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Iron mediated pecipitation of phenol: protein aggregates from sugar cane juice

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IRON MEDIATED PRECIPITATION OF PHENOL:PROTEIN AGGREGATES FROM SUGAR CANE JUICE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF TERMS

Affination	The process of mingling crystalline sugar with a saturates sugar solution with the aim of washing colored contaminants from the crystal surfaces
AU	Arbitrary units, most frequently used to describe wavelength specific absorbance relative to concentration derived using Beer's law
Brix	A measure of dissolved dry solids, reported in degrees or percentage of dry solids, g/100g
Cane	Sucrose laden grass, *Saccharum Officinarum*
Catechol	*o*-hydroxyphenol
Color	ICUMSA, the measure of absorbance at 420nm of a solution at pH 7.
Cuticle	A film covering the surface of plants, the underside of the rind in cane.
Crusher	Mixed juice expressed from a milling tandem
D	Dextrorotatory, carbohydrate nomenclature which indicates that the penultimate hydroxyl of the sugar is facing right in the Fischer projection. This is not equivalent to the R designation given by the Cahn-Ingold-Prelog selection rules.
DAD	Diode array detector, photodiode array detector (PAD), frequently a detection mode in HPLC
Diffuser	Means of expressing sucrose from cane using solid-liquid extraction with hot water, also describes the sort of juice expressed
DRI	Differential Refractive Index, frequently a detection mode in HPLC
EI	Electron impact, ionization method frequently used with GC-MS
FTIR	Fourier transform infrared spectroscopy
GAC	Granular activated carbon
GC	Gas chromatograph, Gas-Liquid Chromatograph, usually hyphenated with the means of detection, vis. GC-mass spectrometry, GC-MS

GPC	Gel permeation chromatography, a technique which separates materials on basis of molecular weight and conformation in solution
HMF	5-hydroxymethyl-2-furaldehyde
HPLC	High pressure liquid chromatography usually hyphenated with the means of detection, vis. HPLC-diode array detection, HPLC-DAD
Hydroquinone	*p*-hydroxyphenol
Imbibition	The practice of washing cane in a counter-current fashion during crushing in order to maximize extraction. Usually applied as a percentage on fiber, eg. Imbibition at 250% of fiber.
Inversion	Hydrolytic cleavage of sucrose to yield one molar equivalent each of glucose and fructose
ICUMSA	International Commission for the Uniform Methods of Sugar Analysis
IV	Indicator Value, Effect of pH on the observed color of a compound or mixture
L	Levorotatory (laevorotatory), carbohydrate nomenclature which indicates that the penultimate hydroxyl of the sugar is facing left in the Fischer projection. This is not equivalent to the S designation given by the Cahn-Ingold-Prelog selection rules.
mDTC	mixed dithiocarbamate mill biocide
Mill	Also milling tandem, a series of up to five three-roll mills used to squeeze juice, and hence sucrose from cane.
MS	Mass Spectrometer, a detector used to measure the fragmentation pattern specific to a given compound
Melt	A solution of sucrose or commercial sugar
Mixed Juice	Diluted Juice extracted from cane via milling or diffusion; it includes water used for inbibition.
MRP	Maillard reaction product
NTU	Nephelometric turbidity units, standardized against colloidal formazin

ODS	C_{18}, Octadecylstyrene
PCS-3102	A polyelectrolyte; cationic polyamine flocculant supplied by Ecolab, Inc.
Pol	Sucrose measured via polarimetry, g/100g
PT	Proton transfer, the movement of a hydrogen atom connected to a carbon separated not more than 1 bond away or the movement of this hydrogen ion as facilitated by a nucleophilic molecule present in excess.
Quinone	conjugated α,β or α,δ-diketone resulting from a o or p-hydroxyphenol
REDOX	Reduction/Oxidation couple, reaction or cycle
SAC	Strong Acid Cation exchange resin, usually a sulfonic acid modified styrene-divinylbenzene copolymer; also made from acrylic polymer.
SDS-PAGE	Sodium dodecyl sulfate poly acrylamide gel electrophoresis
STP	Standard temperature and pressure; 25 °C, 1 atm (1.01E5 Pa).
Sugar	sucrose, saccharose, α–D-glucopyranosyl-(1→2)-β-D-fructofuranoside
RF Brix	Refractometer brix, determined via prism or total internal reflectance
RI	Refractive index, determined using Snell's law
RP	Reversed phase
UV-VIS	Spectroscopy measuring either absorbance or transmission of light in the ultraviolet through the visible wavelength continuum (190-700 nm)

ABSTRACT

The Louisiana cane sugar industry is moving toward a vertically integrated arrangement where raw sugar producers will have an interest in refining. In the sugar refining process, raw sugar is affined to remove ~50 % of the color. The new refinery that will be built in Louisiana, however, will not include affining stations. To reduce costs of refining, either affining stations will need to be installed at each mill or new technologies that provide equivalent color reduction (~50% or ~750 IU) need to be implemented. As part of this dissertation a new technology for color reduction at raw sugar mills is introduced.

Color in raw sugar juice was assessed using chromatography, mass spectrometry, spectroscopy (UV-VIS, FTIR) and wet chemical means. The colored materials were phenolic and conformed with the lignin-monomer classes. It was found that this color can be removed from raw juice by applying Fe^{3+} and cationic polyacrylamide at ambient temperature and settling. The decanted juice was clarified via cold-liming. This is a novel, inexpensive method, which yielded clarified juice with up to 70% less color than that provided using the hot-liming method.

Using modeled juice, it was found that protein, dihydroxybenzoic or propenoic acids, carboxylic acid salts, and Fe^{3+} were required for color removal. The optimum quantities of each component conformed with the average composition of cane juice.

It was hypothesized that the phenolics were oxidized by the iron, engaging in a REDOX cycle which led to oligomerization. Stoichiometry indicated a degree of polymerization of ~8. Oligomerization ceased at this length which appeared sufficient to facilitate cross-linking and/or capping of the protein. The aggregates of iron, lignol(s) and protein were insoluble and precipitated.

The method was tested at pilot-scale using a 151 L (40 gal.) settling clarifier which was operated in pulsed and continuous (7.6 L/min, ~2 gpm) modes. The method scaled well and the product juice exhibited 50-60% less color than a cold-limed control when Fe^{3+} was applied in quantities ranging from 100-200 μg/mL. Cationic flocculants increased the settling rates.

CHAPTER 1. INTRODUCTION

"With heroic determination, then, speed the plow; bear in mind that to go ahead without ever taking the difficulties into account, and by that means to succeed when others dare not undertake, is emphatically the AMERICAN SYSTEM."

--Pierre Rost's address to the LCSA noted as minutes in Debow's Review (DeBow 1847) and quoted by Heitmann (1983a).

1.1. A Brief History of Sugar Technology

According to Indian history, the cane sugar industry dates to approximately 400 B.C. It was noted (Stillman, 1924a) that in approximately 77 A.D.:

"Arabia too produces saccharon (sugar), but that of India is the most esteemed. This substance is a kind of honey which collects in reeds, white like gum, and brittle to the teeth."

The oldest reference found by this author which clearly deals with the processing of cane, is a treatise from the tenth century by Isaac Judaeus (Stillman, 1924b) regarding diet called, "*Dieta*". From there it was quoted:

"Zucarum or zucara is made from certain canes and reeds which grow in swamps near the Nile, and it is the juice of these canes called sweet cane (cana mellis) from which is made zucarum by boiling, just as salt is made from water. For the ground canes are first placed in a cauldron and cooked with a slow fire until it (the juice) is thickened, and first there is seen to pass off from the whole mass [a portion] in foam, and afterwards the thicker and better residue sinks to the bottom, and what is light and foamy remains above and is porous and less sweet and does not crackle between the teeth when masticated, but disappears quickly. But the good is the opposite (econverso), for the good, placed in round vessels in the sun, is made hard and white."

From this were derived the processes now known as crushing, defecation (clarification), evaporation, and, even though the product was likely "loaf sugar", crystallization.

These processes, passed along through Europe, eventually found their way to Louisiana. From Stillman's accord, it is not surprising that cane would be fruitful in the swamps that, at the time, constituted much of the Louisiana territory.

Going back at least as far as 1753 (Sitterson, 1953), the cane processing industry relied on animal power, including mule, ox and horse. The animals were coupled to vertical or horizontal mills, as in **figure 1.1**, which would be used to grind whole-stalk cane. This practice persisted through 1830 when animal power was rapidly supplanted by steam engines. In 1828, 128 out of 691 (18.5 %) mills in Louisiana were powered using steam. (Heitman, 1983b). By 1848, 408 of 762 (53.5 %) mills had converted to steam power (Heitman, 1983c). The industry was growing at an astonishing rate of (based on 20 years) 3.5 mills per year.

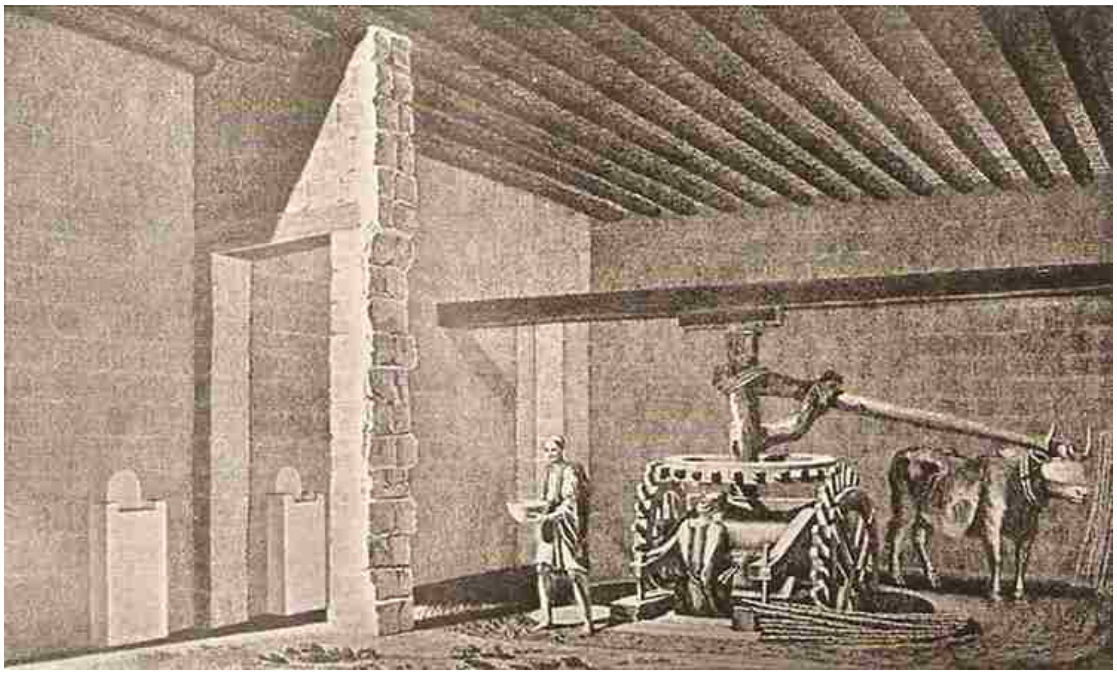


Figure 1.1. An ox-driven Egyptian horizontal sugar cane mill (Deere, 1949a).

During this time, the practice of open-kettle boiling, which was referred to by Judaeus persisted. The French evolution of this technology had been brought to the new-world in the form of a multiple-kettle boiling scheme.

Using this scheme, called a “battery”, “equipage” or “kettle-train” (Heitman 1983), the juice expressed from the cane was clarified or “defecated”, concentrated to syrup, and seeded or “struck”. These functions, which are discrete unit-processes in the modern cane sugar manufacturing industry, were, albeit with less efficiency, conducted across this kettle-train beginning with the first and largest kettle which was called the “grande”. This operation can be seen in **figure 1.2**.

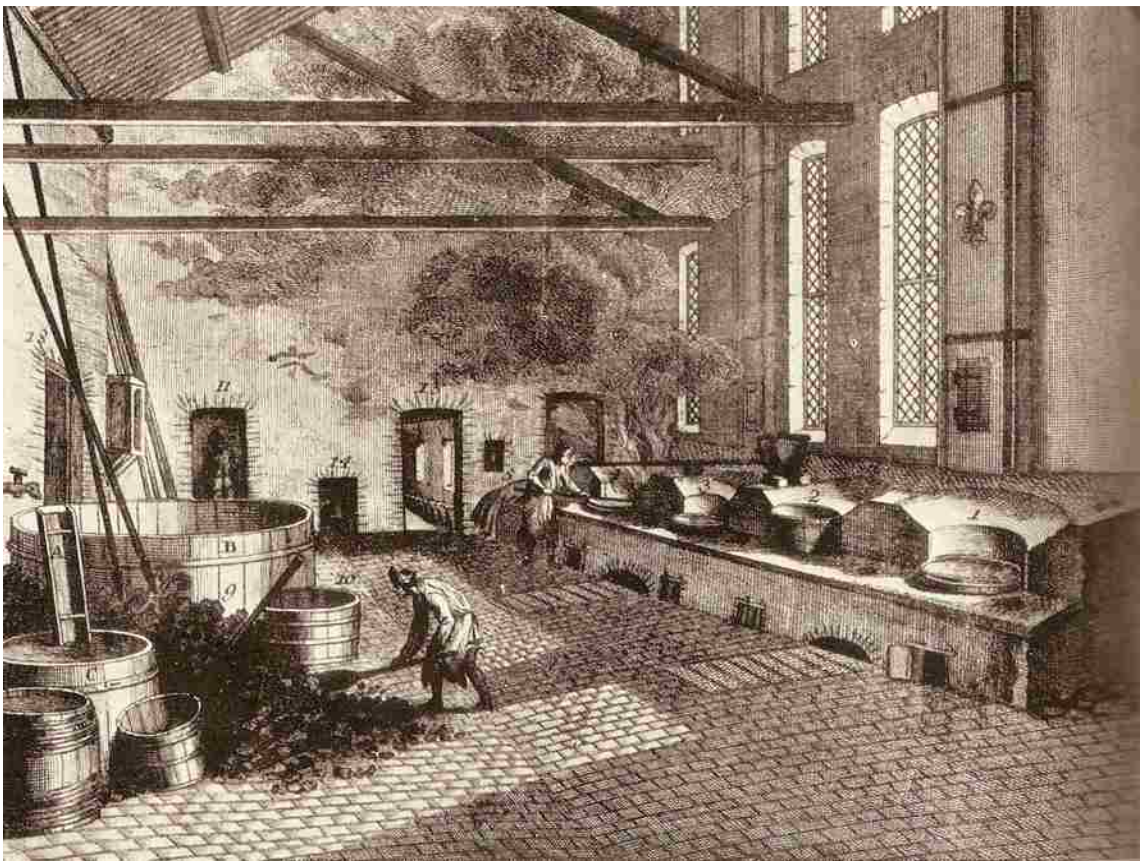


Figure 1.2. The equipage or kettle train (Deere, 1950).

Heated directly with fire fueled using cord wood, the grande could hold between 300-500 gallons of juice. This juice was brought close to boiling and a defecating agent was added. Then, as it is today, lime (CaO) was the clarifying agent of choice. The lime would cause a frothy scum (not unlike that mentioned by Judaeus) to float in the grande.

In this kettle, and for subsequent steps, the frothy material was removed (“brushing off”) and the juice, was transferred manually (“skipping out”), by means of a ladle, into the next kettle in the train.

When moving from the beginning, the next (second) kettle to be encountered was called the flambaeu.

So named because it was heated directly by flame, the juice was brought to boil in the flambaeu and considerable thickening of the juice took place. The contents of this kettle were ladled into a third kettle, which depending upon the mill, would constitute the third of a four or five kettle system. In the case of the former, the kettle was referred to as the “sirop” and is where the juice was evaporated to a thick syrup. In the case of the latter, the kettle was called the “proper clear” because it was here that the syrup was sufficiently pure as to be nearly transparent. In this case, the contents of the kettle would be less concentrated, and would be ladled into a “sirop kettle” for further evaporation. In either case, the content of the sirop kettle was transferred into the final kettle, which was between 70-100 gallons in size and was referred to as the “batterie”.

In the batterie kettle, the syrup was evaporated to very high concentration and, when the conditions were “right” this vessel was “struck” to initiate the crystallization of the sugar. It was not made clear whether this strike was the result of intentional addition of sugar “seed” or the result of spontaneous nucleation, a phenomenon referred to by trade as “false graining”. The acknowledgement of the conditions proper for this to occur were not monitored with analytical tests or any kind and relied upon the experience of the sugar boiler. For example, from document 62 from the U.S. Congress in 1831, quoted by Heitmann (1983d):

“It was eye alone that was to determine the ripeness of cane, and it was the eye and touch alone that were to determine the point at which the syrup was sufficiently boiled to granulate, experience and individual judgement [sic] were alone relied on, and we will know how variable, and how little faith can generally be placed in either.”

Regardless of the method used in the strike, this material or “massecuite” which now contains growing sugar crystals was transferred into barrels. From these barrels would slowly drain the mother liquor or “molasses”. Much molasses remained in the sugar and decreased its value.

Nevertheless, the scale and production continued to increase. During this period (1822-1843), barring disastrous events (hurricanes, etc.) in 1835, 1839 and 1843, the amount of raw sugar produced (Bouchereau, 1917 cited by Heitmann, 1983e) increased from 13,392 to 71,878 long tons (2,240 lb or 1016.06 kg) per year.

The technology of sugar production remained more-or-less consistent from the tenth to the nineteenth century (900-1000 years, a millennium). The advent of the steam engine, and the increased reliance of the sugar industry upon it (appx. 1928-1938) led to the technology which would be used to revolutionize the industry for the first time since the casting of a boiling kettle. Norbert Rillieux invented the multiple effect evaporator.

Despite being, perhaps, the most significant contribution to sugar processing technology (some would argue this point over the vacuum pan, which will be discussed later), the multiple-effect evaporator system was not, at once, accepted. Designed to use steam to generate both the heat and vacuum needed to evaporate juice to yield syrup, the multiple-effect evaporator consists of several stages also known and “bodies” or “effects”. As the juice is concentrated it is transferred to the next effect which is heated by the vapor from the effect which precedes it. Because the system is “closed”, the cooler effect will thermodynamically be under greater vacuum.

The major benefits of this system include lower color syrup with less loss of sugar to inversion (discussed later) and greatly improved steam economy. A diagram of a multiple effect evaporator is given in **figure 1.3**.

At the time (1831-1835), Rillieux was forced to abort a trial (orchestrated at his own expense) because the apparatus was “in the way”. It was, according to Heitmann, 1983 quoting DeBow, 1849, who recorded a letter from McCulloch regarding the progress of Rillieux’s project, whereby it was “…deemed absurd that the cane juice of Louisiana could be boiled by steam.”

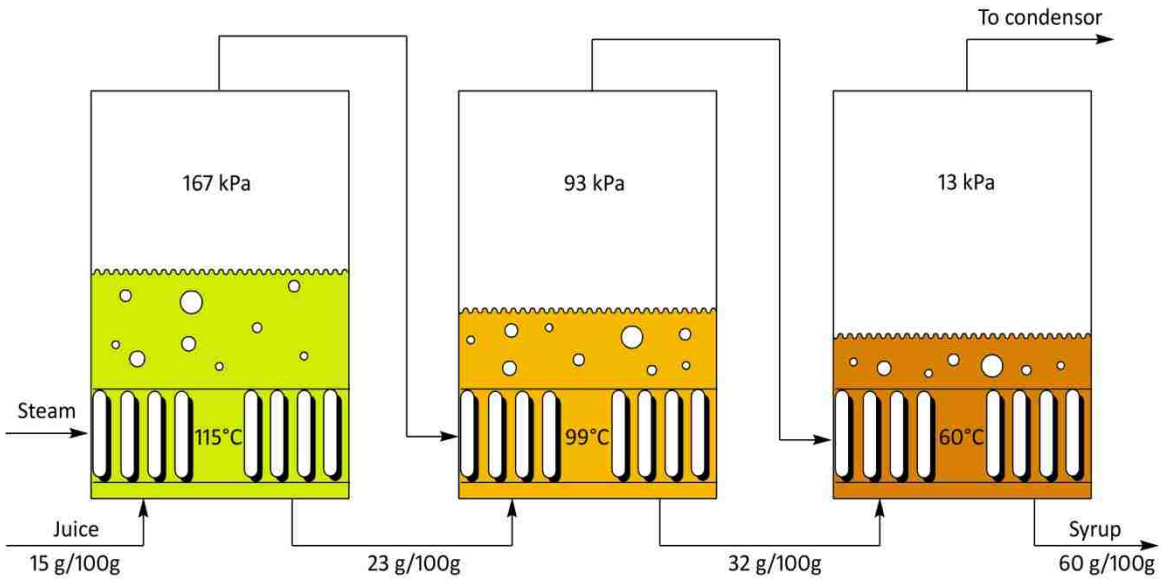


Figure 1.3. Diagram of a triple-effect evaporator train.

In the meanwhile, the 1830's marked the entry of the "vacuum-pan" to the Louisiana sugar processing industry. Patented far earlier, in 1813, by Howard, this piece of equipment that allowed use of steam and vacuum for the boiling of the cane syrup at lower temperature.

Essentially a sealed kettle with steam heating tubes, the "pan" granted the boiler a greater degree of control (it would not overheat like fired kettles and temperature is under nearly instantaneous control) while removing water at a greater rate and at a lower temperature. The increased throughput (and energy efficiency) minimized losses of sugar due to inversion (a process described in detail, later) to produce a superior sugar with significantly less color.

"With the exception of the vacuum pan, attempted improvements in the sugar making process initiated during the 1830's usually ended in frustration and failure." (Heitman, 1983f). Nevertheless, with sugar bringing in 5 cents/lb*, the 1840's were marked with a much greater drive to accept and implement new technologies. With the promise of producing larger amounts of higher quality sugar from their canes, many of the producers at the time were able to sheath their biases with the promise of wealth.

The 1950's marked perhaps the last major technological innovation that brought the sugar processing industry to where it is today. A furnace designed to burn bagasse (the fibrous material remaining after the saccharine juice has been expressed from the cane) was patented by Alfred Stillman (Heitmann, 1983g) in 1955. This equipment facilitates the suspension firing of wet (~50 g/100g moisture) materials, such as bagasse, to provide the steam needed to run the factory. In this way, much of the energy needed for processing was rolled into the price of the cane (which already included transport).

Today, the bagasse furnace provides nearly all of the operational energy for a cane process. This eliminates the overhead for fuel which can consume 2/3 of the profit margin of approximately $0.21 /lb on refined sugar. Because 1/3 of the bagasse is usually leftover from a fully powered mill (at 10,000 tons cane/day this is ~476 tons of fiber[**]), the closed-cycle system of the sugar mill represents the greatest possible return for a Biorefinery enterprise based upon bagasse feedstock (or vertically integrated refining process).

[*] 1.08 USD in 2008 calculated using an inflation index of 21.688 (Austin Genealogical Society, 2003).
[**] 10,000 tons cane/d (9090.9 mt/d) * 0.14 (g/100 g/fiber on cane) = 1,400 tons fiber/d (1272.72 mt/d) * 2/3 (fraction typically burned/d) = 924 tons/d (840 mt/d); 1,400 tons fiber – 924 tons burnt = 476 tons residual fiber/d (432.72 mt/d).

1.2. Research Objectives

The goal of the sugar industry is to extract sucrose from cane (*Saccharum Officinarum*) in such a way as to produce a consumer grade product with both very high purity and very low color (VLC). Louisiana currently produces a raw-grade sugar (~800-2500 ICUMSA color units, IU) which is sold to a refiner who produces a refined product with a color of 15-50 IU (www.buysugarnow.com).
This arrangement is currently in a state of flux as global competition encourages the raw producers and the refineries to cooperate and combine (Brady, 2005).

The profit achievable from sugar is a balance between the quality of the raw material and the cost of the fuel required to refine it. Refineries use natural gas to power their operations.

The white sugar premium is approximately 6.6 cents (Todd, 1997) per kg but, the cost of natural gas can offset this by as much as 39% (www.wtrg.com). Because the fuel used to process raw sugar is integrated with the price of the cane, and the direct production of white sugar can lead to the recovery of approximately ~ 8% of additional sugar (Fechter, 2001), the industry can reap both savings and increased profit on the refinery-end.

The goal of this work was to conceptualize and implement a method to enable a raw sugar mill to manufacture a sugar with 50 % less color without the use of affination.

The use of iron was described in a patent (Madsen, 1984) to improve the clarification of juice from sugar beets. It made use of a hybrid decolorization/clarification method which is superficially similar to the work presented here. Iron salts were used to effect clarification and decolorization when used in tandem with ultrafiltration (UF), peroxide, sulfitation or conventional liming procedures. The description involves the use of ferric chloride as a chelant/oxidant. The agent served to create floc which was removed via ultrafiltration, then followed by hot-liming (Madsen, 1984).

Described in this work is the development and implementation of a process whereby ferric iron is used, in conjunction with a cationic flocculant and followed by conventional cold-liming protocol (without incubation of the juice at ambient temperature) to effect clarification/decolorization *without* the use of carbon, resin or ultrafiltration.

CHAPTER 2. LITERATURE REVIEW

2.1. Sucrose and Other Relevant Carbohydrates

Sucrose (α-D-glucopyranosyl-(1$\rightarrow$2)-β-D-fructofuranose), glucose and fructose are the three primary simple carbohydrates found in sugarcane juice (*Saccharum Officinarum*). The behavior of these carbohydrates relative to any treatment is of critical consequence to the success of any process designed to use sugarcane juice as feedstock. The work described in this dissertation requires a fundamental knowledge of the chemistry of carbohydrates. A mini-review of fundamental and relevant properties of carbohydrates is given in **Appendix A**. Likewise, a review of "Inversion", a specific reaction whereby the disaccharide sucrose is hydrolyzed to the reducing sugars (monosaccharides), D-glucose and D-fructose, is detailed in **Appendix B**.

2.2. The Composition of Cane Juice

Cane juice is a complex mixture containing large amounts of non-carbohydrates. In order to properly understand of any treatment involving this juice, the overall composition must first be defined.

Sugarcane juices and the products created during sucrose extraction are complicated mixtures composed of various natural products and their products of reaction. The plant composition varies throughout the growing season, from season-to-season, with the weather (Legendre, et al, 2007), and, of course, between varieties. In Louisiana, one variety, LCP 85-384 was predominant over the time frame of this research. The newer varieties, L 99-226 and L 99-233 are replacing this strain because they have sturdier stalks, produce denser stands and have higher sucrose yields (Bogren, 2006).The composition of juice, all else being equal, can vary depending on the methods employed for extraction. Expression of juice from cane is conducted either by milling or by diffusion. 10 of 11 operating sugar factories in La. are using milling tandems. Two diffusers are in use and only one mill uses the technology exclusively.

The milling process is a counter-current or “compound” process which involves grinding of the cane in a series of mills, called a ”tandem”, with added water (imbibition). The amount of imbibition water depends upon the fiber content of the cane and contributes to the final quantity of juice. The quantity of imbibition water should be applied in the range of 250-280 % fiber in cane (Rein, 2007) and it is balanced to achieve the highest possible extraction from the cane while using the least amount of water. A typical five-roll milling tandem with compound imbibition is given in figure 2.1 with extraction figures provided by Rein (2007).

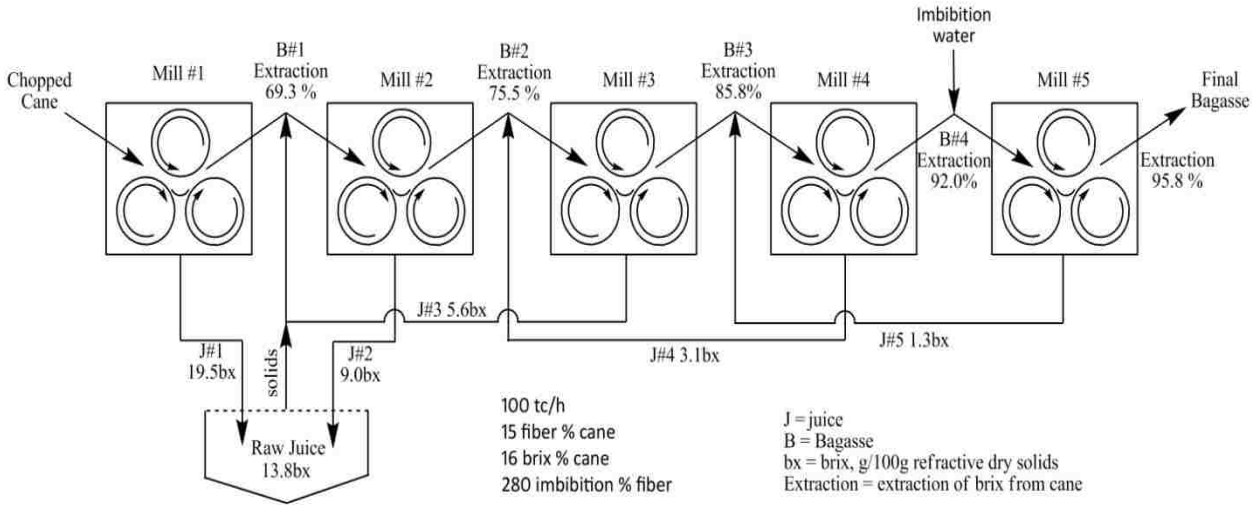


Figure 2.1. A five-roll milling tandem achieving 95.8% extraction.

The diffuser involves moving a bed of chopped cane through a tunnel with a series of 10-18 cells beneath. Hot water, usually 85°C, is sprayed onto the cane bed fed at 300 tons/h which can be 50-60 m in length and ~10 m thick. As the bed moves, the water and juice percolates though the bed and collects in the cell beneath. The contents of each cell are re-heated and pumped onto the cane over the preceding cells. The final bagasse exiting the diffuser is de-watered using a conventional mill.

In either case, the ash (salt content), approximated by conductivity, is surprisingly consistent at 4.4±1.3. The “true” purity (sucrose, $g \cdot 100g^{-1}$/brix, $g \cdot 100g^{-1}$ * 100) is also relatively consistent. Purities averaged 87.3±2.0 %. The other major components are given in **table 2.1**, below.

Table 2.1: Average composition of cane and juices obtained in La. From 2004-2005. (Polanco, et al., 2006).

Year/ Extractor:	Pol* in Juice, g/100g:	Sucrose** in Juice, g/100g:	Reducing*** sugar, g/100g:	Color, IU:	pH:	Gum† mg/kg:
2004						
Mill	11.5 ±0.2	12.0±2.9	4.6±3.7	12200±2400	5.5±1.0	2050±2530
Diffuser	11.3±0.2	11.8±3.9	4.9±3.9	13500±2800	5.2±0.4	1140±1810
2005						
Mill	11.8±0.4	12.3±2.1	5.1±1.7	11000±2300	6.1±1.2	1200±2210
Diffuser	11.4±0.2	11.9±2.5	5.5±1.7	12200±2800	5.5±0.5	670±1160

± indicates the range of values acquired from weekly sampling over a 90 d season.
*sucrose via polarimetry
** sucrose via HPLC vs. external standards
***sum of glucose and fructose via HPLC vs. external standards
† sum of starch via precipitation/colorimetry and dextran via antibody method.

It is assumed, for the purpose of this work and unless otherwise stated, that cane has a gross composition of, based upon whole cane, brix, 14 %(purity, 87 %); fiber, 14 %; ash, 4% and other, 72%. This does not add up to 100% because the fiber measurement, as it is measured, also includes the ash (Madsen, 2003).

"Brix" refers to the g/100g of sample which is determined as refractive dry solid. The constituents of brix are sucrose, 12 g/100g and reducing sugar, 5.0 g/100g. The glucose/fructose ratio is, on average, 1.03. These values were corroborated by an independent laboratory (Iqbal, et al., 2000).

"Ash" is primarily composed of salts of Na^{1+}, NH_4^{1+}, K^{1+}, Mg^{2+} and Ca^{2+} with (mostly) Cl^-, SO_4^{2-}, CO_3^{2}, SiO_x and PO_4^{3+}. The normal ranges, which were, within error, equivalent for both diffuser and mill juices are given in **table 2.2**.

Table 2.2. Mono and di-valent cations in cane juice (Polanco, et al., 2006)

	Mill 2004	Diffuser 2004
Cation:	**mg/kg*:**	**mg/kg*:**
Na	389	291
K	8112	9470
Mg	2036	2167
Ca	2898	2572

*Determined via HPLC/ECD.

"Other" refers mostly to water with the balance consisting of nitrogenous bases, plant pigments, polyphenols, "gums" (starch, dextran, indigenous cane polysaccharides), carboxylic acids and cellulose. Significant quantities of amino acids and amides are found in juice. The concentrations of each are given by Meade and Chen (1977) and are presented in **Table 2.3**.

Table 2.3. Amino acid constituents of cane juice and protein isolate.

Compound:	Free, % DS:	Protein, % DS.
Asparagine	0.71	n/a
Glutamine	0.19	n/a
Aspartic acid	0.11	0.06
Glutamic acid	0.05	0.08
Alanine	0.06	0.05
Valine	0.03	0.04
g-aminobutyric acid	0.03	0.03
Threonine	0.02	0.04
Isoleucine	0.01	0.03
Glycine	<0.01	0.04
Leucine	Trace	0.03
Lysine	Trace	0.04
Serine	Trace	0.03
Arginine	Trace	0.02
Phenylalanine	Trace	0.02
Tyrosine	Trace	0.02
Histidine	Trace	0.01
Proline	Trace	0.01
Total Protein	Trace	0.49

Commercial cane juice is a mixture of juices expressed from both the stalks and the "cane leaf material" (CLM). CLM contains a much larger quantity of pigmented materials than the stalks, and juices expressed from it reflect this (Gil and Saska, 2005). The ratio of leafy materials to stalks entering the mill are unknown, but have increased since the State of Louisiana instituted greater control over field burning (Legendre, 2001). A break-down of the composition of "phenolic" materials found in in stalks, CLM and product sugars are given in **table 2.4**.

The bulk of phenolic materials seem to be monomers derived from lignin and flavonoids.The phenolic compounds found in sugarcane juice remain throughout the production of both raw and refined sugars.

Table 2.4. Phenolics, by compartment, in cane and sugar (Farber, et al, 1971; Godshall, et al, 1988 cited in Meade and Chen, 1977).

CLM Juice	Raw Sugar	Refined Sugar
Coniferin	Caffeic acid	Chlorogenic acid
Coumarin	Quinic Acid	p-coumaric acid
3,4-dihydroxybenzaldehyde	Kaempferol	Ferulic acid
3,4-dihydroxybenzoic acid	Syringic acid	Sinapic acid
Esculin	Umbelliferone	
4-hydroxybenzaldehyde		
4-hydroxybenzoic acid		
Vanillin		
Vanillic acid		
Rutin		
Quercetin		

Chlorogenic acid found in refined sugar likely correlates with caffeic and quinic acids observed in the raw sugar. This is likely an artifact of the analytical techniques as chlorogenic acid is the quinic acid ester of caffeic acid, not vice-versa. It should also be noted that chlorogenic acid is difficult to detect in juice because it is labile to both hydrolysis and oxidation. This makes it unlikely that it would survive the sugar production process to end up in raw sugar. This is reflected by the fact that it is quinic acid which is found in the raw sugar.

2.3. Clarification

"As most sugar produced by simple clarification is sold as raw sugar, which is subsequently refined, no great value should be attached to a clear, sparkling juice" (Payne, 1953).

At present, there is still no premium value ascribed to raw sugar of especially low color. A cooperative refinery may offer a premium value for VLC (very low color) sugar if it saves them money. VLC has the potential to increase profit on the refining end by decreasing expenses associated with the use of natural gas for fuel. In such a case, great attention will likely be paid to the process of clarifying cane juice because as it can be expected that good clarification will lead to a product with less color.

2.3.1. Defecation

Very few words used in the sugar industry will raise the occasional eyebrow more than "defecate".

A verb by definition, the archaic term used in much of the early literature, describes the action of removing “impurities, as in a chemical solution” or to “clarify” (www.dictionary.com) the mixture.

From this comes the modern convention wherein is described the act of defecating sugarcane juice to yield a clarified product. Today, the unit process is referred to as clarification, and with the exception of quoted work, shall be used henceforward.

The various unit processes found in a modern sugar mill are derived from the various kettles used in the early “equipage”. Of these, the addition of lime and conditions prevailing in the “proper clear” leads to modern clarification technologies.

2.3.2. Colloids

Cane juice is a mixture containing a dispersion of particles with a wide range of sizes. These particles were referred to as “dispersoids” by Von Wiemarn, et al., in 1908 who subdivided them by size (Payne, 1953).

Table 2.5. Designation of particle size

Coarse dispersion or suspension	Diameter >0.1 μm
Colloidal solutions	0.1-0.001 μm
Molecular or ionic dispersoids	<0.001 μm

Barring large solids (rocks, bagacillo, fibers, cigarette-butts, etc.), which can be removed by filtration though a fine screen or loose-packed glass wool, cane juice contains particles with diameters up to 6 μm (Bennet, 1957a). The density of these particles ranges between 2.5E8 and 1.2E9 particles/mL. The quantity of total dispersed solids amounts to approximately 0.25 g/100g. According to Bennett, cane juice can be categorized as a “dilute suspension” where 85 % of the particles have diameters of approximately 2 μm (Trinidad, variety B37172).

The particles were subdivided by Bennett into three main classes which differ by composition. The bulk 50-75 % of the total particulates, are waxy and composed mainly of a mixture of ceryl esters (large linear alcohols, Koonce and Brown, 1944) and long chain fatty acids (cane wax).

These particles appear to be present as discrete granules in the cane, as removal of the cuticle (and hence, rind) prior to milling did not significantly decrease the detected quantity (Bennet 1957b).

Type-2 particles are derived from organelles which are released when the plant cells are disrupted during milling. They include chloroplasts and polysaccharide granules (starch); these are referred to by Bennett as "plastids". It was noted by Payne the quantity of polysaccharide present (most likely referring to dextran, Madsen and Day 2005) can increase as a consequence of microbial depredation. Type-3 particles correspond with suspended silicacious particles which are related to field soil and clay. These particles usually range in size between 4-6 μm. It is noteworthy that, in Louisiana, in particular, a large amount of field soil often enters the mill. In some cases, cane may contain >22 g/100g of soil (Madsen, et al. 2003). Most of this will end up in the juice as a consequence of diffusion or milling. This is especially true since the practice of washing the cane prior to milling has largely been discontinued since it was noted that it led to microbial infestation (Day and Kampen, 2003), corrosion due to increased levels of organic acids and loss of sucrose, both inverted and/or consumed (Endres, 2003).

2.3.3. Goals of Clarification

The goal of clarification is to remove the coarse and colloidal materials without losing sugar to either microbial or chemical effects.

Microbial losses result from direct consumption of sucrose by microorganisms including bacteria (e.g. *Leuconostoc sp.,* Eggleston, 2006) and fungi (e.g. *Saccharomyces sp.,* Saska, 2002) which will invert the sucrose and then consume the resulting reducing sugars, producing acids (primarily lactic and acetic), polymers (dextran, levan, etc.) and ethanol.

The acidic metabolites lead to chemical inversion, which is more pronounced at higher temperatures and increases with time. Inversion is discussed in detail in **Appendix B**. Additionally, the acidic products can lead to severe (and costly) corrosion of mild steel.

This is especially so for volatile products, such as ethanoic or butanoic acids, which end up in evaporator condensate and can cause corrosion of the return lines.

Ideally, there should be an increase in juice purity resulting from the removal of non-sugar impurities with very little increase in color, if any. The following criteria for clarification were condensed from the five points given by Payne (1953). The brief notes which follow were added by this author.

1. The clarification should be as complete as possible, clear juice should result--the overall measures of success are the reduction of color and turbidity whilst increasing purity.

2. The mud should settle as quickly as possible—the more quickly the juice is processed, the less time there is for the sugar it contains to be destroyed.

3. The mud should settle into the densest possible pack to minimize volume—a greater quantity of mud tends to indicate a greater amount of removed impurity, but exceeding the filtration capacity can shut down a factory.

2.3.4. Clarification Agents

The use of lime as a clarification agent and pH adjustment chemical predominates in industrial practice, because it is cheap (~$15 US/short ton, Watson and Little, 2002) and the technology is well known and reliable. It was noted by Bennett (1957) that the efficacy of hydrolyzable metal compounds increases with increasing oxidation number. For example, Th^{4+} and Al^{3+} are more effective than Ca^{2+}, which requires approximately 200 mM (1.56 g/dL as $Ca(OH)_2$, 1.20 as CaO) before eliciting an isoelectronic (charge balance on the particles is zero) condition causing the dispersoids to coagulate. This is why, in order to make sure that a sufficient quantity of Ca^{2+} is present, $Ca(OH)_2$ is often applied at 5.00 g/dL (3.2 times the minimum quantity). By the same token, the amount of K^+ required to elicit a similar effect exceeds that which will saturate water at STP (standard temperature and pressure). Regardless, lime is the cheapest, and it is minimally toxic (and is not radioactive), so it remains the coagulant of choice for food related operations. Other clarification agents have been tested, including some 622 materials listed by Spencer and Meade in 1948. Because this reference is difficult to find, and the tables were not carried into the subsequent editions, they are included as **Appendix C**.

Adjunct processes (Rein, 2007) such as sulfitation (treatment/bleaching with SO_2), phosphatation (H_3PO_4, coupled with flotation of scum, frequently used with syrup) and carbonatation (neutralization of the lime with carbonic acid ($H_2O + CO_2 \leftrightarrow H_2CO_3$)) are not practiced in the United States for the manufacture of raw sugar from cane (carbonatation is standard when processing beets). Methods such as these are used widely elsewhere, in places such as India and Brazil, but because they are not currently in use in the U.S. they will not be discussed in detail here.

2.3.4.1. Lime

The first reaction involved with lime clarification is given below.

$$3\ Ca(OH)_2 + 2\ H_3PO_4 \longrightarrow Ca_3(PO_4)_2 + 6\ H_2O$$

This is an oversimplification. The mechanism, at present is a nearly incomprehensible admixture of both chemical and physical phenomena. Chemically, the states of "calcium phosphate" which can simultaneously exist are complex, and led to a series of reactions, some of which were summarized by Greenwood, et al. (2007) in the following series of equations. He also noted that the hydrolyzed tetra calcium phosphate, $Ca_4(OH)_2(PO_4)$ may also form.

$$Ca^{2+} + 2H_2PO_4^- \longrightarrow Ca(H_2PO_4)_2$$

$$Ca^{2+} + HPO_4^{2-} \longrightarrow CaHPO_4$$

$$2CaHPO_4 + 2Ca_3(PO_4)_2 \longrightarrow Ca_8H_2(PO_4)_6$$

$$Ca_3(PO_4)_2 + 2Ca^{2+} + HPO_4^{2-} + H_2O \longrightarrow Ca_5(PO_4)_3OH + 2H^+$$

Physically, there are non-chemical effects which can occur, for example, the following empirical set of equations (Holt, 1925):

$$Ca^{2+} + HPO_4^{2-} \longrightarrow CaHPO_4$$

$$3Ca^{2+} + 2PO_4^{3-} \longrightarrow Ca_3(PO_4)_2$$

The first equation yielding monobasic calcium phosphate is fast, and proceeds almost to completion before the second reaction takes place at significant rate.

The monobasic product, however, is a sticky gel and adheres to the reacting particle hindering access and physically limiting the rate of reaction that yields the final product.

For the aforementioned reasons, the reaction between $Ca(OH)_2$ and H_3PO_4 is quite slow, taking approximately 10 hr to reach equilibrium (Holt, et al., 1925). It follows then, because the retention time of clarified juice rarely exceeds 1 hr, that equilibrium will never be practically be reached during the course of sugar manufacture.

The "calcium phosphate" so formed is a colloidal suspension of miniscule particles ranging from 1-1000 nm in size (IUPAC, 2001). These particles, when charged, will electrostatically repel one-another unless the electrical double layer surrounding them is minimized by neutralizing the charge. Once neutral, van der Waals forces (Whayman, 1975) will cause the particles to aggregate, yielding larger structures. This phenomenon is referred to as coagulation. The application of an electrical field gradient to neutralize charge has been used to increase coagulation (Freeland, et al, 1979; Kampen, 2000) in clarified cane juices.

Below is a description of one of three prevailing models which have arisen to explain coagulation/flocculation phenomena. This model is referred to as the "charge neutralization" model (Dentel, 1988), and is illustrated in **figure 2.2**.

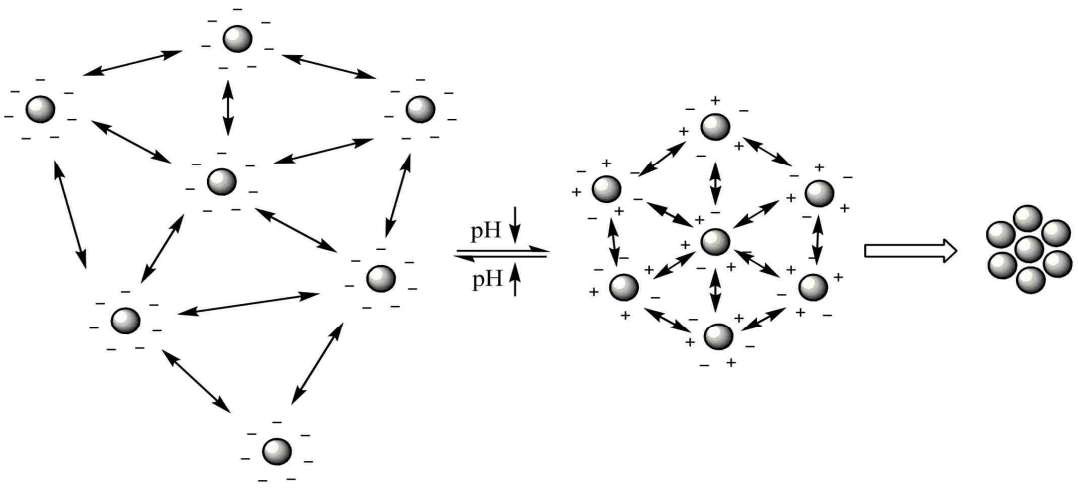


Figure 2.2. Neutralization of electrostatic repulsion via either a change in pH or an applied electrical field leads to coagulation of charged colloidal particles.

The converse is also true. If excess coagulant is added, the charge can re-establish, that is, if all of the particles represented in **figure 2.2** carry a net positive charge, they will repel one-another. This will disrupt the coagulum leading to a re-dispersion of the particles. This is why coagulant dosage is critical, and must be maintained within a narrow window which, under many circumstances, is near the isoelectronic point of the particle or macromolecule.

The macromolecules in question, in cane juice, are primarily proteins categorized within the albuminoid class (Ball, et al, 1943). Proteins are amphiphilic, exhibiting both hydrophilic and hydrophobic interactions. The extent of hydrophobicity of a protein depends upon both the net charge and the conformation of the molecule. The point of zero net charge is referred to as the isoelectronic point. When this condition is reached, the protein will transition from solution, becoming colloidal. The hydrodynamic radius of the polymer increases with temperature. This is demonstrated with BSA in **figure 2.3** (Waldmann, 2005). This effect is attributed to the disruption of the quaternary $\alpha_2\beta_2$ structure of the BSA as it denaturesThis effect is not observed with monomeric proteins, such as lysozyme, where the r_h/T is parabolic rather than sigmoidal.

The other coagulation models are “bridging” (Schmitt, et al, 1998) and “charge-patch” (Wu, 2007) models. In the bridging model, the insoluble, swollen, protein molecules act as an “adhesive” which string the coagula together. For an excellent treatise on the bridging mechanism of flocculation, see Fellow and Doherty (2006). The charge-patch model functions on the assumption that the charge may not be equally distributed upon the surface of a particle. This model provides that charged domains or “patches” exist which will serve as points where charge neutralization or bridging might occur.

Although the isoelectronic point of a protein can be readily measured, and is usually between pH 3.0-5.5 (Chaiyasut and Tsuda, 2001), the presence of sugar tends to stabilize proteins in solution. In the presence of sucrose, the coagulation of protein becomes less dependent upon isoelectronic point.

The presence of sucrose increases the temperature required to denature a given protein (Christ, et al, 2006) and this effect is expected to extend to similar effects caused by addition of acid or base. The observed stabilization increases with the sucrose concentration until the effect of low water activity becomes pronounced.

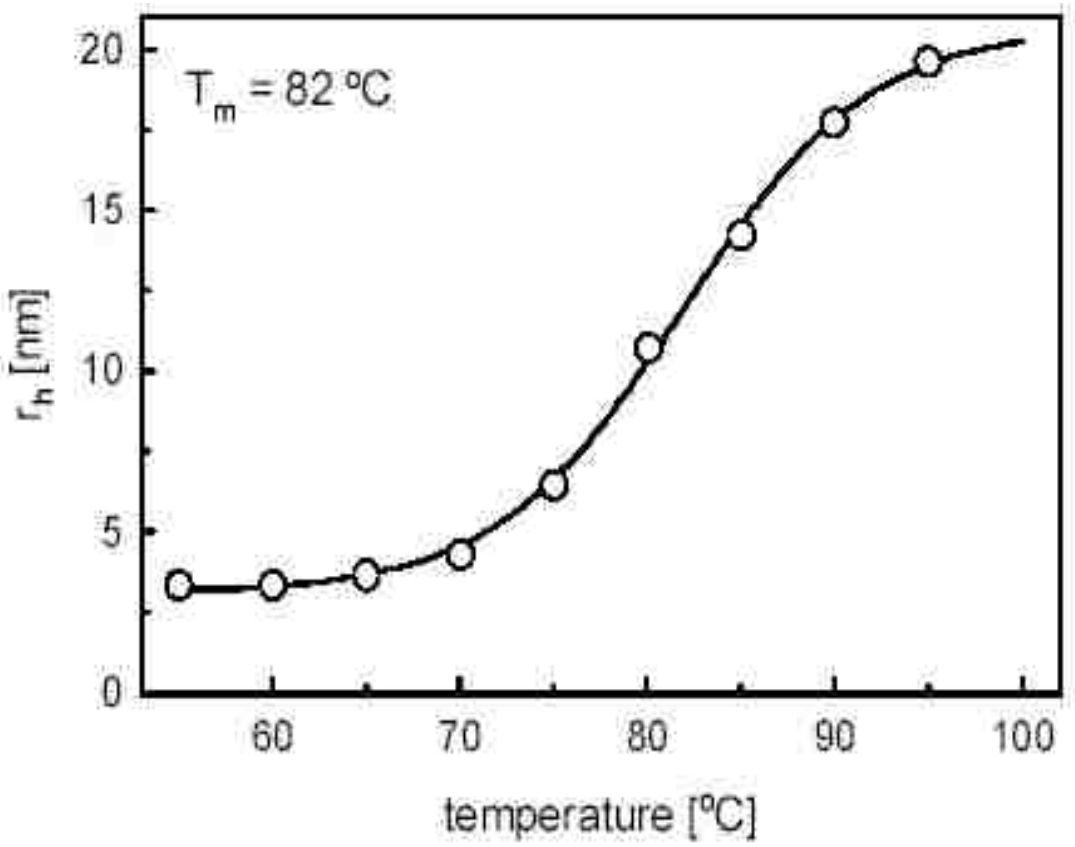


Figure 2.3. Increase in hydrodynamic radius of isoelectronic BSA with increasing temperature (Waldmann, 2005). Note that the minimum size is ~3.5 nm = 0.0035 μm.

Cane juice contains approximately 200 mg/mL of phosphate (Mahadevaiah et al, 2007). Because the tendency of sugarcane proteins to coagulate (and the juice to clarify) is predicated on the presence of phosphate (Paine et al, 1928), phosphoric acid may be added to sugarcane juice improve clarification.

Lime also serves to precipitate compounds which are not especially soluble when alkaline. This can include free-base proteins. These particles can be attracted to and aggregated with the coagulum. A slightly alkaline pH (7.1-7.2) helps protect the sucrose from damage (inversion) as it is rapidly heated to over 100 °C in the next step.

After lime addition, the juice is rapidly heated to greater than 100°C (104-110 °C is common). The superheated juice is flashed to atmospheric pressure (760 Torr, 1.01E5 Pa)The juice, now somewhat more concentrated (by flashing) has undergone some profound changes.

Superheating the juice denatures protein in the juice. At 0.5 g/100g (Chen and Meade, 1977), this is a significant amount of material. In fact, in the Deming process (anon, 1899) heat alone, via injection of steam directly into juice is frequently successful, and once found wide use. Once denatured, the protein is no longer soluble and precipitates from solution. These large particles will attract the coagulum to create macro-structures known as “flocs”, presumably via the bridging mechanism. To speed the settling rate of these flocs, and additional charged polymer is added.

The polyelectrolyte, usually an anionic polyacrylamide (see Magnafloc LT-340) is added after the flash. A contact time of a few seconds is all that is needed for the polyelectrolyte to form macro-flocs which settle rapidly after the juice is admitted into a settling clarifier. It is likely that the protein in the milk or blood added in the Colonies process (McGinnis, 1982) served a similar purpose. After some period of time, the clarified juice separates from the compact floc, which is commonly referred to as “mud”.

The application of lime as a clarifying agent has been tried using a variety of methods (Honig, 1953, specifically Payne, J.H.). These include:

1. Cold Liming
2. Hot Liming
3. Fractional Liming
4. Fractional Liming and Double Heating
5. Compound Clarification
6. Saccharate Liming

Currently, only cold, hot or saccharate liming processes are used in industrial practice; they will be described in greater detail.

“Milk of lime” is a slurry of $Ca(OH)_2$ in saturated aqueous solution (0.12 g/100g of water at 25 °C).

Well mixed (via continuous circulation) milk of lime contains 2.5-7.5 g/100g (5 g/100g, by convention) of added "CaO" (5 g CaO = 6.59 g/100g $Ca(OH)_2$ at 0.089 mol/dL). For information regarding the origins of lime, see **Appendix D**.

The solubility of $Ca(OH)_2$ decreases when temperature is increased. The plot given by Myerson (1990) for the solubility of $Ca(OH)_2$ in aqueous solution at increasing temperature indicates that the $\Delta c/T$ for 0 to 100 °C is ~ -0.000137 g/L. These numbers do not agree with those of Watkins (1983) who published a table, which when plotted appears as seen in **figure 2.4** and gives a $\Delta c/T$ for 0 to 100 °C of -1.14 g/L with 1.65 g dissolving in 1L at 20 °C. This value agrees exactly with the figures given by Cheney Lime Co. (1.65 g/L at 20°C, Cheney Lime). From this, in water, at a given temperature, the number of grams of $Ca(OH)_2$ which will dissolve in water is given by the following linear equation:

Eqn. 2.1

$$g = -0.011T + 1.862$$

Where:

g= $Ca(OH)_2$, grams
T = °C

The presence of sucrose, via complexation of Ca^{2+} with the carbohydrate diol structures, significantly increases the solubility of $Ca(OH)_2$, presumably through the formation of "tricalcium sucrate" (or saccharate). The complexes are likely linear short chain polymer-like aggregates of at least two sucrose units and $Ca(OH)_2$ (Pannetier et al, 2001). Saccharate of lime" is jargon used by the sugar industry and does *not* describe the "calcium saccharate" of trade which is the Ca^{2+} salt of D-glucaric acid. Sucrose can be recovered from the tricalcium complex by treatment with CO_2 (carbonitation, Pigman, 1957). The rule-of-thumb given by Honig (1953) is that a 10 g/100g solution of sucrose will dissolve 1.5 g/100g of $Ca(OH)_2$. This amounts to a 12.5 fold increase in solubility when all else is equal. The behavior of $Ca(OH)_2$ solubility in solutions containing sucrose is given in **figure 2.5**. The solubility increases until the sucrose concentration exceeds ~34 g/100g, after which, the solubility drops parabolically (Musa, 2005).

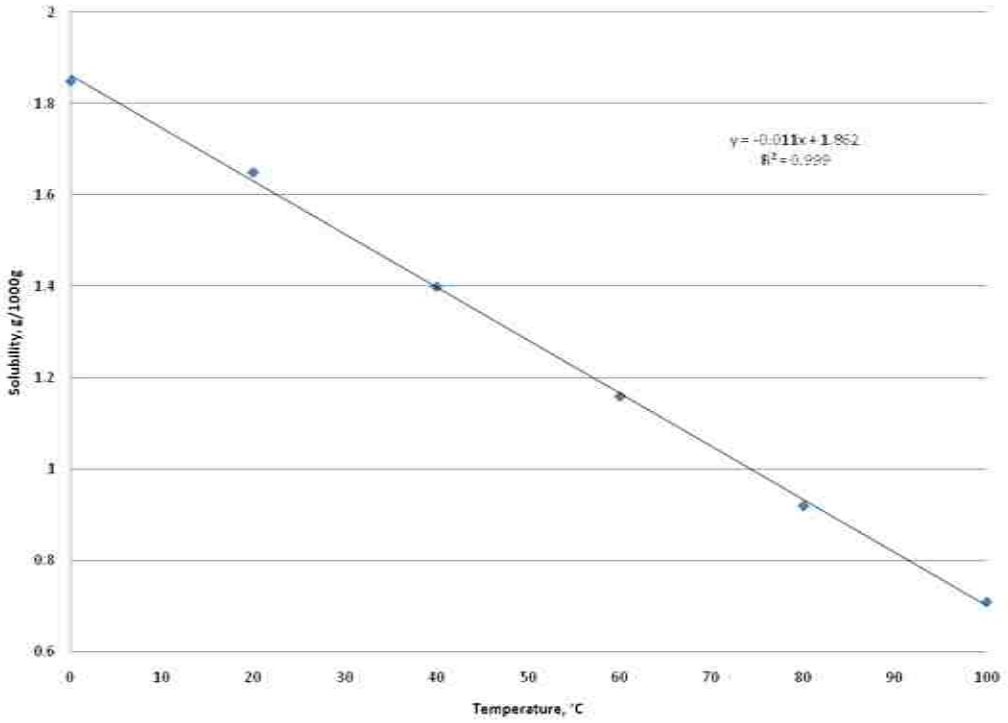


Figure 2.4. Solubility of $Ca(OH)_2$ in water at increasing temperature (Watkins, 1983).

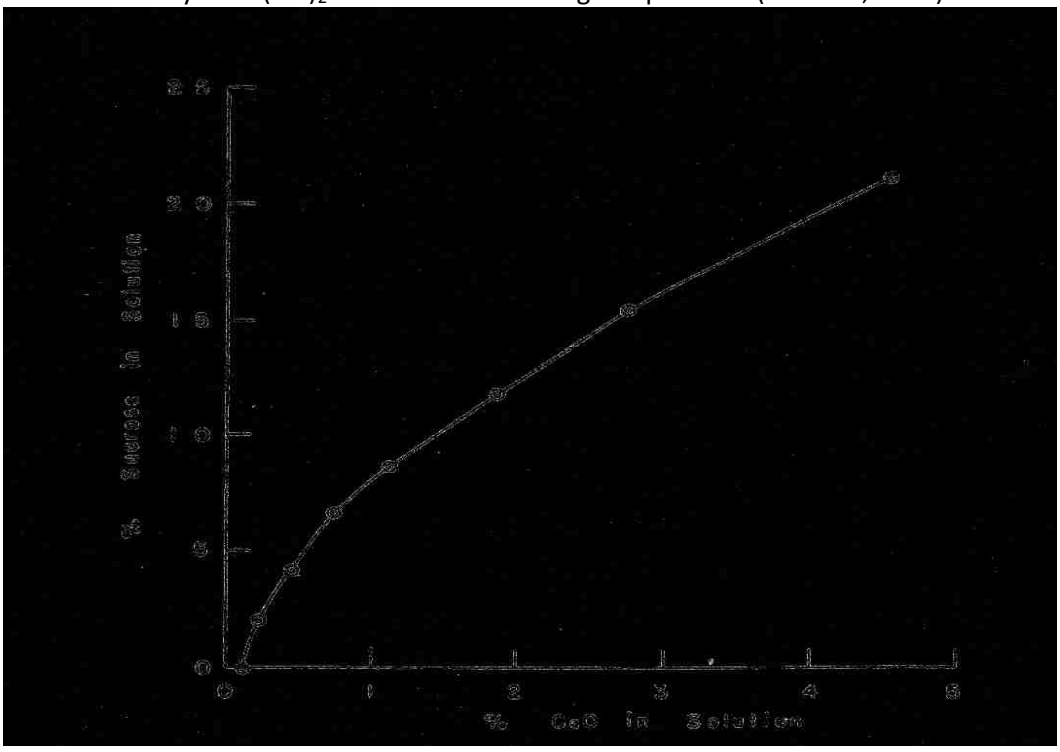


Figure 2.5. Solubility of $Ca(OH)_2$ in solutions containing Sucrose (Honig, 1953 citing Seidell, 1940).

In the simplest of terms, a cold lime procedure involves adjusting juice pH to 7.2-8.6 (Payne, 1953) using milk of lime, incubating for ~20 min and then rapidly heating to 100-102 °C prior to settling.

Hot liming involves first heating the juice to flash then adding milk of lime to pH 7.6-8.0. This seemingly trivial order of operations can have profound consequences, both good and bad, and neither method is without disadvantages. The superiority of either method is the subject of considerable debate. On one-hand, cold and intermediate liming procedures are known to provide better clarification while yielding a product of lower color. On the other, the hot-lime regime requires less lime. This has the two-fold benefit encompassing both lower chemical costs and less build-up of scale on heat transfer surfaces (which improves the overall factory energy balance).

It was recently noted by Zossi, et al (2009) that ~50% of the color of raw juice can be removed by hot-liming at bench-scale. She noted that equivalent results are usually not seen at industrial scale. It appears that bench-scale tests are not necessarily reliable indicators of how well a clarification or decolorization process will work under "real-world" conditions. In order to evaluate the nature of cold and hot liming regimes, Eggleston (2000a) conducted a series of full-scale mill tests. Because this body of work is most definitive for clarification practices in Louisiana, these works shall be analyzed in greater detail. This author believes that it characterizes the current state-of-the-art.

Eggleston (2000a) ran four replicates, which spanned the crushing season, of juices (mixed (MJ), clarified (CJ), etc.) produced at two mills. One of these mills practiced cold-liming and the other hot-liming. Observations from this work suggest that the juice at the cold liming mill had a higher initial purity. The difference in sucrose measured via polarimetry (pol) was, on average, marginal. The hot-liming mill simply was removing more non-sugar than the cold-lime mill, but there was more to remove. It is important to note that while the purity difference (CJ purity – MJ purity) observed in the hot-liming mill was greater than that seen for the cold-lime process, it closely parallels the starting purity difference (cold MJ – hot MJ).

The control of the brix appears spurious in a hot-lime process. The hot lime brix varied by a factor of approximately 6.7 while the mixed juice brix differed by only 1.0.

Thus, the difference seen in the clarified juice as a consequence of process was most likely significant. It was also noted that the brix was measured using refractive index at the cold-liming mill and by spindle at the hot liming mill. The use of the spindle (hydrometer) may have been a source of the bias observed in the hot-lime data. Because the brix is used to calculate the purity, this inconsistency can be expected to propagate though the rest of the data. Either way, the differences in purity, judged vs. a differential in polarimetric sucrose (and uncertain brix) was not definitive, and appears to be, when normalized with respect to starting purity, inconclusive. Neither method appears to be clearly superior.

The differences observed between the CJ and MJ pol (if brix were equal) indicate that the cold lime-process demonstrated a 0.38 g/100g increase in pol while the hot-limed CJ increased by 0.28; these were not significantly different

The pH was measured for both processes. In particular, the cold sample was limed to pH 7.2 at room temperature (rt) whilst the hot sample was limed to pH 6.8 at operating temperature. Because the pH decreases as temperature increases due to effects intrinsic to the pH probe, it can be expected that the limed pH at rt. would be even higher than the cold limed sample. Using the Vukov-Schaffler approximation (Rein 2007, Madsen 2007; see **Appendix B**) the calculated pH at operating temperature (assumed to be 100°C) for the cold lime sample was ~6.15 and that the pH at rt. of the hot limed sample was ~7.3. This would indicate that more $Ca(OH)_2$ was added in the hot limed process than was used during the cold-lime tests.

When these effects were noted for the final evaporator syrup (FES) it appears that the hot limed juice was over-limed (7.31-7.14), but, the lower brix (13.56 cold, 13.03 hot) made it invert more rapidly. Manipulation of this data with the Vukov-Schaffler approximation indicates that for either method, the sucrose inverted over time was almost exactly equal, that is, ~0.25 g/100g of sucrose was inverted either way. It was noted, however that the majority of sucrose losses during cold-lime clarification occurred in the incubation tank (at ambient temperature) and likely resulted from microbial action.

With regard to color, in all cases, the cold-liming procedure produced CJ and FES that exhibited ~20 % less (relative to the incoming MJ color) color than that made by hot-liming. The increase in color from MJ to FES was significantly higher in syrup produced by hot-liming. It is of interest that the mill using the cold-liming process was using a Stockhausen (Evonik Industries) flocculant (presumably Praestol 2640 SL) at 3 ppm on CJ and that the hot-liming mill was using TalosepA3 (Tate and Lyle, Kampen, 2000) at 0.013lb/ton cane. Assuming 86 % juice on cane, this correlates to 3 mg/kg of the Stockhausen flocculant used in the cold lime process vs. 5-8 (7.55) mg/kg of the TalosepA3 that was used in the hot lime process. The effect this difference might have on the color of the resulting clarified juices is unknown, but 5 mg/kg is the regulatory limit for the use of anionic polyacrylamide (21CFR173.5) in cane processing. Polyelectrolyte flocculants will be discussed in greater detail in a later section.

Eggleston (2000b) investigated the properties of cold and hot liming by assessing residual Ca^{2+}, conductivity ash, turbidity and mud settling characteristics. Because of the insolubility of lime at higher temperatures, it would be expected that less residual Ca^{2+} would be present in juice that was limed hot (Farnell, 1924) which explains the lower residual Ca^{2+} detected after hot liming . It appears that the overall Ca^{2+} in the CJ product is not significantly higher than that found in the MJ feed, regardless of whether it was limed hot or cold.

The difference appears to be in the intermediate stage, which is referred to as “hot limed juice” or HLJ. Here, there are significant differences. The Ca^{2+} levels were higher in the cold limed material. It was assumed that the Ca^{2+} which disappeared between this intermediate point and the CJ product precipitated during the process, likely onto heat transfer surfaces in either the juice heaters, evaporators, or both.

“Ash” (non-volatile inorganic material) is frequently defined using a truncated method which correlates ash content with conductivity. This is called “conductivity ash” and an empirical calculation is used to convert the conductivity to the representative value describing ash content.

Regardless of efficacy or accuracy of definition, this measure is frequently used in the sugar industry and there is an ICUMSA certified method for its use; this method was used in the work described below.

It appears that the hot-liming process removes ~1 % more turbidity than the cold-lime process. There appears to be no advantage to using either process.

The turbidity appears to decrease as conductivity increases in mixed juice whilst there appears to be no significant correlation with clarified juice .This type of behavior would beexpected if the mineral material was dissolving rather than precipitating.

In summary, it appears that the superiority of one liming procedure over the other depends upon the desired outcome and can be summarized thus:

1. Hot liming is superior if the desire is to make the largest quantity of a raw-grade product. The smaller quantity of lime needed will minimize operational costs including lime, evaporator and juice heater cleaning chemicals (EDTA, sulfamic acid, HCl, NaOH, etc.) and down-time. The CJ is of equal purity, but suffers in terms of control of brix. The product has greater color and turbidity than that observed with a cold-liming process.

2. Cold liming is superior if the desire is to make a higher grade sugar at the expense of yield, extra lime, chemicals for cleaning and the down-time attendant thereto. This appears to be feasible if there is a premium offered for a finer grade of sugar.

2.3.4.2. Hydrolyzing Metal Salts

In addition to lime, hydrolyzing metal salts are frequently used coagulants. This type of coagulant is not used in the sugar industry. They are most commonly used for waste water treatment, and are frequently salts of either aluminum or iron.

The format of a hydrolyzing metal salt involves the sequential formation of the hydroxyl complex of the metal. This involves ionizing water to add a hydroxyl which results in a proton entering the bulk solution. The rate for each step is variable and depends upon both the metal and salt-form (of both the coagulant and the colloidal particles; Matijevic, et al, 1964). The set of reactions (Gregory and Duan, 2001) for a trivalent metal is given here.

$$M^{3+} + H_2O \rightleftharpoons M(OH)^{2+} + H^+$$

$$M(OH)^{2+} + H_2O \rightleftharpoons M(OH)_2^{+} + H^+$$

$$M(OH)_2^{+} + H_2O \rightleftharpoons M(OH)_3 + H^+$$

$$M(OH)_3 + H_2O \rightleftharpoons M(OH)_4^{-} + H^+$$

$$M^{3+} + 4H_2O \rightleftharpoons M(OH)_4^{-} + 4H^+$$

The rate at which each species formed, relative to pH for Al^{3+} is different from that observed for Fe^{3+}. The differences in coagulating properties are related to this difference, and will be discussed in the following two sections.

2.3.4.2.1. Aluminum Salts

Although not applied, aluminum salts, both neat and partially (aluminum chlorohydrate) or completely pre-hydrolyzed (poly-aluminum chloride, PAC) have been studied extensively for use in the sugar industry. It was demonstrated by Oliveira (2006) that a direct white sugar could be made using PAC (400-800 μg/mL) in conjunction with sulfitation. At roughly the same time, Godshall, et al. (2006) tested the efficacy of the pre-hydrolyzed aluminum salt. They noted that a blend of aluminum polymers (PAC?) and between 25-50 % poly-quaternaryamine (cationic polyacrylamide) flocculant achieved the best results. Specifically, it was stated that "better color reduction was obtained with the PAC containing polyamine, and…the color removal was proportional to the amount of polyamine."

Godshall noted that the flocs settled more slowly, relative to lime, when PAC was used. A lime floc settled to 36 % of the original volume in ~5 min while, over the same time, the mud pack was only 50-60% when PAC was used (either with lime or soda ash). The removal of color ranged from 51-61 % at bench scale, but was unpredictable when scaled removed ~16 %.

It is known that beet juice, treated with PAC no longer foams when agitated.
The stabilization of foam in beet processing is attributed to the juice containing a high concentration of protein (1.56 %) which tended to emulsify the pectin (foaming, Funami, et al, 2007).

This indicates that, on removal of 50-57 % of the polysaccharides, the protein which acts as a mediator between the emulsified domains and the pectin (which facilitates the stability of the emulsion in aqueous media) was also likely removed.

The aqueous solubility of aluminum hydroxide is very small. Since this is the predominant species (**figure 2.6**) at alkaline pH, the residual aluminum was noted, by Godshall, to be lower in the treated products than in the raw juice (by a factor of ~ 16 (2 vs. 32 ppm).

This is positive, as aluminum has been implicated (but not proven unequivocally) with the etiology of several disease states including bone fragility (Mjoberg, et al, 1997), possibly caused by renal damage (Deitl, et al, 1997) and may modulate the formation of neurofibrillary tangles (via binding to Tau protein) which are related to Alzheimer's dementia (Shin, et al, 1994). This was debated by Mizoroki, et al. (2007) who argued that aluminum induces the formation of Tau aggregates in-vitro, but not in-vivo.

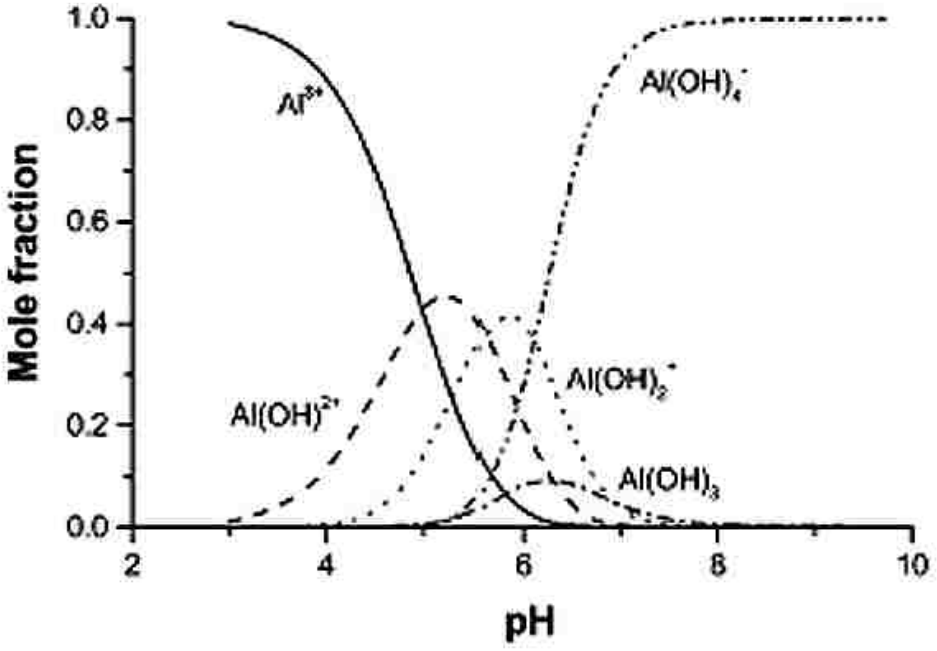


Figure 2.6. Mole fractions of the various hydrolyzed species derived from Al^{3+} at pH 2-10.

From **figure 2.6**, it was noted that the predominant species present at juice pH (~5.8) were $Al(OH)^{2+}$ and $Al(OH)_2^+$ at ~35% each, Al^{3+} and $Al(OH)_4^-$ at ~0.1% each and $Al(OH)_3$ at 0.05%. At liming pH (~7.2) 99% of the Al was $Al(OH)_4^-$. In neither case was Al greatly solvated. This pointed us toward a charge neutralization mechanism centered about Al gel coagula rather than covalent effects.

