids carry less water with them than do di-carboxylic acids (Kertes and King, 1986).

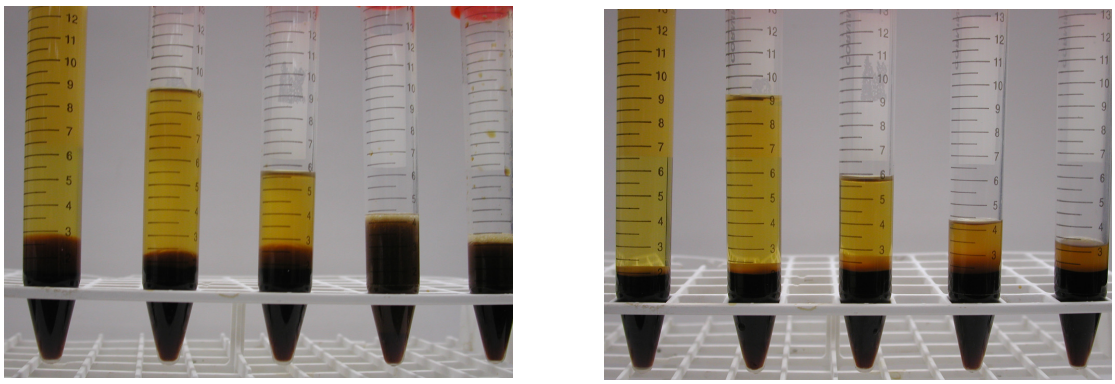

Figure 4.13 Phase separation for the TBP and dodecane - CLM stillage system. OA: 3.5, 2, 1, 0.5, 0.25 (left to right). a) after sedimentation for 10 min. b) after centrifugation

The mutual solubility between an aqueous solution and a solvent at a fixed temperature depends on the kind of components extracted and its total concentration in the system. Solutions containing weak organic acids such TAA, citric and iso-citric acids to be extracted, mutual solubilities cause significant volume changes. The trend is that the volume of the organic phase increases as the total concentration of the acid in the system increases. There is a characteristic behavior between hydrocarbon solvents and those with functional groups. For example, solvent-water pair such as isobutyl alcohol-water (with significant mutual solubility) will accept lower carboxylic acid concentrations before a complete miscibility sets in than a solvent- water pair with low solubility such us TBP - water (Kertes and King, 1986). The last system would have an ideal behavior in term of volume changes at low carboxylic acid concentrations.

Carboxylic acids extracted with carbon-bonded oxygen, such as butanol are strongly hydrated by varying number of water molecules (Kertes and King, 1986). The solvation number, which is number of water molecules joined with carboxylic acid is undetermined, but large, so a large number of butanol molecules is required to compete with the water molecules that hydrate the acid molecules at the interface. No dimerization is observed in solvents with an oxygen donor functional group (Kertes and King, 1986).

Comparing the solvents, it can be concluded that ethyl acetate was the solvent that gave faster separation and lower color transferred from the acidified CLM stillage to the organic phase (Figure 4.14) than the other solvents. The second fastest in separation was butanol, but it was first on color transfer. Water and the other compounds of CLM stillage were more soluble in butanol than in other solvents, which were reflected in the reduction of volume of CLM stillage when the amount of butanol was increased. Finally, the mixture TBP-dodecane had the slowest separation rate, due to its relatively high viscosity. In this mixture, a third phase was observed probably due to the insolubility of water in dodecane and the low solubility of water in TBP The transference of color was similar to that in butanol.

Aqueous Phase: CLM Stillage 0.5 g DS/100g Solution

The results corroborated the high solubility of water in butanol, evidenced by the significant reduction in the volume of aqueous phase with increasing of OA. Complete miscibility was observed in the system of diluted CLM stillage-ethyl acetate, at 0.25, the lowest OA. For all the ethyl acetate- diluted CLM stillage ratios and for TBP and dodecane – diluted CLM sitllage ratios, two phases were observed with no perceptible changes in their volume (Figure 4.15).

The relative change in raffinate volume was calculated to compare the effect of DS in CLM stillage and OA on raffinate volume reduction using the equation shown below,

$$\text{Change of raffinate volume, \% initial} = (VR/VRo)\times 100$$

Where:

VR= Raffinate volume after phase separation, mL

VRo= Initial raffinate volume, mL

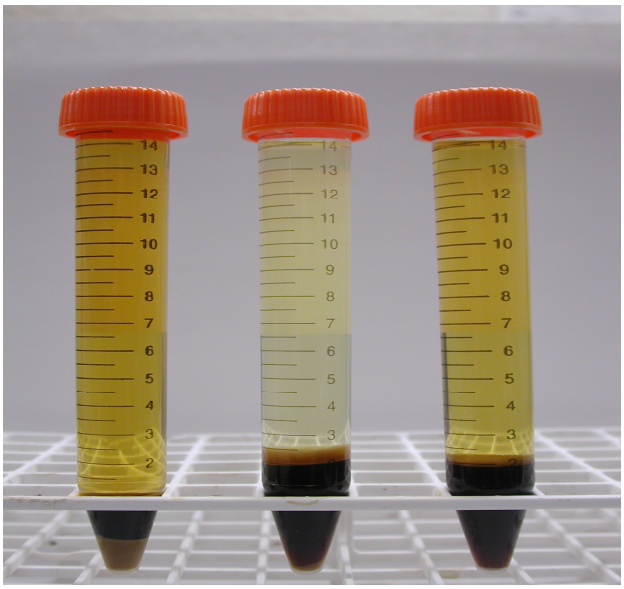

Figure 4.14 Comparison among butanol (left), ethyl acetate (middle) and TBP- dodecane, at OA 3.5

The results, for the systems CLM stillage 35 g DS/100g – butanol and diluted CLM stillage 0.5 g DS/100 g –butanol, show similar behavior. The reduction in raffinate volume increased at higher OA (Figure 4.16). The amount of solids in CLM stillage had a slight effect on solubility. The percent of change in raffinate volume in concentrated CLM stillage was always lower than that observed in diluted CLM stillage (Figure 4.16). The difference was significant at high OA.

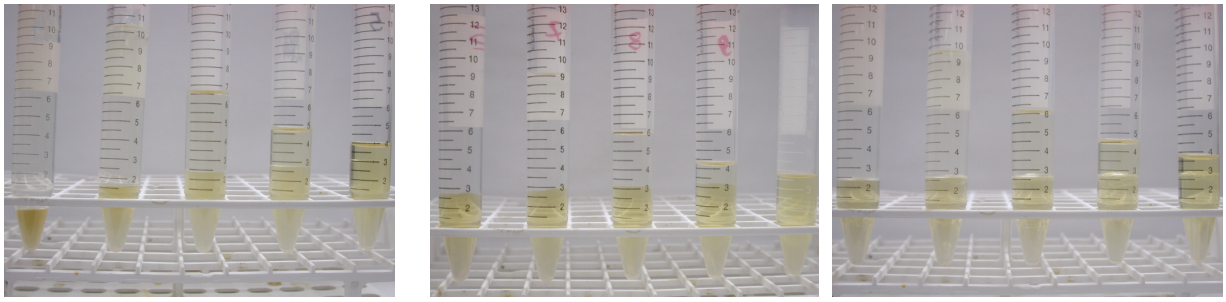

Figure 4.15 Comparison among diluted CLM acidified stillage in butanol (left), ethyl acetate (middle) and TBP- dodecane. OA=3.5, 2, 1, 0.5 and 0.25 (left to right)

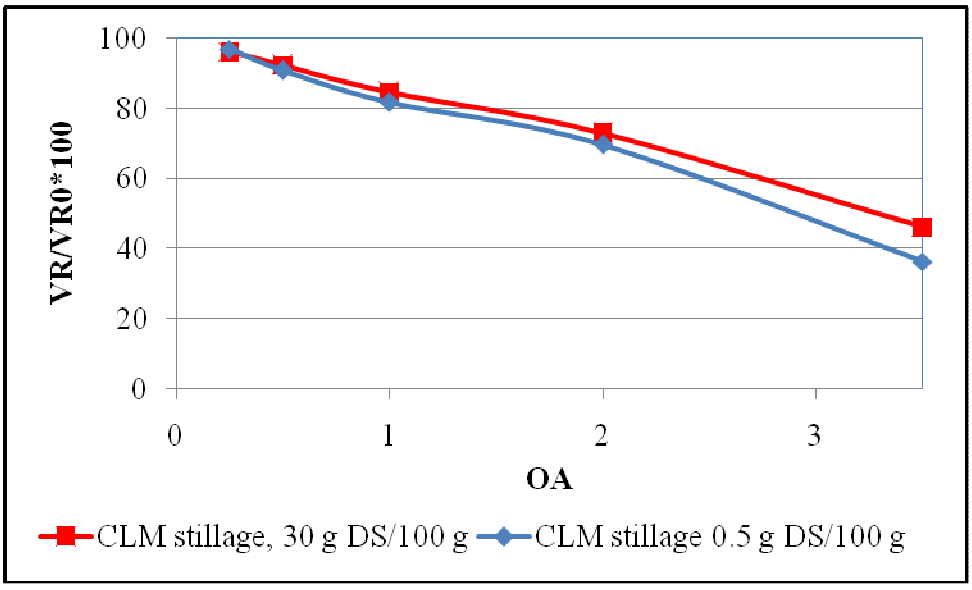

Figure 4.16 Comparison of raffinate volume change between CLM stillage 30g DS/100-butanol and CLM stillage 0.5 g DS/100g at different OA

Sulfuric Acid Consumption

Sulfuric acid is needed during: 1) TAA extraction, to lower pH below pK_a of TAA 2.6, and 2) during esterification, as a catalyst. To estimate sulfuric acid consumption during TAA extraction, CLM stillage containing 33 g DS/100 g, with 4 g TAA/100 g DS, at an initial pH 4.9, was acidified using 1% diluted sulfuric acid. The sulfuric acid consumption referred to the amount of CLM stillage and TAA is illustrated in Figure 4.17 To acidify 100 g of the DS

CLM stillage to pH 2.6; 15.6 g of sulfuric acid, corresponding to 96 % of TAA, was required. At pH 2, the consumption of sulfuric acid is equal to 1.32 times the amount of TAA in CLM stillage (Figure 4.17).

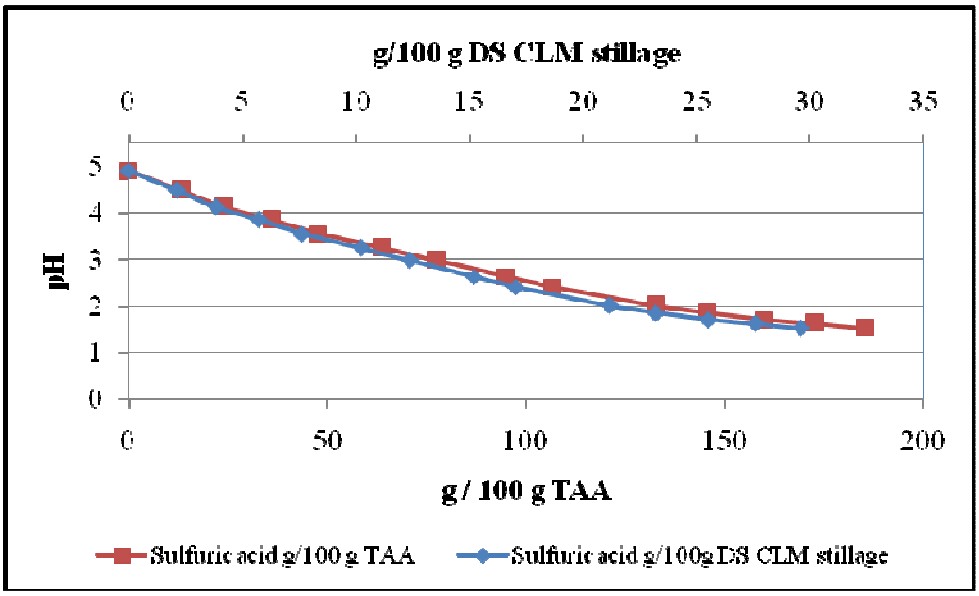

Figure 4.17 Sulfuric acid consumption

Liquid – Liquid Extraction

The anion composition of acidified CLM stillage at pH 2.6, is presented in, the same order as eluted from the IonPac AS11 column. Table 4.6 clearly shows the higher amount of TAA with respect to the other anions, with the only close values corresponding to sulfate as result of the acidification with sulfuric acid. Notice that the anions represent less than 40 g/100 g CLM stillage in dry basis. Although TAA is the anion in the highest proportion, 11.3 g/100 g DS, it represents only the main impurity in this complex matrix. Typical chromatograms, of extract and raffinate phase after LLE, are shown in Figures 4.18 and 4.19. Mostly, all the

sulfate, chlorate and phosphate were retained in raffinate phase showing the affinity of these anions for aqueous phase.

Table 4.6 Anion composition of acidified CLM stillage

Anion	g/100 g DS
$Lactate^{-}$	0.34
Acetate/shikimate	0.41
$Chloride^{-}$	4.50
$Malate^{2-}$	0.11
$Sulphate^{2-}$	9.49
$Oxalate^{2-}$	0.25
$Phosphate^{3-}$	1.95
$Citrate^{3-}$	0.80
$Iso\text{-}citrate^{3-}$	2.61
$Cis\text{-}aconitate^{3-}$	1.53
TAA^{3-}	11.30

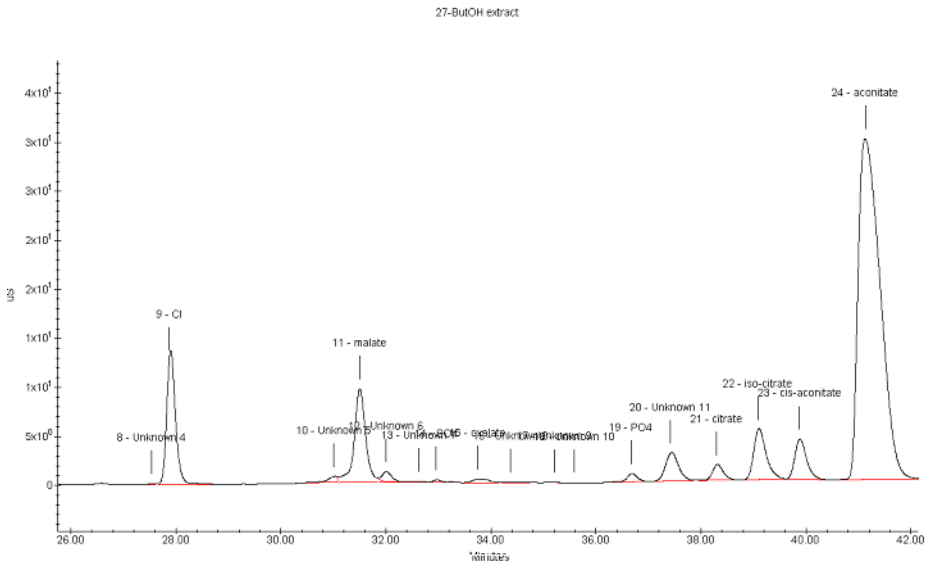

Figure 4.18 Anion chromatogram for butanol extract phase

Tributyl Phosphate

A mixture of TBP (70 mL/100 mL) and dodecane (30mL/100mL) was contacted with acidified CLM stillage at 3 OA: 1, 2.5 and 5 in duplicate (Table 4.6). All tests were conducted

with CLM stillage 33.9 g DS/100 g at 20± 2°C. After extraction, samples with lower and middle OA presented the formation of a third gelatinous “middle phase”. This phase has been attributed to the limited solubility of the extracted materials-ligand complex in the non-polar organic phase (Borkowski et al. 2002). The composition of the middle phase was assumed to be the same as that of the extract phase for balance purpose. The initial and final weights of each phase are shown in Table 4.7.

