COLOR FORMATION IN WHEAT STARCH BASED GLUCOSE SYRUPS AND USE OF ACTIVATED CARBONS FOR SUGAR DECOLORIZATION

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Approval of the Graduate School of Natural and Applied Sciences

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ABSTRACT

COLOR FORMATION IN WHEAT STARCH BASED GLUCOSE SYRUPS AND USE OF ACTIVATED CARBONS FOR SUGAR DECOLORIZATION

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Glucose syrups were produced from wheat starch at 45-90 min liquefaction times followed by 18 h saccharification to study the effect of liquefaction time on color formation and the use of several amounts (0.25%-1%) and types (NORIT, commercial; and hazelnut husk, apricot stone, hazelnut shell based; prepared in Chemical Engineering Department) of activated carbons for color removal. The fractional conversion values and color levels of glucose syrups increased with increasing liquefaction time. However, to reduce the color level to 100 ICUMSA units, the smallest amount of all types of activated carbons were required for, the glucose syrups with highest level of original color,

which were produced at 90 min liquefaction time. Comparison of the performances of the activated carbons showed that hazelnut husk based one was as good as NORIT, while apricot stone based and hazelnut shell based activated carbons showed similar performances, which were somewhat poorer than that of NORIT and hazelnut shell based activated carbon.

Key Words: Glucose syrups, activated carbon, decolorization, adsorption

ÖZ

BUĞDAY NİŞASTASI BAZLI GLİKOZ ŞURUPLARININ ÜRETİMİNDE RENK OLUŞUMU VE RENGİN GİDERİMİ İÇİN AKTİF KARBON KULLANIMI

Çelebi, İpek
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Buğday nişastası bazlı glikoz şurupları, sıvılaştırma işleminin renk oluşumu üzerindeki etkisini incelemek ve farklı miktarda (0.25-1%) ve türdeki (ticari, NORIT; ve Kimya Mühendisliği Bölümü'nde üretilmiş olan; fındık çotanağı, kayısı çekirdeği, fındık kabuğu bazlı) aktif karbon kullanımıyla renk giderimi sağlanmak amacıyla, 45-90 dk sıvılaştırma süresini takiben 18 saat boyunca sakkaritleri parçalama işlemi ile üretilmiştir. Glikoz şuruplarının % dönüşüm değerinin ve renk düzeyinin artan sıvılaştırma zamanı ile arttığı gözlenmiştir. Ancak renk düzeyini 100 ICUMSA değerine düşürmek için, hangi türde olursa olsun, en az miktarda aktif karbona en fazla renk düzeyine sahip olan, 90 dk'lık

sıvılaştırma ile üretilen glikoz şuruplarında gereksinim duyulmuştur. Aktif karbon performansları karşılaştırıldığında, fındık çotanağı bazlı olanın NORIT kadar iyi olduğu, buna karşılık fındık kabuğu ve kayısı çekirdeği bazlı aktif karbonlar birbirine yakın, ancak NORIT ve fındık çotanağı bazlı aktif karbondan biraz daha düşük bir performans sergiledikleri görülmüştür..

Anahtar kelimeler: Glikoz şurupları, aktif karbon, renk giderme, adsorpsiyon

To My Mummy, & Müşkom

&

Loving Memory of My Dearest Grandfather Saim Erkolçak

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NOMENCLATURE

AC : Activated Carbon

AS : Apricot Stone Based Activated Carbon

C final : Color Level of Glucose Syrups after Decolorization

Co : Color Level of Glucose Syrups before Decolorization

C removed : Removed Color level of Glucose Syrups

DP : Degree of Polymerisation

FWS : Freshly Prepared Wheat Starch

HS : Hazelnut Shell Based Activated Carbon

HH : Hazelnut Husk Based Activated Carbon

ICUMSA : International Commission for Uniform Methods of Sugar Analysis

LIQ. : Liquefaction

M : Amount of Activated Carbon, g/L

PIY : Commercial Wheat Starch, PİYALE

CHAPTER 1

INTRODUCTION

Starch syrups are sweet edible products that are widely used in confectionery and other food products. Non food uses represent only a minor proportion of the total market for syrups, but there is considerable diversity in their applications. In United States these syrups are known as ‘corn syrups’ as they are produced by acidic or enzymatic hydrolysis of corn starch. However, in Europe, by using different starches as starting material, perhaps more accurate description ‘glucose syrups’ is preferred although they do of course contain glucose polymers in addition to glucose. The enzymatic hydrolysis is mostly carried out in two consecutive steps as liquefaction and saccharification.

The glucose syrups produced industrially from starch, contain colored materials which form during processing. Since these materials have negative effect on syrup quality, the color removal by several techniques of which activated carbon is the most preferable one should be applied. As well as the commercial ones, agricultural by-product based activated carbons which might have the advantage of offering an effective, lower-cost processing are also used for decolorization of glucose syrups.

Since wheat production is widespread in Turkey, and it is produced as a by-product of gluten manufacture, production of glucose syrup from

wheat starch is worth studying. The aim of the present study was to produce wheat starch based glucose syrups using different liquefaction times and the use of several types and amounts of activated carbons for color removal.

1.1 Starch

For periods of dormancy, germination, growth and propagation, and for survival under adverse conditions, both animals and plants lay down energy reserves. The most common energy reserve in plants is starch (Kearsly and Dziedzic, 1995). Starch is a polysaccharide in which D-glucopyranose, cyclized form of glucose, units are bonded with α linkages and is one of the principal carbohydrates that occur as a product of photosynthesis which is the most important chemical event on the face of our planet since tonnages of materials are reacted and produced (Storz and Steffens, 2004).

$$6n\ CO_2 + 12n\ H_2O \longrightarrow (C_6H_{10}O_5)_n + 6n\ O_2 + 7n\ H_2O \quad \text{.....(1)}$$

Commercial starches are obtained from seeds; particularly corn, waxy corn, high amylose corn, wheat and various rices; and from tubers and roots; particularly potato, sweet potato, cassava and arrowroot (Knight, 1969).

The property which makes starch unique is occuring naturally as discrete particules called granules. Starch granules are relatively dense, insoluble and hydrate only slightly in room temperature water. The starch granule is a small individual entity and it has a hilum; a nucleation point where

the development occured. The size and shape of starch granules are changible with respect to botanical origin of starch. Wheat starch granules have a large range of granular sizes. The smallest have 2 μm diameter while largest have about 55 μm. The larger granules are not spherical but rather lenticular, only smaller ones tend to sphericity (Storz and Steffens, 2004).

Starch granules are composed of a mixture of two polymers called as amylose and amylopectin. Amylose (Fig. 1.1) is a straight chain glucose polymer with a conformational helical structure. Although it is known that it is composed of α(1→4) linked α-D glucopyranosyl units; a very small amount of branching may be present (Knight, 1969).

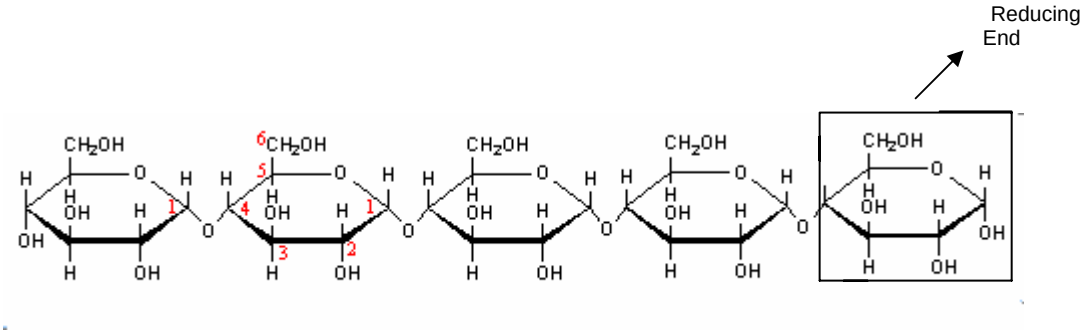

Fig. 1.1 Structure of Amylose

Amylopectin (Fig. 1.2) is a very large molecule which has glucopyronasyl units joined by α(1→4) also α(1→6) linkages, which makes it branched (Knight, 1969), that occur for every 10-12 glucose units (Kearsly and Dziedzic, 1995). Most starches have 18-30% amylose (Godon, 1994) but amylose/amylopectin ratio differs with respect to botanic origin of starch (Knight, 1969). Wheat starch has 25% amylose and 75% amylopectin.