2.3.4.2.2. Iron Salts

Used quite extensively for the treatment, particularly as coagulants, of industrial and municipal waste water (Metcalf and Eddy, 1979; Kemmer, 1988), ferric chloride and sulfate are common and inexpensive chemicals recoverable as waste from other industrial uses (Patterson, 1985), such as iron pickle liquor. Iron is present in sugar cane, and some 30-40% (Seip, 1947) of it is extracted into the juice during milling. Additional iron enters the juice as soluble salts (Subbarao, 1935) via the action of acidity upon mild steel piping and equipment; the total amount of iron in juice is normally ~10-20 μg Fe/L (Seip, 1947; van der Poel, 1998; Riffer, 1986; Prasad, 1989).

This iron is implicated in the formation of color in cane juice, presumably by formation of both colored complexes and oxidation products (quinones and polymer) with intrinsic phenolic compounds. Zerban (1921) noted that iron applied at 20 μg/mL of juice demonstrated no coagulating effect, but did lead to a marked increase in color. At higher concentrations, this is the mechanism was exploited by Madsen (Madsen, 1984) to cause coagulation of phenolic colorants. The hydrolysis of Fe^{3+} at varying pH's is given in **figure 2.7**.

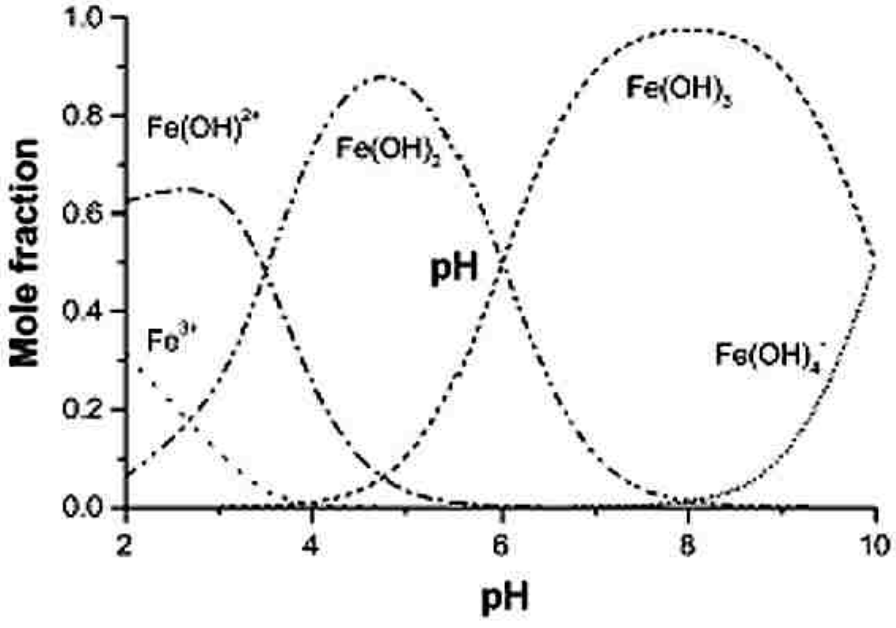


Figure 2.7. Mole fractions of the various hydrolyzed species derived from Fe^{3+} at pH 2-10.

When applied to juice (pH ~5.8), the primary species would be a 60:40 % mix of $Fe(OH)_2$ and $Fe(OH)_3$. The $FeCl_3$ should rapidly hydrolyze and drop the pH to the 3-4 range where we should observe a 55:40:10 % mixture of $Fe(OH)^{2+}$, $Fe(OH)_2$ and Fe^{3+}. If this mixture was limed to pH 7.2, the ~94% of the Fe would be $Fe(OH)_3$. From this, a $FeCl_3$ added to juice will yield soluble and redox active Fe^{3+}/Fe^{2+}. Liming will yield insoluble colloidal $Fe(OH)_3$ which should act in a manner similar to $Al(OH)_3$ coagula.

2.3.5. Flocculants/Polyelectrolytes

The earliest reference that was found where a polyelectrolyte was used in clarification of sugar juice (in this case for beets, *Beta vulgaris*) was the "Colonies Process" which was in use ca. 1811 in the West Indies. Milk or blood (McGinnis, 1982) was added to enhance clarification. Both blood and milk are colloidal suspensions of large, lyophobic macromolecules, specifically proteins.

Polyelectrolytes are applied to enhance both the speed and extent of flocculation of the calcium phosphate-derived coagula. These polymers are by definition charged and are sold commercially in both cationic and anionic forms.

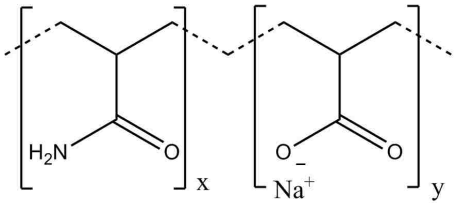
While most are derivatives of polyacrylamide (poly(2-propenamide) in varying states of hydrolysis), other polymers have been used for this purpose, including chitosan (poly-β-glucosamine) which is also water soluble in acidic solutions where it is protonated.

Below is given a representative structure (**figure 2.8**) of an anionic polyelectrolyte with the equation used to express the degree of hydrolysis, and hence, relative charge density of the polymer. The molecular weight of the commercial anionic polyacrylamides are within the 10^7 g/mol (Rein, 2007), and an inexpensive Chinese product cites assay values of 13-15.5E6 g/mol (Dongying Guangzheng, 2008). This material was also cited to contain 88-90.6 (g/100g) of active product and had a degree of hydrolysis of 20-30 %. At least three popular brands, Magnafloc LT340 (Ciba), Praestol 2640SL (Stockhausen) and TaloSep (Tate& Lyle) A3 or A5 are commonly used as process flocculants in the sugar industry. These hydrolyzed polymers are statistical copolymers of acrylamide and acrylic acid.

Preastol 2640SL is sold with a cited molecular weight of >20E6 g/mol whilst TaloSep flocculants are smaller, and usually have a molecular weight between 1 and 5E6 g/mol.

Polymers of this type can also be made by co-polymerization of acrylamide and acrylic acid (or acrylate). The degree of substitution is calculated the same way.

The distribution of functionality in the copolymerized product, unlike the hydrolyzed material, is not solely dependent upon the length and extent of hydrolysis of the parent chain. It is dependent upon the reactivity ratios (r) of the monomers (M).



$$DH = \frac{100\,y}{(x+y)} \qquad \textbf{Eqn. 2.2}$$

Where:

DH = degree of hydrolysis, %
y =number of hydrolyzed, carboxyl-substituted units
x= number of intact amide substituted units

Figure 2.8. Representative structure of an anionic polyacrylamide flocculant.

The reactivity ratios for the copolymerization of two monomers are derived by observation of how often each monomer adds itself to a growing chain. This is measured in terms of the rate constants (k) that are observed for M_1 adding either M_1 or M_2. The ratio of k_{M1+M1} divided k_{M1+M2} is known as the reactivity ratio for M_1 with respect to M_2. r_2 is calculated in the same way. Frequently, $r_1 \neq r_2$. This information can be used to classify the copolymer as ideal (perfectly random, $r_1=r_2=1$), alternating ($r_1=r_2=0$) or block ($r_1>1$, $r_2>1$). Reactivity ratios can also be used to determine the molar composition that can be expected for a given copolymerization.

The quantity of each monomer incorporated into the copolymer of acrylamide and acrylic acid as described by the coplymerization equation which is given below (Odian, 1981).

$$\frac{d[M_1]}{d[M2]} = \frac{[M_1]([r_1[M_1]+[M_2])}{[M_2]([M_1]+r_2[M_2])}$$ **Eqn. 2.3**

Where: $d[M_1]/d[M_2]$ = molar ratio of the monomers where M_1 and M_2 are the moles of monomers 1 and 2.

r_1, r_2 = reactivity ratios for monomers 1 and 2.

With polar monomers, the reactivity ratios can sometimes be controlled by variation of pH (Paril, et al., 2006). It was found that at pH 5 and 2, the predominant monomer was either acrylamide or acrylic acid, respectively.

This feature makes copolymerization of acrylamide and acrylic acid a tunable process providing control over the final properties of the resulting polymer. For example, it is possible to make a copolymer with a relatively low molecular weight and a high charge density. While ideal (no monomer is favored which yields a random copolymer) at pH 3-5, a copolymerization of acrylamide and acrylic acid carried out at pH 7.5 should give a nearly alternating (r_1r_2=0.01), substitution pattern (Paril, et al., 2006). This property makes it possible to calculate the active sites for a given mass of polymer which can be tailored not only to solubility (which is greatly enhanced at lower molecular weights) but also to provide the needed charge density with a quantity of polymer that is within the regulatory limits dictated in 21CFR173.5 (5 μg/mL).

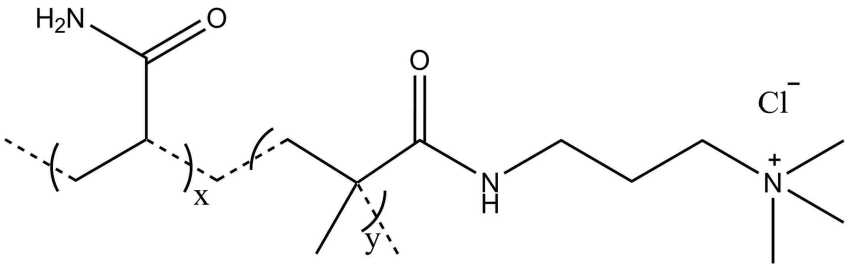
These polymers can be made to contain from 0-100 % acrylate substitution, but the common Talo products are designed to bear between 5 and 50 %, or, approximately one acrylate group per 20 monomeric units to a statistically alternate polymer (Tate & Lyle, 1975). The large amounts of K^+, Cl^- and Ca^{2+}in juice can cause the extended conformation of the polyamine to collapse which decreases the solution viscosity as the polymer is rendered insoluble. Cross-linking is specifically avoided because this limits the water solubility of the polymer.

Anionic polyacrylamide flocculants are available at modest cost and are approved for use in food products in amounts not to exceed 5 mg/kg (anionic) or 100 mg/kg (cationic).

The common process involves the introduction of <5 mg/kg (total polymer) of prepared solution (the process of solution is slow, frequently greater than 2 hr) into the juice after it has reached flashing temperature (directly before settling). The polymer is frequently added directly into the flash tank. While used in sucrose refinery operations (syrup flotation), cationic flocculants are not, at present, used in the production of raw-grade sugars.

Cationic flocculants are more complex. These are usually based on polyacrylamide, but they can also be natural or biomimetic in origin.

For example, chitosan (poly-β-glucosamine) will serve as a cationic polyelectrolyte when solvated under acidic conditions (Pinotti, et al, 2001; Izvozchikova, et al, 2002). In this case, the amino group is protonated, and is thus carries a quaternary ammonium moiety per monomeric unit. Represented in **figure 2.9**, the quaternary ammonium group ($^{+}NR_3H$) is the mainstay of cationic polyacrylamide functionality. Reproduced in one form or another, either in imine or amine form, pyridinium or pyrolium cationic form, the protonated nitrogen is the key.



poly-acrylamide-co-(3-(methylacrylamido)propyl)trimethylammonium chloride

Figure 2.9. Representative structure of a cationic flocculant polymer (MAP-TAC, Shubin and Linse, 1995)

These polymers are usually provided in dry salt form and the counter –ion has been demonstrated to have a profound effect on the amount and type of anionic contaminants that can be removed from suspension. (Liu, et al. 1999).

2.4. The Nature of "Color" in Sugar Processing

The ICUMSA (International Commission for Uniform Methods of Sugar Analysis) color unit (IU) is used for quality control at the raw sugar production level.

The higher this is, the greater the color and, hence, the lower the quality of the sugar so produced. ICUMSA color is defined as the measure of absorbance of light at 420 nm (ICUMSA, 2007) of some material when adjusted to pH 7.0±0.1. Considered arbitrary by many (Singhe, 2006), IU is the standard unit of measure in the raw-sugar factory.

Color follows Beer's law and is calculated as follows. The density of the sucrose solution measured can be referenced from a table or approximated via calculation. An equation for calculating the density of technical sucrose solutions given in **Appendix B**. Density is then used to calculate the concentration of the material:

Eqn. 2.4

$$c = \frac{RDS \cdot \rho}{10^5}$$

Where:

RDS = refractive dry solids or °brix
ρ = density, kg/m^3
c = concentration, g/mL

IUs are then calculated using Beer's Law: **Eqn. 2.5**

$$IU_{7.0} = \frac{1000 \cdot A_S}{bc}$$

Where:

A_s = absorbance at 420 nm
b = cell path length, usually 1 cm
c = concentration, g/mL
$IU_{7.0}$ = ICUMSA color measured at pH 7.0±0.1

Sugar refineries are aware of the dependence of color on pH; color increases with pH. The refineries examine the sugar that they buy at 420 nm, but at pH 8.5. This difference in methodology creates confusion between the raw manufacturer and the refiner. That is, the raw producers do not necessarily know what they are selling, color-wise. It is possible for two 2000 IU raws, to give 2400 and 4800 "IU" respectively, when they are measured at pH 8.5. The type of colorant present, as well as the quality, has a bearing upon the ultimate color of the sugar.

2.4.1. Chromophoric Groups in Sugar Processing

Either produced during processing of cane to make sugar, or entering the process with the sugar cane, the colored compounds related to cane processing are poorly characterized.

Loosely, the colored materials which evolve as a consequence of processing are segregated into three main groups. These groups are referred to as hexose alkaline degradation products (HADP), melanoidins (Maillard reaction products or MRPs) and caramels. All of these colorant classes are formed in the presence of reducing sugars.

The bulk of the carbohydrate in sugar cane juice is sucrose, which is non-reducing, and hence, unreactive. Sucrose must first hydrolyze or "invert" prior to color formation. Because sugar cane is not processed under alkaline conditions (unlike beet processing) and the pH during liming should never exceed pH 7.2, HADP will not be discussed in detail. The processes involved with the synthesis of caramel and melanoidin colorant-classes will be briefly discussed in following sections.

Intrinsic to the sugar cane, chlorophylls, xanthophylls, polyphenolics and flavonoids enter the mill with the cane. These compounds are known to form transition metal complexes, particularly with iron, which are highly colored (Zerban, 1921). Seen in table 2.6, the phenolics are not sufficiently ionized to be removed via clarification with lime. Compounds representing the other colorant classes are sufficiently ionized, but are not present in raw cane juice. The following table (Chou, 2000) gives the percentage ionization for the major colorant classes.

Table 2.6. Ionization of the main colorant classes at clarification pH (6.8-7.2), Chou, 2000.

Colorant Class:	Ionized, %:
Caramels	99.7
Melanoidins	66.7
Hexose Alkaline Degradation Products (HADP)	99.2
Polyphenolics (Flavonoids, cinnamyl derivatives)	0.6

From **table 2.6**, it appears that melanoidins and polyphenolics are likely to pass though the clarification process.

Because caramels and melanoidins are made in-process where temperature and brix are both high, the formation of these classes of colorant must be inhibited. The phenolics entering the process, however, represent the class of compounds that are neither removed nor neutralized.

It is clear that pH's significantly higher than that encountered during lime clarification would be required in order to ionize the phenolic compounds.

2.4.1.1. Caramels

"Caramels" are colored molecules that result when reducing carbohydrates are heated to the point of decomposition. Caramel, represents the "simplest" case and it is best described as a series of events beginning with the establishment, at pH>7.0 (Wolfrom, 1951), of an equilibrium (Clarke, 1997), between glucose, fructose, and mannose. From here, fructose is dehydrated to yield 5-hydroxymethyl-2-furaldehyde (HMF, Wolfrom, 1951; Shaw, et al., 1967; Wunderlin, et al., 1998). Antal also used D_2O to confirm that the dehydration did not involve the open chain form of the carbohydrate. The intermediate **A** (4-hydroxy-5-(hydroxymethyl)-4,5-dihydrofuran-2-carbaldehyde), in **figure 2.10,** was confirmed by Amarasekara, et al (2008) via ^{1}H and ^{13}C NMR using DMSO-d_6 as a solvent and catalyst.

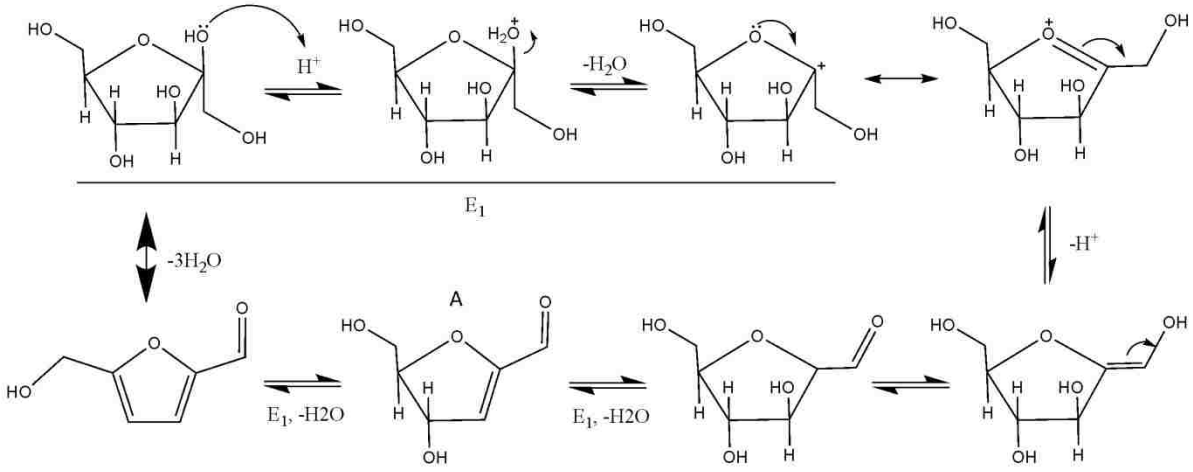


Figure 2.10. Dehydration of fructose to yield HMF and subsequent hydrolysis.

The HMF can be cleaved, *in-situ*, to yield one molecule each of levulinic and formic acid (Antal, 1990).

The mechanism for this reaction is poorly characterized, but empirical models involving acid catalyzed hydrolysis have been made (Chun, et. al. 2006). Accumulation of these organic acids lowers the pH. Because both the synthesis of HMF and its hydrolysis are catalyzed by acid, the reaction sequence is self-catalyzed and cumulative (Kuster, 1977).

With increasing acidity and decreasing water activity, viz. evaporation, a heterogeneous polymer forms which consists of up to 15-28% of difructose dianhydrides (Defaye, et al., 1995).

During this process, the polymerization of some of the HMF also occurs. HMF cannot self-condense (Pine, 1987), but, it can condense with other carbonyl compounds present in the system. It has been demonstrated that reducing sugars can yield reactive α-dicarbonyl intermediates including glyoxal, methyl glyoxal, and dihydroxyacetone (Antal, 1990) via enolization andreverse-aldol scission (Clarke, et al., 1997; Antal, 1990).Fragmentation facilitated by free radical intermediates has been observed using electron-spin resonance (ESR, Namiki, et al., 1983). Reverse aldol scission is exemplified for D-glucose via the alkali-catalyzed mechanism in **figure 2.11**. The acid catalyzed route would first involve protonation

of the carbonyl.

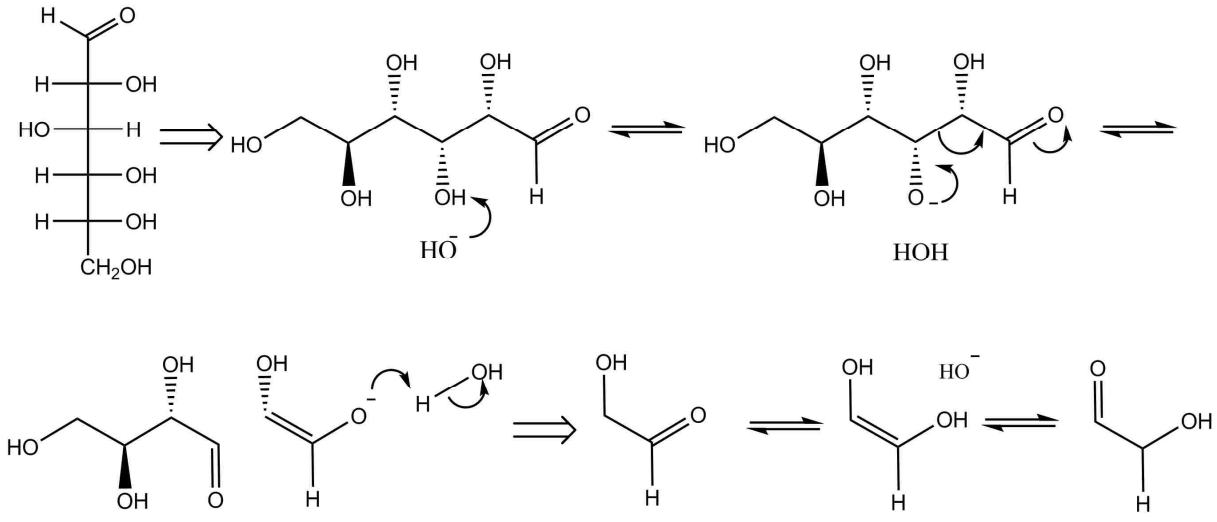


Figure 2.11. Reverse-aldol scission o f D-glucose.

These reactive carbonyl compounds can condense with the HMF yielding first fluorescent precursors, then a yellow/brown (depending on extent of reaction) material of relatively high molecular weight known as “caramel”. The scent and flavor profile of the mixture is mainly composed of pyranones or maltol analogues formed via cyclization and dehydration of fructose which is similar to that for HMF, but begins with the pyranose-form; Antal et al. (1990) present an excellent review of the mechanisms involving the formation of HMF from fructose.

Although not documented as such, the chemistry indicates that HMF will likely oxidize when heated in the presence of air to yield 5-hydroxymethyl-2-furoic acid (HMFA), which can self-condense to yield the corresponding polyester (Lichtenthaler, 2002). A reaction scheme for this is given in **figure 2.12**.

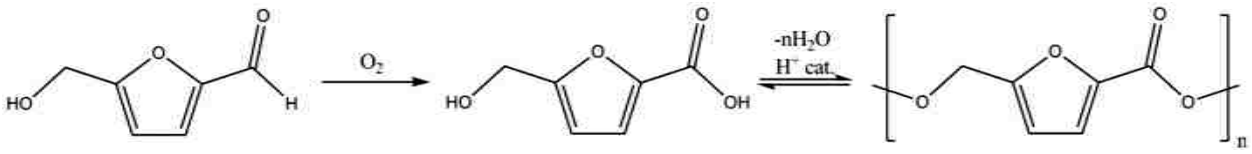


Figure 2.12. Oxidation of HMF and condensation of HMFA to poly-(5-hydroxymethyl-2-furoic acid).

2.4.1.2. Melanoidins

“Melanoidins” are colored molecules that are produced when a reducing sugar is heated in the presence of amino compounds, specifically amino acids. In addition to caramelization, if amino acids, amines, or NH_3 are present, a cascade of reactions known collectively as the “Maillard reaction” can occur with reducing sugar to yield dark brown insoluble polymer, frequently in excess of 20 kDa (Godshall, et al., 1987; Lindeman, 2001). The “reaction” is general in that reducing sugars will react with practically any amine to yield a multitude of products. The amine reacts with the open chain carbohydrate to yield an unstable intermediate which dehydrates to yield the corresponding “Schiff base” or imine (Hodge, 1953a). Under acidic conditions, the imine is protonated, which then promotes the isomerization of an aldose to a ketose (Amadori rearrangement, **Figure 2.13**) or vice versa (Heyns rearrangement), to yield in the Amadori case, an amino-deoxyketose.

From here, deamination can occur via elimination (hydrolysis) of the protonated amine to yield a “deoxyosone” or dicarbonyl compound. The amine can then go on to further catalyze this series of reactions. Noted by Hodge (1953b), the amine is eventually integrated into the colored material, but not until the later stages, and thus is called a “pseudo-catalyst”. The amine pseudo catalyst is regenerated when it is hydrolyzed from the aminoketose (**figure 2.13** center, bottom).

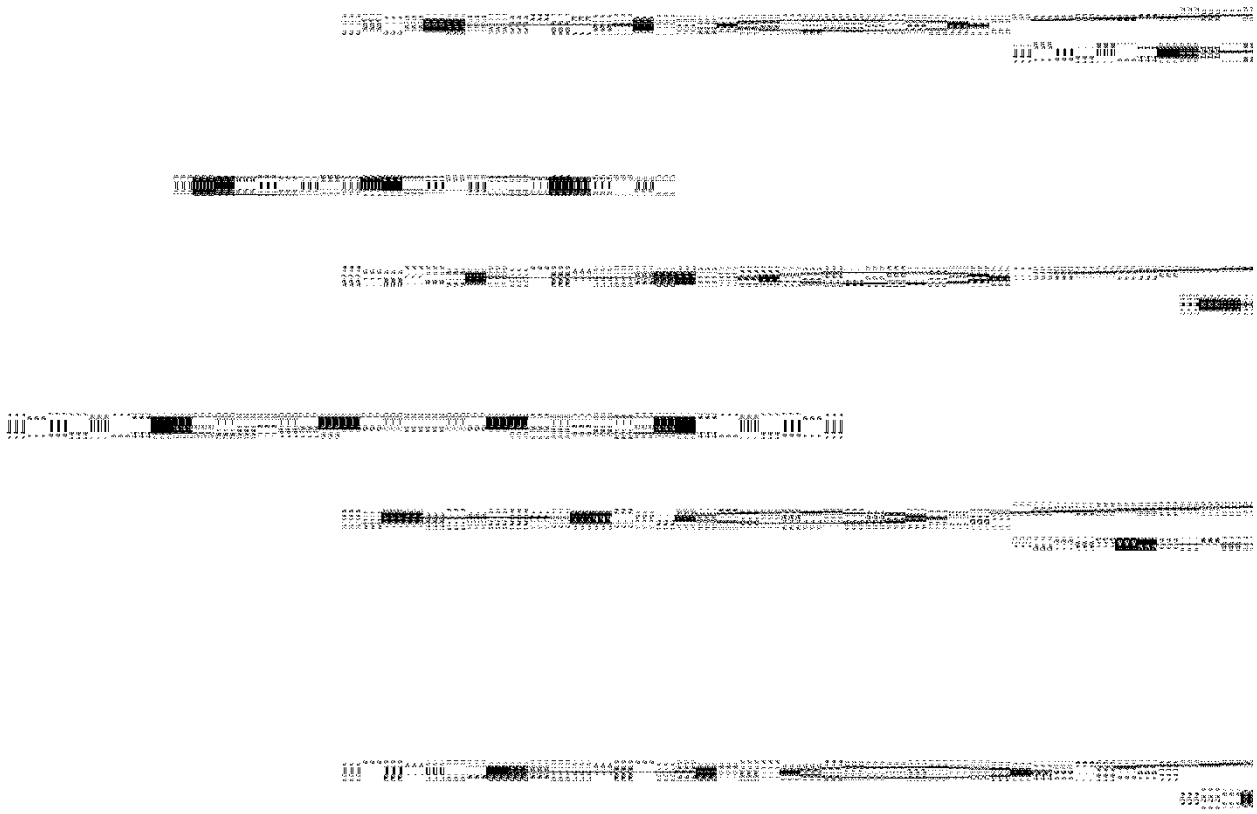


Figure 2.13. Amadori rearrangement of D-glucose to D-fructose via Schiff base, II.

These dicarbonyl compounds are the keystone of color formation via Maillard reaction. They are universally reactive and can lead to the evolution of aldehydes from amino acids via Strecker degradation. The Strecker degradation of phenylalanine assisted by D-glucose and subsequent condensation of the amino-ketone intermediate to yield a pyrazine is given in **figure 2.14**.

These aldehydes can condense with HMF, each other, deoxyosones, proteins, and many other compounds, leading to a wide distribution of products.

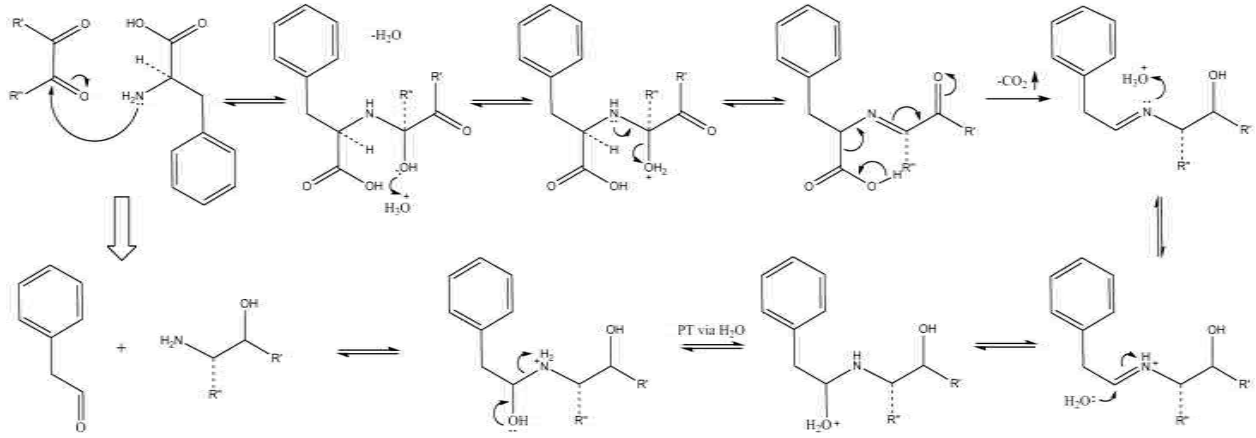


Figure 2.14. Strecker degradation of phenylalanine with D-glucose.

The Strecker degradation is the mechanism by which the amine is finally sequestered. Here, the amino acid decarboxylates and deaminates (Ho, 1994) yielding the aldehyde described above, either NH_3 or an amino ketone (Hoffmann, et al., 2000) and CO_2. These amino ketones then can condense into pyrazines (Shibamoto, 1977; Koehler, et al., 1969) and many other heterocyclic ring structures which are responsible for a large fraction of the flavor and odor profile of cooked foods (Hoffman, et al., 2000; Baltes, 1987). The ammonia can react as cited previously, or can be removed by distillation.

The formation of the dicarbonyl "deoxyosone" intermediate compound is reversible, but decarboxylation is permanent. This is key in the formation of the bonds that can lead to the formation of larger, colored structures. If some agent were introduced to interfere with this intermediate, a significant amount of color formation could likely be inhibited.

It was first noted by Hodge (1953b) that browning reactions were retarded in the presence of bisulfite, and that large amounts of completely inhibited the reaction.

2.4.1.3. Polyphenols

"Polyphenol" is the term used to describe hydroxyphenolic and flavonoid natural products.

More commonly, the term is used to describe a family of compounds with antioxidant potential, within which, the hydroxyphenols and flavonoids belong. Phenolic compounds serve two main purposes in plants. Lignin is the adhesive that holds the cellulose bundles together in plants. It is a heterogeneous cross-linked polymer composed of various phenolic compounds, primarily 4-hydroxy, 4-hydroxy-3-methoxy and 4-hydroxy-3,5-dimethoxy derivatives of cinnamic acid. It is made by the plant from the amino acid phenylalanine by the enzymes phenylalanine ammonia lyase (PAL) and coumaryl methyltransferase (COMT).

Breakage of stalks in wind, cracking from freeze, herbivory or boring from cane-borer moths (or other insects) will cause sugarcane to produce polyphenoloxidase enzymes which lead to the formation of *o*-hydroxyphenyl species and their quinone forms. These polyphenolic compounds, called phytoalexins (in the context of defensive weaponry), are frequently toxic to insects (and many bacteria, including symbiotic gut flora) and can decrease the digestability of protein in the herbivore gut (Falco, et al, 2001).

A wide variety of such compounds have been isolated from cane including 4 and 3,4-dihydroxy derivatives of benzoic and cinnamic acids. Chief among these are caffeic (3,4-dihydroxycinnamic acid) and chlorogenic (5-(3,4-dihydroxycinnamoyl) quinic acid) acids. A comprehensive list of phenolic species found in cane and sugar is compiled in **table 2.7**.

Polyphenyloxidase or *o*-diphenol:O_2 oxidoreductase (PPO, Coombs and Baldry, 1978) is released when cane tissue is exposed to air. It causes the native phenolics (sp. chlorogenic acid) to polymerize and yield colored materials. It was noted by Vickers, et al. (2005) that overexpression of this enzyme in sugarcane results in both darker juice and raw sugar.

They noted that there were linear correlations of juice color (IU) to PPO (U/mL, R^2 =0.8831) of and of juice color to sugar (crystal) color (R^2 = 0.8793). Further, in all cases, 90.51±1.90 % of the color did not end up in the sugar. This means that ~9.5 % remained.

It was indicated that engineering a cane with lower PPO activity would lead to product sugar with lower color. This also suggests that removal of the phenolic materials entering with the cane will result in the production of a sugar with less color.

The activity of the PPO isolated from cane was specific for the oxidation of *o*-dihydroxyphenols (specifically chlorogenic acid, Coombs et al, 1974). Caffeic acid exhibited a level inhibition of 43% upon the oxidation of chlorogenic acid. Of the compounds tested, Purpurogallin was the poorest inhibitor (14 %) and DOPA was the most efficient, inhibiting the reaction by 87 %. Cane was found to not contain a tyrosinase-type PPO (which adds a hydroxyl adjacent to the existing phenol) which operates in tandem with the *o*-diphenoloxidoreductase-type to create larger, more highly conjugated products). Because of this, the flavonoids bearing the *o*-dihydroxy moiety become the most important when considering the potential to form pigments. In table 2.7, these compounds are marked in bold italics and are marked primarily by either the caffeoyl or luteolinoyl groups. Note the *o*-hydroxyphenyl moieties common to the three structures given in **figure 2.15**.

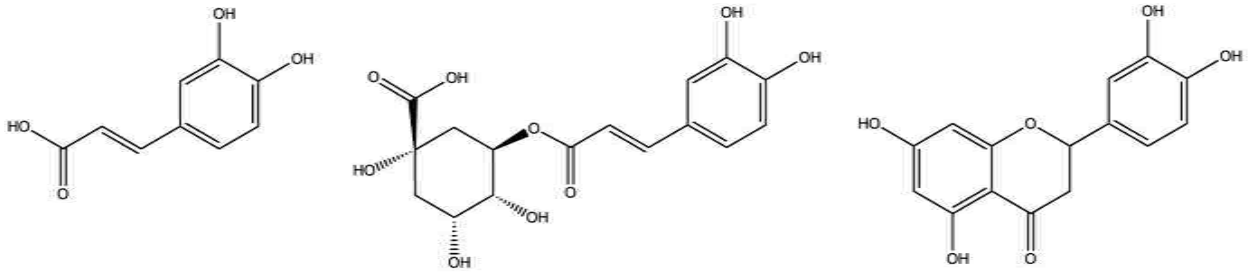


Figure 2.15. The structures, from left-to-right, of caffeic acid, chlorogenic acid and luteolin.

Most of the literature on phenolic compounds deal with the enzymatic color forming process. There is little detailed work in the area of non-enzymatic formation of colored materials from phenolic precursors. The *o*-phenolic compounds can react with amines, specifically those present in protein.

The formation of complexes of between bovine serum albumin (BSA) and protocatechuic (3,4-dihydroxybenzoic acid, 3,4-DHBA) and caffeic acids was noted by Bartolome, et al. (2000).

Table 2.7. Polyphenols isolated from cane or sugar.

Compound:	Type:	Origin:	Method:
p-hydroxybenzoic acid	Phenolic acid	Cane leaf[a]	TLC[a]
4-hydroxy-3-methoxybenzoic acid (vanillic acid)	Phenolic acid	Cane leaf[a]	TLC[a]
4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid)	Phenolic acid	Raw sugar[a]	TLC[a]
3,4-dihydroxycinnamic acid (caffeic acid)	Phenolic acid	Raw sugar[a]	TLC[a]
7-hydroxycoumarin (umbelliferone)	Chromenone	Raw sugar[a]	TLC[a]
3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (Kaempferol)	Flavanol	Raw sugar[a]	TLC[a]
4-hydroxy-3,5-dimethoxycinnamic acid (sinapic acid)	Phenolic acid	Refined sugar[a]	TLC[a]
4-hydroxy-3-methoxycinnamic acid (ferulic acid)	Phenolic acid	Refined sugar[a]	TLC[a]
5-(3,4-dihydroxycinnamoyl) quinic acid (chlorogenic acid)	Phenolic acid	Refined sugar[a,b]	TLC[a], RP-HPLC[b]
p-hydroxycinnamic acid (p-coumaric acid)	Phenolic acid	Refined sugar[a]	TLC[a]
Neocarlinoside (luteolin)	Flavonoid	Juice[b]	HPLC[b]
Vicenin 2 (apigenin)	Flavonoid	Juice[b]	HPLC[b]
Tricin 7-glucoside sulfate	Flavonoid	Leaves[b]	HPLC[b]
Iso schaftoside (apigenin)	Flavonoid	Juice[b]	HPLC[b]
Iso orientin (luteolin)	Flavonoid	Juice[b]	HPLC[b]
Schaftoside (apigenin)	Flavonoid	Juice[b]	HPLC[b]
Swertiajaponin (luteolin)	Flavonoid	Juice[b]	HPLC[b]
Iso orientin triglycoside	Flavonoid	Leaves[b]	HPLC[b]
Swertisin (apigenin)	Flavonoid	Juice[b]	HPLC[b]
Tricin 5-glucoside	Flavonoid	Leaves[b]	HPLC[b]
6-methoxy luteolin	Flavonoid	Juice[b]	HPLC[b]

a. Farber and Carpenter, 1971
b. Paton and Duong, 1992

It was noted, but not covered in any detail, that for 3,4-DHBA and caffeic acid that 24.3 and 33.5 % of each were bound to BSA, respectively.

It was determined by Cavalieri, et al. (2002) that quinones prepared from o-hydroxyphenolic species can be attacked (nucleophilic) by the tertiary amines of purine bases to yield the N-hydroxyphenyl derivatives. No hypothetical mechanism was given. Modifications such as this could modify (mutate) DNA directly and could potentially lead to the evolution of cancer and neurodegenerative disease.

Charlton, et al (2002) studied the interaction of salivary proteins with catechin in the presence of EDTA (which served to sequester cations). They found that complexes with proline-rich proteins proceeded in three phases. In the first phase, a reversible binding occurs, presumably via hydrophobic interaction. This occurs until sufficient catechin has bound to the peptide such that the probability of interaction between coated peptide increases to where interaction is likely to occur. Until this occurs, the complexes are soluble. Then, weak intermolecular bridging occurs through proximity of the polyphenols on one protein to those on a similar particle This was posited to double the size and lead to precipitation. In the third phase, these dimers aggregate into larger structures (flocs). A model considering covalent bonding was not suggested.

2.5. Decolorization

There are two primary strategies for dealing with color in sugar processing:

1. ***Remove*** *the* colorants that enter the mill with the cane.
2. ***Inhibit*** the formation of caramel/melanoidins during processing.

Inhibition, could lead to a more efficient means of color reduction in product sugars. In principle, the idea is to disrupt, out-compete, or otherwise nullify the path of the color forming reactions that can take place during processing. In order to undermine color formation, it is necessary first to know the limiting steps in the chemistry involved.

Polyphenolic colorant materials that are not removed in clarification can account for a large proportion of the color observed in sugar and are not made (appreciably) in-process.
Removing them before they can react or carry though the process is a desirable goal. The decolorization processes often used for refining raw sugar, are discussed briefly below.

2.5.1. Decolorization Processes

Although hot-liming with settling clarification is the most popular technique in raw sugar processing, the quality of the sugar so produced is limited to a VHP (very-high purity; not VLC) raw sugar.

Other technologies exist, which are adapted from current refining processes. In reality, many of these practices including the use of char (bone derived carbon), carbonatation and sulfatation are borrowed from the sugar producers of the 19th century. More recent technologies involve the use of polymeric ion exchange resins, activated carbon and ultrafiltration.

In order to produce either very-low color raws or white sugar directly, a number of technologies have been tried. These methods can be categorized into schemes which include membrane and ceramic ultrafiltration, (Kwok, 1996; Saska, 2001; Willet, 1997; Chou and Iqbal, 2006; Rossiter, et al, 2002; Bekker and Stolz, 2001) granulated activated carbon (GAC) or char (Godshall, et al, 1992; Rein, et al, 2007), chromatography (Kochergin, 2000; Stolz, 2001) and/or ion exchange resins (Rease, 1999) and a variety of additives such as ozone (Davis, et al, 1998; Moodley, et al, 1999; Godshall and McKee, 2004; Charlet, 2002; Davis, 2001), hydrogen peroxide(Mane, et al, 1992; Mane, et al, 1998, Mane, et al, 2000, Davis, et al, 2000;Duffaut, 2002; Mendoza, 2002), sulfur dioxide/sulfite (Oliviero, 2006) and N-acetylcysteine (Madsen, 2006b). All of the above listed techniques have problems associated with them.

2.5.1.1. Ultrafiltration

Ultrafiltration processes are expensive, provide only a small amount of decolorization, and lead to significant recovery costs associated with sucrose in the retentate. Retentate is very dilute, requiring increased energy expense to concentrate (Kochergin, 2000). Membranes are also known to foul when used in raw sugar operations (Clarke, 2006).

2.5.1.2. Carbon/Char

At present, there are no industrial scale decolorizers operating in raw sugar mills in the United States. The base technology is present in the form of carbon and resins used in refined sugar facilities. The color load of incoming cane juice rapidly saturates decolorizing carbon necessitating frequent and costly regeneration.

The overall cost efficiency of a decolorizer is limited most sharply by the necessity of frequent regeneration of GAC and/or resins. This rapidly effects the fiscal viability of the process as saturated carbon conventionally requires kilning (thermal desorption) for regeneration. This difficulty was surmounted by a process (Bento and Rein, 2006) whereby the saturated GAC can be chemically regenerated. Although this allows for recovery of the adsorbed materials, viz. an antioxidant blend (Saska, 2002b), there is currently no market in place to provide an offset for the financial burden associated with the frequent regeneration schedule required when operated with clarified juice as feed. Fouling by particulates can be avoided via installation of a guard column or filtration apparatus, at extra cost.

2.5.1.3. Ion Exchange

Ion-exchange resins are used extensively in sugar refining for deashing, softening and/or decolorization. It has been noted that the larger portion of the colored materials found in sugar cane are negatively charged (Deerr, 1916). As such, they can be removed via exchange using a weak anionic resin, usually in Cl^- form (Broadhurst and Rein, 2002). It has been noticed, however, that a large amount of colored material seems to be removed, along with a large amount of "ash" using a cationic resin (sulfonated styrene-divinylbenzene) (Kearney, 2003). With a strong-acid cationic resin, the low pH necessitates the use of refrigeration (which is an energy intensive process) in order to avoid inversion of sucrose. A strong cation exchange resin is used at high temperature commercially, to hydrolyze sucrose yielding a 1:1 glucose:fructose product called "invert sugar".

There is also significant evidence that a large amount of the colored material removed using either type of resin may be more an effect of hydrophobic interactions with unsubstituted parts of the resin than of ion exchange or electrostatic attraction.

Extension of this technology to raw sugar production can be problematic.

In the refinery, the product stream is consistent in terms of composition, purity, pH and temperature, and, the overall colorant load is very small when compared to clarified cane juice. Clarified juice, on the other hand, is the opposite in every respect. Not only are the composition, pH and temperature variable, but the colorant and ash load is large enough to rapidly saturate the active sites on the GAC/resin surfaces. This creates a frequency in regeneration exceeding what is normally seen in a refinery operation.

2.5.1.4. Adjuvants

In general, the use of additives to remove (bleaching) or prevent the formation of (inhibition) color in processing streams, or on sugar (Saska, 2006) can be expensive. For example, the use of H_2O_2 as a pretreatment method for decolorization involves a continuous dosage (if applied as a 70% solution) of approximately 1000μg/g on dry solids (Rein, 2007). A mill crushing 9091 MT cane/d can produce ~9400 MT clarified juice/d. 1000μg/mL of peroxide amounts to approximately 1.5 MT on solids (at 15 °brix) or ~2.2 MT of 70%, per day. The unit cost of this peroxide solution is $1.54/kg ($0.70/lb, Daly, 2007) which corresponds to a daily cost of approximately ~$3,300/d or $300,000 per (90d) season.

2.6. Autooxidation

Molecular oxygen, O_2, exists in its most stable form as a triplet "diradical species". It is therefore capable of existing in highly reactive singlet states as well (Puglia, et al. 1984). Both molecular and singlet oxygen are capable of triggering the formation of various oxidative species including peroxyl radical ($R\text{-}O\text{-}O^{\cdot}$), superoxide ($O_2^{-}$) and hydroxyl radicals ($HO^{\cdot}$). In order to combat this oxidative stress, plants and animals have developed protective mechanisms which serve to scavenge these reactive oxygen species (ROS).

2.6.1. Anti-oxidant/pro-oxidant behavior

In the most fundamental sense, any compound which can lead directly to the formation of radical species is called a pro-oxidant.

Conversely, any compound which is capable of stabilizing the radicals, thus removing them from the reactive theatre is called an anti-oxidant. Somewhat less known however, is that an anti-oxidant compound which is effectively "saturated" with stable radicals can theoretically operate as a destructible reservoir, and hence source of radical species. The two may also operate in tandem to initate catalytic REDOX cycling that can yield reactive oxygen species (ROS).

2.6.2. Oxidation of *o*-Hydroxyphenyl Derivatives

Ortho and para substituted dihdroxybenzene derivatives can be readily oxidized to yield their corresponding quinones. This has been done in a number of ways including the use of Jone's reagent (potassium dichromate in sulfuric acid; chromic acid), AgO, $AgCO_3$, $Pb(OAc)_4$, HIO_4 and air (March, 1992) (with and without catalytic metal ions). The mechanism is not well characterized, but studies involving the oxidation of catechol in $H_2{}^{18}O$ using sodium periodate observed that the product was not labeled. They suggested the following mechanism:

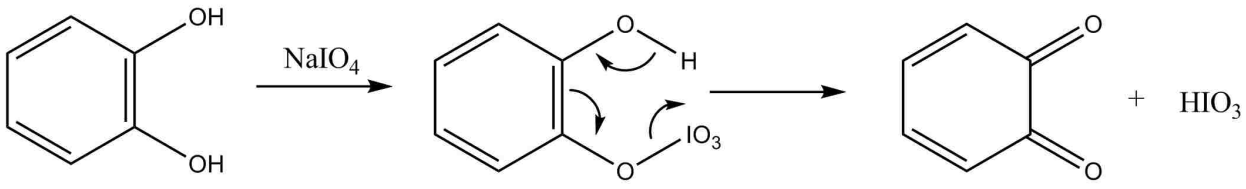


Figure 2.16. Mechanism proposed for the oxidation of catechol using $NaIO_4$.

For the scope of the work described in this dissertation, we are more interested in reactions where O_2 is the oxidant, and a transition metal is leading to a radical (semiquinone) intermediate. The reaction involved is chemically reversible, and can be described as a series of single-electron transfer reactions which can be carried out electrochemically (without catalytic species). The electrochemical REDOX of quinoid species is well documented (Huang, et al. 1998, Danilewicz, 2003). The scheme for this is given in **figure 2.17**.

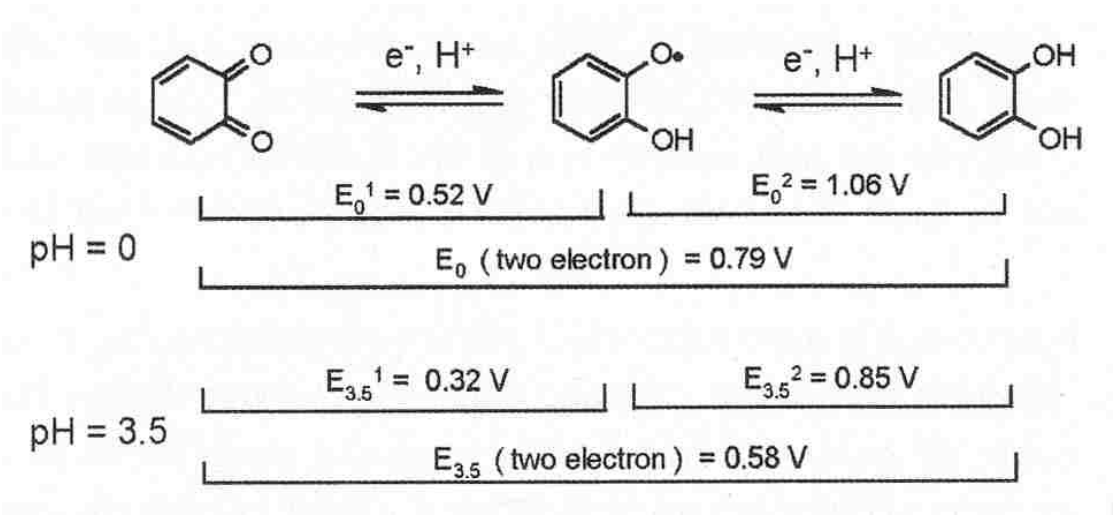


Figure 2.17. REDOX potentials of catechol at pH 0 (standard) and 3.5 (Danilewicz, 2003). Note the semiquinone intermediate.

2.6.2.1. Nucleophilic addition

The addition of an electron rich attacking species (nucleophile) to an electron poor target (electrophile) is known as a nucleophilic addition. The converse is known as electrophilic addition. Nucleophilic additions can appear, kinetically, either first or second order. The order of the reaction is determined by the transition state which is dictated by the steric environment of the target molecule, the "hardness" of the nucleophile and the choice of solvent.

Where polar, protic solvents such as water favor S_N1 reactions, polar aprotic solvents such as N,N'-dimethylformamide (DMF) or dimethylsulfoxide (DMSO) favor the S_N2 type. In highly concentrated solutions, viz >65 g/100g sucrose, the water activity drops off quite sharply (Malmberg and Maryott, 1950). The dielectric constant of the medium can become as low as 40 (from 80, for pure water) at the concentrations typically found in a vacuum pan or crystallizer (> 80 g/100g, 65 °C). The results that will be presented do not consider water activity, but would be fit to a mass percentage of sucrose in the mixture.

2.6.2.2. Michael Addition

The conjugate addition of a nucleophile to an α,β-unsaturated system is frequently referred to as a Michael addition. This usually takes place under alkaline conditions whereby the reaction is driven by the removal of a proton from the attacking species.

This reaction has been demonstrated to occur with quinones (March, 1992). The reaction occurring in acidic environments has not been well studied, however. It is possible, that under acidic oxidative conditions (e.g. with $FeCl_3$), the quinone thus formed is immediately protonated creating a electrophilic electron sink negating the need for a deprotonated enolate. Such a route is given here:

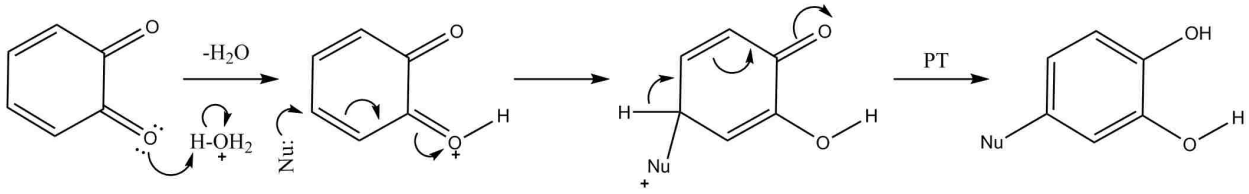


Figure 2.18. "Michael addition" of a nucleophile to an protonated α,β-unsaturated carbonyl

In a bio-based system such as sugarcane juice, the primary nucleophiles will be amino and sulfhydryl groups.

Under aerated conditions, such as those prevailing with processed cane juice, the sulfhydryl groups on non-denatured protein will likely be oxidized to yield disulfide linkages. These linkages remove the sulfhydryl group and leave the dangling N^{ε}-groups from lysine as the primary nucleophilic species. This group is separated from the rest of the molecule by three carbon units, which allows us to ignore inductive effects from either the α-amino or the carboxylic groups. Assuming that the N^{ε} groups of lysine act as primary aliphatic amines, using methylamine as a model, the pKa of the conjugate acid is ~10.6. At any pH lower than ~10.6 amino groups of this type will begin to protonate. At lower pHs, 4-6, such as those prevailing in cane juice, these groups have a very high probability of being completely protonated to yield the quaternary ammonium species. Quaternary ammonium derivatives are non-nucleophilic.

Thus, at low pH (1-3), the acidic Michael-type mechanism is limited by the concentration of the nucleophile (which is very small). We suggest that a mechanism similar to that given for the electrochemical model in **figure 2.17** or a similar system with alternative free radical initiation can be expected to prevail. The likelyhood of this is given in **table 2.8,** below.

Table 2.8. REDOX potentials of phenolic compounds at pH 0 (standard) and 3.5 (Danilewicz, 2003).

Quinone + 2e + $2H^+$ ◦ Polyphenol	E_0	$E_{3.5}$
Catechol	0.79 V[a, b]	0.58 V[c]
Caffeic acid	0.79 V[a]	0.59 V[c], 0.62 V[d]
(+)-Catechin	0.79 V[e]	0.59 V[d]
Pyrogallol	0.68 V[a]	0.47 V[c]
Ascorbic acid	0.41 V[b]	0.19 V[b]
O_2/H_2O_2	0.78 V[f]	0.57 V[f]

From the data given in **figure 2.17**, it appears that the REDOX potentials for most quinoid species decrease at lower pHs. The rate of the analogous reaction of catechol in D_2O with singlet oxygen generated photochemically with rose Bengal dianion as a photosensitizer is 5.4E7 L/mol.s (Martire, et al., 1991). The rate for caffeic acid (3,4-dihydroxycinnamic acid is 5.4E5 L/mol.s in CD_3OD (Scurlock, et al., 1990) when excited at 532nm in the presence of hematoporphyrin IX (2.5E-3 mol/L). From this, it appears that the rate of reaction of catechol exceeds that of caffeic acid by a factor of at least 10 with respect to singlet oxygen.

2.6.2.3. Specific Oxidation Reactions

Here, phenol is o-hydroxylated by H_2O_2 with Cu^{2+} acting as a catalyst. Reactions of this sort are referred to as “assisted “electrophilic aromatic substitutions (Stewart, 1964). The mechanism for this reaction is given below. The arrows were not included in the cited figure and were added by this author. In this case, the oxidizing species is the cupryl peroxo ion. This system is can undergo further oxidation to yield the quinone.

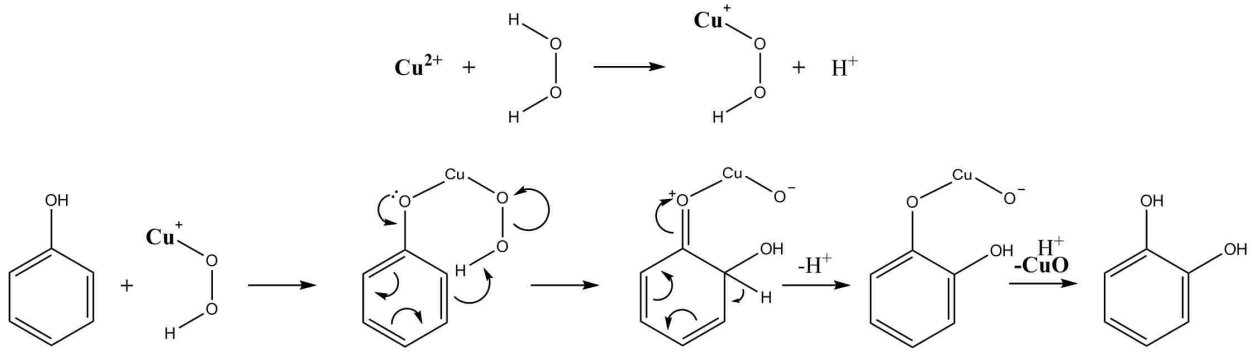


Figure 2.19. Assisted electrophillic hydroxylation of phenol (Stewart, 1964).

From the mechanism given by Stewart (1964), a small reservoir of quinone is required to achieve reasonable quantities of the semiquinone intermediate. The needed species likely arrives as a consequence of trace contamination in the media (Isenberg, 1961). Since some small amount of quinoid species will be found in any unpurified hydroquinone (or any purified material that has been stored for any appreciable amount of time in contact with air). This mechanism could be expected to function in mixed media, such as cane juice which has been, by consequence of process, aerated.

Following suit, a REDOX cycle of this sort may be established with Fe^{3+} so long as a small amount of Fe^{2+} is present or made *in-situ* from contaminants in the substrate.

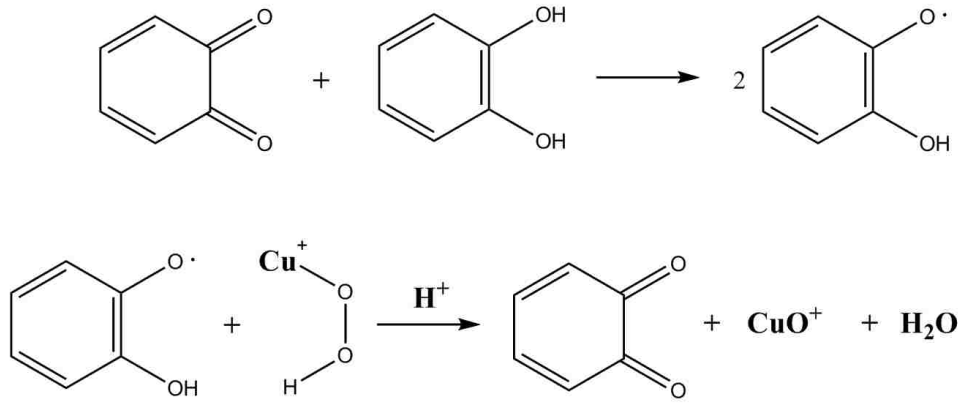


Figure 2.20. In-situ oxidation of catechol to *o*-benzoquinone (Stewart, 1964).

Iron is capable of oxidation states up to 6+ (Stewart, 1964). The Fe^{6+} is most commonly encountered as the perferryl ion, FeO_2^{2+} (George, 1954) or, simply “ferrate”. This material is available as the potassium salt from Aldrich Chemicals (K_2FeO_4, #480010).

It can be made via this reaction: $Fe^{2+} + O_2 \rightarrow FeO_2^{2+}$. Fe^{2+} can react with H_2O_2 to give $FeOH^{2+}$ and $HO^{\cdot}$ (Stewart, 1964). In a fashion analogous to Cu, $Fe^{3+} + H_2O_2 \rightarrow FeO_2H^{2+} + H^+$. Following this, we can expect the ferryl (Fe^{4+}) ion to behave in a way similar to Cu.

The REDOX potential of the important half-cell reactions are given by Bard and Faulkner(2004):

p-benzoquinone + $2H^+ + 2e^- \leftrightarrow$ 0.6992 V

$Fe^{2+} + 2e^- \leftrightarrow Fe°$ -0.44 V

$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$ 0.771 V

Attempts have been made to differentiate between the oxidative activity of ferryl and purported hydroxyl radical solutions (Rush, et al, 1990). It was noted that ferryl is not acting as the oxidant species whilst in acidic media (Walling, 1975). This strengthens the case for the transient existence of hydroxyl radical in connection with oxidations involving trivalent iron and hydroquinoid species.

Similar reactivity has been noted with aliphatic α-hydroxy ketones (Fieser and Fieser, 1967). Here, acidic $FeCl_3$ has been observed to lead to the oxidation of 2-hydroxycyclohexanone to give cyclohexane-1,2-dione in high yield (90%). For the original article see L. De Borger, et al, 1964.

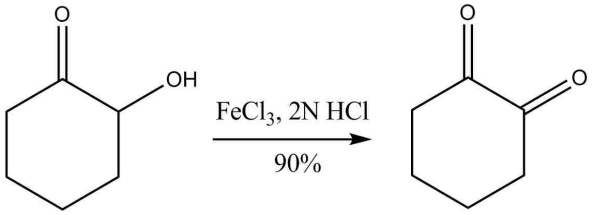


Figure 2.21. Oxidation of α-hydroxyketone

An intermediate complex of catechol with Fe^{3+} and subsequent oxidation to yield the quinone was posited by Hamilton (1963) to proceed via an ionic mechanism. In this case, anisole is hydroxylated via the catechol:iron complex. The proposed scheme for this reaction is given in **figure 2.22**.

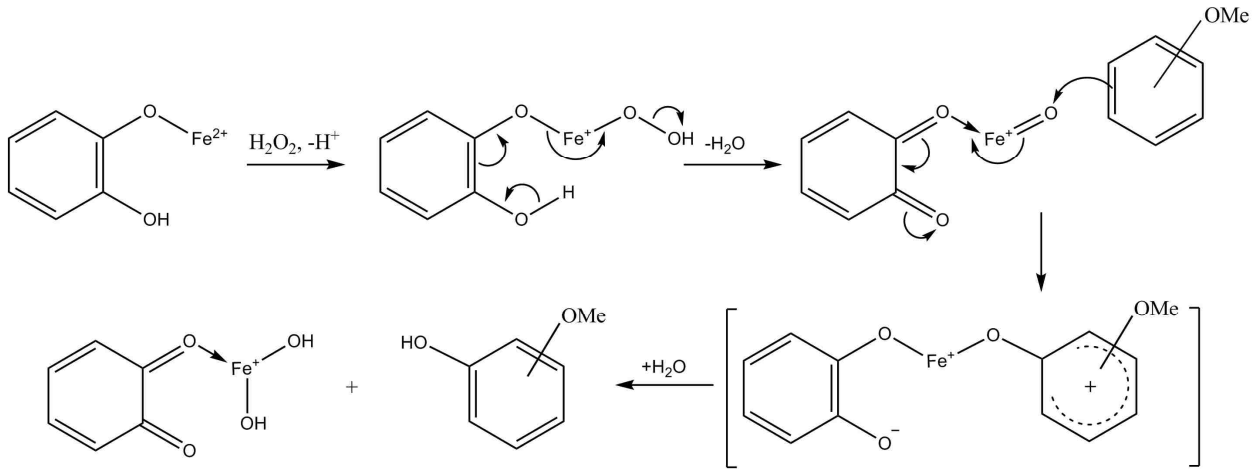


Figure 2.22. Proposed ionic mechanism for the oxidation of o-hydroxyphenol using Fe^{3+} and H_2O_2.

The formation of the iron complex proceeded at a rate 25 times faster than that of the corresponding oxidation (Xu, 1988b) and two equivalents of Fe were required. As such, the complex formation precedes the oxidation reaction, and can be thought of as a “rapid preequilibrium”. This can be observed by color change.

During the reaction of 2,3-DHBA with Fe^{3+}, blue color is first observed, followed, in several minute by yellow which turned, in several hours, brown. It appears that the initial complex involving the carboxylic acid group and the 2-hydroxyl was blue and the semiquinoid 2,3-dihydroxy iron complex was yellow.

The brown color would be consistent, as outlined previously, with polymerization of the quinone thus formed with remaining dihydroxy substituted species. The scheme for this is given in **figure 2.23** (Xu, 1988a).

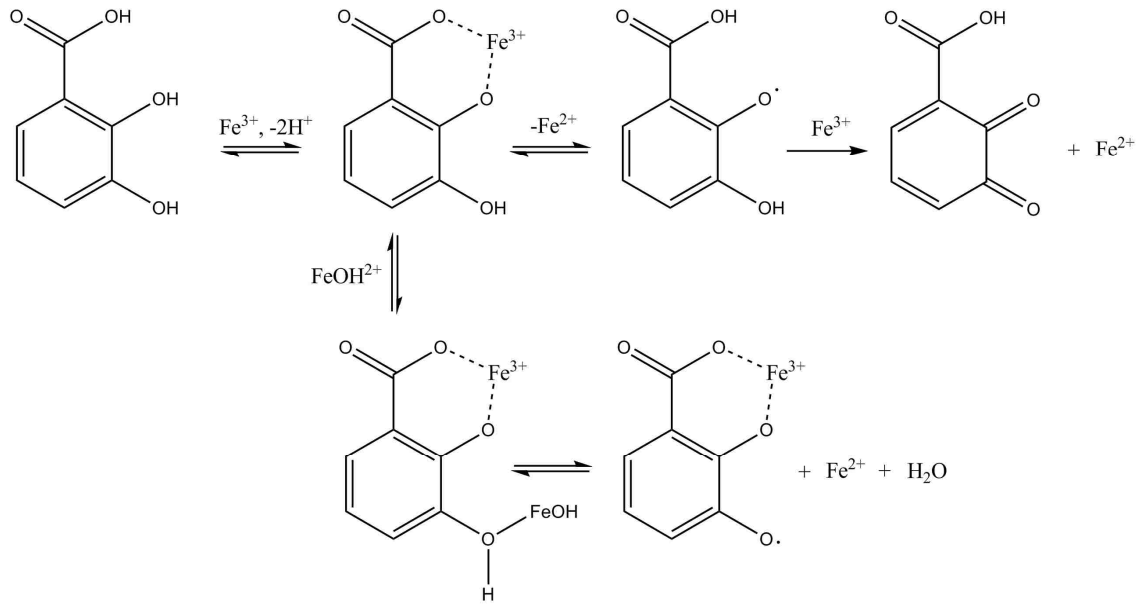


Figure 2.23. Proposed radical mechanism for the oxidation of *o*-hydroxyphenol using Fe^{3+} and H_2O_2. Note the semiquinone intermediate.

2.7. Iron and Fenton's Reagent

Published first by Henry J. Horstman Fenton in 1876 (Fenton, 1876), was the serendipitous (Koppenol, 1997) observation that ferrous iron (sulfate or chloride) will react with tartaric acid (L or D-2,3-dihydroxybutane-1,4-dioic acid) in the presence of either chlorine water (HOCl) or hydrogen peroxide to yield a violet product under alkaline conditions. Fenton continued to work with this reaction and determined that the action of iron in the oxidation of tartaric was catalytic (Koppenol, 2000). Ultimately, the resulting product was dihydroxymaleic acid and a detailed account was given by Fenton in 1905. The identity of the product was questioned by Hartree (1953) who determined that the free acid was, specifically, *trans*-dihydroxyfumaric acid.

Fenton observed that the reaction with tartaric acid was oxidative and only took place when ferrous iron was present. In 1894, Fenton noted that alkaline mixtures containing tartaric acid, Fe^{2+}, and H_2O_2 were decolorized via addition of acid (Fenton, 1894). It was also noted that, as with similar mixtures containing $FeCl_3$ and phenolic materials (pyrocatechin or phloroglucin), readjustment of pH toward alkaline restored the violet color.

Further, it was found that excess iron or oxidant likewise decolorized the mixtures. This indicates that there may exist, in these mixtures, some product or intermediate that operates as a pH indicator.

Alternative oxidants were attempted including chlorine water (as per the original note), hypochlorites, $Ba(OH)_2$, NaOOH and $KMnO_4$; these were found to be inferior to H_2O_2. Nitrous and nitric acids and ozone (O_3) were likewise unreactive. Passing O_3 through ether (implied to be diethyl ether), however, yielded a highly reactive material, likely diethyl peroxide, which further strengthened the case that the observed reaction was peroxide-specific.

The reaction was determined to be (although not called such) electrochemically REDOX active as the aforementioned solution would be yellow about the anode and violet at the cathode of a cell using Pt electrodes. The reaction was also found to be sensitive to air when ferrous tartrate, made alkaline, turned purple when exposed to air (of interest, Fenton thought that fresh air was more reactive than the air in his laboratory).

Two *key* observations were noted in this early work, first, the iron need be used only in catalytic amounts, that is, changing the iron ratio from 1 to 1/16 to one mole of tartaric acid did not affect the maximum color developed so long as the air exposure remained fixed. Second, the extent of reaction was found to be *greatly* enhanced by the addition of Fe^{3+} salt. It was noted by Koppenol (2000), citing Manchot and Lehman (1928) that the stoichiometry was concentration dependent. The equivalence of iron to H_2O_2 was 2:1 when the iron was present in excess.

When the converse is true, the amount of iron oxidized is greater than two equivalents. It was hypothesized that the excess iron was oxidized to yield Fe_2O_5 (Fe^{5+}) which was cycled via reduction to Fe^{3+} by either Fe^{2+} or H_2O_2.

These observations were explained in a chain of transactions which are reviewed in chronological order by Koppenol (2001). The relevant features are outlined here, and were initiated by the work of Haber and Willstatter(1931).

Originally designed to elucidate the mechanistic function of catalase, this work ultimately led to these equations:

$$HO\cdot + H_2O_2 \longrightarrow H_2O + O_2^{\cdot -} + H^+$$

$$\underline{H_2O_2 + O_2^{\cdot -} + H^+ \longrightarrow O_2 + HO\cdot + H_2O}$$

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

George (1947) demonstrated that Fe does not appear to effect superoxide via experiments involving K_2O (superoxide). The observed evolution of O_2 from K_2O was stoichiometric. He noted that O_2 evolved from systems containing $O_2^{\cdot -}$ and Fe^{2+} regardless of the presence or concentration of H_2O_2. George concluded that superoxide will disproportionate spontaneously in the presence of iron. The reactions are given below.

$$2O_2^{\cdot -} + H_2O(H_3O^+) \longrightarrow [O_2^{\cdot -}\cdot O_2H] + {}^-OH(H_2O)$$

$$\underline{[O_2^{\cdot -}\cdot O_2H] \longrightarrow O_2 + {}^-O_2H}$$

$$2O_2^- + H_2O(H_3O^+) \longrightarrow O_2 + 2^-OH + HOOH$$

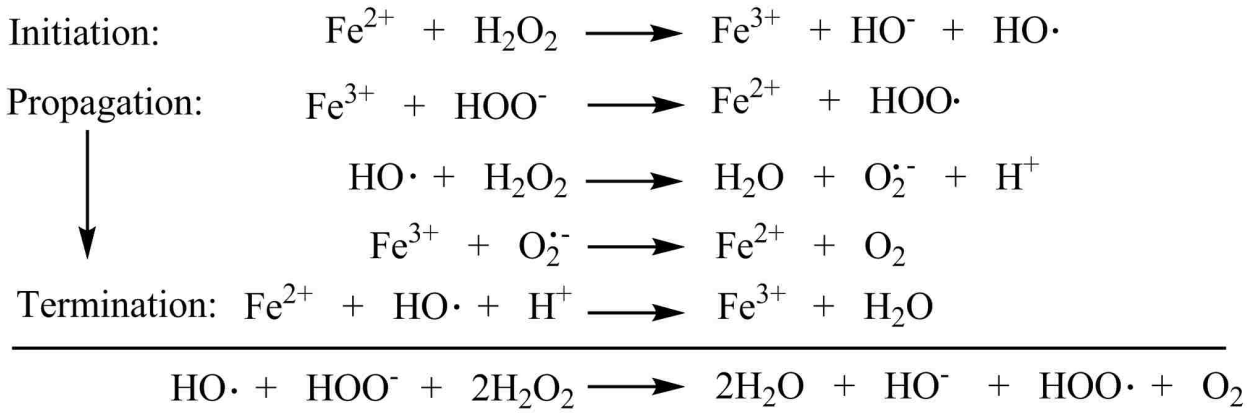
The originally published work of Haber and Weiss (1932) demonstrated that the action of iron upon the decomposition reaction of H_2O_2 was catalytic and that the reaction proceeded via a chain-propagation-type mechanism. This is reflected in the following equations where the cycling of iron cancels out on both sides.

$$Fe^{3+} + O_2^{\cdot -} \longrightarrow Fe^{2+} + O_2$$

$$\underline{Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^- + HO\cdot}$$

$$O_2^- + H_2O_2 \longrightarrow O_2 + HO^- + HO\cdot$$

The chain reaction initiated with Fe^{2+}, with the corrections which were added after considerable debate between Weiss and George, are given here:



It would not be until 1951 that reactions of this sort would be connected to Fenton's work (Barb, et al, 1951a). Barb et al. described, in great detail, the reaction cascades and behavior of both ferrous and ferric (Barb, et al, 1951b) species.

2.7.1. **Reactive Intermediates Resulting from Fenton's Reaction**

An additional feature of Fenton's work with the oxidation of tartaric acid (Fenton, 1894) was that many of the products were too reactive (air, pH, temperature, evaporation under vacuum) to be isolated. A powerful reducing agent was isolable from ether under very dry conditions (H_2SO_4, P_2O_5, or silica gel).

A white product remained after removal of the solvent which reacted with Fe^{3+} to yield a violet color. This suggests that the compound is likely capable of keto-enol tautomerization. Of further interest, is that the isolate readily decarboxylates at 50 °C.

The oxidative power exerted upon the substrate, tartaric acid, became apparent when Fenton noted that the reaction of his isolate with ethanolic (?) phenylhydrazine yielded at least three phenylhydrazones. This indicated that the products were either ketones, aldehydes, or both. The reductive nature of the product appeared to indicate the presence of an aldehyde.

When regarding the products associated with the Maillard reaction, we observed the formation of compounds which confirm Fenton's findings. We refer to compounds of this sort as "reductones". The term "reductone" has been used rather ambiguously and includes any sort of reducing keto (or tautomeric) species. These compounds fall into many classes, all of them representative of the sort of color-related compounds that we will find throughout the course of sugar processing. It is these compounds that can show up as reducing sugar under conventional REDOX assays where no such complementary amount of reducing sugar is to be found using GC (Eggleston, 2006).

Among them are the antioxidant phenolic compounds of (unsurprisingly) the quinoid class such as hydroxyhydroquinone (Yasuhara, 2002). Certain intermediates in the Maillard reaction cascade are likewise antioxidative, and are referred to as amino-reductones. Examples of this type include the enamine, 3-hydroxy-4-(morpholino)-3-butene-2-one and "amino hexose reductone" (Dittrich et al, 2003). Thus, a mixture of glucose and amino acid (glycine, lysine or arginine, in Dittrich et al's work) will yield a mixture of antioxidative intermediates which will eventually condense to yield colored materials. This also suggests that the intermediate products associated with phenolic and amino reductones can interact.

Fenton's reagent establishes an oxidative cycle whereby Fe^{3+} is reduced to Fe^{2+} and a reactive substrate is oxidized.