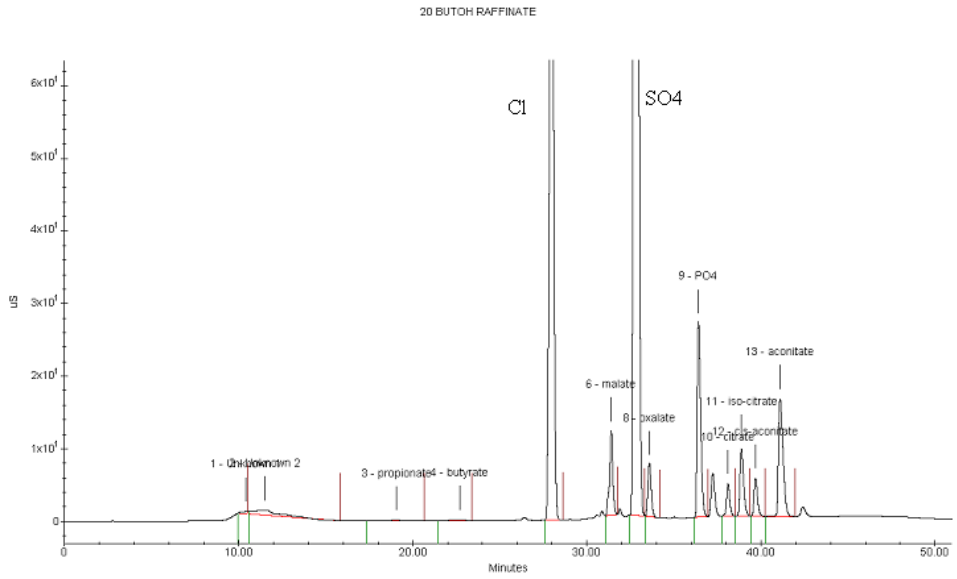

Figure 4.19 Anion chromatogram for butanol raffinate phase

Significant amounts of middle phase are formed at lower OA. Borkowski et al. (2002) found that third phase formation may depend on the limiting organic concentration (LOC) which is the highest extracted compounds concentration in the organic phase without the formation of a third phase (Borkowski et al. 2002). Third phase formation makes it difficult to operate multiple counter- current solvent extraction systems and creates errors in the material balance (Kedari et al. 2005). Third phase formation is also a function of the carbon chain length and the degree of branching of the diluents. Short chain, aliphatic compounds have

higher LOC. However, the low vapor pressure of these diluents, their flammability, and toxicity limit their applications in commercial process (Kedari et al. 2005). Dodecane, a nonpolar and a long straight chain diluent, can adopt a relatively regular structure with strong Van der Wall's forces; therefore it does not contribute to increasing the LOC when blended with TBP.

The extraction yield of TAA, together with its isomer, citric and iso-citric is illustrated in Table 4.8. On average, lower yields were obtained at low OA even though no significant yield differences were observed between the middle and high ratio. The formation of intermediate phase at middle OA prevented its use.

Table 4.7 Initial and final weight (g) of the phases during liquid-liquid extraction with TBP - dodecane

OA	CLM stillage	Organic phase	Extract	Raffinate	Middle phase
1	70	70	45.5	54.7	35.3
1	70	70	44.2	60.3	29.4
2.5	57	143	140.0	47.7	10.8
2.5	57	143	137.8	52.2	8.2
5	33	168	168.7	28.5	0
5	33	168	164.4	31.3	0

TAA and cis-aconitic extraction yield was higher than citric and iso-citric acids. This difference in the extraction yield of citric and iso-citric acids is explained by the hydroxyl functional group which makes these acids more polar and reduces their lipophilicity (McMurray and Griffin, 2002). The TAA purity ranged between 25 and 37 g/100 g DS, indicating that not only organic acids are transported from CLM stillage to the extract phase but also other compounds. None of these were identified in this work. TAA purity is lower than those reported by McMurray and Griffin (2002); extracting TAA from final molasses, using supported membranes immersed in a mixture of TPB and Shellsol with purities, ranged between 40 and 60 g/100 g DS.

The back extraction of TAA from the TBP-dodecane loaded phase (Table 4.8) with water, NaOH 0.1 M and NaOH 0.2 M is illustrated in Table 4.9. From the results, it can be concluded that water is not a good stripping agent for TAA, which corroborates the observations by Malmary et al. (1995). NaOH works better but at higher concentration. Extraction conditions play an important role; obtaining the best results at high TBP/CLM ratio (Table 4.9). The overall recovered yield, including extraction and back extraction process, was around 80 g/100 g. The main disadvantage of this procedure is that the final product is the aconitate salt instead of the free acid. Further processes are required to obtain and purify the acid, decreasing the yield and increasing the cost.

Table 4.8 Extraction yield of some organic acids and TAA purity in extract using TBP-dodecane

OA	Extraction yield, %				TAA, g /100 g DS in extract
	Citric	Iso-citric	Cis-aconitic	TAA	
1	99	44	49	88	34
1	99	35	43	84	28
2.5	63	58	92	95	25
2.5	14	31	39	87	35
5	55	67	91	94	35
5	67	73	93	95	37

Table 4.9 Recovery of TAA, g/100 g in TBP-dodecane loaded phase, from back extraction with different stripping agents

OA	Stripping agent		
	Water	NaOH, 0.1 M	NaOH, 0.2M
1	5.8	12.5	25.0
1	5.1	9.4	20.4
2.5	5.9	25.4	48.5
2.5	4.0	22.3	49.1
5	4.0	41.2	84.0
5	3.5	40.0	76.2

Ethyl Acetate

This solvent was evaluated in two tests conducted at an OA of 3.5, 20°C and 1 h contact time. The effect of pH was studied at 2 pH levels: 2.0 and 2.6. In both tests 10 g of

CLM stillage were used. No significant changes were observed in the weight of the phases after LLE (Table 4.10). The DS of the raffinate slightly decreased in comparison with acidified CLM stillage, which was 36.2 g/100g DS and 37.7 g/100 DS at pH 2.6 and 2.0 respectively.

Table 4.10 Weight and concentration of phases after LLE with ethyl acetate

	Raffinate		Extract	
pH	Weight, g	DS, g/100g	Weight, g	DS, g/100 g
2.6	9.8	35.7	34.6	1.29
2.6	11.2	34.0	33.9	1.17
Average	10.5	34.9	34.2	1.23
2.0	9.8	36.8	34.7	1.48
2.0	9.8	36.5	34.7	1.41
Average	9.8	36.7	34.7	1.45

Ethyl acetate is a highly selective TAA extractant. On average, at pH 2.6, for each 100 g DS in extract phase, 54 g correspond to TAA. The selectivity decreases with decreasing pH (Table 4.11). Although with ethyl acetate, the selectivity is high, the extraction yield is quite low. On average, 64 g TAA were extracted per 100 g of TAA in CLM stillage acidified to pH 2.0. The extraction yield decreased from 64 to 46.8 in CLM stillage acidified to pH 2.6 (Table 411).Hill and Kancharla (1999) recovered 81% of TAA from sugar molasses, after acidifying the feedstock to pH 1.3. This corroborates the effect of pH on extraction yield. The amount of sulfuric acid required to acidify CLM stillage to 1.3 pH would be twice than the amount of TAA expected to be extracted (Figure 4.17) without considering the decrease in TAA selectivity at this low pH.

Butanol

The variables studied during the extraction of TAA with butanol were the OA, pH, time, temperature and CLM stillage solids concentration. The variables were adjusted in agreement with the best results obtained.

Table 4.11 Extraction yield of some organic acids and TAA purity in the extract using ethyl acetate

pH	Extraction yield, %				TAA, g /100 g DS in extract
	Citric	Iso-citric	Cis-aconitic	TAA	
2.6	5.2	6.3	11.5	46.8	53.7
2.6	5.7	6.9	12.9	49.9	55.2
Average	5.5	6.6	12.2	48.4	54.4
2.0	11.3	17.8	26.1	61.6	50.2
2.0	13.4	20.2	31.5	66.3	52.7
Average	12.4	19.0	28.8	64.0	51.5

Organic/Aqueous Ratio (OA)

CLM stillage with a low TAA concentration of 4 % DS was concentrated to 55.9% DS and acidified to pH 2.5. The effect of OA (Table 4.12) indicated that a ratio of 2 was very low to reach an acceptable yield. No significant difference in extraction was observed between OA 3 and 4. TAA concentration in DS basis was incremented from 4% in CLM stillage to 12% in the extract.

Table 4.12 Effect of the OA on the extraction of some organic acids and TAA purity in the extract with butanol. Feed composition: DS: 55.9g/100g, pH: 2.5, TAA % DS: 4. T= 50 °C, t= 3h. Some duplicate results are shown

OA	Extraction yield, %				TAA, % Anions	TAA, % DS in extract
	TAA	Cis-aconitic	Citric	Iso-citric		
2	47, 51	44, 58	20, 26	13, 20	54, 57	12, 13
3	67	74	45	1.4	65	15
4	69, 70	42, 73	22, 31	13, 52	57, 60	8, 12

Under the same conditions, but with CLM stillage concentrated to 28 % DS (Table 4.13), extraction yield showed a similar behavior to that observed at 55.9% DS. CLM stillage concentration would not appear to have a large effect on TAA extraction.

pH

Four levels of pH 2.0, 2.2, 2.4, and 2.6 were studied under the conditions indicated in Table 4.14. The results showed that the extraction yield is higher at lower pH, as the un-

dissociated form of the organic acids becomes more predominant. The extraction yield of TAA, citric, and iso-citric were incremented. The relative increase in the last two acids was significant. The concentration of TAA on a dry solid basis in the organic phase showed a tendency to decrease as consequence of a higher extraction of other components. The TAA values in the extract were higher than previous tests (Tables 4.12 and 4.19) because the CLM stillage had a larger concentration of TAA.

Table 4.13 Effect of the OA on the extraction of some organic acids and TAA purity in the extract with butanol. Feed composition: DS: 28.2 g/100 g, pH: 2.5, TAA %DS: 5, T= 50 °C, t= 3h

OA	Extraction yield, %				TAA, % Anions	TAA, % DS in extract
	TAA	Cis-aconitic	Citric	Iso-citric		
2	47	44	26	20	54	12
4	70	42	31	52	60	8

Temperature

The effect of three levels of temperature 22, 50 and 80°C is presented in Table 4.15. No differences were observed between 22 and 50°C. In contrast, at 80 °C, a decrease in TAA selectivity was clearly observed. This decrease is probably due to the extraction of other components besides the organic acids. Therefore, 50°C was set as the optimum temperature to perform the extraction. Two reasons to choose 50 °C were the good results obtained and the CLM stillage temperature around 60°C in a step previous to extraction, during vacuum concentration. The TAA concentration increased from 10 g/100 g DS in CLM stillage to 21 – 24 g/100 g DS in the extract.

Other two tests were conducted at 50°C, as before, with the only difference that the CLM stillage was acidified to pH 2.6 instead of 2.45. The results (Table 4.16) were very

similar with those reported in Table 4.15, line 2 for TAA. However, for the other organic acids, the extraction yield was considerably lower; this confirms the big effect of pH.

Table 4.14 Effect of pH on the extraction of some organic acids and TAA purity in the extract with butanol. Feed composition: DS: 30.9 g/100g TAA % DS: 10, OA: 3.5 t= 3h, T= 50 °C

pH	Extraction yield, %				TAA, % Anions	TAA, % DS org. phase
	TAA	Cis-aconitic	Citric	Iso-citric		
2.0	80, 97	42, 50	86, 91	77, 85	60, 66	23, 28
2.2	82, 74	44, 47	67, 71	46, 51	64, 65	25, 27
2.4	74	43	57, 60	36, 40	64, 66	27
2.6	72, 75	73, 74	36, 38	25, 26	62, 65	28

Table 4.15 Effect of temperature on the extraction of some organic acids and TAA purity in the extract with butanol. Feed composition: DS: 34.1g/100g, pH: 2.45, TAA % DS: 10, OA: 3.5 t= 3h.

T, °C	Extraction yield, %				TAA, % Anions	TAA, % DS org. phase
	TAA	Cis-aconitic	Citric	Iso-citric		
22	89, 95	92, 97	57, 81	55, 80	76, 80	24
50	89, 91	82	55, 70	39, 60	79, 80	21, 24
80	89, 90	89, 92	60. 61	65, 67	58, 60	14, 17

Equilibration Time

The time effect was determined in the CLM stillage acidified to pH 2. The results did not show a substantial effect of time on the extraction yield (Table 4.17); therefore, equilibrium conditions were already reached after one hour. This result differs from the reported by Malmary et al. (1995), they claimed that the equilibrium was obtained after 2.5 h. Independently of the time; the extraction yield was the highest for TAA, cis- aconitic and citric acids. The results corroborated the effect of decreasing pH on the distribution coefficients of the organic acids.

In summary, the best results for an extraction process with butanol were: OA= 3.5, CLM stillage concentration 30 g/100g DS, CLM stillage acidified to pH=2, shaker time 1 h, extraction temperature 50 °C. Under these conditions around 90% of TAA can be recovered.

However, it was not feasible to raise the TAA concentration in the extract (purity) to values higher than 32 g/100 g DS. It is important to note that this low purity remains during further decolorization and esterification processes. Barnes et al. (2000) correlated the relatively high solubility of water in pentanol with its low selectivity. Their observation would explain the results obtained with butanol. On the other hand, researchers from Vasantdada Sugar Institute (2002) extracted TAA from a 2% aqueous solution. They correlated the higher extraction yield (more than 65%) with solvents moderately miscible in water, such as butanol and methyl ethyl ketone, and lower extraction yields in solvents with less miscibility in water such as ethyl acetate. This observation is valid for the results obtained with butanol and ethyl acetate, but not for those obtained with TBP - dodecane, solvents with very low miscibility in water and higher TAA extraction yields.

Table 4.16 Effect of temperature on the extraction of some organic acids and TAA purity in the extract with butanol. Feed composition: DS: 30.9 g/100g , pH: 2.6, TAA % DS: 9, OA: 3.5 t= 3h, T = 50 °C.

Extraction yield, %				TAA, % Anions	TAA, % DS in extract
TAA	Cis-aconitic	Citric	Iso-citric		
82	61	38	39	57	21
82	69	42	39	54	23

Table 4.17 Effect of equilibration time on the extraction of some organic acids and TAA purity in the extract with butanol. Feed composition: DS: 30.9 g/100g , TAA % DS: 9.2, pH 2.0, OA: 3.5, T= 50 °C

Time, h	Extraction yield, %				TAA, % Anions	TAA, % DS in extract
	TAA	Cis-aconitic	Citric	Iso-citric		
1	91	80, 81	94	30	69, 70	32
2	90, 92	77, 78	94	25, 26	70, 71	29
3	92, 93	87, 98	92, 97	28, 72	68, 70	30, 31

Comparison among Butanol, Ethyl Acetate and TBP - Dodecane as TAA Extractants

To compare these three solvents, LLE tests were conducted at OA= 3.5, ambient temperature, and a shaking time of 1 h, in duplicate. The only variable was pH at 2.6 or 2.0.