Starch granules consist of both crystalline and amorphous regions which link amylose and amylopectin (Godon, 1994).

Amylopectin occurs in both crystalline and amorphous regions, but it is considered to be solely responsible for crystallinity of starch while amylose is associated with the amorphous regions of granule (Kearsly and Dziedzic, 1995).

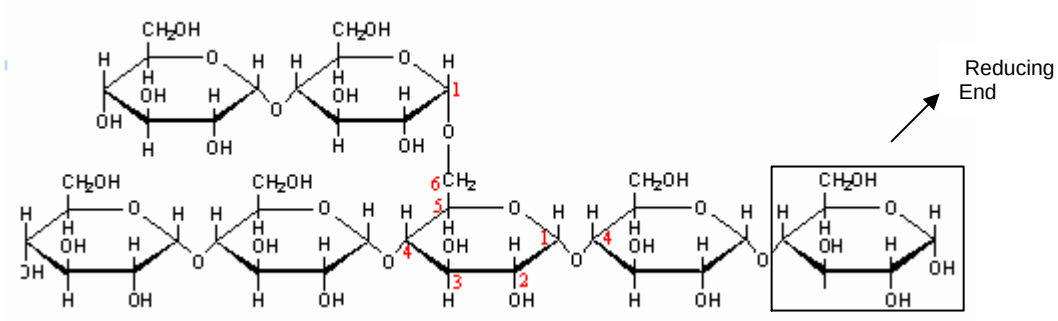

Fig. 1.2 Structure of Amylopectin

There is one reducing end at the end of each chain in starch, which means one end of each is formed by a glucose residue with e free reducing group, while the other end is glucose residue with a non reducing end (Fig. 1.1, Fig. 1.2) (Godon, 1994). Reducing end means, the oxygen atom of hydroxyl group on anomeric carbon is free and can donate its electrons to reduce copper and iron ions in an alkaline media. Starch's effect is too small to produce a positive test, so starch should be hydrolyzed to glucose or maltose to give more reducing ends.

Starch is an important raw material in the pharmaceutical and food industry (Storz and Steffens, 2004). It and its derivatives are already widely employed in the manufacture of paper, textiles and adhesives, and they are increasingly being considered as an environmentally-friendly alternative to the use of synthetic additives in many other products, including plastics, detergents, pharmaceutical tablets, pesticides, cosmetics and even oil-drilling fluids since they are biodegradable and renewable in nature.

1.2 Glucose Syrups

While starch was used to be primarily looked upon as a major source of energy in the human diet, its significance as a polysaccharide has only been discovered in the last three to four decades. In the wake of this development, a significant market has evolved. Thus starch has become an important raw material for the sugar industry which, for centuries, relied exclusively on sugar beet and sugar cane for the production of natural sweeteners.

Glucose syrups, obtained by controlled partial hydrolysis of starch, are purified aqueous solutions of nutritive saccharides (D-glucose, maltose & other polymers of D-glucose) (Kearsly and Dziedzic, 1995).

They consist of glucose, its oligomers (short polymers) and the components of the syrups range in molecular weight from 180 to about 3000 (Kearsly and Birch, 1985). Each syrup contains varying proportions of each component, having different properties, results in glucose syrups having different properties depending on their

composition. They are characterized mainly by dextrose equivalent; DE; which is a measure of total reducing sugars present in a syrup compared to dextrose and calculated on a dry weight basis (Kearsly and Birch, 1985; Storz and Steffens, 2004), and also by their carbohydrate composition. Glucose syrups having same DE but produced by different ways can have different hydrolysate profile and thus having different properties. So, DE gives only a rough idea about the yield; not the product profile (Knight, 1969).

DE shows the extent of hydrolysis since pure starch has a DE value of '0', while glucose has a DE value of '100'. Glucose syrups have a DE value between 20 and 97. The products are called as maltodextrins which have a DE value of 20 and below and hydrols is the name given products having a DE value of 97 and higher (Godon, 1994). Traditional products from 3-45 DE are generally avaliable as spray dried powders and from 30-97 DE as syrups (Kearsly and Dziedzic, 1995).

1.2.1 Production of Glucose Syrups

Glucose syrups are produced by three ways as;

1. Acidic hydrolysis (regular syrups)
2. Acidic hydrolysis followed by enzymic hydrolysis (dual conversion syrups)
3. Enzymic hydrolysis alone

1.2.2 Acidic Hydrolysis

Firstly, the hydrolysis of starch was achieved by boiling raw starch in H_2SO_4 to give sweet syrup by Kirschoff (Paolucci-Jeanjean et al, 1999). Hydrolysis of starch has commonly been carried out using hydrochloric acid at temperatures of 130-170°C with subsequent partial neutralization. Large acid-resistant converters in batchwise are still used for hydrolysis of starch, but there have been a strong tendency over the last two decades to the using of continuous converters. In this process, starch slurry is acidified with hydrocholoric acid and pumped through a series of steam-heated pipes where the conversion of starch into sugars occurs. Temperature, acidity, and retention time are the major factors that govern the extent of the hydrolysis (Whistler et al, 1984). Acidic hydrolysis has some disadvantages as inducing formation of coloring and flavoring substances as well as other contaminants such as furfural, levulinic acid and formic acid (which of all give in high refining cost), being lack of process control, being an unsafety process and also giving low yields (Paolucci-Jeanjean et al, 1999).

1.2.3 Acidic Hydrolysis Followed by Enzymic Hydrolysis

Dual conversion syrups are manufactured by hydrolysis of the starch to a specific DE by acid and completing the hydrolysis by the use of one or more enzymes (Whistler et al, 1984). For example when maltodextrins are obtained by acid catalysis, they tend to retrogradate forming a haze. In a dilute solution, starch molecules precipitated, and it is difficult to redissolve this insoluble material by heating and this return to an insoluble state is called as retrogradation. So acid-catalyzed hydrolysis to

DE 5-10 is followed by enzyme-catalyzed hydrolysis to prevent haze and to produce maltodextrins of low hygroscopicity and high water solubility (Cornell and Hoeving, 1998).

1.2.4 Enzymic Hydrolysis

Since enzymes have efficiency, specific action, ability to work under mild conditions, increasing reaction rate, operation without contamination by microorganisms and having high purification and standardization, they are ideal catalysts for the food industry (Selmi et al, 2000). Enzyme reactions, requiring simple equipment, are easily controlled and can be easily stopped when the desired degree of conversion is reached (Kearsly and Dziedzic, 1995). The preparation of glucose syrups depend on depolymerization of starch and the glycosidic bonds in starch are specifically degraded by the amylolytic enzymes which are widely distributed throughout the microbial, plant, and animal kingdoms (Godon, 1994).

1.2.4.1 Amylolytic Enzymes

These enzymes are separeted into three groups due to their specifity to $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ linkages (Godon, 1994).

1.2.4.2 Enzymes Specific for $\alpha(1\rightarrow4)$ Linkage

According to action pattern; these enzymes; also called as amylases, are subdivided as endoenzymes and exoenzymes.