The cycle is perpetuated by the addition of H_2O_2 which re-oxidizes Fe^{2+} whilst creating a hydroxyl radical which oxidizes the nearest organic substrate. In this way, most organic materials, including very stable compounds such as polynuclear aromatic hydrocarbons (PAH, Flotron et al, 2003) or polychlorinated biphenyls (PCBs, Aronstein et al., 1995) can be oxidized to CO_2. For this reason, Fenton's reagent is often employed in a H_2O_2 fed-batch to remediate especially intractable industrial sludges. It was noted by de S. e Silva, et al. (2008) that endogenous Fe in Brazilian soil (~600 μg/g) can act as a photo-Fenton catalyst.

This reaction was found to be more effective for remediating PAH than the classic Fenton reagent. Cane juice is exposed to very little (usually diffuse) light so, the photo-fenton and like reactions will not be discussed in any detail.

Although Fe^{3+} is not capable of catalyzing the decomposition of H_2O_2, the Fe^{2+}, undoubtedly present as a contaminant, is. The small amount of $^{\cdot}OH$ so produced can yield $O^{\cdot 2-}$ from H_2O_2. This is capable of giving Fe^{2+} from Fe^{3+} and establishing a Fenton cycle. The rate of reaction will be limited by the small concentration of the catalytic species. In order to increase the rate, the concentration of Fe^{2+} in the system must be increased. Because Fe^{3+} is not capable of directly oxidizing a PAH such as naphthalene, a pro-oxidant, such as caffeic acid, will be required to increase the amount of Fe^{2+}. A proposed scheme is given here:

$$Fe^{3+} + PO \longrightarrow Fe^{2+} + PO^{+}$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{-}OH + {}^{\cdot}OH$$

$$Nap + {}^{\cdot}OH \longrightarrow [{}^{\cdot}Nap + H_2O] \text{ or } HONap$$

$$H_2O_2 + Nap + PO \longrightarrow {}^{-}OH + PO^{+} + HONap$$

$$HO_2Nap \longrightarrow HOOC_2Nap$$

$$HOOC_2Nap \longrightarrow CO_2 + Nap\text{-}1C$$

$$Nap\text{-}1C \longrightarrow CO_2 + Nap\text{-}2C$$

$$HO_2Nap \longrightarrow 2CO_2 + Nap\text{-}2C$$

$$2H_2O_2 + Nap + 2PO \longrightarrow 2^{-}OH + 2PO^{+} + Nap\text{-}2C$$

PO = pro-oxidant

Nap = napthalene

HO_2Nap = dihydroxyNap

$HOOC_2Nap$ = phenylenediacetic acid

-nC = - n carbons

From this, it appears that the rate of oxidation of naphthalene (or other substrate) might be controlled by altering the amount of pro-oxidant, and thus, the amount of catalyst which is present. In the system presented, caffeic acid would be oxidized to the quinone in the process of reducing the Fe^{3+} to yield Fe^{2+}.

Because the rate of complex formation is significant and some of the pro-oxidant will be relegated to polymerization with the quinones, the rate increase witnessed will be somewhat self-cancelling and dependent upon the quantity of Fe^{3+}. This also means that if a substoichiometric quantity of Fe^{3+} is added to a biomimmetic system containing pro-oxidants and multiple substrates, that the pro-oxidants will be oxidized first. So long as the amount of Fe^{3+} added is below the threshold for the establishment of a true-Fenton cycle (e.g. in excess over the pro-oxidant) then reactive quinoid species should exist whilst minimizing oxidative damage to the sugars present in the system.

A truly biomimmetic system will not have the provision for added H_2O_2. It will likely have some iron, but that iron is bound to prevent detrimental Fenton-like events from occurring *in-vivo*. When this goes wrong, for example with the genetic disease, hemochromatosis (Figueiredo et al., 1993) the effects can be lethal. Free radicals were noted in rat-liver (secondary hemochromatosis was induced using "saccharated" iron) at pH 5.0 using electron paramagnetic resonance (EPR). The radicals were not detected at pH 7.4. However, certain pro-oxidants, such as CFA can yield H_2O_2 on oxidation. The following scheme demonstrates that with the proper pro-oxidant, a stoichometric amount of H_2O_2 can accumulate which will be decomposed by the Fe^{2+}. The extent of oxidation resulting from the hydroxyl radicals thus formed will be directly dependent upon the quantity of pro-oxidant in the system so long as the Fe is present in excess.

$$\begin{aligned} Fe^{3+} + PO &\longrightarrow Fe^{2+} + PO^{+} + H_2O_2 \\ Fe^{2+} + H_2O_2 &\longrightarrow Fe^{3+} + {}^{-}OH + {}^{\cdot}OH \\ \hline PO &\longrightarrow PO^{+} + {}^{-}OH + {}^{\cdot}OH \end{aligned}$$

2.8. Concluding Statement

During sugar processing, sugarcane is extracted via either milling or diffusion and yields both juice and bagasse. The bagasse is stored and burned for fuel creating a carbon neutral closed-energy system.

The remaining bagasse could be used to generate other products, viz. cellulosic ethanol, but this technology is not ready to deploy. The juice is purified, primarily via hot-liming where it is heated to >100°C, treated with lime to pH ~7.2 and then up to 5 mg/kg of a very high molecular weight anionic polyacrylamide is added. Cationic polyacrylamide is not currently used in the production of raw sugar. The resulting mixture is allowed to settle and the clarified juice is decanted. This yields a clear juice, but it gains color during the process. This color can carry through the process to increase the color of the final sugar.

The removal of these colored materials has been done using GAC and/or ion-exchange, often in tandem with ultrafiltration. Because of the added expense and intrinsic technical difficulties, ultrafiltration is only used to produce certified “organic” sugar which sells for a significant premium.

Additionally, various chemical techniques have been applied with success, including sulfitation, carbonitation and phosphoflotation. These techniques are expensive to implement and maintain and residual processing chemical in product sugar is intolerable. Slim profit margins (~$0.01lb/production cost) and marginal quality control on raw sugar make implementation of these technologies in Louisiana unlikely and ill-advised. These technologies (WSM, NAP, DWISP, etc.) have been used to make a direct white sugar and while commercialized, have yet to be applied in the United States.

Because the new 1E6 lb/y Louisiana refinery is to be built without the benefit of either an affining station or GAC, the quality of the feed is important. A raw feedstock with a lower color will likely produce a final sugar of higher quality. We surmise that it is possible to produce a raw-grade sugar with color low enough to compete with the affined materials. In order to do this, two strategies may be employed.

First, the formation of color (melanoidin and/or caramel) during processing can be inhibited via the addition of nucleophilic traps (such as R-SH). Lowering process temperature and/or processing at greater speed will slow down the formation of the electrophilic reactive intermediates.

Second, the color which passes though the process, which is principally composed of phenolic compounds in various states of polymerization, can be removed before they enter the process.

The chemical reactions involved with the formation of both caramel and melanoidin involve dicarbonyl intermediates. These condense and dehydrate to yield a heterogeneous conjugate polymeric material that is of moderately high molecular weight and dark color. Likewise, *o*-phenolic compounds in quinone form (which are α, β-dicarbonyl species) can react via conjugate addition or condensation reactions with the aforementioned dicarbonyl species resulting from the caramel or Maillard-type reaction series. The polymer which can result resembles humic material and is not soluble in water or common solvents. Polymer of this sort has been found on heat transfer surfaces where the temperature is >100°C. This material impedes heat transfer, which has a negative impact on steam economy.

The phenolic component of the final colorant polymer originates with the cane, and only 0.6% of it is ionized during clarification with lime.

This means that ~99.3 % of these compounds proceed into the process where they are subject to oxidation, dehydration and heat. If these compounds can be removed before this occurs, it is expected that the sugar produced will carry less color with it. Improved heat transfer at exchanger surfaces could be a desirable side-effect.

Adapted from water treatment technology, hydrolyzing metal salts are not used in sugar processing. Preliminary studies using PAC with mixtures of flocullant polymers have observed that excellent color removal and clarification characteristics are observed at bench scale. The technology was found to be unpredictable and unsatisfactory when applied at industrial scale. The Al salts and mixtures thereof are also expensive and Al is implicated with disease in man. There was a patent filed which made use of ferric chloride to remove color and turbidity from cane juice.

It produced very small coagula which required the use of ultrafiltration to remove. The use of ultrafiltration for this purpose would not offset the cost of its use.

The premium for high grade raw sugar is much less than that for refined sugar which is fractional when compared to the per-unit price for an “organic” product.

Hydrated ferric chloride is a good oxidizer and it readily forms complexes with phenols and enols. This is the basis of a common qualitative test (e.g. phenol:Fe^{3+} is purple). The complexation of Fe^{3+} with *o*-hydroxyphenols or hydroxysalicylic acid derivatives involves the radical semi-quinone intermediate. This intermediate is more easily oxidized by air and can be an electrophilic target.

This makes attack from either radical or nucleophilic species possible and creates a route for polymerization of the quinone. The addition of the $FeCl_3$ results in the release of three equivalents of H^+ which can rapidly drop the pH. At lower pH, nucleophilic species such as amines will be protonated. When Fe^{3+} is added to a mixture containing an amine and an *o*-hydroxyphenol, it first hydrolyzes which drops the pH. The hydrolysis competes with complex formation with the *o*-hydroxyphenol. The quinone is evolved more slowly via autooxidiation of the semiquinone by O_2.

The quinone would then be protonated to create an electrophilic target. Unless the addition of the nucleophile to the quinone exceeds the rate at which is protonated, the addition of the remaining nucleophile will be slow. Thus, the rate of conjugate addition to a protonated quinone would be a factor of the pH which is dependent upon the amount of added Fe^{3+}.

The amine can also add to a quinone carbonyl group to yield the imine. The imine makes decarboxylation of precursors (if the target is a phenolic acid, such as 3,4-dihydroxybenzoic acid or the amine is an amino acid) more likely. The imine can also be protonated to create an electrophilic target which is why they are subject to hydrolysis. The existence of these species, minus a reducing agent to trap the imine as the amine, is thus an equilibrium which can be easily reversed by altering the pH.
In highly aqueous and aerated media, such as cane juice, decarboxylation is unlikely and hydrolysis is the most likely route. Imines are likely transient species in this system. It was noted that radicals existed in biological systems, at pH 5.0, when iron exceeded of the chelating ability of the native enzymes.

This pH is very near that of cane juice (5.0-6.0), particularly if $FeCl_3$ has been added (3.4-5.5). Cane juice, when fresh is a living biological system with active enzymes and living cells and should be expected to behave in a similar way.

CHAPTER 3. EXPERIMENTAL

3.1. Color: Significance and Behavior vs. pH

It was indicated previously that many consider both the definition and means of measuring color to be arbitrary. We will attempt to more clearly define what is meant by "color" in the context of sugar processing. The relationship between color in raw mill syrup and pH was defined by UV-Vis spectra obtained by titrating syrup vs. pH adjusted with standard NaOH. For comparison, the same syrup was treated with 160 μg/g of Fe^{3+}.

3.1.1. Materials and Methods

Raw mill syrup (provided by Cora Texas Mfg. Co.) was titrated with 0.01 N NaOH. The titrant was prepared from freshly dried (50°C, 24" Hg, 8 hr) NaOH (Baker, pellets) weighed quickly into degassed (24" Hg, sonication, 20 min) 18 MΩ water (Barnstead nano-pure). Standard HCl was prepared from certified standard 1.005-0.995 N (Fisher) via dilution by mass to provide, assuming 100% dissociation, a titrant H^+ concentration of 0.1010±0.0057 N. A solution of $FeCl_3 \cdot 6H_2O$ (Mallinkrodt) was prepared in degassed 18MW water. The stock solution assayed at 44,600 μg/g Fe^{3+} (phenanthroline).

Weighed aliquots (5.008±0.006 g) of the alkali so prepared were titrated to phenolphthalein (1 drop of 1% g/100mL in EtOH) endpoint using standard (0.1010 N, Fisher) HCl. All volumetric measurements were converted to mass via density correction. The alkali was standardized in triplicate and had a hydroxide concentration of 0.0981±0.0005 N. This material was diluted (1:10), by mass, in a pre-tared 100 mL volumetric flask to give a titration with an alkali concentration of 0.0098 N.

Syrup was vacuum filtered through a 47 mm 0.45mm filter with a Whatman #4 paper prefilter. To 60.42 g of filtered syrup was added 80.04 g of water. 25.0034 g of this were further diluted to a total mass of 50.0838 g. A second batch was prepared similarly, and was treated with 160 μg/g of Fe^{3+}.

Both syrups were adjusted to pH 3.00±0.02 with standard HCl and then titrated vs. pH (Orion, temperature compensated) using standard alkali.

Every time pH increased by +0.5, a 0.5 mL aliquot was removed (Eppendorf). The absorbance spectrum of each aliquot was measured from 200-700 nm using a Beckman-Coulter DU-800 spectrophotometer. Absorbance measured in the UV range exceeded the linear range of the detector (ABS >2.0) so, 25μL aliquots were removed from the previously measured samples and diluted into 1000μL (final, DF = 40) in 1.5 mL 9Q quartz cuvettes. These were scanned similarly.

3.1.2. Results

The UV-range spectra were unremarkable; there were no significant differences observed that were clearly dependent upon pH. A plot of this data is given below. There appears to be a peak in measured color at a pH between 3 and 4.5. Otherwise, the ΔABS/ΔpH when measured at 334 nm seen in **figure 3.1** inset, is flat from pH 5-10.

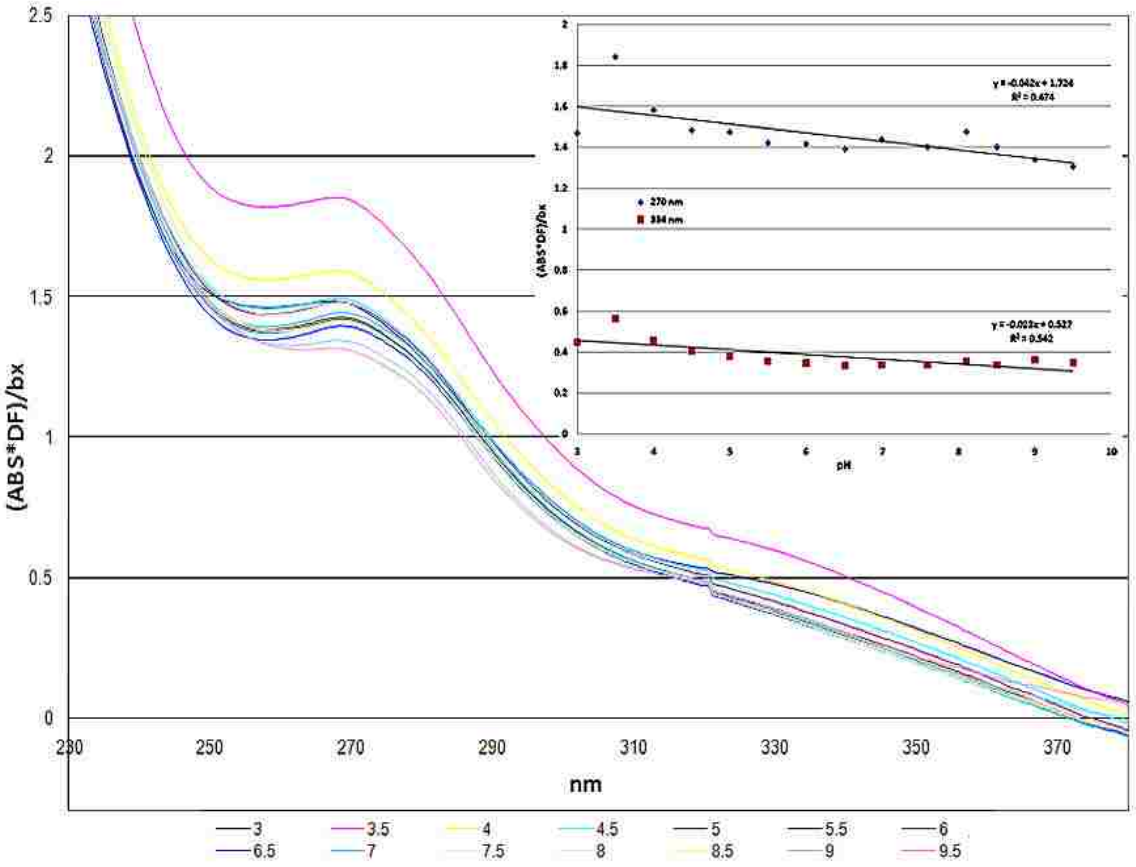


Figure 3.1. UV-range absorbance of cane syrup at various pH. Inset, lack of dependence of ABS_{UV} to pH.

Absorption in the visible wavelengths, however, exhibits significant dependence on pH. The range from approximately 330-520 nm is the only part of the wavelength continuum where pH has a significant effect on absorbance.

A strong, non-linear pH dependence in absorbance, **figure 3.2**, is seen between 380-480nm, the peak has a λ_{max} ~400, and is a shoulder on a much larger peak with a λ_{max} between ~330-380 nm. It interesting to note that the absorbance at 400 nm also exhibits a peak at pH between 3-4, this is likely the result of baseline elevation which occurs as a consequence of some component(s) which absorb in the 330-380 nm band and are pH sensitive in this range.

The behavior of pH relative to the mMol of titrant with the corresponding "color" is given in **figure 3.3**.

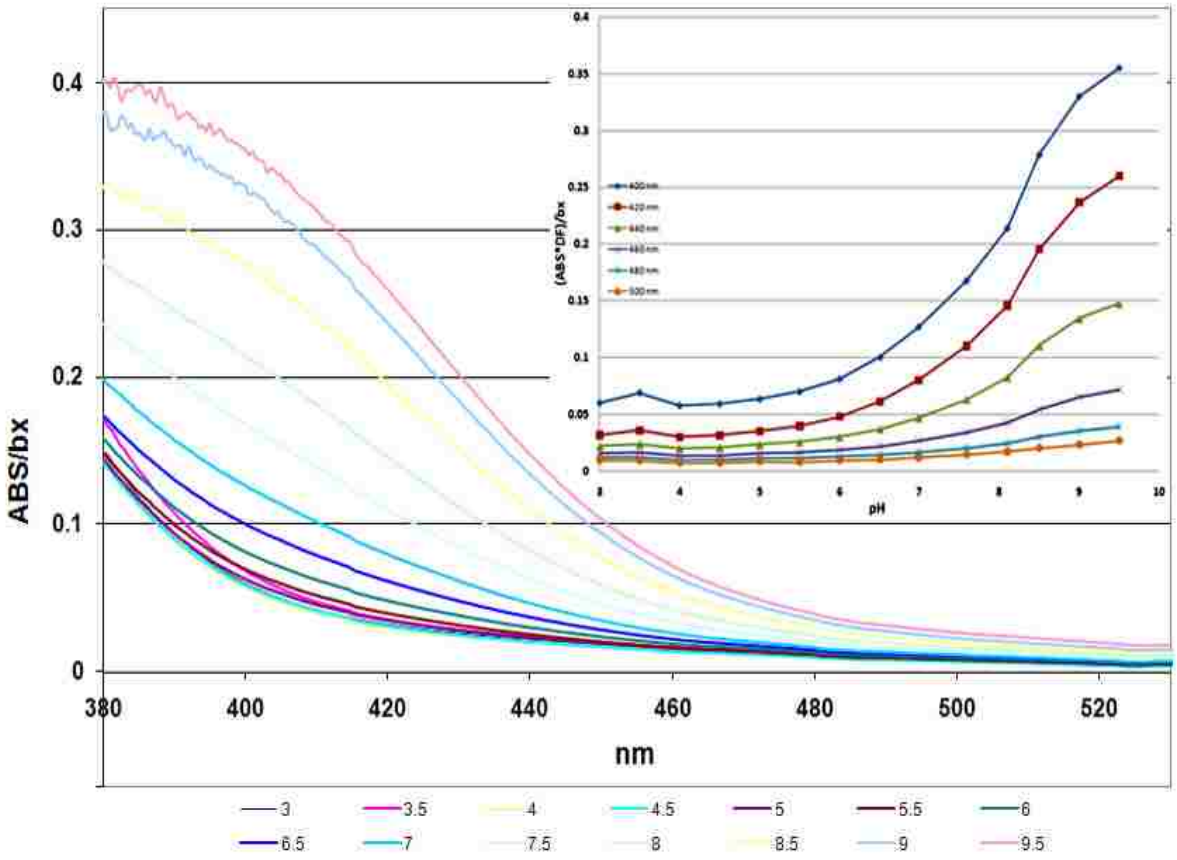


Figure 3.2. Visible-range absorbance of cane syrup at various pH. Inset, dependence of ABS_{VIS} on pH.

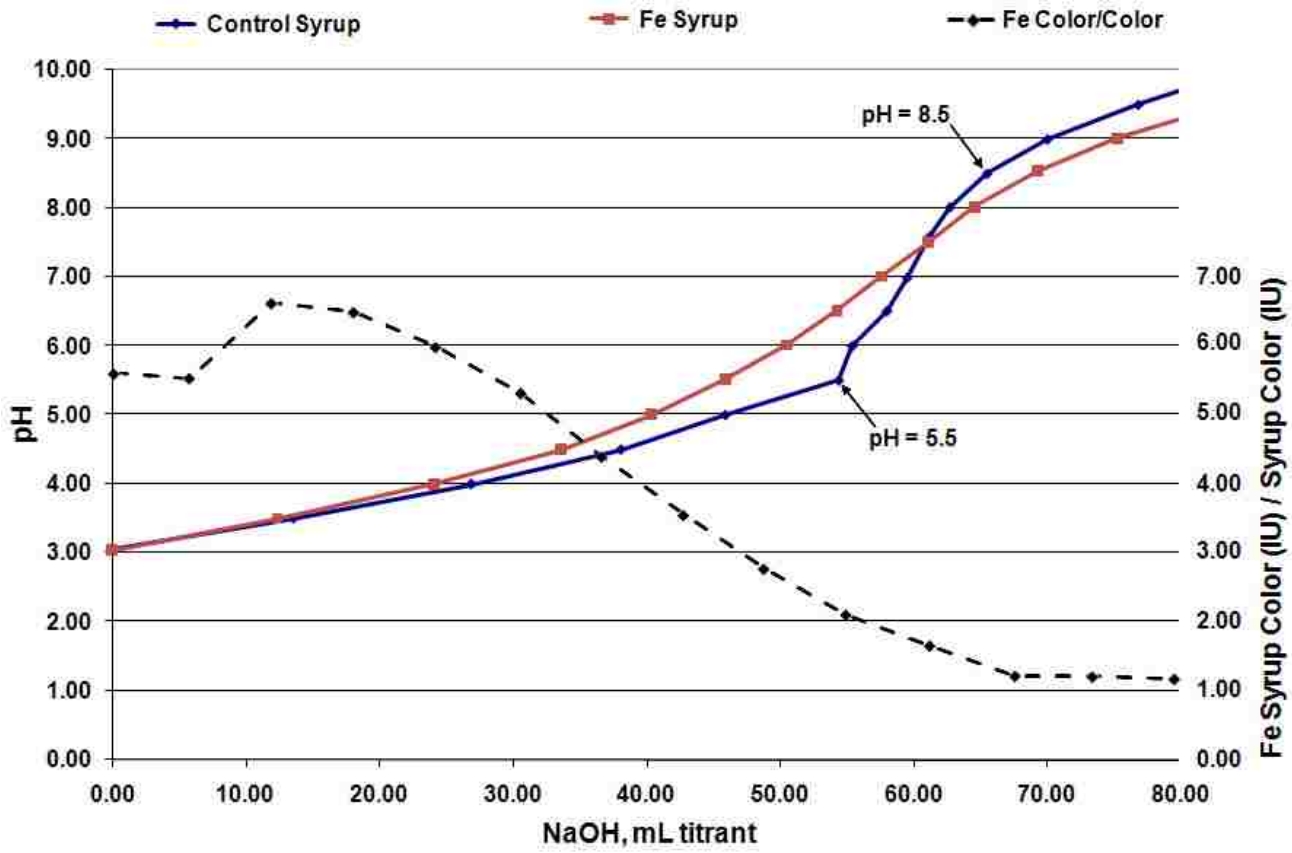


Figure 3.3. The behavior of pH relative to titration with alkali (NaOH). Note the inflection points in the untreated syrup at pH 5.50 and 8.00. The hashed line, with scale on the right is the ratio of color (IU) of the iron treated syrup / untreated syrup.

3.1.3. Conclusion/Discussion

With the exception of a peak in absorbance between pH 3 and 4.5, a pH range which is specifically avoided in sugar processing, there is little if any dependence of absorbance on pH in the UV range (230-380 nm). There is, however, a strong and non-linear dependence which is observed in the VIS range (380-700 nm).

In the VIS range, specifically between 380 and 520 nm, we see that "color" measured as absorbance at 420 nm is very close to the maximum (λ_{max} = 400 nm) of a shoulder on a much larger peak with a λ_{max} at 340 nm. This wavelength range is consistent with phenolic compounds and corresponds specifically with an absorbance which is characteristic of the $\pi \rightarrow \pi^*$ transition of an aromatic system substituted with electron donating group(s) and is conjugated at the benzylic carbon.

This is highly suggestive of components(s) which resemble or contain a 4-vinylphenol core structure.

The results of the titration indicate that a linear relationship exists between pH and added titrant over a pH range from 5.5 to 8.5. There is a parallel relationship between pH and the ”color” measured over this range. Interestingly, the ratio of color in the samples treated with iron to the untreated color is linear, and decreasing between pH 5.5 and 8.5 (Fe IU/IU = -1.5748*mL titrant + 13.915; R^2 = 0.9930).

At present, a number which is the ratio between the absorbances measured at pH 9 and 4 is used to provide a measure of the quality of a raw sugar or syrup. This value is known as an“indicator value (IV)” and is attributed primarily to the phenolic compounds present in the material (Paton, 1992). From this work, it appears that determination of IV should be made between pH 5.5 and 8.5.

It also appears that the iron not only increases the color, but that the color formed is less subject to the effect of increasing pH. The ratio of observed color in iron treated syrup to untreated syrup ranges from 5.5 (pH=3.00) to 6.7 (pH = 3.5) and then decreases to 1.32 at pH 8.5. The ratio does not change as pH is increased past this point. It appears that some small amount of iron present in the native syrup may contribute to the peak in color seen at pH 3.5. The pH vs titrant for the iron treated syrup lacks the distinct inflection points that are seen in the untreated syrup. This suggests that the behavior of whatever colored material formed when iron is added is likely more complex than the simple protonation-deprotonation-type pH dependence that is observed with the native material.

It appears that, if the decision to use 420 nm for measurement of “color” was indeed arbitrary, then it was fortuitous. However, raw sugar factories measure their sugar at pH 7 and the refinery, who purchases the sugar, measures at pH 8.5.

Because the quantity and type of colorant present can vary significantly from day-to-day and/or mill-to-mill, the definition of what a 1500 IU raw sugar actually is remains the subject of debate.

3.2. Analysis of Cane Juice and Sugar Using GC-MS

As much of the colored material that finds its way into sugar is likely phenolic in origin, we sought to identify, as completely as we could, the phenolic compounds found in cane processing streams. We were also interested in the behavior of phenolic compounds with respect to the process of clarification using heat and lime. Juices, syrups, molasses and sugars were extracted then the products separated using gas-liquid chromatography with mass selective detection (GC-MS).

3.2.1. Materials and Methods

Samples of cane juice, clarified juice, syrup, molasses, raw and refined sugars were extracted using dichloromethane (DCM). Each matrix was spiked with a known quantity of 3-phenylphenol, prior to extraction to aid in evaluation of possible matrix-related effects on recovery. Liquids (100g, each) were extracted at pH 2 and 10, each with 3 X 25 mL DCM to yield separate acid and base-neutral fractions. These extracts were dried over $Na_2SO_{4(anh.)}$ and concentrated to 1 mL using a Zymark Turbovap. Solid sugars were weighed into 500 mL Erlenmeyer flasks and extracted using 3 X 50 mL DCM with shaking and ultrasonic treatment. The resulting extracts were dried and concentrated as before.The solid products were then dried completely in-vacuo at 45°C, 24" Hg, 4 hr. These products were then extracted as before using 10 % methanol in ethanol (absolute). As before, the extracts were concentrated and subject to GC-MS.

The resulting extracts were analyzed using gas-chromatography with mass selective detection (GC-MSD). The operating parameters for the instrument are given in **table 3.1**.

Table 3.1. Operating parameters for the GC-MSD.

Gas chromatograph	Agilent 7890, Injector 250°C, 2 μL, split 50:1 (He, 50 L/min). split/splitless liner.
MSD	Agilent 5975C, inert source EI 71 eV, solvent delay: 3 min., scan 40-550 u, threshold 150, source 250°C, quadrupole 150°C.
Column	DB-5XLB 30m X 0.25mm X 0.25 μm, He, 1 mL/min (7.36 psi, 54 mL/min total).
Temperature Program	Init. 45°C, total run-time: 36.17 min. 15°C/min to 250°C hold 10 min. 20°C/min to 300°C hold 10 min.

The total ion chromatograms (TICs) were integrated with a threshold area of 50 counts. The integrated peaks were compared with library searches against the NIST and WILEY spectral data bases. Spectra with significant similarity to those found in the database were examined for accuracy using a mixture of fragment analysis and familiarity with the source material (for example, we know that mescaline, 3,4,5-trimethoxyamphetamine is impossible while the structurally similar syringic acid, or 4-hydroxy-3,5-dimethoxy benzoic acid is reasonable).

3.2.2. Results

The compounds listed in **table 3.2** were found both in clarified juice and in raw sugars at relative concentrations of 0.1 % or greater (GC, % of total area). The relevant library search results are given in **Appendix E**.

Table 3.2. Major compounds identified in DCM extract of clarified juice. *likely a folic acid analogue.

Tr:	Compound:	Q%:	Area %:
5.51	benzoic acid	87	1.149
6.05	benzeneacetic acid	83	0.118
6.53	2-methoxy-4-vinylphenol	91	0.558
6.83	4-hydroxy benzaldhehyde	91	1.191
7.14	4-hydroxy-3-methoxy benzaldehyde	94	0.563
7.25	3,4-dimethoxyphenol	95	0.145
7.7	1-(4-hydroxy-3-methoxyphenyl)-ethanone	97	0.355
8.38	3,4,5-trimethoxyphenol	96	0.111
8.74	4-hydroxy-3,5-dimethoxybenzaldehyde	95	1.641
9.3	3,4,5-trimethoxybenzoic acid	95	0.246
9.92	3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid	93	2.027
10.07	3,4-dimethoxycinnamic acid	98	1.324
10.26	n-hexadecanoic acid	96	0.629
10.46	3,5-dimethoxy-4-hydroxycinnamaldehyde	91	1.634
10.69	5,6,7,8-tetrahydro-1,3,6,7,8-pentamethyl-2,4(1H,3H)-Pteridinedione?*	83	0.611
11.06	4,5-dimethoxy-4-hydroxycinnamic acid	93	1.392

3.2.3. Conclusion/Discussion

The extracts of clarified juice contained both more numerous and larger quantities of phenolic constituents than extracts of equivalent masses of raw juice. Most of the compounds identified were phenolic and appeared to be representative of the lignin monomers commonly seen in grasses (Boerjan, et al., 2003).

Coumaryl (4-hydroxy), guiacyl (4-hydroxy-3-methoxy) and syringyl (4-hydroxy-3,5-methoxy) substituted species of both benzoic and cinnamic acids were tentatively identified. Of the 31 compounds assigned reasonable identities, 16 of them were present in quantities of 0.1 % or greater (based upon total chromatographic area). Of these, 14 were phenolic and possibly originated from or were precursors for lignin.

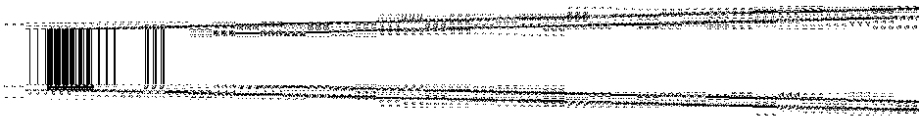


Figure 3.4. Cinnamic monolignols, from left, caffeiyl, p-coumaryl, guiacyl or coniferyl and sinapyl alcohols.

The high levels of lignin-like phenolic compounds in clarified juice can be attributed to the pH (high) and temperatures (100-110°C) that are employed in the clarification process. It is known that these conditions can solvate lignin (Thring, et al., 1989) and it is a solvolysis involving the hydrolysis (or ethanolysis in organosolv processes) of the lignin backbone. It is likely that the increase in color across clarification,is more an issue of solvated lignin than of the formation of hexose alkaline degradation products. An overlaid chromatogram comparing the complexity of extracts obtained from equivalent quantities of mixed and clarified cane juice is shown in **figure 3.5**.

It was also noted that the bulk of the material removed during lime clarification (hot or cold) consisted of waxes and sterols. This is consistent with the waxy type I particles observed by Bennett (1957b). The main wax component was identified, vs. the bona-fide compound, to be n-octacosanol (a ceryl alcohol).

A secondary wax was unidentified, but the fragmentation pattern strongly suggests that it too is a linear waxy alcohol, likely $C_{26}H_{54}O$ (382.71 g/mol).

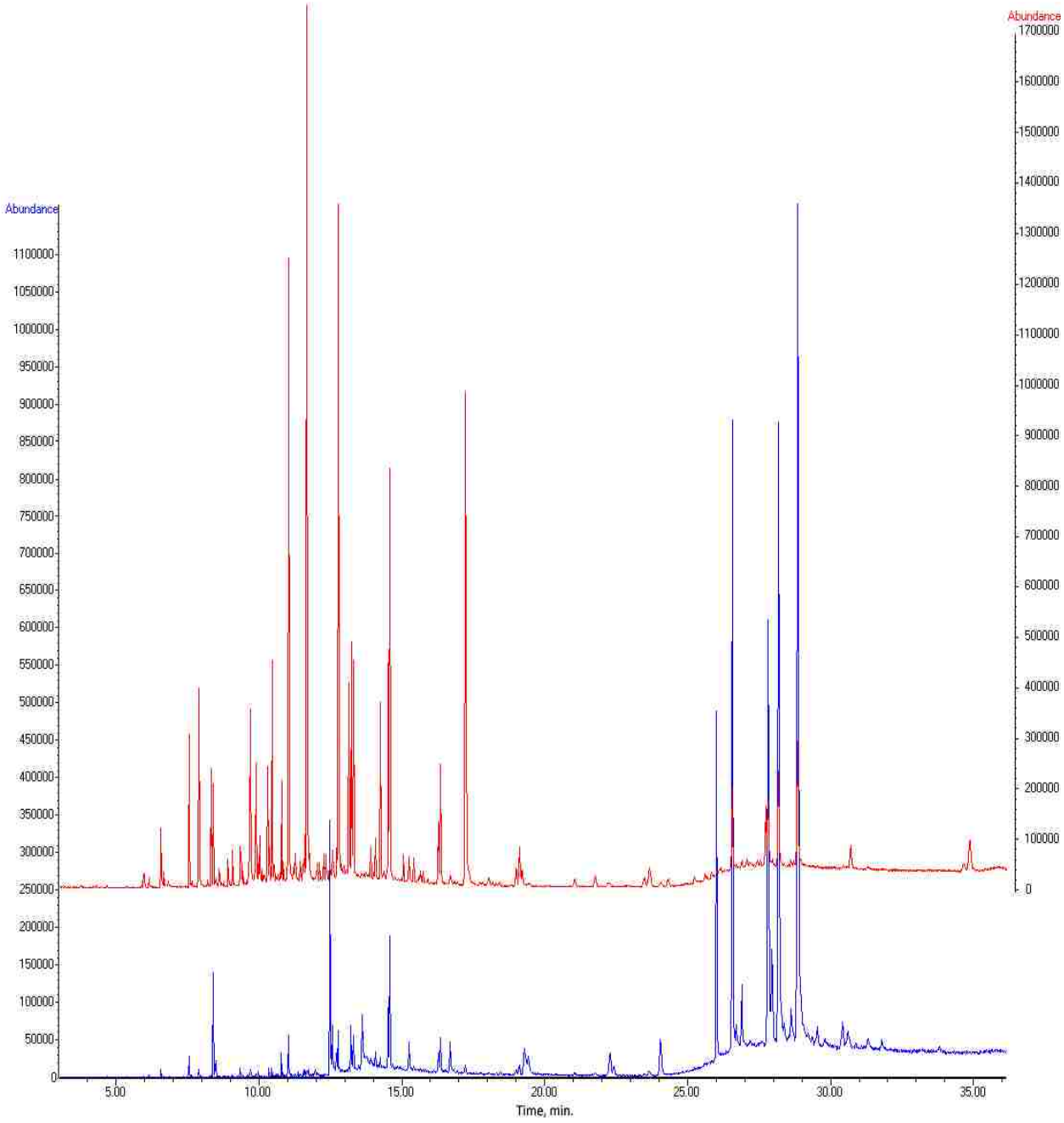


Figure 3.5. GC-MS TIC, extracts from equivalent volumes of raw, blue and clarified cane juice, offset with scale on the right, red.

The main sterol components, given **in figure 3.6**, were γ-sitosterol ((3-β-24S)-Stigmast-5-en-3-ol), stigmasterol (β-(E)-23-ethylcholesta-5,22-dien-3-ol), and campesterol (β-ergost-5-en-3-ol) .If an

efficient and inexpensive isolation method can be derived on a large scale (400 tons/hr), then a nutritive (Awad and Fink, 2000) product derived from these materials could be produced.

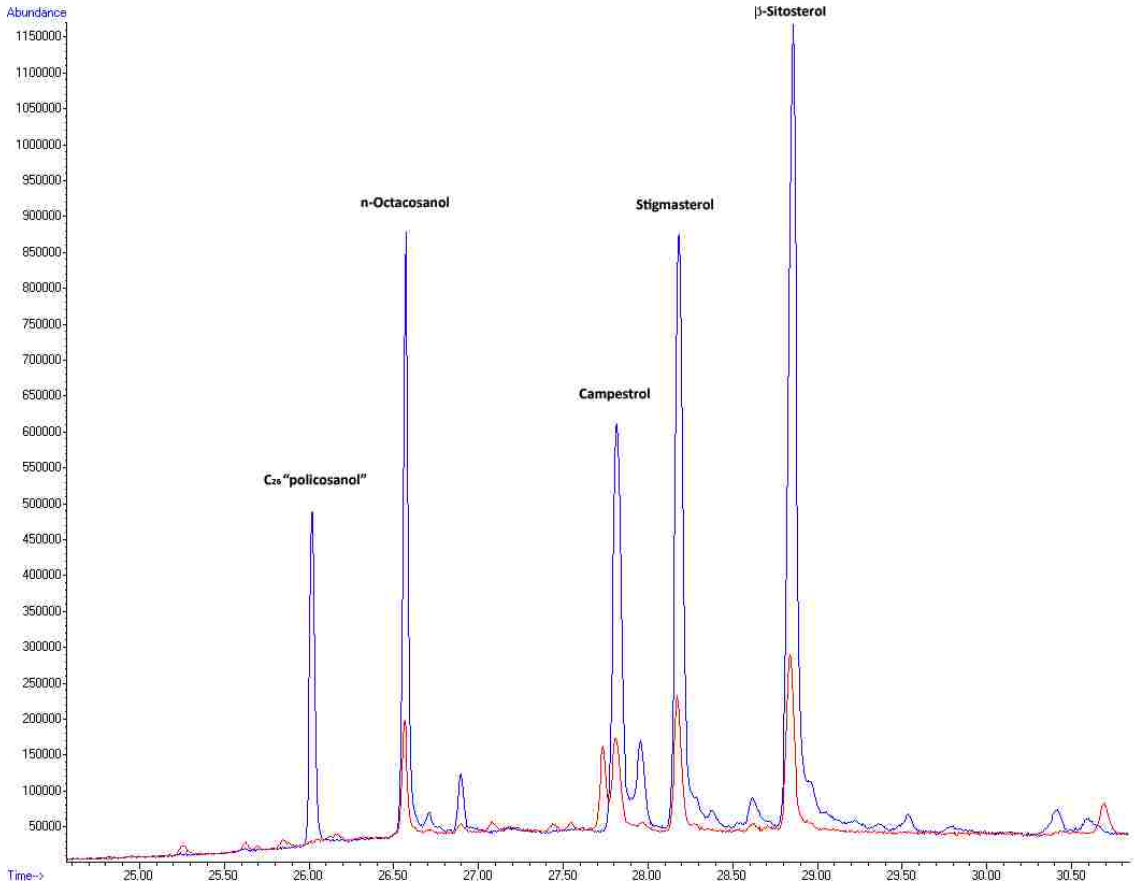


Figure 3.6. Policosanols and phytosterols identified in raw (blue) and clarified (red) cane juice.

The component profile of raw sugar was simpler than that of clarified juice. While it contained fewer phenolics (that were present in quantities we could detect), it also contained compounds which were consistent with the formation of caramel which included 5-hydroxymethyl-2-furaldehyde (HMF) and maltol. These materials were fluorescent under long-wave ultraviolet light (365 nm) and color decreased the observable fluorescence; most likely due to a quenching effect.

In refined sugar, the profiles are even simpler, and the quantities of each color component carried through the refining process are very small. The contaminants resembled caramel-type intermediates and were most highly represented by HMF, maltol and other furanoid analogues. An overlay of chromatograms from clarified juice, raw and refined sugar is given in **figure 3.7**.

The chromatograms are not normalized to the indicated scale.

The phenolic compounds present the greatest overall (detectable) contribution to the color to raw sugar. It was noted that the clarification process increased the quantity and variety of these compounds in the juice. It was decided, from this point forward, to focus on the phenolic compounds which are inherent in the cane when considering methods for producing a raw sugar with very low color.

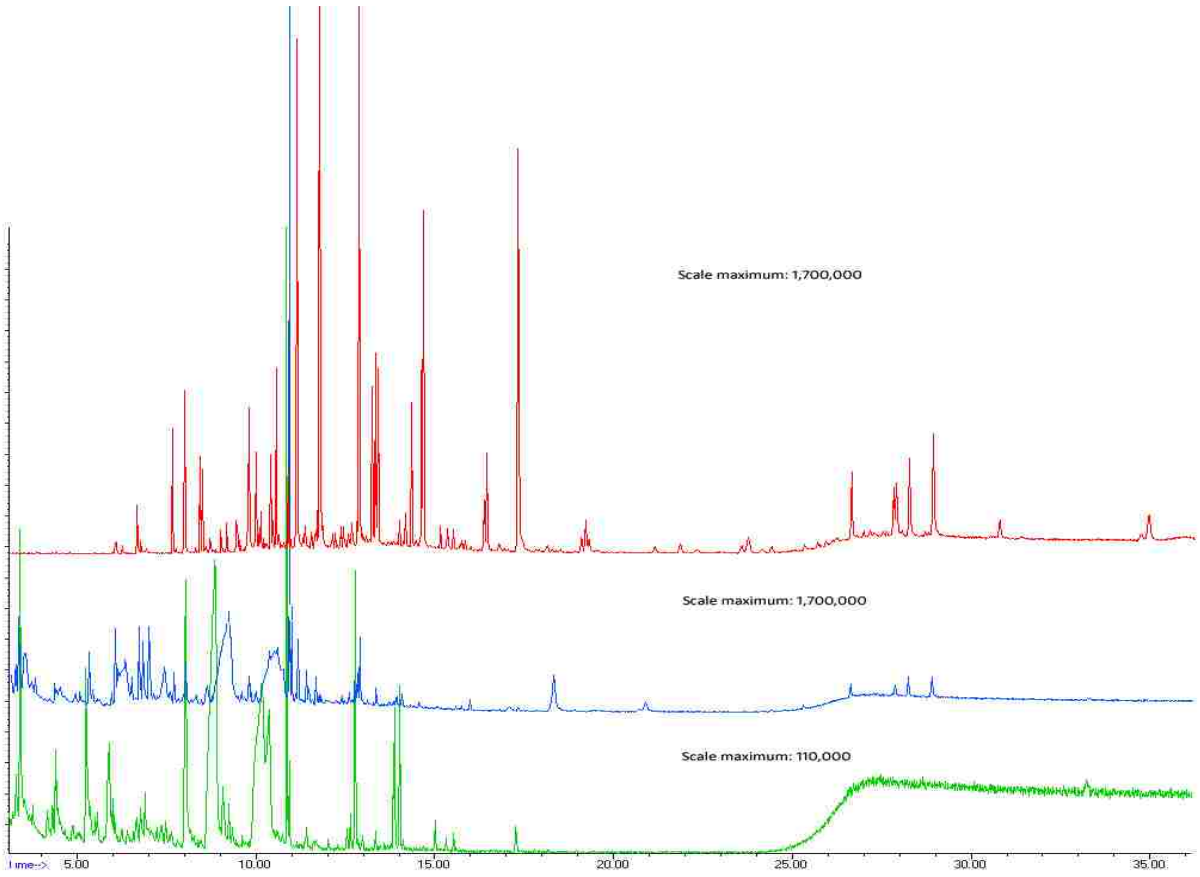


Figure 3.7. TICs of clarified juice (red), raw (blue) and refined (green) sugar.

3.3. Behavior of Clarified Cane Juice and Syrup in the Presence of Iron (III).

As previously described (Bennett, 1953a), hydrolyzing salts containing transition metals in higher oxidation states are effective coagulants. As the higher oxidation states may promote the formation and precipitation of aggregated phenolic contaminants, ferric ion, Fe^{3+} was tested for efficacy in removal of color from clarified cane juice and syrup.

Models containing and aqueous mixture of "phenolic surrogate" (caffeic acid) and L-glutamine (sugarcane contains a large quantity of this free amino-acid) were micro-titrated with Fe^{3+}.
The changes in the UV-Vis spectra were recorded for each level of added Fe. The binding of Fe^{3+} was tested via addition of ethylenediaminetetraacetic acid (EDTA) to the samples.

3.3.1. Materials and Methods

Clarified cane juice syrup were obtained from Cora Texas Mfg. Co., Inc. An aqueous solution of $FeCl_3$ was prepared ($FeCl_3 \cdot 6H_2O$, Mallinckrodt, 99.8 %) to contain approximately 1.4% Fe^{3+}(x_j = 0.207) . Using a Hamilton syringe, ten μL (140 μg/mL Fe^{3+}) of this solution was applied directly to 1 mL each of the clarified juice and syrup. The samples were visually inspected and then the UV-VIS spectra (200-700nm) were acquired (Beckmann Coulter DU-800).

Following this, model solutions were made to contain either 3,4-dihydroxycinnamic acid (caffeic acid, Aldrich 99%), L-glutamine (Sigma, 99%), or both. To one mL each of these solutions was added the $FeCl_3$ solution, in 1 uL aliquots. After the addition of each aliquot, the UV-Vis spectrum was acquired as before. After the addition of 14 μL (196 μg/mL) of $FeCl_3$ solution, EDTA (Sigma-Aldrich, ACS 99.4%) was added in excess.

3.3.2. Results

The addition of Fe^{3+} to clarified juice resulted in an instantaneous four to six–fold increase in color. An example of this is given in **figure 3.8**, where W = water, CJ = clarified juice, CJFe = clarified juice with added Fe^{3+}.

UV-Vis matrices against Fe^{3+} indicated the appearance of a strongly colored complex with a peak absorbance of 300-350 nm with a concomitant increase in absorbance at 210nm, which is consistent with an oxidation of *o,p*-phenolic materials to yield quinones ($n \rightarrow \pi^*$, carbonyl). Analogous experiments made with models containing sugar, caffeic acid and L-glutamine behave similarly, and examples of the spectral matrices are given in **figure 3.9**.

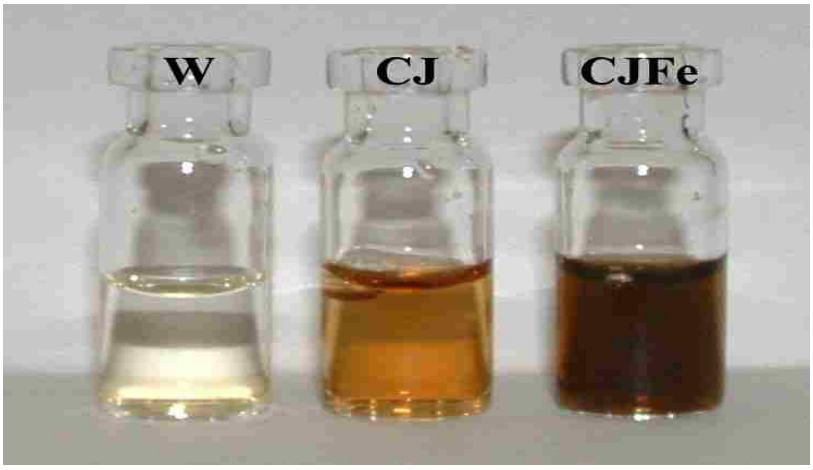


Figure 3.8. The effect of iron on the color of clarified juice.

EDTA added to this mixture diminished the color (presumably via disruption of non-covalent Fe^{3+}-caffeic acid complexes following sequestration of the Fe^{3+} ions), but, the color of the product was significantly higher than the starting material indicating that some permanent, covalent colored material had been formed.

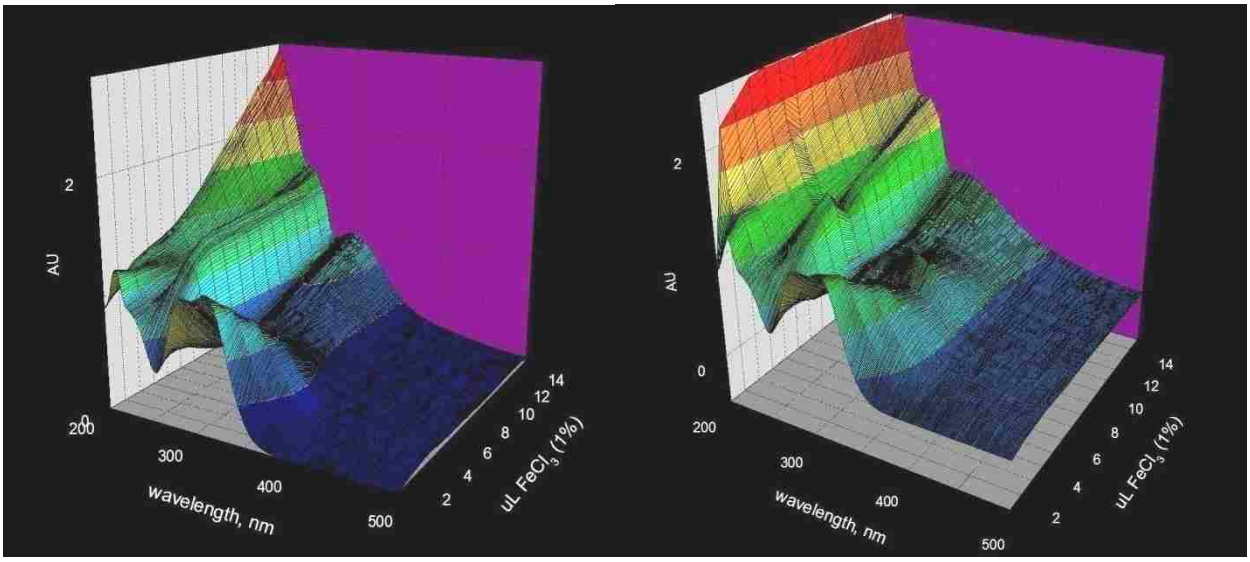


Figure 3.9. Caffeic acid with, right and without, left, glutamine in a sucrose matrix treated with Fe^{3+}

3.3.3. Conclusion/Discussion

Attempts in our laboratory failed to polymerize the phenolic compounds to a molecular weight sufficient to precipitate them from clarified cane juice.

These tests demonstrated that Fe^{3+} can markedly increase the color of clarified juice and syrup (Anjal, et al., 1974), which is in agreement with the literature (Riffer, 1986). The observed spectra are consistent with the oxidation of the dihydroxy moiety of CFA to yield quinoid species.

The evolution of an absorption band at ~300 nm at the expense of the carbonyl absorbance at 210 nm is consistent with reaction of the quinoid carbonyls. This reached a maximum when 140 μg/mL of Fe^{3+} had been added. The peak at 300 nm decreases upon addition of further Fe^{3+}. We suppose that the increasing absorbance at 400 nm reflects increased conjugation resulting from polymerization of the quinones and un-oxidized *o*-dihydroxyphenolic species. These systems did not precipitate color when treated with neither anionic nor cationic polymeric flocculants.

Exploration into the limitation and mechanistic operation of this technique emphasized the complexity of cane juice and the difficulties which lie in the deconvolution of any particular chemical system operating within it.

In order determine how this system works, we examined models which were made to resemble cane juice, in terms of composition. Following this, the optimized system was tested on raw cane juice.

3.4. Behavior of Raw Cane Juice in the Presence of Iron (III).

It was hypothesized that the reason the application of iron to syrup failed to precipitate phenolics was that either the concentration of participating species was too low, or that the kinetics were too slow to offer the desired result in a useful span of time. In either case, the material removed from the juice during the clarification process must be accounted for. It was realized that the primary component which is removed during hot-lime clarification was protein. Raw juice contains bulk albuminoid protein which is not found in clarified juice.

We supposed that quinones, which are susceptible to nucleophilic attack by other phenolic species, might also be labile toward the addition of other nucleophilic species. The free amino groups (Kroll and Rawel, 2001), specifically the N^{ε}-amino groups present of lysine, and the sulfhydryl moieties of cysteine fill that niche.

The hypothesis was that the free amino groups in the protein would lend themselves to cross-linking via *in-situ* induction of quinoid species (**figure 3.10**). To test this, Fe^{3+} (Mallinkrodt, $FeCl_3$-$6H_2O_{(aq)}$) was added to raw juice in increasing concentrations ranging from 0-1600 μg/mL.

Figure 3.10. Conversion of *o*-dihydroxybenzenoid compounds to *o*-quinones via REDOX cycling of Fe with O_2.

The iron treatment was followed by liming step. Two permutations of the liming step were tested. They were both two-step processes whereby the juice was treated with iron while at either ambient (22-24 °C) or boiling temperature (100-101 °C). After dosing with Fe^{3+} (2 minutes) both were boiled, neutralized with lime and treated with 5 μg/mL of LT-340 (Ciba) anionic polyacrylamide.

3.4.1. Materials and Methods

To mixed cane juice was added, with stirring, an aqueous solution of $FeCl_3 \cdot 6\ H_2O$ made to contain 1.35 g/100g of Fe^{3+}. For the initial tests, the Fe^{3+} was added drop-wise until an effect was observed. Later, these drops were weighed to establish that Fe^{3+} in a range of 400-500 μg/g routinely gave satisfactory results. Following these tests, Fe^{3+} in the range of 50-1600 μg/g was tested on juices

provided by three Louisiana sugar mills. The juices were treated with Fe^{3+}, incubated for 20 minutes at ambient temperature (24 °C) and centrifuged at 4 kRPM for 10 min.

The centrifugate was quickly brought to a boil and treated with lime to a pH of 7.2 (electrode with temperature compensation). The hot mixtures were treated with 5 μg/mL of an anionic polyacrylamide, Magnafloc LT-340 and allowed to settle.

The juice was decanted and re-centrifuged, as before.

The color was determined by the ICUMSA method and pH was assayed using an electrode.

3.4.2. Results

When applied to *raw juice* at ambient temperatures, Fe^{3+} led to a removal of color with a corresponding reduction in turbidity. The color removal was concentration-dependent. This behavior, seen in **figure 3.11,** is an average of three replicates (mean RSD, % =14.5), represented by one sample each from three different Louisiana sugar mills. ICUMSA color and percent decolorization are given in **figure 3.12**. In all cases, there was a significant reduction in the amount of the pH dependant colored material. This decrease in the indicator values (IV pH 9/3) are given (inset) for iron dosages of 0-500 mg/kg.The observed behavior suggests that phenolic components were removed.

It can also be observed from **figure 3.12** that at higher doses, the amount of color removed appears to decrease. In fact, the color observable with the eye increases over the control. Beside the increase in ICUMSA color, the absorbance at 590 nm (violet) also increased. This was observed as the appearance of an absorption band for phenol:iron complexes (Tomiyasu, et al., 2000). It should be noted that the peak at 590, though broad, is not large enough to interfere with the absorbance measured at 420. The net result is a global increase in color which is sepia (dark brown/black) in color.

When the juice was treated with 450 mg/kg Fe^{3+} at ambient temperature and limed, 62 %, on average, of the color was removed. A sample of this juice, made using 322 μg/g of Fe^{3+} at ambient temperature followed by hot-liming, is compared with a hot-limed control in **figure 3.13**.

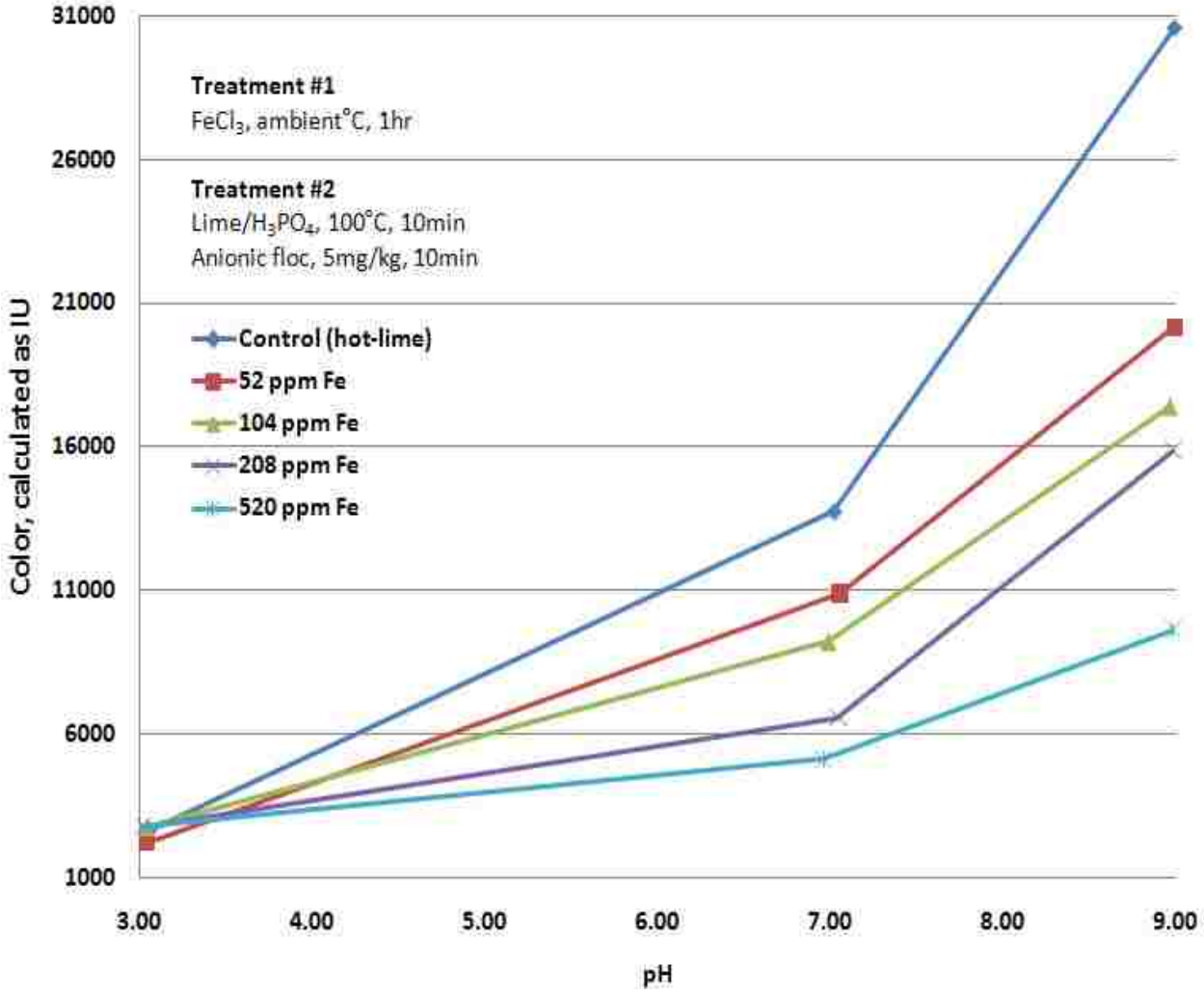


Figure 3.11. Average of three replicates of raw cane juice treated with increasing concentrations of Fe^{3+} at ambient temperature.

Adding the iron when hot, however, yielded the expected result at first, but then the juice darkened rapidly upon exposure to air after liming. The increase in color is empirically similar to that observed with the larger iron dosages (500-1600 mg/kg). The pH of the final first (ambient-$FeCl_3$) and second (Hot-Lime) stage juices are shown in **table 3.3**.

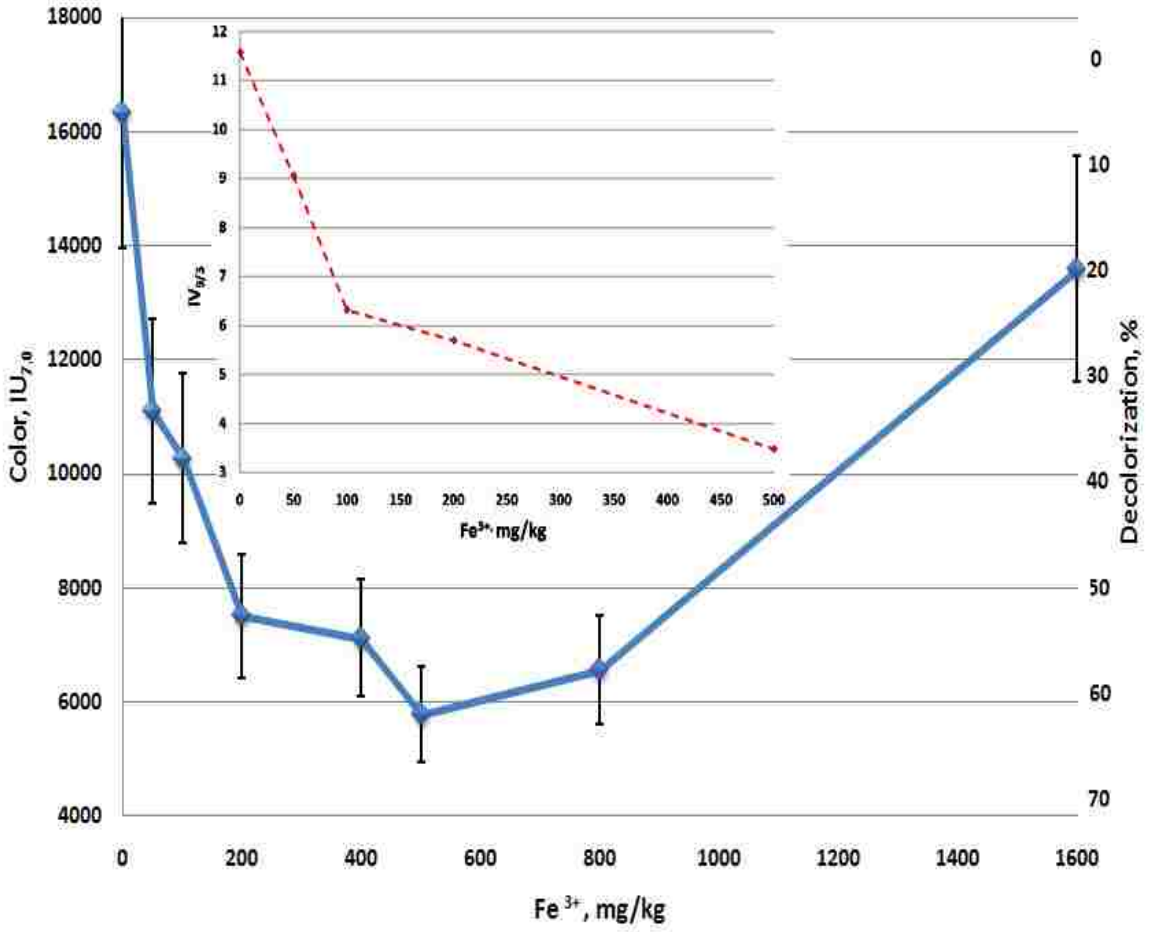


Figure 3.12. ICUMSA color of three juices from three mills with respect to added Fe^{3+}.

Table 3.3. pH of first and second stage juices relative to iron dosage.

Sample:	Fe^{3+}, ug/g:	Stage 1 pH:	Stage 2 pH:	Color, IU:
Juice	0	5.98	6.31	8806
50	45.82	5.54	6.21	9177
100	91.63	5.20	6.27	6875
150	137.45	4.80	5.89	6993
200	185.18	4.42	5.81	6038
250	230.99	4.18	6.10	5721
300	276.81	3.84	6.23	5580
350	322.63	3.55	7.07	2632
400	368.44	3.30	6.16	3260
450	414.26	3.04	7.17	4038
500	461.98	2.80	6.77	4383
600	553.62	2.53	6.00	7711

Figure 3.13. Left, raw juice clarified normally via hot liming and right, treated with Fe^{3+} the hot-limed.

3.4.3. Conclusion/Discussion

The color responded to the dose of Fe^{3+} and led to a reduction in color which, reached a maximum, on average, of 62 %, relative to the control The sample in **figure 3.13** exhibited a reduction in color of 70%; this appears to be upper limit of the color that can be removed by the addition of $FeCl_3$ whilst ambient followed by hot-liming. Addition of the iron to boiling juice initiated a situation whereby the profound color formation would occur upon liming and exposure to air.

It is hypothesized that the larger polyphenolic molecules are being oxidized to quinones which then serve to crosslink the native protein (probably via the ε-amino group of lysine residues). Such cross-linking has been described with salivary proteins and tannin (Cai and Bennick, 2006), bovine serum albumin (BSA) and epichatechin (Chen and Hagerman, 2005) and protocatechuic acid (Bartoleme, et al., 2000), catechol and both S- and N- terminal groups of protein (Schwiegert, et al., 2001), and chlorogenic acid, a known component of cane juice, with lysozyme (Rawel, et al., 2000).

These large adduct molecules are likely charged under the conditions employed and thus serve as flocculent/coagulants themselves, viz. a coagulant synthesized *in-situ* from the native materials found in the raw juice.

This type of relationship was noted between protein and indigenous cane polysaccharides in refined sugar (Du Boil, 1997), but not with cane polyphenolics in raw juice. Since the protein is largely removed during hot lime clarification (Martinez, et al., 1987), most likely the result of denaturing at clarification temperatures, this process *does not work* with clarified juice and subsequent products.

In order to explain the increase in color that occurs at high temperature and low pH, we hypothesize that it was possible that at least four concomitant reaction-types were occurring. First, the protein was denatured and released any iron that was chelated. Second, larger phenolic adducts were hydrolyzed (or otherwise disrupted) to cause the bulk release of small, reactive phenolic species. The free iron formed complexes with the phenols to yield green iron-semiquinone species. These species then reacted with air and re-polymerized to yield a brown/black polymer.

The observed color was consistent with that witnessed upon reaction of CFA with glutamine in the presence of air (described later). The polymer so formed was of insufficient size or charge to precipitate or form floc.

The involvement of air in the formation of color suggests that a radical mediated reaction is taking place. Because Fe^{3+} is added to our mixtures which contain reducing/antioxidant substances (o-phenols), we reason that Fenton-type reactions were established. At room temperature, and acidic pH (optimum pH=4.5, range 3.0-6.0), Fenton-type chemistry is relatively slow (Bishop, 1968; Cheves, 1975). This is consistent with the stability, relative to air, of the samples when at low pH. This could be offset by addition of large quantities of iron, which would lead to higher concentrations of radical species, even at low temperatures.

When the pH is adjusted using lime, the model enters the optimal range for the Fenton reaction.

Since the lime is added hot, the chemistry should demonstrate an Arrhenius dependence on rate (approximately 16 times faster than at rt.). We suspect that some amount of the iron bound to free phenols facilitating autooxidation via O_2 and the rest was reduced to Fe^{2+}, which initiated a Fenton cycle top yield hydroxyl radicals which proceeded to either oxidize and damage the nearest substrate (perhaps a protein, where the Fe was initially bound) or to disproportionate yielding peroxyl radical.

3.5. A Representative Model

In order to ascertain whether our hypothetical mechanisms were correct, it was decided to use models to mimic the behavior witnessed with cane juice.

3.5.1. Materials and Methods

Two sets of samples were prepared, one contained Fe^{3+} (220 μg/g) and the other was an untreated control. Assuming a dependence upon protein and phenolic material, both sets contained water and either 0.5 g/100g bovine serum albumin (BSA,Sigma, Cohn fraction V, 69 kDa), 900 μg/mL caffeic acid (CFA, Aldrich, 99+%) or both.

Initially, it was supposed that both calcium and phosphate would be required, so these samples were prepared to contain approximately 400 μg/mL Ca as $CaCl_2$ (Fisher, ACS) and 300 μg/mL phosphate, applied as H_3PO_4 (Fisher, 85%).

The test was repeated in a matrix containing 50 g/100g of sucrose.

3.5.2. Results

When the iron was added, both samples immediately became turbid. The sample containing caffeic acid turned green/black. Centrifugation of all samples (4 kRPM, 15min) yielded the results seen in **figure 3.14**, below.

The reaction of CFA with GLN can be seen in **figure 3.15**. The CFA/Fe^{3+} was initially green, but on storage (16 hr) turned brown and contained particulates. The sample with GLN immediately turned black and formed stable particulates.

Glutamine, alone, did not react with Fe^{3+}. Other amines, eg. 2-aminopropanol, behaved in a similar way with respect to color.

Figure 3.14. Effect of Fe^{3+} on BSA both with and without caffeic acid, and controls (right).

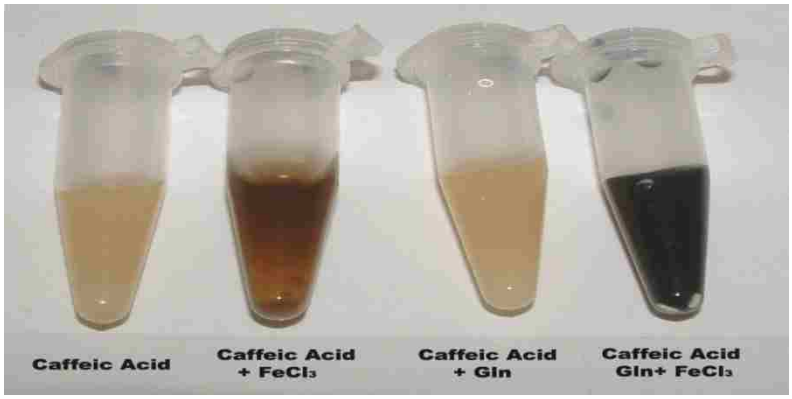


Figure 3.15. The appearance of caffeic acid treated with iron either alone, or in the presence of L-glutamine.

The replicate which contained sucrose did not react when iron was added. These samples turned green and remained transparent, regardless of the content of amino nitrogen.

They slowly developed into green, turbid mixtures. The material was colloidal and did not coagulate. Centrifugation at 4 kRPM for 20 min was insufficient to sediment the particles.

3.5.3. Conclusion/Discussion

It appears that the $Ca_3(PO_4)_2$ precipitates regardless of BSA or CFA content. The Fe^{3+} caused the BSA to precipitate. The color change in the sample was consistent with CFA reacting with an amine, not merely the formation of the green iron semi-quinone complex (which becomes reddish brown, slowly, in the presence of air as polymerization occurs).

From this point forward, we continued to operate under the assumption that the caffeic acid, the phenolic surrogate, is being oxidized (via Fe and O_2), and is removed along with the protein (BSA) which is serving as the vehicle.

The addition of sucrose stabilized the complex(es) forming stable, non-colored colloidal material which failed to settle, even with centrifugal assistance (4 kRPM/10 min was insufficient).

3.5.3.1. Triggering the Precipitation of BSA:CFA from Solutions Containing Sucrose

In order to determine why the BSA:CFA failed to precipitate when in the presence of sucrose, and, more importantly, to find a way around it, we consulted the "known" composition of cane juice. It was noted that cane juice contains a large amount of carboxylic acids. The addition of various carboxylic acids and other carbonyl compounds (aldehydes and ketones) was tested to determine if the precipitation of sucrose-stablized protein could be triggered.

3.5.3.1.1. Materials and Methods

All compounds save pentanoic acid, sodium salt (sodium valerate), were purchased at ACS or analytical grade and were used as is. The amount of each chemical was adjusted to compensate for purity and adsorbed moisture and/or water of hydration (determined via gravimetry).

Sodium valerate was prepared from valeric acid (pentanoic acid, Aldrich, 99%, 10g, 0.1M) via slow, stoichiometric addition, with magnetic stirring, of chilled NaOH (ACS) diluted to 1M.

The precipitate was filtered (Buchner), washed with water until pH ~ 4.5 and dried in-vacuo (26" Hg, 60 °C) overnight. The product, a pure white (microcrystalline) material releases heat upon solution, and assayed 99+% via HPLC-HAEC (suppressed gradient HPLC anion exclusion chromatography).

Many attempts and permutations which involved 16 compounds, were tested and are listed, with the model parameters (quantities of sucrose, BSA, CFA and iron), in **table 3.4**.

The samples were made to contain, in order, sucrose (prepared as a 50 g/100g solution), BSA (prepared to contain 6050 μg/g), CFA (an aqueous suspension containing 8780 μg/g), the test compound (each diluted to 10 mMol) and Iron (29,170 μg/g as Fe^{3+}, $FeCl_3 \cdot 6H_2O$, Fe $x_j = 0.20$). The samples were kept at ambient temperature (24 °C) for 20 minutes and then centrifuged for 10 min at 4 kRPM. Success was measured by the presence of a dense pack of green/black precipitate in the bottom of the tube.

Table 3.4. Compound test matrix.

Test Compound:	Sucrose, g/100g:	BSA, mMol:	CFA, mMol:	Fe^{3+} mMol:	Agent, mMol:
AcONa Buffer, 1M*	31	3.9E-05	9.3E-04	1.00E-02	0.35
AcONa Buffer, 1M*	31		0.0E+00		0.35
AcONa Buffer, 1M*	31		9.3E-04		2.35
Methanal	31				0.35
Propanal	31				
Isobutyral	31				
Isopentanal	31				
Benzaldehyde	31				
Phenylacetaldehyde	31				
Methanoic acid	31				
Ethanoic acid	31				
Propionic Acid	31				
Butanoic acid	31				
Pentanoic Acid	31				
Citric Acid	31				
Aconitic Acid	31				
Acetone	31				
Acetylacetone	31				

3.5.3.1.2. Results

It was found that neither the n-aldehydes (methanal-isopentanal) nor the neat acids (methanoic-pentanoic) caused BSA to precipitate from solutions of sucrose.