The highest extraction of TAA was obtained with butanol, followed by TBP- dodecane and ethyl acetate (Figure 4.20). The same order was observed for the two pH levels studied (Figure 4.20). In terms of TAA purity in the extract, results shows that ethyl acetate extracts TAA more selectively and butanol less so (Figure 4.21). Both, TAA extraction yield and TAA purity were dependent of pH, but in opposite ways, as the TAA extraction yield increased at low pH, while the contrary was true with the TAA purity.

In addition to extraction yield and selectivity, butanol shows the advantage that the final product (TBA) can be obtained directly after LLE. A significant disadvantage is the high solubility of water and other CLM stillage compounds in butanol. This decreases TAA purity in the extract and would affect the handling of raffinate due to its relatively high viscosity. The disadvantages of TBP-dodecane are: 1) the aconitate is obtained after back extraction; further processes are required to obtain the free acid, and 2) a the high energy demand to recover the solvent due to the high boiling point of these solvents. The disadvantages for ethyl acetate are: 1) the high volatility of this solvent, which could make its manipulation dangerous and 2) the experiments conducted to crystallize TAA in acetic acid solution were unsuccessful. A process to recover TAA from ethyl acetate must be developed.

From an economic point of view, butanol also has a significant advantage. It is currently marketed at about US$0.99/l in bulk and US$ 1.80 in 208 liter drums (www.butanol.com), while the price for TBP varies between US$ 4.2-7.4/l (CLP Chemicals Inc., Houston, TX), and for ethyl acetate it is US$ 1.4/l (www.icispricing.com).

Another difference among the solvents was the amount of color extracted from acidified CLM stillage. The color was measured as the relationship between the absorbance at 420 nm, and dry solid concentration, without adjusting the pH. This ratio is used in sugar

industry to calculate the color of a solution after adjusting the pH to 7.0. Ethyl acetate was the solvent with a lesser affinity for color bodies, its AU and AU/DS ratio were the lowest (Table 4.18 and Figure 4.22). Butanol was the solvent with a higher AU. However, the AU/DS ratio showed that TBP-dodecane extracted more color for unit of DS (Table 4.18).

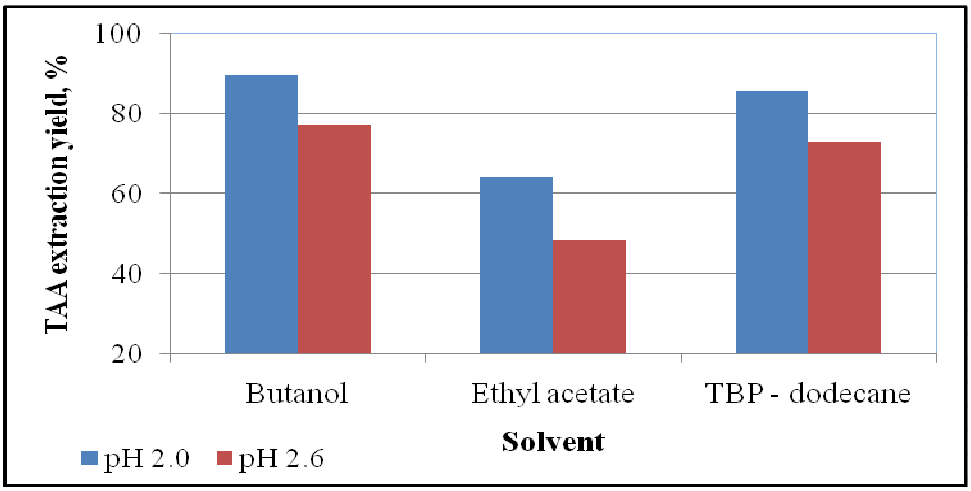

Figure 4.20 Effect of solvent and pH on TAA extraction yield

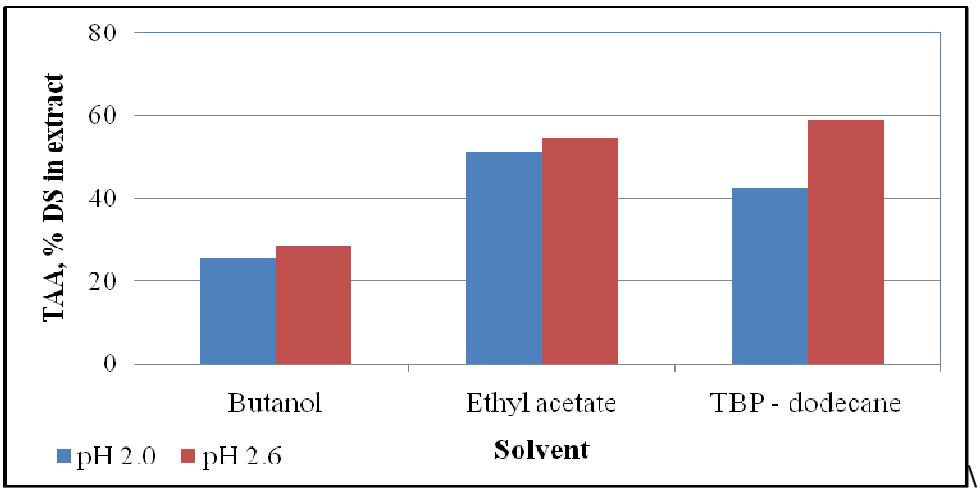

Figure 4.21 Effect of solvent and pH on TAA % DS in extract

Color in Acidified CLM Stillage

LLE not only extracts TAA and other organic acids from CLM stillage but also co-extracts other compounds, some of them color bodies. The color of CLM stillage determined

at pH 7 is showed in Table 4.19. Color for samples 1 and 3, CLM stillage at low Brix, are practically the same as reported by Lionnet (1992) as the average for color in CLM from 4 sugarcane varieties in South Africa.

Table 4.18 Dry substance (DS) and absorbance (AU) values of extract phase

Solvent	DS, %	AU, 420 nm	AU/DS
Butanol	3.12	1.502	0.48
Ethyl acetate	1.45	0.543	0.37
TBP + dodecane	1.61	1.260	0.78

CLM stillage color is 10 to 15 times higher than the typical value of color reported in sugarcane mixed juice. To identify the kind of color bodies in CLM stillage, the IV value was determined. Low IV's between 1 and 3 (Table 4.19) are associated with high molecular weight colorants, and with compounds such as caramels, alkaline degradation products of fructose and melanoidins (Lionnet, 1992). However, this classification can not be followed rigorously, because phenolic compounds are found typically in green leaves at concentrations 7 times higher than in clean stalks, (Lionnet, 1992) have IV values between 5 to 13. Godshall et al. (1998) found that TAA has a particular attraction for a high molecular weight dark brown colorant with high concentration of leucoanthcyanidin pigment. Apparently this colorant would be responsible for the dark brown color observed in the extract.

Table 4.19 Color determination (IU) and index value (IV) in CLM stillage

Sample	pH 7	IV
1	133,286	3.39
2	186,429	2.24
3	139,750	2.97
4	181,000	2.77

Color Transfer from Acidified CLM Stillage to Butanol Extract Phase

The transfer of color from acidified CLM stillage to the butanol extract occurred almost immediately. After one minute, its absorbance was around 1.0 (Figure 4.23). During

the first 20 minutes the major changes occurred. The rate of change for dry substance was higher than for color (Figure 4.23). Although AU values varied with time, its variation is small when compared with its value at the first minute. Therefore, the transference of color bodies from acidified CLM stillage to butanol extract depended more on the solvent than on the contact time.

Figure.4.22 Qualitative comparison of the color in extract phase, left to right: butanol, ethyl acetate, tri-butyl phosphate-dodecane

High affinity of color bodies for butanol was verified in CLM stillage and butanol extract by measuring and expressing the phenol concentration as vanillin. The measurements showed that 40 % of the phenols were simultaneously extracted with TAA. In their experiments to recover TAA, Hanine et al. (1992) found that sodium aconitate, polyphenols, and colored substances were eluted at the same time from the anionic resin column. In order to reduce the color, butanol extract was decolorized before and sometimes after esterification with powdered activated carbon.

Esterification of TAA Recovered in Butanol Extract

Impurities in the butanol extract represent at least 70 g/100 g DS and have an inhibitory effect on esterification yield. When sulfuric acid at a concentration of 0.8 g/100 g solution, was used as catalyst, a y conversion yield of 100% was reached for pure TAA, but only 81% for a butanol extract (Figure 4.24). For resin, the negative impact of impurities was even higher. Although the amount of resin was 2 g/100 g solution; the conversion yield decreased to only 50% for pure TAA and 19 % for butanol extract (Figure 4.24).

Apparently the performance of the resin is affected not only by the impurities but also by the large excess of butanol. When TBA was produced using resin as catalyst at a butanol/TAA ratio of 2 the esterification yield was 100% (see chapter 5), but when this ratio was increased up to 125, to simulate the butanol/TAA ratio found in butanol extract, the esterification yield decreased to half.

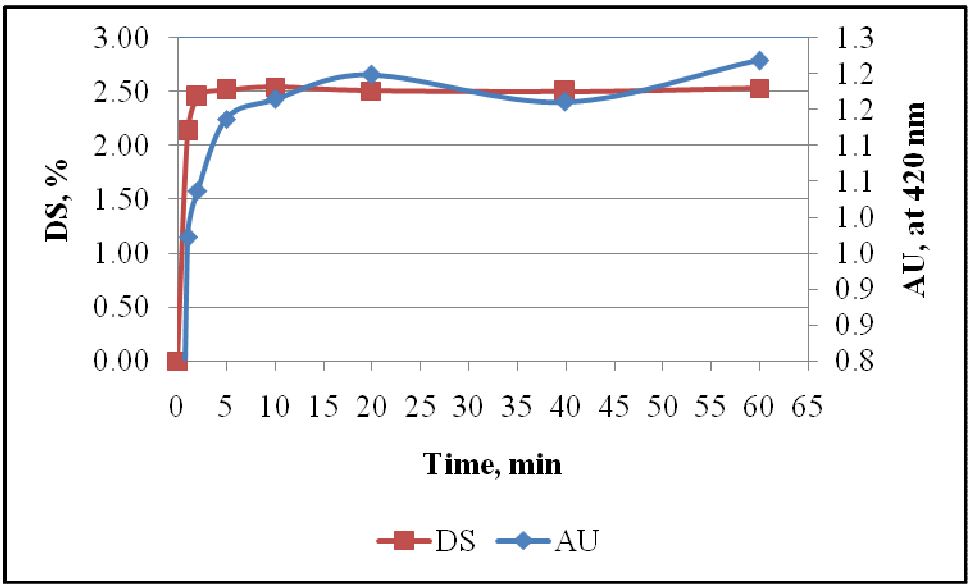

Figure 4.23 Changes in DS and absorbance in butanol extract phase with the time

The GC chromatogram, of butanol extract esterified using sulfuric acid as catalyst, shows not only the TBA peak eluted at 30 min, but also the peaks of other compounds. The complexity of this matrix is evident in Figure 4.25.

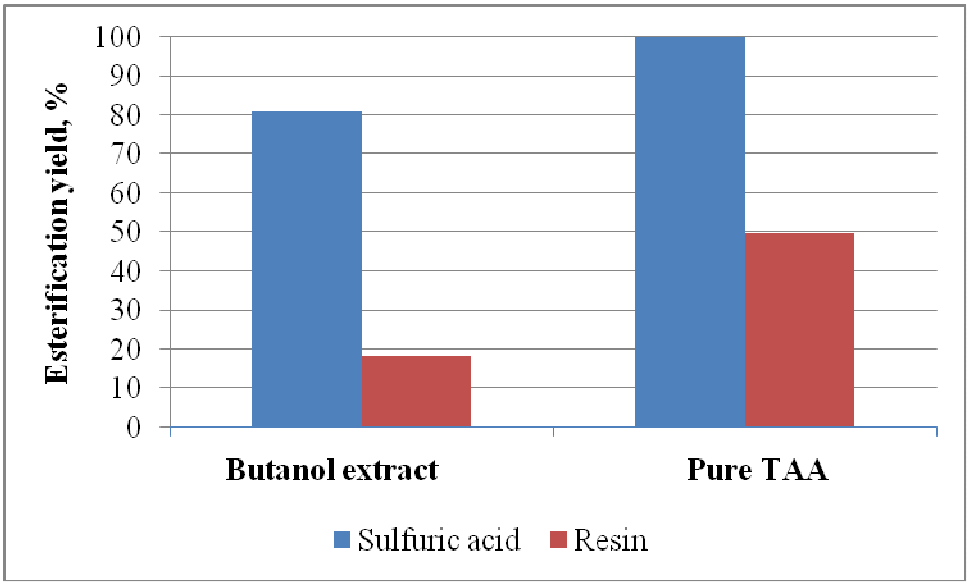

Figure 4.24 Comparison of the esterification yield of butanol extract and pure TAA

Additional esterification trials were made with and without partial butanol removal under vacuum. Sulfuric acid and resin in different doses were used as catalysts. The results showed the strong effect of time and sulfuric acid doses on esterification yield. Esterification is a chemical reaction, which requires a certain time to occur. In some experiments where the temperature increase was too rapid, apparently the time was not enough to allow esterification of TAA. Because of this the yield was lower, independent of the amount of sulfuric acid used. In sample 5, with a dose of sulfuric acid of 0.79 g/ g TAA and esterification time of 105 min, the conversion yield was 90%. In sample 4, with a sulfuric acid dose of 0.74 g/ g TAA and esterification time 70 min, the conversion yield was only 66% (Table 4.20). The samples 1 and 3 show the combined effect of sulfuric acid doses and esterification time on conversion

yield. The results also corroborated the poor conversion when using resin as catalyst, which is independent of the amount of resin used (Table 4.20).

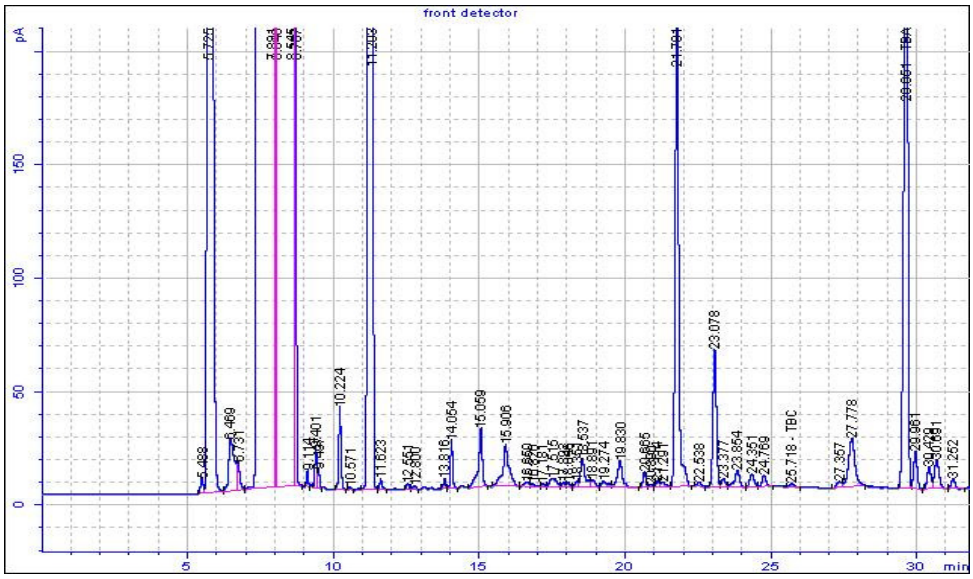

Figure 4.25 GC chromatogram of butanol extract esterified

The overall results indicated that a dose of sulfuric acid of 0.8 g/g TAA will be enough to assure a complete esterification, taking in account that the esterification time is a very important variable and it will depend directly on the partial removal of butanol.