Endoenzymes

The α-amylase (E.C 3.2.1.1) is an extra cellular enzyme which is widespread among higher plants, animals and microorganisms. It has an ability to hydrolyze α(1→4) linkages in starch components and related carbohydrates. Thermostable α-amylases which are very important for industrial uses are bacterial in origin and are derived from *Bacillus* species as *B. amyloliquefaciens, B. licheniformis, B. streothermophilus* and *B. subtilis* (Demirkan et al, 2005; Ramesh and Lonsane, 1989). Calcium is often added to enhance enzyme stability (Marchal et al, 1999c). Action pattern on amylose (Fig. 1.3): α(1→4) linkages along the same chain are hydrolyzed randomly releasing glucose and oligosaccharides of two to seven glucosyl units with an anomeric reducing end.

The hydrolysis products are essentially maltose and maltotriose of which hydrolysis into maltose and glucose takes place later, since this oligosaccharide is more resistant to hydrolysis. Two main mechanisms are effective for hydrolysis of amylose as multiple attack and multichain attack. In multiple attack mechanism, all bonds have equal chance to be hydrolyzed. After hydrolysis, only one molecule is released while the retained part is ready for latter hydrolysis. Finally chain is released by several repetitions of this process. In the multichain attack mechanism, all bonds are not equally susceptible to enzymatic hydrolysis due to the particularly resistant ends of the chains.

When α-amylase acts on amylopectin (Fig. 1.3) products are glucose, maltose, maltotriose and branched α-limit dextrins having α(1→6) and adjacent α(1→4) linkages with a resistance to enzymatic hydrolysis since these enzymes do not have a capability to hydrolyze that α(1→6) linkages. The α-limit dextrins may be composed of three, four or five glucosyl units (Godon, 1994).

The susceptibility of starch granules to degradation by α-amylase, so the product spectra, depends on sources of starch and of α-amylase (Colonna et al, 1988; Moore et al, 2005) also operating conditions. Colonna et al, (1988) has reported that when wheat starch granules were subjected to α-amylase derived by *B. substilis*, enzymatic degradation occured granule by granule and crystalline fractions were completely degraded by α-amylase.

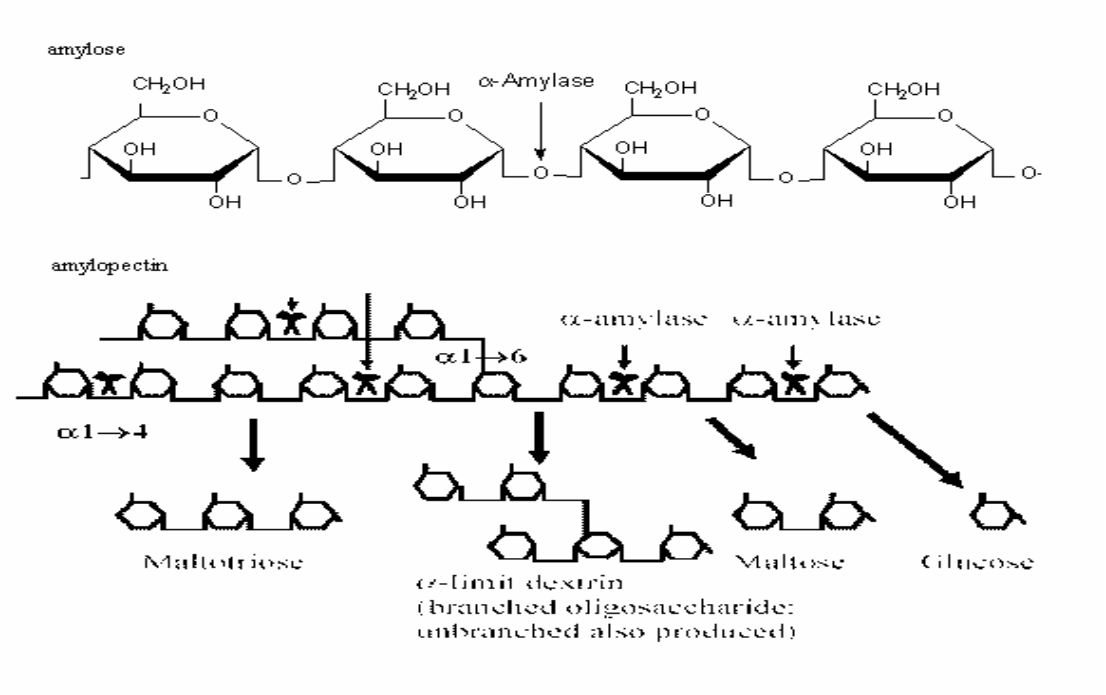

Fig. 1.3 Attack of α-amylase on Starch; Amylose and Amylopectin

It has been also reported by Franco et al, (1988) that enzymatic degradation pathway of α-amylase was strongly affected by botanic origin of starch, enzymatic corrosion mainly occured at surface for cassava granules while radial corrosion was also observed near surface corrosion for corn starch granules.

Exoamylases

The exoenzymes are mainly represented by β-amylase group (E.C. 3.2.1.2) although new microbial exoenzymes that produce one specific type of oligosaccharide has been recently discovered. These enzymes act on starch starting from non reducing end removing maltose units (Godon, 1994).

1.2.4.3 Enzymes Specific for α(1→6) Linkage

These enzymes are present essentially in higher plants and microorganisms which are specific for hydrolysis of α(1→6) linkages and called as *pullulanases* (E.C 3.2.1.41) and *isoamylases* (E.C 3.2.1.68) (Godon, 1994).

1.2.4.4 Enzymes Specific for α(1→4) and α(1→6) Linkages

The amyloglucosidases (E.C 3.2.1.3) are exoenzymes that have ability to hydrolyze both types of linkages in starch removing glucose units from non reducing ends and the name given to them can be one of them; glucoamylase, glucamylase, amyloglucosidase or γ-amylase. They are

mainly derived from microorganisms, especially by molds such as *Aspergillus, Penicilium, and Rhizopus*.

Table 1.1 represents the usage enzymes used in starch industry. When these enzymes are used in combined form, efficient results are obtained. For example; the role of α-amylase is to supply new substrate molecules for glucoamylase by endowise random splitting of large molecules and also not only they are used followingly as in the production of glucose syrups, but also it has been reported that, when the mixture of them is used, the reducing end formation was more than twice as much as the sum of sugar formation by each corresponding enzyme due to synergism which is peeling the molecule from surface and revealing new glucoside bonds on the next layer of the granule by glucoamylase which is reacted by α-amylase (Franco et al, 1987; Fujii et al, 1988; Ayernor et al, 2002).

Table 1.1 Usage of Enzymes in Sugar Industry

Enzyme	**EC number**	**Source**	**Action**
α-amylase	3.2.1.1	*Bacillus amyloliquefaciens*	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins and predominantly maltose (G2), G3, G6 and G7 oligosaccharides
		B. licheni formis	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins and predominantly maltose, G3, G4 and G5 oligosaccharides
		Aspergillus oryzae, *A. niger*	Only α-1,4 oligosaccharide links are cleaved to give α-dextrins and predominantly maltose and G3 oligosaccharides
Saccharifying α-amylase	3.2.1.1	*B.subtilis*	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins with maltose, G3, G4 and up to 50% (w/w) glucose
β-amylase	3.2.1.2	*Malted barley*	Only α-1,4-links are cleaved, from non-reducing ends, to give α-dextrins and maltose
Glucoamylase	3.2.1.3	*A. niger*	α-1,4 and α-1,6-links are cleaved, from the non-reducing ends, to give glucose

1.2.5 Factors That Affect Enzymic Hydrolysis

The enzymatic hydrolysis of starch is mainly affected by botanic sources of starch (Colonna et al, 1988; Franco et al, 1988; Gorinstein, 1993; Sarikaya et al, 2000; Yook and Robyt, 2002) including amylose/amylopectin ratio (Cone and Walters, 1990; Lauro et al, 1993a), crystallinity (Colonna et al, 1988; Franco et al, 1988) and size of granules (Atkins and Kennedy, 1985a; Franco et al, 1988; Manelius et al, 1997; Bertoft et al, 2000). Not only botanic source has an importance on the hydrolysis but also operating conditions as starch concentration (Arasaratnam et al, 1998; Doyle et al, 1999; Marchal et al, 1999b), temperature (Ramesh et al, 1989; Marchal et al, 1999a), pH (Ramesh et al, 1989), enzyme type (Moore et al, 2005; Atkins and Kennedy, 1985a, Atkins and Kennedy, 1985b; Fujii et al, 1988; Holm and Björck, 1988; Lauro et al, 1993a; Gaouar et al, 1997; Doyle et al, 1999; Sarikaya et al, 2000; Brandam et al, 2003; Demirkan et al, 2005), enzyme concentration (Gorinstein, 1993; Slominska et al, 1998; Marchal et al, 1999 c; Paolucci-Jeanjean et al, 2000a; Ayernor et al, 2002) are important.