The salts of these acids (acetic and propionic), however, triggered the immediate formation of color and concomitant precipitation of protein/phenolic adducts. The pH attributed to the addition of the various salts was consistent, averaging 4.5. Other carboxylic salts were subsequently investigated.

Sodium salts of formic, acetic, propionic, butyric and valeric acids triggered the precipitation of BSA:CFA adduct from solutions containing sucrose. Ammonium acetate triggered precipitation of BSA at a concentration equivalent to an effective dose of the Na^+ salt. The observation that ammonium and sodium acetates possess equivalent activity indicates that the choice of counter ion is of little consequence. The carboxylate ligand is most likely the active principle.

It was noted that the amount of sodium acetate required, 0.5 mMol, was significantly less than that needed for sodium formate, 0.68 mMol. It was subsequently determined, as in **table 3.5**, that the length of the carbon backbone was inversely related to the minimum mole-equivalent of salt that was needed to trigger the precipitation of BSA. A plot of the effective quantities, in both mass and moles, is given in **figure 3.16**. Other materials conform with **table 3.4**. The minimum concentrations that triggered precipitation of the BSA were made bold.

Table 3.5. The quantity of each carboxylic acid salt needed to precipitate BSA from sucrose solution.

Test Compound:	mMol:	mMol:	mMol:	mMol:
Sodium formate	0.25	0.40	0.55	**0.69**
Sodium acetate	0.25	0.38	**0.50**	0.63
Ammonium acetate	0.25	0.38	**0.51**	0.64
Sodium propionate	0.25	0.33	**0.40**	0.48
Sodium butyrate	0.25	0.27	0.32	**0.36**
Sodium valerate	0.25	**0.32**	0.50	0.60
Sodium oxalate	**0.25**	0.38	0.51	0.64

3.5.3.1.3. Conclusion/Discussion

Because of the threshold in the length of the carbon "tail", the carboxylates described here are too small to be creating a formal micelle (Ueno at al, 1981). The critical micelle concentration (CMC) reaches an asymptotic value at chain lengths of ~10 (CMC = $85.91e^{-1.146carbon\#}$). The extrapolated CMC for NaOEt would be ~8.74 mol/L or ~7.6%; the effect we are observing takes place at concentrations ~24,000 times less. Thus, It is possible that the salts accommodate either ligand assisted phase transfer or are acting as chain transfer catalysts (Fevola, 2003), perhaps to propagate $^{\cdot}OH$ or $^{\cdot}OOH$ radicals. Sodium oxalate also causes BSA to precipitate and it does so at the lowest concentration of all of the compounds tested (½ of that needed with acetate).

Because oxalic acid is both small (MW = 90.03 g/mol) and a dicarboxylic acid, it has two polar ends and very little of the hydrophobic character associated with a large alkyl "tail". The 2:1 stoichiometry of oxalate, relative to acetate, tends to rule out micellar activity and adds credibility to the ligand assisted phase-transfer hypothesis.That is, one-half of oxalate = acetate, in terms of efficiency in this system. Because the destabilizing effect is observed with terminal carboxylates, and the effect is quantitative, it is unlikely that the oxalate is behaving as a difunctional bridging species. This is of special consequence because in cane juice, oxalic acid constitutes, along with citric (2-hydroxypropane-1,2,3-tricarboxylic), aconitic (prop-1-ene-1,2,3-tricarboxylic), succinic (1,4-butanedioic), glycolic (2-hydroxyethanoic) and malic (2-hydroxy-1,4-butanedioic) acids, approximately 13 % of the nonsugar fraction (Payne, from Honig, 1953). The sodium salt-forms of these acids have yet to be tested in the outlined model system.

Since chemicals are sold on weight, rather than moles, sodium propionate appears to be optimal. This behavior is described in **figure 3.16**.

It was initially thought that protein stabilization via sucrose should only be a factor in highly concentrate sucrose solutions, viz. >50%, as the polarity decreases with increased concentration.

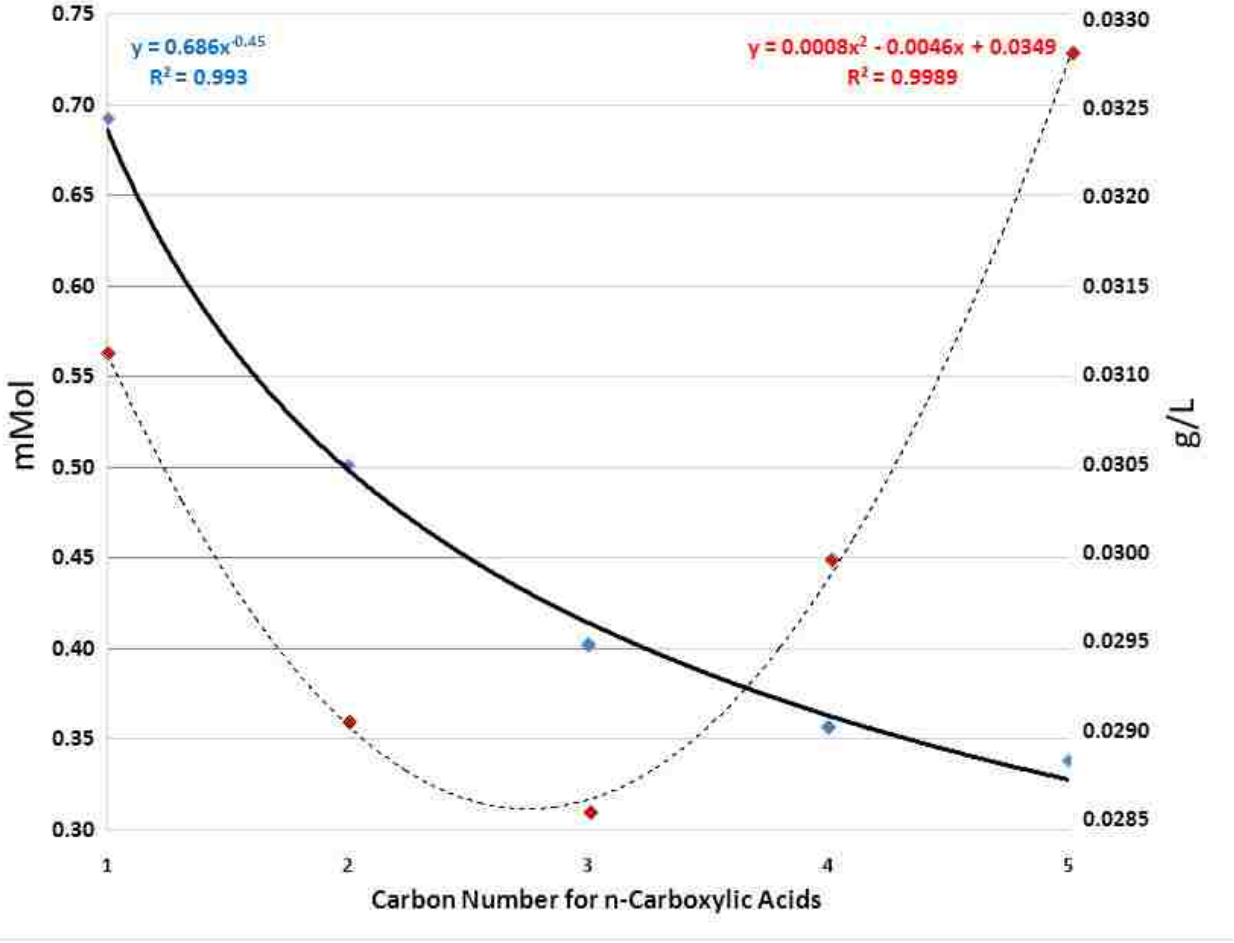


Figure 3.16. The dependence of the chain length of carboxylic acid Na^+ salts on the amount, in mMol, blue and g/L, red, on the Fe^{3+} that triggered formation of insoluble aggregates of caffeic acid and BSA.

The calculated values from 0 to 85% $sucrose_{(aq)}$ predict a change in dielectric constant from ~80→40. This is a function of free water/total water, which decreases markedly at sucrose concentrations in excess of 60g/100g where much of the available water is involved in the sucrose hydration sphere (Barbosa-Canovas, 2003; Starzak and Mathlouthi, 2005).

This was found to be less significant when further tests revealed similar behavior in more dilute solutions. In solutions containing sucrose approximate to juice (10-15 g/100g, polarity approximate with water) the precipitation required the carboxylic acid salt. It was concluded that if sucrose is present in *great* excess (the threshold appears to be ~10 g/100g) over the other components, particularly the protein, then the observed stabilization effect (and salt requirement) will occur.

3.5.3.2. Optimization of the Model

We now knew that four components, iron, protein, lignol and carboxylic acid salt were required to initiate coagulation of protein in the presence of sucrose. We did not, however, know how much of each is required. Toward this, experiments were conducted to determine not only the optimum concentration of each component, but also to discern the practical limitations of the method. Because the colored materials found in raw sugar have propagated through the entire cane sugar manufacturing process, these experiments took place in a dilute raw sugar solution.

3.5.3.2.1. Materials and Methods

We optimized the concentration of salt required for our process to work. We chose to use sodium acetate because it is abundant in cane juice. Ferric chloride (assayed 15,960 μg/mL Fe^{3+}), CFA (8501 μg/g), BSA (0.60 g/100g) and sucrose (50.08 g/100g) were sourced and prepared as described. All volumetric deliveries were made using Eppendorf pipetters that were pre-calibrated by weight.

Into 9 x 15 mL polyethylene conical centrifuge tubes was added 8 mL of a raw sugar solution prepared to contain 50.08 g/100g. To each tube was added 4 mL of BSA (1846.2 μg/mL) and 0.6 mL of CFA (392.4 μg/g). In the case where sodium acetate (NaOAc) was the dependent variable, 0, 25, 50, 75, 100, 125, 150 and 200 μL of NaOAc (1M, pH 5.0) was added to tubes 2-9. Tube 1 was a control which contained neither BSA nor NaOAc. The tubes were sealed and mixed by inverting slowly three times. To each tube was added 200 μL of $FeCl_3$ solution (245.5 μg/mL). The tubes were sealed, inverted slowly once to mix, and allowed to sit at ambient temperature (22 °C) for 20 minutes prior to centrifuging at 4 kRPM for 10 min.

The samples were photographed and aliquots were corrected to pH 7.0±0.1 (electrode) using either dilute HCL or NaOH, filtered through 0.45 μm nylon membrane filters.
The absorbance of the samples was measured at both 420 and 590 nm (Beckman Coulter DU-800).

These steps were repeated with each variable to be tested, namely CFA, AcONa and Fe^{3+}.

Each model set was built upon the prior set, eg. The optimum value for NaOAc from set one was carried to the next set. The sets are given in **table 3.6**.

Table 3.6. Optimization of Model parameters, empirical quantities, A and mMol, B. Non-title bold numbers indicate the optimization range, A or maximum value tested, B.

			g/mol:
BSA:	0.6	g/100g	69200
Sucrose:	50.08	g/100g	342.3
Fe^{3+}	15960	μg/mL	55.845
CFA:	8502	μg/mL	180.16
NaOAc:	1	M	84.05

A

Set:	**BSA, mL:**	**$FeCl_3$, μL:**	**Sucrose, mL:**	**CFA, μL:**	**NaOAc, μL:**
NaOAc	4	200	8	600	**0-200**
CFA	4	200	8	**0-600**	200
Fe^{3+}	4	**0-294**	8	301	200
BSA	**0-5.2**	148	8	301	200

B

	BSA	**$FeCl_3$**	**Sucrose**	**CFA**	**NaOAc Max**
Set:	**mMol:**	**mMol:**	**mMol:**	**mMol:**	**mMol:**
NaOAc	0.00035	0.057	11.70	0.028	**0.017**
CFA	0.00035	0.057	11.70	**0.028**	0.017
Fe^{3+}	0.00035	**0.084**	11.70	0.014	0.017
BSA	**0.00045**	0.042	11.70	0.014	0.017

3.5.3.2.2. Results

Each optimization trial is outlined and discussed in order.

3.5.3.2.2.1. Carboxylic Acid Requirement Modeled Using NaOAc

This sample set was made with raw sugar to contain sucrose, 50.08g/100g; BSA, 0.17 %; CFA, 360 mg/kg; Fe^{3+}, 230 mg/kg and acetate, variable. A picture of this series is given in **figure 3.17.**

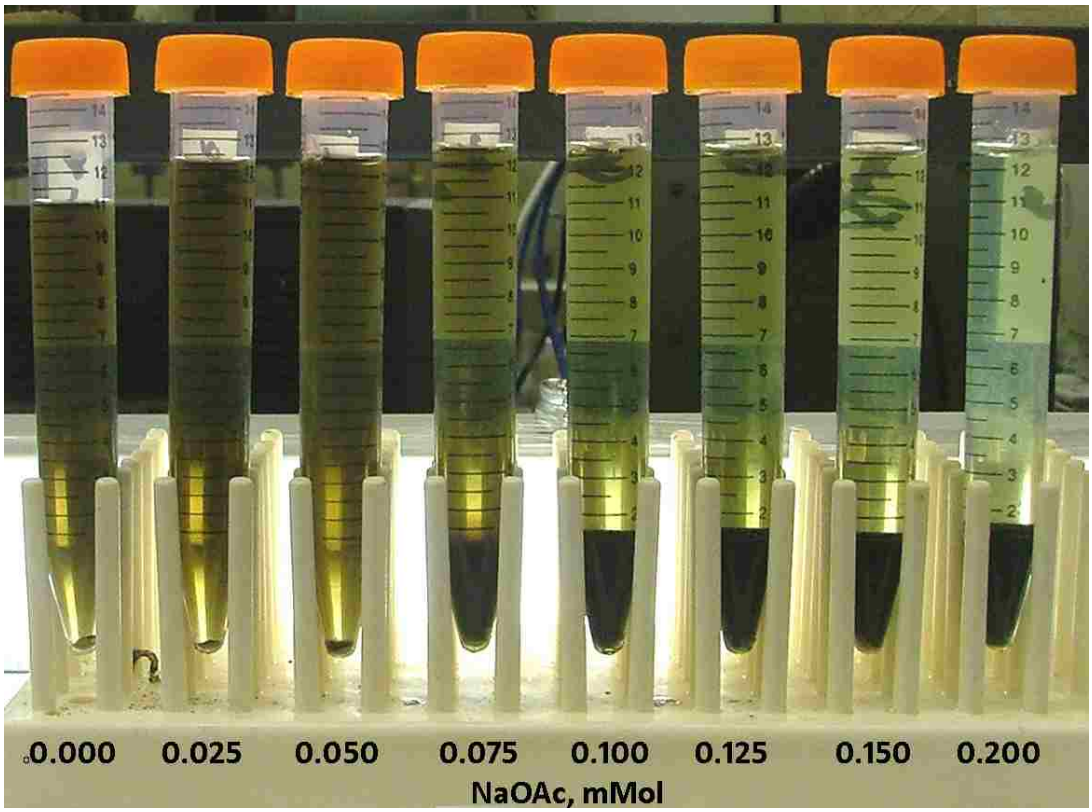


Figure 3.17. Samples treated with increasing amounts of sodium acetate.

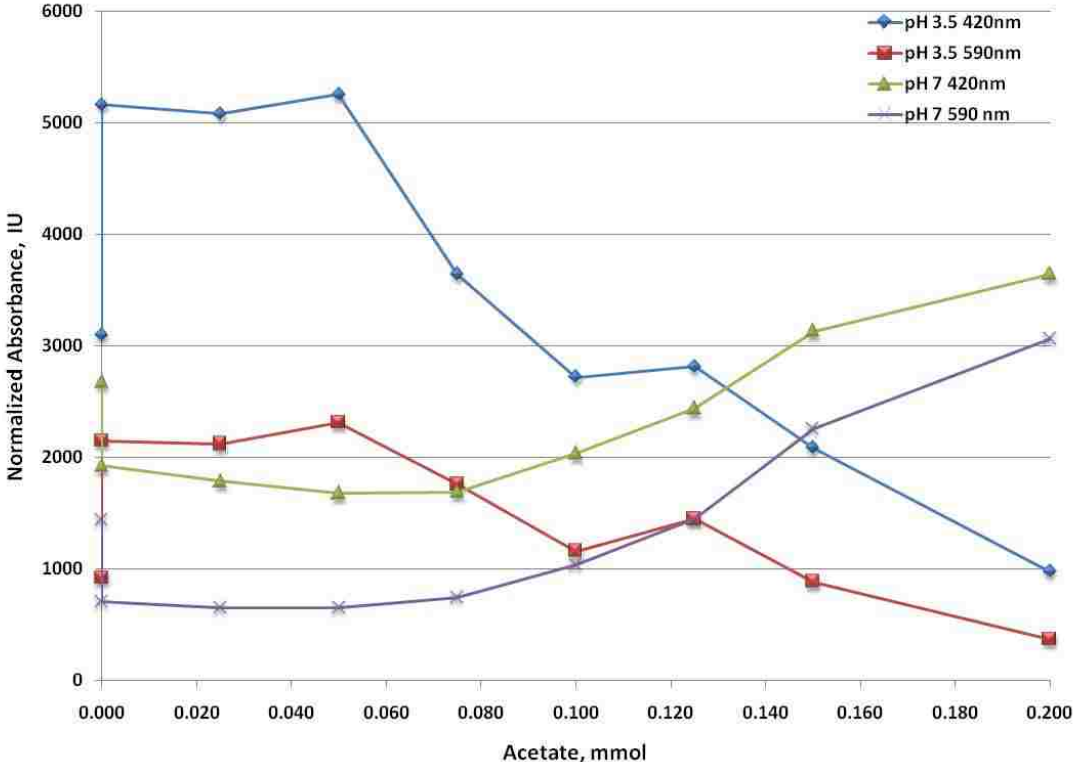


Figure 3.18. Color of samples in **figure 3.17** after pH adjustment and filtration.

3.5.3.2.2.2. *o*-hydroxyphenol Requirement Modeled Using CFA

Precipitation of BSA is dependent upon the quantity of phenolic material. This is seen in **figure 3.19** in solutions made to contain Sucrose, 50.08 % (50 bx), BSA, 0.17 %, Fe^{3+}, 226 mg/kg and variable CFA. It is apparent from this series that, from **figure 3.19**, there is a clear relationship between the amount of phenolic compounds present and the volume of sediment which evolves. From **figure 3.20**, however, it appears that there is also a fairly narrow range where sedimentation is abundant and the color remains manageable.

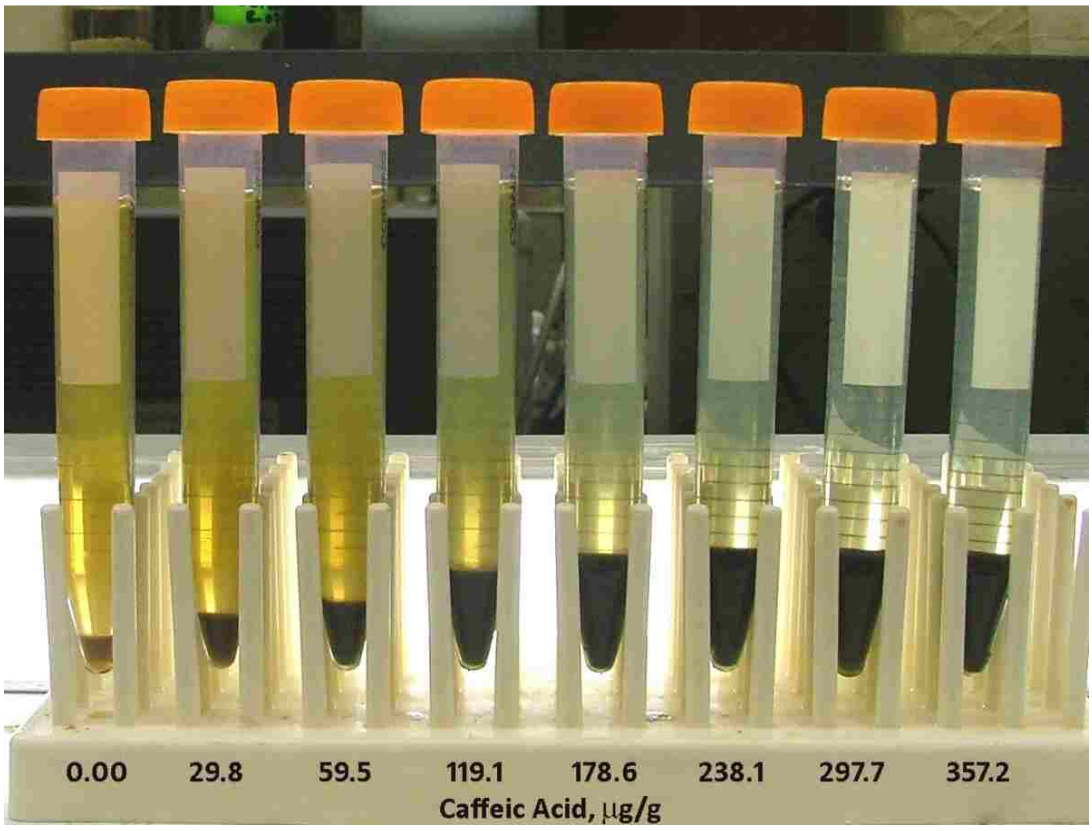


Figure 3.19. Samples treated with increasing amounts of caffeic acid.

Further tests have indicated a strong relationship of this quantity (of CFA) with the amount of protein present in the system.

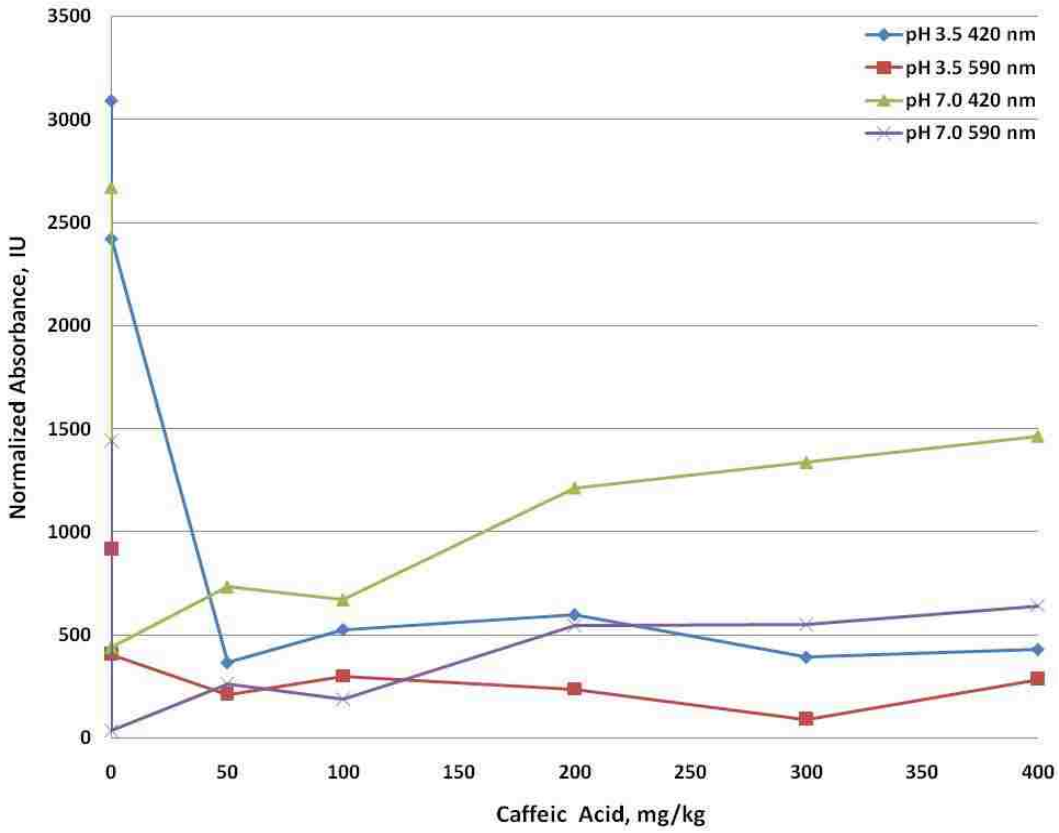


Figure 3.20. Color of samples in **figure 3.19** after pH adjustment and filtration.

3.5.3.2.2.3. Ferric Iron Requirement Modeled Using $FeCl_3$

Raw sugar solutions made to contain sucrose, 50.08 % (50 bx), BSA, 0.17 %, Caffeic acid, 60 mg/kg and variable Fe^{3+} were tested. The expected increase in sediment and concomitant color removal was seen up to ~226 mg/g Fe^{3+}. After this, seen in **figure 3.21**, the material remained quite dark. Adjustment of the materials to pH 7 and subsequent filtration via 0.45µm (nylon) removed the color yielding a consistent dose response curve which is given in **figure 3.22**.

3.5.3.2.2.4. Protein Requirement Modeled Using BSA

The optimal concentration of protein (BSA), relative to CFA, was determined as previously described. **Figure 3.23** was taken of a system optimized based on the previous tests (in terms of iron, CFA and acetate). The values used were Sucrose, 50.08 % (50 bx), BSA, variable, caffeic acid, 183 mg/kg and Fe^{3+}, 198 mg/kg.

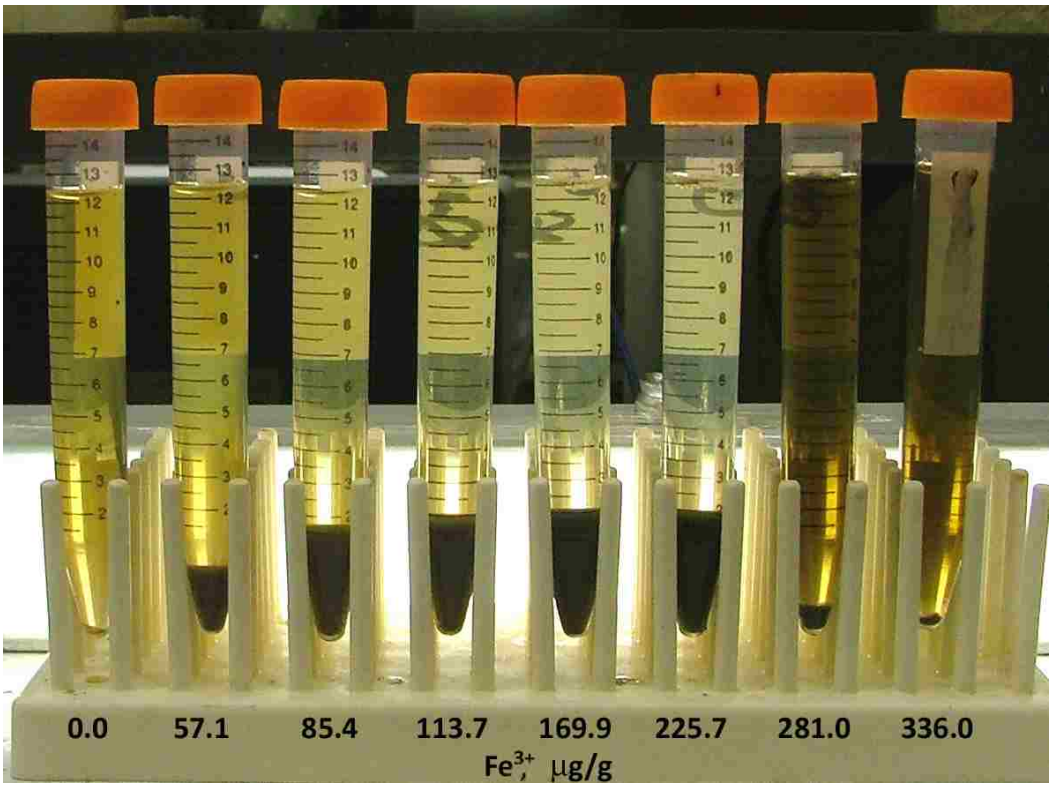


Figure 3.21. Samples treated with increasing amount of Fe^{3+}.

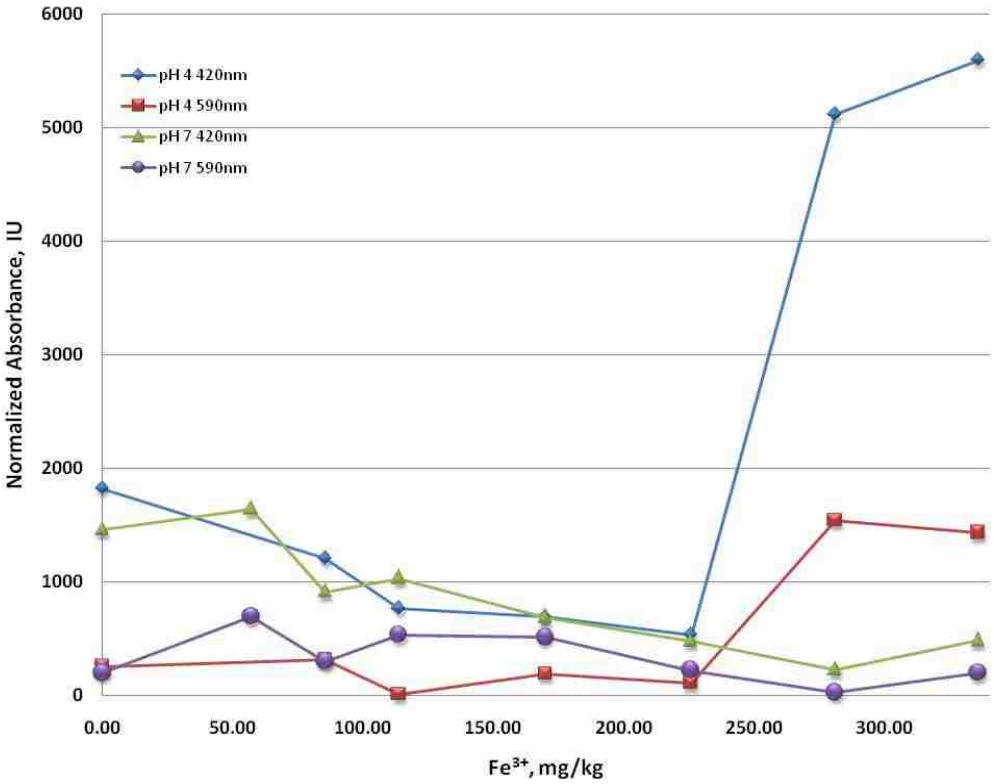


Figure 3.22. Color of samples in **figure 3.21** after pH adjustment and filtration.

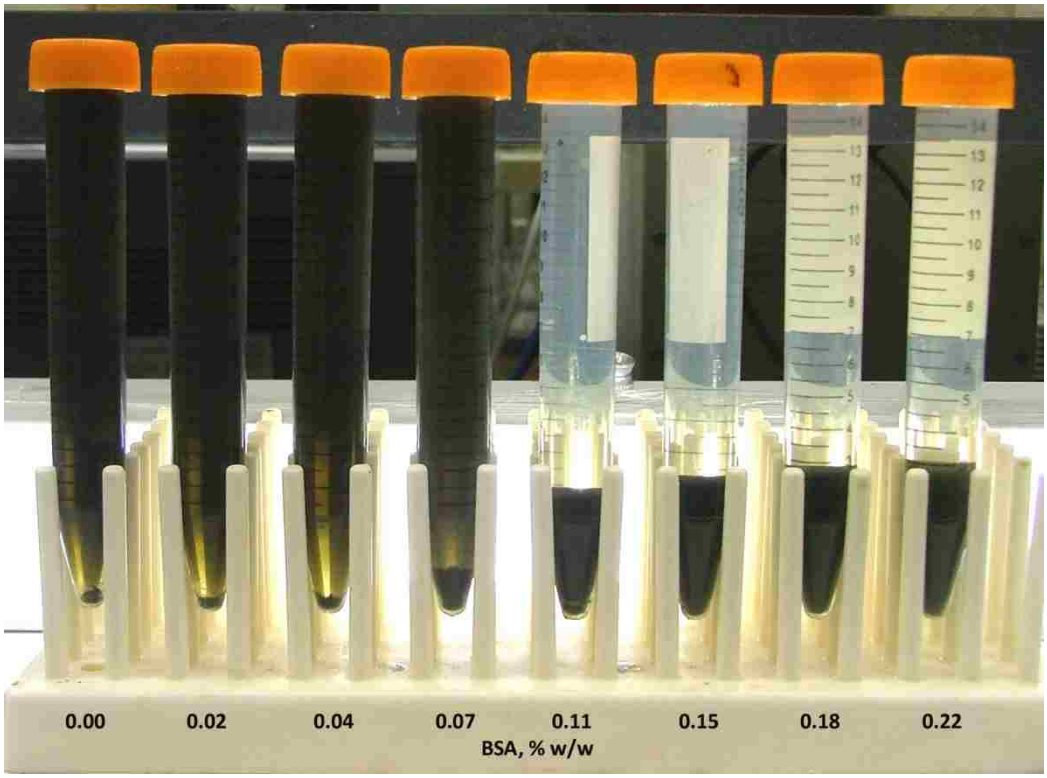


Figure 3.23. Samples treated with increasing amounts of BSA.

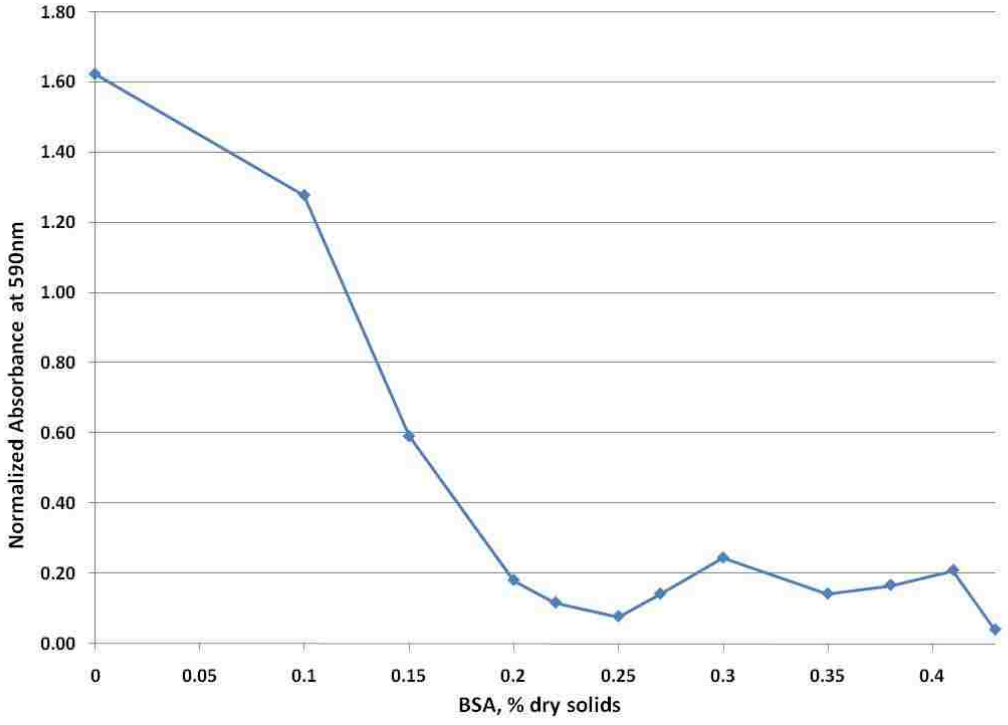


Figure 3.24. Removal of CFA:Fe complex (ABS_{590nm}) relative to quantity of BSA.

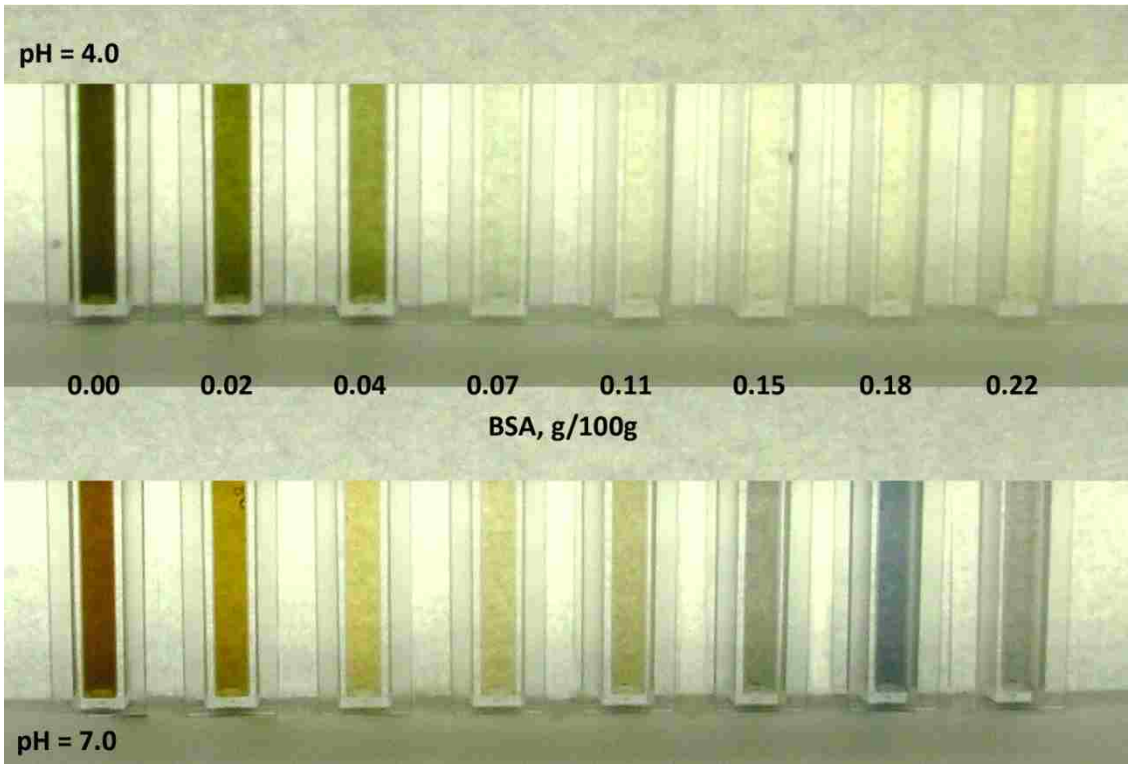


Figure 3.25. Samples from **figure 3.23** at pH 4, top, and 7, bottom.

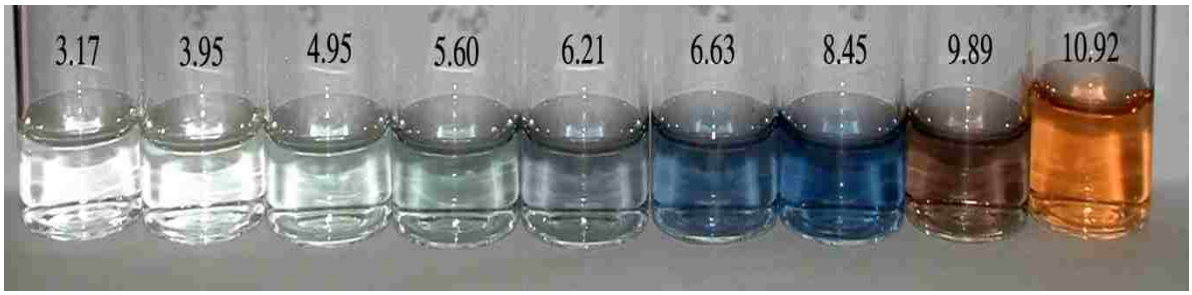


Figure 3.26. CFA:Fe^{3+} complex as an indicator from pH 3-11.

3.5.3.2.3. Conclusion/Discussion

In terms of NaOAc, the optimal value is not obvious from the acidic solutions. Upon neutralization and filtration, we can see that the optimum value is actually the point where we get the maximum volume of sediment (**Fig. 3.17**, 0.075-0.200 mMol NaOAc) and where the color is also acceptable. In this case, this value, seen in **figure 3.18**, is between 0.075 and 0.125 mMol. It appears, however, that the amount of material which is strongly colored at acidic pH is removed to an extent of approximately 80% and correlates well with the amount of acetate present.

For CFA, the optimum value lies between 60-120 μg/g (CFA in this idealized system). The amount of CFA, 119-180 μg/g, which is required for good precipitation to occur is rather close to the point of diminishing return. Nevertheless, in models containing sucrose, caffeic acid appears to be required at a minimum molar ratio, relative to BSA, of ~ 35:1.

The color which was discharged via liming and filtration was attributed to residual iron which was present at the higher iron dosages. Under these conditions, the pH adjustment did not increase the color, which appears to be $Fe(OH)_3$ (Gregory and Duan, 2001) which, upon precipitation, carried the remaining color, via adsorption, with it. Regardless of ultimate color removal, this iron level (>250 μg/g) is undesirable for our purposes. Where pH adjustment is routine, membrane filtration is *not*. Therefore, the optimum range established for the iron dosage should be between 150 and 225 μg/g.

Figure 3.23 demonstrates the efficacy of BSA quantities greater than or equal to 0.11 g/100g. The overall removal of iron:phenolic complexes is evidenced in **figure 3.24** by absorbance at 590 nm, and was approximately 90.6 % when BSA was present at 0.11 g/100g.

When adjusted to pH 7, the samples with protein ranging from 0.04-0.11 g/100g gave the best results, color-wise (absorbance at 420 nm). Of interest is that some residual iron must be present from 0.04-0.07 g/100g which precipitates as $Fe(OH)_3$ at pH 7.

While the initial colored materials appear to be removed, all concentrations greater than 0.11 g/100g led to the evolution of some blue material (**figure 3.25**). This nature of this material is unknown at present, but is consistent with the CFA:Fe complex at similar pH which is seen in **figure 3.26**.

In summary, the optimized values for this quaternary system (regarding raw sugar solutions at 50g/100 dry solids) are given in **Table 3.8**.

It is interesting to note, that when adjusted for the increased color of juice, (15,000 IU) the quantities of each of the four parameters modeled during this study are similar to those found in cane juice (van der Poel, et al., 1998).

Table 3.8. The quantities of each component needed for optimal color removal.

Component:	mg/kg:	In cane juice. mg/kg:	mMol eq. to BSA:
BSA	1500	Protein: 700-2200	1
CFA	150	Phenolics: 50-400	35
AcONa	2000	Carboxylic acids: 4100-6200	1450
Fe^{3+}	150-200	~10	117

3.6. Residual Iron

Residual iron can lead to green sugar (Riffer, 1986) which is known to deteriorate (color-wise) rapidly upon storage (Prasad, et. al., 1989). In order to determine if this will be a problem, raw juice was treated with iron (0-554 μg/g) and the juices and sediment were assayed for residual iron.

3.6.1. Materials and Methods

Briefly, 8 mL samples of raw cane juice (Cora Texas) were treated at ambient (24°C) temperature with $FeCl_3$ (aqueous, 16,641 mg/g Fe^{3+}) to contain Fe^{3+} ranging from 0-554 μg/g of juice. The samples were mixed by inversion and 10 μg/g of cationic polyacrylamide (Cytec-C445) was added. The samples were incubated at ambient temperature for 20 minutes and centrifuged at 4 kRPM for 10 min. Each liquor was decanted (liquor 1) and sampled.

The sediment was filtered through a tared filter paper (Whatman, medium, ashless), washed and dried *in-vacuo* (26" Hg/40 °C, overnight).

Liquor 1 was subject to hot liming and the product, liquor 2 was decanted. The residue from stage 2 was recovered as before. The weighed filter papers (with residues) were digested whole in aqua regia first via boiling, and then via ultrasonic treatment. The digest was filtered and diluted to 25 mL (weights were used for expression of results on mass).

The digests and juices were filtered, diluted in acetate buffer (1M, pH 5) and the background absorbance (510nm) was measured. To each sample was added hydroxylamine-HCl and *o*-phenanthroline. Briefly, to a 1.5 mL polystyrene cuvette was added acetate buffer, 600 μL, water, 500 μL, sample, 50 μL and then 150 μL each of hydroxylamine hydrochloride (Aldrich, metals grade 99.999%, 1% w/v) and *o*-phenanthroline (Sigma, spectro-grade, 0.2% w/v).

The cuvettes were sealed with parafilm and inverted to mix. The samples were allowed to react for 20 minutes prior to reading the absorbance at 510 nm (Spectronic Genesys 5).

The absorbance for each sample was read and compared against a six point curve prepared with standard Fe^{3+} (EM) to contain 0-7.5 μg/g Fe. Final values were calculated on mass basis.

3.6.2. Results

The composite results for this test (as total Fe) are given in **figure 3.27**. The stages indicated in the legend are defined as follows and are given as the total per cent of Fe in the dried solids.

Stage 1 liquor: the filtered liquid after treatment with iron and cationic polyacrylamide

Stage 1 residue: the material remaining on the filter

Stage 2 liquor: the filtered liquid after treatment of stage 1 liquor with lime and anionic polyacrylamide while hot

Stage 2 residue: the material remaining on the filter

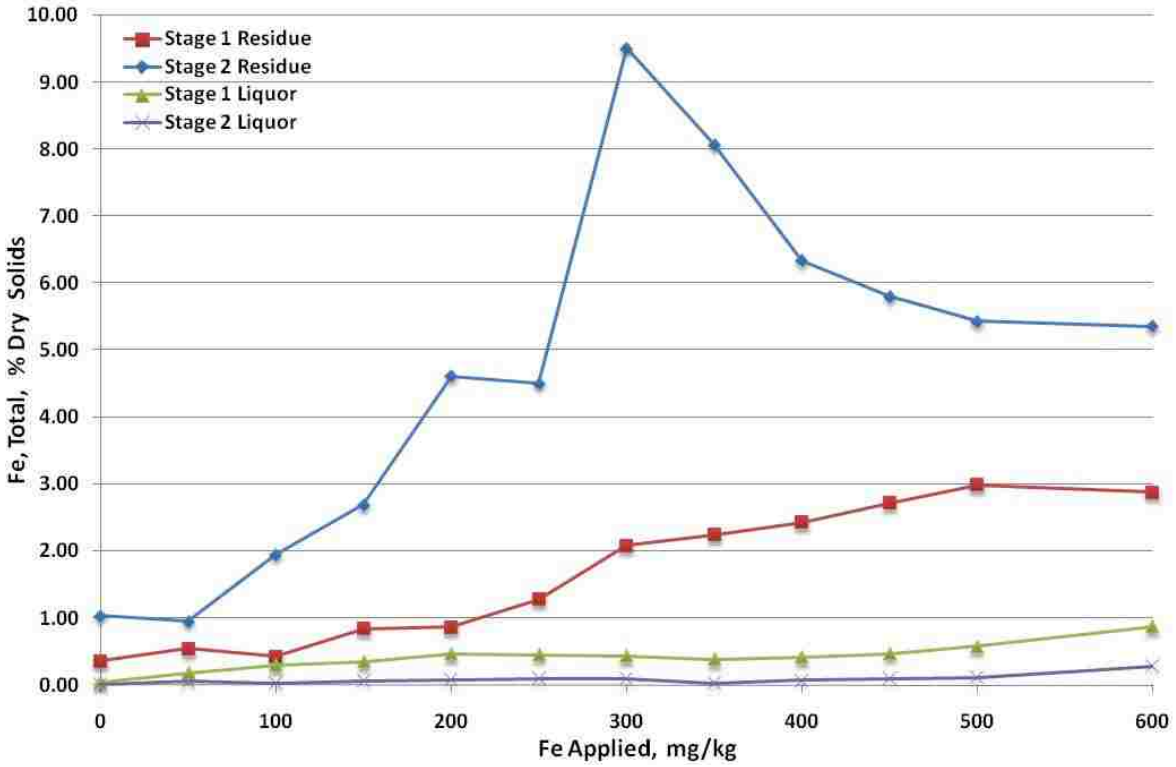


Figure 3.27. Residual iron in liquors 1 and 2 and in the precipitate collected from each stage.

3.6.3. Conclusion/Discussion

Significant iron, up to 3g/100g of dry residue, co-precipitated with the protein during the first, acidic stage. The remainder in solution, up to 9.5g/100g, was removed during the liming phase to produce a final juice with levels of iron similar to the intrinsic level found in milled raw juice. A compartmental accounting of the iron is given in **figure 3.27**. Blank filter papers digested similarly were negative for Fe.

Of interest is that in samples where the iron concentration exceeded the optimum range between 150-300 μg/g, the residual iron decreased and the liquor iron increased (it is not as apparent from the plot as the residue is 100% solids and the juice is perhaps 15%).

This suggests that once the protein:CFA "capacity" is exceeded, free iron accumulates, and leads to a rapid increase in color, particularly on exposure to air. This suggests the initiation of, perhaps, unchecked Fenton type chemistry.

3.7. Decolorization Relative to Temperature at Varied Iron Dosages

It was noted that the final, hot step operated in a hot-liming mode, that is, heated to boiling then treated with lime and anionic poly-acrylamide, was unpredictable. A study was made to determine how this system behaved relative to temperature.

3.7.1. Materials and Methods

Because there is insufficient protein in syrup for iron decolorization to work, diluted (15 g/100g sucrose) syrup was fortified with BSA (the optimized amount, 0.11 g/100g,). The pH of the starting material was measured (electrode) and 30 g samples were equilibrated to the testing temperatures (25, 45, 65, 75, 85 and 95 °C) for 5 minutes. The equilibrated samples were then treated with 500, 1000, or 2000 mg/g of Fe^{3+} and allowed to react for exactly 2 minutes (stopwatch). The pH was then adjusted to 6.8-7.5 (Hydrion papers) using lime and 5 μg/g of LT-340 anionic polyacrylamide was added. The liming operation took 5 minutes, and the samples were cooled and allowed to settle. The final juice was gravity filtered through fluted paper and assayed for residual iron as previously described.

An aliquot of each sample was diluted by weight to contain 0.25 g/100g refractive dry solids (Bellingham and Stanley critical angle refractometer). These samples were filtered through 0.45 μm nylon membrane syringe filters and analyzed via HPLC-DRI calibrated for sucrose, glucose and fructose. The HPLC results were compared against the Vukov-Schaffler inversion model which is described in **Appendix B**.

3.7.2. Results

A plot of the effect of temperature on color is given in **figure 3.28** (the control syrup color was 8000 IU), and residual iron is plotted in **figure 3.29.** The amount of sucrose (HPLC-DRI) hydrolyzed is described in **figure 3.30**.

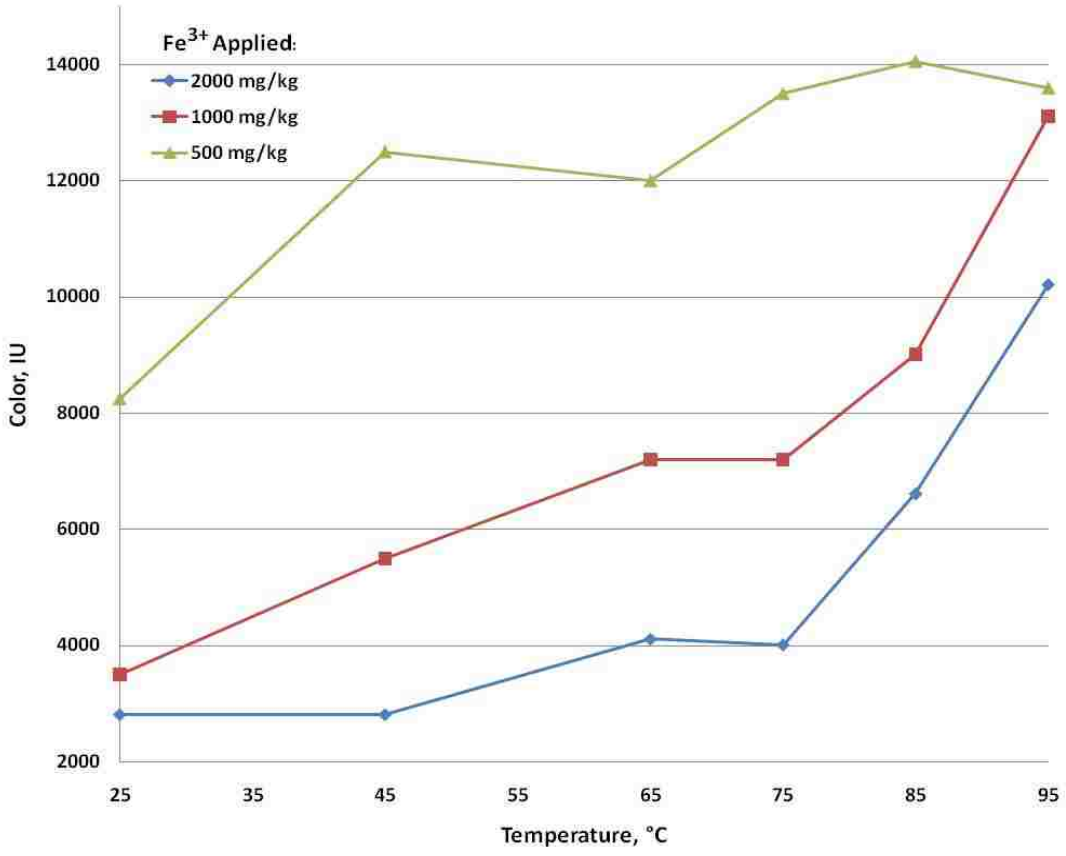


Figure 3.28. Color relative to temperature and iron dosage.

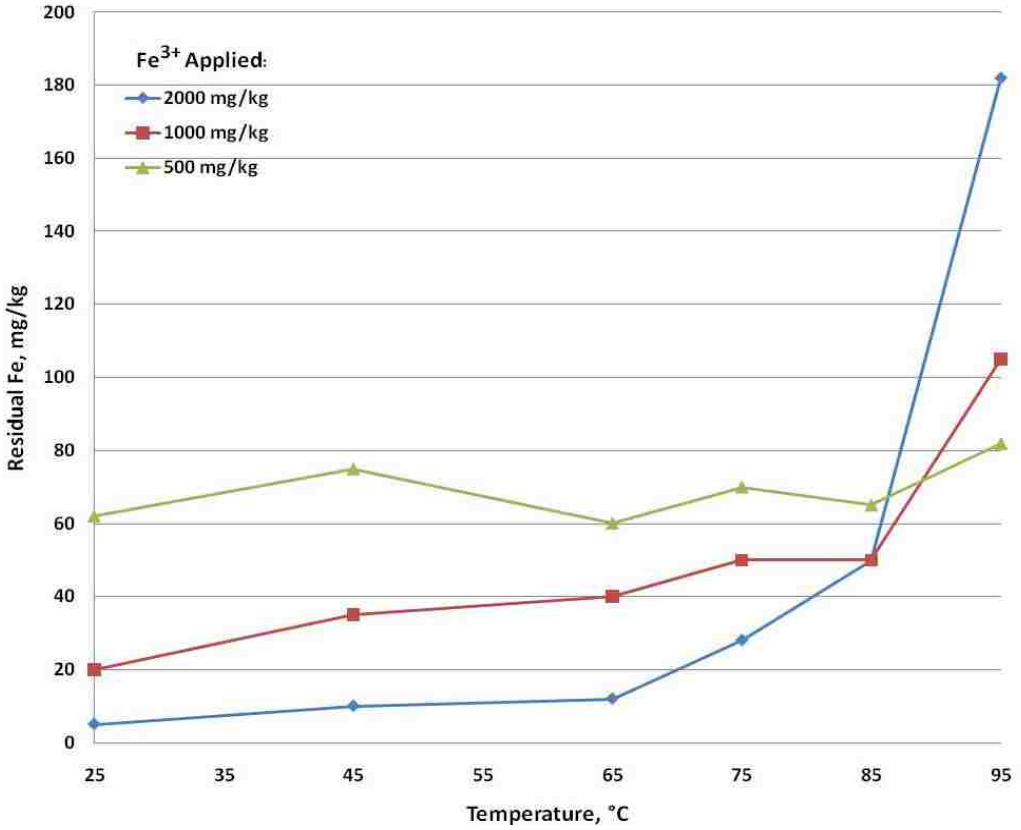


Figure 3.29. Effect of temperature on residual iron.

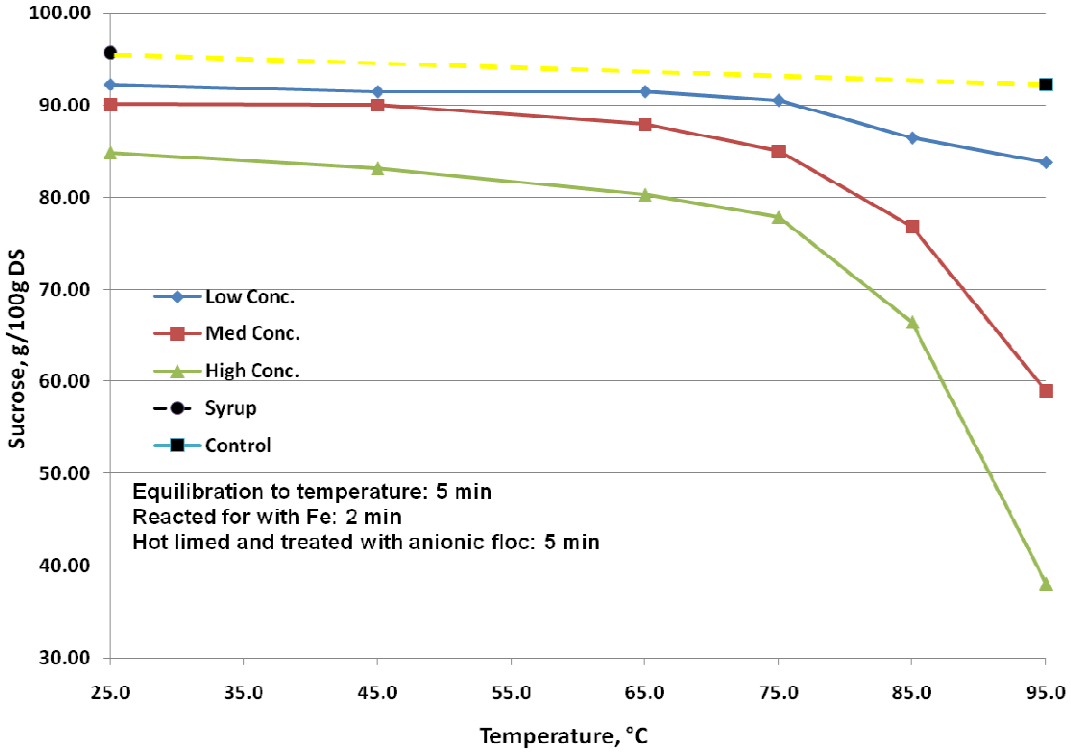


Figure 3.30. Sucrose as a function of operating temperature and iron dose.

3.7.3. Conclusion/Discussion

The amount of iron required to decolorize the diluted syrup (15 brix with BSA at 0.11 g/100g) was 2 to 4 fold larger than was required to obtain equivalent behavior in juice. This could be due to the fact that syrup contains caramel/melanoidin precursors as well as phenolic compounds. There is also the possibility that the indigenous cane protein is more amenable to precipitation via Fe^{3+} than BSA. Because these samples were not fortified with NaOAc, this effect might also be attributed, at least in part, to distillation of the volatile organic acids during the evaporation process.

From **figures 3.28-3.30** it appears that, given equivalent time, increased temperature leads to increased levels of unbound iron, increased color (above and beyond the control) and an increased rate of sucrose hydrolysis (inversion). Curiously, as the applied iron dosage is increased, the residual iron in the juice decreases. This implies that it is removed with the mud. As the temperature is increased the residual iron in the juice increases, and with it the rate of inversion. It is not known if the iron is a direct variable upon which the inversion rate is dependent.

The rate of inversion exceeds that which would normally be estimated as a result of pH and temperature alone (Vukov, 1965; Schaffler, 1987). The ratio of the observed to calculated (observed/calculated) inversion is given in **table 3.9**. The behavior of the observed inversion came closer to "ideal" as both iron dosage and temperature were increased. Because the pH decreased when $FeCl_3$ is added, this may be explained by some mechanism which ceases to function at very low pH, below which, classical inversion predominates. At very low pH, Fenton-type chemistry is slow. This may be attributed to the increased stability of H_2O_2 at acidic pH. At lower iron dosages, and thus higher pH, it is possible to reach the range where Fenton-type reactions can occur at significant speed, namely pH = 3.0-6.5. It is possible that the amount of Fe^{2+} produced from the oxidation of the phenolic compounds may reach a concentration which is sufficient to quench the radical species to yield, hydroxyl radical, Fe^{3+} and HO^- (Halliwell and Gutteridge, 1990). At any rate, this implicates the iron, at lower temperatures and iron dosages, with an anomalous sucrose inversion. It also provides a possible explanation for why

the color increases when the mixtures containing excess Fe (at low pH) and/or treated with Fe while hot and then limed.

Table 3.9. Ratio of the observed inversion (%) over that calculated using the Vukov-Schaffler model.

T, °C:	obs./calcd. Fe^{3+} 500 mg/kg:	obs./calcd. Fe^{3+} 1000 mg/kg:	obs./calcd. Fe^{3+} 2000 mg/kg:
25	19679.71	3529.73	1182.82
45	1407.91	184.53	56.97
65	121.62	18.19	4.12
75	45.01	7.63	1.35
85	29.46	4.36	0.75
95	13.99	3.01	0.73

It appears likely that at high temperatures, the rate of REDOX cycling of iron with the phenolic materials (in air) is greatly accelerated. This might act to deplete the antioxidant potential of the mixture until it is unable to quench the remaining free radicals which then proceed to react with the first available target.

If this system was at very low pH, there would be an accumulated reservoir of Fe^{2+} and H_2O_2. If the pH is suddenly increased, as it would be during a liming procedure, the H_2O_2 would become more reactive and subject to decomposition catalyzed by Fe^{2+} (which would be present in relatively large amounts). This could result in an "avalanche" of $HO^{\cdot}$. The target of the hydroxyl radicals so produced may be phenol:iron and/or protein:iron complexes (or aggregates of the two). Destruction/denaturing of these complexes might release the iron and increase the load of soluble colored material (this would account for both the increase in color and iron).

3.8. The Dependence of pH and Temperature on Autooxidation in the Presence of Air

Because $FeCl_3$ hydrolyzes to yield the hydrated ion and 3-4H^+, the pH drops when it is added to water. Cane juice is more complex than water, and it has a buffering capacity that is not well characterized. To provide a plot for reference, a sample of juice was titrated against pH (electrode) using a $FeCl_3$ solution.

We determined that under hot liming regimes, the juice would darken over time (minutes) on exposure to air. We supposed that the initial pH of the Fe^{3+} might contribute to the autooxidation in air which leads to the undesirable formation of color. To test this, $FeCl_3$ was applied, at various levels of neutralization, to juice pre-equilibrated to 95 °C.

3.8.1. Materials and Methods

Using a manual burette, 50 g samples of raw juice were titrated vs pH using standardized HCl (Fisher, Certified 0.1010 N).

Mixtures were prepared via titration of a pre-determined quantity (to deliver 400 μg/g) of the $FeCl_3$ solution which amounted to 500 μg/g Fe^{3+}, on-sample. The pH of each of five mixtures was adjusted using 0.1N NaOH (See **3.1.1.**) vs electrode to 3, 4, 5, 6 and 7±0.1, respectively. The appearance of the mixtures is seen in **Figure 3.31**.

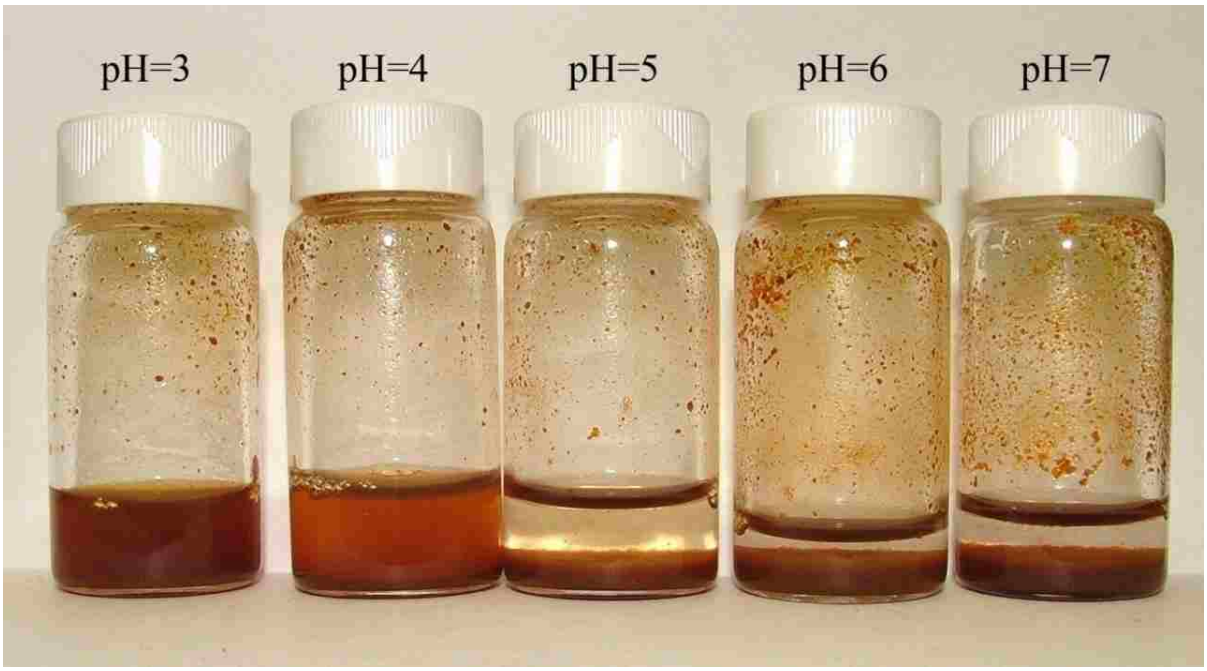


Figure 3.31. Ferric chloride adjusted to various pH.

Samples (50 g) of raw juice were equilibrated to temperature on a hot-plate with a thermocouple feedback loop. The set point was set to 95°C.

This was checked using a NIST (National Institute of Standards and Technology) certified digital thermometer. The juice was allowed to equilibrate to bath temperature for 5 minutes. The "neutralized" $FeCl_3$ was added and allowed to react for 1 minute. The juices were then limed to pH 7.2, allowed to boil for 2 minutes and then 5 mg/mL of Magnafloc LT-340 (Ciba) anionic polyacrylamide was added. The samples were cooled and centrifuged at 3.6 kRPM for 10 minutes.

Color was measured by ICUMSA method. Aliquots were removed from each sample, diluted with water and the carbohydrate profiles were assayed via HPLC-DRI.

3.8.2. Results

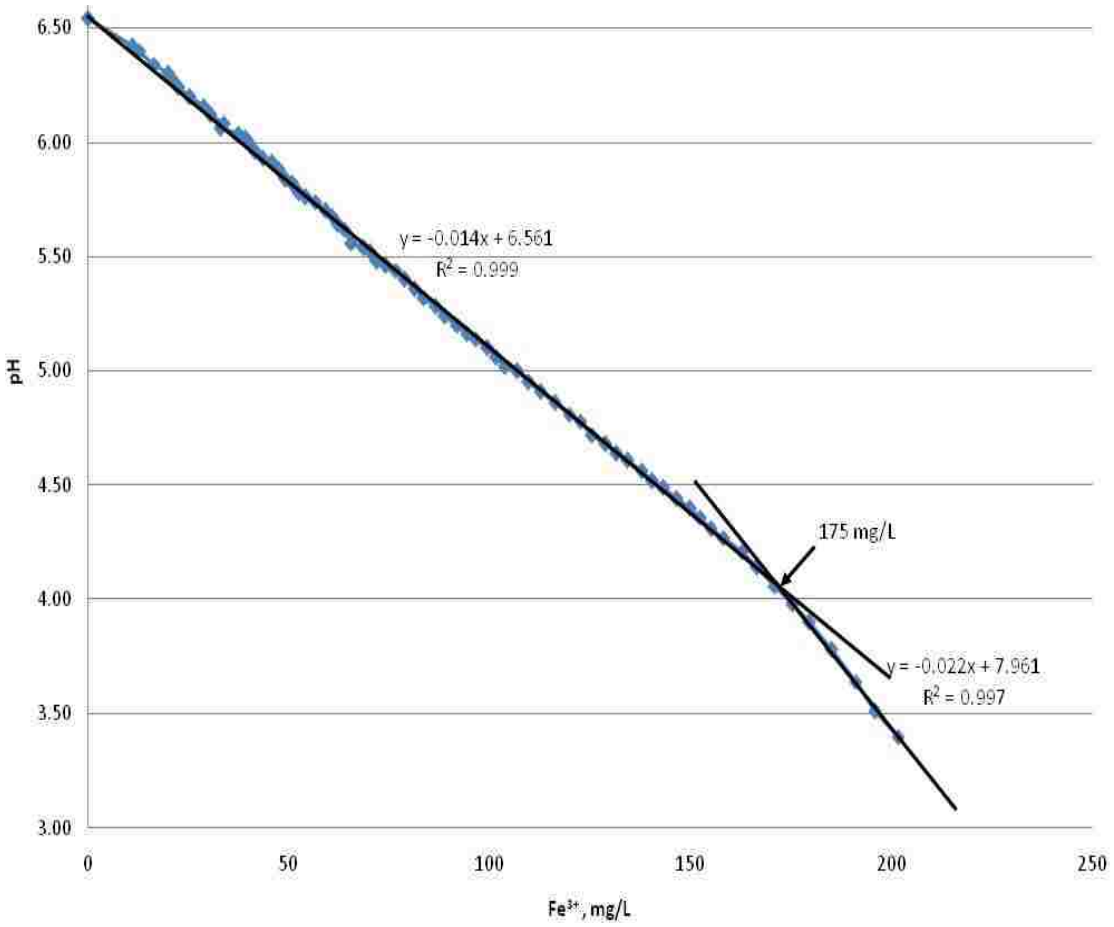


Figure 3.32. The effect of added $FeCl_3$ on the pH of raw cane juice

The titration of juice with $FeCl_3$ was straightforward and demonstrated two discrete linear ranges (pH 6.5-4.1-3.3). The inflection point was sharp at pH 4.1 (175 μg/mL Fe^{3+}).

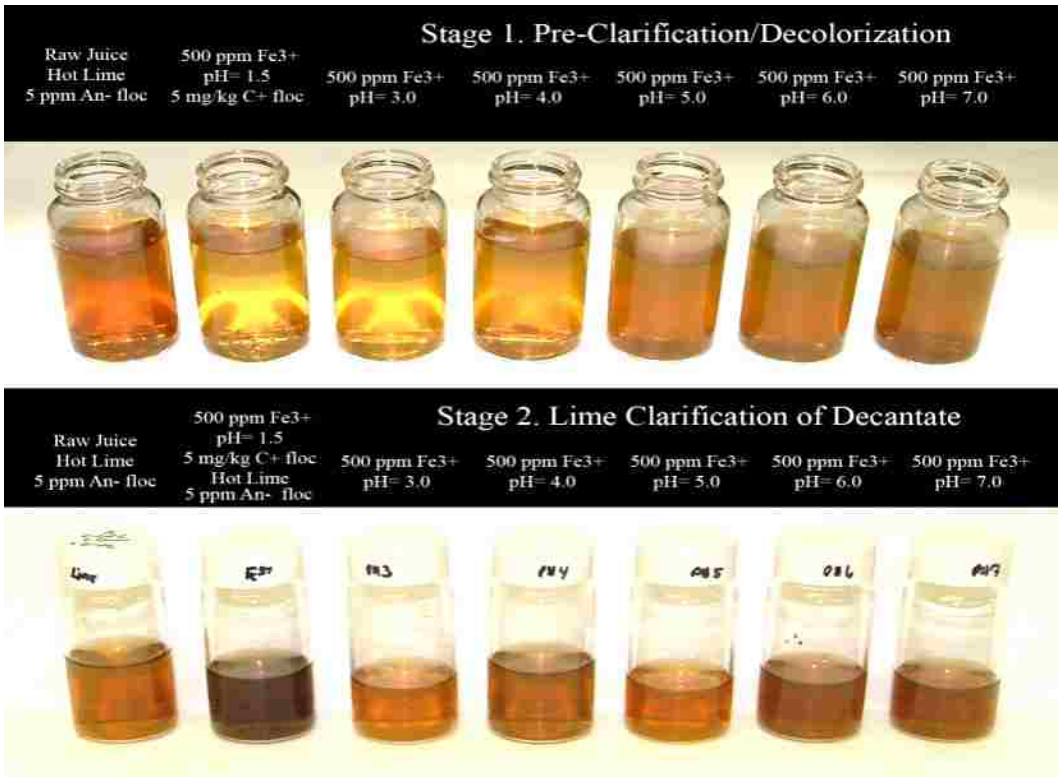


Figure 3.33. The appearance of cane juice clarified hot with Fe^{3+} (top) and then hot-limed (bottom).

3.8.3. Conclusion/Discussion

The behavior of pH relative to added $FeCl_3$ is not similar to that observed with the previous titration of acidified juice vs. standard alkali. There is only one inflection point, and it occurs upon addition of 175 μg/g of Fe^{3+}. The titration is presented in **figure 3.32**. pH was not a discrete dependant variable. Adjustment of juice to equivalent pH (based on $FeCl_3$ dosage) with HCl affected neither clarification nor color.

All resulting juices, except the Fe^{3+} at pH 1.5 (the native pH of the stock solution) were stable color-wise on exposure to air; this is seen in **figure 3.33**, bottom. The color removal was marginal when compared with that observed when the iron is applied at ambient temperature. Adjusting the pH of raw juice to <2 with H_3PO_4 affected neither color removal nor formation of floc. It is apparent that the optimal pH of the $FeCl_3$ "reagent" for this "hot" treatment is approximately 3.

Above this pH, color removal decreases and turbidity increases.

Below this point (pH = 1.5), in the presence of air, a reaction begins that causes a rapid increase in color.