Decolorization

The decolorization of a butanol extract prior to and after esterification was carried out using powder activated carbon at 50°C. The first decolorization was made before esterification adding in most cases 10 g activated carbon/100 g butanol extract (Table 4.21).

Except for sample 1, decolorized with 5% of powder activated carbon and sample 7, the absorbance at 420 nm showed values between 0.07 and 0.15. In the latter samples the removal of butanol prior to decolorization concentrated the color bodies. Prior to acidification and esterification, samples 7, 8 and 9 were submitted to a second decolorization. After that,

the absorbance was very low. However, after esterification, the absorbance again increased (Table 4.21).

Table 4.20 Esterification of butanol extract

Sample	Partial butanol removal before esterification	Sulfuric acid, g/ gTAA	Time, min	TBA, g/100 g expected
1	Yes	0.21	25	34
2	No	0.60	90	64
3	Yes	0.73	35	93
4	No	0.74	70	66
5	No	0.79	105	90
6	No	19.3 (resin)	120	23

Table 4.21 Decolorization of butanol extract prior and after esterification

	1st decolorization		2nd decolorization		AU at 420 nm, after esterification
Sample	Activated carbon, g/100g extract	AU at 420 nm	Activated carbon, g/100g extract	AU at 420 nm	
1	5	0.250			1.739
2	10	0.143			1.313
3	11	0.081			1.434
4	11				0.952
5	11				1.242
6	11	0.107			1.566
7	10	0.678	10	0.201	1.668
8	10	0.136	5	0.050	0.976
9	10	0.105	5	0.020	1.212
10	10	0.152	5, after esterification	0.227	0.227
11	10	0.123	5, after esterification	0.232	0.232
12	10	0.078	5, after esterification	0.325	0.325
13	10	0.114	5, after esterification	0.270	0.270

A second decolorization was conducted after esterification, in samples 10 to 13; the absorbance was acceptable. However, it is uncertain whether the absorbance will change when the material is heated again. The overall process for CLM juice extraction, fermentation, TAA extraction from CLM stillage, and esterification of TAA to TBA is

presented in Figure 4.26. CLM juice extraction and its fermentation, TAA extraction and its esterification were covered in detail in this study. The purification of TAA, butanol recovery, and the final dispose of the raffinate were no researched. The composition and production of CLM bagasse was discussed in chapter 3

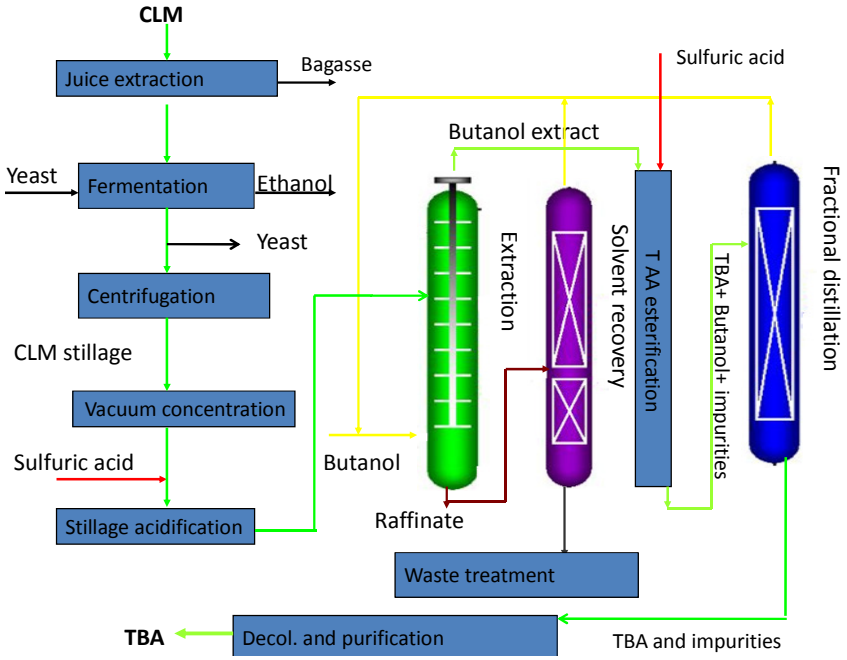

Figure 4.26 Flow chart processes for the extraction of TAA from CLM and the esterification of TAA to TBA

Solid-Liquid Extraction

505 g of CLM stillage (30%DS) were spray dried and yielded 110 g of CLM powder stillage (Figure 4.27). Although the expected amount of CLM powder stillage was around 150 grams, some losses occurred due to the amount of CLM that adhered to the walls of the equipment.

The anion composition of the powder CLM stillage is shown in Table 4.22. The attempts to esterify TAA from powder CLM stillage were unsuccessful. The highest conversion yield was only 2.5%, which indicates that most of the TAA remained in the

powder CLM stillage rather than be esterified. Reasons for low conversion yield could be that extraction and esterification of TAA may not be occurring simultaneously, and the low purity of TAA in the powder CLM stillage. TAA purity in CLM stillage is 12 g/100 g DS compared to 60 g/100g DS as reported by Moore and Sanborn (2004).

Figure 4.27 Spray dried CLM stillage

Table 4.22 Anion composition of spray dried CLM stillage

Organic acid	g/100 g DS
$Lactate^{-}$	0.51
$Acetate^{-}$	0.49
$Formate^{-}$	0.15
Cl^{-}	4.93
$Malate^{2-}$	2.02
$SO4^{2-}$	7.01
$Oxalate^{2-}$	0.22
$PO4^{3-}$	2.22
$Citrate^{3-}$	1.01
$Iso\text{-}citrate^{3-}$	2.74
$Cis\text{- }aconitate^{3-}$	1.34
TAA^{3-}	12.43

Evaluation of Final Molasses as a TAA Source

The amount of TAA in final molasses is relatively high. Its concentration depends mainly on the content of CLM in the sugarcane delivered to the mill. The feasibility of extracting TAA from final molasses was evaluated using a composite sample from the 2005 season prepared at the Audubon Sugar Institute. The best conditions found for extraction of TAA from CLM, with butanol as solvent, were used. Two pH levels were studied, 2.0 and 2.6 (Table 4.23). The extraction yield for the organic acids was higher at pH 2 than at 2.6, corroborating the results obtained with CLM (Table 4.14).

Table 4.23 Effect of pH on the extraction of some organic acids and TAA purity in the extract from molasses stillage with butanol. Duplicate experiments. Feed composition: 29 % DS, TAA= 6 % DS, OA= 3.5 t= 3h, T= 50 °C

pH	Extraction yield, %				TAA, % Anions	TAA, % DS in extract
	TAA	Cis-aconitic	Citric	Iso-citric		
2.0	93, 93	95, 95	66, 67	73, 73	48, 59	17,18
2.6	82, 81	82, 80	32, 31	45, 43	62,66	20, 19

Conclusions

This chapter describes the efforts made to recover TAA from CLM stillage, as well as, its subsequent esterification and decolorization. The conclusions for each step are:

CLM juice and final molasses were fermented under the same conditions. The conversion of TFS in ethanol was 92% for CLM and 96% for final molasses. The differences in yield were lower than expected based on the reported negative effect of TAA on fermentation. This result gives an alternative to the ethanol industry to increase its production using CLM, biomass that has significant level of TFS after mechanical harvest (See chapter 3).

Three solvents with dissimilar characteristics were used to extract TAA from CLM stillage. TBP had a slightly higher selectivity for TAA than butanol and it is almost immiscible in CLM stillage. However, its LOC is very low and a higher OA is required. Another disadvantage is a more complex process is required to obtain the free acid from its salt. Ethyl acetate had the highest selectivity for TAA, and the lowest extraction of color bodies. The main disadvantage of this solvent was its low TAA extraction. Butanol had the highest TAA extraction and lowest TAA selectivity. When compared with TBP, butanol has the following advantages: 1) a higher LOC that avoided a third phase formation even at low OA, 2) a lower price, and 3) butanol can be used for two purposes, not only as solvent extractant but also as main component in the esterification of TAA to produce TBA (a plasticizer evaluated with good results for PVC). The disadvantage of butanol is its relatively high solubility in CLM stillage. The solvent must be recovered from the raffinate before disposal. However, all solvents not only extract TAA but also other compounds. This brings up two problems: 1) a decrease in the purity of TAA in extract phase, which was no higher than 53g/100 g DS in all trials and 2) high color content of the extract phase. These two problems will limit the purification of TAA and drop the yield obtained.

Esterification of butanol extract was conducted with success; 90 to 93% of the TAA was esterified. The purity of the ester was quite close to those measured for TAA in butanol extract. Decolorization was done using powder activated carbon. Although the color was reduced by a factor of 8, the final product increased its color three fold as soon it was heated.

The attempts to esterify TAA from powder CLM stillage were unsuccessful; the highest conversion yield was only 2.5 %.

TAA was extracted also from final molasses using butanol as its extractant. The results indicated a lightly higher extraction yield than those obtained with CLM stillage, due to the higher viscosity of CLM stillage. The problems with color and low purity were also present. Moreover, the TAA concentration in final molasses was lower than CLM, therefore having higher impurities to remove.

In summary, a methodology was developed to extract TAA from CLM juice, with an overall yield, after esterification, around 84%. This methodology is also applicable to final molasses. However, future work is required to complete the decolorization of the product and its purification.

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CHAPTER 5 SOME OBSERVATIONS ON FEASIBILITY OF RECOVERING ACONITIC ACID FROM LOW PURITY CLM STILLAGES*

Introduction

T*rans*-aconitic (1,2,3-propene tri-carboxylic) acid (TAA) (Figure 5.1) occurs naturally as a minor component of plants, at about 1 to 2 g/L in the juice extracted from sugarcane (Balch, 1945). Its recovery by precipitation of its alkaline earth (Ventre, 1949, Hanine, 1991) or ferric (Fort and Smith, 1955) salts, adsorption with ion-exchange resins (Liggett and Wimmer, 1953; Regna and Bruins, 1956; Anon, 1957; Hanine, et al. 1992) liquid-liquid extraction (Regna and Bruins, 1956; Anon, 1957; Hill and Kancharla, 1999; Barnes, 2000) and even, distillation (Blinco, 2000) has been a subject of much research. Of the tested procedures, only precipitation temporarily reached commercial status (Azzam and Radwan, 1986; Godchaux, 1949), yet interest in its recovery persists within an industry eager to find new marketable products.

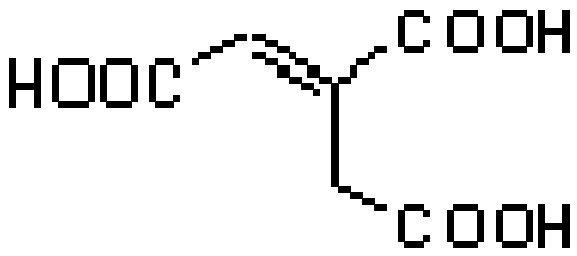

Figure 5.1 Trans-aconitic acid

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Saska, M., and Gil, N (2006) Some Observations of feasibility of recovering aconitic acid from low purity CLM stillages. International Sugar Journal, V.108, No. 1288: 203-208.

With exception of the alkaline regenerants from resin adsorption tests, the cis isomer representes only a small part of the total aconitic acid, with the trans/cis ratio in the range 7 to 10, is common in sugarcane juice.

Materials and Methods

In most cases, the feedstock for the TAA recovery trials was dilute stillage (vinasse) prepared by fermentation of juice extracted from tops of the sugar cane plant. The sugarcane tops are known to contain TAA in much higher concentrations than juice expressed from the stalk of the plant, and in about an equal quantity overall as the conventional final molasses (Gil and Saska, 2005). After juice extraction with a sample mill, in order to maximize the TAA concentration (on the solids basis), the juice containing about (15% sucrose and 35 % invert sugars on dry solids basis) was fermented with standard baker's yeast. After 6 to 8 hours of batch fermentation, some 95% of sugars were converted to ethanol that was recovered and the stillage further concentrated with a RE71 laboratory rotary evaporator (Yamato, Orangeburg, New York). The suspended solids were removed from the stillage by centrifugation. Typically, the stillage contained 10-15 g/100 g DS of TAA, about three to five times more than usually found in sugar cane juices (Table 5.1). The details of this process are discussed more fully in other sections.

Table 5.1 Color (IU) and anion composition (g/100 g DS) of the sugar cane liquor feed in the aconitic acid recovery tests.

Color	$Chloride^{-}$	$Sulfate^{2-}$	$Phosphate^{3-}$	$Citrate^{3-}$	Trans-$aconitate^{3-}$
106,098	4.8	2.6	1.5	0.86	10.5

The ion analysis was done with a Dionex (Sunnyvale, California) HPIC system equipped with an IonPac AS11 column, with a sodium hydroxide eluent and suppressed-conductivity detectors. Sugars were determined with a HPLC system with a Bio-Rad Aminex HPX87K potassium-based column and a Waters (Milford, MA) 410 refractive index detector. Some variations of the retention times, noted in Figures 5.2, 5.3 and 5.4 are not uncommon in anion chromatography, as the retention times are very sensitive to the strength of the mobile phase and the condition of the chromatography column. Any ambiguity in identifying the major peaks was avoided by frequent calibrations with standard solutions containing all anions of interest at known concentrations. Brix of the solutions was measured with a precision PTR46 refractometer (Index Instruments, Kissimmee, FL).

Results and Discussion

Adsorbent Testing

Several ion exchange resins were selected from among the many commercially available products and their capacity and selectivity for TAA were tested in bench-scale batch and column tests. In the batch tests, 5 g of wet resin (with 60 to 65 % moisture content) and 10 g of CLM stillage were contacted overnight in sealed 100 mL heavy glass bottles at about 60 ˚C in a shaker water bath. Next day, the solution was separated from the resin and analyzed. Its color was calculated from the absorbance (AU), that was measured at wavelength 420 nm, in a 1 cm path length cell, typically on a 1g/100 mL diluted sample and after 0.45 micron filtration as 100,000 x AU/ c, where c is the DS concentration of the diluted sample in g/100 g.

Some of the data related to the capacity and selectivity of six resins, three strong base anion (SBA) resins, Dowex Marathon MSA, Purolite MN-400 and Thermax' Tulsion A-30 in

both chloride and sulfate forms and three weak base anion (WBA) resins, Dowex 66, Duolite A-7 and Amberlite IRA67 in the free base form are given in Table 5.2 each at two different solution concentrations. The relative reduction (or increase) was calculated from the color and the concentration of the aconitate anion, expressed on the dry solids basis, prior and after equilibration with the resins. The sulfate form of the SBA resins appears preferable to the chloride form in terms of the aconitic acid uptake. The Dowex Marathon in the sulfate form and the Dowex 66 have the highest capacity, adsorbing, at lower concentration levels, some 86 and 88 % of the total amount of aconitic acid, but at the same time reducing the solution color by 91 and 82 % respectively.