The effect of time on hydrolysis and enzyme stability was reported by Apar and Özbek, (2005). It was found that; when rice starch was hydrolyzed by α-amylase derived from *B.subtilis* with processing time (from 0 to 90 min), a decrease in the activity of α-amylase was observed with the time of exposure. The degree of hydrolysis reached a value of 84.51% and 81.82% the efficiency of α-amylase was lost after 90 min.

Not only sole effect of these parameters but also interactions between them should also be taken into account (Marchal et al, 1999c) on hydrolysis of starch.

1.2.6 Industrial Starch Hydrolysis

Conversion of starch into sugar syrups and dextrins forms the major part of the starch processing industry. Sugar syrups obtained by starch are sweet edible products that are widely used in confectionery and other food products, also solid glucose can be prepared by crystallization from completely hydrolyzed liquors. In United States these syrups are known as corn syrups since they are produced by acidic/enzymatic hydrolysis of corn starch. Other starches, such as those from wheat, potato and rice can, of course, be used to manufacture such products. (Whistler et al, 1984). In our country since wheat starch production is widespread, and it is produced as a by-product of gluten manufacture, production of them from wheat starch gains importance.

The industrial hydrolysis of starch into glucose syrups is generally performed in two following steps as liquefaction and saccharification (Paolucci-Jeanjean et al, 1999; Storz and Steffens 2004; Ayernor et al, 2002; Akberg et al, 2000). After saccharification, fructose syrups are obtained from glucose syrups by isomerization if desired (Godon, 1994; Kearsly and Dziedzic, 1995).

Liquefaction is a process of dispersion of insoluble starch granules in an aqueous solution followed by partial hydrolysis using thermostable amylases. α-amylase behaves as a thinning agent which results in

reduction in viscosity and partial hydrolysis of starch. The traditional thinning agent used in starch technology was acid; hydrochloric or oxalic acids; the introduction of stable α-amylase gave in milder operation conditions, reduction in refining and recovery costs. Two distinct types of thermostable α- amylase are extensively used in starch hydrolysis; firstly α-amylase derived from *Bacillus amyloliquefaciens* was used on large scale and followed by the usage of a more temperature stable one *Bacillus licheniformis.* Liquefaction can be done by two ways as acid-enzyme liquefaction and single stage enzyme liquefaction (Prasanna, 2005).

Acid-enzyme liquefaction: Starch slurry containing 30-40% dry solids is cooked at a high temperature for about 5 min. A jet cooker is used is used for mechanical thinning due to shearing. The pH may be in the range 2-5, but if it is too low, by-product formation will be significant however too high pH values result in increased color formation. After cooking, the slurry is flash cooled to about 100°C and before the addition of enzyme, pH is set to 6 to 6.5. Enzyme consumption is slightly reduced and since better fat/protein separation is achieved, fitration properties are also improved. On the other hand, due to high temperature cooking; there is an increase in steam consumption, so increase in fuel costs (Prasanna, 2005).

Single stage enzyme liqueafaction: is performed by use of stirred tank reactors, continuous stirred reactors (CSTR) or a jet cooker (Kearsly and Dziedzic, 1995).

Single dose jet cooking process is the one that is mostly used in many plants. In this process, α-amylase is metered into starch slurry in feed tank after pH adjustment, and the slurry is pumped through a jet cooker of which temperature is raised to 105°C by steam injection, and a subsequent passage through a series of holding tubes provides a 5 min residence time (Kearsly and Dziedzic, 1995) for *gelatinization* which is essential to improve the limited effect of α-amylases on native starch granules (Lauro et al, 1993b; Göksundur and Güvenç, 1994).

When starch granules are heated in presence of water, *gelatinization* takes place. Starch gelatinization corresponds to a combined mechanism of semicrystalline polymer melting and hydration (Selmi et al, 2000). It is the disruption of molecular order within the starch granules which include irreversible granule swelling, loss of birefringence and loss of crystallinity (Whistler and Bemiller, 1999) occuring over a temperature range. Larger granules gelatinize earlier than smaller ones. The apparent temperature of initial depends on the starch-water ratio, granule type, and heterogeneities within the granule population (Franco et al, 1988, Whistler and Bemiller, 1999). If starch granules are continued to heat in excess water, further granule swelling and additional leaching of soluble components (primarily amylose) takes place. The reason why gelatinization occurs is when the temperature of starch-water suspension increases, molecules within granules vibrate and twist so violently that intermolecular hydrogen bonds break and are replaced with hydrogen bonds of water molecules and this produce more extensive hydration. Thus starch polysaccharides become sheeted in the layers of molecules that plasticize them and allow them to move more freely. Eventually, molecular segments move distances and positions that make it

impossible for them to return to their original positions upon dehydration (Whistler and Bemiller, 1999).

Gelatinization is applied not only prior to liquefaction but also along with it. It has been reported that gelatinization and liquefaction was performed in a single step process involving agitation, 20% starch slurry with 1% α-amylase solution was heated at 95°C and pH 6.5 (Lee and Kim, 1990).

In jet cooking process, by flashing the partially liquefied starch, temperature is reduced to 90-100°C and for 1-2 h, until desired DE is obtained; the enzyme is allowed to react further. The operating conditions for that process are dry substance 30-35% (w/w), pH 6.0-6.5 and Ca^{+2} content of 20-80 ppm (w/v). The enzyme dose is 0.5-0.6 kg per ton of starch.

Using batch reactors is the classical way for enzymatic hydrolysis of starch (Paolucci-JeanJean et al, 1999; and Paolucci-JeanJean et al, 2000b) although jet cooking process is the most common one to produce glucose syrups. After pH adjustment to 6-7, the starch substrate is mixed with the enzyme at temperatures > 80°C (Godon, 1994). Heating-up of the suspension, 30-35% w/w dry substance concentration (Godon, 1994; Paolucci-JeanJean et al, 1999), combined with enzyme addition initiates the starch hydrolysis process. The product of liquefaction is maltodextrins, having DE value of 15-20 (Kearsly and Dziedzic,1995) sometimes till 25, are obtained as the product of liquefaction and are used as carrier or bulk agents, texture provider, spray-drying aid, fat

replacer, film former, freeze control agent, for preventing sugar crystallization, or supplying nutrional value (Marchal et al, 1999 b). Maltodextrins are hydrolyzed into low molecular sugars such as glucose, maltose, maltotriose by the process, saccharification, which is catalyzed by one or several types of enzymes (glucoamylase, pullulanase, or β-amylase) depending on the type of product desired (Godon, 1994). The glucose syrup production is generally achieved by glucoamylase (Paolucci-JeanJean et al, 1999) at about 50-60°C and pH values 4.5-5, due to optimum contions of the glucoamylase used, in a batch reactor (Kearsly and Dziedzic, 1984) for 24-96 h. It is also possible to perform that process in continuous way as using a plug flow (tube) reactor; series of, including minumum eight tanks, small continuous flow stirred tanks reactors (CSTR); and a membrane reactor which is modeled as a simple CSTR requiring higher enzyme dosages compared to the batch reactor (Kearsly and Dziedzic, 1995).