It also appears that essentially all of the Fe^{3+} is in the insoluble hydrated form ($Fe(OH)_3$) when pH >4. The direct application of the colloidal $Fe(OH)_3$ did not lead to the removal of color or phenolic species. It is supposed that the oxidative species must be in solution at the time of addition and that this cannot occur with $FeCl_3$ solutions prepared at pH much greater than 3.

A process using the ambient first-stage appeared to be superior, but may be subject to microbial depredation. It was supposed that the lower pH may mitigate this effect. Lower pH and faster clarification, such as that promised by fractal designs (Kearney, 2006), could help prevent loss of sucrose either to inversion or dextran.

3.9. Iron Mediated Clarification and Decolorization (FeMCaD).

In order to evaluate which compounds are either added or removed from raw juice during clarification, a composite raw juice was created (Raceland and Lafourche Sugar Mills).
We discovered, using small spot-tests, that liming the iron-treated juice prior to heating it yields satisfactory clarification and color removal whilst eliminating the problem with stability in air. This set of tests evaluates the treatment of cane juice with iron and 15 μg/g PCS 3106 (Ecolab) cationic polyacrylamide, liming to pH 6.8-7.2, bringing to boil, adding 5 μg/g LT-340 Magnafloc (Ciba) anionic polyacrylamide and settling.

3.9.1. Materials and Methods

The raw juice was examined as-is. A 500 mL portion of composite raw juice was cold-limed to pH 7 (litmus), rapidly brought to a vigorous boil (microwave oven on “high” for approximately four minutes), and then 5 μg/mL of LT-340 anionic polyelectrolyte was added. The resulting material was settled in a 1L Pyrex Imhoff cone. The settling rate was measured with a stopwatch and volumes were determined using a syringe. The mud was gravity filtered through coarse paper and the resulting filtrate was added to the decanted juice to yield a composite sample.

The composite juice was treated, in triplicate (3 x 100 mL, 104 g), with 350 μg/g Fe^{3+} (450 μL of $FeCl_3$ assayed at 49,000 mg/g Fe^{3+} via phenanthroline method) and 15 μg/g PCS-3106 (Ecolab) cationic polyamine flocculant. They were settled and the rates were measured in the same manner that was used for the hot-limed sample. As for the cold-limed sample, the mud was filtered and the resulting filtrate was added to the decanted juice. The stage-1 juice was then limed to pH 7 and brought to boil, treated with 5 μg/mL LT-340 (Ciba) anionic polyacrylamide and settled as before. All juice products were subject to assay via HPLC-DRI for content of sucrose, glucose and fructose, absorbance at 420 nm (pH = 4.5, 7.0 and 8.5), and dry solids via refractometry.

The muds from each stage were decanted into tared centrifuge tubes and spun down at 4 kRPM for 10 min. The resulting pellets were suspended with deionized water and re-spun twice. The tubes were loosely capped and the water was removed in-vacuo (26" Hg, 50°C, overnight). The residue weights were determined by difference from the tare weight of the tube. Because the quantity of mud was very small when dry, the resulting muds were combined (stage 1 and 2 composites).

The composite mud samples were pulverized using a mortar and pestle. The powdered muds were extracted with 3 X 10 mL hexanes via sonication for 20 minutes. Each time, the solvent was removed with a pipette and transferred into a tared aluminum dish. The solvent was evaporated and an extract residue weight acquired by difference. This step was repeated using dichloromethane. Once weighed, the residues were dissolved in DCM, concentrated to 1mL using a N_2 blow-down technique and analyzed via GC-MS.

3.9.2. Results

The analytical data for this test are given in **table 3.10**. Results from the GC-MS are **Appendix E**.

Table 3.10. Analytical parameters and composite data table for a bench-scale test of FeMCaD method.

Sample:	Brix, g/100g	Sucrose, Bx %:	Glucose, Bx %:	Fructose, Bx %:	color pH:	"Color", IU:	Decol., %:	mud, mg/g:	hexane, mg/g:	DCM, mg/g:
					4.52	6662				
Raw juice	13.52	84.91	2.72	6.10	6.96	9890	0.00			
					8.50	14943				
					4.50	4301				
Cold-Lime	12.44	90.36	2.90	2.74	7.03	9507	3.87	3.517	0.072	0.021
					8.49	15942				
					4.49	6073				
stage 1 A	13.46	91.69	3.11	2.81	6.99	9209	6.88			
					8.48	13263				
					4.51	4885				
stage 1 B	13.51	90.91	2.93	2.84	7.02	9342	5.54			
					8.48	13456				
					4.52	4475				
stage 1 C	13.51	92.93	3.08	3.01	7.04	9758	1.33	9.721	0.064	0.046
					8.46	13787				
					4.53	1835				
stage 2 A	12.62	87.72	2.85	2.74	7.03	4025	59.30			
					8.49	7994				
					4.52	2332				
stage 2 B	12.68	86.26	2.80	2.65	6.99	4724	52.23			
					8.49	8563				
					4.52	2394				
stage 2 C	12.72	95.47	2.99	2.78	6.95	4559	53.90	0.003	0.015	0.007
					8.51	9102				

3.9.3. Conclusion/Discussion

The resulting juices, filtered through 1.2 μm cellulose acetate membranes with a coarse ashless paper pre-filter, are shown in **figure 3.34**, below. The degree of decolorization (Decol. %) was relative to the cold-limed control and for the set shown in **figure 3.34**, the FeMCaD either removed or prevented the addition of, on average, 57.88±3.7 % of the colored material.

Inversion did not appear to be a significant factor when the acidic, iron-laden juice was limed prior to heating.

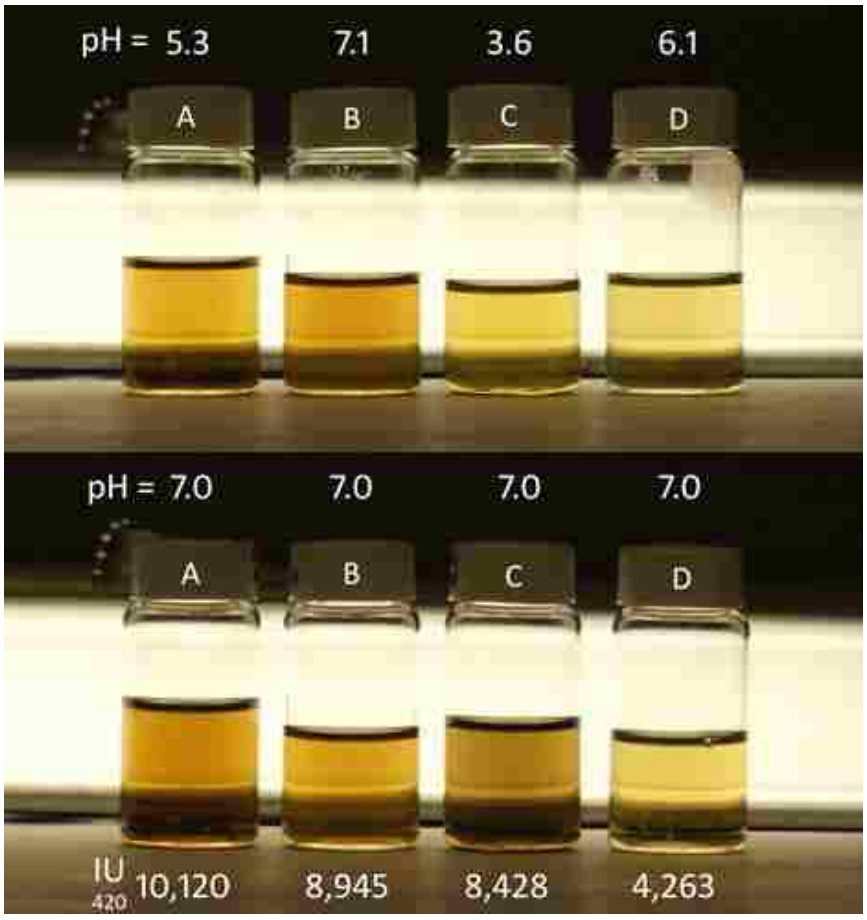


Figure 3.34. Raw juice, A, cold-limed juice, B, stage 1, C and FeMCaD, D, juice at process pH, top and pH 7±0.1, bottom.

It is interesting to note that the stage-1 juice still contains pH sensitive material that increases in color when brought from the operational pH of 3.5-4.0 to the pH used for determination of ICUMSA color (7.0). The stage-two juice does not exhibit this pH sensitivity and is stable to air. The example of this given in **figure 3.35** demonstrates a color removal of approximately 60 per cent. The path-length was approximately 2 cm.

It was concluded that the first stage of iron mediated clarification removed the bulk of the colored material, but left behind some pH sensitive iron-complex(s) which polymerized upon neutralization of pH. The colored materials thus formed were removed as colloidal $Fe(OH)_3$, formed *in-situ* during the liming stage. In other words, the method described using Fe^{3+} incorporates at least two discrete mechanisms.

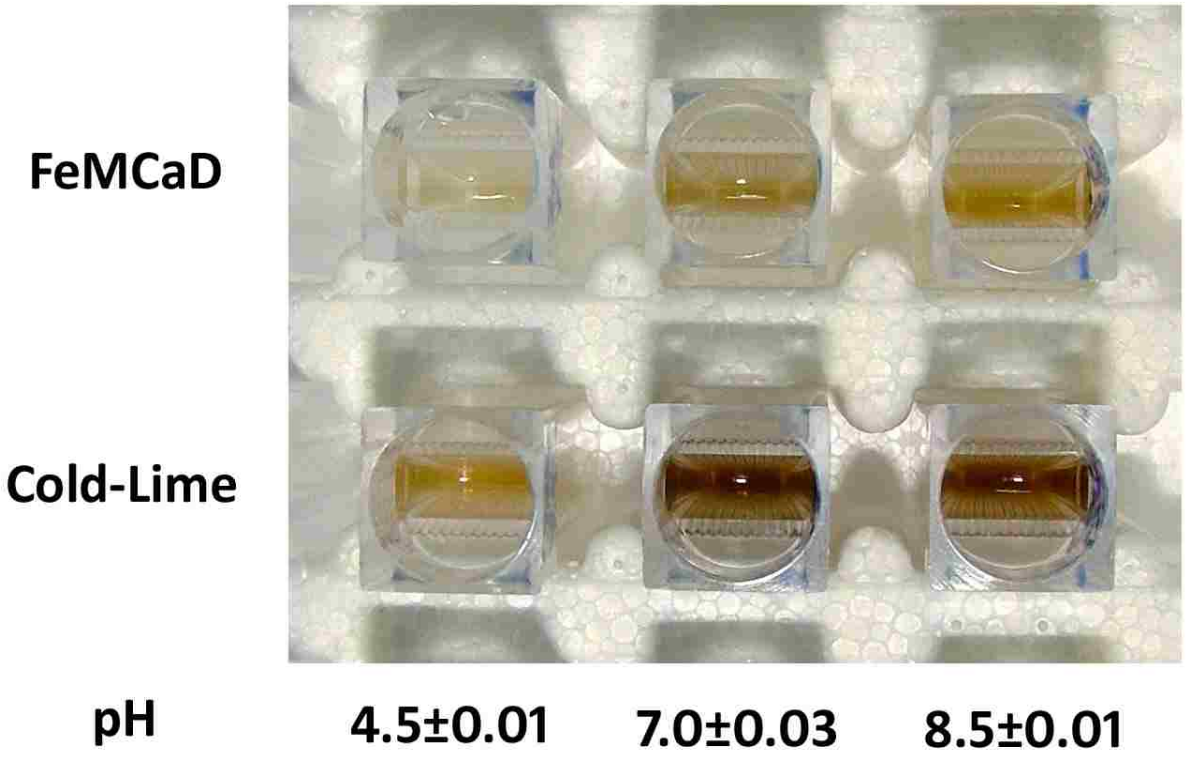


Figure 3.35. Clarified cane juice produced via a two-stage iron mediated clarification and decolorization (FeMCaD) process, top and a cold-limed control, bottom at pH 4.5, 7.0 and 8.5.

When acidic, the phenolics are oxidized by the soluble Fe^{3+} and react with the protein. If the protein reservoir is exceeded, which occurs much of the time due to the small stoichiometric ratio relative to CFA, some oxidized or conjugated material still exists. This material is then removed via $Fe(OH)_3$ either by adsorption, coagulation, or both. The two-stage process yielded approximately 2.5-3.0 times more mud, on dry mass, than the cold-limed control.

The kinetics involved with the settling of the floc from lime is also quite different from that observed with the first stage of the iron process. The juice clarified with lime settles logarithmically, and the iron is removed sigmoidally. An example of this is given in **figure 3.36** and suggests that the iron-based system requires an induction period (~5 min) before large flocs form. At 20 minutes, the mud resulting from the iron-based process had settled to only ~83 % of that for lime.

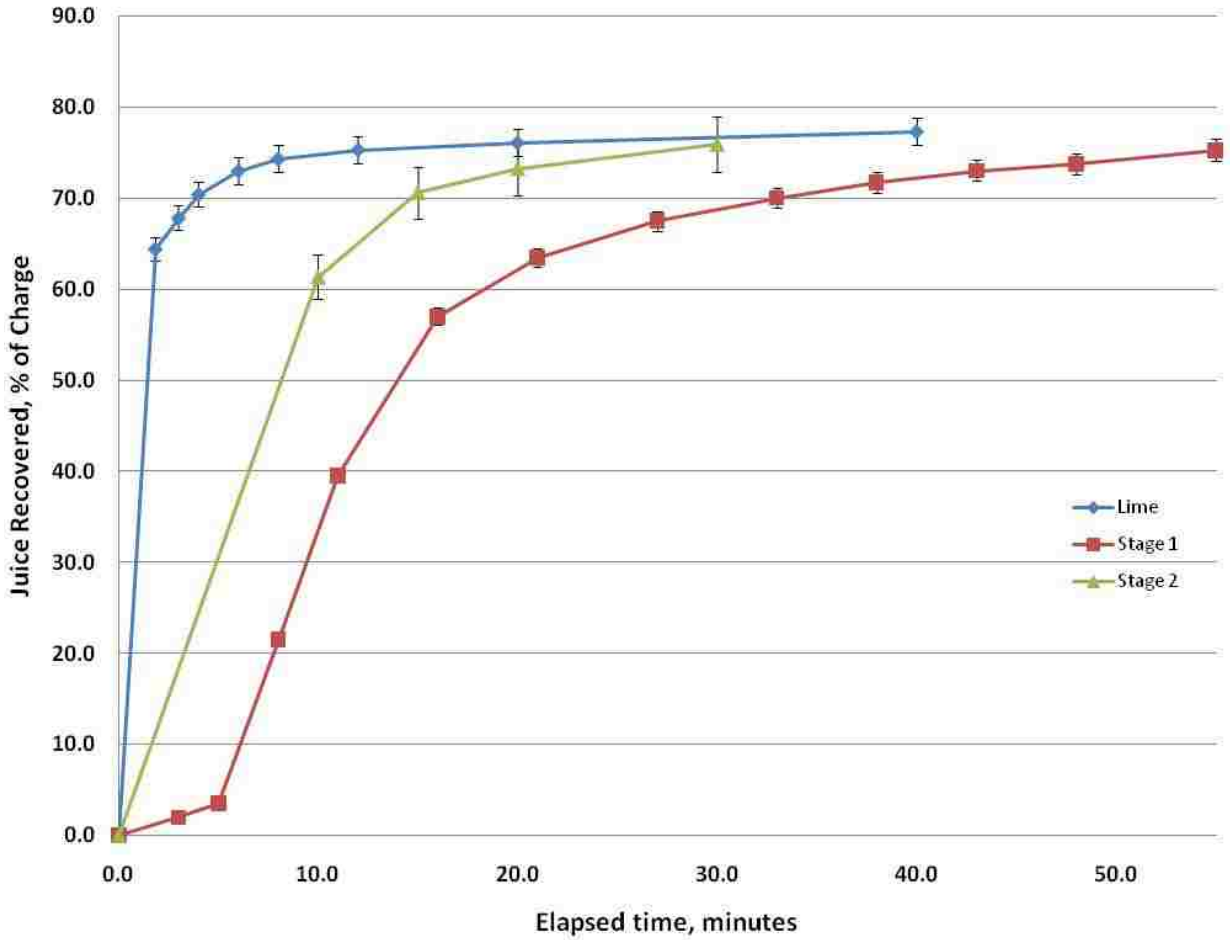


Figure 3.36. Settling characteristics of the iron-based process compared with a cold-lime process.

As noted previously, the materials which can be discerned via GC-MS become more numerous during clarification. An overlay of the TICs from extracted muds (hot lime, stage1 and 2) is given in **figure 3.37**. It should be noted that the FeMCaD removes significantly greater quantities of the policosanol and phytosterol components than hot liming. The FeMCaD process was observed to remove (or otherwise convert to quinoid derivatives) many phenolic compounds while hot-liming appears to strip them from lignin and add them to solution. The selective isolation of the policosanal/sterol fraction may lead to the cost effective recovery of these value-added products.

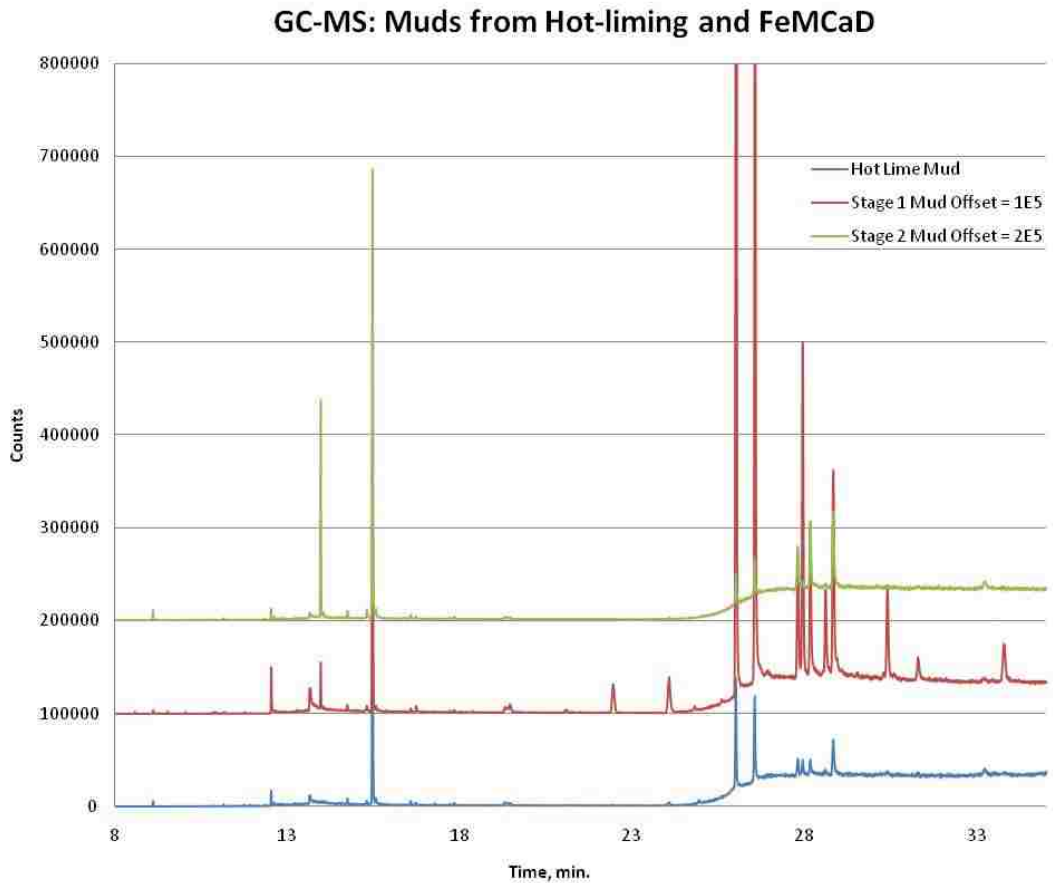


Figure 3.37. GC-MS of extracts from hot-lime and stage 1 and 2 FeMCaD juice.

3.10. Stoichiometry and Hypothetical Mechanisms

Previously, the stoichiometry of BSA to CFA was assessed. Here, we duplicated that test with tighter control and GPC (gel permeation chromatography) support. From literature (SwissProt) we know that BSA contains 60 lysine residues, which, barring translational modification, can behave as nucleophiles with respect to oxidized quinoid species. In this test, Fe^{3+} and BSA are fixed. The objective was to elucidate a reasonable mechanism explaining how the phenolic compounds interact with BSA when in the presence of Fe^{3+}.

3.10.1. The Stoichiometry of CFA and BSA in the Presence of Iron

The objective of this section is to attempt to establish the stoichiometry that exists between BSA and CFA:Fe^{3+} at steady-state. From this it should be possible to derive the nature and length of the bridges that appear to be made between protein molecules when irreversible coagulation occurs.

3.10.1.1. Materials and Methods

A solution was prepared with analytical grade sucrose (Fisher, ACS, 99.8% $[\alpha]_D^{25}$=+66.5°) and 18 MΩ de-ionized water (Barnstead Nanopure with 0.2μm capsule filter) . This solution contained 40 g/100g (brix) and was standardized by refractometry (Bellingham and Stanley RFM340).

Ferric chloride (Mallinckrodt, hexahydrate, 99.8%) solution was made with 18 MΩ, degassed and de-ionized water to contain 79,923 μg/g of Fe^{3+}. This material was assayed via absorbance of the tris-*o*-phenanthroline complex at 510 nm. The solution so prepared was found to contain 49,232 μg/g of iron, 1.71 % of which was Fe^{2+}. The difference was likely water taken up from humid air.

Fraction V bovine serum albumin (BSA, Cohn fraction V, Sigma, 96-99%) was used as is to make up a solution to deliver 9.04E-8 mMol/μL (5853 μg/g) in de-ionized water.

1M Acetate buffer was prepared from 18 MΩ de-ionized water and sodium acetate (Fisher, ACS, anhydrous, 99.4%); it was adjusted to pH 5.00±0.05 (Oakton 11 series with Ag/AgCl probe) using glacial acetic acid (Mallinkrodt, AR).

3,4-dihydroxycinnamic acid (caffeic acid, CFA, Sigma, 99 %) was used as-is to make a solution of 4.95E-5 mMol/μL (8825 μg/g) in a degassed (26 ”Hg/sonication) matrix consisting of ethanol (Aaper, absolute-200 pf) and water, 1:1.

A 0.2M phosphate buffer solution (PBS) was prepared by dissolving NaH_2PO_4 (EM, GR, 99 %), 9.36 g and Na_2HPO_4 (Baker, ACS, 99+ %), 32.73 g into 1 L (volumetric flask) of 18 MΩ de-ionized water. Eluent for gel permeation chromatography (GPC) was prepared using 250 mL of this solution and 17.53 g NaCl (Mallinckrodt, ACS, 100%) diluted to 1 L. The eluent contains PBS 50mMol and NaCl, 0.3M and pH= 7.0±0.1. This solution was degassed under vacuum (24” Hg) with sonication (Branson 5210) prior to use.

The aforementioned materials were used to prepare a set of samples according to the matrix given in **table 3.11**.

The amount of BSA applied to each sample equates to 9.04E-5 mMol of protein or 5.42E-3 mMol of N^{ε}-NH_2-lysine. Fe^{3+} is added at 5.65E-3 mMol, a slight excess over the BSA amino equivalent. Samples CFA 3 and 7 (bold text) represent one and two equivalents of CFA, respectively.

To 15 mL polyethylene centrifuge tubes was added, in this order: water, sucrose solution, BSA solution, CFA solution, AcONa buffer and, finally, $FeCl_3$ solution.

Table 3.11. Sample matrix

Sample:	BSA, µL:	$FeCl_3$, µL:	AcONa, µL:	Sucr., 40bx:	CFA, µL:	water, µL:	Total, µL:	µg/mL:	CFA mMol:
CFA 0	1000	22	125	2500	0	1353	5000	0.00	0.00000
CFA 1	1000	22	125	2500	59	1294	5000	104	0.00289
CFA 2	1000	22	125	2500	85	1268	5000	150	0.00416
CFA 3	**1000**	**22**	**125**	**2500**	**111**	**1242**	**5000**	**196**	**0.00544**
CFA 4	1000	22	125	2500	137	1216	5000	242	0.00671
CFA 5	1000	22	125	2500	163	1190	5000	288	0.00798
CFA 6	1000	22	125	2500	189	1164	5000	334	0.00926
CFA 7	**1000**	**22**	**125**	**2500**	**221**	**1132**	**5000**	**390**	**0.01083**
CFA 8	1000	22	125	2500	241	1112	5000	425	0.01181
CFA 9	1000	22	125	2500	261	1092	5000	461	0.01278
CFA 10	1000	22	125	2500	281	1072	5000	496	0.01376

The samples were sealed and swirled (vortex mixer) to mix and allowed to stand at room temperature (24°C) for 10 minutes. The samples were centrifuged at 3.6 kRPM for 10 min.

The tubes were sampled and analyzed via GPC. The operational parameters for the GPC are given in **table 3.12**. The instrument was standardized (**figure 3.38**) for molecular weight using (6.5 kDa), carbonic anhydrase (29 kDA) and BSA (66.4 kDa) via absorbance at 280 nm.

A quantitative calibration, in **figure 3.39**, was made using a mixed standard containing BSA and CFA. Since only one wavelength is available at a time, 330 nm was chosen because it is an absorbance maxima for caffeic acid. BSA has a very small absorbance at this wavelength, so it was thought that quantitation could be made using the DRI.

Table 3.12. GPC parameters

Pump	Waters 510
Detector 1	Thermo Differential Refractive Index (DRI), 45°C
Detector 2	Applied Biosystems UV-VIS, 330 nm
Detector hold-up time	1 minute, DRI to UV-VIS
Column	Shodex Protein KW-903, 300mm X 4.2 mm(ID), 5μm particles
Column Heater	40°C
Eluent	50mMol phosphate buffer, pH 7.00±0.05 and NaCl, 0.3 M
Degasser	Perkin Elmer, Vacuum
Flow	1.0 mL/min
Autosampler	BioRad AS3500
Acquisition/run-time	Dionex ACI, Dionex Peaknet 5.2, 30 min
6.5 kDa, Aprotinin, min.	16.17
29 kDa, Carbonic anhydrase, min.	12.53
66.4 kDa, BSA, min.	9.45
Quant. BSA, monomer at 7.29 min.	491, 978, 1949 μg/g, RF= 1.12E-3, R^2 =0.995
Quant. CFA, 15.02 min	115, 203, 397 μg/g, RF= 1.26E-5, R^2 =0.997

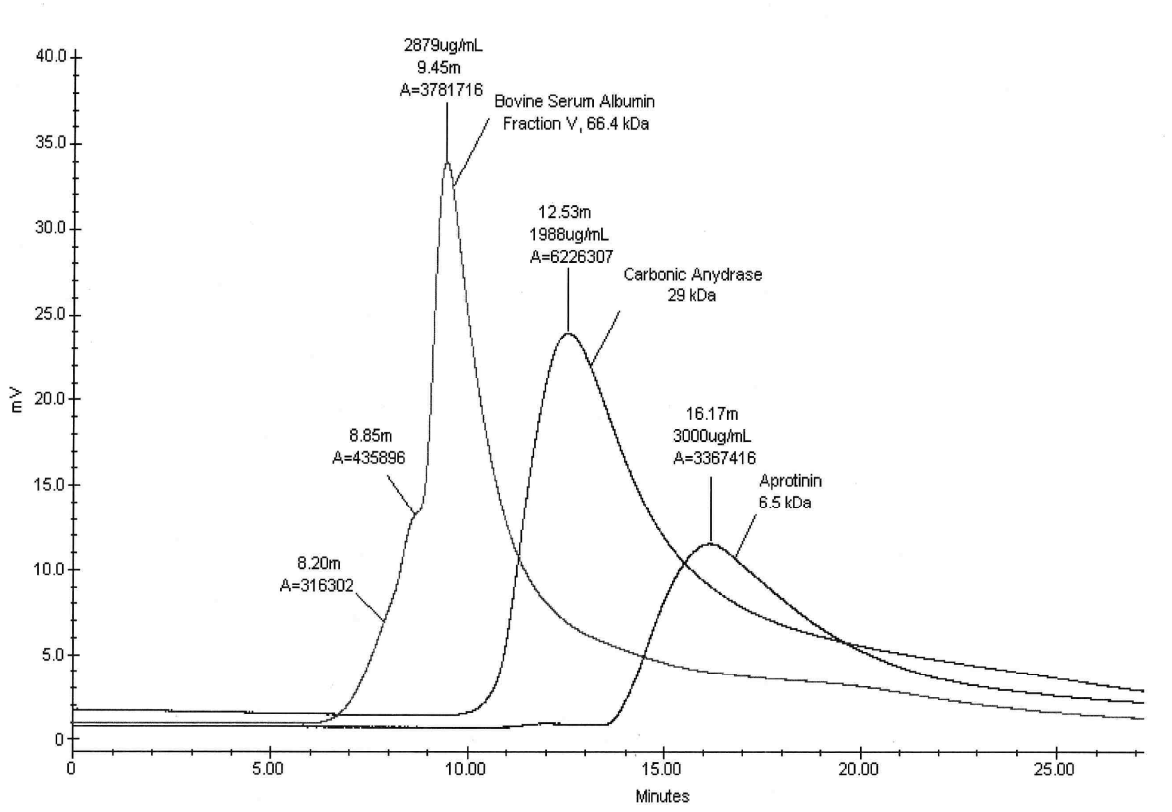


Figure 3.38. Molecular weight calibration; ABS 280 nm.

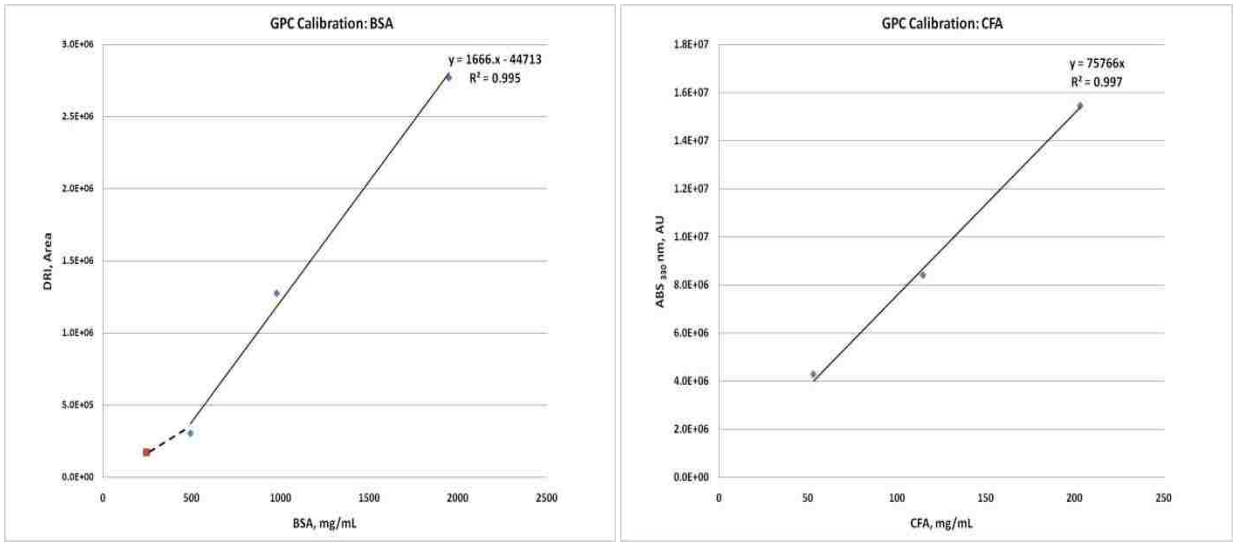


Figure 3.39. Curves used for quantitation.

3.10.1.2. Results

As seen in **Figure 3.40A**, the samples appear to be identical, with the exception of the color of the increasing amount of CFA. Upon the addition of the iron, it was immediately noted that samples containing 2 BSA equivalents (1.1E-2 mMol) of CFA precipitated immediately. This was not obvious without inspection against a bright light source, so the samples in **figure 3.40B** were centrifuged. The precipitation of colored material in **figure 3.40C** is striking and increases with the amount of added CFA. Further, upon standing sealed for three days, all samples containing at least 1 BSA equivalent (5.4E-3 mMol) of BSA precipitated completely. The precipitate pellets in **Figure 3.40D** appear smaller because the mixtures were re-centrifuged to settle the fluffy flocs which had formed.

In order to determine when the system “saturates” which should be evident as a lack of CFA consumption, it was decided to test a second set of samples with higher concentrations of CFA. It was hoped that, when coupled with figures describing the external free Lys-amino groups, that this figure could be used to calculate an approximate chain length or degree of polymerization (X_n).

The samples shown in **figure 3.40C** were analyzed via GPC. The plots acquired using the DRI were not used because the BSA rapidly fell below our limit of quantitation. Further, the sucrose and buffer created an immense smear across much of the chromatogram.

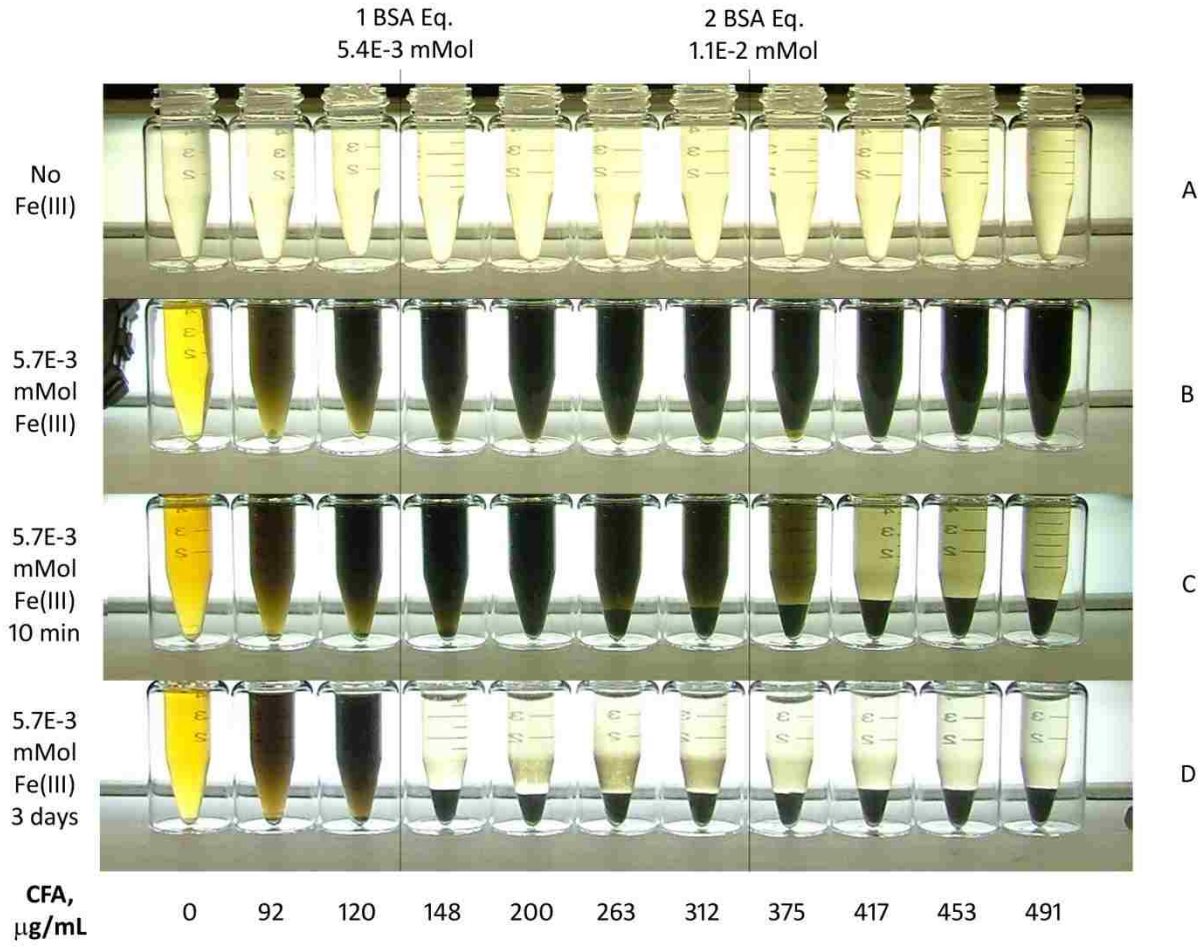


Figure 3.40. Addition of iron and centrifugation of model samples; 148 and 375 μg/mL CFA correspond to CFA 3 and 7.

This smear appeared to be consistent for all samples. The results from these plots were not used. The UV-VIS plots taken at 330 nm were useful. The absorbance of the BSA at 330 nm was too small to give a response, but the CFA was linear across all concentrations used here. The following results were derived from the absorbance plots and were based on the quantities of CFA detected.

Under the conditions specified in **table 3.11**, caffeic acid had a retention time (or elution volume at 1 mL/min) of 14.68±0.04 minutes.

A response factor (amount/area) was used for quantification; this value was 6.022E-05±4.7%. The CFA detected versus the amount applied is plotted in **figure 3.41**. The points at 200, 390, 568, 780, 992, and 1204 μg/mL represent 1.0, 2.0, 3.0, 4.0, 5.1 and 6.2 BSA (60 * LYS) equivalents, respectively.

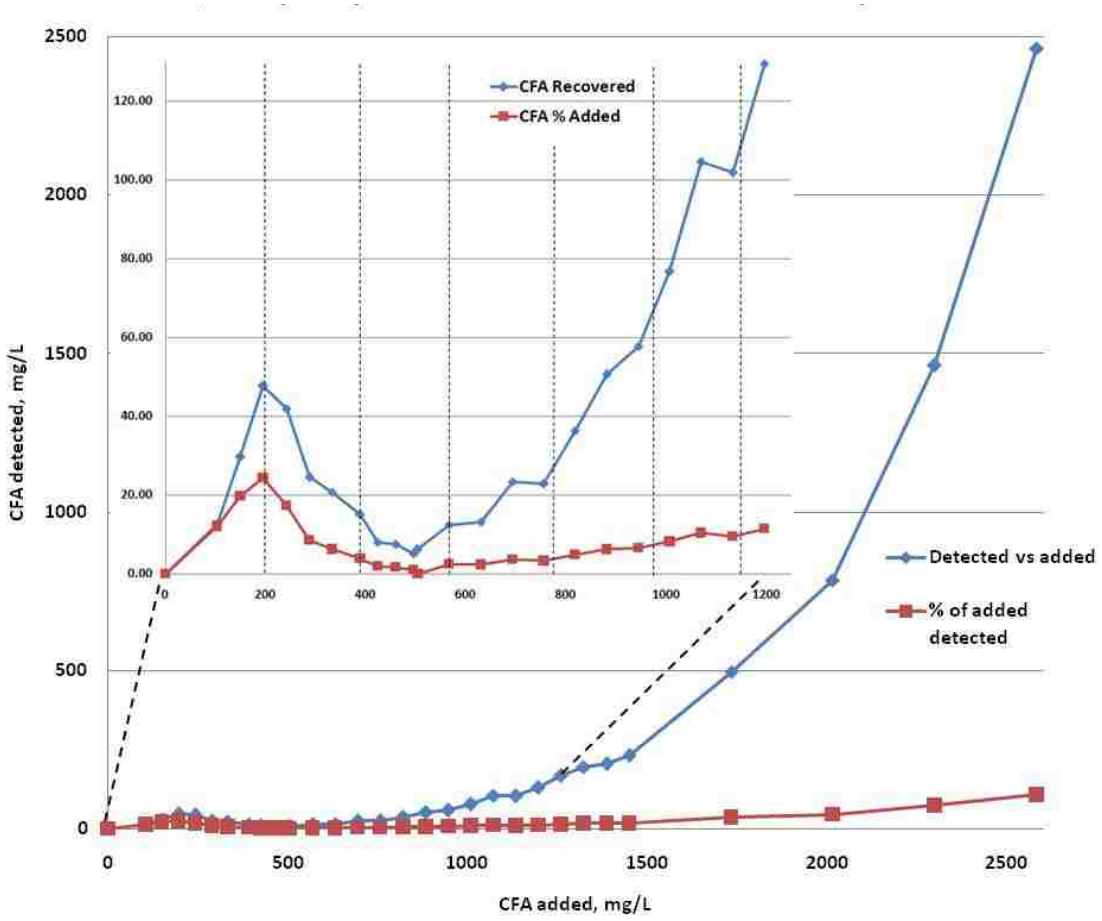


Figure 3.41. CFA detected relative to the initially amount (blue) and the percent of the initial amount (red). BSA Equivalents are marked with dotted vertical black lines.

The results in **figure 3.41** appear to be rather puzzling. It is indicated that the CFA detected increases until some critical point and then drops off, even though the amount of CFA added to each sample is increasing. Later, after three BSA equivalents, the CFA levels begin to increase relative to the amount applied. In order to more clearly understand what is happening, the same data is plotted in terms of the amount of CFA consumed (amount added – amount detected) relative to the dose. This is given in **figure 3.41**.

The discrete peak in **figure 3.41** reached maximum at 200 and was resolved at 500 μg/mL CFA. This is equal to 0.005 mMol and is very close to 1 N^{ϵ}-lysine BSA equivalent (0.0054 mMol). It appears that the inflection point in consumption at the same point is in **figure 3.42** is a real feature.

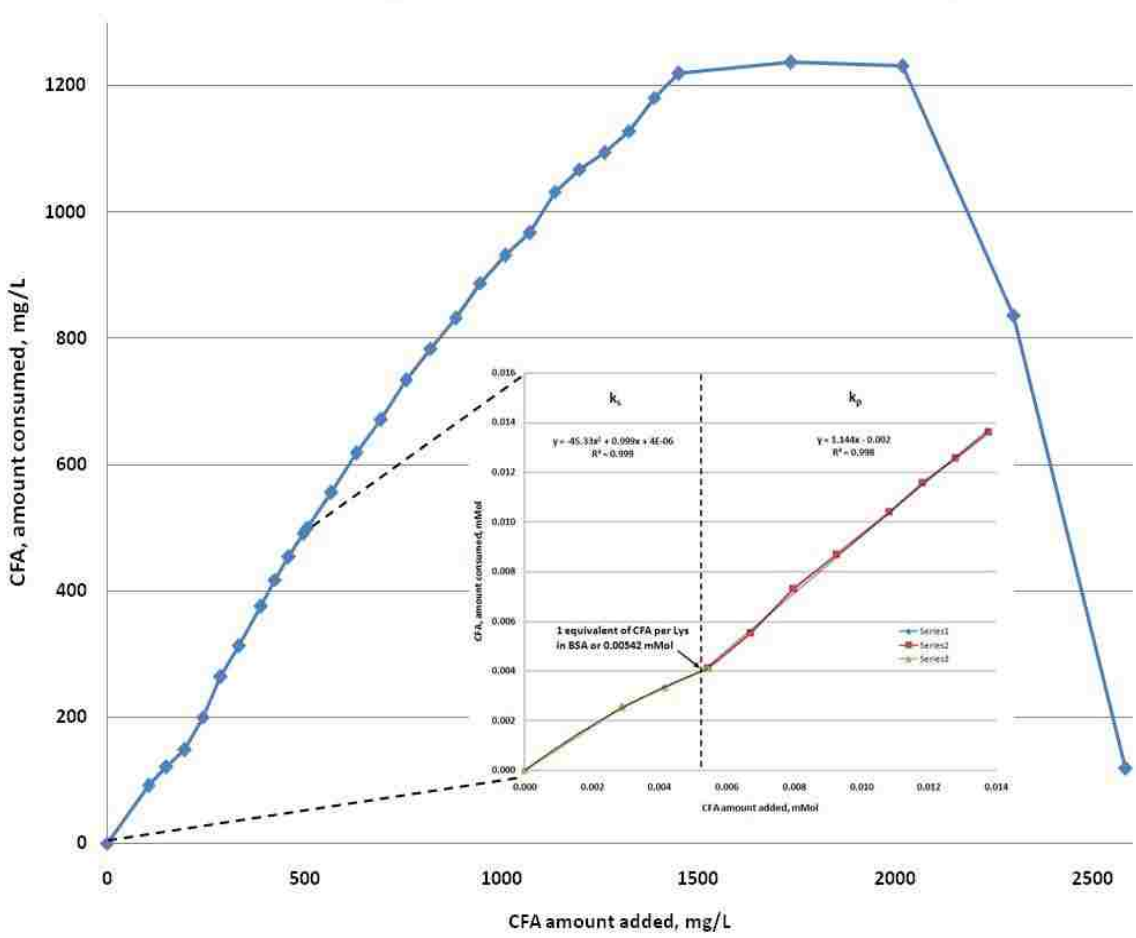


Figure 3.42. Amount of CFA consumed vs that which was added.

At least two, simultaneous reactions were occurring. The first suggested a saturation effect and the second, polymerization. Note the plateau (steady state) and point of saturation at ~2000 mg/L CFA.

Between 7.971 and 8.333 BSA equivalents of CFA are needed before the rate of consumption stabilizes. Further, at approximately 14.5 equivalents are needed before the amount of CFA detected equals the amount that was applied.

Upon examination of the stage-1 (acidic Fe^{3+}) process under a dissecting microscope, it was found that the application of 6M urea completely failed to disrupt the floc, and the addition of EDTA caused only a marginal disassociation. Addition of *o*-phenanthroline caused the flocs to become larger and more robust. This is demonstrated in **figure 3.43**.

GC-MS of mixtures of juice and phenanthroline with and without Iron indicated that the ligand was either removed from solution or otherwise destroyed.

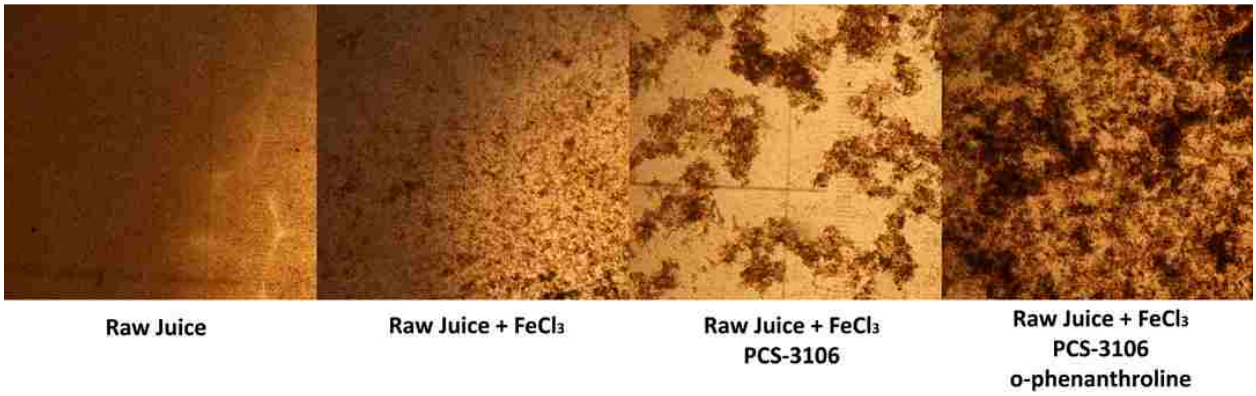


Figure 3.43. FeMCaD trial in a juice droplet, 25X magnification.

3.10.1.3. Conclusion/Discussion

The plot in figure 3.41 suggests that there is a fast initial consumption of CFA which ends when ~1 lys-BSA equivalent, or 0.05 mMol of CFA has been added. This suggests that the free sites are reacting/binding until saturation. Following this, there is a short induction period, possibly the accumulation of an undetectable intermediate, followed by a linear increase in consumption. This implies that either the residues added in the prior saturation step are reacting with further CFA and removing it from the bulk solution or the CFA is homopolymerizing.

It appears from this data that first, the free N^{ε}-amino-lysine groups are reacting with the phenolic species, probably in the quinone form. Then, the quinone end-groups are reacting further to extend phenolic chains outward. This continues until either, the CFA reservoir is exhausted and/or the chains extending from two separate proteins meet and couple.

From the stoichiometry given in **figure 3.42**, where the maximum CFA consumed amounts to 0.0343 mMol, it can be derived that the equivalence to BSA is approximately 380:1. On free amino-groups this amounts to a ratio of 6:1. Since there appears to be, depending upon the cross-linker used, of 60 lysine residues, approximately 8-12 free lysine residues in BSA are able to crosslink though space (Huang, 2004). We can approximate that the number of chains per protein is, on average, 10.

The CFA to amino group ratio then becomes 38:1. Dividing this figure by two, for two interacting protein molecules gives a bridge length or degree of polymerization of approximately 8.

The failure of 6M urea to disrupt the floc tends to argue that little in the way of hydrogen bonding is responsible for the stability of the BSA:CFA aggregates. The very slight disruption observed when the aggregate was treated with EDTA indicates that some, but not all of the structure is likely dependent upon chelation of iron. The effect of added *o*-phenanthroline was unexpected.

Rather than causing the material to disintegrate (at least partially) due to sequestration of any bound iron that might be structural, the addition of *o*-phenanthroline caused the flocs to become larger. Unsure of this result, we treated juice with "excess" *o*-phenanthroline with and without added $FeCl_3$. These samples were analyzed via GC-MS and revealed a marked (>90%) removal of the ligand. In order to explain this, it was found that Cavalieri, et al (2002) had reported on the electrophilic addition of *o*-quinone species to purine bases in DNA. The reaction involves the radical semiquinone and is functional over a relatively wide range of pH, including physiological. An adapted scheme is given in **figure 3.44**.

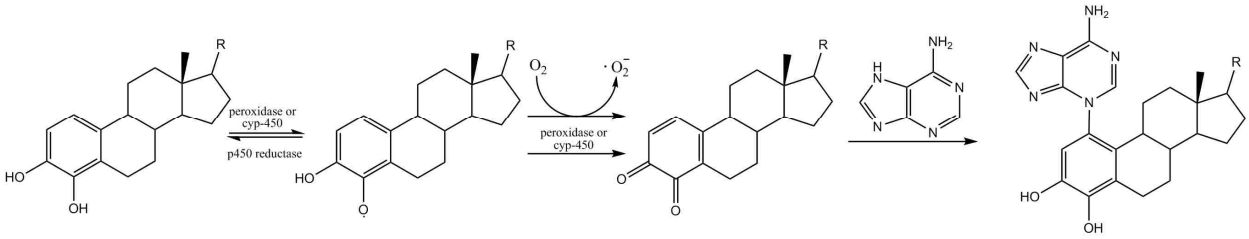


Figure 3.44. Synthesis and reaction of an *o*-quinone with a purine base (Cavalieri, et al. 2002).

The scheme in **figure 3.44** is in-line with what we have observed. Instead of using cyp (cytochrome P) 450, Fe^{3+} serves as the initial oxidant to yield the semiquinone which is subsequently subject to autooxidation via O_2. The resulting quinone then reacts with the purine base to yield the adduct.

We suspect, but were unable to confirm that the *o*-phenanthroline was behaving in a similar way. Further, it is suspected that the compound, being difunctional, might be serving a crosslinking species.

It was noted that with cold liming, it might be possible to do both steps, sequentially, in one reactor.

3.10.2. The Stoichiometry of CFA and Iron

From the previous set of experiments, it was decided to review the dependence of the system on the quantity of iron used.

3.10.2.1. Materials and Methods

Tubes were made to contain BSA, 0.0052 mMol (LY –eq.) AcONa buffer, 0.125mMol, CFA, 0.0096 mMol in 20 % $sucrose_{(aq)}$.

To each tube was added an increasing amount of $FeCl_3$, in a range from 0 - 0.0565 mMol. The experiment, with CFA substrate (the samples without CFA contained water-to-balance), was structured as described in **table 3.13**.

Table 3.13. Sample matrix

Sample:	BSA, µL:	$FeCl_3$, µL:	AcONa, µL:	Sucr., 40bx:	CFA, µL:	water, µL:	Total, µL:	Fe, µg/mL:	mMol:
CFA 0	1000	0	125	2500	220	1155	5000	0	0.0000
CFA 1	1000	5	125	2500	220	1150	5000	79	0.0071
CFA 2	1000	10	125	2500	220	1145	5000	158	0.0141
CFA 3	1000	15	125	2500	220	1140	5000	237	0.0212
CFA 4	1000	20	125	2500	220	1135	5000	316	0.0283
CFA 5	1000	25	125	2500	220	1130	5000	395	0.0353
CFA 6	1000	30	125	2500	220	1125	5000	474	0.0424
CFA 7	1000	35	125	2500	220	1120	5000	552	0.0495
CFA 8	1000	40	125	2500	220	1115	5000	631	0.0565

3.10.2.2. Results

Samples in **figure 3.45** (A, BSA without CFA and B, BSA and CFA), below, containing 0.014-0.021 mMol, correspond to dosages of 158 and 237 µg/mL Fe^{3+}, respectively.

The samples seen in **figure 3.45** were scanned from 300-1100 nm using a Beckmann/Coulter DU-800 spectrophotometer. It was thought that the samples not containing CFA would be used for background correction.

It appears, however, that the increase in absorption caused by the iron, or of some BSA:iron complex changes when CFA is included in the system.

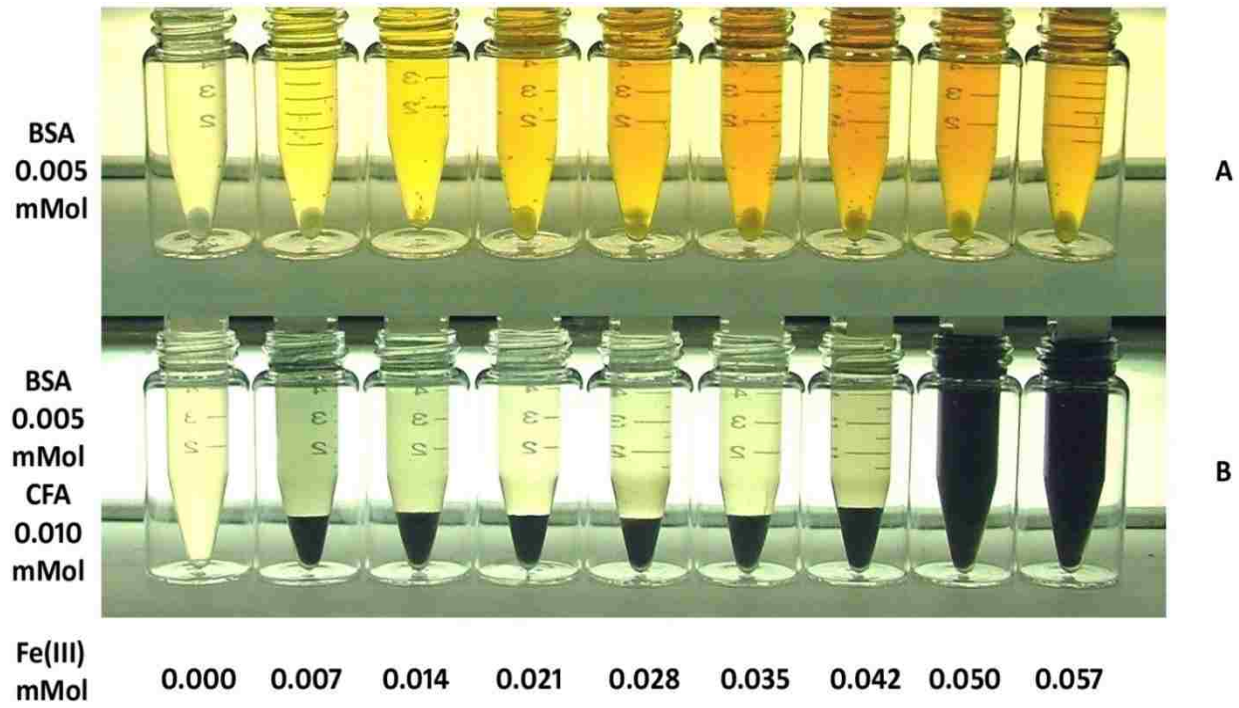


Figure 3.45. Model system with increasing amounts of iron.

Seen in **figure 3.46**, the absorbance at 315 nm (near the second strong absorbance maxima for caffeic acid) decreases exponentially ($ABS_{315} = 1.034e^{-180\,Fe}$, R2 = 0.989) as iron is increased until the quantity exceeds that of CFA (0.01 mMol) by a factor of approximately 2.2.

When the quantity of iron exceeds 2.2 equivalents (on CFA) the peak at 315 begins to return and increases in a linear fashion ($ABS_{315} = 33.52Fe_{mMol} - 0.683$, $R^2 = 0.990$) until a Fe:CFA ratio of 4.4:1 exists. At this point, it appears that the increased absorbance at 315 nm heads toward an asymptotic value somewhere between 0.80 and 1.00 AU, which corresponds with the original CFA dose.

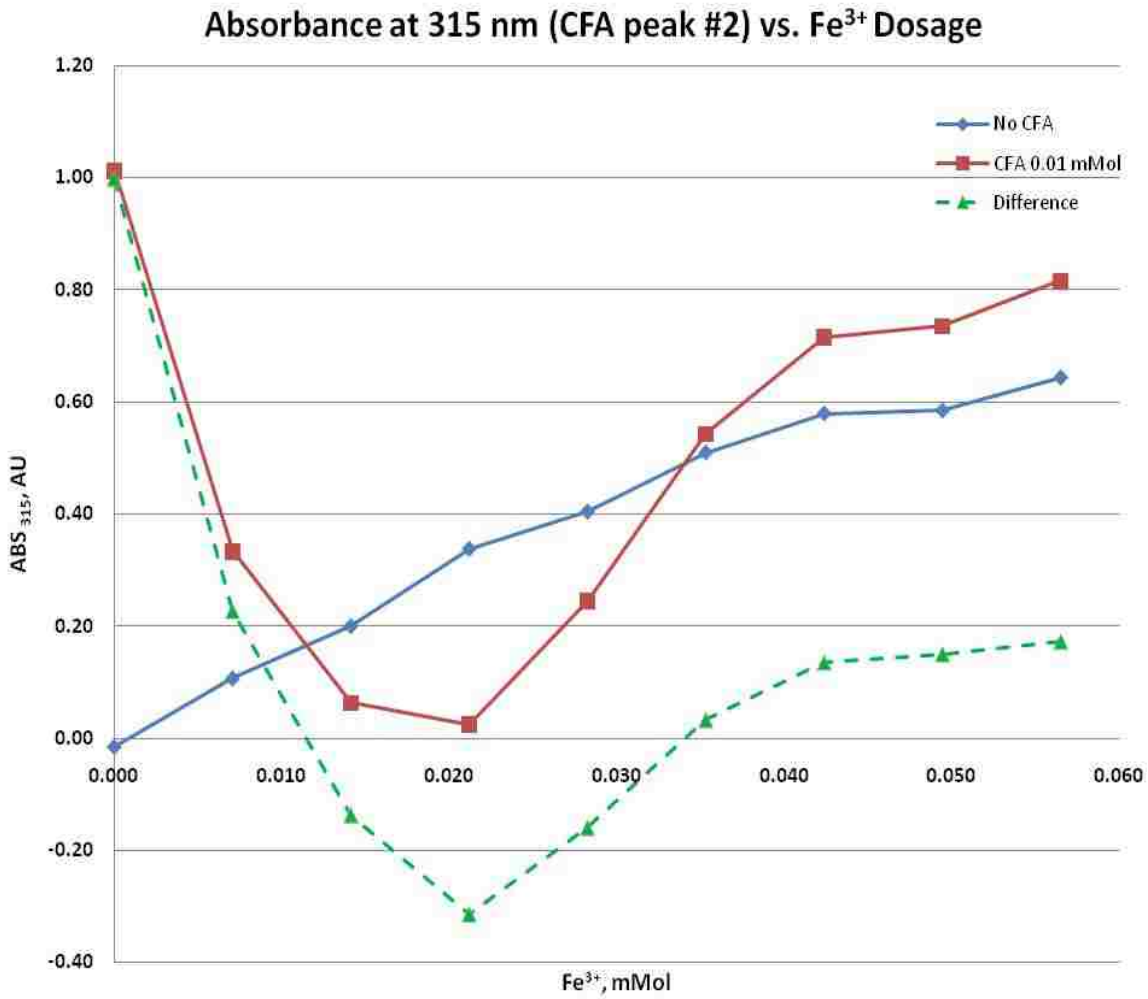


Figure 3.46. The absorbance at 315 nm vs Fe^{3+} of samples with (red) and without (blue) CFA and the difference (green).

3.10.2.3. Conclusion/Discussion

The optimum quantity of iron was re-confirmed to be between 150-350 μg/g (237 μg/g was optimal) and the correct molar ratio of Fe^{3+} to BSA appears to be 4.08. The point of saturation, where free iron appears is twice that, when the ratio is ~8.15 (474 μg/g).

The dashed line in **figure 3.46** illustrates that the background absorbance of the Fe^{3+}:BSA mixtures disappears completely, and consistently ($\Delta ABS_{315control}$ = -0.5073±0.084) when CFA is present at the tested dose and iron is present at a 2.2:1 ratio. Prior to the establishment of this ratio, ΔABS increases ($y = 2898.x^2 - 138.6x + 0.999$, $R^2 = 0.997$) with Fe^{3+} dose which suggests that it is being bound.

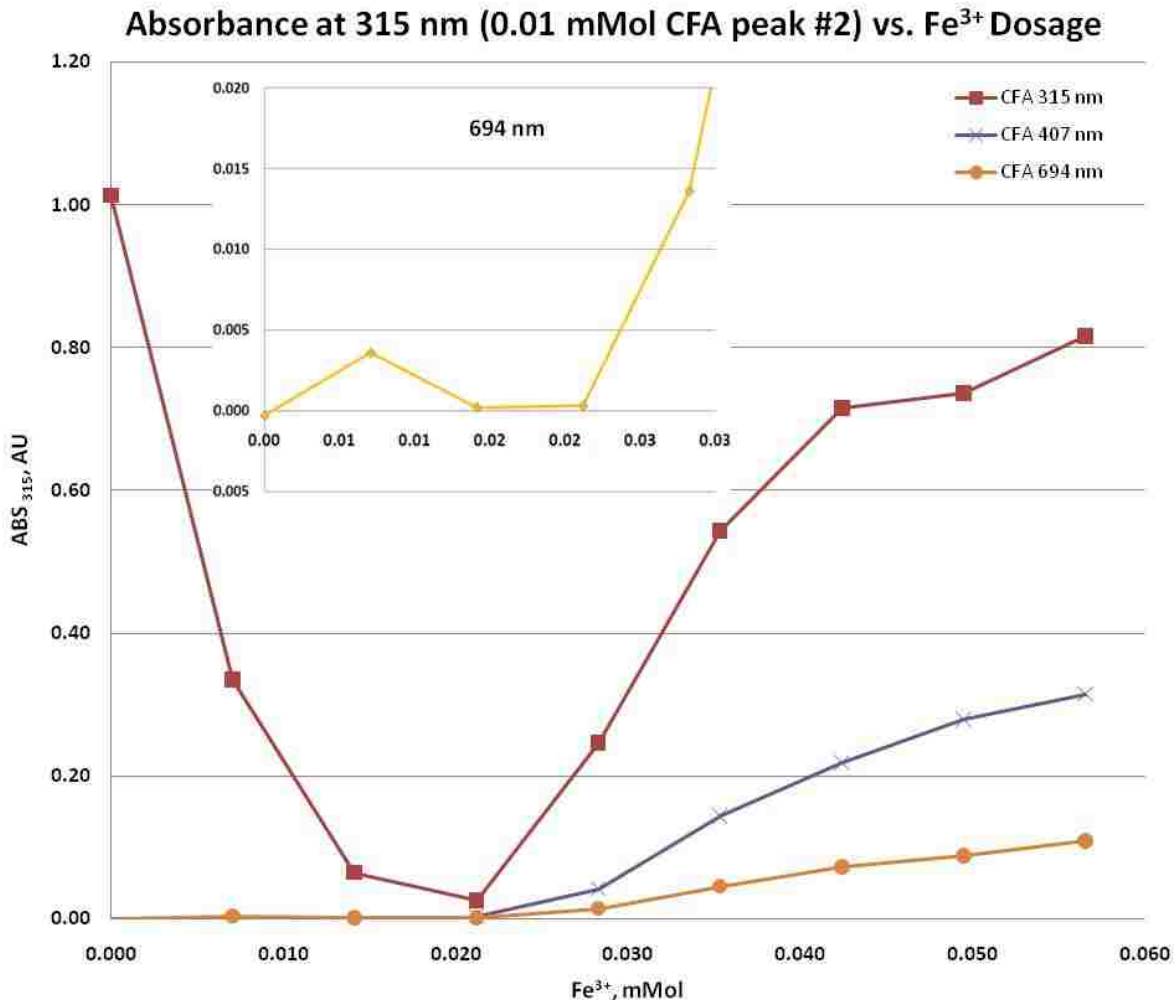


Figure 3.47. UV and VIS absorbing products from BSA:CFA when iron dosage exceeds 0.02 mMol.

The amount of "re-appearing" CFA that is not accounted for via background correction originated from product(s) which evolve when Fe^{3+} exceeds 0.02 mMol. The traces in **figure 3.47** demonstrate the evolution of absorbance at 315 and 694, which overlap and elevate the baseline. These absorbance bands may arise from conjugated compounds, such as phenol:quinone adducts and iron semiquinone complexes, respectively.

3.10.3. Inhibition of Coagulation/Flocculation of BSA by Surrogate Amines

The involvement of the BSA amino groups is consistent with our observations. If we assume that this is true, then we can suppose that the reaction of the protein-bound N^{ε}-lysine groups should be relatively slow due to the steric bulk of the macromolecule. Smaller, kinetically favored amines should react with the quinone species at a greater rate than those attached to the protein. In order to test this, the much smaller amines, L-lysine and 3-aminopropanol, were added to a model system. If the protein amino groups are involved, then the addition of a smaller, more soluble amine should retard, or inhibit altogether, precipitation of the phenol:protein aggregates.

3.10.3.1. Materials and Methods

The samples were prepared as before (see **tables 3.11**, **3.13**), but now were made to contain a gradient of either L-Lysine (Sigma) or 3-amino-1-propanol (Sigma Aldrich) along with the BSA. BSA, CFA and Fe^{3+} were added at 0.0053, 0.010 and 0.031 mMol, respectively. The samples were centrifuged (3.6 kRPM, 10 min) after incubation at ambient temperature (24 °C) for 10 minutes. The centrifugation was repeated after 24 hr had elapsed. A control was made in either case which contained only the inhibiting amine at 1 BSA Lysine equivalent or 0.0053 mMol.

3.10.3.2. Results

For both experimental series, the samples containing only the inhibiting amine yielded precipitate first. The volume of precipitate was smaller than for those samples also containing BSA. The samples fortified with lysine demonstrated a break-point between 0.0037 and 0.0046 mMol. This was not observed with 3-aminopropanol. Both sets exhibited complete precipitation after 24 hr.

3.10.3.3. Conclusion/Discussion

The samples containing the lysine gradient precipitated immediately between 0.0037 and 0.0046 mMol. The quantity of precipitate in the control without protein was <1/2 of that observed in the other samples.

This was attributed to the formation of phenol:amine aggregates that were not as bulky as the protein equivalent. Rather than inhibiting the aggregation, the lysine made it more efficient.

The samples containing lysine appeared to precipitate at approximately twice the rate as those containing 3-amino-1-propanol. At present, we suspect that this was observed because lysine is an α, ε diamine which can participate in cross-linking reactions to trigger, rather than inhibit, precipitation of the aggregate material.

The samples containing 3-aminopropanol improved only subtly across the concentration gradient. We suspect that inhibition was taking place, but that the "capped" protein was also unstable in solution. It was supposed that the 2-amino-1-propanol was reacting with the phenolics and bridging some, but not all, of the protein reversibly via hydrogen bonding.

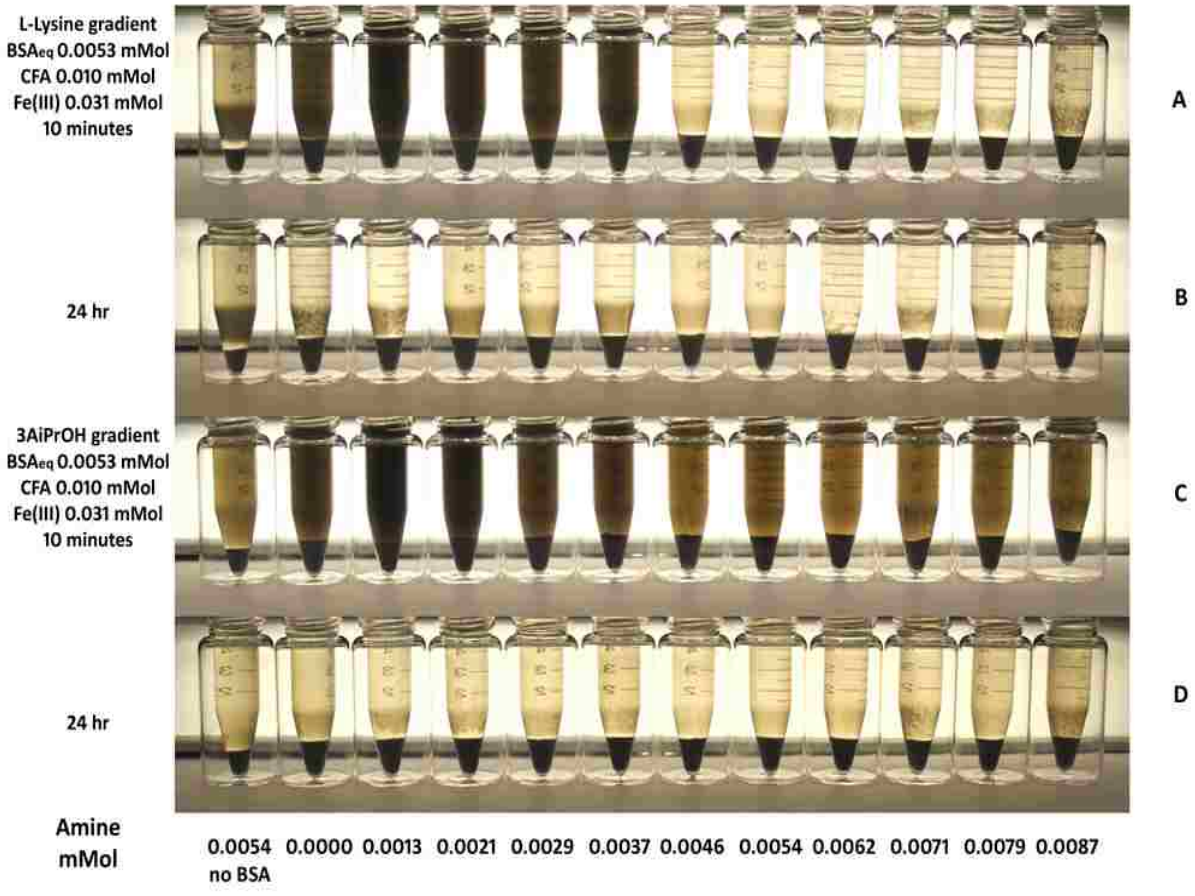


Figure 3.48. Inhibition of protein:phenol coupling in the presence of a more reactive amine.

3.11. Pilot Tests

This work describes the pilot-scale test of the iron mediated clarification and decolorization (FeMCaD) method using two reactors. The method is inexpensive and yields product juice with less color than conventional hot-liming. The bench-scale (15 mL – 1L) experiments, described previously, were scaled to 152 L. The pilot was operated in both pulsed and continuous modes at Raceland Raw Sugar Corp. during the 2007 crop season.

3.11.1. Two-Reactor Configuration

At bench scale, up to 70% of the colorant material entering the mill in the cane juice can be removed using this method. Exploration of the clarification mechanism with modeled juice and syrup revealed that four components, protein, phenolic compounds, carboxylic acid salts, and iron were required. The quantities of each of these components, except iron, are in the range found in cane juice.

It was noted during the bench scale trials that, although the decolorization continues to improve as iron levels are increased (up to a point), the settling rates decrease and mud-pack volume increases. This can be seen, for juice settled for 20 minutes, in **figure 3.49** where less juice recovered equates to a greater volume of mud. In the laboratory, we can centrifuge or filter the juice in order to measure the color.

Because this is impossible to do in the mill, it was decided that dosing to 150-200 mg/L to provide a decolorization of up to 50% would be our practical limit.

The aim of this testing was to evaluate the overall color removal which is possible both in batch (single-tank of juice dosed at once) and continuous modes of operation (steady-state with in-line addition of chemicals).