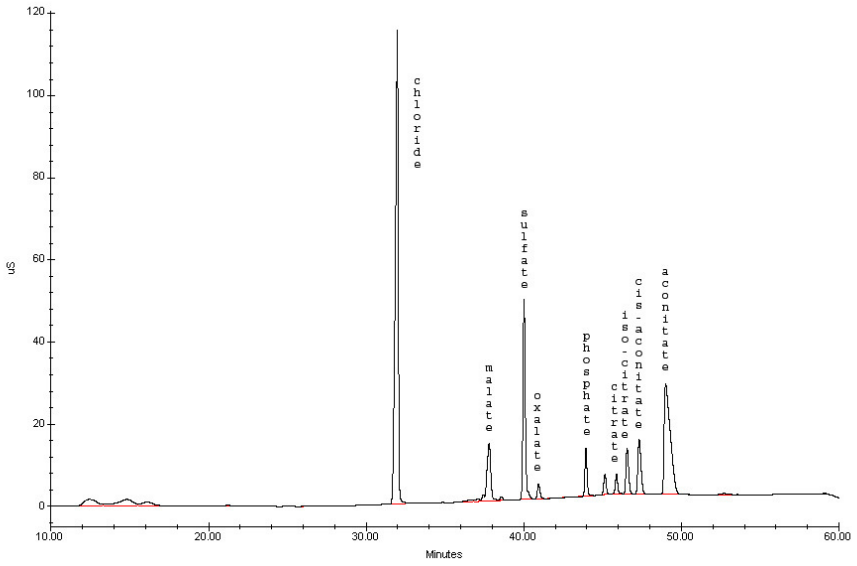

Figure 5.2 An anion chromatograph of a sample of the CLM stillage used for the resin tests.

The capacity of these two resins is in the range 50 – 70 g TAA per kg wet resin, consistent with capacities reported previously (Regna and Bruins, 1956). The apparently unavoidable competition with the weakly acidic colorants at the resin surface is undesirable as it may

reduce the resin's capacity for TAA and, unless de-sorption can be done selectively, will lead to the need for further decolorization of the TAA extract. Because of the higher thermal stability reported by the manufacturer, the Dowex Marathon MSA strong base anion resin was chosen for further testing.

Table 5.2 Resin characteristics, final solution concentration and the relative reduction (%) of color and aconitate in solution.

Resin / type	Ionic form	Final concentration DS, g/100 g	Color reduction	Aconitate Reduction
Dowex Marathon MSA SBA type I styrenic macroporous	chloride	12.5 24.3	81 56	37 18
	sulfate	16.2 20.5	91 71	86 47
Purolite MN-400 SBA styrenic	chloride	10.0 22.1	95 80	-11 -12
	sulfate	10.5 22.5	94 81	8 -1
Tulsion A-30 MP SBA acrylic macroporous	chloride	13.3 26.8	21 -20	32 15
	sulfate	12.2 23.7	40 -1	53 29
Dowex 66 WBA acrylic macroporous	free base	10.7 20.5	82 61	88 63
Amberlite IRA67 WBA acrylic gel	free base	12.8 24.1	34 -18	59 42
Duolite A-7 WBA phenolic	free base	10.4 19.8	90 83	76 52

In addition to the Dowex Marathon MSA, two non-ionic polymeric adsorbents known for their affinity for sugarcane colorants were included in the follow-up testing of the pH effects (Tables 5.3 and 5.4) and regeneration conditions (Figures 5.2 and 5.3). Two levels of pH were tested: pH ~ 5.1, the "natural" pH of the liquor and a pH lowered with sulfuric acid to about 2.8, close to the pK_{a1} value of the acid (pK_{a1}(25 °C) = 2.90 $pK_{a\,2}$ (25 °C) = 4.46) where the

un-dissociated form is favored. While for the Marathon strong base anion resin, good capacity (low residual TAA) was found even at the higher pH, it was nil at high pH for the non-ionic Optipore SD-2 adsorbent but equally high at the low pH. Obviously, only the un-dissociated form of the acid is adsorbed on the non-ionic adsorbent. Results for the other non-ionic adsorbent that was tested, Rohm & Haas XAD-4 are not reported here as its capacity was found equally low at high pH and much inferior to Optipore's at low pH (2.8).

Table.5.3 Final solution composition, ions in g/100 g DS. CLM stillage at the "natural" pH 5.1.

	Final conc. % Brix	Color	Cl^-	$SO4^{2-}$	$PO4^{2-}$	Citrate $^{3-}$	TAA $^{3-}$
Marathon MSA (SO4)	5.8	7,238	1.9	22.7	1.3	0.3	0.7
	10.4	10,577	1.9	19.4	1.3	0.6	2.1
	13.5	22,037	2.8	15.1	1.7	0.7	3.8
	19.6	36,607	2.6	9.8	1.5	0.7	4.2
Optipore SD-2	11.2	1,786	8.1	3.6	1.3	1.0	11.3
	21.3	4,695	7.3	3.7	1.3	1.0	11.2

Stripping of TAA from the Resin

The two sorbents, Dowex Marathon MSA (in the SO_4 form) and the Optipore SD-2 were loaded with TAA by slowly passing either a solution of an analytical grade acid (Catalog No. 122750, Sigma-Aldrich, St. Louis, MO) or the CLM stillage through a jacketed glass column packed with the resin. The analytical grade acid is specified by the manufacturer to be 98% pure and its chromatograms showed only traces of the cis-isomer. Based on the inlet and outlet concentrations, loadings of about 65 and 50 g/L resin respectively were estimated for Marathon and Optipore SD-2 with pure TAA aqueous solutions (pH 2.2). Stripping in column tests or batch contacting were done with water, dilute (~ 0.5 %) sulfuric acid, dilute (~ 0.5 %) sodium hydroxide, butanol and butanol acidified with sulfuric acid (~ 2 g of 1:1 diluted sulfuric acid to 100 mL butanol), at a range of temperatures from about 80 to 120 ˚C.

Examples of representative effluent compositions from stripping about 15 mL of the CLM stillage-loaded Optipore packed in a 0.9 x 50 cm jacketed glass column at 95 ˚C, with 50 mL of the dilute acid and hydroxide, respectively are in Figure 5.2. Obviously, of the two (the data for regeneration with water is quite similar to that with acid and is not shown for the sake of clarity) for a non-ionic adsorbent the dilute NaOH solution is the superior regenerant, not only in terms of aconitic acid recovery but also the recovery of colorants. This is consistent with recommendations by the manufacturer for regeneration of this resin in decolorization of sugarcane juice, and is reflected in the absorptivities (420 nm, 1 cm, 0.45 micron filtration) of the three eluents that were 0.104, 0.020 and 0.786, for water, acid and caustic, respectively. Notable is the increase of the *cis* isomer in the NaOH eluant; the *trans*/*cis* ratio is 5.9, 7.1 and 2.4 in the aqueous, acid and caustic eluents, respectively. Although this is in apparent contradiction to a previous report (Walford, 1998) of pH effects on *trans/ cis* isomerization (low pH promotes the trans to cis isomerization; on the other hand, the opposite is expected at high pH), it may be a result of the overriding positive effect of sodium (Walford, 1998) on the trans/cis isomerization.

As the free form of the acid (rather than its salt) is likely to be the preferred product, hydroxide regenerant is at a disadvantage and focus was, therefore, placed on testing regeneration of the resin with a recoverable solvent, butanol, to avoid formation of a salt. Furthermore, the past (Roberts et al. 1954) and present (Gil et al. 2006) works indicate the tri-butyl aconitate (TBA) to be an excellent plasticizer for PVC that may likely represent the most immediate commercial market for the acid.

Table 5.4 Final solution composition, ions in g/100 g DS. CLM stillage acidified to pH 2.8.

	Final conc. DS, g/100g	Color	Cl^-	$SO4^{2-}$	$PO4^{2-}$	Citrate $^{3-}$	TAA^{3-}
Marathon MSA (SO4)	4.2	12,500	2.3	28.7	1.3	0.3	0.4
	9.6	19,531	2.2	23.5	1.2	0.4	0.8
	14.5	21,724	2.4	21.6	1.2	0.5	1.1
	18.7	36,096	2.7	19.6	1.4	0.7	1.9
Octipore SD-2	7.8	2,885	10.6	18.5	2.6	0.9	1.0
	15.9	3,774	8.8	15.5	2.3	1.0	2.4

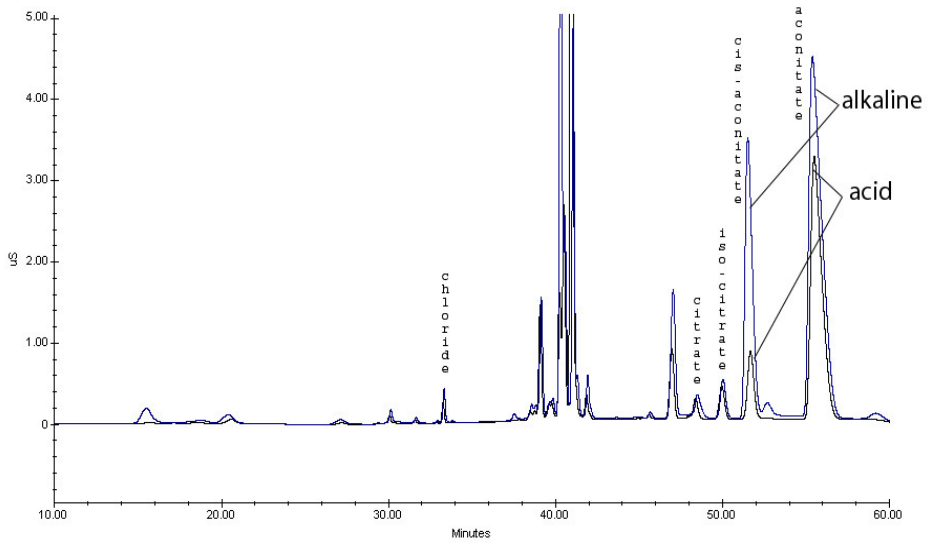

Figure 5.3 An overlay of anion chromatographs of two eluents from regenerating the Dowex Optipore-SD 2 resin with dilute sulfuric acid and dilute sodium hydroxide.

Fisher esterification of TAA to TBA, *post* regeneration, or even during regeneration was among the objectives of the present work. No significant recovery of TAA with butanol was observed from the SBA resin (Dowex Marathon MSA - SO_4 form) but a high recovery of the acid at a high purity was found with the non-ionic Optipore SD-2 (Figure 5.4), and was independent of temperature in the 80 – 100 ˚C range. Monitoring the esterification reaction with GC and GC-MS during boiling of the Optipore SD-2 resin previously loaded with TAA under reflux with acidified n-butanol (~ 117 ˚C) revealed a degree of esterification and may

present an alternative to simply stripping the resin with butanol, concentrating the acid in the butanol phase by reusing the regenerant and esterifying the acid in the concentrated extract separately over an acid catalyst.

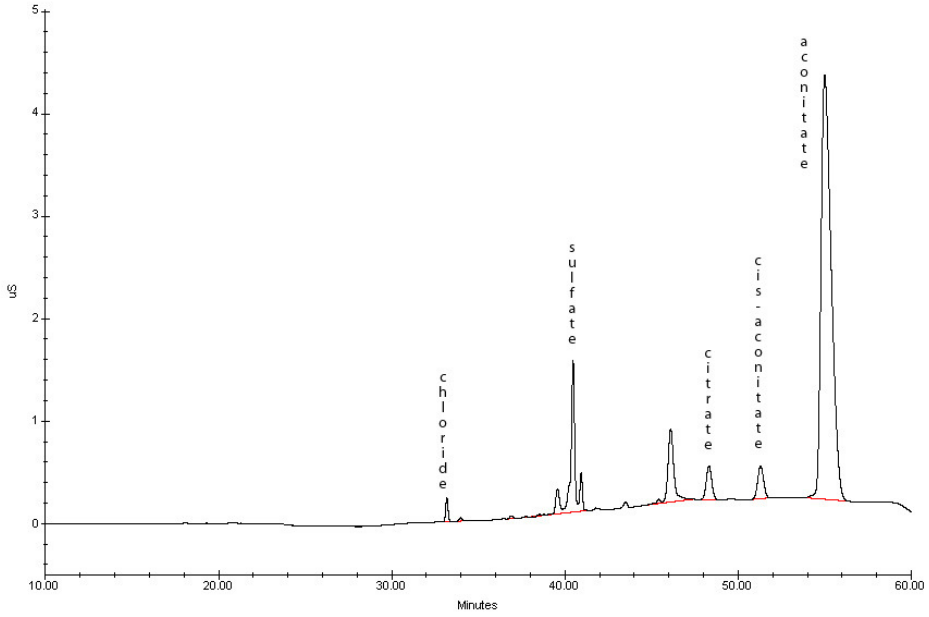

Figure 5.4 An anion chromatograph of the eluent from regenerating the Dowex Optipore SD-2 resin with butanol

Volatility of Aconitic Acid

Azzam and Radwan (1986) reported a 90% recovery of the TAA from Egyptian sugarcane molasses in a laboratory arrangement that implied entrainment of vapors of TAA with those of ethyl acetate, rather than simple liquid-liquid extraction as it was interpreted and tested by Hill and Kancharla (1999). This attractive process, while simple, could reasonably be expected to be quite selective for the acid and a few tests were therefore performed to assess the volatility of the acid under a range of conditions. In an arrangement similar to that of Azzam and Radwan (1986) but with a condensate withdrawal port some 10 cm above the

liquid level, high enough to minimize any carry-over of the liquid, the two-phase TAA solution – ethyl acetate mixture was slowly boiled at atmospheric pressure, at some 75 – 80 ˚C at the following conditions:

- 50 g of the CLM stillage (as in Table 5.1Table) at about 10% dry solids, at a pH "as is" and after acidification to pH 1.3 with H_2SO_4, respectively, with 50 g ethyl acetate
- 5 g of the CLM stillage pre-concentrated to 57 % DS, at an "as is" pH, and at a pH 1.7, respectively, with 50 grams ethyl acetate
- 2 g of analytical grade TAA, dissolved in 10 g of de-ionized water (pH 2.1), with 50 g ethyl acetate
- 2 g of analytical grade aconitic acid, dissolved in 10 g of De-ionized water, acidified with H_2SO_4 to pH 1.3, with 50 g ethyl acetate

In each case the condensate was collected over a period of 1 to 3 hr, the time that it took to distill off all organic phase and the solvent was then removed from the distillate under vacuum at about 70 to 80˚C. Any residue, imperceptible in most cases was re-dissolved in de-ionized water and analyzed for TAA. In none of the tests more than mere traces of TAA were found in the condensate, and it must therefore be concluded that liquid carry-over led to the observations of Azzam and Radwan (1986). Our finding of less than accurate description in that paper are supported by the report of Hill and Kancharla (1999) that contrary to the claims of Azzam and Radwan recovery by extraction with ethyl acetate at a neutral pH is not feasible.