Also it has been reported that conversion of starch is achieved by one-step process either using saccharifying enzymes able to attack native starch, derived from *Rhizopus niveus* and from barley may, or strong liquefying enzymes such as Termamyl in a in a batch reactor (Paolucci-JeanJean et al, 2000b) or in a continuous recyled membrane reactor (Paolucci-JeanJean et al, 1999) since the batch reactor has some disadvantages such as having long retention times, large volumes, poor enzyme utilization, batch to batch variations and labor costs (Gaouar et al, 1997).

At the end of saccharification although there are many variations possible due to used type and concentration of enzyme and processing

time, three main types of syrups are interest commercially which are produced using different enzyme combinations (Table 1.2) (Kearsly and Dziedzic, 1984). The obtained syrup is filtered then to remove protein, purified with activated carbon to remove color and solubilised protein, treated with ion exchange to remove ash, and concentrated with evaporation (Kearsly and Dziedzic, 1984; Bowler et al, 1985). The process outline of glucose syrup production is given in Figure 1.4.

Recently; Sakintuna, (2001) studied the hydroysis of commercial, PIY, and freshly prepared, FWS wheat starch for the production of glucose syrups. Extent of hydrolysis was determined both by Nelson Somogyi method, and HPLC. Liquefaction was carried out using three different α-amylases; Orbamil-T *(Bacillus licheniformis)*, Orbamil-BHT (*Bacillus stearothermophilus)* and Sigma amylase *(Bacillus licheniformis).* In order to observe effect of starch concentration on the hydrolysis behaviour, different concentrations of FWS, 20-34% (w/w), were studied for 15-120 minutes at 97 ^{o}C with Orbamil-T and Orbamil-BHT (0.1% of dry matter) in batch system. Highest conversion was obtained with 27% starch concentration. When hydrolysis of FWS (27% (w/w)) was carried out with Orbamil-T, Orbamil-BHT, and Sigma amylase at 97 ^{o}C for 60 min and products were analyzed by HPLC, it was seen that, Orbamil-T and Sigma amylase gave quite similar extent of hydrolysis and product profiles. Also PIY (27% (w/w)) was hydrolysed under the action of Orbamil-T for 60 min at 97 ^{o}C and it was seen that, the extent of hydrolysis was significantly lower than FWS wheat starch. These results showed the effect of starch and enzyme type on the hydrolysis. To understand the effect of temperature on the hydrolysis of FWS, experiments were conducted with Orbamil-T and Orbamil-BHT at four

different temperatures as 40, 60, 80 and 97 ºC for 15-120 minutes. Although it was seen that the highest conversion, analyzed by Nelson Somogyi method, was obtained at 60 ºC for both enzymes, which might be due to non-purity of enzymes, HPLC results showed that, highest glucose unit conversion was observed at 97 ºC. In order to observe the influence of enzyme concentration on starch hydrolysis, 0.5% /dm enzyme concentration was studied in batch system with Orbamil-T, Orbamil-BHT and Sigma amylase. Although enzyme concentration was increased five times for the hydrolysis of FWS for 0-60 min at the same experimental conditions, the conversion increased 3.5 times, again obtaining no significant difference between Orbamil-T and Sigma amylase. In addition to batch system, hydrolysis behaviour of wheat starch in flow system was also studied at 27% starch concentration at 110 ºC for different retention times, 0.7-6.6 minute, which were obtained by changing the coil lengths (1-6 m) and flow rates (12.5-62.5 ml/min). Reproducibility of flow system was better than batch system and it was seen that the product profiles seem to depend less on the system, than enzyme type. Similar result was obtained by Sakintuna et al, (2003) and quite different hydrolysis behaviour of FWS and PIY starches was also confirmed. Sakintuna, (2001) reported that, following liquefaction, saccharification studies were carried out using two glucoamylases, obtained from ORBA and Sigma in batch system. Liquified starch obtained by flow system using 5 meter coil and 12.5 ml/min flow rate to obtain 5.5 minute retention time by the hydrolysis with Orbamil-T. Then liquefied starch was saccharified by ORBA glucoamylase or Sigma glucoamylase for 0-72 hours in batch system at 60 ºC. Sigma glucoamylase resulted in faster hydrolsis than ORBA glucoamylase and also FWS gave higher conversion results than PIY.

Table 1.2 Saccharification Products (Kearsly and Dziedzic, 1984)

Syrup Type	***DE***	***Glucose %***	***Maltose %***	***Sacch. Enzyme***
High Maltose	40-50	2-7	45-60	A. oryzae
High Conversion	63-67	34-40	33-37	A. oryzae-A. niger
Dextrose	95-97	95-98	1.5-2.0	A. niger

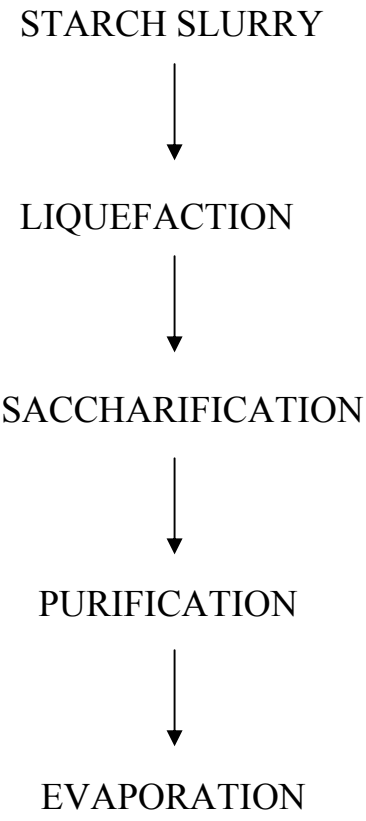

Fig. 1.4 Production of Glucose Syrups

1.2.7 Properties and the Usage of Glucose Syrups

A glucose syrup should have the properties given in Table 1.3 in order to have commercial value according to Turkish Standard TS 10752.

Table 1.3 Properties of Glucose Syrups Required by the Turkish Standard, 10752 (Anon, 1995)

Properties	***Values***
DE (D-glucose on db, min)	20
pH, max	6.5
Sulphate ash, %, max	1
SO_2, mg/kg, max	20
Arsenic, mg/kg, max	1
Copper, mg/kg, max	5
Lead, mg/kg, max	0.5
Mercury, mg/kg, max	0.03
Cadmium	0.15

Table 1.4 shows classification of glucose syrups according to their DE values by the Turkish Standard 2066.

Table 1.4 Classification of Glucose Syrups by the Turkish Standard, 2066 (Anon, 1993)

Degree of Glucose Syrups	***DE***
Low degree glucose syrups	28-37
Normal degree glucose syrups	38-47
Medium degree glucose syrups	48-57
High degree glucose syrups	58-67
Very High degree glucose syrups	min. 68

Glucose syrups effect the characteristics of many food products they added and provide the quality and shelf-life which the manufacturer desire. The physical, chemical and functional properties of them, that are the sum of the characteristics of the each component composing them, determine the effect of glucose syrups on the food industry.

DP, Degree of Polymerization

This term is widely used in the glucose industry to describe the number of glucose units in individual components of glucose syrup. Thus DP_1 = glucose (1 unit), DP_2= maltose (2 units), DP_3= maltotriose (3 units) (Kearsly and Dziedzic, 1984).