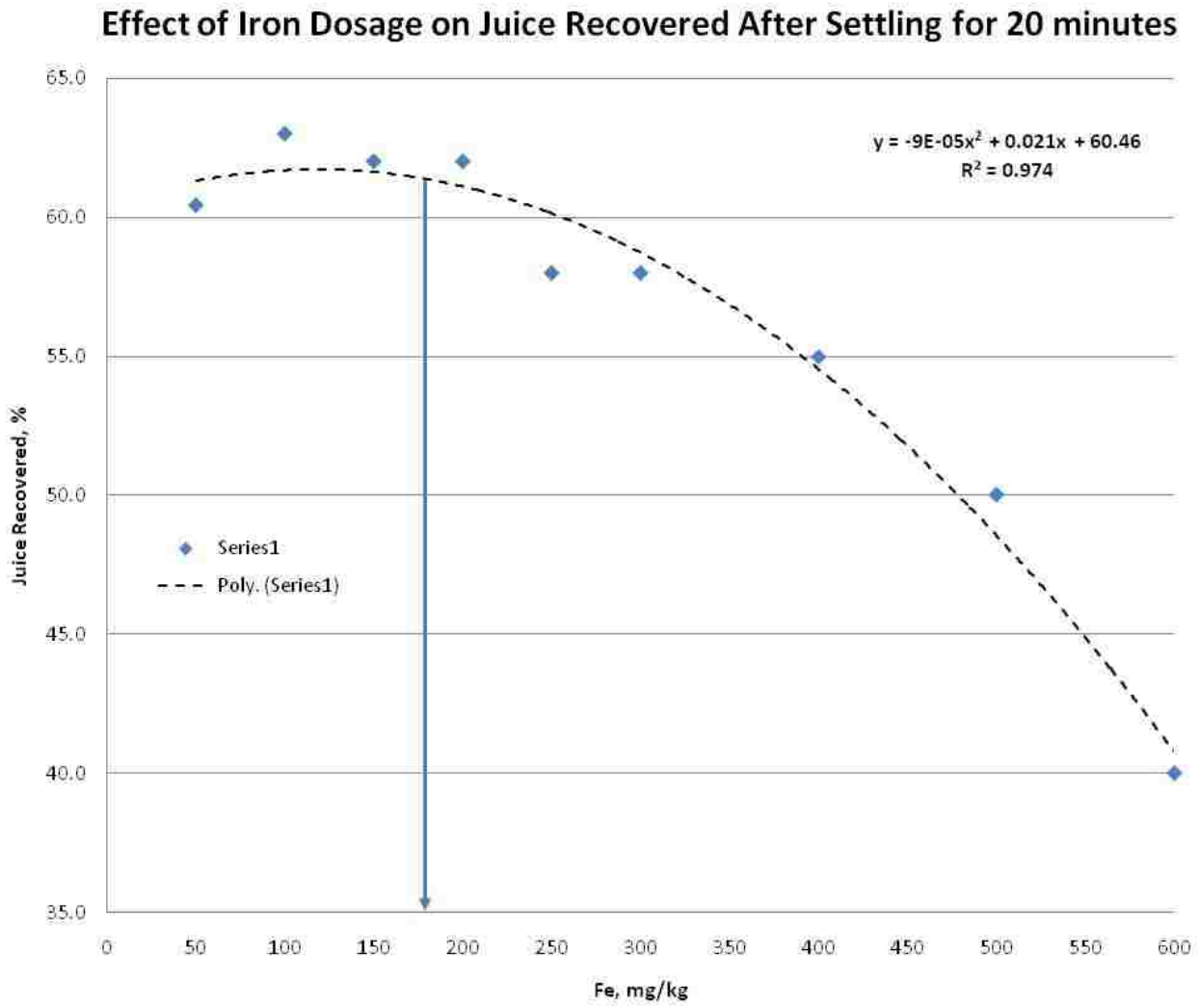


Figure 3.49. Increase of mud volume witnessed when increasing iron dosages beyond 200 mg/L.

Briefly, the two-reactor or "dual clarification" method involves:

1. Concomitant treatment of raw juice with Fe^{3+} at approximately 150 mg/L and a cationic polyacrylamide flocculant applied at 10-15 mg/L at ambient temperature.

2. Settling of this mixture to yield "stage-1 juice".

3. Stage 1 juice is limed "cold" to pH 7 and rapidly heated to boil.

4. To this is added up to 5 mg/L conventional anionic flocculant and the mixture is settled to provide a decolorized, "stage-2" juice.

3.11.1.1. Batch Tests

The clarifier was operated in batch mode.

A fixed quantity of raw juice was bolus-dosed with $FeCl_3$ and cationic polyacrylamide to give the desired concentrations of $Fe^{3+}$and flocculant. The color removal and settling characteristics were noted.

3.11.1.1.1. Materials and Methods

The bench-scale process (4-14 mL) was scaled-up, using a pilot-scale settling-clarifier constructed to hold approximately 40 gallons (151.6 L) of juice and was designed to operate continuously at two gal/min (7.58 L/min). The clarifier was used to test the efficacy of the two-reactor iron-mediated decolorization (FeMCaD) technology which was developed from this research. The set up used for this work can be seen in **figure 3.50**.

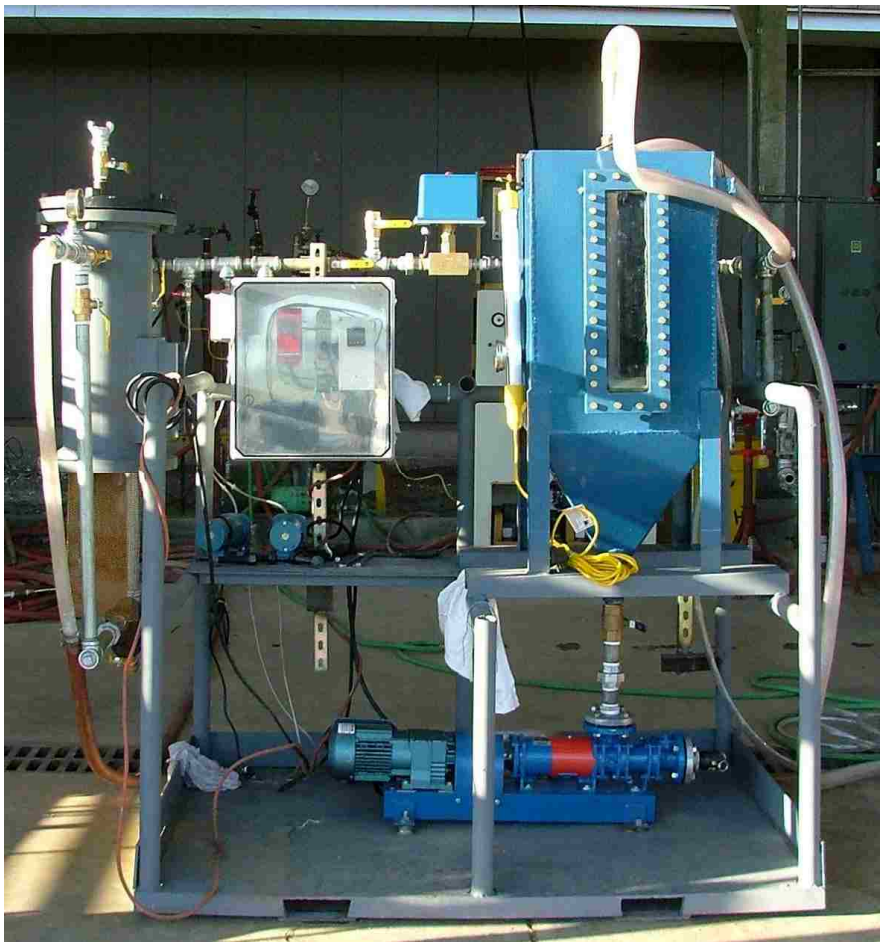


Figure 3.50. Pilot clarification module tested at Raceland (2007 campaign).

$FeCl_3 \cdot 6H_2O$ was applied directly as shipped (55 gal, 38.4 % w/w, water purification grade, Harcros Chemicals) using a small peristaltic pump.

Ecolab PCS 3106 cationic polyacrylamide (supplied as a solution containing 60% polymer) was likewise added from a solution diluted to 5100 μg/g polymer.

In the batch tests, the amount of iron added was bolus-dosed and was incremented from 0 to 50, 100, 150 and 200 mg/L in 40 gallons (152 L) of raw juice. Samples of the incoming (untreated) juice and treated juice were taken simultaneously. The incoming juice was cold-limed and rapidly boiled (microwave, high, 4 min.), treated with 5 mg/g LT-340 (Ciba) anionic polyacrylamide then settled. The iron-treated sample was cold-limed to pH 6.8-7.1, brought to boil, and treated with LT-340. Color was measured by ICUMSA method.

3.11.1.1.2. Results

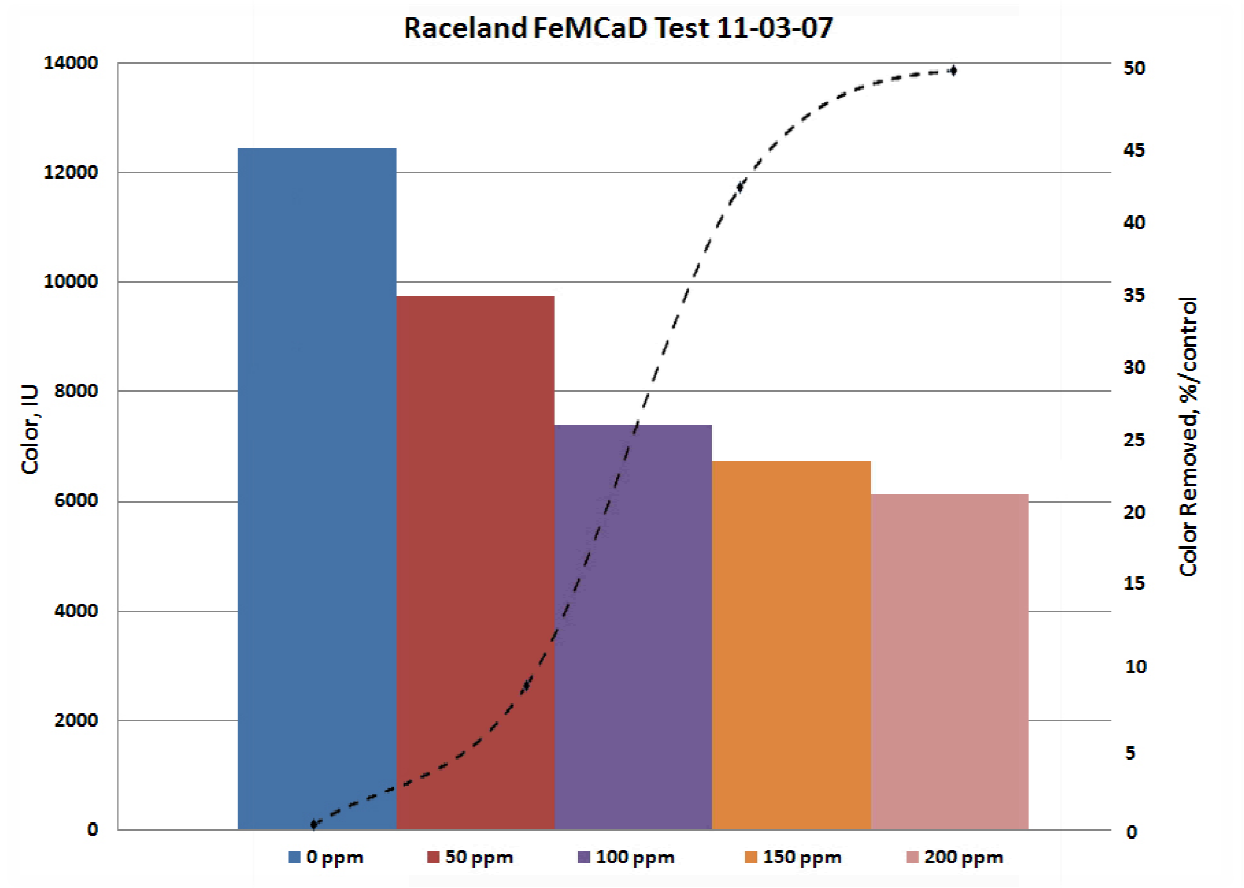


Figure 3.51. 152 L batch tests with incremental amounts of Fe^{3+}. CTRL samples were cold limed.

It was found that the color (ICUMSA) reduction was dependent on amount of iron applied. This relationship appears to follow the following empirical equation, where x is the amount of iron added in mg/L. Color removal appeared asymptotic at 50 % when iron was applied at 200 μg/g on juice.

Eqn. 3.1.

$$y = 35289x^{-0.33}$$

A chart displaying the removal, relative to a control taken simultaneously for each iron dosage is given in **figure 3.51**.

3.11.1.1.3. Conclusion/Discussion

The overall color reduction, versus raw juice clarified by cold-liming (control) was approximately 50% when the iron was applied at 150-200 mg/L. When applied iron exceeded this amount, the settling rate decreased. Thus, a dosage of 150 mg/L of iron was chosen for the test where the clarifier would be operated continuously.

The results were consistent with those achievable in the lab with a nominal average color removal of ~50%. It was noted that ~1/2 of the iron was required to achieve the same result with very fresh juice. This was attributed to state of the protein (unfrozen, etc.) relative to that observed in the laboratory.

3.11.1.2. Continuous Test

For this test, the clarifier was run under flow with continuous dose of iron and cationic polyacrylamide until steady state (a clear and static liquid:solid boundary with a bulk solution composition that remained more-or-less the same) was reached. Then, the juice produced was cold-limed and the color was assayed relative to controls, as before. The settling characteristics were noted.

3.11.1.2.1. Materials and Methods

The clarifier was brought to steady-state; this took approximately one hour. The 40 gallon (151.6 L) vessel was fed raw juice at 2.04 gallons per minute (gpm, 7.7 L/min).

Iron and cationic flocculant were dosed to supply 150 and 15 mg/L, respectively. The stage-1 juice was taken off at a rate of 1.8 gpm (6.7 L/min) and mud was removed via a progressive-cavity pump at a rate of 0.26 gpm (1.0 L/min). Samples were taken with controls as previously described. Color was measured by ICUMSA method, and dry solids were determined via gravimetry.

3.11.1.2.2. Results

After 45 minutes, the mud had a settled volume of 26 mL mud/100 mL of raw juice. This volume of mud contained approximately 1.2 g of dry solids (~4.6 g solid/100g mud). When run at bench scale, different juice (acquired on a day outside of the pilot test) from Raceland results very similar to those observed with the pulsed tests. This figure should vary somewhat, depending upon location, cane and the conditions prevailing through harvest, transport, storage and milling.

3.11.1.2.3. Conclusion/Discussion

The clarifier operated without incident for approximately 6hr (~734 gallons). During this time, the juice was collected, along with a concomitant control, limed, brought to boil using a microwave, flocced (5 mg/L anionic flocculant) and settled to yield stage-2 juice which was assayed for color. The juice examined in this way exhibited a level of decolorization which was comparable to that observed in the batch test at equivalent dosage.

It was concluded that the two-reactor method scales well and can provide a clarified juice with ~50% less color than that achievable with normal hot or cold-liming procedures.

During the course of this test it was realized that heating the juice after liming (cold liming) was quite effective. This was contrary to prior tests, involving the heating then liming of the juice (hot-liming) which led to juice which colored on exposure to air. Likewise, it was noted that cationic flocculant allows for higher dosages of iron to be used whilst still achieving reasonable mud settling rates. From this, it was found that a single reactor process, negating the need for a second clarifier, is possible.

We have found that if cane juice is treated with iron, cationic flocculant and cold-limed, up to 50% of the color in the juice can be removed at the clarifier. The liming step *must* take place *before* the juice is heated. If this order is reversed, the sucrose inverts and the juice will form color on contact with air. The discovery of this fact led to the single reactor process which negates the need to install a second clarifier.

3.12. A "Dual-Stage" Process Using One Reactor

A one-reactor process has been tested at bench scale. Briefly, the one-reactor process entails:

1. Concomitant treatment of raw juice with Iron (III) at approximately 150 mg/L and a polyamine cationic flocculant at 10-15 mg/L at ambient temperature.

2. The juice is limed "cold" to pH 7 and rapidly heated to flash.

3. To this is added up to 5 mg/L conventional anionic flocculant and the mixture is settled to provide a decolorized juice.

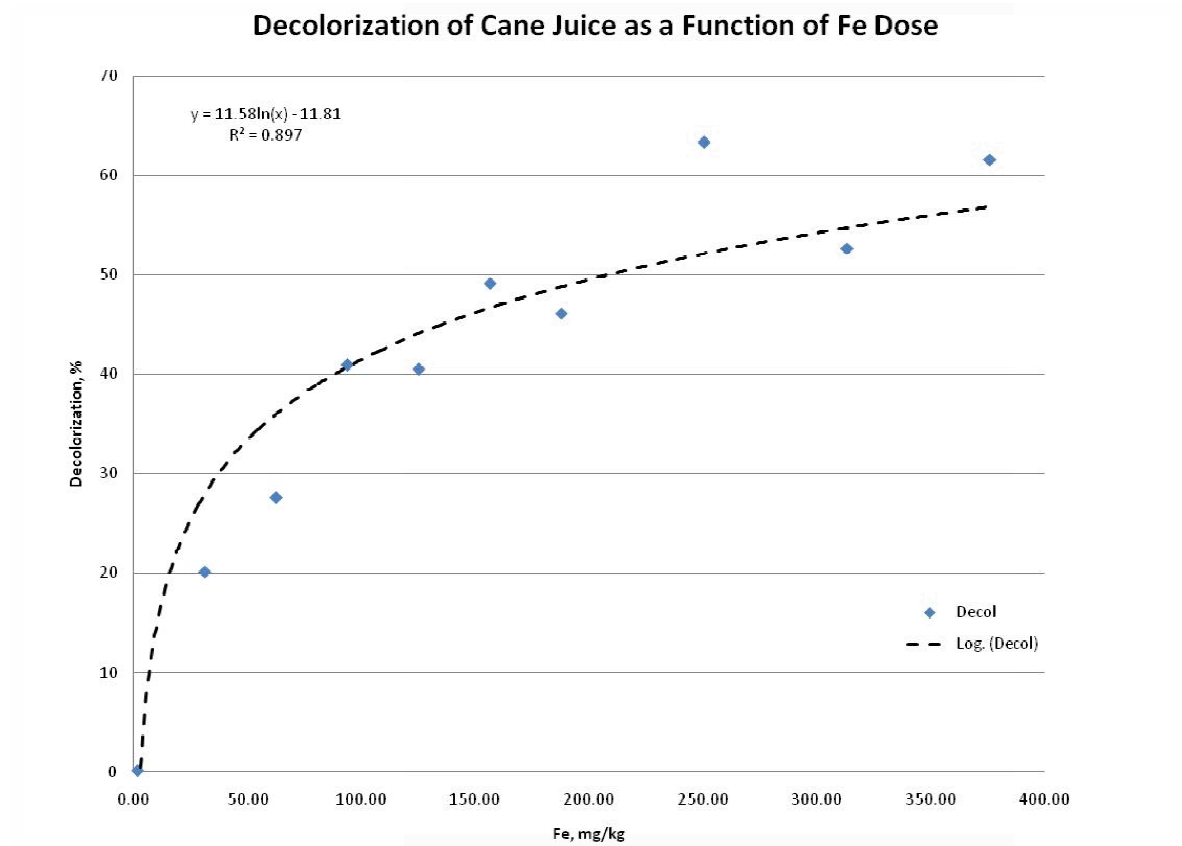


Figure 3.52. Decolorization of raw juice using the one-pot process at bench scale.

The decolorization potential, in **figure 3.52**, is similar to that seen in the 2-pot process, but suffers, as in **figure 3.49**, from similar issues with settling when operated at iron concentrations greater than 200 mg/L.

It appears, from the preliminary work, that the one-reactor process can yield results that are comparable to (decolorization, settling characteristics, etc.) those achieved using the two-reactor process.

We expect that this process could be implemented in a cold-liming mill with only the costs involved being chemicals, dosing pumps, and plumbing.

In order for this procedure to work at a mill, iron must be dosed at the appropriate rate, concurrently with a cationic flocculant. This can be done at the level of the first mill. *Immediately* thereafter, the treated juice can be limed and clarified normally. A minimum time of about a minute (it can be less, but we have observed that this time is safe) is required after iron addition, prior to liming.

3.12.1. Conclusion/Discussion

Iron (150-200 mg/mL) was found to remove approximately 50% of the color that entered the mill with the cane. The method produced reproducible results with mixed juices acquired from three Louisiana mills and the dose-response was consistent from batch-to-batch.

At present the iron mediated process is embodied in the form of two processes. The first is a two-stage process which involves the treatment of the juice with the iron and a cationic flocculant (cationic polyamine, 10-15 mg/L, active ingredient) and settling the material at ambient temperature. The resulting juice is decanted and cold-limed (it was found that hot-liming led to a rapid increase in both residual iron and color). This method has been tested at pilot scale (40 gal at 2 gpm) and yielded results similar to those seen in the more controlled laboratory-scale tests.

The second embodiment of the technology has only been tested at laboratory-scale, and can be done in one reactor.

This method has more promise for use in a sugar milling operation because it will necessitate far fewer expensive changes in the factory configuration. In this case, the juice, at ambient temperature, is treated with the iron and cationic agent and allowed to react for 1-5 minutes. To this is added lime to pH 7-8. If additional removal of turbidity is desired, the juice can be over-limed to pH 8 and titrated to pH 7 using H_3PO_4. The limed juice is rapidly heated to boiling and is treated with up to 5 mg/L anionic flocculant. This material is then settled and decanted normally. The only changes to a cold-liming mill involve the addition of dosing pumps and, perhaps a static mixer.

CHAPTER 4. SUMMARY

A new sugar refinery is to be built in Louisiana. Cooperation of the refinery with the raw sugar producers would help to insure the liquidity of the industry in the global market by providing a profit share on refined sugar (0.41-0.43 $/lb). Because raw sugar is produced at roughly 0.01$/lb (0.19-0.24 $/lb) over cost of production, there is significant motivation for the raw producers to participate. However, the refinery could import less expensive raw sugar from abroad (Brazil, Mexico, etc.). There must be a net increase in profit in order for this cooperative to work. The required incentive can be derived from the cost of fuel.

When cane is processed, the juice is removed from it leaving behind a lignocellulosic material called “bagasse”. The production of raw sugar from cane is a “green” process which is powered by combusting approximately 2/3 of the daily yield of bagasse. The remaining third is retained, and might find use in ventures involving the production of cellulosic ethanol. The price of the bagasse is rolled into the price of cane. Since this price is established on the theoretically recoverable sugar (TRS, lb sugar/ton cane), the fuel is free.

The refinery is powered using natural gas and the associated cost can offset up to 2/3 of the profit on refined sugar. A refinery is an industrial decolorizer. If the raw producer can make sugar with less color, then the refinery will need to burn less gas to operate. Therefore, for a cooperative venture to be profitable, the feed sugar must be of significantly less color. The threshold color reduction of at least 50 % (1500-700 IU) relative to conventional raw sugar would be required.

To do this, the raw producer could use carbon, ion exchange resins or ultrafiltration at the front end on either clarified juice or syrup. The initial and operational expenses associated with these operations are prohibitive unless a high-value specialty product is made (eg. Organic sugar). Alternatively, the factory could install affining stations which mingle the crystals in a solution pre-saturated with sugar. This serves to wash the mother liquor (molasses) off of the crystals.

Affination has been found to remove ~50% of the color. These stations cost approximately 30 million USD each, and this tends to increase the interest in new alternatives.

Toward this, we investigated the "color" found in the various stages of sugar processing. First, we observed that the choice of 420 nm for measurement of color at pH 7.0 was not arbitrary. Titration with standard alkali and subsequent UV-Vis spectrophotometry revealed that "color" was a shoulder with a λ_{max} = ~400 nm on a much larger (order of magnitude) peak at λ_{max} = ~370 nm. This range 9400-560nm), unlike the UV portion of the continuum, was pH sensitive and increased with the application of alkali. Relative to the peak at 400 nm, 420 nm exhibits an absorbance low enough to, in many cases, avoid dilution which contributes to the practical nature of this measurement. It was interesting to note that, all else equal, the ratio of color with iron over that without, relative to mMol of NaOH, was linear between pH 5.5 and 8.5. The equation defining this line was (R^2 = 0.9970):

Eqn. 4.1.

$$\frac{IU_{Fe}}{IU} = -892.87 \cdot mMol + 15.43$$

A linear range in color was also demarcated between pH 5.5 and 8.5; it is given here:

Eqn. 4.2.

$$pH = 1114.9 \cdot mMol - 9.24$$

It was concluded that an indicator value determined between these 5.5 and 8.5 would bear more theoretical significance than convention dictates, namely, the ratio of absorbance at 420 nm measured at pH 8.5 and 4.

The bulk of the color in sugar processing results either from reactions between carbohydrates alone (caramels) or with amines (melanoidin) via the Maillard-type reactions or, it enters the mill with the cane. Analysis via GC-MS of juice, syrup, molasses and raw sugar indicate that a many phenolic compounds enter in juice and survive processing to arrive in raw sugar. Very small amounts survive the refining process.

Focus was placed on the clarification process, and it was found that the clarification of hot juice (100-104 °C) with lime causes an increase in the number and quantity of phenolic compounds present in the juice. Because the majority of the phenolic compounds were from the monolignol class (coumaryl, guacyl or syringyl derivatives of benzoic or cinnamic acid), we concluded that alkaline delignification of the fine vegetable matter (bagacillo) present in the juice was causative. Compounds of this sort, specifically the 1,4 and 3,4-dihydroxybenzenoid derivatives, once oxidized, can become quinones.

Because it is widely known to be correlated with color, and was explained by Riffer (1986), iron has been regarded as anathema in sugar industry circles. Nevertheless, we considered that trivalent iron may be used to cause the polyphenols to polymerize to an extant sufficient to remove them via precipitation.

Because current research regarding decolorization dealt with clarified juice or syrup, we tested Fe^{3+}, applied as $FeCl_3.6H_2O$ on those first. We observed a 4-6 fold increase in color, which justified the industry-wide fear of iron. The next day, we observed that the color had decreased and there was a brown precipitate. Our idea was valid, but the time required was so long that microbial destruction and/or inversion of sucrose would be irreconcilable. Why did this not work?

It occurred to us that amines could react with quinones, and that protein is made up, in part, by the amino acid L-lysine (LYS). An α,ε-diaminocarboxylic acid, LYS, when part of a protein, will have a tethered (ε) amino group which could function as a nucleophile through space. Clarification with lime and heat removes the protein from the cane juice. We supposed that quinones could serve as cross-linking agents with respect to the free-amino functionality that is intrinsic to cane albumin. Without protein to rapidly increase the molecular mass of the aggregates, the addition of Fe^{3+} triggered the immediate formation of green-black, water-soluble phenol:iron complexes which eventually (hours) polymerized. We supposed that Fe^{3+} would increase the color of any product downstream from the clarifier. It is also why we applied Fe^{3+} to raw juice, protein and all.

Raw juice turned black when 500 μg/g Fe^{3+} was applied. The black material soon coagulated, and settled slowly. Upon centrifugation, ~90 % of the color appeared to have been removed. After hot-liming to pH 6.8-7.2, the material regained (indicator value) some color, but, final decolorization was observed to be ~70%. When tested on three raw juices, representing three Louisiana mills, we demonstrated a decrease in color that was dose responsive. The response when the juice was measured at pH = 7 is described here (R^2=0.9703):

Eqn. 4.3.

$$Decolorization,\% = 19.935 \cdot \ln\left(Fe^{3+}\mu g / mL\right) - 58.313$$

The relation given above applied well to iron doses of 500 μg/mL, or less. When more than this was applied, a large increase in color occurred. This color thus formed resembled that observed when iron was applied to clarified juice or syrup. A similar effect was observed after liming when raw juice was treated with iron while hot. The color formation was observed to occur upon exposure to air. More troubling is that when the color forming reactions were taking place, sucrose was being inverted at a rate exceeding that predicted by the Vukov-Schaffler model.

It was noted that the accelerated effect was more profound at the lower temperatures and that it tended to agree more with the model at higher temperatures and with higher iron doses. The behavior correlated well with the power law described here. The results are given in **table 4.1.**

Eqn. 4.4.

$$\frac{I_{observed}}{I_{predicted}} = a \cdot x^{-y}$$

Table 4.1. Inversion in FeMCaD models relative to the Vukov-Schaffler approximation.

Where: x = T °C

Fe^{3+}μg/mL:	***a*:**	***y*:**	**R^2:**
500	1.00E+12	5.529	0.9931
1000	2.00E+11	5.468	0.9966
2000	2.00E+11	5.912	0.9886

At low temperature (25°C), the model predicts that very little inversion should be taking place during the allotted 12 minutes. Thus, even a *very small* amount of inversion will provide a very large ratio. We suppose that the tendency toward ideal (model-like) behavior at higher temperature involves the point at which "true" inversion becomes the dominant reaction. The tendency for this to happen faster, when observed at equivalent temperature, is likely dependant upon iron dosage because H^+, which is catalytic, results from hydrolysis of the $FeCl_3$. The nature of the reaction which leads to the destruction of sucrose at low temperature is unknown, and would be an interesting subject of further research.

It became clear that there were factors at work in the juice that we did not understand. In order to gain control of this system, so that it could be made industrially useful, we modeled the system. Drawing on the findings of the cited works and our study using GC-MS, we decided to use caffeic acid, the prototype 3,4-dihydroxycinnamic acid found in cane as our phenolic surrogate. For protein, we chose to use BSA (Cohn fraction 5) because it is well characterized and readily available in purified form.

Our initial tests took place in water, and it was found that BSA will precipitate when acted upon by $FeCl_3$. HCl when added to equivalent pH (4.5) failed to elicit the same response. With CFA in the system, the BSA precipitated, but it had turned black. CFA, like chlorogenic acid (chlorogenic means "giving birth to green") treated with Fe^{3+} yields a green complex.

We suspected that the CFA was reacting with the protein in some way. We suspected that the amine functionality from lysine ($^{\varepsilon}NH_2$) was involved. Because L-glutamine is known to exist in cane juice, we tested mixtures of CFA and GLN with $FeCl_3$.

These samples containing the amino acid also turned black. When spectrophotometrically titrated against $FeCl_3$, a peak arose at ~ 300 nm at the expense of a peak at ~230 nm. The peak at 300 nm disappeared at the expense of a broad elevation of absorbance in the visible range when larger doses of iron were added.

A large increase in absorbance at 210 nm was attributed to an increase in carbonyl (quinone) functionalty. We attributed these observations to the reaction of quinones with amines either via Schiff-base imine formation with the quinone carbonyls or oxidative addition to the quinoid rings. The disappearance of the peak at higher iron dosage was attributed to polymerization of the quinoid materials to yield larger more highly conjugated structures.

When tested in sucrose solutions ranging from 10 – 50 g/100g, Fe^{3+} failed to precipitate BSA regardless of whether or not CFA was present. Other than the color of the $FeCl_3$ solution, there was very little, if any change in color. It was found that the salts of small carboxylic acids triggered the immediate formation of colored precipitate. The counter ion was found to be of no consequence as the NH_4^+ salt of acetic acid was found to be equivalent to the Na^+ form. The free acids were not effective. There was a dependence of chain length of the acid to the amount which was required, and it decreased with increased carbon number. This dependence followed a power law and is given here (R^2 = 0.9930, Cn = carbon number for n-alkanoic acids):

Eqn. 4.5.

$$Carboxylate_{g/L} = 0.686 \cdot Cn^{-0.45}$$

Because of increasing molecular weight, the relation in mM is parabolic (R^2 = 0.9989):

Eqn. 4.6.

$$Carboxylate_{mMol} = 0.0008 \cdot Cn^2 - 0.0046 \cdot Cn + 0.0349$$

From the parabolic relation, it appears that the propionic acid salts are the most effective relative to the mass required. This is offset by the fact that there is a large amount of acetate present in cane juice.

It was noted that the quantity of sodium oxalate required to trigger precipitation was almost exactly ½ of that for sodium acetate.

Because the counter ion was found to be unimportant (if monovalent), we concluded that the carboxylate moiety was required, and that oxalate was behaving as two equivalents. It is not known if the oxalate was bridging.

The mechanism is unknown, but because these carboxylates are too small to form micelles, we are concluding that the mechanism involved may be related to ligand assisted phase transfer. This hypothesis would provide an excellent starting point for future work in this area. Further, oxalate is present in juice, along with other di and tri-carboxylic acids. It would be of interest to test the sodium forms of these acids for efficacy.

Now that we had models that worked, we were able to test them. The model was optimized in terms of the quantities of each component, BSA, CFA, acetate and iron that provided both adequate clarification and removal of color. The optimum quantities were determined via spectrophotometry and were compiled into **table. 3.8**. The quantities of each component corresponded with those normally found in cane juice, the optimized quantity of Fe^{3+} (~225 mg/mL or 0.05 mM) was routinely removed 60-70 % of the color from raw juice. Our model was found to be representative of juice with respect to flocculation with hydrolyzing iron salts.

Residual iron decreases the quality of raw sugar and can cause the sugar to degrade more rapidly over time. We tested the juices and muds resulting from the iron and lime stages of the two-step process. We determined that significant iron is removed in the initial stage, which suggest some removal via complex formation.

The bulk of the residual iron was removed during the liming stage where it was removed as the insoluble hydroxide. Significant decolorization, presumably via different mechanisms occurred at each stage.

A plateau in the iron recovered from stage 1 mud occurred at dosages exceeding 500 mg/kg. This was attributed to a “saturation” of the existing chelating material. The iron began to increase in the juice at the point directly following the plateau. This is consistent with the previous conclusion.

It appears that the Fe^{3+}:CFA equivalence is ~2.2:1. Binding of Fe^{3+} directly to the protein was also observed, and this appears to be optimal at a molar ratio totaling ~4.1:1. Free iron began to appear at ratios exceeding 8.2:1.

An ancillary experiment that was not detailed in this work, demonstrated that while both caffeic and prototcatechuic (3,4-dihydroxybenzoic) acids rapidly led to floc formation in optimized systems, catechol (2-hydroxyphenol) *did not*. Upon examination of these samples after 24 hr, it became apparent that the catechol did yield precipitate, but that it took far longer—hours rather than seconds. This information, coupled with the equivalents on iron, pH of the solutions, knowledge of "capping" that was derived though the inhibition experiments helped us to imagine the theoretical structure in **figure 4.1**, which is one of many, that fits the observations that we have made.

From this figure we imagine that Fe^{3+} complexed with, in this case, CFA and facilitated the oxidation via O_2 to yield the electrophilic quinone which was attacked by the N^{ε}-amino group from lysine. The total Fe bound and needed for single electron transfers adds up to very nearly 4:1 on CFA which is very close to the observed 3.8-4.1. Alternatively, the radical from the semiquinone delocalized to the 6 position and participated in the bond formation. In this way, the protein is first "capped". It is unknown if catechol caps the protein and then gets "stuck there" relying on a dilute concentration of monomer to grow.

The initial "caps" then add further CFA which is held in place by salicylate-type bonding. This stage should be reversible, but, at room temperature, was too fast to test. Held in place by the Fe^{3+}, which simultaneously facilitated the autooxidation, the addition of the new CFA residue occurs. Growth would continue in this way until the CFA is exhausted, the iron is totally bound, the chains from opposing proteins meet or otherwise terminate, and/or the capping oligos become large enough to precipitate the aggregate.

It is not known, but is possible, that metal assisted hydroxylation (via Fe^{3+}/O_2) of tyrosine (also, possibly, tryptophan) residues might occur. This would explain some of the deviations in the stoichiometry that we were unable to account for. That is, on-board, reactive phenolic species could lead to inter-chain bonding by creating a quinone to serve as an attachment point.

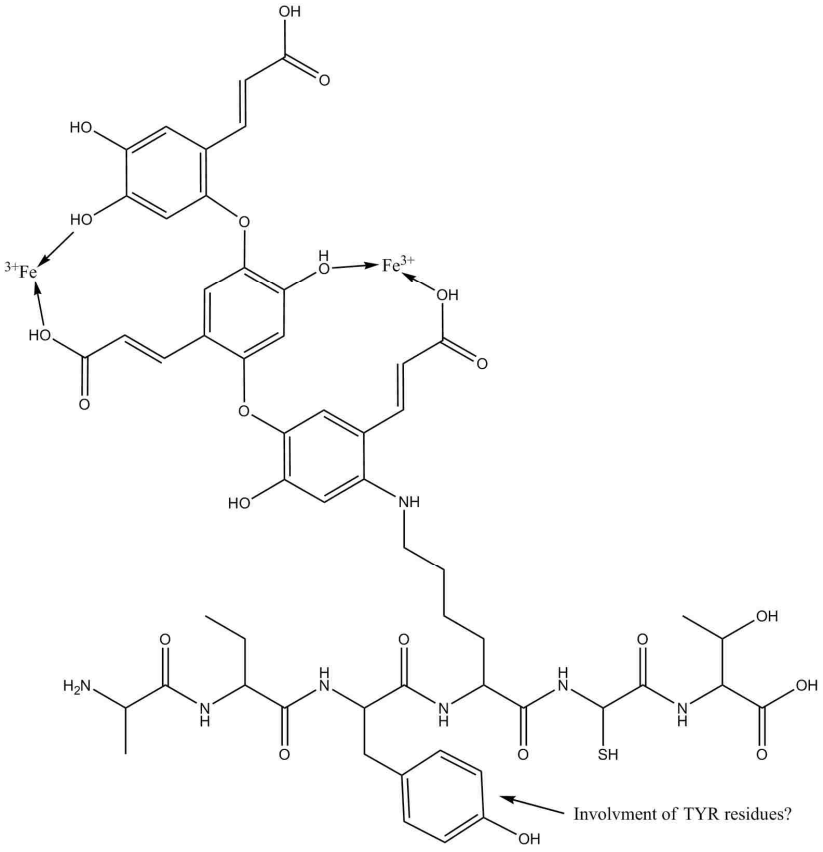


Figure 4.1. A theoretical aggregate intermediate.

Once they are exposed to lime, the fate of the materials formed during treatment with iron is unknown. What is the partition coefficient for iron between the phenolic and proteinaceous fractions? That would be an excellent question to answer with further work.

It appears that the iron mediated stage involves reversible, but fast, coagulation via charge neutralization. Irreversible bridging resulted from crosslinking of protein via phenol-oligomers grown *in-situ* which were flocked via a combination of electrostatic and covalent bridging with cationic polyacrylamide (c-PAC).

The coagulation/flocculation which occurred both before and after addition of c-PAC was non-reversible. Addition of further c-PAC did not re-disperse the flocs and increased floc volume. When centrifuged, higher c-PAC dosages facilitated decolorization in the higher ranges (60-80%), but the suspensions did not settle, unassisted, within a useful frame of time (20 min or less). Because this limits the decolorization potential to ~50%, doses of c-PAC greater than 10-15 μg/mL should be avoided.

Changing pH did not disrupt the stage-one flocks. They survived pH 2-11 intact. Because the equilibria governing imine formation can be reversed by changing pH, this tends to rule out a Schiff-only covalent mechanism. Whether a reduction occurs stabilizing the imine as the amine, which would render it stable to changes in pH is unknown. A study testing mixtures of various antioxidants with pre-formed imino compounds in the presence of Fe^{3+} and then looking for formation of the corresponding amines would be interesting.

At this time we have a two stage system which can remove up to 70% of the colored material which enters the mill with the cane. The method is chemically inexpensive, but the implementation of a two-stage process includes the addition of a second clarifier which will make implementation difficult or impossible. The method works well, but it was found that treatment of heated juice (hot-liming protocol) with iron led to the initation of a reaction sequence which rapidly increases the color when the juice is exposed to air. This effect was attributed to the quantity of free iron detected in the juice.

Concisely, at higher temperatures, a smaller quantity of iron will contribute a similar destructive influence as an excessive dose added at lower temperature. When this condition occurs, color is formed and destruction of sucrose results at a rate exceeding that predicted by calculation.

Experiments conducted to understand why this was happening with the hot-liming system system revealed that the addition of iron to juice at ambient temperature (19-30°C) and then liming (a *cold*-liming protocol) produced a decolorized juice which was stable on exposure to air. Neither added color nor inversion resulted when the iron was applied to cold juice, limed then heated.

The stoichiometry of CFA to BSA demonstrates a rapid saturation where nearly all available amino groups are “capped” via reaction with quinone species. Consumption of CFA increases in a linear fashion which suggests polymerization. The steady state consumption of CFA just prior to termination, which is abrupt, indicates that each protein would have, attached to it, ~10 phenolic chains of DP = ~8.

Aside from this, there are at minimum, four components required for this process to work. There must be soluble iron in 3+ oxidation state, some protein (or amino-substituted polymer; both chitosan and poly-allylamine work very well as protein substitutes), phenolic material, and carboxylic acid salt. The ranges required are 150-300 mg/g, 0.04-0.15g/100g, 60-150mg/g and 0.075-0.125mM, respectively.

It appears that cane protein behaves differently than BSA in diluted syrup matrices, requiring up to an order of magnitude more iron to achieve the same result. Work is on going to characterize gross cane protein such that a greater understanding of this system can be derived.

Methods geared toward the measurement of antioxidant activity are severely hampered by the fact that the samples contain iron, and if the samples also contain phenolics, then some of that iron is Fe^{2+}. Fe^{2+} quenches, via single electron transfer, most stable free radicals that are customarily used as probes (such as 1,1’-diphenyl-2-picrylhydrazyl). Effort toward the development of a viable assay for these matrices is underway and the initial results look promising.

It has been demonstrated (Godshall, et al. 2006; Zossi, et al. 2009) that what works on the bench is not always equally effective when brought to industrial scales. The iron mediated decolorizing and clarification (FeMCaD) process is the embodiment of a cold-liming procedure involving a pretreatment of the juice with $FeCl_3$ (less effective, $Fe_2(SO_4)_3$). On the bench, with centrifugation, it can provide a juice with ~70 % less color than a hot-limed control. When left to settling only, with the aid of a cationic polyacrylamide, the method reliably delivers a 50 % reduction in color while settling within 20 minutes.

The method was scaled up to 40 gallons (151.6 L) using a specially constructed pilot scale settling clarifier that was operated in both pulsed and continuous (2 gpm, 7.6 L/min) modes with increasing iron dosages. It was demonstrated that the color removal, relative to a cold-limed (removal vs. hot-limed juice would appear to be greater) control was dependant upon the dosage of iron (unlike PAC where color removal was proportional to the amount of flocculant; Godsall et al, 2006). The dependence is given by **equation 3.1**. The color removal reached a maximum near 50%. When run in continous mode, at a fixed iron concentration of 150 mg/L, the color removal was again ~50 %, relative to a cold-lime control. If this translates into sugar with less color, this process is competive with affining.

We concluded that the FeMCaD process can be run at intermediate scale with efficacy similar to that seen on the bench. We have finished the addition of a tube-sheet heat exchanger, hold up tank and additional dosing pumps to our pilot clarifier. We intend to run this module for several weeks, in a sugar mill, to determine how well color removal from juice translates to sugar.

Methods, like this one, meeting the benchmark of 50% color removal, can extend the time between regeneration cycles in decolorizer modules using carbon and/or ion exchange. Since the profit achievable from sugar is a balance between the raw sugar quality and the cost of the fuel needed to refine it, it is our intent to fully develop this new technology so that it can be eventually applied to the economic benefit and stability of our state.

REFERENCES

1. Amarasekara, A.S., Williams, L.D. and Ebede, C.C. (2008). **Mechanism of the dehydration of D-fructose in dimethylsulfoxide at 150 °C: an NMR study**. *Carb. Res.343, pp. 3021-3024.*

2. Angyal, S.J. (2001). **The Lobry de Bruyn-Alberda van Eckenstein Transformation and Related Reactions**. *Top. Curr.Chem. 215, pp. 1-14.*

3. Antal, M.J., Mok, W.S.L. and Richards, G.N. (1990). **Mechanism of Formation of 5-(hydroxymethyl)-2-furaldehyde from D-Fructose and Sucrose**. *J. Carbohydr. Res. 199, pp. 91-109.*

4. Aronstein, B.N., Paterek, J.R., Kelly, R.L. and Rice, L.E. (1995). **The Effect of Chemical Pretreatment on the Aerobic Microbial Degradation of PCB Congeners in Aqueous Systems**. *J. Ind. Microbiol. 15 (1), pp. 55-59.*

5. Arrhenius, S. (1889).**On the Reaction Velocity of the Inversion of Cane Sugar by Acids.** *Zeitschrift für Physikalische Chemie **4**, 226; translated and published in Margaret H. Back & Keith J. Laidler, eds., Selected Readings in Chemical Kinetics (Oxford: Pergamon, 1967).*

6. Austin Genealogical Society (2003). **Calculate Consumer Price Index (CPI) from 1665-2012**. http://www.austintxgensoc.org/calculatecpi.php.

7. Awad, A.B. and Fink, C.S. (2000). **Phytosterols as Anticancer Dietary Components: Evidence and Mechanism of Action.** *J. Nutr. 130, pp. 2127-2130.*

8. Ball, C.D., Hardt, C.R. and Duddles, W.J. (1943). **The Influence of Sugars on the Formation of Sulfhydryl Groups in Heat Denaturation and Heat Coagulation of Egg Albumin**. *J. Biol. Chem. 151 (163), pp. 163-169.*

9. Ballash, N.M. and Robertson, E.B. (1972). **The Mutarotation of Glucose in Dimethylsulfoxide and Water Mixtures**. *Can. J. Chem. 51, pp. 556-564.*

10. Baltes, W. and Bochmann, G. (1987). **Model Reactions on Roast Aroma Formation.1. Reaction of Serine and Threonine with Sucrose Under the Conditions of Coffee Roasting and Identification of New coffee Aroma Compounds**. *J. Agric. Food. Chem. 35(3), pp. 340-346.*

11. Barb, W.G., Baxendale, J.H., George, P and Hargrave, K.R. (1951a). **Reactions of Ferrous and Ferric Ions with Hydrogen Peroxide: Part 1-The Ferrous Ion Reaction.** *Trans. Faraday Soc. 47, pp. 462-500.*

12. Barb, W.G., Baxendale, J.H., George, P and Hargrave, K.R. (1951b). **Reactions of Ferrous and Ferric Ions with Hydrogen Peroxide: Part 2-The Ferric Ion Reaction**. *Trans. Faraday Soc. 47, pp. 591-616.*

13. Barbosa-Canovas, G.V. (2002). **Water Activity Prediction**. *Annual Meeting of the Institute of Food Technologists, Anaheim, California.*

14. Barbosa-Canovas, G.V., Fernandez-Molina, J.J., Alzamora, S.M., tapis, M.S., Lopez-Malo, A. and Welti Chanes, J. (2003). **Handling and Preservation of Fruits and Vegetables by Combined Methods for Rural Areas**. *Food and Agricultural Organization of the United nations, Technical Manual, FAO Agr. Serv. Bull. 149. ISBN# 92-5-10486-4, Chapter 4. Full text online: http://fao.org/docrep/005/Y4358E/y4358e00.htm*

15. Bard, A.J. and Faulkner, L.R. (2004). **Electrochemical Methods : Fundamentals andApplications**. *John Wiley & Sons, Inc. QD553.B37 2000. pp. 808-809.*

16. Bartoleme, B., Estrella, I. and Hernandez, M.T. (2000). **Interaction of Low Molecular Weight Phenolics with Proteins (BSA).** *J. Food. Sci. 65 (4), pp. 617-621.*

17. Bekker, P.I. and Stolz, H.N.P. (2001). **Enhancement of Sugar Production Part I: Production of White Sugar in the Raw House.** *Proc. S. Afr. Sug. Technol. Ass. 75, pp.341-345.*

18. Benedict, S.R. (1908). **A Reagent for the Detection of Reducing Sugars.** *J. Biol. Chem. 5, pp. 485-487.*

19. Bennett, M.C. (1957a). **Behavior of Adsorbed Protein in Cane Sugar Juice**. *J. Colloid Sci. 12, pp. 434-452.*

20. Bennett, M.C. (1957b). **The Physical Nature and Behavior of Cane Sugar Juice, Part I**. *Int. Sug. J. 59 (703), pp. 176-177.*

21. Bento, L.S.M. (2006). **Activated Carbons: Adsorption of Sugar Colorants and Chemical Regeneration.** *Proc. Sug. Ind. Technol. 65.*

22. Bishop, D.F. et.al. (1968**). Hydrogen Peroxide Catalytic Oxidation of Refractory Organics in Municipal Waste Waters**. *Ind. Eng. Chem., Process Design & Development, 7, pp. 1110-1117.*

23. Bochek, A.M. (2003). **Effect of Hydrogen Bonding on Cellulose Solubility in Aqueous and Nonaqueous Solvents**. *Rus. J. Appl. Chem. 76 (11), pp. 1711-1719.*

24. Boerjan, W., Ralph, J. and Baucher, M. (2003). **Lignin Biosynthesis**. *Annu. Rev. Plant Biol. 54, pp. 519-546.*

25. Bogren, R. (2006). **New Varieties, Cane Energy Highlight LSU Field Day**. *Delta farm Press, Penton Media, Inc.Sept. 8. http://deltafarmpress.com/mag/farming_new_varieties_cane/.*

26. Brady, B. (2005). **Cargill, Louisiana Sugar Producers Team Up in Production and marketing Alliance: New Refinery to be built on Gulf of Mexico**. *Press Release. http://www.cargill.com/news/news_releases/2005/051101_lcspi.htm*

27. Broadhurst, H. A. and Rein, P.W.(2002). **The Characterization of Cane Sugar Colorant**. *Proc. Sug. Proc. Res. Inst. pp. 292-310.*

28. Budavari, S., O'Neil, M.J., Smith, A. and Heckelman, P.E., Eds. (1989). **The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. 11th Ed**. *Merck & Co., Inc. Rahway, N.J., USA. ISBN # 911910-28-X, pp. 4183.*

29. Cai, K. and Bennick, A. (2006). **Effect of Salivary Proteins on the transport of Tannin and Quercetin Across Intestinal Epithelial Cells in Culture**. *Bioch. Pharmacol. 72, pp. 974-980.*

30. calculated manually from **thermochemical data tables**: http://en.wikipedia.org/wiki/Standard_enthalpy_change_of_formation_(data_table)

31. Cameco Industries. (1995). **ABC Process**. *Proc. Int. Soc. Sugar Cane Technol. 22, pp.*

32. Cavalieri, E.L., Li, K-M., Balu, N., Saeed, M., Devanesan, P., Higginbotham, S., Zhao, J., Gross, M.L. and Rogan, E.G. (2002). **Catechol ortho-quinones: the electrophilic compounds that form depurinating DNA adducts and could initiate cancer and other forms of diseases**. *Carcinogenesis 23 (6), pp. 1071-1077.*

33. Chaiasut, C. and Tsuda, T. (2001). **Isoelectronic Points Estimation of Proteins by Electroosmotic Clow: pH Relationship Using Physically Adsorbed Proteins on Silica Gel**. *Chromatography 22 (2).*

34. Charlet, P.G., Colodette, J.L., Xavier, C.R and Mascioli, J.C. (2002). **Process for Preparing Improved Sugar Product**. *U.S. Patent # 6,368,413. Apr. 9.*

35. Charlton, A.J., Baxter, N.J., Khan, M.L., Moir, A.J., Haslam, E., Davies, A.P., Williamson, M.P. (2002). **Polyphenol/peptide binding and precipitation**. *J. Agric. Food. Chem. 50 (6), pp. 1593-1601.*

36. Chen, Y. and Hagerman, A.E. (2005). **Reaction pH and Protein Affect the Oxidation Products of β-pentagalloyl Glucose**. *Free. Rad. Res. 39 (2), pp. 117-124.*

37. Cheney Lime and Cement Co. **Lime Facts**. http://www.cheneylime.com/limefact.htm

38. Chenier, P.J. (1992). **Survey of Industrial Chemistry, 2nd Ed**. *VCH Publishers, ISBN# 1-56081-622-8. pp. 80-86.*

39. Cheves, W. (1975). **Fenton's Reagent Revisited**. *Accts. of Chem. Research. 8, pp. 125-131.*

40. Chou, C.C. (2000). **Handbook of Cane Sugar Refining: A manual for the Design and Operation of Sugar Refining Facilities**. *John Wiley & Sons, Inc. TP377.H27 2000, ISBN# 0-471-18357-1, pp. 63.*

41. Chou, C-C., Iqbal, K., Min, Y-G., Gao, D-W. and Duffaut, E. (2006). **SAT Process as a Replacement for Sulfitation in Mill White Sugar Production.** *Int. Sug. J. 108 (1289), pp. 247, 249-251, 253.*

42. Chou, C-C., Min, Y-G., Gao, D-W. and Theoleyere, M.A. (2002). *U.S. Patent # 6,485,574. Nov. 26.*

43. Christ, D., Takeuchi, K.P. and Cunha, R.L. (2006). **Effect of Sucrose Addition and Heat Treatment on Egg Albumin Protein Gelation**. *J. Food Sci. 70 (3), pp. E230-E238*.

44. Chun, C., Xiaojian, M.A., Peilin, C.E.N. (2006). ***Kinetics of Levulinic Acid Formation from Glucose Decomposition at High Temperature****. Chin. J. Chem. Eng. 14 (5), pp. 708-712.*

45. Clarke, S.J. (2006). **Some Hard Lessons with Membrane Filtration**. *Proc. I.S.S.C.T. Processing Workshop, June 1. Lod-Cook Conference Center, L.S.U.*

46. Clarke, M.A., Edye, L.A. and Eggleston, G. (1997). **Sucrose Decomposition in Aqueous Solution, and Losses in Sugar manufacture and Refining**. *Adv. Carbohydr. Chem. Biochem. 52, pp. 441-469.*

47. Coombs, J., Baldry, C., Bucke, C. and Long, S.P. (1974). **o-Diphenol:Oxygen Oxidoreductase from Leaves of Sugar Cane**. *Phytochem. 13, pp. 2703-2708.*

48. Coombs, J. and Baldry, C.W. (1978). **Formation of colour in cane juice by enzyme-catalyzed reactions Part 1. Mechanism and chemical inhibition**. *Int. Sug. J. 80, pp. 291-294.*

49. Cotton, F.A. and Wilkinson, G. (1980). **Advanced Inorganic Chemistry: A Comprehensive Text. 4th Ed**. *John Wiley and Sons, Inc. ISBN#: 0-471-02775-8, pp. 283.*

50. Cumpstey, I. (2008). **Carbohydrate Chemistry Course – Synthesis : Lecture 2-Protecting groups in carbohydrate chemistry**. *www.casper.organ.su.se/chochem/synthesis2.pdf*

51. Daly, J.D. (2007). **Pricing scheme for hydrogen peroxide is to be based on dry weight for all purchases made in NAFTA countries.** *http://www.solvaychemicals.us/static/wmp/pdf/8/6/2/8/HPconversionscr.pdf*

52. Danilewicz, J.C. (2003). **Review of Reaction Mechanisms of Oxygen and Proposed Intermediate Reduction Products in Wine: Central Role of Iron and Copper.** *Am. J. Enol. Vitic. 54 (2), pp. 73-85*.

53. Davis, S.B. (2001). **Experiences with Oxidative Decolourising Processes in the Southern African Sugar Cane Industry**. *Proc. Int. Soc. Sug. Cane. Technol., 24, pp. 112-115.*

54. Davis, S.B., Moodley, C.L., Govender, B. and Xaba, M. (2000). **Preliminary Results of Hydrogen Peroxide Trials at Gledhow Refinery.** *Proc. S. Afr. Technol. Ass.74, pp. 358-360.*

55. Davis, S.B., Moodley, M., Singh, I. and Abendorff, M.W. (1998). The **Use of Ozone For Colour Removal at the Malelane Refinery**. *Proc. S. Afr. Technol. Ass.72, pp. 255-260.*

56. Day, D.F. and Kampen, W.H. (2003). **Organic Acids in the Sugar Factory Environment**. *J. Am. Soc. Sug. Technol. 23, p. 111.*

57. Day, D.F. and Chung, C-H. (2007). **Isomaltooligosaccharides from Leuconostoc as neutraceuticals**. *US Patent # 7291607. http://www.freepatentsonline.com/7291607.html*.

58. Deerr, N. (1916). **The Clarification of Cane Juice in the Manufacture of Raw Sugar**. *Int. Sug. J. 18 (215), pp. 502-506.*

59. Deerr, N. (1949). **The History of Sugar Vol. 1**.*Chapman and Hall, LTD. Cat# 346/4. Plate 7 facing p.79.*

60. Deerr, N. (1950). **The History of Sugar Vol. 2**.*Chapman and Hall, LTD. Cat# 349/4. Plate 24 facing p.451.*

61. Defaye, J. and Garcia Fernandez, J.M. (1995). **The Oligosaccharide Components of Caramel**. *Zuckerind. 120 (8), pp. 700-704.*

62. Deitl, K.H., Winterberg, B., Bertram, H.P. Still, F.H., Spieker , C. and Kisters, K. (1997). **Aluminum Bone Disease Improved After Kidney Transplantation: Determination by Atomic Absorption Spectroscopy.** *Trace Elements and Electrolytes, 14 (3), pp. 113-115.*

63. Dentel, S.K. (1988). **Application of the Precipitation-Charge Neutralization Model of Coagulation**. *Environ. Sci. Technol. 22, pp. 825-832.*

64. de S. e Silva, P.T., Locatelli, M.A.F., Jardim, W.F., Neto, B.B., de Motta, M., de Castro, G.R. and da Silva, V.L. (2008). **Endogenous Iron as a Photo-Fenton Reaction Catalyst for the Degradation of Pah's in Soils.** *J. Braz. Chem. Soc. 19 (2), pp. 329-336*.

65. Dittrich, R., El-Massry, F., Kunz, K., Rinaldi, F., Peich, C., Beckmann, M.W. and Pischetsrieder, M. (2003). **Maillard reaction products inhibit oxidation of human low-density lipoproteins in-vitro**. *J. Agric. Food Chem. 51 (13), pp. 3900-3904.*

66. Dongying Guangzheng Chemicals Co., Ltd. (2008). **Features Specifications: Anionic Polyacrylamide**. *http://www.alibaba.com/product-gs/51085859/Anionic_Polyacrylamide.html. See also, specifically, http://www.dygzchem.com.*

67. Eggleston, G., Vercelotti, J., Edye, L. and Clarke, M. (1996). **Effects of Salts on the Initial Thermal Degradation of Concentrated Aqueous Solutions of Sucrose**. *J. Carb. Chem. 15, pp. 81-94*.

68. Eggleston, G. (2000a). **Hot and cold lime clarification in raw sugar manufacture I: Juice quality differences.** *Int. Sug. J. 102 (1220), pp. 406-416.*

69. Eggleston, G. (2000b). **Hot and cold lime clarification in raw sugar manufacture II: Lime Addition and settling behavior**. *Int. Sug. J. 102 (1221), pp. 453-457.*

70. Eggleston, G. (2002). **Sources and Indicators of Cane Deterioration**. *Proc. Sug. Process. Res. Conf. March 10-13, pp. 227-242.*

71. Eggleston, G. (2006). **General Subject 4: Molasses**. Proc. ICUMSA, Yasahura, Y., Ando, K., Koyama, K., Hiramoto, K. and Kikugawa, K. (2002). **Effect of Supplementation of a Reductone in Coffee,**

72. Eggleston, G., Basso, L.C., Amorim, H., De Lima Paulillo, S.C. and Basso, T.O. (2006). **Mannitol as a Sensitive Indicator of Sugarcane Deterioration and Bacterial contamination in Fuel Alcohol Production.** *Proc. Sug. Proc. Res. Inst. Sept. 17-20, Águas de São Pedro, S.P., Brazil, pp. 199-213.*

73. Endres, T.D. (2003). **Experiences with Unwashed Cane at Raceland***. J. Am. Soc. Sug. Technol. 23, p. 112.*

74. *Falco, M.C., Phellippe, A.S., Marbach, P.A.S., Pompermayer, P., Lopes, F.C.C. and Silva-Filho, M.C. (2001).**Mechanisms of sugarcane response to herbivory**. Genet. Molec. Biol. 24 (1-4), pp. 113-122.*

75. Fales, H.A. and Morrell, J.C. (1922). **The Velocity of Inversion of Sucrose as a Function of the Thermodynamic Concentration of Hydrogen Ion**. *J. Am. Chem. Soc. 44 (10), pp. 2071-2091.*

76. Farnell, R.G.W. (1924). **Report on a Preliminary Investigation of the Colloids of Cane Juice and Molasses.** *Int. Sug. J. 26 (308), pp. 420-425.*

77. Fechter W.; Rajh S.; Reimann M.; Ahmed F.E.; Jensen C.R.; Schorn P.M.; Walthew D.C. (2001): **Direct production of white sugar and high-grade molasses by applying membrane and ion-exchange technology in a cane sugar mill.** *Proc. Int. Soc. Sugar Cane Technol. 24, pp. 100-107.*

78. Fehling, H. (1849). **Die quantitative Bestimmung von Zucker und Stärkmehl mittelst Kupfervitriol**. *Annalen der Chemie und Pharmacie 72 (1), pp. 106-113*.

79. Fellows, C.M. and Doherty, W.O.S. (2006). **Insights into Bridging Mechanisms**. *Macromol. Symp. 231, pp. 1-10.*

80. Fenton, H. J. H. (1876). **On a New Reaction of Tartaric Acid.** *Chem. News. 33, pp. 190.*

81. Fenton, H. J.H. (1894). **Oxidation of Tartaric Acid in presence of Iron**. *J. Chem. Soc. 65, pp. 889-910.*

82. Fenton, H.J.H. (1905). **Further Studies on Dihydroxymaleic Acid**. *J. Chem. Soc., Trans., 87, pp. 804-818.*

83. Fevola, M.J., Roger, H. and McCormick, C.L. (2003). **Molecular weight control of polyacrylamide with sodium formate as a chain-transfer agent: Characterization via size exclusion chromatography/multi-angle laser light scattering and determination of chain-transfer constant**. *J. Polym. Sci. A, 41 (4), pp. 560-568.*

84. Fieser, L.F. and Fieser, M. (1967). **Reagents for Organic Synthesis**. *John Wiley and Sons, Inc. LCCC#66-27894. pp. 391.*

85. Figueiredo, M.S., Batta, O., Barbieri-Neto, J. and Zago, M.A. (1993). **Liver Injury and Generation of Hydroxyl Free Radicals in Experimental Secondary Hemochromatosis.** *Res. Exp. Med. 193 (1), pp. 27-37.*

86. Flotron, V., Delteil, C., Bermond, A. and Camel, V. (2003). **Remediation of Matrices Contaminated by Polycyclic Aromatic Hydrocarbons: Use of Fenton's Reagent**. *Polycyclic Arom. Comps. 23 (4), pp. 353-376.*

87. Freeland, D.V., Riffer, R. and Penniman, J.G. (1979). E**lectrokinetics Applied to Sugar Refining.** *Int. Sug. J. 81 (196), pp. 196-199.*

88. Funami, T., Zhang, G-Y., Hiroe, M., Noda,S., Nakauma, M., Asai, I., Cowman, M.K., Al-Assaf, S. and Phillips, G.O. (2007). Effects **of the Proteinaceous Moiety on the Emulsifying Properties of Sugar Beet Pectin.** *Food Hydrocolloids, 21 (8), pp. 1319-1329.*

89. Gadre, S.R. (2002).**Century of Nobel Prizes, 1903 Chemistry Award: Svante Arrhenius (1859-1927)**. *Resonance, May.*

90. George, P. (1947). Some **Experiments on the Reactions of Potassium Superoxide in Aqueous Solutions**. *Trans. Faraday. Soc. 42 (155), pp. 196-205.*

91. George, P. (1954). **The Oxidation of Ferrous Perchlorate by Molecular Oxygen**. *J. Chem. Soc. pp. 4349-4359.*

92. Gil, N. and Saska, M. (2005). **Composition and Processing of Sugarcane Green Leaves and Tops**. *J. Am. Soc. Sug. Cane Technol. 25, pp. 125.*

93. Giunta, C. (2003). **Classical Calculations, Arrhenius Kinetics.** *http://web.lemoyne.edu/~giunta/classicalcs/arrkin.html*

94. Godshall, M.A., Clarke, M.A., Dooley, C.D. and Roberts, E.J. (1987). **Large Colorant and Polysaccharide Molecules in Raw Cane Sugars**. *Proc. Sugar Ind. Technol. 46, pp. 193-211.*

95. Godshall, M.A., Clarke, M.A., Miranda, X. and Blanco, R.S. (1992). **Comparison of Refinery Decolorization Systems.** *Proc. Sug. Proc. Res. Conf., pp. 281-305.*

96. Godshall, M.A., and McKee, M. (2004). **The Effect of Ozone, Hydrogen Peroxide and Sulfite on Cane and Beet Macromolecules**. *Proc. Sug. Proc. Res. Conf., pp. 111-127.*

97. Godshall, M.A., McKee, M., Triche, R. and Moore, S. (2006). **Laboratory and Mill Studies on the Action of Polyaluminum Coagulants in Cane and Beet Juice**. *Proc. Sug. Proc. Res. Inst. Sept. 17-20, Águas de São Pedro, S.P., Brazil, pp. 373-381.*

98. Greenwood, J., Rainey, T. and Doherty, W.O.S. (2007). **Light Scattering Study on the Size and Structure of Calcium Phosphate Hydroxyapatite Flocs Formed in Sugar Solutions**. *J. Colloid. Interface. Sci. 306, pp. 66-71.*

99. Gregory, J. and Duan, J. (2001). **Hydrolyzing Metal Salts as Coagulants**. *Pure. Appl. Chem. 73 (12), pp. 2017-2026.*

100. Grenthe, I. and Wanner, H. (2000). **Guidelines for the Extrapolation to Zero Ionic Strength. v. 6**. *Thermochemical Data Base Project, TDB-2, OECD Nuclear Energy Agency. Le Seine-St. Germain, 12, Bd. Des Iles, F-92130 Issy-les-Moulineaux, France.*

101. Haber, F. and Weiss, J. (1932). **Uber Die Katalyse des Hydroperoxydes**. *Naturwissenschaften, 20 (51), pp. 948-950.*

102. Haber, F. and Willstatter, R. (1931). **Unpaarigheit und Radikalketten im Reaktion-Mechanismus Organischer unds Enzymatischer Vorgange**. *Chem. Ber., 64, pp. 2844-2856.*

103. Halliwell, B. and Gutteridge, J.M.C. (1990). **Role of Free Radicals and Catalytic Metal Ions in Human Disease: An Overview**. *Meth. Enzymol. 186, pp. 1-85.*

104. Hamilton , G.A. and Friedman, J.P. (1963). **A Hydroxylation of Anisole by hydrogen Peroxide Requiring Catalytic Amounts of Ferric Ion and Catechol**. *J. Am. Chem. Soc. 85 (7), pp. 1008-1009.*

105. Harp, D.L. (2002). **Current Technology of Chlorine Analysis for Water and Wastewater**. *Hach Co., USA, Lit # 7019, L21.5. Technical Information Series, Booklet 17, pp. 8*

106. Hartree, E.F. (1953). **On the Configuration of the So-Called Dihydroxymaleic Acid**. *J. Am. Chem. Soc. 75 (24), pp. 6244-6249.*

107. Heitman, J.A. (1983). **Scientific and Technological Change in the Louisiana Sugar Industry***, 1830-1910. Ph.D. Dissertation. Johns Hopkins University. University Microfilms International. 300 N. Zeeb Rd., Ann Arbour, MI. 48106. pp. 2, 4-6, 19, 22, 35-36, 386-388,*

a. Wilson Jr., S. (1980). **Architecture of Early Sugar Plantations.** *Chapter in Green Fields: Two Hundred years of Louisiana Sugar. The Center for Louisiana Studies, Lafayette, La. pp. 51-82.*

b. Silliman, p. 31

c. Leon, J.A. (1848). **On Sugar Cultivation**. *John Oliver, London, I, p. 4.*

d. U.S. Cong., House, Letter from the Secretary of the Treasury, in Reply to a Resolution of the House of Representatives…, **upon the subject of the cultivation of the sugar cane, and the manufacture and refinement of sugar**. *21st Cong., 2nd sess., Doc. No. 62, 21 January, p. 47.*

e. Bouchereau, A. (1917). **Bouchereau's Revised Directory for 1917**. *New Orleans, La. p. 38.*

f. DeBow (1849). DeBow's Review, 7. P. 58. A letter from McCulloch, Feb. 5, 1849 detailing Rillieux's account during the 1830's.

g. Stillman, A. (1855). **Stillman's Patent Bagasse Furnace**. Times Picayune, New Orleans, La.

108. Ho, C-T., Zhang, J., Hwang, H-I. and Riha, W.E. (1994). **Release of Ammonia from Peptides and Proteins and Their Effects on Maillard Flavor Generation**. *Maillard Reactions in Chemistry, Food, and Health. Labuza, T.P., Reineccius, G.A., Monnier,V., O'Brien, J. and Baynes, J. Eds. Proceeding of the 5th International symposium on the Maillard Reaction. The Royal Society of Chemistry. ISBN# 0-85186-802-9. pp. 126-130.*

109. Hodge, J.E. (1953a). **The Amadori Rearrangment under New Conditions and its Significance for Non-Enzymatic browning Reactions**. *J. Am. Chem. Soc. 75, pp. 316-322.*

110. Hodge, J.E. (1953b). **Dehydrated Foods: Chemistry of Browning Reactions in Model Systems**. *J. Agric. Food Chem. 1 (15), pp. 928-943.*

111. Hofmann, T., Munch, P. and Schieberle, P. (2000). **Quantitative Model Studies on the Formation of Aroma-Active Aldehydes and Acids by Strecker-Type Reactions.** *J. Agric. Food Chem. 48 (2), pp.434-440.*

112. Holt, E., La Mer, V.K. and Chown, B.H. (1925). **Studies in Calcification. I. The solubility product of secondary and tertiary calcium phosphate under various conditions**. *J. Biol. Chem. 64 (3), pp. 509-565. Cited by Honig (1953) as: J. Biol. Chem. 44 (1925), 509-567.*

113. Honig, P. (1953). **Principles of Sugar Technology Vol. 1**. *Elsevier Publishing Co.*

a. Payne, J.H. Chapter 13. Fundamental Reactions of the Clarification Process. pp. 501-535.
b. Citing specifically: Seidell, A. (1940). Solubilities of Inorganic and Metal Organic Compounds, 3rd Ed., Van Nostrand.

114. *http://www.buysugarnow.com/sugarspec.pdf*

115. http://www.danisco.com/cms/connect/fructofin/technical+benefits/sweetness/.

116. *http://dictionary.reference.com/browse/defecate*

117. *http://www.sugaralliance.org/desktopdefault.aspx?page_id=54*

118. *http://www.wtrg.com/daily/gasprice.html*

119. Huang, X., Xu, R., Hawley, M.D., Hopkins, T.L. and Kramer, K.J. (1998). **Electrochemical Oxidation of N-Acyldopamines and Regioselective Reactions of their Quinones with N-Acetylcycteine and Thiourea.** *Arch. Biochem. Biophys. 352 (1), pp. 19-30.*

120. Hugot, E. (1986). **Handbook of Cane Sugar Engineering 3rd Ed**. *Elsevier. pp. 541-543.*

121. ICUMSA, (2002). **Determination of the Solution Colour of Raw Sugars, brown Sugars and Coloured Syrups at pH 7.0 – Official.** *GS1/3-7. Verlag Dr. Albert bartens KG, Berlin. ISBN# 978-3-87040-552-6.*

122. ICUMSA. (2007). **The Determination of Conductivity Ash in Raw Sugar, Brown Sugar, Juice, Syrup and Molasses-Official**. *GS1/3/4/7/8-13. Verlag Dr. Albert Bartens KG. ISBN# 978-3-87040-552-6, pp. 1-2.*

123. ICUMSA (2007). **Turbidity in Clarified Cane Juice-Official**. *GS7-21 (1994). Verlag Dr. Albert Bartens KG. ISBN# 978-3-87040-552-6, pp. 1-2.*

124. Isenberg, I. (1961). **Some Comments on Broad Electron Spin Resonance Absorptions Observed I Nucleic Acid Preparations.** *Biochem. Biophys. Res. Comm., 5 (2), pp. 120-124.*

125. Iqbal, K., Andrews, L.S. and Godshall, M.A. (2000). **The Non-sucrose Profile in Two Louisiana Cane Sugar Mills**. *Proc. Sug. Proc. Res. Apr. 9-12, Porto, Portugal. pp. 261-273.*

126. IUPAC (2001). Definition and Classification of Colloids. http://old.iupac.org/reports/2001/colloid_2001/manual_of_s_and_t/node33.html

127. Izvozchikova, v.A., Pastukhova, N.V., Ryabov, S.A., Semchikov, Y.D., Smirnova, L.A. and Mochalova, A.E. (2002). **Flocculation Power of Chitosan and Its Derivatives in Mixtures with Anionic Flocculants**. *Rus. J. Appl. Chem. 76 (11), pp. 1784-1786.*

128. Jack Li, J. (2003). **Name Reactions: A collection of detailed reaction mechanisms. 2nd Ed.** *Springer-Verlag Berlin Heidelberg New York, ISBN 3-540-40203-9. pp. 260.*

129. Kampen, W.H., Njapau, H. and Monge, A. (2000). **Electro-clarification of clarified juice to minimize evaporator scale**. *Int. Sug. J. 102 (1223). pp. 579-584.*

130. Kearney, M. (2006). *Proc. SPRI, Aguas de Sao Pedro, Brasil*.

131. Kearney, M. and Rearick, D.E. (2003). **Weak Cation Exchange Softening: Long Term Experience and Recent Developments.** *J. Am. Soc. Beet. Technol.* *http://www.arifractal.com/weak%20cation%202003%20assbt%20.pdf*

132. Kemmer, F.N. (1988). **The Nalco Water Handbook, 2nd Ed**. *McGraw-Hill Book Co. QD169.W3N34 1987. ISBN # 0-07-045872-3. pp. 6.13-6.19, 8.9-8-10, 17.5-17.4, 26.6, 45.2.*

133. Kenner, J. and Richards, G.N. (1954). **The Degradation of Carbohydrates by Alkali. Part IV. 1-O-methylfructose, Glucose and Fructose**. *J. Chem. Soc. pp. 1784-1789. Reprint order No. 5054.*

134. Kochergin, V., Kearney, M., Jacob, W., Velasquez, L., Alvarez, J., and Baez-Smith, C. (2000). **Chromatographic Desugarization of Syrups in Cane Mills**. *Proc. S. Afr. Sug. Technol. Ass. 74, pp. 62-69.*

135. Koehler, P.E., Mason, M.E. and Newell, J.A. (1969). **Formation of Pyrazine Compounds in Sugar-Amino Acid Model Systems**. *J. Agric. Food Chem. 17 (2), pp. 393-396.*

136. Koonce, S.D. and Brown, J.B. (1944). **An Historical Review of the Chemistry of Carnauba Wax**. *J. Am. Oil Chem. Soc. 21 (6), pp. 167-170.*

137. Koppenol, W.H. (1997). **The Chemistry of Peroxynitrate, A biological Toxin**. *Quimica Nova, 21 (3), pp. 326-331.*

138. Koppenol, W.H. (2000). **Facts and Fiction in Free Radical Biochemistry: The Haber-Weiss Cycle. Proc**. *Oxygen Soc., San Diego, CA, Nov. 17-20.*

139. Koppenol, W.H. (2001). **The Haber-Weiss Cycle-70 Years later**. *Redox Report, 6 (4), pp. 229-234*.

140. Kroll, J. and Rawel, H.M. (2001). **Reactions of Plant Phenols with Myoglobin: Influence of Chemical Structure of the Phenolic Compounds***. J. Food Sci. 66 (1), pp. 48-58*.