Conclusions

Of the several resins and adsorbents that were selected for testing recovery of TAA from CLM stillages, two, Dowex Marathon MSA, a strong base anion resin in sulfate form and a non-ionic adsorbent, Dowex Optipore SD-2 were found good prospects and were tested in

more detail. The adsorption capacity for the acid on both was found to be enhanced by lowering the pH of the liquor. The capacity of the non-ionic adsorbent for the acid at the natural pH of the liquor was found nil but comparable to that on the anion exchange resin at a pH of 2.8 As the recovery of the TAA from the regeneration effluents, either by acid or alkaline regeneration would add significantly to the complexity of the TAA production, regeneration of the two resins with butanol that could be readily recovered by distillation was studied and found feasible for Dowex Optipore, the non-ionic adsorbent. The consumption of sulfuric acid to acidify the liquor to pH 2.8 is estimated to be about 14 g/100 g DS of the CLM stillage, or some 100 – 140 % on TAA for the high acid liquor employed here, still a substantial cost item but less so than in the case of recovery from cane molasses with typically 3 to 5% acid on dry solids. The volatility of the TAA in presence of ethyl acetate was found too low for its recovery by distillation.

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CHAPTER 6 ACONITATE ESTERS AS PLASTICIZERS OF POLY(VINYL-CHLORIDE)*

Introduction

After polyethylene, poly (vinyl chloride) is the most widely used plastic. Some 25 to 35 % of the total PVC production goes into flexible consumer products such as wire and cable insulation, flooring, wall covering and packaging materials. For these applications, before molding or extrusion, PVC must be blended with a suitable plasticizer, a compound designed to weaken intermolecular bonds in the polymer in order to increase its workability, toughness and flexibility (Shah and Shertukde, 2003). In addition to good miscibility with the polymer, other attributes of a good plasticizer are a high boiling point to prevent or reduce its loss during processing and a low rate of migration out of the polymer to avoid loss of its properties (Brydson, 1982) and contamination of the materials or consumers in contact with it if it is a part of food grade or personal use products.

Until now, plasticizers produced from phthalic anhydride, such as, di-isononyl phthalate (DINP) (Figure 6.1), di-2 ethylhexyl phthalate (DEHP) and, di-isodecyl phthalate (DIDP) have been the most widely used. In a recent work (Shah and Shertukde, 2003) the effects were evaluated of several phthalate plasticizers and their blends on mechanical, thermal and electrical properties of PVC. However, growing concerns about their toxicity (Latini, 2000; NIH, 2001; EPA) in consumer products made of PVC have led to a search for alternatives that would not elicit the same public health concerns.

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Gil, N., Saska, M., and Negulescu, I. (2006) Evaluation of the effects of bio-based plasticizers on thermal and mechanical properties of poly(vinyl-chloride), Journal of Applied Polymer Science, V.102, No. 2: 1366-1373.

Alternatives to phthalates have been esters of bio-derived citric acid, such as tri-butyl citrate (TBC), acetyl tri-butyl citrate (ATBC) (Figure 6.1), tri-ethyl citrate, acetyl tri-ethyl citrate and tri-(2-ethylhexyl)-citrate. The U.S. Food and Drug Administration (FDA) approved both the acid and its esters as additives in food (Wilburn, 2002). Nevertheless, the price of these esters is about three times higher than that of phthalates so their application has been limited to small niche markets.

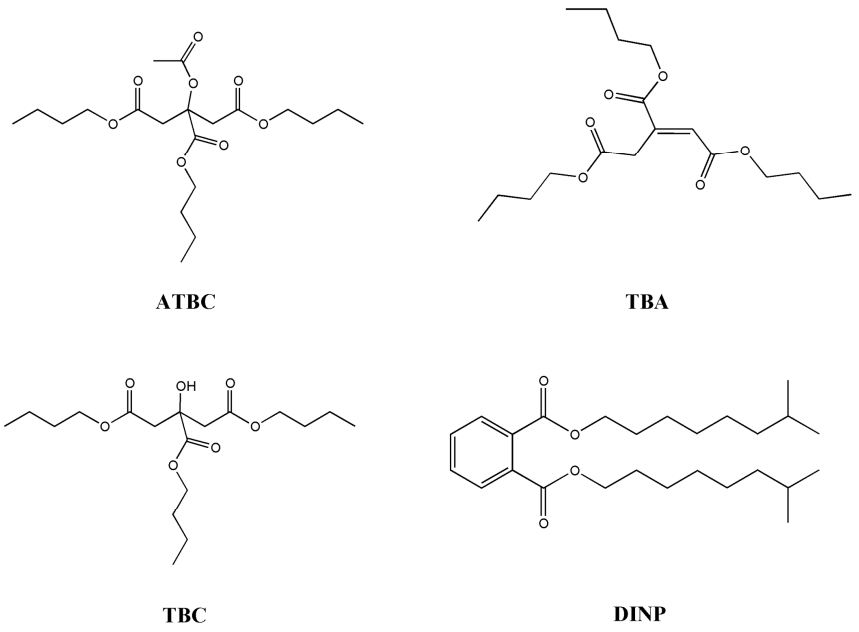

Figure 6.1 Chemical structure of the four plasticizers tested in this work, esters of citric, aconitic and phthalic acids

Alternatives that were proposed in the past were esters of aconitic (1,2,3-propene tri-carboxylic) acid, and in particular tri-butyl aconitate (TBA) (Figure 6.1). Several hundreds of tons annually of calcium magnesium aconitate were produced in Louisiana from 1946 to about 1956 from by-products of sugar cane processing (Balch, 1945, Godchaux, 1949). No published reports are known to the authors about the processes that employed the aconitate at

that time but its use was presumably based on two U.S. patents (Hanson and Coggin, 1942, Cox, 1947) that without much detail disclosed ways of preparing co-polymers of aconitate esters with PVC. Both the aconitic esters and tri-carballylic acid esters (the hydrogenated variants of aconitic esters) were evaluated as vinyl plasticizers in the early 1950's (Roberts et al. 1954). Among other effects, it was claimed that TBA protected PVC from negative effects of light and heat. Nevertheless, it appears that after several years of production the market for crude aconitate disappeared, replaced with lower cost esters of sebacic and phthalic acids.

In view of the advances in technologies for recovering aconitic acid from sugarcane (Blinco, 2000) that should lower its cost and the continued need within the sugar cane industry to find alternative products, the study was undertaken to re-evaluate the industrial potential of TBA, and provide its comparison as a PVC plasticizer with TBC, ATBC and DINP.

Materials and Methods

Trans-aconitic acid (TAA), ATBC and PVC (M_n 60,000, M_w 106,000) were purchased from Sigma Aldrich (St. Louis, MO). Citric acid monohydrate was obtained from Fisher Scientific (Fair Lawn, NJ) and n-butyl alcohol was purchased from Mallinckrodt (Paris, KY). Both were used as received. DINP was donated by Dow Chemical Company. Drapex 6.8 (epoxidized soybean oil) and Mark 6711 (a barium zinc stabilizer) were purchased from Crompton (Hahnville, LA). Calcium stearate and PE (H)-100 (non-oxidized polyethylene homopolymer wax) were obtained from Struktol (Stow, Ohio). Calcium carbonate filler was received from Omta Vermont (Lucerne Valley, CA) and Paraloid K 120 N (stearic acid) from Rohm & Haas Company (Philadelphia, PA).

TBC and TBA were prepared in our laboratory (ASI) from the respective acid and alcohol by Fisher esterification with a cation exchange resin, AG 50W-X4, 100-200 mesh, hydrogen form (Bio-Rad, Sunnyvale, CA) as a catalyst. Thirty grams of the acid, a 50% excess n-butanol (52.1 g for citric acid monohydrate, 57.5 g for trans-aconitic acid) and 4 g of the ion exchange resin were mixed and boiled in a 250 mL round bottom flask at atmospheric pressure under reflux in an arrangement similar to the Dean-Stark apparatus allowing continuous removal of the water of reaction. After about one and half hours of boiling when temperature of about 140°C was reached and no more water was being produced, the reaction was considered complete. The resin was screened off from the liquid product, the ester returned in the distillation apparatus and any residual alcohol removed by heating to about 130°C for another hour under vacuum.

Purity of the products was evaluated with an Agilent Technologies (Foster City, CA) 6890 N gas chromatograph with a flame ionization detector and a DB-5 capillary column (30m, 0.25μ film) under the following conditions: 0.2 % solution in ethyl acetate, oven temperature 250°C, run time 32 min, He carrier gas at 30 mL/min, and a 2μL injection volume (Figure 6.2). TBC and TBA prepared as described above were used in blends with PVC without any further purification.

PVC Plasticization

PVC in a powder form, plasticizers and other ingredients (Table 6.1) were weighed and mixed according to a modified Large 55 formulation used by the industry (Titow, 1990). Three series of samples were prepared for each plasticizer, at the plasticizer to PVC ratios 15:100, 30:100 and 40:100, respectively; or 15, 30 and 40 parts per hundred parts of resin (phr).

Fifty grams batches of the well mixed ingredients were processed in a Haake (Coesfeld, Germany) torque rheometer at 60 rpm, at a temperature between 170 to 180^{o}C. The blending was continued for 15 to 20 minutes and stopped when the torque reading dropped and then stabilized after the polymer had melted. The exact blending temperature depended upon the plasticizer and its content, but in no case exceeded 180^{o}C. After partial cooling, the polymer was easily removed from the rheometer as small irregularly shaped granules of light yellow to light brown color.

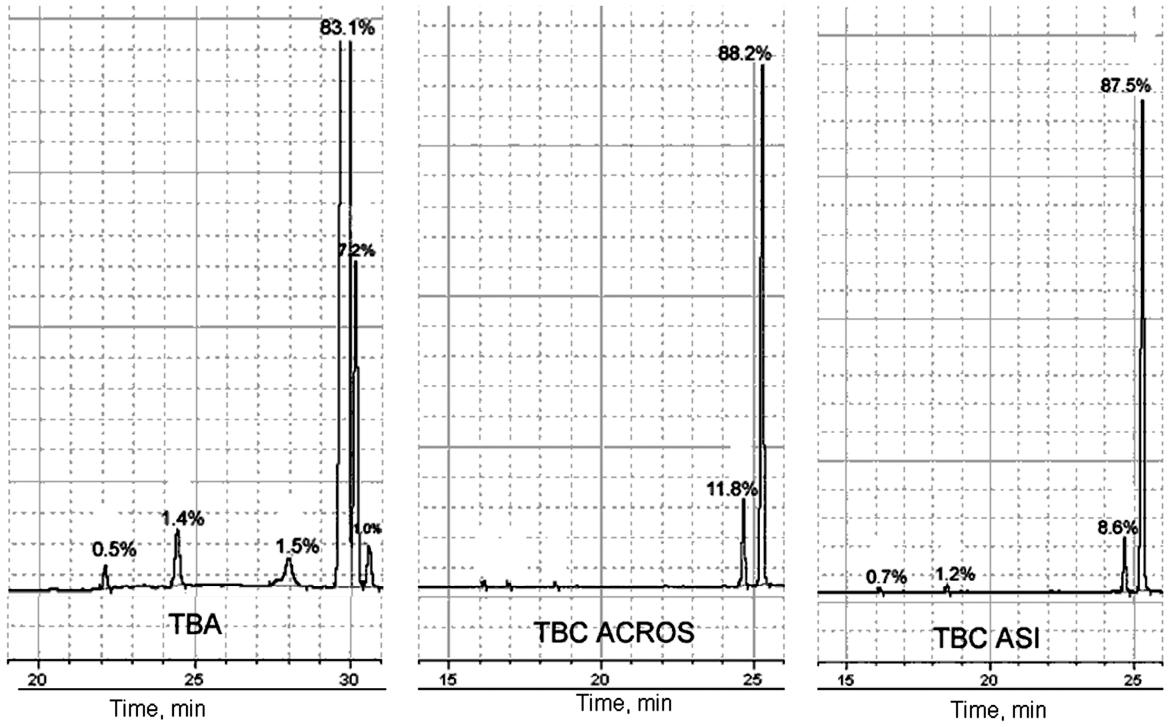

Figure 6.2 Gas chromatograms of ethyl acetate (EtAc) solutions of TBA, TBC supplied by Acros Organics - Fisher Scientific and TBC-ASI

Film Preparation

Samples of PVC films for mechanical and dynamic-mechanical analyses were prepared by pressing the plasticized polymer granules in a metallic frame using a Carver

(Wabash, IN) laboratory press equipped with heated plates. The pressing temperature and pressure for each composition were 180°C and 0.77 MPa for 15 phr, 175 °C and 0.62 MPa for 30 phr, and 170°C and 0.48 MPa for 40 phr. The samples were kept in sealed plastic bags at room temperature until testing. After the initial round of tests, remaining samples were stored for up to eight months under the same conditions.

Table 6.1 Formulations for PVC plasticization, given as phr or parts per hundred parts of resin (g per 100 g of PVC)

Materials	Plasticizer content, phr		
	Low	Middle	High
Plasticizer	15.0	30.0	40.0
Heat Stabilizers			
Drapex 6.8	4.43	5.01	5.40
Mark 6711	1.32	1.49	1.60
Lubricants			
Calcium stearate	0.90	1.02	1.09
PE (H)-100	0.45	0.51	0.55
Paraloid K 120 N	0.45	0.51	0.55
Filler			
Calcium carbonate	17.7	20.0	21.5

Testing

Mechanical properties of the plastics were evaluated by determining the modulus and ultimate strength in tensile mode with an Instron (Grove City, PA) tester Model 4301 provided with a 1kN load cell. The crosshead speed was set at 25mm/min. The sheets were cut into 76.2 mm × 13.5 mm x 1.3 mm strips, measured with a digital caliper. The samples were conditioned at 21-23°C and 50% relative humidity for at least 40 hours prior to testing.

The glass transition temperature, T_g was determined by dynamic mechanical analysis (DMA), in a tensile mode with a Seiko Instruments (Torrance, CA) DMS 200 system using sample films of approximately 25 x 7 x 1.3 mm. The film length under tension was 20 mm. The runs were carried out between 25°C and 75°C (for low plasticizer ratio) and – 30°C to

75°C for middle and high plasticizer ratios using a heating rate of 1°C/min and a train of frequencies from 0.1 to 10 Hz. Alternatively, a TA Instruments (New Castle, DE) DMA Q800 and a AR2000 high torque rheometer were used for determination of the glass transition temperature at the same conditions.

Thermo-gravimetric analysis (TGA) was conducted with a TGA 2950 (TA Instruments) thermo-balance to assess thermal stability of the pure components and of the blends at the standard processing temperatures for plastics. Samples of approximately 10 mg were analyzed in nitrogen with a heating rate of 5°C/min over the 25 to 600°C range.