Carbohydrate Composition

Carbohydrate composition of a glucose syrup depends conversion process (acid, acid-enzyme, enzyme-enzyme hydrolysis, type of enzyme used) and DE (Godon, 1994). As mentioned before, two glucose syrups, having the same DE, may have highly different composition due to method of manufacturing as seen in Table 1.5 (Godon, 1994; Kearsly and Dziedzic, 1984). So, in order to determine the suitibility of a glucose syrup to the desired aim, not only DE, but also carbohydrate composition should also be taken into account.

The fuctional properties of glucose syrups are sweetness, preventation of crystallization, control of humidity, boiling point elevation and freezing point depression, control of osmotic pressure, viscosifying power, glaze formation, foam development and stabilization, and fermentability. The increase or decrease of these properties due to DE, or molecular weight is seen in Table 1.6.

Table 1.5 Carbohydrate Composition of Glucose Syrups (Kearsly and Dziedzic, 1984)

DE	***Glucose %***	***Maltose %***	***Maltotriose %***	***Higher Saccharides%***	***Method***
21	2	5	6	87	enzyme/enzyme
30	10	9	10	71	acid
42	19	14	12	55	acid
45	25	18	12	45	acid
42	6	45	12	37	acid/ enzyme
42	3	56	16	25	enzyme/enzyme
63	37	32	11	20	acid/ enzyme
65	34	47	3	16	enzyme/enzyme
95	93	2	1	4	enzyme/enzyme

Table 1.6 Functional Properties of Glucose Syrups (Kearsly and Dziedzic, 1995)

Functional Property	***Type of Glucose Syrup***	
	High MW/Low DE	***Low DE/ High MW***
Bodying Agent	←	
Fermentability		→
Foam Stabiliser	←	
Freezing Point Depression		→
Glazing Agent	←	
Humectancy	←	
Hygroscopicity		→
Osmotic Pressure		→
Preventation of crystallization	←	
Sweetness		→
Viscosity	←	

CHAPTER 2

COLOR IN GLUCOSE SYRUPS

The goal of any production process is to produce as large a quantity of product within large quality criteria. One of the most important criteria in glucose syrups is color since it has negative effect not only on sensorial characteristics (flavor, appereance) but also on nutritional value.

The standard color of glucose syrups when offered for sale is usually described as ‘water white’ or colorless (Kearsly and Dziedzic, 1995).

Color formation (browning) in glucose syrups may occur as a result of two types of reaction:

(i) Maillard Reaction
(ii) Caramelisation (Usually ignored)

2.1 Maillard Reaction

Maillard reaction is a complex set of reactions that take place between reducing sugars, having reducing ends, and amino groups of proteins or free aminoacids during thermal processing or storage, usually requiring

addition of heat (Kearsly and Dziedzic, 1995; Carabasa-Gibiret and Ibaraz-Ribas, 2000).

Thus all glucose syrups are potentially reactive as they contain free aldehyde groups, and when they are converted to fructose syrups groups by isomerization, those are also reactive with free ketone groups. Glucose syrups having high DE are more reactive towards proteins/aminoacids (Kearsly and Dziedzic, 1995). During Maillard rection, which cause development of first yellow then brown coloration, a wide range of, almost infinite (Clarke, 1984), reaction products are formed with a significance importance for nutritional value of foods (Bostan and Boyacıoğlu, 1997; Martins et al, 2001).

This reaction was described for the first time by Louis Maillard in 1912 however the first coherent scheme was put forward by Hodge in 1953 that is seen in Fig. 2.1 (Martins et al, 2001).

The chemistry of the Maillard reaction is very complex. However, it is generally divided into three stages (Coca et al, 2004):

First stage:

The Maillard reaction is initiated by a condensation reaction between the carbonyl group of the aldose, such as glucose, and the free amino group of an amino acid to give an N-substituted aldosylamine (Fig. 2.2). This is the result of a nucleophilic attack group by the amino (NH2) group of the amino acid on the electrophilic carbonyl groups of sugar. The

condensation product rapidly loses water and is converted into a Schiff base.

MAILLARD REACTION

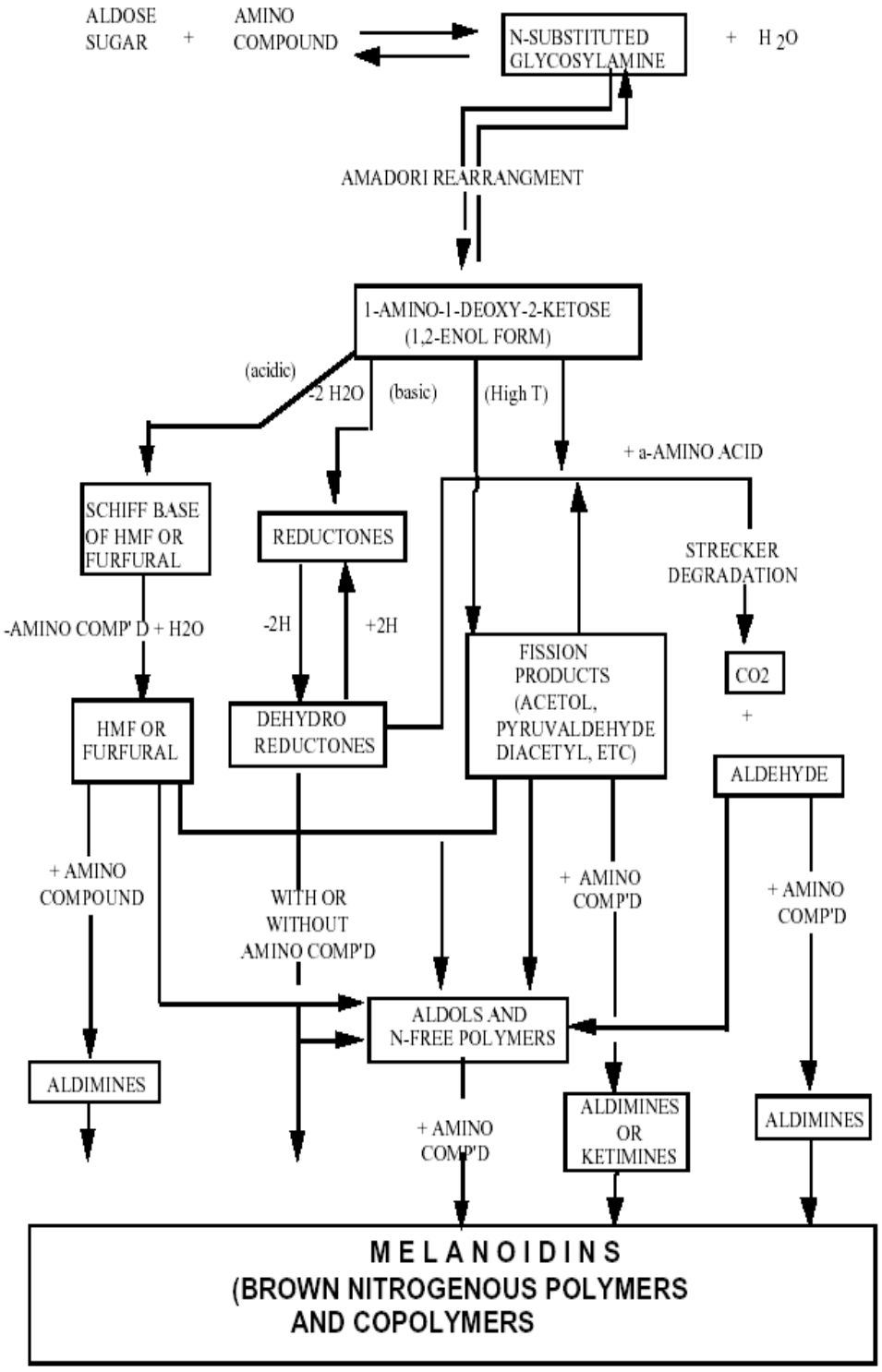

Fig. 2.1 Scheme of Maillard Reaction (Hodge, 1953)

This reaction is reversible and acid-base catalyzed. The Schiff base then cyclizes into the aldosylamine. The *Amadori rearrangement* follows (for aldoses; Heyns rearrangement for ketoses) to form a ketosamine (Fig. 2.3).