141. Kuster, R.M. and Temmink, H.M.G. (1977**). Influence of pH and Weak-Acid Anions on Dehydration of D-Fructose**. *J. Carbohydr. Res. 54, pp.185-191.*

142. Kwok, R.J. (1996a). **Production of Super VLC Raw Sugar in Hawaii**. *Int. sug. J. 98 (1173), pp. 490-494.*

143. Kwok, R.J., Lancrenon, X. and Theoleyere, M.A. (1996b). **Process of Manufacturing Sugar From Aqueous Sugar Juice Such as Cane Juice or Sugar Beet Juice**. U.S. Patent # 5,554,227. Sept. 10.

144. L. De Borger, M., Anteunis, H., Lammens, H. and Verzele, M. (1964). *Bull. Soc. chim. Belg., 73 (73).*

145. Legendre, B. (2001). **Prescribed Burns Help the Sugarcane Industry and Reduce Smoke and Ash Problems. Louisiana Agriculture, Fall**. *http://www.lsuagcenter.com/en/communications/publications/agmag/Archive/2001/Fall/Prescribed+Burns+Help+the+Sugarcane+Industry+and+Reduce+Smoke+and+Ash+Problems.htm.*

146. Legendre, B., Tew, T., Birkett, H., Eggleston, G., Finger, C., Stein, J. (2007). **Impact of Subfreezing Temperatures on the 2006 Louisiana Sugarcane Harvest** (abstract). *Sug. J. 70(1):27-28*.

147. Lichtenthaler, F.W. (2002). **Unsaturated O- and N-Heterocycles from Carbohydrate Feedstocks**. *Acc. Chem. Res. 35, pp. 728-737.*

148. Lindeman, P.F. and O'Shea, M.G. (2001). **High Molecular Weight (HMW) Colorants and Their Impact on the Refinability of Raw Sugar. A Study of Australian and Overseas Raw Sugars.** *Proc. Aust. Soc. Sugar Cane Technol. 23, pp. 322-329.*

149. Liu, S., Jia, H., Yang, D. and Ji, F. (1999). **Electrical conductivity and Counter ion Association of cationic polyacrylamide Derivatives in Aqueous Solution**. *Polym. Int. 48 (11), pp. 1080-1084.*

150. Lunn, J.E. (2002). **Evolution of Sucrose Synthesis.** *Plant. Physiol. 128, pp. 1490-1500.*

151. Lyle, O. (1957). **Technology for Sugar Refinery Workers. 3rd Ed. London**, *Chapman and Hall. TP 377 L96 1957.*

152. Madsen, L.R. (2002). **Mass Balance of Cane Stalks: Data produced for Prof. Michael Saska via HPLC, HPIC and HPAEC**. *Unpublished data, Sept. 6th.*

153. Madsen II, L.R., Rein, P.W. and White, B.E. (2003). **Evaluation of a Near Infrared Spectrometer for the Direct Analysis of Sugar Cane**. *J. Am. Soc. Sug. Technol. 23, pp. 80-92.*

154. Madsen, L.R. and Day, D.F. (2005). **Mixed Dithiocarbamates for the Preservation of Sugar Cane Juice**. *Int. Sug. J. 107 (1282), pp. 576-580.*

155. Madsen II, L.R. (2006a). **From Arrhenius to you: Sucrose Inversion and Practical Kinetics**. *Proc. Am. Soc. Sug. Cane Technol. 26. June, St. Petersberg, Fl.*

156. Madsen II, L.R. (2006b). **Simulated Multiple Effect Evaporation of Clarified and Decolorized Sugarcane Juices and Accelerated Storage Tests of the Syrups:Evolution of Color and Behavior of Carbohydrates and Related Compounds**. *Proc. Sug. Proc. Res. Inst. Sept. 17-20, Águas de São Pedro, S.P., Brazil, pp 149-172.*

157. Madsen, L.R. (2006c). **Analytical Data: Total Iron in Cane Juice via o-phenanthroline complex.** *Unpublished data, Jul. 21st.*

158. Madsen, R.F., Nielsen, W.K. and Kristensen, S. (1984). **Method of Purifying Sugar Juice Prepared by Extraction of Sugar beet Material**. *U.S. Patent # 4,432,806. Feb 21.*

159. Mahadevaiah, M.S., Kumar, Y., Abdul Galil, M.S., Suresha, M.S., Sathish, M.A. and Nagendrappa, G. (2007). **A Simple Spectrophotometric Determination of Phosphate in Sugarcane Juices, Water and Detergent Samples**. *E-Journal of Chem. 4 (4), pp. 467-473*.

160. Malmberg, C.G. and Maryott, A.A. (1950). **Dielectric Constants of Aqueous Solutions of Dextrose and Sucrose.** *J. Res. Natl. Bur. Stds. 45 (4), pp. 299-303.*

161. Manchot, W. abd Lehman, G. (1928). **Uber Die Einwirkung von Hydroperoxyd auf Ferrosalz**. *Liebig's Ann. Chemie, 460, pp. 179-201.*

162. Mane, J.D., Pachpute, S.P. and Pahdnis, S.P. (1998). **Effects of Hydrogen Peroxide Treatment on Cane Syrup**. *Int. Sug. J. Vol. 100 (1193), pp. 210-212.*

163. Mane, J.D., Phadnis, S.P. and Jadhav, S.J. (1992). **Effects of Hydrogen Peroxide on Cane Juice Constituents.** *Int. Sug. J. Vol. 94 (1128), pp. 322-324.*

164. Mane, J.D., Phadnis, S.P., Jambhale, D.P. and Yewale, A.V. (2000). **Mill Scale Evaluation of Hydrogen Peroxide as a Processing Aid: Quality Improvement in Plantation White Sugar**. *Int. Sug. J. Vol. 102 (1222), pp. 530-533.*

165. March, J. (1992). **Advanced organic Chemistry: Reactions, Mechanisms, and Structure. 4th Ed**. *Wiley-Interscience Publications, John Wiley and Sons, Inc. ISBN 0-471-58148-8.pp. 1171-1172.*

166. Martinez, N., Fabregat, P., Bobrovnik, L. D., Nemirovich, P. M. and Nikolaev, A. P. (1987). **Purification of Sugarcane Juice by the Action of Direct Steam**. *Izvestiya Vysshikh Uchebnykh Zavedeniĭ, Pishchevaya Tekhnologiya, 2, pp. 120-121.*

167. Martire, D.O.; Braslavsky, S.E.; Garcia, N.A. (1991). *J. Photochem. Photobiol., A **61**, 113-124.*

168. Matijevic, E., Janauer, G.E. and Kerker, M. (1964). **Reversal of Charge of Lyophobic Colloids by Hydrolyzed Metal ions I. Aluminum Nitrate**. *J. Colloid Sci. 19, pp. 333-346.*

169. McCarty, C.G. and Vitz, Ed. (2006). **pH Paradoxes: Demonstrating That It Is Not True that pH=-log[H+]**. *J. Chem. Ed. 83 (5), pp. 752-757.*

170. McGinnis, R.A. (1982). **Beet-Sugar Technology, 3rd Ed**. *Beet Sugar Development Foundation, Ft. Collins, Colo. Ch. 7, Juice Purification, I. History and Fundamentals*.

171. Meade, G.P. and Chen, J.C.P. (1977). **Cane Sugar Handbook, 10th Ed**. *Wiley-Interscience, John Wiley & Sons, Inc.*

a. Roberts and Martin, BCRP 1959

b. Farber and Carpenter, ISSCT, 1971, pp. 1589-1600

c. Godshall and Roberts, CSRRP, ARS-S-88, pp. 51-65

172. Mendoza, J.J. and Espejo, D. (2002). **Update on the Use of Hydrogen Peroxide at Central El Palmar Refinery.** *Proc. Sug. Proc. Res. Conf., pp. 178-188.*

173. Metcalf & Eddy, Inc. Revised by Tchobanoglous, G. (1979). **Wastewater Engineering: Treatment, Disposal, Reuse, 2nd Ed**. *McGraw-Hill Book Co. TD645.M57. ISBN# 0-07-041677-X. pp. 258-267.*

174. Mizoroki, T., Meshitsuka, S., Maeda, S., Murayama, M., Sahara, N. and Takashima, A. (2007). **Aluminum Induces Tau Aggregation in vitro but not in vivo**. *J. Alzheimers Dis. 11 (4), pp. 419-427.*

175. Mjoberg, B., Hellquist, E., Mallmin, H. and Lindh, U. (1997). **Aluminum, Alzheimer's Disease and Bone Fragility**. *Acta Orthop. Scand., 68 (6), pp. 511-515.*

176. Moodley, M., Davis. S.B. and Abendorff, M. (1999). **Full Scale Decolourisation Trials With Ozone**. *Int. Sug. J. 101 (1203), pp. 165-170***.**

177. Morel Du Boil, P.G. (1997). **Refined Sugar and Floc Formation, a Survey of the Literature**. *Int. Sug. J. (Cane Ed.). 99 (1182), pp. 310-312.*

178. Musa, I.A., Shide, E.G. and Gimba, C.E. (2005). **Calcium Hypochlorite from Nigerian Limestone**. *ChemClass J. 2. pp. 47-50.*

179. Myerson, A.S., Lo, P.Y., Kim, Y.C. and Ginde, R. (1990). *Proc. 11th Symp. Ind. Cryst. (Mersmann, A. Ed.). Eur. Fed. Chem. Eng., Munich, Germany. P. 847.*

180. Namiki, M. and Hayashi, T. (1983). **A New Mechanism of the Maillard Reaction Involving Sugar Fragmentation and Free Radical Formation**. *The Maillard Reaction in Foods and Nutrition, Waller, G.R., and Feather, M.S. Eds. ACS Symposium Series 215, American Chemical Society, Washington DC. TP372.55.M35M34, ISBN# 0-8412-0769-0. Pp, 21-46.*

181. National Library of Medicine – Medical Subject headings, MeSH Descriptor Data, Oligosaccharides. (2008). http://www.nlm.nih.gov/cgi/mesh/2008/MB_cgi?mode=&term=Oligosaccharides.

182. Nordstrom, D.K.; Alpers, C.N., Ptacek, C.J. and Blowes, D.W. (2000). **Negative pH and Extremely Acidic Mine Waters from Iron Mountain, California.** *Environ. Sci. Technol. 34, pp. 254-258.*

183. Oliveira, D. T. (2006). **Plantation White Sugar Production in Brazil**. *Proc. Int. Soc. Sug. Cane. Technol. Processing workshop. May 29-June 1, Baton Rouge, La., http://www.issct.org/processabst06.htm#Plantation%20White%20Sugar%20Production%20in%20Brazil*.

184. Oliverio, J.L. and Boscariol, F.C. (2006). **DRD-Dedini Refinado Direto (Dedini Direct Refined) – The Refined Sugar Without a Refinery**. *Int. Sug. J. 108 (1289), pp. 239-246.*

185. Paine, H.S., Keane, J.C. and McCalip, M.A. (1928). **Influence of Phosphate and Colloid Contents of Cane Juice on Defaction**. *Ind. Eng. Chem. 20 (3), pp. 262-267*.

186. Pannetier, N., Abdel, K. and Francois, J. (2001). **Physico-chemical study of sucrose and calcium ions interactions in alkaline aqueous solutions.** *Int. Conf. Polymer-Solvent Complexes and Intercalates. Besancon, 28-30 Aug. Vol. 166, pp. 203-208.*

187. Papacosta, K. (2002). Turbidity **Calibration Standards Evaluated From a Different Perspective**. *Turbidity and Other Sediment Surrogates Workshop, April 30-May 2, Reno, NV, pp. 1-3. http://water.usgs.gov/osw/techniques/TSS/kemon-test.pdf.*

188. Parker, K.J. (1970). **Chemical Problems in the Sucrose Industry**. *Sucr. Belge, 89, 119-126.*

189. Paton NH, Duong M. (1992). **Sugar-cane phenolics and 1st expressed juice color. 3. role of chlorogenic acid and flavonoids in enzymatic browing of cane juice**. *Int. Sug. J. 94 (1124), pp. 170-176.*

190. Patterson, J.W. (1985). Industrial **Wastewater Treatment Technology, 2nd Ed.** *Butterworth Publishers. TD745.P32 1985 ISBN# 0-409-90002-8. pp. 155-172.*

191. Pigman, W. (1957). **The Carbohydrates: Chemistry, Biochemistry, Physiology**. *Academic Press, inc. New York. LCCC.# 57-8379.*

192. Pine, S.H. (1987). **Organic Chemistry**, *5th Ed. McGraw-Hill. QD251.2.P47, ISBN#: 0-07-050118-1. Pp. 404-405.*

193. Pinotti, A., Bevilacqua, A. and Zaritzky, N. (2001). **Comparison of the Performance of Chitosan and a Cationic Polyacrylamide as Flocculants if Emulsion Systems**. *J. Surfact. Deterg. 4 (1), pp. 57-63.*

194. Polanco, L.S., Rein, P.W. and White, B.E. (2006). **A Comparison of Sugarcane Juice Quality from a Mill and a Diffuser**. Sug. J. March 2006, pp. 12-20.

195. **Potassium Ferrate(VI):** *http://www.sigmaaldrich.com/catalog/search/ProductDetail/ALDRICH/480010*

196. Prasad, M., Jain, P. K. and Nigam, G.D. (1989). **Effect of Iron on Sugar Crystals During Storage.** *Int. Sug. J. 91 (1091), pp. 222-226.*

197. Puglia, C.D. and Powell, S.R. (1984). **Inhibition of Cellular Antioxidants: A Possible mechanism of Toxic Cell Injury**. *Env. Health. Persp. 57, pp. 307-311.*

198. Rawel. H.M., Kroll, J. and Riese, B. (2000). **Reactions of Chlorogenic Acid with Lysozyme: Physiochemical Characterization and Proteolytic Digestion of the Derivatives**. *J. Food. Sci. 65 (6), pp. 1091-1098.*

199. Rease, S.F. (1999). **Process for Purifying Sugar Solutions**. *U.S. Patent # 5,893,947. Apr. 13.*

200. Rein, P.W. (2007). **Cane Sugar Engineering, 1st Ed**. *Verlag Dr. Albert Bartens KG., pp. 219-244.*

201. Rein, P.W. and Bento, L.S.M. (2006). **Direct Production of White Sugar.** *Proc. I.S.S.C.T. Processing Workshop, June 1. Lod-Cook Conference Center, L.S.U.*

202. Rein, P.W., Bento, L.R.S.M. and Cortes, R. (2007). **The Direct Production of White Sugar in a Cane Sugar Mill**. *Int. Sug. J. Vol. 109 (1301), pp. 286-299.*

203. Riffer, R. (1986). **The Chemistry of Iron in the Sugar Refinery**. *Proc. Sug. Proc. Res., 50, pp. 231-251.*

204. Rodriguez-Saona, L.E. (2005). FSC 605-Food Chemistry. *http://class.fst.ohio-state.edu/FST605/lectures/lect14.html*

205. Rossiter, G., Jensen, C. and Fechter, W. (2002). **White Sugar from Cane at the Factory: The Impact of WSM**. *Proc. Sug. Proc. Res. Conf., pp. 162-177.*

206. Rush, J.D., Maskos, Z. and Koppenol, W.H. (1990). **Distinction Between Radical and Ferryl Species**. *Meth. Enzymol. 186, pp. 148-156.*

207. Saska, M. (2001). **Method of Producing White Sugar**. *U.S. Patent # 6,228,178. May 8.*

208. Saska, M. (2002). **Ethanol and Lactic Acid Levels as a Measure of Post Harvest Cane Deterioration in Different Harvesting Schemes**. *Int. Sug. J. 104 (1237), pp. 10-14.*

209. Saska, M. (2006). **Bleaching Sugar with Hydrogen Peroxide**. *Proc. I.S.S.C.T. Processing Workshop, June 1. Lod-Cook Conference Center, L.S.U.*

210. Saska, M. and Chou, C-C. (2002). **Antioxidant Properties of Sugarcane Extracts**. *Proc. 1st Biannual World Conference on Recent Developments in Sugar Technologies, Delray Beach, Fl. May 16-17.*

211. Sato, K-I., Sakai, K., Tsushima, K. and Akai, S. (2007). **The first total synthesis of telephiose A**. *Tetrahedron Lett. 48 (21), pp. 3745-3748.*

212. Saxena, I.M., and Brown, M. (2005). **Cellulose Biosynthesis: Current Views and Evolving Concepts.** *Annals of Botany 96, pp. 9-21.*

213. Schaffler, K.J. (1987). **Estimation of pH of Sugar Cane Juices at High Temperature**. *Proc. S. Afr. Sug. Technol. June. pp. 14-17.*

214. Schmitt, A., Cabrerizo-Vilchez, M.A., Hidalgo-Alvarez, R. and Fernandez-Barbero, A. (1998). **On the Identification of Bridging Flocculation: An extended Collision Efficiency Model**. *Prog. Colloid and Polym. Sci. 110, pp. 105-109.*

215. Schwiegert, N., Zehnder, A.J.B. and Eggen, R.I.L. (2001). **Chemical Properties of Catechols and their Molecular Modes of Toxic Action in Cells, from Microorganisms to Mammals**. *Env. Microbiol. 3 (2), pp. 81-91.*

216. Scurlock, R.; Rougee, M.; Bensasson, R.V. (1990). *Free Radical Res. Commun.* ***8****, 251-258.*

217. Seip, J.J. (1947). **Studies on Cane Juice Clarificants**. *M.S. Thesis #378.76 L930 1947 Seip. L.S.U. pp. 8.*

218. Shaw, P.E., Tatum, J.H. and Berry, R.E. (1967). **Acid-Catalyzed Degradation of D-Fructose.** *Carbohyd. Res. 5, pp. 266-273.*

219. Shibamoto, T. and Bernhard, R.A. (1977). **Investigation of Pyrazine Formation pathways in Sugar-Ammonia Model Systems**. *J. Agric. Food Chem. 25 (3), pp. 609-614.*

220. Shin, R.W., Lee, V.M. and Trojanowski, J.Q. (1994). **Aluminum Modifies the Properties of Alzheimer's Disease PHF Tau Proteins in vivo and in vitro**. *J. Neurosci. 14, pp. 7221-7233.*

221. Shubin, V. and Linse, P. (1995). **Effect of Electrolytes on Adsorption of Cationic Polyacrylamide on Silica: Ellipsometric Study and Theoretical Modeling**. *J. Phys. Chem. 95, pp. 1285-1291.*

222. Singh, K. and Sharma, K.K. (2006). **Selection of pH for Measurement of Sugar Solution Colour**. *Proc. ICUMSA Sept. 17-20, Águas de São Pedro, S.P., Brazil, pp 1-7.*

223. Sitterson, J.C. (1953). **Sugar Country. The Cane Sugar Industry in the South 1753-1950**. *University of Kentucky Press, Lexington Ky.*

224. Solomons, T.W.G. (1994). **Organic Chemistry, 5th Ed**. *John Wiley and Sons, Inc. ISBN 978-0471050285. Pp. 218,227.*

225. Spencer and Meade (1948). **Cane Sugar Handbook, 8th Ed**., John Wiley and Sons, New York. pp. 798-811.

226. Starzak, M. and Mathlouthi, M. (2005). **Water Activity in Concentrated Sucrose Solutions: Consequences for the Availability of Water in the Film of Syrup Surrounding the Sugar Crystal**. *Sug. Rev. 0 (0), pp. 1-19.*

227. Stewart, R. (1964). **Oxidation Mechanisms: Applications to Organic Chemistry**. *W.A. Benjamin, Inc. LCCC# 63-19978, pp. 159-161.*

228. Stillman, J.M. (1924). **The Story of Early Chemistry**. *D. Appleton and Company, New York; London, MCMXXIV. 540.9S81s. pp. 54, 236-237.*

a. Plinius, S.C. (77). **Natural History**. *Book 33 of 37. Tr. Bostock, J. (1856-93) 6 vols. H.T. Riley, London.*

b. Judaeus, I., Cf. Haeser, H. (1875). **Lehrbuch der Geschichte der Medicin***, Jena Vol. I, p. 573.*

229. Subbarao, M.R. (1935). **A Study on the Composition of Raw Cane Sugars: Its Importance in Refining.** *M.S. Thesis #378.76 L930 1935 Rao. L.S.U. pp. 2.*

230. Sumner, J.B. and Graham, V.A . (1921). **Dinitrosalicylic Acid: A Reagent for the Estimation of Sugar in Normal and Diabetic Urine**. *J. Biol. Chem. 47, pp. 5-9.*

231. Tate & Lyle. (1975). **TALO Flocculants in Sugar Manufacture**. *Tate & Lyle Enterprises, Ltd. Bromley, BR2 9NA, England, pp. 1-27. www.iberiasugar.com/.../TEC-058%20-%20TALO%20flocculants%20in%20sugar%20manufacture%201975.pdf*.

232. Thring, R.W., Chornet, E., Bouchard, J., Vidal, P. and Overend, R.P. (1989). **Characterization of Lignin Residues Derived from the Alkaline Hydrolysis of Glycol Lignin**. *Can. J. Chem. 68, pp. 82-89*.

233. Todd, M. (1997). **Prospects for the World White Sugar Market**. *Proc. Sug. Ind. Technol. 56, pp. 1-16.*

234. Tomiyasu, T., Yonehara, N. and Kawashima, T. (2000). **Kinetic Study of the Iron-catalyzed Reaction of the Oxidative Coupling Reaction of 3-methyl-2-benzothiazolinone hydrazone with N,N-dimethylaniline**. *Analytica Chimica Acta, 424 (1), pp. 127-137.*

235. Ueno, M., Takasawa, Y., Miyashige, H., Tabata, Y. and Meguro, K. (1981). **Effect of Alkyl Chain Length on Surface and Micellar Properties of Octaethyleneglycol-n-alkyl ethers**. *Colloid. Polym. Sci. 259 (7), pp. 761-766.*

236. Van den Ende, W. and Van Laere, A. (1996). **De-novo synthesis of fructans from sucrose in vitro by a combination of two purified enzymes (sucrose: sucrose 1-fructosyl transferase and fructan: fructan 1-fructosyl transferase) from chicory roots (*Cichorium intybus* L.).** *Planta, 200 (3), pp. 1432-2048.*

237. van der Poel, P.W., Schiweck, H. and Schwartz, T. (1998). **Sugar technology: Beet and Cane Sugar Manufacture**. *Verlag. Dr. Albert Bartens KG. TP390.S8 pp. 151-157.*

238. Van't Hoff, J.H. (1884). **Études de Dynamique Chimique**. *Frederik Muller, Amsterdam. Reproduced in microform 1972.*

239. Vickers, J.E., Grof, C.P.L., Bonnett, G.D., Jackson, P.A. and Knight, D.P. (2005). **Overexpression of Polyphenol Oxidase in Transgenic Sugarcane Results in Darker Juice and Raw Sugar**. *Crop. Sci. 45, pp. 354-362.*

240. Von Weimarn, P.P. and Ostwald, W.O. (1908). *Kolloid-Z. 3, pp. 26.*

241. Vukov, K. (1965). **Kinetic Aspects of Sucrose Hydrolysis.** *Int. Sug. J., June pp. 172-175.*

242. Waldmann, L. (2005). **Effect of L-arginine and guanidinium chloride (GdmCl) on the unfolding and refolding of hen egg-white lysozyme (HEWL)**. *Dissertation zur Erlangung des akademischen doctor rerum naturalium vorgelegt an der Mathematisch-Naturwissenschaftlich-Technischen Fakultat der Martin-Luther-Universitat Halle-Wittenberg. 03-18-05. http://sundoc.bibliothek.uni-halle.de/diss-online/05/05H053/t7.pdf.*

243. Walling, C. (1975). Acc. Chem. Res. 8, p. 125.

244. Watson, M. and Little, C. (2002). **Understanding Value in Lime. Extension Fact Sheet, ANR-9-02**. *The Ohio State University Extension; Agriculture and Natural Resources, 2120 Fyffe Rs. Columbus, OH. pp. 1-6. http://ohioline.osu.edu/anr-fact/0009.html.*

245. Whayman, E. and Crees, O.L. (1975). **Mechanistic Studies of Cane Mud Flocculation**. *Sug. J. November. pp. 20-24.*

246. Wilhelmy, L.F. (1850).**The Law By Which the Action of Acids on Cane Sugar Occurs.** *Poggendorf's Annalen der Physik und Chemie **81**, 413-433, 499-526; [from Henry M. Leicester and Herbert S. Klickstein, eds., A Source Book in Chemistry, 1400-1900 (Cambridge, MA: Harvard, 1952)]*

247. Willet, C.C. (1997). **The ABC Process: Direct Production of Refined Quality Sugar from Cane Mized Juice.** *Int. Sug. J. 99 (1177E), pp. 7-8.*

248. Wittwer, E. (1984). **Pressure Evaporation in the cane Sugar Industry: Advantages and some Problems Related to its Introduction**. *Int. Sug. J., 86 (1025), 139-145.*

249. Wolfrom, M.L. and Shilling, W.L. (1951). **Action of Heat on D-Fructose. III. Interconversion to D-Glucose.** *J. Am. Chem. Soc.73, pp. 3557-3558.*

250. Wrodnigg, T.M. and Eder, B. (2001). **The Amadori and Heyns Rearrangements: Landmarks in the History of Carbohydrate Chemistry or Unrecognized Synthetic Opportunities?** *Top. Curr. Chem.215, pp. 115-152.*

251. Wu, X., Ge, X., Wang, D. and Tang, H. (2007). Distinct Coagulation Mechanism and Model Between Alum and High Al_{13}-pACl. Colloid. Surf. A, 305, pp. 89-96.

252. Wunderlin, D.A., Pesce, S.F., Ame, M.V. and Faye, P.F. (1998). **Decomposition of Hydroxymethylfurfural in Solution and Protective Effect of Fructose**. *J. Agric. Food Chem. 46, pp. 1855-1863.*

253. Xu, J. and Jordan, R.B. (1988a). **Kinetics and Mechanism of the Oxidation of 2,3-Dihydroxybenzoic Acid by Iron (III)**. *Inorg. Chem. 27 (25), pp. 4563-4566.*

254. Xu, J. and Jordan, R.B. (1988b). **Equilibrium and Kinetic Studies of the Complexing of Iron (III) by 1,2-dihydrozybenzene Derivatives**. *Inorg. Chem. 27 (8), pp. 1502-1507.*

255. Zerban, F.W. (1921). **Relative Importance of Some Colouring Matters in Cane Juices and Syrups**. *Int. Sug. J. 23 (265), pp. 32-35.*

256. Zhang, Q., Lu, Z., Hu, H., Yang, W. and Marszalek, P.E. (2006). **Direct Detection of the Formation of V-Amylose Helix by Single Molecule Force Spectroscopy.** *J. Am.Chem. Soc., 128 (29), pp. 9387-9393.*

257. Zossi, S., Liu, H., Zhao, Z-G. and Saska, M. (2009). **Color Profiles in Sugar Factory Streams**. *Annual Meeting of the Am. Soc. Sug. Cane Technol., La. Division. Baton Rouge, La. Feb. 2-3.*

APPENDIX A: FUNDAMENTALS OF CARBOHYDRATE CHEMISTRY

A.1. Nomenclature

Carbohydrates are naturally occurring polyols. They are usually denoted via the end group, carbon number, and the suffix “ose”. Thus, carbohydrates containing six carbons are referred to as “hexoses”. Further, the functionalization of the end group also adds a prefix for aldehydes and ketones, specifically, “aldo-“ or “keto-“. It follows then that a six carbon moiety may be referred to as either an aldo or ketohexose. The numbering system for a simple aldohexose, glucose, is given in **figure A.1**. Individual carbohydrate residues are also referred to as “monosaccharides”.

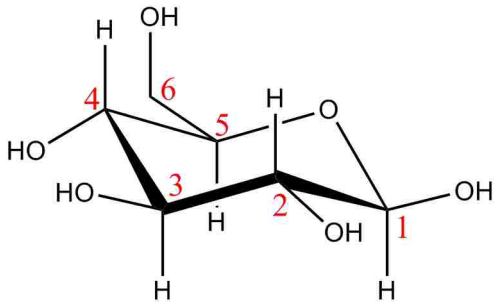


Figure A.1. The numbering system used for monosaccharides.

The family tree of aldoses is given in **figure A.2**. Frequently, the keto form is differentiated by adding “-ulose” onto the name. Examples of this include dextrose (an aldohexose, glucose) and levulose (a ketohexose, fructose). This brings us into dealing with the stereochemistry of carbohydrates, beginning with L and D nomenclature.

Note the red, blue and green highlighted positions in **figure A.3**. By convention, sugars and amino acids are referred to as either D or L (dextro or levorotatory). If the hydroxyl group attached to the penultimate carbon (the fifth position) is on the left side of the fisher projection, the carbohydrate is designated as L. If the 5-hydroxyl is on the right, it will be called a D sugar. Although this nomenclature does in no way predict the optical rotation of the molecule, it does refer, structurally, to the progenitor carbohydrate, D-glyceraldehyde.

A clear example of this, which will be described in detail later, can be observed when glucose (D-glucopyranose) and fructose (D-fr uctofuranose) are polarized. The D-glucose is dextrorotatory (positive) and the D-fructose is levorotatory (negative). These conventions are not interchangeable with the Cahn-Ingold-Prelog priority rules where R (*rectis*) and S (*sinister*) are most commonly used.

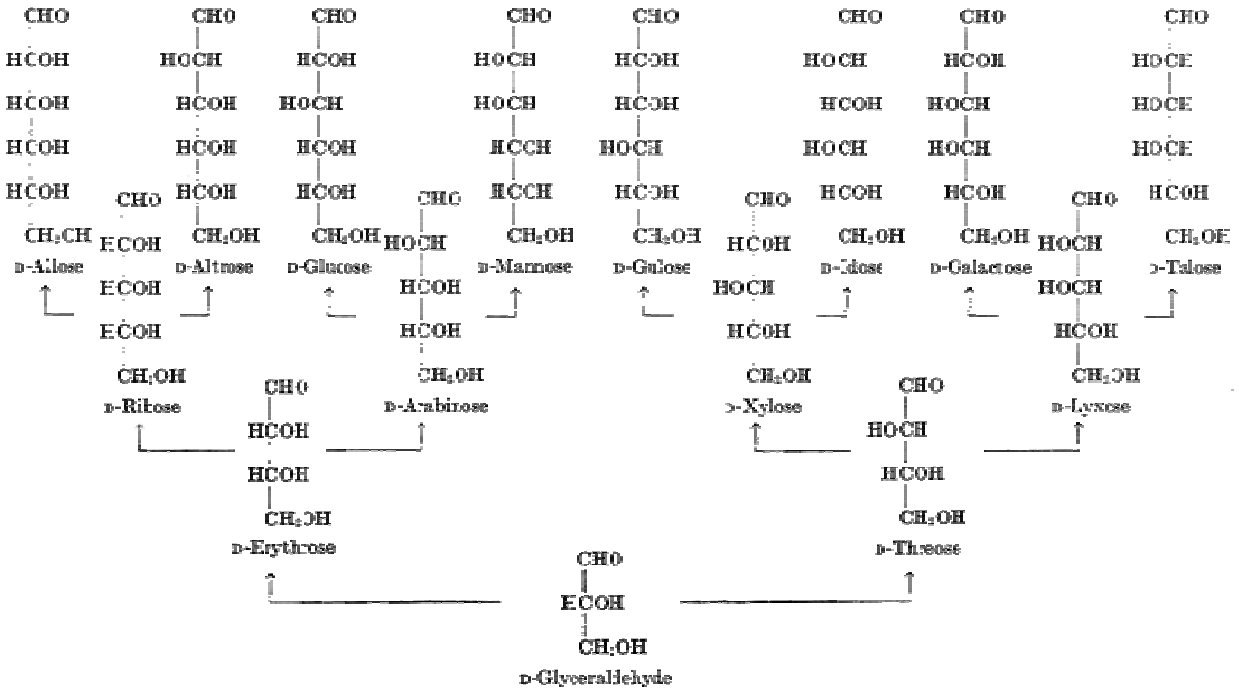


Figure A.2. The D-family of aldoses (Pigman, 1957).

Nearly all naturally occurring carbohydrates are D in configuration (the opposite is true for amino acids where the L form predominates in nature). Interpreting carbohydrate structures, like the Fisher projections given in **figure A.2** can be difficult. In order to more clearly define how these structures are interpreted, the most common are given in **figure A.3**. The structure **A** is the Fisher projection of the aldohexose, glucose. As shown in **B**, all of the bonds given in the Fisher projection are assumed to be facing out of the plane of the page. Staggering the bonds into the sterically favorable form **C** and bending it around through a structure similar to **D** which leads to the Haworth structure, **E**. **F**, the most stable "chair form", is the standard used most today. The green centers in **figure A.3** represent anomeric carbons.

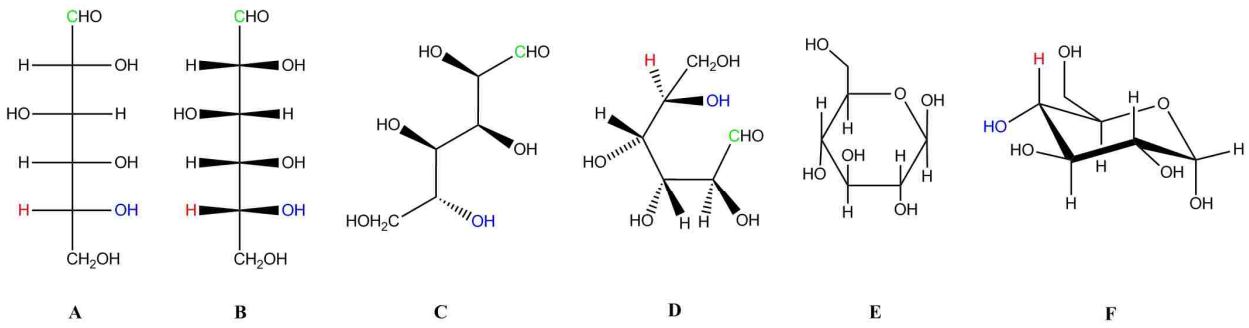


Figure A.3. Fisher projection and the literal interpretation, A and B; the bent form of B, C the Haworth structure, E and the chair form, F.

A.2. Mutarotation

Also known as saccharose or, simply "sugar", sucrose (α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside) is a dissacharide composed of α-D-glucose and β-D-fructose. They are connected by a 1→2 glycosidic bond. This bond serves to protect the hemiacetal of both residues which prevents the carbohydrate from behaving as a reductant. The reducing potential of a carbohydrate results from the linear form which bears an aldehyde group. The linear form is usually present only in small amounts at equilibrium and is due to an isomerism about the anomeric carbon which known as mutarotation.

Mutarotation is the process whereby a carbohydrate achieves anomeric equilibrium. That is, β-D-glucose in aqueous solution will experience opening of the hemiacetal. The resulting open chain aldehyde then acts as an electrophile whereby nucleophilic attack by the 5-OH (for a hexose) may occur. When this happens, the hydroxylic nucleophile has the opportunity to add to either side of the carbonyl. This results in the formation of two anomeric forms, α or β. The general mechanism for this is given below in **figure A.4**.

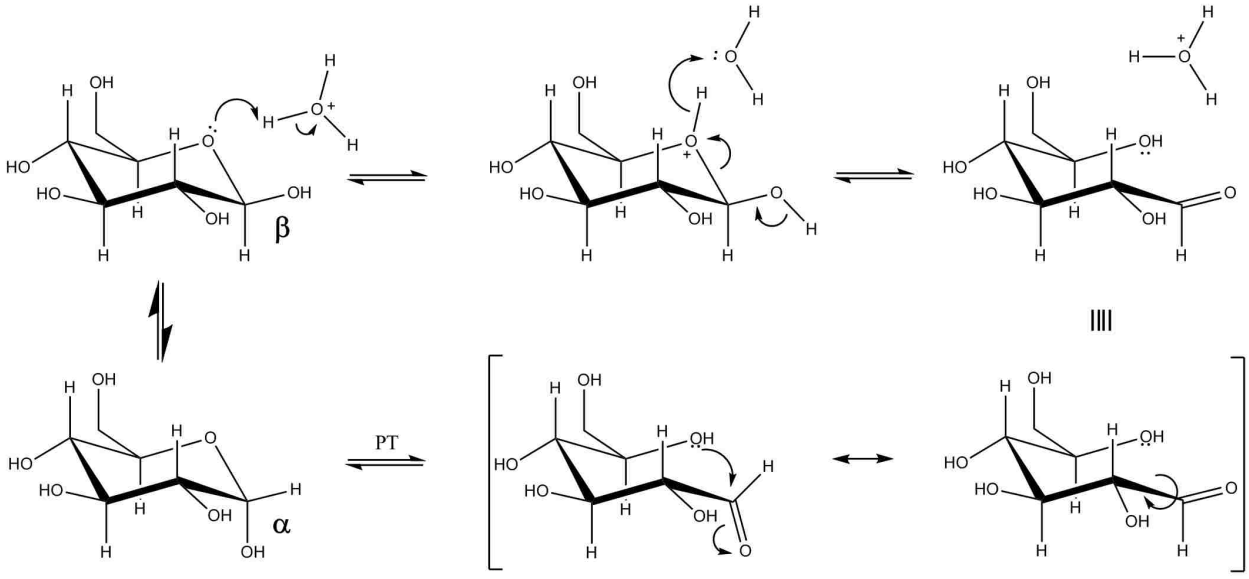


Figure A.4. Mutarotation of glucose

At neutral pH and room temperature (25°C), mutarotation of glucose is slow or, in the case of fructose, non-existent. Mutarotation is accelerated by increased temperature. This is why fructose is only used as a sweetener in cold-beverages; when heated, the predominant furanoid anomeric form is much less sweet. Acidic pH, when cold tends to favor mutarotation which yields the sweeter pyranose forms (Danisco, 2005). Evidence of increased carbonyl, and hence open-chain form were given using fourier transform infrared (FTIR) spectroscopy (Rodriguez-Saona, 2005). It was demonstrated that an exponential increase in open-chain fructose begins at 20°C and increases with temperature. Fructose displays anomalous mutarotation resulting from the isomerization of the keto-form which is the rate determining step. This is because, unlike glucose (which is either open-chain or, predominantly α (35%) or β (64%) pyranose), fructose has appreciable amounts of each of 5 possible anomeric forms (open-chain, α,β furanose (0.76 and 28-32%) and pyranose (4 and 68 %)) at equilibrium. This can be sped up geometrically via alkaline catalysis (Budavari, 1989). Because heating a reducing sugar can lead to evolution of acidic products, and acid can catalyze mutarotation (Ballash and Robertson, 1972), heat will cause "self-catalysis" to occur. Arrhenius kinetics are obeyed.

The anomeric forms are not strictly equivalent and the proportions are influenced by steric hindrance and hydrogen bonding of the intermediates (which differ by the arrangement of hydroxyl groups which is peculiar to the carbohydrate). Ergo, one form usually predominates. Because the optical rotation of the α and β anomer are not necessarily the same, the quantities of each, from pure carbohydrate can be approximated using polarimetry. The optical rotation attributed to each anomer, for both glucose and fructose, and of their mixtures at equilibrium are given in **Appendix B**, which deals with inversion.

A.3. Glycosidic Bonding

When one or more monosaccharides are connected to one another, we say that there is glycosidic bond between them. The naming convention is based upon the number of units (residues) that are connected. For example, two units gives a disaccharide, four a tetrasaccharide and so-on. Generally, chains of more than 4 residues and less than 10 are called oligosaccharides (National Library of Medicine, 2008). Larger chains are called polysaccharides and can, in nature, easily exceed 40,000 kDa in molecular weight.

The name of the saccharide is derived from the loci of bonding as well and even apparently small differences can have profound structural (and hence, chemical) consequences. For example, consider the disaccharides maltose and cellobiose. Both are constructed from two glucose residues, and both are connected with 1→4 glycosidic bonds. A glycosidic bond results when the hemiacetal of one saccharide condenses with a hydroxyl of another. This makes the glycosidic bond and completes the acetal.

Because a carbohydrate exists in a constant state of tautomeric flux, even mild conditions will likely damage the saccharide before it has the opportunity to form a glycosidic bond. Some of the transformations include dehydration via the E_1 mechanism and fragmentation via reverse (or retro) aldol (Kenner and Richards, 1954).

Under either condition, the small, highly reactive product molecules (usually carbonyl species) can condense to form a multitude of conjugated products.

Reactions like these, coupled with the difficulties associated with the selective modification, protection and deprotection (Cumpstey, 2008) of any particular hydroxyl group make modifications of carbohydrates especially challenging. Usually, many steps (Sato 2007) using expensive and/or harsh reagents are needed. Even so, there is frequently a complex mixture of products which then leads to a laborious (often "impossible") task of separating a useful quantity of the desired product. Because of this, enzymes, such as α-1→4 glucosyltransferase or Sucrose 6-phosphate-synthase (Lunn, 2002) are usually required to effect a reliably specific modification of a carbohydrate with reasonable yield. Even using enzymes, the molecular weight of the product so obtained can be polydisperse (Van den Ende, 1996) which can make product isolation tedious and expensive (Day and Chung 2007). It follows that the naming conventions, particularly IUPAC (International Union for Pure and Applied Chemistry), while systematic, are complex. Practitioners have defined a unique nomenclature to help mitigate this

Usually, for naming purposes, the residue with carbonyl involvement is chosen to be the starting point. Following the numbering convention given in **figure A.1**, maltose (diglucose) would be called, by IUPAC rules, "6-(hydroxymethyl)-5-(3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)-oxy-oxane-2,3,4-triol". The stereochemistry of each glucose residue would be given as 2R, 3S, 4R, 5R. Assignment of the R/S stereochemical descriptors for glucose is given in **figure A.5**. Since this sort of nomenclature leads one toward names which increase in complexity with the number of residues, the carbohydrate Chemist would call this molecule, more simply, "D-glucopyranosyl -(1→4)-α- glucopyranoside". In practice, the linking atom need not necessarily be oxygen; the linkage atom is sometimes also given. Thus, maltose can also be called 4-O-α-D-glucopyranosyl glucose. Abbreviations are also used, and following this "short-hand" maltose is also known as "D-Glc(1→4)-α-D-Glc". This sort of linkage is also frequently referred to as an "α(1→4)" linkage.

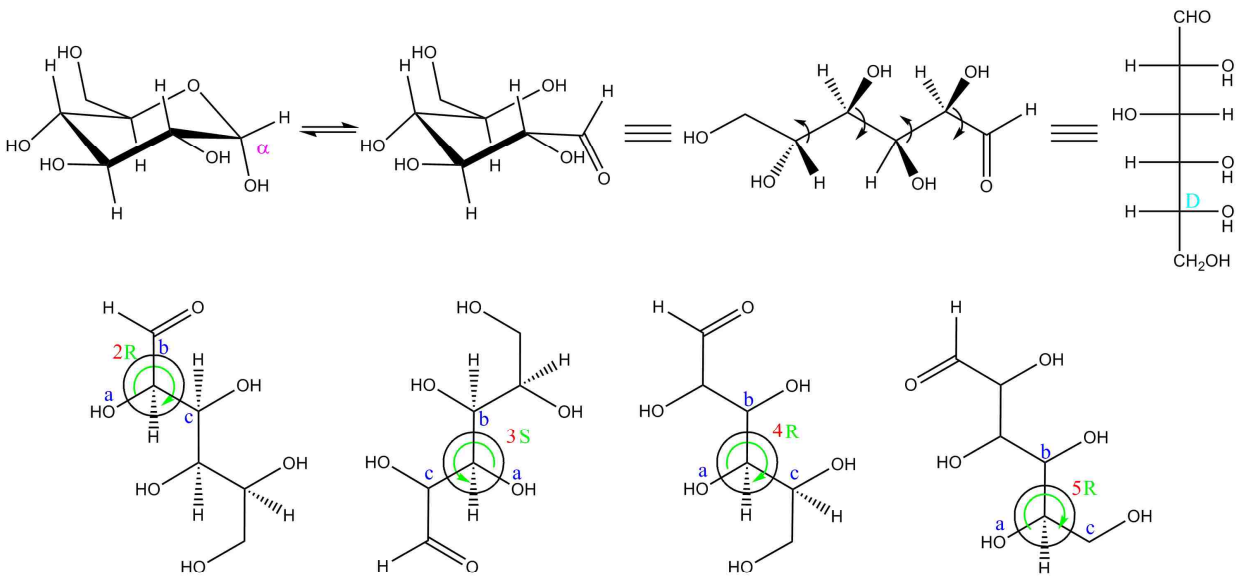


Figure A.5. Assignment of stereochemistry to a carbohydrate exemplified using α-D-glucose.

Maltose and cellobiose are constitutional isomers differing only with the anomerism about the glycosidic bond. For maltose, this bond is α and for cellobiose, it is β. This yields two structures which differ greatly in how they are conformed in space; this is exemplified in **figure A.6**.

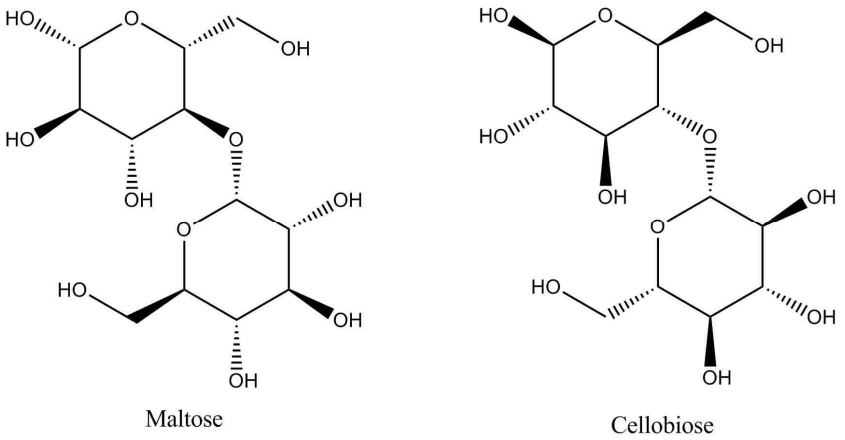


Figure A.6. Maltose and cellobiose.

The spatial conformation effects the inter and intramolecular hydrogen bonding options which effect crystallinity and hence, the solubility and reactivity of the molecule.

While maltose and cellobiose are both hydrolytically cleaved by acid, more profound differences are seen when additional residues are added to yield, from maltose or cellobiose, starch and cellulose, respectively.

A principle component of starch, amylose is an $\alpha(1\rightarrow4)$ polyglucan which self-orders via intra-chain hydrogen bonding into discrete helices (Zhang et al, 2006) whilst cellulose is linear (Saxena and Brown, 2005) and crystalline due to inter-chain hydrogen bonding (Bochek, 2003). Examples of this phenomenon are given in **figure A.7**, exemplified using α and $\beta(1\rightarrow4)$ octasaccharides. This explains why amylose is begrudgingly soluble in water whilst cellulose, for which plants would be thankful, if they could, is not.

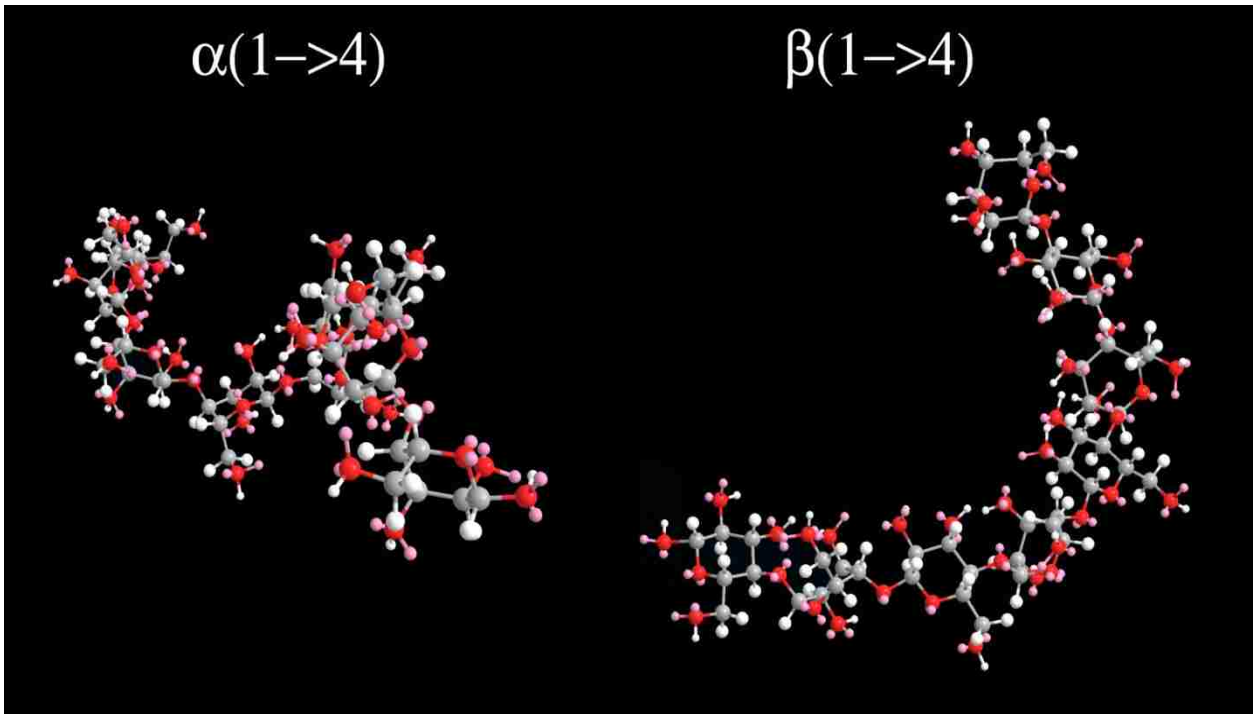


Figure A.7. Conformation of maltooctaose, α and cellooctaose, β. Note the helical conformation of the α form. The figures were drawn using ChemBioDraw Ultra v.11, imported into Chem3D Pro v.11 and the energy was minimized using MM2 *ab-initio* to 300 K. The resulting files were given a black background, spliced, labeled and cropped using Adobe Photoshop CS.

A.3.1. Sucrose: α-D-glucopyranosyl-(1→2)-β-D-fructofuranose

The main carbohydrate of commerce is known as "(2R,3S,4R,5R)-2-(3S,4R,5R)-3,4-dihydroxy-2,5-bis(hydroxymethyl)tetrahydrofuran-2-yloxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol". More commonly referred to as "D-glucopyranosyl-(1→2)-β-D-fructofuranose", "saccharose", "sucrose" or simply "sugar", this material serves , with high fructose corn syrup (HFCS) as a primary bulk sweetener. Sucrose is used as the standard material for what is considered to be "sweet".

Like maltose, sucrose is a disaccharide. Unlike maltose, it is composed of one residue each of D-glucose and D-fructose. D-fructose or levulose, is the keto isomer of D-glucose and its formation via reversible isomerization is given here in **figure A.8**. The numbering and anomeric convention applied to furanoses differs from that used for the corresponding pyranoses. For example, an anomeric hydroxyl pointing "down" is α for a pyranose and β for a furanose. Further, the first carbon on the hydroxymethyl group connected to the anomeric carbon is designated as 1. Thus, both a (1→2) furanoside and a (1→1) pyranoside would both be full acetals.

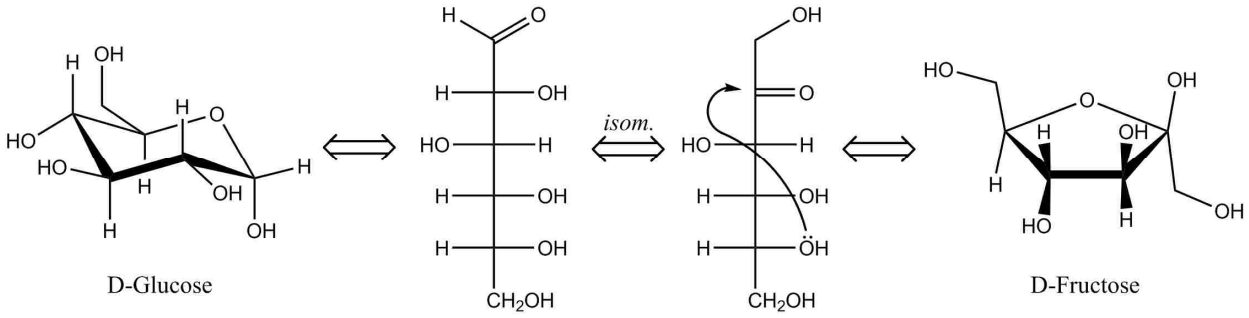


Figure A.8. Isomerization glucose to fructose

The Isomerization of carbohydrates from aldo to keto form and *vice-versa* is frequently referred to as the Lobry de Bruyn-Alberda van Ekenstein rearrangement and an excellent review is presented by Angyal (2001). This reaction can be accelerated via acidic or alkaline catalysis, although the alkaline route is both faster and more synthetically useful. The rearrangement can be pseudo-catalyzed by the presence of amino species, including amino acids. This can segue into the Maillard reaction sequence.

Sucrose is an $\alpha(1\rightarrow2)$ glycoside, and as such, has a completed acetal. Sugars like this are not reducing. That is, an oxidizer added to a mixture containing sucrose will not be reduced. This is the basis for many time-tested assays for "reducing sugar". These assays include, but are not limited to Fehling's (Fehling, 1849) and Benedict's (Benedict, 1908) copper reagents and the popular dinitrosalicylic acid assay (Sumner and Graham, 1921). The ability of some carbohydrates to be reducing is also principle behind why electrochemical methods (PAD/HPLC) are widely used and it is the reason why maltose [$\alpha(1\rightarrow4)$]will reduce Tollins reagent (Solomons, 1994) and sucrose [$\alpha(1\rightarrow2)$] will not. The structure of sucrose is given in **Appendix B.**

Another fine example, yet another disaccharide containing two D-glucosyl units, trehalose, is isolated from mushrooms, and is thus, frequently called "mushroom sugar" (Pigman, 1957). Like sucrose, it is non-reducing. Trehalose is an $\alpha(1\rightarrow1)$ sugar and is thus useful as a unique, non-reducing material that is useful as an internal standard for sucrose in quantitative methods using HPLC (ICUMSA, 2007). The structure of trehalose and the REDOX chemistry typical of a reducing sugar is given in **figure A.9** where the green oxygen atoms represent complete acetals that are non-reducing. Red atoms indicate incomplete acetals that are capable of opening via equilibrium or acid/base catalysis to the aldehyde which can be oxidized by an added reagent. Any carbohydrate end group that is not protected in acetal form will be referred to as a "reducing sugar".

Either route is further accelerated by the addition of an amine "pseudocatalyst" (acts to minimize the needed Ea, but is eventually consumed to yield reaction products). Rearrangements of this sort are called, depending upon whether the starting carbohydrate is an aldose or a ketose, the Amadori or Heyns rearrangement, respectively (Wrodnigg and Eder, 2001). These reactions lead to the Maillard reaction sequence which is discussed in **chapter 2**.

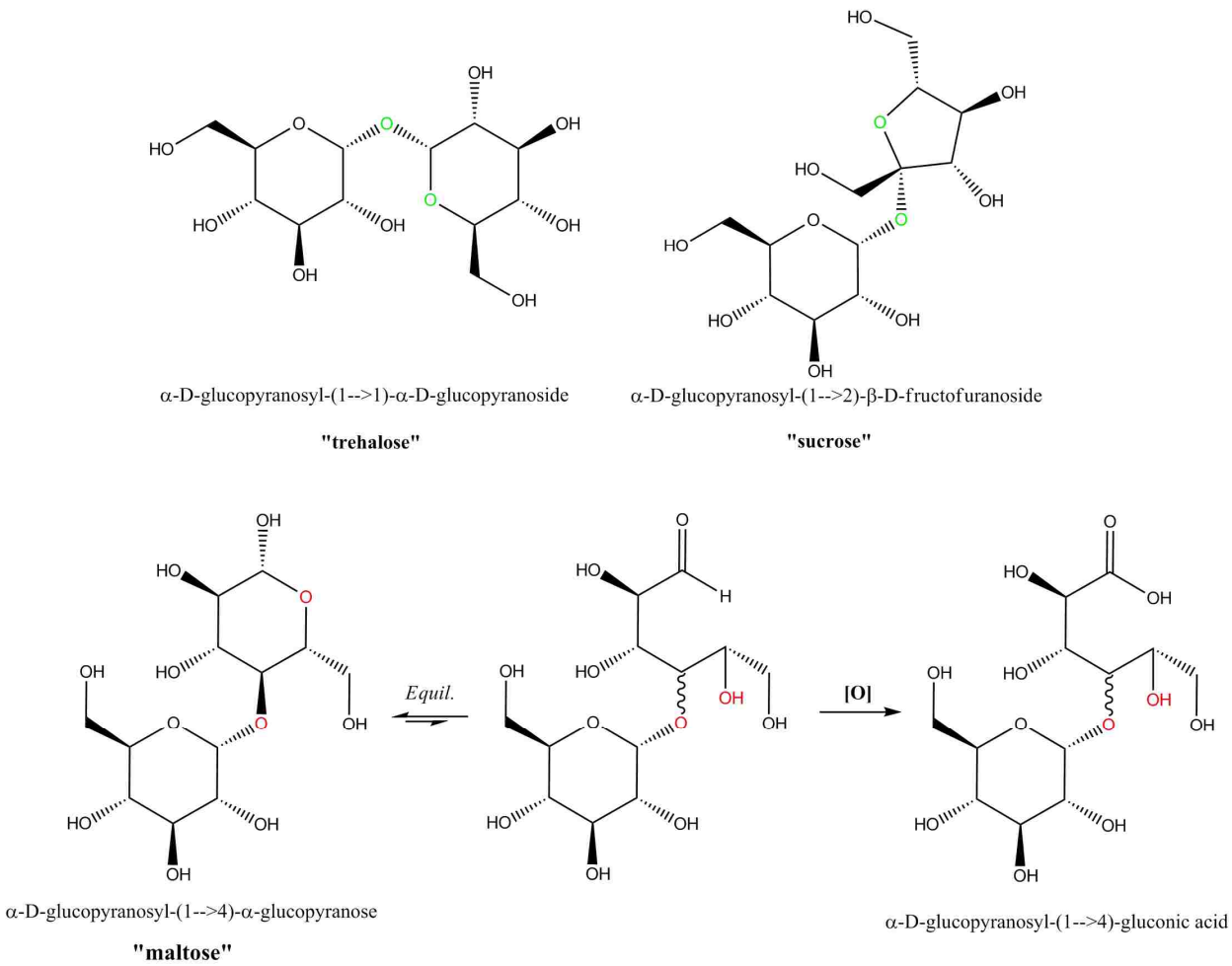


Figure A.9. Structure-activity relationship between sugars which are reducing and those that are not (top) and an example, below.

APPENDIX B. INVERSION

B.1. Definition and Measurement

Sucrose “inversion” is a hydrolysis reaction whereby one equivalent of sugar is made to yield one equivalent each of glucose and fructose. This reaction is given in **figure 2**, below:

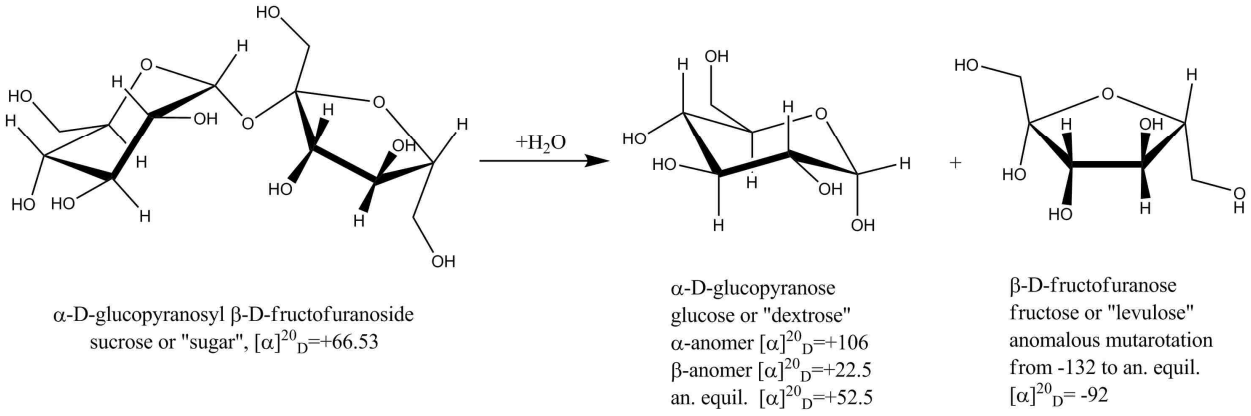


Figure B.1. Hydrolytic “inversion” of sucrose; note optical rotation (Budavari, 1989; Pigman, 1957).

From **figure B.1**, it can be seen that the hydrolysis of sucrose yields a mixture with a negative net contribution, viz. (glucose $[\alpha]^{20}_{D}$= +52.5°) + (fructose $[\alpha]^{20}_{D}$= -92°) = -39.5° to the optical rotation. This means that, when observed with a polarimeter, a mixture of sucrose and water while heated with some catalyst, viz. invertase or acid, will become progressively negative as time goes on. This change in the optical rotation from positive to negative is why sucrose hydrolysis is frequently called “inversion”.

Since “inversion” is a process that destroys our product, and the conditions governing the phemonema are frequently encountered during routine processing of cane, a number of methods for estimating the magnitude of loss have been determined and have found wide use. Among these methods, the assembled tables of Stadler (Stadler, 1932, Honig, 1953) and mass balance approaches (Schaffler, et al., 1985) are popular. Unfortunately, regardless of the utility of the Stadler tables, there is no accommodation for the effect of sucrose concentration on the rate of inversion. The mass balance approach produces adequate results, but it requires that the compositional sugar analysis be known before-hand.

In answer to this, this work serves to outline the derivation of a method which can be used to approximate the amount of sucrose theoretically inverted under varied conditions of temperature, brix and pH whilst requiring no compositional information besides the material purity.

The first detailed kinetic observation of sugar "inversion" via acid catalysis was made by Wilhelmy in 1850 (Wilhelmy, 1850). His work established that, at ambient temperature and with added acid, the concentration of sugar did not effect the rate of inversion. He did discover, however, that changes in the ambient temperature *did* effect inversion, but he did not endeavor to create a model.

B.2. Dependence of temperature on the rate constant.

The defining work in this area was performed by the Swedish scientist Svante' Arrhenius in 1889 (Arrhenius, 1889). Amongst many seminal works including the concept of ionic dissociation of salt(s) in solution, for which he was awarded a Nobel Prize in 1903 (Gadre, 2002), and the concept of "global warming", his observations on inversion of cane sugar are most important to us.

Arrhenius determined experimentally that increasing the temperature of the mixture by 10 K (Kelvin, or °C+273) resulted in a two-fold increase in the rate of inversion. Building on the work of Van't Hoff (1884), he conceived of an energy barrier, called the "activation energy". This activation energy or E_a describes the energy that need by introduced into a system in order to form an "activated complex". Which way the equilibrium shifts depends on an independent "collision number", A, which represents the probability that this activated complex will dissociate to form either products or reactants. From this, the Arrhenius equation was formulated, and was found to agree with the data sets provided by many contemporaries including Urech (1884) and Spohr (1888) (Giunta, 2003):

Eqn. B.1.

$$k = Ae^{\left(\frac{-E_a}{RT}\right)}$$

Where,

k = rate constant, mole/second
A=frequency constant, or collision number
R= gas constant, 8.314 J°K/mole
T= Temperature, °K
E_a=Activation energy, J/mole

E_a, or the activation energy peculiar to sucrose has been experimentally observed by many, and a comprehensive table of values is given by Vukov (1965). From this table the average value of the E_a of sucrose is 25.92±0.74 kcal/mole (108.45 kJ/mole). E_a was found to be *essentially* constant within the temperature range of 20-130°C.

Since E_a is constant for sucrose within the given temperature range, Vukov found it simpler to derive a decadic exponent that to calculate the values of the Arrhenius exponent continuously. As a result, for sucrose, the value for E_a/R just slightly forward of equilibrium is ~5670 ($10^{3.7536}$). Dividing this by the temperature in °K gives, 5670/T, the change in the rate constant relative to the temperature for systems composed of sucrose and water.

B.3. pH Definition and Measurement

Fales and Morrell (1922) addressed the issue of catalysis of sucrose inversion with acid. In particular, they examined the effect of the thermodynamic H^+ ion concentration or activity on the rate of inversion. They were able to support the prior findings of Jones and Lewis ( ) that even though the concentration of H^+ ion was constant, the activity of the H^+, a_{H+} (activity = $[H^+]\gamma$, where γ is the activity coefficient, usually calculated using the extended Debye-Huckel equation) was not; it increased with added sucrose!

Indeed, this phenomenon plagued earlier researchers including Ostwald and Arrhenius, both of whom conclude that the effect must be the result of the neutral salt effectively increasing the relative concentration of sucrose available for reaction (Fales and Morrell, 1922).

This problem exists today, and has been investigated by Eggleston et al (1996), who concluded that salts actually do increase the H^+ ion concentration by cleavage of water to yield metal hydrate complexes and a H^+ ions. The truth likely involves both effects, where salts on one hand, serve to augment or quench the H^+ ion concentration and on the other where they effect the activity of those ions; it is possible, for example to add LiCl to HCl (pH=1.0) and observe a decrease in H^+ ion concentration with a concomitant decrease in pH (McCarty and Vitz, 2006). Examples of this are given in **table B.1**, below with values obtained at The Audubon Sugar Institute (Madsen, 2002) verifying that the range of values is representative.

Table B.1. Effect of salt addition on the pH of aqueous solutions.

Salt:[a]	**$\Delta pH/\Delta C_m$:**[a]	**Cane Juice, % solids:**[b]
KCl	-0.10	as K_2O, 1.31[14] as K^+ 4.63[15]
NaCl	-0.20	As Na^+ 0.12[15]
$CaCl_2$	-0.45	as CaO, 0.29[14] as Ca^{++} 0.37[15]
$MgCl_2$	-0.48	as MgO,0.28[14] as Mg^{++} 0.24[15]
Cl^-	n/a	as Cl^- 0.22[c14] as Cl^- 1.19[15]
SO_4^{2-}	n/a	as SO_4^{-2} 0.52[14] as SO_4^{-2} 0.70[15]
PO_4^{3-}	n/a	as P_2O_5 0.40[14] as PO_4^{3-} 0.39[15]
Fe^{3+}	n/a	Fe_2O_3 0.01[d14] Fe_2O_3 0.008[17]

[a]McCarty and Vitz, 2006, [b14]Chen and Meade, 1977, [b15]Madsen, 2002, [b17]2006c, [c]Clarified juice, [d]Syrup

It is interesting to note that most of the salts given are not likely to increase the H^+ ion concentration in solution, viz. by formation of complex aqua-cations. In fact, it is stated specifically for Mg^{2+}, ($MgCl_2$), that $Mg(H_2O)_6^{2+}$ is *not* acidic (Cotton, 1980). Why does a one molal (mole/kg or ~9.6% w/w) addition of $MgCl_2$ to an aqueous solution reduce the pH by 0.48?

Cations require significant water to become solvated. This leads to an overall reduction in the amount of water available for the solution of the existing H^+ ions. This results in an increase in the reactivity or activity of the H^+ ions.

As for the augmentation of the H^+ ion concentration, ions such as K^+ or Mg^{2+} are not suitable alone, but in the presence of other cations such as Fe^{3+} and anions, viz. SO_4^{2-}, can lead to significant H^+ ion evolution (Nordstrom, et al., 2000):

$$Fe3^+ \text{ alone:} \quad [Fe(H_2O)_6]^{3+} = [Fe(H_2O)_6(OH)]^{2+} + H^+$$

$$Fe^{3+}, K^+, \text{ and } SO_4^{2-}: 3Fe^{3+} + 2SO_4^{2-} + K^+ + 6H_2O = Kfe^{3+}{}_3(SO_4)_2(OH)_2 + 6H^+$$

The H^+ ion activity of even simple solutions containing one or two species can be difficult to approach theoretically. Since cane juice contains multiple salts and likely hundreds of unknown non-sugar components (and this composition varies constantly), a reliable mathematical approximation of H^+ ion activity is practically impossible at this time. Theory derived for approaching this problem only applies to *dilute solution* (Grenthe and Wanner, 2000), viz. it involves extrapolation through zero concentration (k_o, or the rate constant at infinite dilution). The current theory falls apart when concentrations approach what a sugar technologist would call "dilute", viz. juice where the minimum concentration of solute would be ~10-15% w/w. In order to correct measured pH_{20} to true pH at operating conditions, an *empirical* approximation is required.

In 1987, Schaffler (1987) explored the effects of temperature on the pH measured in cane juices. In this work, it was made clear that the pH used in the calculations must be the same as the operating pH for the results of Vukov's approximation to be correct. Following this, Schaffler explored the pH values for cane juice at temperatures of 30-35, 50-55, and 80-85°C. These values were subject to regression analysis yielding a 2nd order polynomial equation approximating the change in pH relative to the instantaneous temperature. As such, it will eventually become parabolic yielding increasingly inaccurate results. This figure may only be used for pH in the range of 4.5-8.5. This equation is given here; pH_{25} is the pH measured at 25°C:

$$\frac{dpH}{dT} = -0.0339 + 0.015\,pH_{25} - 0.0017\,pH_{25}{}^2 \qquad \textbf{Eqn. B.2.}$$

Now, this figure is used to calculate the pH at operating temperature:

$$pH_{op} = pH_{25} + (T - 25)\frac{dpH}{dT}$$ **Eqn. B.3.**

B.4. Water and Inversion

Vukov (1965) reasoned that, even though water is consumed 1:1 with sucrose, the overall effect on the water quantity was negligible. For example, 100g of a 50% brix (w/w) solution contains 50g each of water and sucrose, but the molar ratio is ~19:1 and an excess of water is virtually insured. This seems to be less true as concentration exceeds 60% w/w, the Norrish equation (Barbosa-Canovas, 2002), indicates that for sucrose, water activity falls off sharply at concentrations exceeding 60% w/w. The Norrish equation accounts for the measure of "free" water which is bound solvating the sucrose.

For now, let's call $\log_{10}(\rho\text{-}c)$ or simply "W" the fraction of the composition that is water. W can be calculated by first using an equation which approximates the density of sucrose solutions at various levels of saturation. Although there are more rigorous (and slightly more accurate) approximations of sucrose solution density (Hugot, 1986; see Bubnik), the equation below approximates the density of sucrose solutions with fidelity sufficient for our needs (Lyle, 1957) while requiring that only the % dry solids and temperature (°C) be known.

Eqn. B.4.

$$\rho = 1000 \cdot \left[1 + \frac{w_{DS}\left(w_{DS} + 200\right)}{54000}\right] \cdot \left[1 - 0.036\left(\frac{T - 20}{160 - T}\right)\right]$$

Where:

ρ = density, kg/m^3
w_{DS}=g dry solids/100g of material
T = Temperature, °C

Using this density, we can calculate the fraction of our material that is water:

Eqn. B.5.

$$W = \frac{\rho(100 - bx)}{100}$$

Where:

ρ = density, g/cm^3
bx = brix, g sugar/100g

B.5. Assembly of Working Equations

Parker (1970) derived an equation which enables the direct calculation of k_a using temperature, pH, and concentration.Vukov (1965) assembles a nearly identical equation to approximate the rate constant of sucrose inversion under varying conditions of temperature, concentration, and pH. Vukov's equation is given here. The differences with Parker's equation are discussed afterward:

Eqn. B.6.

$$\log_{10} k_a = k_u + \log_{10}(\rho - c) - \frac{E_a}{RT} - pH$$

Where:

k_a= Rate constant, min-1
k_u= Empirical constant, 16.91min^{-1}
ρ= density of the solution, g/mL
c= solute, g/mL
T= Absolute temperature, °Kelvin
pH = -$\log_{10}(a_{H+})$

Substituting in the previously derived decadic exponent, water fraction, and pH correction gives us:

Eqn. B.7.

$$\log_{10} k_a = k_u + \log_{10} W - \frac{5670}{T} - pH_{op}$$

The equation derived by Parker differs in three respects, 1, k_u is given as 15.30 rather than 16.91 min^{-1}, 2, the decadic expression is given as 5810/T rather than 5670/T, and 3, water fraction is given by the $\log_{10}$(water moles – sucrose moles) rather than $\log_{10}(\rho\text{-}c)$. Described by Wittver (1984), this results in two equations both useful for pH ranges of 1-6.0, but for temperature ranges of 25-85°C (Parker) and 20-130°C (Vukov). The broad temperature range of Vukov's approximation appears most appropriate for our use, and most references shall be made accordingly.

Although simpler to calculate by parts using a program such as Microsoft[tm] Excel, the complete equation is given here: **Eqn. B.8.**

$$\log_{10} k_a = k_u + \log_{10}\left(\frac{\rho(100 - bx)}{100}\right) - \frac{5670}{T} - pH_{25} + (T - 25)\left(-0.0339 + 0.015\ pH_{25} - 0.0017\ pH_{25}^{\ 2}\right)$$

Then we solve for k_a: **Eqn. B.9.**

$$k_a = 10^{\log_{10} k_a}$$

Since we can now calculate the rate constant k_a, we need to be able to express this in a practical form. Integrating the previous rate equation with respect to time yields an equation that approximates the proportion of original sucrose that has been "inverted". This equation (Honig, 1953) is given here:

Eqn. B.10.

$$I = \frac{(c_o - c)}{c_o} = 1 - e^{-k_a t}$$

Where:

I=fraction of sucrose inverted
c=concentration of sucrose at time t
c_o=Initial concentration of sucrose
e= base of natural logarithms, 2.718…
t=time, minutes

The equation set we have derived here can be used to approximate the level of sucrose inversion relative to time.