Results and Discussion

Plasticizer is a compound incorporated in a polymer matrix to lower its rigidity and improve the properties of the commercial plastic product. Therefore, addition of the plasticizer often lowers the melt viscosity, glass transition temperature and the elastic modulus of the polymer. In a simplified way, properties and effects of a plasticizer can be compared with those of a solvent. A solvent (or *plasticizer*) for a given polymer may be judged by either of two separate standards, one kinetic and the other thermodynamic. A kinetically good solvent (*plasticizer*) is one that will dissolve (*in*) the polymer rapidly. In the thermodynamic sense, a good solvent (*plasticizer*) must be capable of strong interactions with the polymer. Thermodynamics dictate that a compound will be a solvent for a particular polymer when the free energy of solution (F) is negative. The factors which contribute to the free energy of solution – temperature (T), enthalpy (H), and entropy (S), are related by the familiar equation

$$\Delta F = \Delta H - T\Delta S$$

Because of the many degrees of local motion permitted to an amorphous polymer, its entropy of solution usually is small but positive, and the sign of ΔF depends upon the magnitude of ΔH. The enthalpy of mixing, i.e., the overall heat of mixing for two liquids is given by the Hildebrand's equation (Van Krevelen, 1990):

$$\Delta H = \phi_s \phi_p (\delta_s - \delta_p)^2$$

where ϕ_s and ϕ_p are the volume fractions of the solvent and polymer, respectively and δ_s and δ_p are their respective solubility parameters. It is obvious that as $(\delta_s - \delta_p)^2$ approaches 0, dissolution or, in the present case, incorporation of the plasticizer in the polymer matrix is assured by the entropy factor. One may therefore conclude that as a requirement for the solubility of polymer P in a solvent S, or *vice-versa* when the plasticizer is substituted for the solvent, $(\delta_s - \delta_p)^2$ has to be small.

The molecular weight of PVC and of the plasticizers used in the present work, the polarity reflected by the dielectric constant and the solubility parameter were used as predictors of compatibility between PVC and the plasticizer (Table 6.2).

While the dielectric constants of TBC and ATBC are quite different and higher than those of PVC, DINP and TBA, their solubility parameters are similar and close to that of PVC. In the case of TBA, both the dielectric constant and the solubility parameter are very close to, if not the same as those of PVC. DINP has a dielectric constant between that of the PVC and of the citric esters; however, using the $(\delta_s - \delta_p)^2$ criterion, it should have the lowest miscibility in PVC. These data, even if not exact because of the approximations made in the calculation of the solubility parameters, are very encouraging, pointing to the fact that the bio-

based esters considered in the present work should behave at least as well as DINP in plasticizing PVC.

Thermal Stability of Plasticized PVC

The weight loss for all blends at 100°C, 150°C and 200°C is given in Table 6.3. The data at temperatures above 200°C are not shown as they are not relevant for processing PVC.. The loss is proportional to the level of the plasticizer in the PVC blend.

Table 6.2 Predictors of compatibility of plasticizers and PVC

Materials	M_n	Molecular formula	Dielectric constant	Solubility parameter (J/cc) ^{½}
PVC	60,000	$(C_2H_3Cl)_n$	3.13[a] 3.10[17]	19.4[17]
TBA	342.2	$C_{18}H_{30}0_6$	3.02[a]	19.4[b]
ATBC	402.3	$C_{20}H_{34}O_8$	6.05[17]	18.4[17]
TBC	360.2	$C_{18}H_{32}O_7$	10.00[17]	18.5[17]
DINP	418.3	$C_{26}H_{42}O_4$	4.64[17]	15.7[b]

[a] Calculated by assimilation of groups (Van krevelen, 1990).

[b] Calculated as the ratio between the product of the density and the molar attractions constants and the molecular weight of the materials (Brydson, 1982)

Table 6.3 Weight loss in PVC / plasticizer blends

Plasticizer	Phr	Weight loss (%)		
		100°C	150°C	200°C
TBA	15	0.11	0.38	1.90
	30	0.15	0.51	3.82
	40	0.31	0.86	5.97
ATBC	15	0.07	0.16	1.09
	30	0.05	0.21	2.42
	40	0.26	0.64	4.99
TBC	15	0.19	0.49	2.67
	30	0.15	0.49	3.43
	40	0.18	0.87	6.19
DINP	15	0.03	0.08	0.38
	30	0.01	0.08	0.51
	40	0.05	0.23	0.97

At 170°C, the actual processing temperature for the 40 phr formulations, the weight loss from pure PVC and the 40 phr DINP blend (Figure 6.3) was around 0.30%, while the weight loss from the PVC blended with TBC and TBA was between 1.4 and 1.9 %.

An onset temperature was defined as the intersection of the extension of zero weight TG loss line with the tangent to the TG curve drawn at the temperature corresponding to the highest rate of weight loss, i.e., the temperature of the maximum on the DTG curve (Figure 6.4). The boiling points of these esters are very high (234^{o}C at 17 mmHg for TBC, 174 ^{o}C at 1 mm Hg for ATBC, 246°C at 5 mm Hg for DINP (Sears and Darby, 1982) and 126^{o}C at 0.1 mm Hg for TBA (Roberts, et al. 1954)) and it is therefore expected that at atmospheric pressure the onset temperature in these blends refers mostly to the thermal degradation of the esters.

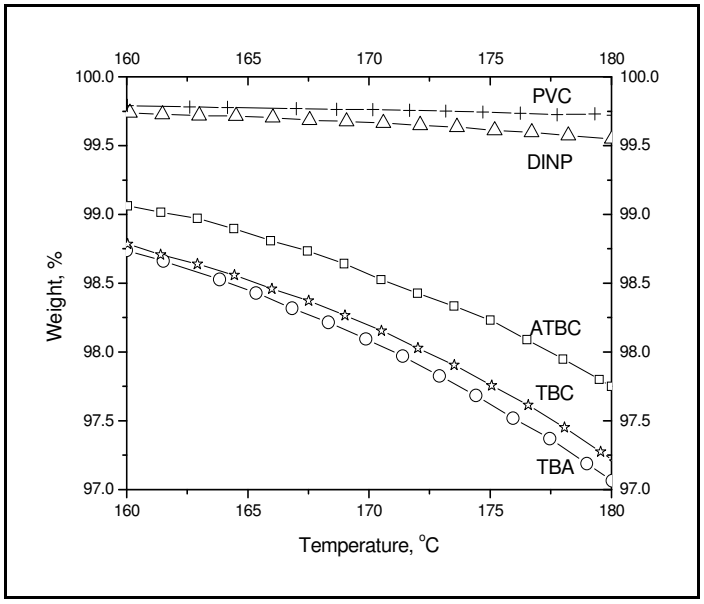

Figure 6.3 The weight loss in pure PVC and PVC/plasticizer blends at 40 phr in the range of temperatures in industrial processing of PVC.

The onset temperature of PVC blends plasticized with any of the esters at 15 phr was some 20-25°C higher than the onset temperature of pure PVC (251°C) because of the effect of

heat stabilizers in the PVC/plasticizer blends. The effect of the plasticizers is to lower the onset temperature of PVC blends. After six months, the onset temperature in the same samples increased presumably as a result of plasticizer migration. The largest changes were observed in the PVC / DINP blends with 40 phr of the plasticizer (Table 6.4).

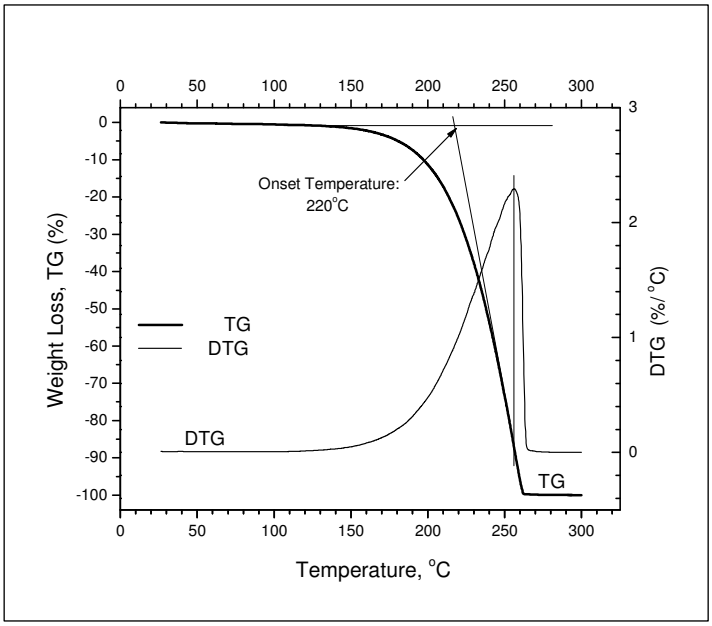

Figure 6.4 A graphical definition of the onset temperature.

Table 6.4 Changes in the onset temperature of the fresh PVC samples and after aging for six months

Plasticizer	Phr	Onset temperature, °C		
		Fresh samples	After aging	Difference
TBA	15	276	283	7
	30	266	279	13
	40	263	272	9
ATBC	15	273	282	9
	30	258	275	17
	40	248	267	19
TBC	30	258	276	18
	40	257	274	17
DINP	15	275	285	10
	30	266	279	13
	40	254	278	24

Dynamic Mechanical Analysis (DMA)

The actual values of dynamic mechanical characteristics of materials are related both to the temperature and frequency of the determination. According to the superposition time (frequency)-temperature principle, a certain transition, such as glass transition, can have a multitude of values, each one corresponding to a certain frequency (Carreau, 1997).

Figures 6.5, 6.6 and 6.7 show the variations of tan δ (the ratio between the viscous and elastic moduli) as a function of temperature at 1Hz for the four PVC samples containing 15, 30 and 40 phr levels of plasticizers, respectively. The T_g values that correspond to the maxima of the curves were grouped in a narrow 5°C temperature range at 15 and 30 phr, but a wider range of glass transition temperatures was observed at 40 phr.

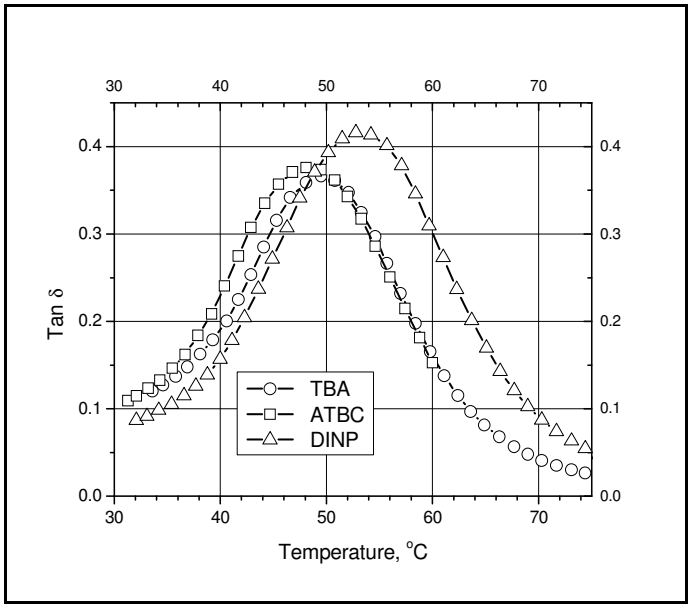

Figure 6.5 Glass transition temperatures (maxima of the tan δ curves) of the fresh PVC blends made with 15 phr of plasticizer. Seiko Instruments DMS 200 at 1 Hz.

The glass transition temperature decreased dramatically when increasing the plasticizer level from 15 to 30 phr, with the lowest value found for the ATBC blends. As a rule, narrow peaks

of the tan δ v. temperature curves indicate good miscibility between PVC and the plasticizing agent1. In our experiments, the peaks generally became broader as the plasticizer level increased. The tan δ dependence of the 15 phr TBA sample exhibited the narrowest peak while the contrary was found for the 15 phr DINP blend, in good agreement with the solubility values listed in Table 6.2 At 30 phr, the width of all peaks became larger (Figure 6.6). At 40 phr, the curves of bio-based plasticizers exhibited multiple maxima (Figure 6.7). The major peak was taken as the glass transition temperature (Table 6.5), but one could also consider the minor peaks as glassy transitions.

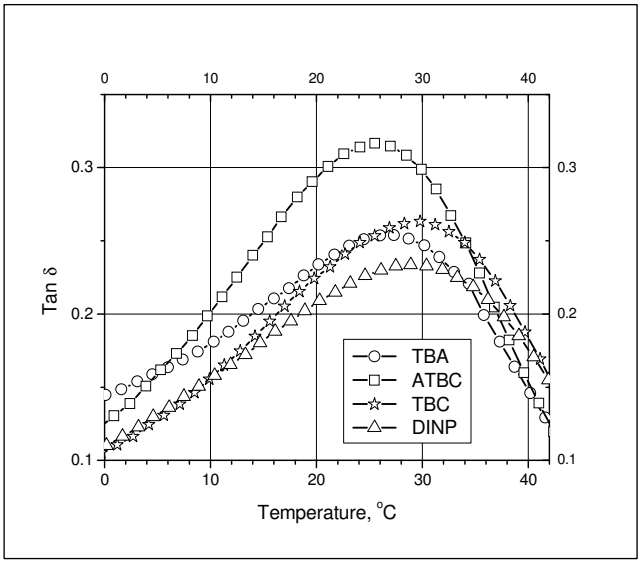

Figure 6.6 Glass transition temperatures (maxima of the tan δ curves) of the fresh PVC blends made with 30 phr of plasticizer. Seiko Instruments DMS 200 at 1 Hz

Because of instrumental problems, the 40 phr samples were only measured three months after they were prepared, on an AR2000 rheometer, and some plasticizer migration within this period may have occurred. Multiple peaks were also observed in the same samples (bio-based plasticizers at 40 phr) after six months (not shown), measured with a TA

Instruments Q800. DINP at 40 phr and all samples at 15 and 30 phr showed only one maximum, both fresh and after six months of aging.

Table 6.5 Frequency effect on T_g values at low and middle plasticizer ratios

Plasticizer	Phr	Glass transition, °C			
		0.1 Hz	1 Hz	5 Hz	10 Hz
TBA	15	46	50	52	54
	30	23	27	30	32
	40	-	30	-	-
ATBC	15	45	48	52	54
	30	20	26	30	32
	40	-	22	-	-
TBC	30	25	30	33	34
	40	-	32	-	-
DINP	15	49	53	57	59
	30	24	29	33	35
	40	-	31	-	-

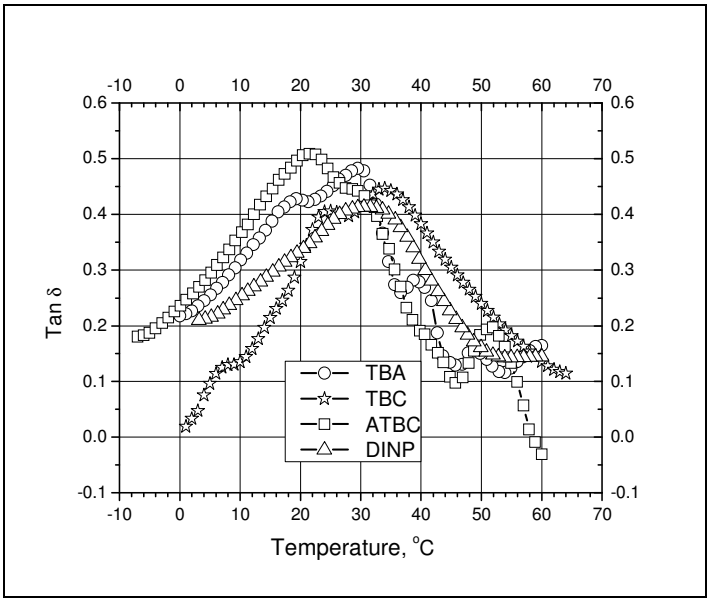

Figure 6.7 Glass transition temperatures (maxima of the tan δ curves) of three months old samples with 40 phr of plasticizers. AR 2000 rheometer at 1 Hz.