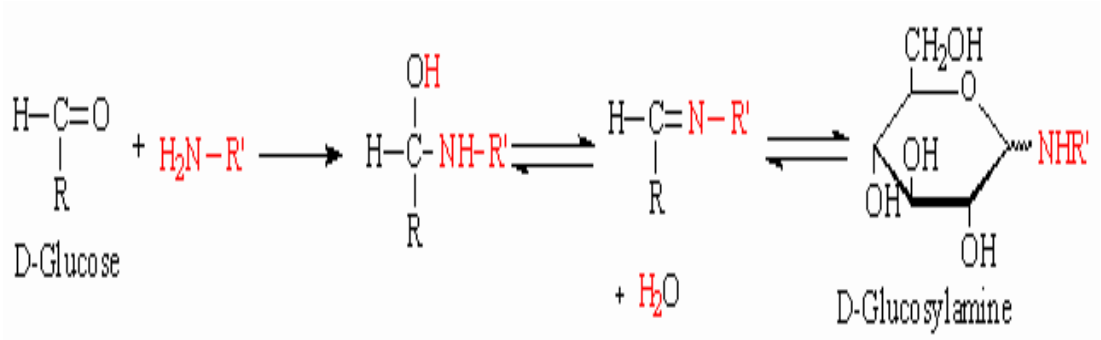

Fig. 2.2 Condensation Reaction between Carbonyl group and Free Amino group

The Amadori rearrangement is considered to be the key step in the formation of major intermediates for the browning reaction.

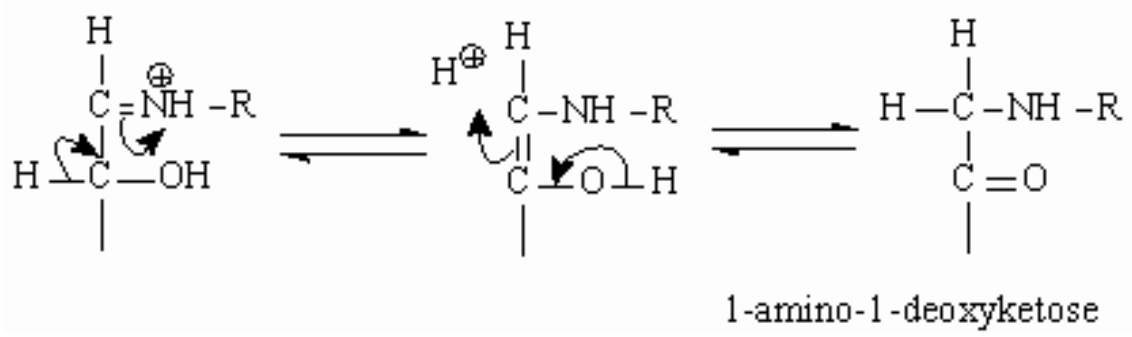

Fig. 2.3 Amadori Rearrangment

Ketoses such as such as fructose, react with amines to form aminoaldoses, this is called the Heyns reaction. Aminoaldoses are not very stable and readily react forming the Amadori compound (Ledl and Schleicher, 1990). No browning occurs at that stage.

Second stage:

The Amadori product degrades by one of three main pathways depending on the conditions.

1) The free hydrogen of the amino group of the ketosamine may react with a second molecule of aldose to form a diketosamine. This compound is less stable than the monoketosamine and decomposes to give a monofructoseamine and nitrogen-freecarbonyl compounds (Anet, 1959), e.g. 3-deoxyosuloses (Anet, 1964) and the cis- and the trans-forms of 3,4-dideoxyosulos-3-ene (Anet, 1962) which are probably the most important intermediates in the Maillard reaction (Wedzicha and McWeeny, 1974). The decomposition of the diketosamine has a maximum rate at pH 5.5 (Anet, 1959).

2) At pH 7 or below, an enolization of the Amadori product results in formation of furfural (when pentosans are involved), or hydroxymethylfurfural (HMF) (when hexoses are involved). At pH above 7, the degration of Amadori product is thought to mainly due to 2,3 enolisation which involves formation of 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (HMF^{one}), and a variety of fission products, including acetol, pyruvaldehde and diacetyl are formed. All these

products are highly reactive and take part in further reactions (Martins et al, 2001).

3) Carbonyl groups can condense with free amino groups, which results in the incorparation of nitrogen into the reaction products. Dicarbonyl groups will react aminoacids with the formation of aldehydes and α-aminogruops. This reaction is called as Strecker degradation (Fig. 2.4) and it is characterized by the production of CO2 (Martins et al, 2001; Coca et al, 2004).

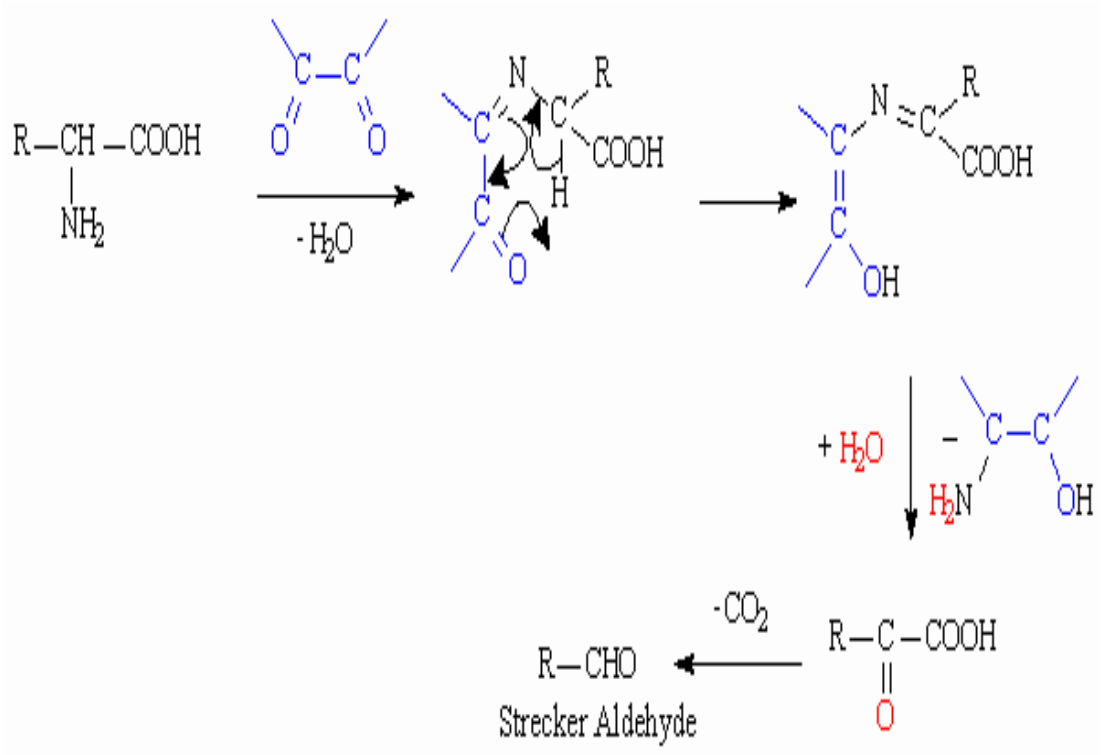

Fig. 2.4 Strecker Degradation

Third Stage

In the final stage, colored intermediates and other reactive precursors (enaminol products, low-molecular weight analogues, unsaturated cabonyl products) condense and polymerse to form brown polymers. A wide range of reactions take place, including cylications, dehydrations, retroaldolisations, rearrangments, isomerizations and further condensations which lead to formation of these brown nitrogenous polymers and copolymers called as *melanoidins* (Mauron, 1981; Martins et al, 2001; Coca et al, 2004) although nitrogen free polymers are also formed from furfurals or dehydroreductones (Morales and Boekel, 1999). Table 2.1 summarizes Maillard reaction.