APPENDIX C. CHEMICALS APPLIED TO IMPROVE THE CLARIFICATION OF CANE JUICE

Index of Substances that Are or Have Been Used for Purifying, Decolorizing, and Clarifying Sugar-Containing Solutions

Compilation by Prof. Dr. Edmund O. von Lippmann, printed in Deutsche Zuckerindustrie, Vol. XXXIV, page 9 (Jan., 1909).—Translation supplied through the courtesy of Dr. Charles A. Browne, Bureau of Chemistry, Washington.

See also supplementary Table 51*a*, p. 812, extending this list to 1937.

(A list of the abbreviations of references is given at the end of this index.)

I. Sulphur; its acids, compounds and derivatives

1. Sulphur (Leuchs III, 86; 1836).
2. Hydrogen Sulphide (Sievier, 1847. in Woodcroft, 94; Hlavati, Chz., 28, 1180).
3. Hydrogen Persulphide (Hlavati, Chz., 28, 1180).
4. Sulphuric Acid (Achard, about 1800, Gesch., 407; Kessler, Z., 16, 760; Hagemann, D. Z., 12, 491).
5. Sulphuric Acid with Lime (Mége, D., 115, 215).
6. Sulphuric Acid with Zinc Chloride (Thiele, Chz., 20, 404).
7. Sulphuric Acid with Zinc Sulphate (Terry, 1833, in Woodcroft, 54).
8. Potassium or Sodium Sulphate (Macfadyen, 1830, Gesch., 423).
9. Ammonium Sulphate (Dullo, D., 155, 71; Beanes, D., 167, 220).
10. Sulphuric with Sulphurous Acid (Possoz, D., 170, 64).

11. Sulphurous Acid (Drapiez, "Bull. de la Société d'encourag.," Paris, 1811, X. 56; Perpere, 1812, and Dubrunfaut, 1829, Zerban, 1908.
12. Sulphurous Acid and Hydrogen Sulphide (Hlavati, Bl. Ass., 16, 759).
13. Sulphurous Acid with Calcium Bisulphite (Stolle, D., 114, 305).
14. Sulphurous Acid with Chloride of Lime and Phenol (Meiner, Bl. Ass., 10, 165).
15. Sulphurous Acid and Phenol (Kowalski, Z., 55, 396).
16. Sodium Sulphite (Perrier and Possoz, Z., 12, 128; Rümpler, N.Z., 30, 204).
17. Potassium Sulphite (Cassel and Kempe, S. ind., 47, 684).
18. Ammonium Sulphite (Beanes, D., 167, 220).
19. Calcium Sulphite (Prout, 1810; Melsens, C. r., 55, 729; Calvert, Z., 12, 500).
20. Barium Sulphite with Ozygen (Bouillant, S. ind., 50, 189).
21. Magnesium Sulphite (Mehay, Z., 23, 27; Drost and Schulz, Oe., 1885, 891; Degener, D. Z., 24, 203).
22. Lead Sulphite (Scoffern, 1847, in Woodcroft, 98).
23. Ferrous Sulphite (Englert and Becker, N. Z., 16, 70).
24. Aluminum Sulphite (Boulin, 1846, in Zerban, 15; Brandé, 1846, Z., 44, 455; Mehay, Z., 23, 27).
25. Aluminum Sulphite with Calcium Hydrate (Schubarth, Z., 2, 129).
26. Aluminum Sulphite with Manganese Sulphate (Massé, Z., 10, 256).
27. Acid Potassium Sulphite (Z., 1, 254; Cassel and Kempe, S. ind., 47, 684).
28. Acid Sodium Sulphite (Perrier and Possoz, Z., 12, 128).
29. Acid Alkali Sulphite with Calcium Bisulphite (Allabard, Engl. Patent No. ?).
30. Acid Calcium Sulphite (Stolle, 1838, in Zerban, 15; Melsens, D., 117, 136; Reynoso, Z., 12, 500).
31. Acid Calcium Sulphite with Alum (Leyde, Z., 1, 365).
32. Acid Calcium Sulphite and Calcium Hydrate and Alum (Lapeyrere, S. ind., 27, 568).
33. Acid Barium Sulphite, also with Alum (Lapeyrere, see above).
34. Acid Strontium Sulphite (Melsens, S. ind., 9, 379).
35. Acid Magnesium Sulphite (Mehay, Z., 23, 26; Hulwa, Oe., 13, 465; Saillard, S. ind., 42, 82).
36. Acid Iron Sulphite (Becker, N. Z., 16, 6).
37. Acid Aluminum Sulphite (Stolle, 1838, S. ind., 8, 295; Becker, Z., 35, 924).
38. Acid Aluminum Sulphite with Aluminum Phosphate (Schiller, Z. B., 12, 509).
39. Calcium Trisulphite (?) (Labarre, Oe., 18, 36).
40. Basic Magnesium Sulphite (Berggreen, B., 16, 2542).

41. Hyposulphurous Acid (Talamo, N. Z., 29, 211; Baudry, Z., 53, 260).
42. Sodium Hyposulphite (Thiele, Chz., 20, 204).
43. Sodium Hyposulphite with Lime and Aluminum Acetate (D. Z., 33, 912).
44. Sodium Hyposulphite with Phosphoric Acid or Phosphates (Stein and Crosfield, Z., 53, 1334).
45. Hyposulphites of the Alkaline Earths and Magnesia (Reece and Price, 1849, in Woodcroft, 106).
46. Hydrosulphurous Acid (Ranson, Oe., 26, 737).
47. Ammonium Hydrosulphite (Descamps, S. ind., 65, 673).
48. Sodium Hydrosulphite (Thiele, Chz., 20, 404; Schiller, Z. B., 22, 683).
49. Calcium Hydrosulphite with Barium Hydrate (Descamps, S. ind., 65, 673).
50. Hydrosulphite of Calcium, Barium or Strontium (Descamps, S. ind., 65, 673).
51. Magnesium Hydrosulphite (Becker, Z., 36, 978).
52. Cadmium Hydrosulphite (Urbain, S. ind., 50, 31).
53. Zinc Hydrosulphite (Urbain, see above).
54. Double Salt of Zinc Hydrosulphite with Sodium Chloride or Bromide and Ammonium Chloride or Fluoride (Harding, S. ind., 66, 742).
55. Iron or Manganese Hydrosulphite (Descamps, S. ind., 65, 673).
56. Aluminum Hydrosulphite (Descamps, see above).
57. Hydrosulphite of Alumina (Becker, Z., 36, 978).
58. Hydrosulphurous Acid and Phenol (Kowalski, Z., 55, 396).

II. Phosphorus; its acids, compounds and derivatives

59. Phosphorus Sulphide (Hlavati, Chz., 27, 254).

60. Phosphoric Acid (Stammer, Z., 9, 433).
61. Sodium Phosphate (Kuhlmann, Z., 2, 130).
62. Potassium Phosphate (Blanchard, B., 6, 153).
63. Ammonium Phosphate (Kuhlmann, Z., 2, 92; Beanes, Amer. patent, 1862).
64. Sodium Calcium Phosphate (Gwynne, Z., 3, 292).
65. Calcium Phosphate (Oxland, Z., 2, 130; Ostermann, S. ind., 40, 598).
66. Barium Phosphate (Heffter, Oe., 22, 71).
67. Strontium Phosphate (Heffter, see above).
68. Magnesium Phosphate (Kessler, Z., 15, 525).
69. Phosphate of Alumina (Oxland, Z., 2, 130).
70. Acid Ammonium Phosphate (Packert, S. ind., 25, 25).
71. Acid Ammonium Phosphate with Barium Hydrate (Chameroy, S. ind., 51, 173).
72. Acid Calcium Phosphate (Richter, 1834, Z., 44, 446; Schott, N. Z., 14, 314).
73. Acid Calcium Phosphate with Calcium Bisulphite (Barthelémy, S. ind., 52, 468).
74. Acid Calcium Phosphate with Magnesium Sulphate (Kessler, Z., 15, 51).
75. Acid Barium Phosphate (Manoury, J. Fabr., 29, 24).
76. Acid Magnesium Phosphate (Kessler, Z., 15, 51).
77. Acid Phosphate of Alumina (Oxland, Z., 2, 130).
78. Calcium Superphosphate (Maguin, J. Fabr., 29, 23).
79. Superphosphate of Alumina (Daubeny, 1857, in Ling-Roth, 23; Stubbs, Bl. Ass., 9, 912).
80. Commercial Superphosphate (Casamajor, Z., 34, 1269).
81. Tribasic Calcium Phosphate, also with Sulphurous Acid (Packert, S. ind., 25, 25).
82. Tribasic Calcium Phosphate with Alum (Kessler, Z., 15, 51).
83. Tribasic Calcium Phosphate with Ammonium Phosphate (Leplay, Z., 12, 193).
84. Tribasic Phosphate of Alumina with Sulphurous Acid (Packert, S. ind., 25, 25).
85. Manganese Phosphate (Lefranc, S. ind., 58, 410).

86. Metaphosphoric Acid (Bielmann, S. C., 28, 386; Müller, S. ind., 47, 410).

87. Sodium Calcium Metaphosphate (Gwynne and Young, 1836, in Woodcroft, 59).
88. Sodium Calcium Pyrophosphate (Gwynne and Young, see above).
89. Phosphorous Acid (Hlavati, Chz., 27, 254).
90. Phosphite of Alumina (Spence, Z., 31, 231).
91. Acid Phosphites and Sulphites (Kühnel, Prager Marktb., 1888, 168).
92. Phospho-sulphites of the Alkalies and Alkaline Earths (Prangey and Grobert, S. ind., 54, 425).
93. Hypophosphorous Acid (Hlavati, Chz., 27, 254).

III. BORON, SILICON, CARBON, THEIR ACIDS, COMPOUNDS AND DERIVATIVES

94. Boric Acid (Payen, 1828, in Weber I, 565).
95. Boric Acid with Sulphur Powder (Fancher and Clarke, Bl. Ass., 9, 912).
96. Boric Acid and Borates of the Alkaline Earths (Oppermann, Z., 30, 533; Brear, B., 15, 1224).
97. Ammonium Borate (Besson, J. Fabr., 43, 1).
98. Borax (Brear, B., 15, 1224).
99. Hydrofluoboric Acid (Hlavati, Z., 53, 258).

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100. Silicon Fluoride (Hlavati, Z., 52, 758).
101. Silicic Acid (Leuchs III, 86, 1836).
102. Silicic Acid (Kieselguhr), (Heddle, Oe., 16, 441).
103. Kieselguhr and Saw-dust (Soxhlet, Z., 43, 972).
104. Hydrated Silicic Acid (Schubarth, Z., 2, 92).
105. Potassium Silicate, also with Gypsum (Schott, D., 251, 91).
106. Sodium Silicate (Wagner, Z., 9, 331).
107. Polysilicates of Magnesium and Aluminum (Hlavati, Chz., 28, 1180).
108. Zinc Silicate (Hlavati, see above).
109. Silicate of Alumina, *e.g.*, Brick Dust (Maumené, textbook).
110. Silicate of Alumina, *e.g.*, Brick Dust, with Caustic Lime (Breyer, Z., 54, 1271).
111. Hydrofluosilicic Acid (Kessler, Z., 16, 760; Gin, Z., 46, 627; Schoonjans, Chz., 30, 382).
112. Ammonium Hydrofluosilicate (Mills, N. Z., 39, 115; Whiteman, S. C., 1903, 565).
113. Ammonium Hydrofluosilicate with Lime (Hlavati, Chz., 28, 1110).
114. Hydrofluosilicic Acid with Calcium Carbonate (Marix, Bl., 1869, 346).
115. Magnesium Hydrofluosilicate (Kessler, Z., 16, 760).
116. Zinc Hydrofluosilicate (Rivière, Bl. Ass., 25, 603).
117. Lead Hydrofluosilicate (Vivien, Bl. Ass., 8, 24; Sokol, Chz., 21, R., 68).
118. Basic Lead Salt of Hydrofluosilicic Acid (Hlavati, Chz., 28, 1180).
119. Aluminum Hydrofluosilicate (Rivière, J. Fabr., 49, 18).
120. Iron Hydrofluosilicate (Lefranc, Z., 41, 498; Drost Patent, 54, 372).
121. Hydrofluosilicic Acid with Powdered Iron or Aluminum (Mertens, S. ind., 63, 659).
122. Manganese Hydrofluosilicate (Kessler, Z., 16, 760).
123. Hydrofluosilicate of Alumina (Kessler, Z., 15, 525).
124. Hydrofluosilicic Acid with Alumina (Gin, S. ind., 46, 48).
125. Carbonic Acid (Barruell, 1811; Oe., 23, 946; Leuchs, 1836, III, 86).
126. Potassium Carbonate, also with Fuller's Earth (Freund, 1827, Gesch, 369).
127. Sodium Carbonate (Dubrunfaut, about 1830 (?), Clémandot in Weber, III, 568).
128. Sodium Potassium Carbonate (Richard, 1856, in Woodcroft, 211).
129. Ammonium Carbonate (Payen, 1828, in Weber, I, 565; Nind, Z., 1, 595; Stammer, Z., 9, 430).
130. Acid Sodium Carbonate (Perrier and Possoz, St. J., 1863, 350).
131. Acid Sodium Carbonate with Alum (Salisbury, Z., 54, 849).
132. Acid Ammonium Carbonate (Dubrunfaut, about 1830 (?),).
133. Potassium Percarbonate (Bismer, Oe., 38, 534).

IV. HYDROGEN, OXYGEN, HALOGENS, NITROGEN, THEIR ACIDS, COMPOUNDS AND DERIVATIVES

134. Nascent Hydrogen, from Hydroperoxide with Zinc, Lead or Manganese (Manoury, Z., 48, 140).
135. Hydrogen Peroxide (Frank, Z., 11, 392).
136. Hydrogen Peroxide with Phosphoric Acid or Alkaline Phosphates (Stein and Crosfield, Oe., 28, 181).
137. Hydrogen Peroxide with Phosphoric Acid and Magnesia (Pechnik and Bögel in Z., 25, 127).
138. Hydrogen Peroxide and Bone Black (Ranson, Oe., 26, 737).
139. Oxygen Gas (Reboux, S. ind., 36, 150; Wayland, S. C., 1893, 611).
140. Ozonized Air (Schneller and Wisse, S. ind., 39, 467).
141. Air and Ozonized Air (Steffens, S. ind., 72, 214).
142. Ozone (Beanes, 1866, in Woodcroft, 392; Lee, B., 2, 64).
143. Ozone with Chlorine and Soda (Brin, Engl. Patent, 2297).
144. Ozonized Chlorine (?) (Lewicki, Z., 54, 245).
145. Ozone with Sulphurous Acid and Barium Hydrate (Verley, S. ind., 53, 301).
146. Ozone with Chloride of Lime and Alumina (Brin, Engl. Patent, 2297).

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147. Chlorine Gas (Strathing and Smit, 1820, Z., 49, 370; Z., 1, 258; Siemens, 1859, Z., 44, 458; Duncan, St. J., 1882, 274).
148. Liquefied Chlorine (Reboux, S. ind., 36, 150).
149. Chlorine with Carbonic Acid (Bismer, Oe., 38, 532).
150. Chlorine with Acetylene (Carlee, D. Z., 33, 738).
151. Chlorine with Ethylene (Kitsee, S. C., II, 2, 49).
152. Hydrochloric Acid (Margueritte, S. ind., 8, 71; Kessler, Z., 16, 761; Erk, Z., 26, 288).
153. Hydrochloric Acid with Metallic Powders (Hlavati, Z., 52, 758).
154. Hydrochloric Acid with Alum (Thiele, Chz., 20, 404).
155. Ammonium Chloride (Macfadyen, 1830, Gesch., 423; Reboux, Z., 84, 94; Licht, St. J., 24, 415).
156. Potassium Chloride (Macfadyen, 1830, Gesch., 423).
157. Sodium Chloride (Nash, 1852, in Woodcroft, 151).
158. Hypochlorous Acid (Z., 1, 255; Bismer, Oe., 34, 532).
159. Hypochlorous Acid Anhydride (Lagarigue, S. ind., 35, 549).
160. Hypochlorites of Alkalies (Dobler, S. ind., 66, 517; Hafner, Oe., 37, 86).
161. Hypochlorites of Alkaline Earths (Herapath, 1862, in Woodcroft, 320).
162. Hypochlorite of Alumina (used in England about 1880).

163. Bromine (Maumené, S. ind., 1895, 577).
164. Hydrofluoric Acid (Frickenhaus, Z., 15, 43; Schoonjans, Chz., 29, 889).
165. Ammonium Fluoride (Besson, Chz., 27, 863, Bartz, 125).
166. Ammonium Fluoride with Aluminum (Voss, Z., 50, 438).
167. Magnesium Fluoride (Kessler, S. ind., 1, 363).
168. Calcium Fluoride (Kessler, S. ind., 1, 363; Abraham, C. Z., 11, 886).

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169. Nitrous Oxide (Melsens, 1849, D. Z., 25, 1360; Hlavati, Chz., 28, 1180).
170. Nitrous Acid (Drapiez, in Blachette-Zoega, 1833, 264; Newton, 1849, in Woodcroft, 111).
171. Nitrites of the Alkalies and Alkaline Earths (Decastro, Z., 29, 270).
172. Nitric Acid (Kessler, Z., 16, 61).
173. Calcium Nitrate (Decastro, Z., 29, 270).
174. Potassium Nitrate (Macfadyen, 1830; Gesch., 423).

V. ALKALIES, ALKALINE EARTHS, AND THEIR COMPOUNDS

175. Ammonia (Nash, 1852, in Woodcroft, 152; Michaelis, Z., 2, 448).
176. Ammonia, also with Caustic Lime (Marot, B., 9, 643).
177. Ammonia with Magnesium or Aluminum Sulphate (Hlavati, S. ind., 65, 673).
178. Ammonia with Oxalic Acid (Hlavati, Z., 56, 300).
179. Ammonium Sulphide (Bandris, 1853, in Ling-Roth, 107).
180. Caustic Potash with Alkali Carbonate (partially causticated plant ash), about 700 in Egypt, Gesch., 134 and 287).
181. Potassium Sulphide or Sodium Sulphide (Bandris, see 179).
182. Sodium acetate (Margueritte and Maumené, Z., 28, 845).

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183. Calcium Peroxide (Hlavati, Chz., 27, 254).
184. Caustic Lime and Hydrated Lime (in Egypt about 700, Gesch., 134 and 287).
185. Calcium Hydrate with Soda (Beuster, J. Fabr., 32, 2).
186. Calcium Hydrate with Gypsum (Nathusius, in Bley, 75).
187. Calcium Chloride (Balling, 1837, Z., 44, 452; Michaelis, Z., 2, 65).
188. Chloride of Lime (Brandes, 1824, Z., 44, 447, Z, 7, 423).
189. Chloride of Lime with Sulphurous Acid (Hafner and Bismer, Oe., 37, 199).
190. Calcium Chloride with Lime or Magnesia (Guignard, Z., 53, 446).
191. Calcium Carbonate (Maumené, J. Fabr., 17, 22).
192. Calcium Carbonate with Milk of Lime (Dabrowski, Z., 50, 615).
193. Calcium Bicarbonate (Reece and Price, 1849, in Woodcroft, 106).
194. Calcium Nitrate with Sulphate of Alumina (Pape, Chz., 12, 30).
195. Calcium Sulphate (Howard, 1810, Gesch., 368; Druke, 1816, in Woodcroft, 23; Leyde, Z., 1, 378; Duquesne, D., 196, 83).
196. Calcium Sulphate with Lime (Kassner, D. Z., 29, 2151).
197. Calcined Gypsum with Lime (Lelsy, D. Z., 29, 919).
198. Calcium Acetate (Barth, 1832, Z., 44, 449; Durieux, St. J., 8, 334).
199. Calcium Borate (Klein, B., 9, 1433).
200. Calcium Sulphide (Drapiez in Blachette-Zoega, 1833, 264).
201. Calcium Sulphide with Magnesium Sulphate (Drummond, D., 203, 325).
202. Calcium Persulphide (Talamo, S. ind., 40, 57).
203. Calcium Sulphuret (Reece and Price, 1849, in Woodcroft, 106).
204. Polysulphurets of Calcium or Calcium Sulphide with Ammonia and Sulphurous Acid (Hlavati, S. ind., 72, 487).
205. Calcium Carbide (Rivière, Bl. Ass., 15, 583).

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206. Barium Oxide Hydrate (Lagrange, J. Fabr., 14, 34; Du Beaufret and Manoury, Z., 40, 590).
207. Barium Oxide Hydrate with Ammonium Phosphate (Lagrange, J. Fabr., 14, 34).
208. Barium Oxide Hydrate with Soda (Oppermann, Z., 40, 592).
209. Barium Oxide Hydrate with Iron Vitriol (Curley, S. ind., 43, 361).
210. Barium Peroxide (Beaudet, D. Z., 18, 1824).
211. Barium Peroxide with Phosphoric Acid (Stein and Crosfield, Z., 53, 1334).
212. Barium Peroxide Hydrate (Ranson, S. ind., 47, 251).
213. Barium Chloride (Licht, B., 15, 1471).
214. Barium Chloride with Caustic Soda (Plique, D. Z., 2, 51).
215. Barium Carbonate (Seyferth, Z., 25, 611; Heffter, Oe., 22, 71; Weisberg, S. ind., 64, 429).
216. Barium Carbonate with Sodium Phosphate and Sulphurous Acid (Packert, S. ind., 25, 25).
217. Barium Carbonate with Sulphate of Alumina (Eisenstuck, St. J., 3, 244).
218. Barium Carbonate with Potassium Permanganate (Talamo, N. Z., 29, 210).
219. Barium Sulphate with Barium Chloride and Lime (Haesendonck, S. ind., 43, 598).
220. Barium Sulphide and Sulphuret (Reece and Price, 1849, in Woodcroft, 106; Weisberg, S. ind., 64, 429).
221. Barium Sulphide with Caustic Soda (Romiguiéres, S. ind., 26, 682).
222. Barium Sulphide with Magnesium Sulphate (Drummond; D., 203, 325).
223. Barium Manganate (Lefranc, Bl. Ass., 18, 962).
224. Barium Silicate (Hlavati, S. ind., 65, 675).
225. Barium Carbide (Rivière, Bl. Ass., 15, 583).
226. Barium Carbide with Barium Hydrate (Battistoni, S. ind., 68, 198).

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227. Strontium Oxide (Moureaux, Bl. Ass., 19, 1483).
228. Strontium Oxide Hydrate (Scheibler, Z., 32, 986).
229. Strontium Oxide Hydrate with Iron Sulphate (Curely, S. ind., 43, 361).
230. Strontium Chloride (Kottmann, Z., 32, 899).
231. Strontium Carbonate (Heffter, Oe., 22, 41).
232. Strontium Sulphide and Sulphuret (Reece and Price, 1849, in Woodcroft, 106).
233. Magnesium Alloyed with Potassium, Sodium, Copper, Mercury, Tin, Zinc, or Antimony (Besson, Oe., 36, 466).
234. Magnesium Powder with Alkalies (Ranson, Chz., 21, 1033).
235. Magnesium Powder with Acids (Manoury, S. ind., 51, 103).
236. Magnesium Oxide (Thénard, Z., 13, 128).
237. Magnesium Oxide Hydrate (Rümpler, D. Z., 4, 52; Oppermann, N. Z., 18, 216).
238. Magnesium Oxide Hydrate with Sulphuric Acid and Lime (Koebig, S. C., 1894, 274).
239. Magnesium Oxide Hydrate with Magnesium Carbonate (Rümpler, D. Z., 4, 180).
240. Magnesium Calcium (Dolomite), Oxide Hydrate (Oppermann and Manoury, S. ind., 1888, 240).
241. Magnesium Chloride (Nash, 1852, in Woodcroft, 151; Kessler, Z., 16, 760; Z., 23, 74; Drenckmann, D. Z., 17, 1468).
242. Magnesium Carbonate (Reich, Z., 6, 173; Spreckels, Chz., 28, 1070).
243. Magnesium Subcarbonate (Stenhouse, 1856, in Woodcroft, 216).
244. Magnesium Bicarbonate (Reece and Price, 1849, in Woodcroft, 106; N. Z., 25, 91).
245. Dolomite (Dubreul, B., 6, 155).
246. Magnesium Sulphate (Bayvet, Z., 10, 256).
247. Magnesium Sulphate with Lime or Baryta (Manoury, S. ind., 26, 680).
248. Magnesium Sulphate with Alcohol (Degener, Chz., 12, 174).
249. Magnesium Sulphide (Dubreul, J. Fabr., 13, 27; Rivière, Bl. Ass., 15, 583).
250. Magnesium Sulphide and Sulphuret (Reece and Price, 1849, in Woodcroft, 106).
251. Magnesium Silicate (Hlavati, S. ind., 65, 674).
252. Magnesium Carbide (Rivière, Bl. Ass., 15, 583).
253. Radium (?) (C. Z., 1902, 466).

VI. METALS AND THEIR COMPOUNDS

254. Aluminum Dust (Ranson, Chz., 21, 1033).
255. Aluminum Dust with Alkalies (Ranson, see above).
256. Aluminum Dust with Ammonium Sulphite (Besson, Bl. Ass., 19, 800).
257. Aluminum Dust with Hydrofluoric Acid or Hydrofluosilicic Acid (Mertens, Z., 54, 118).
258. Aluminum Alloys, also with Copper or Zinc Dust (Bessen, Chz., 28, 529).
259. Aluminum Chloride (Nash, 1852, in Woodcroft, 151; Heffter, Oe., 22, 71).
260. Aluminum Chloride with Lime (Siemens, St. J., 18, 256).
261. Aluminum Fluoride (Kessler, Z., 15, 525).
262. Alumina (about 700 in Egypt, Gesch., 135 and 295; Murray, about 1802, Gesch., 368).
263. Hydrate of Alumina (Howard, 1810, Gesch., 368; Z., 2, 92).
264. Colloidal Alumina (Löwig, Z., 29, 905).
265. Fuller's Earth (Fritsche, Z., 35, 361).
266. Sodium Aluminate also with Sulphurous Acid (Besson, Bl. Ass., 25, 733).
267. Aluminate of the Alkaline Earths (Plicque, D. Z., 2, 51).
268. Calcium Aluminate (Oxland, Z., 2, 92).
269. Basic Calcium Aluminate (Gui, Z., 46, 202).

270. Tetra- and Hexa-Basic Aluminate of Calcium or Barium (Gin and Leleux, Bl. Ass., 16, 707).
271. Aluminate of Barium or Strontium (Jacquemart, French Patent, 51, 908, 1861; Rembert, Bl. Ass., 20, 747).
272. Barium Aluminate with Ammonia Alum (Geistodt, Z., 28, 843).
273. Barium Aluminate with Sulphurous Acid (Jaluzot, S. ind., 63, 690).
274. Barium Aluminate with Aluminum Sulphate (Jaluzot, see above).
275. Magnesium Aluminate (used about 1888; Hlavati, S. ind., 65, 674).
276. Sulphite of Alumina (Kessler, Z., 15, 525; Massé, 1860, Z., 44, 458).
277. Sulphate of Alumina with Phosphoric Acid (Stein and Crosfield, Oe., 28, 183).
278. Basic Sulphate of Alumina (Hunt, Z., 30, 361; Brünjes, D. Z., 25, 19).
279. Alum (about 700 in Egypt, Gesch., 135; Hermbstaedt, "Anleit. z. Fabrik. des Zuckers," Berlin, 1811, 86).
280. Alum with Sodium Carbonate (Salisbury, Z., 54, 1274).
281. Alum with Lime and Alcohol (Derosne, Oe., 23, 948).
282. Alum also with Sulphate of Alumina (Howard, 1812, Z., 44, 446).
283. Aluminum Acetate (Oxland, 1850, in Ling-Roth., 121; Schubarth, Z., 2, 92).
284. Tartrate of Alumina (Dumas, C. Z., 1906, 939).
285. Oxalate of Alumina (Sievier, 1847, in Woodcroft, 94; Mialhe, D., 99, 482; Dumas, C. Z., 1906, 939).
286. Aluminum Phosphate (Oxland, Z., 2, 92, and 2, 130).
287. Aluminum Silicate (Maumené, Lehrbuch).
288. Aluminate Silicates (Gans, Z., 57, 206).
289. Iron- and Quartz-containing Clay (Harm, D. Z., 22, 1104).
290. Aluminum Sulphide (Hlavati, Chz., 27, 254).
291. Ferrous Oxide (Hills, 1850, in Woodcroft, 121).
292. Iron Hydroxide, also with Gypsum (Rousseau, Z., 11, 671).
293. Iron Sesquioxide, also with Ozone (Wayland, Chz., 19, 1519).
294. Iron Sesquioxide Hydrate (Wackernie, S. ind., 47, 251).
295. Iron Peroxide (Reynolds, 1859, in Woodcroft, 250).
296. Iron Ochre (Martineau, 1815, in Woodcroft, 21).
297. Iron Chloride (Sievier, 1847, in Woodcroft, 94; Karl, Z., 18, 317; Licht, N. Z., 11, 63).
298. Ferrous Chloride (Maumené, S. ind., 1895, 577).
299. Iron Oxy-chloride (Spunt and Schachtrupp, N. Z., 30, 216).
300. Ferrous Fluoride (Junius and Gouthiere, Chz., 25, 603).
301. Iron Carbonate (Reynolds, 1859, in Woodcroft, 250).
302. Ferric Sulphate (Sievier, 1847, in Woodcroft, 94; Karl, Z., 18, 317).
303. Basic Ferric Sulphate (Mehrle, Z., 32, 385).
304. Ferric Sulphate (Bayvet, Z., 10, 256; Mehrle, Z., 32, 385).
305. Iron Vitriol with Alkaline Earths (Curely, S. ind., 43, 361).
306. Iron Vitriol with Barium Carbonate (Beaufret, Bl. Ass., 10, 803).
307. Iron Vitriol with Gypsum (Lohmann, 1817, Z., 44, 447).
308. Iron Vitriol with Zinc (Schetke, Chz., 1906, 23).
309. Iron Vitriol with Albuminates (Karl, Z., 18, 317).
310. Iron Nitrate (Sievier, 1847, in Woodcroft, 94).
311. Salts of Ferric Acid, so-called "Ferrites" (Liesenberg, about 1892).
312. Iron Cyanide and Sulphurous Acid (Thompson, Z., 50, 957).
313. Potassium Ferrocyanide (Sievier, 1847, in Woodcroft, 94).
314. Potassium Ferrocyanide, also with Sulphurous Acid (Boot, Java Archiv., 1903, 1046).
315. Calcium Ferrocyanide (Therry, 1833, in Woodcroft, 54).
316. Chromium Peroxide (Piettre, Bl. Ass., 19, 1381).
317. Chromic Acid and Salts of Chromic Acid (Maumené, S. ind., 1895, 577).
318. Acid Chromic Acid Salts (Maumené, see above).
319. Chromium Sulphate (Lefranc, S. ind., 58, 410).
320. Chromium Phosphate (Lefranc, see above).

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321. Manganese Dust with Acids (Manoury, S. ind., 51, 103).
322. Manganese Oxide (Spreckels, Chz., 28, 1270).
323. Manganous Oxide (Eachran, D., 251, 91).
324. Manganese Dioxide (about 1836, Bley, 47; Frickenhaus, Z., 10, 301; Piettre, Bl. Ass., 19, 1351).
325. Manganese Chloride (Manoury, about 1880).
326. Manganese Chloride with Oxalic Acid (Fontenille, S. ind., 54, 425).
327. Manganese Carbonate (Newton, 1859, in Woodcroft, 253).
328. Manganese Sulphate (Massé, Z., 10, 256).
329. Manganates of the Alkalies and Alkaline Earths (Hawes, 1853, in Woodcroft, 163).
330. Sodium Manganate (Knaggs, 1866, in Woodcroft, 384).
331. Manganate of Lime (Z., 1, 256; Lefranc, Bl. Ass., 18, 962).
332. Calcium Permanganate (Maumené, J. Fabr., 1894, 51).
333. Sodium Permanganate (Knaggs, 1866, in Woodcroft, 384).
334. Calcium Permanganate (Fayolle, S. ind., 52, 554).
335. Aluminum Permanganate (Fayolle, see above).
336. Permanganates with Barium Carbonate and Oxalic Acid (Talamo, N. Z., 29, 210).
337. Copper Sulphate with Lime (Hlavati, Z., 56, 300).
338. Lead Dust, also with Acids (Manoury, S. ind., 51, 103).
339. Lead with Sulphides of the Alkalies (Bandris, Ling-Roth, 107).
340. Lead Oxide (about 1836, Bley, 126).
341. Plumbic Hydrate (Gwynne, Z., 3, 392; Lagrange, S. ind., 1892, 468; Wohl and Kollrepp, Z., 55, 60).
342. Litharge (Pfeifer and Langen, N. Z., 19, 131).
343. Lead Peroxide (Maumené, S. ind., 1895, 577; Piettre, Bl. Ass., 19, 1351).
344. Plumbites of the Alkaline Earths (Galloway, 1852, in Woodcroft, 147 and 171).
345. Lead Carbonate (Hills, 1850, in Woodcroft, 121; Besson, Chz., 28, 1270).
346. Lead Sulphate (Scoffern, 1850, in Woodcroft, 115).
347. Lead Nitrate (Lagrange, S. ind., 1892, 468).
348. Lead Nitrate with Sulphate of Alumina (Pape, Chz., 12, 30).
349. Basic Lead Nitrate (Wohl and Kollrepp, Z., 55, 60).
350. Lead Acetate (Scoffern, 1847, in Zerban, 15; Gwynne, Z., 3, 393).
351. Lead Subacetate, also with Sodium Sulphide (Maumené).
352. Lead Subacetate with Chalk (Pajot de Charmes, 1821, Gesch., 369).
353. Lead Subacetate with Sulphurous Acid (Scoffern, D., 117, 265; Ling-Roth, 81 and 82).
354. Lead Triacetate (?) (Gwynne and Young, in Woodcroft, 59).
355. Lead Saccharate (Gwynne, 1850, in Woodcroft, 116; Wohl and Kollrepp, Z., 54, 854).
356. Lead Albuminate (Gwynne and Young, 1836, in Woodcroft, 59).

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357. Zinc Dust with Mineral Acids (Manoury, S. ind., 51, 103).
358. Zinc Dust with Sulphuric Acid and Barium Sulphide (Cripo, St. J., 24, 416).
359. Zinc Dust with Sulphurous Acid, also with Ferrocyanides (Boot, Oe., 27, 717).
360. Zinc Dust with Hydrofluoric Acid (Mertens, Z., 54, 118).
361. Zinc Dust with Tartaric Acid (Koperski, Z., 54, 1271).
362. Zinc Dust with Alkalies (Ranson, Chz., 21, 1033).
363. Zinc Dust with Dolomite (Hlavati, Bl. Ass., 16, 759).
364. Zinc Dust with Ammonium Sulphide (Brünn, Chz., 31, R., 459).
365. Coppered Zinc-Powder (Verley, Chz., 24, 596).
366. Zinc Iron Alloys (Mertens, Z., 54, 118).
367. Zinc Chloride (Gauchy, N. Z., 13, 43; Heffter, Oe., 22, 71).
368. Zinc Fluoride (Hlavati, Z., 53, 258).
369. Zinc Oxide (about 1836, Bley, 126).
370. Zinc Hydrate (Wilson, 1815, Gesch., 368).
371. Zinc Hydrocarbonate (Perrin, Chz., 22, 54; Mittelstaedt, D. Z., 23, 1112).
372. Zinc Hydrocarbonate with Oxalic Acid (Moureaux, Bl. Ass., 19, 1483).

373. Zinc Sulphate (Wilson, 1818, in Woodcroft, 27; Z., 44, 447; Hermbstädt, in Weber, 1829, 100).
374. Zinc Sulphate with Barium Hydrate (Wackernie, S. ind., 53, 201, 61, 718).
375. Zinc Nitrate (Decastro, St. J., 19, 340).
376. Zinc Nitrate with Alkali Sulphide (Decastro, see No. 375).
377. Zinc Nitrate with Calcium Sulphide or Barium Sulphide (Decastro, see No. 375).
378. Zinc Aluminate (Hlavati, S. ind., 65, 674).

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379. Cadmium Oxide (Mouraux, Bl. Ass., 19, 1483).
380. Cadmium Carbonate (Moraux, see No. 379).

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381. Tin Dust (Besson, Chz., 27, 863).
382. Stannic Oxide, also with Soda (B., 19, R., 520).
383. Stannic Hydrate (Wilson, 1815, Gesch., 368).
384. Stannic Chloride (Nash, 1852, in Woodcroft, 151; Maumené, J. Fabr., 20, 7).
385. Stannous Chloride (Nash, 1852, see No. 384; Havemeyer, 1869, in Zerban, 77; Manoury, Z., 34, 1275; Maumené; S. ind., 1895, 577).
386. Stannous Chloride with Sulphuric Acid (Thiele, Chz., 20, 404).
387. Stannous Nitrochloride (Nash, 1852, see No. 384).
388. Stannous Fluoride (Ranson, Chz., 24, 1026).
389. Stannic Sulphate (Anderson, 1856, in Woodcroft, 218).
390. Stannous Sulphate (Oe., 15, 76).
391. Tin Nitrate (Reynolds, 1859, in Woodcroft, 250).
392. Tin Chloronitrate (Reynolds, 1859, see No. 391).
393. Stannic Acid or Metastannic Acid (Reynolds, 1859, see No. 391).
394. Stannates of the Alkalies (Reynolds, 1859, see No. 391).
395. Stannates of the Alkaline Earths (Reynolds, 1859, see No. 391).
396. Metastannates of the Alkalies and Alkaline Earths (Reynolds, 1859, see No. 391).
397. Aluminum Metastannate (Reynolds, 1859, see No. 391).
398. Mercury Peroxide (Piettre, Bl. Ass., 19, 1351).
399. Mercuric Nitrate (S. C., II, 4, 216).

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400. Antimony Dust (Besson, Chz., 27, 863).
401. Antimony Tin Alloy (Mertens, S. ind., 63, 659).
402. Antimony Peroxide (Piettre, see No. 398).
403. Antimony Sulphide (about 1670, Gesch., 311).
404. Bismuth Nitrate (Sievier, 1847, in Woodcroft, 94).
405. Bismuth Salts (Hawes, 1853, in Woodcroft, 163).
406. Ammonium Molybdate (Wichardt, D. Z., 31, 652).

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407. Salts of Tungstic Acid (Reynolds, 1859, in Woodcroft, 259).

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408. Titanic Acid (Employed in England about 1880).
409. Ferrititanite (Liesenberg, about 1892).
410. Thorium- and Monazite-Earths (Browne, C. Z., 1904, 568).

VII. ORGANIC SUBSTANCES AND COMPOUNDS; BONE BLACK AND ITS SUBSTITUTES

411. Extract of Gall-Apples (about 700 in Egypt, Gesch., 135).
412. Tannins and Tanning Liquors (Dorion, 1816, Z., 49, 578; Leuchs, 1836; III. 86; Luther, Chz., 29, 1091).
413. Quebracho, Valonea, and Sumach (Hlavati, Chz., 27, 254).
414. Tannic Acid (Wagner, Z., 9, 331; Walkhoff, 1863, Z., 44, 459).
415. Liquid Tannic Acid (?) (Elias, S. ind., 1895, 20).
416. Tannate of Potassium or Ammonium (Galloway, 1853, in Woodcroft, 171).
417. Tannic Acid with Lime (about 1836, Bley, 126; Heffter, Oe., 16, 442).
418. Tannic Acid with Salts of Barium or Strontium (Heffter, see No. 417).
419. Tannic Acid with Alumina (Heffter, see No. 417).
420. Tannic Acid with Tartaric Acid, Metaphosphoric Acid, and Hydrofluosilicic Acid (Royers, S. ind., 50, 32).
421. Tannic Acid with Glue, Starch or Albumen (Heffter, see No. 417).
422. Pertannic Acid (?) (Meritens, Z., 28, 800).
423. Gallic Acid (Royers, see No. 420; Kowalski, Chz., 26, 972).
424. Gallate of Potassium or Ammonium (Galloway, 1853, see No. 416).
425. Acetic Acid, also with Sulphurous Acid (Z., 20, 741; Stutzer and Wernekinck, S. ind., 51, 114). For acetates look under the list metals.
426. Wood Vinegar (Leidenfrost, Z., 20, 746).
427. Butyric Sulphonic Acid (?) (Spreckels, Chz., 28, 1270).
428. Fatty Acids with Sulphurous or Sulphuric Acid (Spreckels, Z. ang., 1902, 891; Chz., 28, 1072).
429. Stearic Acid (Z., 2, 91; Wagner, Z., 9, 331).
430. Ammonium Stearate (Besson, Chz., 27, 863).
431. Stearic Acid with Sulphites of the Alkalies or Magnesium (Stewart, Z., 57, 268).
432. Stearic-Sulphonic Acid (Spreckels, see No. 427).
433. Palmitic-Sulphonic Acid (Spreckels, see No. 427).
434. Margaric Acid (Pidding, 1853, in Woodcroft, 162).
435. Oleic Acid (Pidding, see No. 434; Thénard, Z., 8, 130).
436. Oleic-Sulphonic Acid (Spreckels, see No. 427).
437. Oxalic Acid (Leuchs, 1836, III, 86; Wagner, Z., 9, 331; Eissfeldt, Z., 21, 1102).
438. Oxalic Acid with Ammonia, Magnesium and Zinc (Besson, Bl. Ass., 18, 616).
439. Ammonium Oxalate (Sievier, 1847, in Woodcroft, 94; Besson, J. Fabr., 43, 1).
440. Oxalic Acid with Barium Carbonate and Permanganates (Talamo, N. Z., 29, 210).
441. Tartaric Acid (Possoz, Z., 23, 27; Stutzer and Wernekinck, S. ind., 51, 114).
442. Ammonium Tartrate (Besson, Chz., 27, 863).
443. Malic Acid with Metallic Bases or Carbonates (Moreaux, Bl. Ass., 19, 1483).
444. Citric Acid with Metallic Bases or Carbonates (Moreaux, Bl. Ass., 19, 1483).
445. Citric Acid, also with Polysilicates (Hlavati, Chz., 28, 1180).
446. Salicilic Acid (Hulwa, Z., 25, 640; D. Z. 9, 7).
447. Resin Acids (Leuchs, 1836; III, 86).
448. Pimaric Acid (Schiller, Z. B., 12, 33).
449. Pectic Acid (Acar in Wagner's Technologie, 12th Ed., 563).
450. Formaldehyde (Boulet, Chz., 20, 12; Friedrich, Chz., 27, 1183; Simpson, Bl. Ass., 25, 531).
451. Acetaldehyde (Newton, 1849, in Woodcroft, 111; Melsens, 1849, D. Z., 25, 1306; Boulet, Chz., 20, 12).
452. Methylalcohol (Trovach, D. Z., 11, 1302).
453. Alcohol (Jennings, 1825, in Woodcroft, 33; Pesier, Z., 11, 522).
454. Alcohol with Chlorine Gas (Duncan, St. J., 22, 274).
455. Alcohol with Acetic Acid (Paulet, 1837; Z., 14, 641; 19, 376).
456. Alcohol with Hydrochloric Acid, Nitric Acid or Sulphuric Acid (Ure, 1830, in Woodcroft, 49).
457. Alcohol with Sulphuric Acid and Gypsum (Duquesne, D., 196, 83).
458. Alcohol with Sulphurous Acid (Stolle, D., 114, 305).
459. Alcohol with Alum and Lime (Derosne, 1810, Oe., 23, 948).
460. Alcohol with Magnesium Sulphite (Degener, Chz., 12, 174).
461. Rum or Gin (Stokes, in Weber, III, 236).
462. Glycerine (Rabe, Z., 14, 124).
463. Glucose and its Salts (?) (Bielmann, S. C., 28, 386).
464. Saccharites of Lead or of the Alkaline Earths (Reece and Price, 1849, in Woodcroft, 106; Gwynne, Z., 3, 392; Stammer, Z., 12, 336).
465. Magnesium Saccharate (Galloway, Z., 3, 31).
466. Starch with Caustic Lime (Steinkamp, 1848, in Woodcroft, 102)
467. Hydrocarbons and Petroleum (Carbonelle, S. ind., 33, 455).
468. Kerosene with Alumina and Metallic Powder (Z., 53, 444).
469. Kerosene Oil (Spreckels and Kern, Z., 53, 878).
470. Tar Oils (Newton, 1849, in Woodcroft, 111).

471. Benzol or Toluol, also with Sulphurous Acid or Hydrosulphurous Acid (Kowalski, Z., 53, 909).
472. Phenol (Fishman, Z., 21, 313; D. Z.,21, 9, 7).
473. Phenol with Benzol or Petroleum (Kowalski, Z., 55, 396).
474. Phenol with Chloride of Lime (Menier, Bl. Ass., 10, 165).
475. Oxybenzol (Kowalski, see No. 471).
476. Oxynaphthalin and Oxyanthracene, also with Sulphurous Acid or Hydrosulphurous Acid (Kowalski, see No. 471).
477. Oxyanthraquinone (Kowalski, see No. 473).
478. Fats with Sulphurous or Sulphuric Acid (Spreckels, Z. ang., 1902, 891; Chz., 28, 1072).
479. Tallow or Lard with Sulphurous or Sulphuric Acid (Spreckels & Kern, Z., 55, 571; 53, 878).
480. Fatty Oils and Mineral Oils (Bouvier, Z. B., 1896, 386).
481. Fatty Oils with Sulphurous or Sulphuric Acid (Spreckels, Z. ang., 1902, 891).
482. Fatty Oils with Soda (Brooman, 1857, in Woodcroft, 232).
483. Wax and Neutral Fat (Leuchs, III, 86).
484. Spermaceti and Spermaceti Oil (Pidding, 1853, in Woodcroft, 162).
485. Stearine and Palmatine (Carlee, D. Z., 33, 738).
486. Fish Oil with Sulphurous or Sulphuric Acid (Spreckels, Z., 55, 571; Z. ang., 1902, 891).
487. Linseed Oil with Sulphuric Acid (Spreckels and Kern, Z., 53, 878).
488. Castor Oil with Sulphuric Acid (Spreckels, Z., 55, 571).
489. Soap (Basset, Z., 7, 381).
490. Ammonia Soaps (Brooman, Z., 8, 449; Besson, J. Fabr., 43, 1).

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491. Turpentine (Newton, 1849, in Woodcroft, 111; Carlee, D. Z., 33, 738).
492. Turpentine and Sulphuric Acid (Spreckels and Kern, Z., 53, 878; 55, 571).
493. Tar with Sulphurous or Sulphuric Acid (Spreckels, Z. ang., 1902, 891).
494. Tar Oil (Pidding, 1853, in Woodcroft, 162).
495. Tar Oil also with Alumina and Metallic Powders (Z., 53, 444).
496. Tar Oils with Sulphurous or Sulphuric Acid (Spreckels, Z. ang., 1902, 891).
497. Resin (Pidding, 1853, in Woodcroft, 162).
498. Resin and Sulphuric Acid (Spreckels and Kern, Z., 53, 878).
499. Pitch (Pidding, 1853, see No. 497).
500. Creosote (Newton, 1849, in Woodcroft, 111).
501. Shellac (Greiger, S. ind., 54, 23).
502. Carbon Bisulphide (Ckiandi, S. ind., 25, 268).
503. Mustard Oil (Leuchs, 1836, III, 86; Newton, 1842, in Woodcroft, 111).
504. Radish Oil (Newton, 1842, see No. 503).
505. Sulphur-containing Ethereal Oils (Spreckels, Chz., 29, 1307).
506. Ethereal Oils with Sulphurous or Sulphuric Acid (Spreckels, Z. ang., 1902, 891; Chz., 28, 1072).
507. Animal Oil (Pidding, 1853, in Woodcroft, 162).
508. Eucalyptol (Fry, Amer. Patent, No. 472,989).
509. Eucalyptus Oil and Sulphuric Acid (Spreckels, Z., 55, 571).
510. Indigo White (Bielmann, S. C., 28, 386).
511. Milk (about 600, in Persia, Gesch., 102; Batley, 1810, Gesch., 369; Hermstadt, 1811).
512. Casein (Krüger, Z., 9, 221).
513. Albumen (about 700, in Egypt, Gesch., 135 and 209; Wilcox, Bl. Ass., 9, 912).
514. Calcium Albuminate (Karl, Z., 18, 317).
515. Calcium Albuminate with Iron Vitriol (Karl, see No. 514).
516. Blood (about 1700, Gesch., 324).
517. Hay or grass (Hlavati, Chz., 27, 254).
518. Bark of Trees, as Elm Bark (Stokes, in Weber, III, 236).
519. Cork (Wagner, Z., 43, 630).
520. Plane Shavings (Leuchs, 1836; III, 86).
521. Wood Dust (Wiechmann, 1885; D. Z., 28, 1544).
522. Sawdust (Hills, 1853, in Woodcroft, 163; Casamajor, 34, 1269).
523. Wood Meal from Mechanical Pulp Process (Soxhlet, Z., 43, 972).
524. Wood Wool (Excelsior) (Müller and Schubert, Z., 44, 233).
525. Wood Pulp or Paper Pulp (Spreckels, Chz., 28, 1270).
526. Linen or Cotton Fabrics and Threads (Ost. Z., 58, 556).
527. Bran (Tyre, Z., 50, 475).
528. Dead Yeast (Clowes, Z., 54, 1286).
529. Yeast with Hydrochloric Acid (Effront, Z., 58, 326).
530. Peat with Calcium Sulphite (Nowak, Z., 53, 988).
531. Peat-Coal or Lignite (Maumené, Z., 4, 452).
532. Calcium Humate (Schmidt and Degener, D. Z., 20, 209).
533. Brown Coal (Böttcher, 1836, Gesch., 377).
534. Brown Coal Coke (Knauer, Z., 11, 350).
535. Wood Charcoal (Lowitz, 1793, Gesch., 368).
536. Charcoal Dust (Remmers, Z., 35, 369).
537. Coal-Dust, also with Alumina (Pajot de Charmes, 1821, Gesch., 369).
538. Lamp-Black (Martineau, 1815, in Woodcroft, 21).
539. Sugar Charcoal (Sievier, 1847, in Woodcroft, 94).
540. Plant-Blood Charcoal (Degener, Z., 46, 492).
541. Bituminous Coal (Martineau, 1815, in Woodcroft, 21; Payen, 1830, Z., 49, 594).
542. Carbon-Alumina (?) (Kachmarkiewicz, C. Z., 1906, 229).
543. Alumina Impregnated with Carbonized Blood (Olschewsky, Z., 32, 525).
544. Carbonized Kieselguhr (Infusorial Earth) (Heddle, Z., 37, 478).
545. Kieselguhr Impregnated with Carbonized Fatty Residues (about 1900, in America).
546. Bone Black (Figuier and Magnes, Gesch., 368).
547. Bone Black with Hydrogen Peroxide (Mastbaum, Z., 37, 704).
548. Bone Black Saturated with Carbonic Acid or Sulphurous Acid (Lach., Z., 46, 497).
549. Bone Meal (Hills, 1853, in Woodcroft, 163).
550. Osteine (Brunon and Rothé; Z., 54, 848).
551. Ferrocyanide Residues (So-called Coal-settlings) (Cavaillon, 1817, in Woodcroft, 25; Gawalowski, Oe., 18, 718).
552. Powder-Settlings of Stearin Factories (Lach and Benies, S. ind., 1895, 20).
553. Graphite with Bone Black and Zinc Bloom (Macherski and Koperski, Z., 57, 1121).
554. Graphite with Sand and Zinc Powder (Macherski and Koperski, Z., 57, 1044).
555. Anthracite (Hlavati, Z., 56, 300).
556. Coal-Tar with Lime (Lemaire, S. ind., 9, 56).
557. Carbonized Scums (Karlik, Oe., 32, 256).
558. Gravel (Bergmann, 1840, Z., 29, 1184; Meyer, 1879, Z., 30, 1149).
559. Bauxite (Hlavati, Z., 56, 300).
560. Calcined Phosphate-Slag (Lachaux, S. ind., 59, 677).
561. Cement (Harm, D. Z., 25, 1946).
562. Brick-Dust with Lime (Breyer, S. ind., 65, 655).
563. Pumice Stone (Saunders, 1835, in Woodcroft, 56).
564. Talc or Meerschaum (Hlavati Chz., 28, 1180).
565. Mica (Hlavati, Chz., 28, 1180).
566. Natural Zeolite (Riedel, S. ind., 70, 230).
567. Permutite = Artificial Zeolite (Riedel, see No. 566).
568. Soil from the Beet Storehouse (Kohlrausch, Z., 28, 215).

VIII. ELECTROLYTIC SUBSTANCES

569. Hydrogen (Kugler, Chz., 32; R., 454).
570. Ozone (Schollmeyer, Chz., 24, 825).
571. Chlorine, Bromine, Iodine, Fluorine (Spillern-Spitzer, Z., 53, 244).
572. Sulphurous Acid (Lallement, S. ind., 53, 301).

573. Sulphurous Acid or Sulphites with Lead, Zinc, Aluminum, Iron, or Tin (Baudry and Charitonenko, Z., 50, 625).
574. Hydrosulphurous Acid (Ranson, Oe., 26, 737).
575. Coal (Despeissis, Battut, Z., 46, 624).
576. Wood Charcoal (Hlavati, Z., 53, 258).
577. Alkaline Earths (Gin and Leluex, Z., 53, 627).
578. Calcium Carbonate (Schwerin, D. Z., 29, 451).
579. Barium Salts (Bonillaut, S. ind., 50, 189).
580. Barium Aluminate (Rembert, Bl. Ass., 20, 966).
581. Magnesium (Urbain, Bl. Ass., 16, 719).
582. Magnalium (Murphy, J. Fabr., 44, 18).
583. Magnesium Hydroxide (Schwerin, Chz., 28, 626).
584. Magnesium Carbonate (Schwerin, D. Z., 29, 451).
585. Zinc (Schollmeyer, Z., 46, 624).
586. Zinc Alloy with Calcium or Antimony (Hlavati, Z., 53, 258).
587. Basic Zinc Salts (Wohl and Kollrepp, D. Z., 27, 1280).
588. Cadmium (Urbain, Bl. Ass., 16, 719).
589. Lead (Javaux, Gallois, Dupont, Z., 46, 626).
590. Lead-Antimony Alloy, also with Manganese Sulphate (Piettre and Nodon, D. Z., 27, 1211).
591. Lead Oxides (Z., 46, 626).
592. Lead Peroxide (Piettre and Nodon, Bl. Ass., 19, 1351).
593. Lead Saccharate (Wohl and Kollrepp, D. Z., 27, 1280).
594. Basic Lead Salts (Wohl and Kollrepp, see No. 593).
595. Aluminum (Z., 46, 626).
596. Aluminum-Magnesium (Browne, Z. ang., 1908, 174).
597. Aluminum Manganate with Zinc Hydroxide or Iron Hydroxide (Delavierre, Z., 53, 1106).
598. Alumina (Z., 46, 626).
599. Iron (Jennings, 1846, Clement, 1848, in Woodcroft, 89 and 103; Maigrot, Z., 46, 625).
600. Iron Bisulphide (Aschermann, Chz., 26, 683).
601. Manganese-Silicon Alloy (Hlavati, Z., 53, 258).
602. Manganese Dioxide (Hlávati, Z., 53, 626).
603. Hydrated Manganese Peroxide (Piettre and Nodon, Bl. Ass., 19, 1351).
604. Manganates of the Alkalies and Alkaline Earths (Lavollay and Bourgoin, D. Z., 25, 330).
605. Chromium Trioxide (Piettre and Nodon, see No. 603).
606. Nickel (Horsin-Déon, Oe., 28, 162).
607. Nickel with Sulphurous Acid or Sulphites (Baudry and Charitonenko, Z., 50, 625).
608. Copper (Görz, Z., 46, 624).
609. Iron (Horsin-Déon, Bl. Ass., 16, 729).
610. Antimony (Piettre and Nodon, D. Z., 27, 1211).
611. Antimony Peroxide (Piettre and Nodon, Bl. Ass., 27, 1315).
612. Mercury (Polaczek, Bl. Ass., 16, 720; Gurwitsch, Z., 54, 1030).
613. Mercury Amalgams (Polaczek, see No. 612).
614. Mercury Peroxide (Piettre and Nodon, Bl. Ass., 19, 1351).
615. Easily-Fluid Mercury Alloys (Palms, Bl. Ass., 17, 274).
616. Silver (Horsin-Déon, Oe., 28, 162).
617. Silver with Sulphurous Acid or Sulphites (Baudry and Charitonenko, Z., 50, 625).
618. Platinum (Collette, Z., 46, 623; Thomas and Howe, S. ind., 66, 624).
619. Platinum Antimony Alloy, also with Manganese Sulphate (Piettre and Nodon, D. Z., 27, 1211).
620. Platinized Copper (Charitonenko, S. ind., 53, 272).
621. Sebonaft = "Solid Mineral Oil" (Nowakowski, C. Z., 17, 277).
622. Straw-Meal (Lenze, D. Z., 33, 937).

Abbreviations of References

Abbreviations	*Reference*
Bartz	Claassen-Bartz's "Zuckerfabrikation" (Leipzig, 1905).
B	Berichte der deutschen chemischem Gesellschaft (R = Referate).
Bl.	Bulletin de la Société chimique.
Blanchette Zoega	"Manuel du fabricant et du raffineur de sucre" (Paris, 1833).
Bley	Bley's "Zuckerbereitung aus Runkelrüben" (Halle, 1836).
Bl. Ass.	Bulletin de l'association des chimistes.
Chz.	Chemiker-Zeitung (R = Repertorium).
C. r.	Comptes rendus.
C. Z.	Centralblatt für die Zuckerindustrie.
D.	Dingler's polytechnisches Journal.
D. Z.	Die Deutsche Zuckerindustrie.
Gesch.	Lippmann's "Geschichte des Zuckers" (Leipzig, 1890).
J. fabr.	Journal des fabricants de sucre.
Ling-Roth	Ling-Roth's "Guide to the Literature of Sugar" (London, 1890).
Leuchs	Leuchs "10,000 Erfindungen und Ansichten" (Nürnberg, 1871).
Maumené	Maumené's "Traité de la fabrication du sucre" (Paris, 1878).
N. Z.	Neue Zeitschrift für Rübensuckerindustrie.
Oe.	Oesterreichisch-Ungarische Zeitschrift für Zuckerindustrie.
Prager Marktb.	Prager Marktbericht.
S. C.	The Sugar Cane.
S. ind.	La sucrerie indigène et coloniale.
St. J.	Stammer's "Jahresbericht der Zuckerfabrikation."
Weber	Weber's "Zeitblatt für Gewerbetreibende."
Woodcroft	Woodcroft's "Abridgments of Specifications relating to Sugar" (London, 1871).
Z.	Zeitschrift des Vereins der Deutschen Zuckerindustrie.
Z. ang.	Zeitschrift für angewandte Chemie.
Z. B.	Zeitschrift für Zuckerindustrie in Böhmen.
Zerban	Zerban's "Louisiana Bulletin No. 103" (Baton Rouge, 1908).

Additional Abbreviations for Table 51*a*

C.	Chem. Centr.
F. a. S.	Facts About Sugar.
I. S. J.	International Sugar Journal.
Newlands	"Sugar" (London, 1909).
S. B.	Sucré Belge.

APPENDIX D. ORIGIN OF LIME

CaO or "lime" is manufactured either via calcination (heating) of limestone or as a byproduct of the hydrolysis of calcium carbide (CaC_2) to yield ethyne (acetylene). $Ca(OH)_2$ can be produced on a small scale directly by the reaction of Ca° with water. The general reactions for the industrial preparation of $Ca(OH)_2$ are given below ([a]Watkins, K.W., 1983; [b]Chenier, P.J., 1992, [c]Thermochemical data tables).

$$CaCO_3 \xrightarrow[1250\ °C^b]{897\ °C^a} CaO + CO_2\uparrow \qquad 178.1\ kJ^a$$

$$CaO + H_2O \underset{>400\ °C^a}{\rightleftharpoons} Ca(OH)_2 \qquad -64.8\ kJ^a$$

$$CaCO_3 + H_2O \longrightarrow Ca(OH)_2 + CO_2\uparrow \qquad 113.81\ kJ^c$$

$$CaCO_3 \xrightarrow[1250\ °C^b]{897\ °C^a} CaO + CO_2\uparrow \qquad 178.1\ kJ^a$$

$$CaO + 3C^b \xrightarrow{2100\ °C} CaC_2 + CO\uparrow \qquad 464.8\ kJ^c$$

$$CaC_2 + H_2O \longrightarrow C_2H_2\uparrow + Ca(OH)_2 \qquad -413.73\ kJ^c$$

$$CaCO_3 + 3C + H_2O \longrightarrow C_2H_2\uparrow + Ca(OH)_2 + CO_2\uparrow + CO\uparrow \qquad 230.0\ kJ^c$$

In either case, a large amount of energy goes into the preparation of CaO which is why hydrating it is a strongly exothermic process. When hydrated, or "slaked", CaO yields $Ca(OH)_2$. The solution of $Ca(OH)_2$ is an exothermic process, but much heat (-64.8 kJ/mol) was released when the CaO was slaked. This does not leave much free energy to accommodate solution; see below. Because of its low solubility in water at STP, the heat of solution is measured as the heat of neutralization which invokes the same ionization, principally:

$$Ca(OH)_2 + H_2O \longrightarrow Ca^{2+} + 2\,\bar{O}H \qquad 11.7\ kJ^a$$

APPENDIX E: GC-MS LIBRARY SEARCH RESULTS FOR EXTRACTS OF CANE JUICE and SUGAR

E.1. Raw Juice Extracted with Dichloromethane; with 3-Phenylphenol Surrogate ($)

Pk#	RT	Area%	Library/ID	Qual
1	5.698	0.21	Phenylethyl Alcohol	94
2	6.666	0.26	2,3-dihydro-Benzofuran	87
5	8.583	1.35	3,4-dimethoxy- Phenol	94
7	10.058	0.78	3,4,5-trimethoxy- Phenol	94
9	10.530	0.40	4-hydroxy-3,5-dimethoxy-Benzaldehyde	94
10	10.862	3.95	[1,1'-Biphenyl]-3-ol **$**	97
11	10.932	0.64	p-Hydroxybiphenyl **$**	97
12	11.107	1.96	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	97
15	12.547	3.06	n-Hexadecanoic acid	99
17	12.786	0.57	3,5-Dimethoxy-4-hydroxycinnamaldehyde	95
20	13.654	0.54	(Z,Z)-9,12-Octadecadienoic acid	90
21	13.677	1.24	(E)-9-Octadecenoic acid	99
22	13.957	0.76	Hexadecanamide	95
23	15.309	5.35	(Z)-9-Octadecenamide	99
24	24.058	1.31	Trifluoroacetic acid, n-octadecyl ester	91
25	26.017	9.17	Hexadecyl-oxirane	90
26	26.570	15.73	Cyclooctacosane	96
30	27.812	7.99	Campesterol	99
31	27.952	2.92	Hexadecyl-oxirane	90
32	28.173	10.75	Stigmasterol	96
34	28.844	14.23	β-Sitosterol	98

E.2. Hot-Limed Juice Extracted with Dichloromethane; with 3-Phenylphenol Surrogate ($)

Pk#	RT	Area%	Library/ID	Qual
1	5.698	0.25	Phenylethyl Alcohol	94
2	6.665	0.36	2,3-dihydro-benzofuran	72
3	7.639	0.94	2-Methoxy-4-vinylphenol	90
4	7.989	0.55	4-hydroxy-benzaldehyde	91
5	8.414	0.35	Vanillin	97
6	8.583	0.80	3,4-dimethoxy-Phenol	94
8	9.714	0.59	4-hydroxy-3-methoxy-benzoic acid	87
11	10.064	0.76	3,4,5-trimethoxy-Phenol	98
12	10.192	0.31	3-Hydroxy-.beta.-damascone	78
14	10.530	1.11	4-hydroxy-3,5-dimeth oxy-benzaldehyde	94
15	10.862	3.71	[1,1'-Biphenyl]-3-ol **$**	97
16	10.932	0.67	p-Hydroxybiphenyl **$**	97
17	11.107	3.25	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	97
18	11.206	0.37	6-chloro-N-ethyl-N'-(1-methylethyl)-3,5-Triazine-2,4-diamine	98
19	11.503	0.37	4-hydroxy-6-trimethyl-4-(3-oxo-1-butenyl)-3,5,2-Cyclohexen-1-one	86

20	11.597	0.18	4-hydroxy-3,5-dimethoxy-benzoic acid	93
21	11.678	0.73	Benzoic acid, 4-hydroxy-3-methoxy-ethyl ester	83
24	12.547	2.42	n-Hexadecanoic acid	98
29	13.654	0.37	7-Pentadecyne	95
30	13.677	0.91	(Z)-9,17-Octadecadienal	98
31	13.957	0.92	Hexadecanamide	94
33	15.315	9.50	(Z)-9-Octadecenamide	95
34	15.508	0.49	Octadecanamide	93
35	24.058	0.82	2-Chloropropionic acid, octadecyl ester	90
37	26.017	5.64	1,19-Eicosadiene	94
38	26.564	8.68	(Z)-9-Tricosene	93
40	26.891	0.57	Vitamin E	87
43	27.812	7.12	Campesterol	99
45	28.173	9.65	Stigmasterol	99
47	28.843	12.77	β-Sitosterol	95

E.3. Fe^{3+} Stage 1 Juice Extracted with Dichloromethane; with 3-Phenylphenol Surrogate ($)

Pk#	RT	Area%	Library/ID	Qual
1	3.267	0.44	p-Xylene	95
2	6.666	0.34	2,3-dihydro-benzofuran	80
4	7.639	1.21	2-Methoxy-4-vinylphenol	90
6	8.583	1.39	3,4-dimethoxy- Phenol	94
8	9.714	0.44	4-hydroxy-3-methoxy-benzoic acid	91
9	9.790	3.91	2,6-Dimethoxybenzoquinone	80
10	10.064	0.88	3,4,5-trimethoxy-Phenol	95
12	10.530	0.79	4-hydroxy-3,5-dimethoxy-benzaldehyde	90
13	10.862	5.69	[1,1'-Biphenyl]-3-ol **$**	97
14	10.932	1.04	p-Hydroxybiphenyl **$**	94
15	11.107	2.37	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	97
16	11.503	0.41	4-hydroxy-3,5, 5-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexenone	90
20	12.547	2.22	n-Hexadecanoic acid	97
25	13.654	0.26	(Z,Z)-9,12-Octadecadienoic acid	99
26	13.677	0.79	7-Pentadecyne	95
28	13.957	1.95	Hexadecanamide	94
30	15.321	19.31	(Z)-9-Octadecenamide	95
31	15.508	1.04	(Z)-9-Octadecenamide	93
32	22.298	0.78	Squalene	91
34	26.011	5.44	1,19-Eicosadiene	94
35	26.564	8.04	Cyclooctacosane	94
38	27.806	5.43	Campesterol	99
40	28.167	7.31	Stigmasterol	99
41	28.838	8.83	β-Sitosterol	95

E.4. Fe^{3+} Stage 2 Juice Extracted with Dichloromethane; with 3-Phenylphenol Surrogate ($)

Pk#	RT	Area%	Library/ID	Qual
1	6.666	0.39	2,3-dihydro-benzofuran	87
3	7.639	1.22	2-Methoxy-4-vinylphenol	90
4	7.989	1.06	4-hydroxy-benzaldehyde	91
5	8.344	0.44	2-methoxy-1,4-Benzenediol,	94
6	8.414	0.38	Vanillin	97
8	8.583	0.57	3,4-dimethoxy-Phenol	94
10	9.714	0.44	4-hydroxy-3-methoxy- Benzoic acid	91
13	10.064	0.69	3,4,5-trimethoxy- Phenol	98
14	10.192	0.56	3-Hydroxy-.beta.-damascone	92
17	10.530	1.42	4-hydroxy-3,5-dimeth oxy-benzaldehyde	81
18	10.862	4.78	[1,1'-Biphenyl]-3-ol **$**	97
19	10.932	0.73	p-Hydroxybiphenyl **$**	97
20	11.107	2.87	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	97
21	11.503	0.36	4-hydroxy-3,5, 6-trimethyl-4-(3-oxo-1-butenyl)- 2-cyclohexenone	80
25	12.541	0.87	n-Hexadecanoic acid	99
27	12.657	1.20	Tetradecanamide	97
28	12.786	0.73	3,5-Dimethoxy-4-hydroxycinnamaldehyde	93
29	12.832	1.89	trans-2H-Pyran-2,2-dicarboxylic acid, 3, 6-dihydro-3,6-dimethyl-diethyl ester	80
34	15.327	26.69	(Z)-9-Octadecenamide	95
35	15.514	1.69	(Z)-Octadecenamide	90
37	26.011	2.04	1,19-Eicosadiene	98
38	26.559	2.36	Pyridine-3-carboxamide, oxime	90
39	27.812	4.56	Campesterol	99
41	28.173	6.34	Stigmasterol	98
42	28.838	8.22	β-Sitosterol	96

E.5. Mud from Hot-liming

Pk#	RT	Area%	Library/ID	Qual
1	12.541	1.76	Hexadecanoic acid	98
2	15.479	36.44	Octadecanoic acid, butyl ester	96
3	26.023	22.83	C_{26} policosanol	99
5	26.570	16.91	n-octacosanol	99
6	27.818	4.54	Campestrol	95
8	28.185	3.66	Stigmasterol	95
9	28.849	9.53	β-sitosterol	95

E.6. Mud from FeMCaD Stage 1

Pk#	RT	Area%	Library/ID	Qual
1	12.541	0.56	Hexadecanoic acid	99
2	13.654	0.25	(Z,Z)-9,12-Octadecadienoic acid	98
3	13.677	0.59	9,12-Octadecadienoic acid	97
4	13.975	0.57	Hexadecanoic acid, butyl ester	99
5	15.479	4.63	Octadecanoic acid, butyl ester	96
6	22.467	1.32	(Z)-14-Tricosenyl formate	91
7	24.087	1.68	(trans)-2-nonadecene	91
8	26.040	33.68	C_{26} policosanol	95
10	26.582	23.83	n-octacosanol	94
12	27.824	3.79	Campestrol	97
13	27.969	8.82	hexadecyl oxirane	90
14	28.185	4.56	Stigmasterol	95
15	28.628	2.71	1-Eicosanol	89
16	28.855	7.07	β-sitosterol	99

E.7. Mud from FeMCaD Stage 2

Pk#	RT	Area%	Library/ID	Qual
1	3.716	0.05	2,5-Cyclohexadiene-1,4-dione (p-Benzoquinone)	76
2	4.200	0.07	Hexanoic acid	80
4	4.387	0.09	(E)-3-Hexenoic acid	97
5	5.564	0.08	Nonanal	91
6	5.704	0.32	Phenethyl alcohol	95
7	6.106	0.12	Benzoic acid	91
8	6.567	0.11	Decanal	91
9	6.666	0.64	4-vinylphenol	91
10	6.957	0.66	2-methoxy-[1,4]-benzoquinone	93
11	7.639	0.82	2-Methoxy-4-vinylphenol	96
13	7.995	1.28	4-hydroxy-benzaldehyde	94
15	8.344	0.37	2-methoxy-1,4-Benzenediol	97
16	8.420	0.44	4-hydroxy-3-methoxy-benzaldehyde (vanillin)	96
17	8.583	0.86	3,4-dimethoxy-phenol	95
20	9.166	0.10	1-(3-hydroxy-4-methoxyphenyl)-ethanone	93
21	9.370	0.19	BHT	97
22	9.469	0.19	3,5-Dimethoxybenzyl alcohol	95
24	9.650	0.14	n-Dodecanoic acid	91
25	9.714	0.40	3-Hydroxy-4-methoxybenzoic acid	93
26	9.802	3.52	2,6-Dimethoxybenzoquinone	86
29	10.064	0.83	3,4,5-trimethoxy- Phenol	95
30	10.192	0.31	3-Hydroxy-.beta.-damascone	78
34	10.530	1.40	4-hydroxy-3,5-dimethoxy-benzaldehyde	93
38	10.868	4.47	m-hydroxybiphenyl	96
39	10.932	0.78	p-Hydroxybiphenyl	97
40	11.043	0.18	β-4-Hydroxy-3-methoxyphenyl)-propionic acid	91
41	11.107	2.57	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	97

42	11.160	0.38	Tetradecanoic acid	95
43	11.288	0.27	1H-Indole-3-ethanol	90
46	11.603	0.21	4-hydroxy-3,5-dimethoxy-benzoic acid	94
50	11.859	0.14	Pentadecanoic acid	93
51	11.970	0.03	3-(4-hydroxy-3-methoxyphenyl)- 2-Propenoic acid	90
53	12.547	1.71	Hexadecanoic acid	99
55	12.786	0.82	3-(3',5'-dimethoxy-4'-hydroxyphenyl)-E-2-propenal	93
59	13.648	0.25	(Z,Z)-9,12-Octadecadienoic acid	99
60	13.672	0.55	9,12-Octadecadienoic acid	94
62	13.957	1.72	Hexadecanamide	96
65	15.327	16.20	(Z)-9-Octadecenamide	99
66	15.508	1.15	Octadecanamide	89
73	22.286	1.81	2,6,10,14,18,22-Tetracosahexaene	99
80	26.011	2.79	1,19-Eicosadiene	99
84	26.559	2.29	Cyclooctacosane	97
91	27.806	4.25	Campestrol	99
93	28.168	5.08	Stigmasterol	99
95	28.832	6.69	β-sitosterol	99

Of interest:

45	11.509	0.35	4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)- 2-Cyclohexen-1-one	87

VITA

Lee R. Madsen, II, was born in New Orleans, Louisiana, in March, 1975. He was awarded his High School Diploma from The St. Paul's School, Covington, Louisiana, in 1992. He began his undergraduate career at Lousiana State University in the fall later that year. In 1995 while attending LSU, he began his career working full-time for Analytical and Environmental Testing, Inc. As his career moved forward, he worked for LSU Agricultural Chemistry performing analytical method development until he was awarded, in 2001, a Bachelor of Science degree in chemistry with a second dicipline in nuclear science. He was hired as a temporary field scientist by the Audubon Sugar Institute (ASI, LSU AgCenter) Sept. 10, 2001. He remains there and is currently the supervisor of the Analytical Department.