After aging, Tg values increased by up to 20 °C (Table 6.6). This behavior is associated with the progressive loss of elasticity of the blends during aging.

The effect of frequency of DMA measurement (Table 6.5) is well fitted with a power model (Figure 6.8).

Tensile Properties

The polymeric materials are classified by ASTM as rigid if the Young modulus is above 700 MPa (the case of un-plasticized PVC); semi-rigid if it is between 70 and 700 MPa and soft if it is below 70 MPa (Stuart, 2002). In agreement with this classification, our blends with the low plasticizer level were semi-rigid and soft at higher levels (Table 6.7). When the content of the plasticizer was doubled from 15 to 30 phr, the modulus dropped roughly ten times, then decreased about two-fold as the plasticizer content increased from 30 to 40 phr.

Table 6.6 Changes in T_g at 1 Hz as a result of PVC aging for six months at ambient temperature

Plasticizer	Plasticizer, phr	T_g, °C		
		Fresh	Aged	Change, %
TBA	15	50	58	16
	30	27	44	63
	40	30[a]	33	10
ATBC	15	48	60	25
	30	26	40	54
	40	22[a]	31	41
TBC	30	30	48	60
	40	32[a]	38	19
DINP	15	53	62	17
	30	29	49	69
	40	31[a]	35	13

[a] three month old

The tensile stress data shown in Table 6.8 represent the maximum stress supported by the plastics. The trend was similar to that of the Young modulus, i.e., the tensile stress decreased as the plasticizer content increased. However, the strength decreased gradually from 30-33 MPa for low ratio to 22-27 MPa for middle and 20-21 M Pa for 40 phr samples. The small differences in tensile data between middle and high plasticizing ratio samples point

to saturation of PVC with the plasticizer, regardless of its nature. TBC at 30 phr displayed, however, a slightly increased strength (higher tensile stress) as compared with the other compositions.

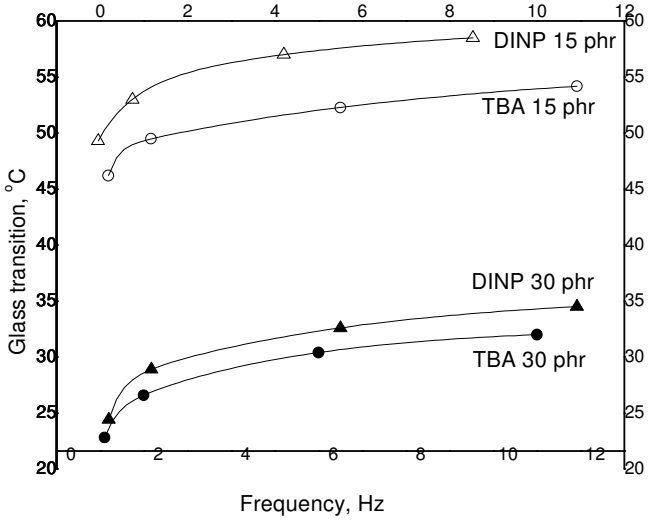

Figure 6.8 Glass transition temperature, T_g at different frequencies of the fresh PVC samples plasticized with TBA and DINP at 15 and 30 phr levels.

Table 6.7 Young modulus of plasticized PVC samples, fresh and after six months of aging

Plasticizer	Plasticizer, phr	Modulus,MPa		
		Fresh	Aged	% change
TBA	15	455.6 ± 60	569 ± 74	25
	30	42.7 ± 7	124 ± 22	196
	40	12 ± 0.2	26 ± 2	114
ATBC	15	479 ± 29	561 ± 34	17
	30	31 ± 2	81 ± 6	162
	40	14 ± 1	19 ± 1	40
TBC	30	44 ± 0.4	174 ± 12	300
	40	25 ± 4	84 ± 10	236
DINP	15	473 ± 64	562 ± 24	19
	30	54 ± 5	126 ± 15	132
	40	23 ± 1	36 ± 6	54

If one considers the Young modulus data, the situation changed, however, after aging. The modulus increased in all formulations, the least for the low plasticizer ratio blends (Table

6.7). If the change of Young modulus with age is used as a relative measure of the plasticizer migration, the results indicate that TBC was more prone to migration followed by TBA, ATBC and DINP. At the same time, the ASTM classification of 30 phr plasticized blends changed from soft to a semi-rigid. A noticeable decrease of the tensile stress occurred after aging only in the 15 phr blends. The other compositions remained within the same limits of tensile strength.

Table 6.8 Stress at break point (tensile stress) of plasticized samples

Plasticizer	Plasticizer, phr	Stress, MPa		
		Fresh	Aged	% change
TBA	15	33.2 ± 0.5	27.1 ± 0.2	-18.4
	30	22.2 ± 0.6	20.4 ± 0.4	-8.1
	40	20.4 ± 0.7	20.6 ± 0.6	1.0
ATBC	15	29.8 ± 3.0	27.7 ± 2.5	-7.0
	30	24.0 ± 2.1	25.0 ± 0.5	4.2
	40	20.0 ± 0.8	20.2 ± 1.0	1.0
TBC	30	27.3 ± 0.6	25.8 ± 1.0	-5.5
	40	21.2 ± 3.2	19.8 ± 1.5	-6.6
DINP	15	32.1 ± 1.4	27.3 ± 0.6	-15.0
	30	21.3 ± 1.6	22.2 ± 2.8	4.2
	40	20.8 ± 0.1	20.3 ± 1.1	-2.4

Conclusions

As expected from the closeness of dielectric constant and solubility parameters, the bio-based esters tested in this work had the same capacity as DINP as plasticizers in PVC. Young modulus of PVC blends with any of the four plasticizers decreased about ten-fold by increasing the plasticizer level from 15 to 30 phr, from the semi-rigid to flexible range in the ASTM classification. A 40 phr level was required, though, for PVC to retain its flexibility beyond six months. At 40 phr, TBA performed better than DINP and TBC, yielding flexible PVC with a lower Young modulus, both in freshly produced samples and after six months.

Changes of the tensile strength with the plasticizer content were less dramatic and no age effects were noticeable.

The effect of the four plasticizers on the glass transition temperature of PVC was similar, lowering T_g to close to ambient temperature at 30 and 40 phr in fresh samples and to between 40 and 60˚C after six months. Thermal stability of the PVC plasticized with DINP was superior among the group, losing less than 1% of its weight in the 170-180˚C range. PVC blended with the bio-derived compounds was less thermally stable with the weight loss about 2% at 170˚C.

Overall, TBA is a good candidate to substitute DINP in consumer products where the alleged toxicity of DINP is an issue and comparable to esters of citric acid in its effects in flexible PVC formulations.

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CHAPTER 7 CONCLUSIONS

This research investigated the composition of juice extracted from sugarcane stalk and cane leaf matter (CLM), in particular the concentration of trans-aconitic acid (TAA), the extraction of TAA from CLM juice after fermentation and ethanol distillation, and its industrial utilization as a plasticizer of poly vinyl chloride (PVC).

CLM represents 10 – 18 % of the total weight of sugarcane. TAA content in immature sugarcane was almost two times higher than in mature sugarcane either with or without the use of a ripener (Polado-L®). In mature sugarcane, TAA was three to six times higher (dry basis) in CLM juice than in clean stalk juice. In addition to the negative effect of maturation on TAA content, it was found that sugarcane exposure to freezing temperatures reduced TAA content in both stalk and CLM.

CLM and leaves together represent up to 50% of the total fiber of cane. The similarity in composition between sugarcane bagasse and CLM makes CLM a possible source to be used as fuel in the boilers or as a cellulosic feedstock for ethanol production. Although the transportation cost double due to the low density of CLM, processing whole sugarcane can be profitable if the whole sugarcane is transported at distance of no more than about 20 miles from the field to the mill. This profit is increased significantly if the mill has a turbo-generator with high efficiency.

Seasonal variations in the TAA content were observed from 2003 to 2006 crushing seasons. The amount of TAA extracted in a pilot mill ranged from 2.2 kg/t CLM to 3.1 kg/t CLM. Therefore, the potential production in Louisiana of TAA at a crushing rate of 12 million tons of cane/year would be approximately 5600 t of TAA from CLM per year.

CLM juice and cane molasses were fermented under the same conditions. The conversion of total fermentable sugars in ethanol was 92% for CLM and 96% for cane molasses. These results give an alternative to the sugarcane ethanol industry to increase its production using CLM as a possible feedstock. More studies are needed to determine the effect of higher content of ash in CLM juice during fermentation on ethanol yield.

Liquid-liquid extraction (LLE), solid-liquid extraction and ion exchange were evaluated for extraction of TAA from CLM stillage. Three solvents were evaluated for LLE, tri-butyl phosphate (TBP)-dodecane, ethyl acetate and butanol. TBP-dodecane was found to be nearly immiscible in CLM stillage. Almost complete extraction (> 92 g/100 g in CLM stillage) of TAA was observed with TBP-dodecane and butanol. However, the mixed solvent, TPB -dodecane, was more susceptible to emulsion and third phase formation. Ethyl acetate had the highest selectivity for TAA and had the lowest extraction of color bodies, but also the lowest TAA extraction yield (64 g/100g in CLM stillage).

The advantages of butanol over TBP-dodecane are its higher limiting organic concentration avoids the third phase formation even at low organic/aqueous ratio, low cost, and that it can be directly esterified to TBA, avoiding the need for back extraction. The disadvantages of butanol are its relatively high solubility in CLM stillage and its low selectivity for TAA. Butanol must be recovered from the raffinate before raffinate can be disposed of.

After consideration of these factors, butanol was chosen as the best solvent of the three to extract TAA from CLM stillage. The optimum conditions to extract TAA were pH 2.0, OA 3.5, temperature 50°C, and 1 hour equilibrium time. These conditions were also used to

extract TAA from cane molasses. The results indicated a slightly higher extraction yield than those obtained with CLM stillage, due to the higher viscosity of the stillage.

The co-extraction of other compounds with TAA decreased the purity of the extract and increased color. These two problems limited the degree of TAA purification and reduced the yields. The color of CLM stillage was found to be between 12 to 15 times higher than the color of sugarcane juice. Phenolic compounds were found in the extract phase.

Direct esterification of butanol extract was conducted with success. Approximately 90 to 93% of the TAA was converted to tri-butyl aconitate. The purity of the ester was quite close to those measured for TAA in butanol extract. Decolorization was done using powder activated carbon. Although the color was reduced by a factor of 8, an increase in color was observed after heating.

The consumption of sulfuric acid to acidify the liquor to pH 2.0 was estimated to be about 21 g/100 g dry solids of the CLM stillage, or 1.3 - 1.5 times higher than anticipated TAA yield. This makes it a substantial cost item, but less so than in the case of recovery from cane molasses with consumption estimated to be 2- 2.3 times higher than TAA yielded.

The attempts to esterify TAA from powder CLM stillage (solid-liquid extraction) were unsuccessful. The highest conversion yield was only 2.5 %.

Dowex Marathon MSA, a strong base anion resin in sulfate form, and Dowex Optipore SD-2, a non-ionic adsorbent, were chosen from several resins and adsorbents and evaluated for the recovery of TAA from CLM stillage. The adsorption capacity for the acid on both was found to be enhanced by lowering the pH of the CLM stillage. Regeneration of the two resins with butanol was studied. Promising results were observed with Dowex Optipore.

The color of CLM stillage after TAA extraction was reduced more than eight fold indicating the high rate of color retention by the resin.

Tri-butyl aconitate, (TBA), tri-butyl citrate (TBC) and acetyl tri-butyl citrate (ATBC), all bio-based esters, were compared as plasticizers of PVC with di-isononyl phthalate (DINP). Young modulus of PVC blends with any of the four plasticizers decreased about 10-fold by increasing the plasticizer level from 15 to 30 part per hundred parts of resin (phr). At 40 phr, TBA performed better than DINP and TBC, yielding flexible PVC with a lower Young modulus, both in freshly produced samples and after six months. A 40 phr level was required, though, for PVC to retain its flexibility beyond six months. Changes of the tensile strength with the plasticizer content were less dramatic and no age effects were noticeable.

The effect of the four plasticizers on the glass transition temperature of PVC was similar. Thermal stability of the PVC plasticized with DINP was superior among the group, losing less than 1% of its weight in the 170-180˚C range. PVC blended with the bio-derived compounds was slightly less thermally stable with a weight loss of about 2% at 170˚C.

In summary, a methodology was developed to extract TAA from CLM juice, with an overall yield, after esterification, of 84%. Further work is required to complete the decolorization of the product and its purification and the overall process design and process economics.

TBA is a good candidate to substitute DINP in consumer products where the alleged toxicity of DINP is an issue and is comparable to esters of citric acid in its effects in flexible PVC formulations.

APPENDIX: LETTERS OF PERMISSION

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September 27, 2007

From: W. David Constant, Associate Dean and
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To: Nicolas Gil
PhD Candidate
Engineering Science Program

The Engineering Science Program approves inclusion of the following papers as chapters in your dissertation, "Evaluation of the effects of bio-based plasticizers on the thermal and mechanical properties of poly (vinyl chloride)" by N. Gil, M. Saska and I. Negulescu, published in the Journal of Applied Polymer Science, Volume 102, Issue No 2, Date: 15 October, 2006, Pages: 1366-1373, and "Some observations of feasibility of recovering aconitic acid from low purity sugarcane liquors" by M. Saska and N. Gil published in International Sugar Journal, Volume 108, Issue No 1288, Date: April, 2006, Pages:203-208.

This approval is subject to approval of your graduate committee to include the above papers in your dissertation.

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I am finishing my Ph.D. at Louisiana State University, and I am planning to include this paper as a chapter in my dissertation. For this reason, I am requesting a written permission to use this published material.

Thank you in advance for your prompt reply.

Nicolás Gil

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Baton Rouge, September 5, 2007

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Dear Sir,

I am the co-author of the paper entitled, "Some observations of feasibility of recovering aconitic acid from low purity sugarcane liquors" published in the Volume 108, Issue No 1288, Date: April, 2006, Pages:203-208.

I am finishing my Ph.D. at Louisiana State University, and I am planning to include this paper as a chapter in my dissertation. For this reason, I am requesting a written permission to use this published material.

Thank you in advance for your prompt reply.

Nicolas Gil Zapata
Ph.D. Candidate
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VITA

Nicolas Javier Gil Zapata was born to Javier Gil and Matilde Zapata, in Medellin, Colombia. He attended the Universidad Industrial de Santander, Bucaramanga campus in Colombia, to obtain a bachelor's degree in chemical engineering in 1988. From 1989 to 1995 he worked as a Research associate at Centro de Investigacion para el Mejoramiento de la Panela, CIMPA, in Barbosa, Santander, Colombia. In September of 1995, he moved to work to Research Center of Sugarcane, Cenicana, in Cali, Colombia. In 2003, with the sponsor of Cenicana and a graduate assistantship offered from Audubon Sugar Institute, he became his doctoral program at Louisiana State University in Baton Rouge. He will receive a degree of Doctor in Philosophy in engineering science with a minor in chemical engineering in December 2007