The products of these various pathways range from colorless to yellow and intensely brown colored depending on the conditions and extent of reaction. They include heterocyclic compounds (mostly pyrazines), nonheterocyclic compounds (such as thiophenes, oxazoles or oxazolines), and pyranones, furanones, and related compounds. The colored compounds are grouped into two groups as low molecular weight compounds, which typically contain two-to-four linked rings having extented double-bond conjugation and *melanoidins* which are brown polymers possesing molecular weight of several thousands daltons and discrete choromophore groups (Morales and Boekel, 1999). These colored compounds are in different molecular weights, structures and properties (Coca et al., 2004). The explanation of chemical structure of melanoidins is diffucult due to the complexity of Maillard reaction (Coca et al., 2004). It is only known that they may contain carbonyl, carboxyl, amine, amide, pyrole, indole, azomethine, ester, anhydride, So

far only partial properties of melanoidins have been elicited.

Table 2.1 Summary of Maillard Reaction

Initial Stage	Reactions: Condensation, enolization, Amadori rearrangement. With proteins, glucose and free amino groups combine in 1:1 ratio	Properties: Reducing power in alkaline solution increases. Storage of colorless 1:1 glucose-protein product produces browning and insolubility
Intermediate Stage	Reactions:1) Formation of diketose amine to give monofructose amine and nitrogen free carbonyl groups 2) At pH below 7, formation of furfural or hyroxymethyl furfural, HMF At pH above 7, formation of 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one, (HMFone) 3) Strecker degradation; carbon dioxide evolves	Properties: Addition of sulfite decolorizes; reducing power in acidic solution develops; pH decreases; sugars disappear faster than amino acids. With proteins, acid hydrolysis fails to regenerate the sugar(D-glucose). Positive Elson-Morgan test for amino sugars (Amadori compounds)
Final Stages	Reactions:A wide range of reactions including, cyclications, dehydrations, retroaldolisations, rearrangments, isomerizations, further condensations and polymerisations which lead to formation of brown nitrogenous polymers and copolymers called as melanoidins	Properties: Acidity; caramel-like and roasted aromas develop; colloidal and insoluble melanoidins form; fluorescence; reductone reducing power in acid solution; addition of sulfite does not decolorize

2.1.1 Factors That Affect Melanoidin Formation

Reaction conditions play an important role in the fundemental structure of melanoidins. The composition of melanoidins depends temperature, heating time, pH, chemical composition of food system and water content during processing or storage of foods (Morales and Boekel, 1999; Carabasa-Gibiret and Ibaraz-Ribas, 2000; Martins et al., 2001; Coca et al., 2004).

Temperature:

The rate of melanoidin formation increases with temperature (Coca et al., 2004). It has been reported that, the effect of temperature on kinetic constants of non-enzymatic browning reaction in apple puree was described by Arrenhenius-type equation and the value of kinetic constant increased with temperature (Carabasa-Gibiret and Ibaraz-Ribas, 2000) and thus increase caused a faster darkening of samples. It has has also been verified by other authors that browning rate followed an Arrenhenius type of dependence upon temperature (Bostan and Boyacıoğlu, 1997; Göğüş et al, 1997; Buedo et al, 2001).

Time:

Several workers have derived theoretical equations to predict the extent of brown pigment as a function time but they can not be solved because of some variables, rate constants of intermediary reactions, are still unknown (Morales and Boekel, 1999) however it is explained by some others that the rate of melanoidin formation increases in proportion to the

square length of heating at a given temperature (Mauron, 1981; Coca et al, 2004).

Color formation in milk resembling model systems (lactose-caseinate or glucose-caseinate solutions) which was heated between 110 and 150 ºC where browning was measured spectrophotometrically (420 nm) by a tristimulus calorimeter. It was seen that the increase in pigments accumulation was due to increase in heating time (Morales and Boekel, 1999).

pH

The pH has a significant effect on the Maillard reaction. In general, the rate and extent of browning increases with increasing pH (Kearsly and Dziedzic, 1984; Bostan and Boyacıoğlu, 1997; Kearsly and Dziedzic, 1995; Martins et al, 2001; Coca et al, 2004). The reaction generally has a minimum is at pH 3 (Lea and Hannan, 1949). At a pH < 3 and > 9, other nonenzymic interactions (i.e. sugar-sugar and protein-protein) compete with the Maillard reaction (Coca et al, 2004). The effect of pH is due to increasing pH increases the reactivity of aminoacids due to acid base equilibrium (Bostan and Boyacıoğlu, 1997).

Chemical Composition of Food System:

The extent of browning seems to vary according to the sugar:amine ratio. At a molar ratio of 1:1, the development of color in a glycine-glucose reaction system was higher than at a ratio of 10 sugar to one amino group (Wolfrom et al, 1974). However, a study by Warmbier et al, (1976) on a glucose-lysine model system showed that the rate of browning increased to a maximum at a ratio of one glucose to three

lysine. The ratio of amino acid to reducing sugar can affect the rate of browning. The increase for both is greater than the relative concentration increase (2 times greater for sugar and 2 to 3 fold times greater for amine) (Baisier and Labuza, 1992).

Type of monomer is also important as glucose and fructose have different chemical reactivities with aminoacids depending on the reaction conditions (Coca et al, 2004).

Göğüş et al, (1997) studied the color formation in boiled grape juice which contains fructose, glucose; aminoacids, glutamine and arginine at 55, 65 and 75 ºC over ten days at pH 3.5. It was concluded that fructose was found to be more active than glucose and with fructose as substrate, glutamine was more reactive than arginine but with glucose, arginine was found to be more reactive than glutamine under the same conditions.

Water Content:

For the first stage of the Maillard reaction to occur, water is essential. Thus, the rate of this and overall Maillard reaction is dependent on the amount of free water available as related to water activity, a_w.

As a consequence of the law of mass action, the reaction occurs less readily in foods with a high a_w value. In addition, at high a_w values the reactants are diluted while, at low a_w, the mobility of reactants is limited, although they present at increased concentration. In practice, the Maillard reaction occurs most rapidly at intermediate a_w values. Maximum browning occurs at 30% moisture (Wolfrom and Rooney, 1953) which corresponds to a water activity of 0.6 - 0.8.

2.2 Caramelisation

Caramelisation of a carbohydrate occurs when it is heated excessively and particularly in the presence of an alkali. The final products are different from the ones produced at Maillard reaction.

Caramelisation has no or negligible effect on glucose syrups since it requires high temperatures > 120°C (Kroh, 1994; Bostan and Boyacıoğlu, 1997), so color in glucose syrups is thought to be due to Maillard reaction.

2.3 Quantification of Color

The color of glucose syrups is is represented by ICUMSA units (Anon, 1994); and a glucose syrup should have a color level below 200 ICUMSA units (Bostan and Boyacıoğlu, 1997; International Starch Institute) defined according to equation (Anon, 1994);

$$ICUMSA = 1000*A/(b*c), \qquad \text{.....(2)}$$

where A = Absorbance at 420 nm of the test sample

b = length (cm) of the adsorbing path

c = concentration (g/ml) of the test sample

In industry saccharification should be followed by purification step which is mentioned in Chapter 3.

CHAPTER 3

DECOLORIZATION OF GLUCOSE SYRUPS

Purity is a subjective concept. Colored materials formed during processing of some foods are desirable in i.e. coffee or bread but it is an impurity in glucose syrups. Purification is carried out by providing effective means of separation which is generally achieved by the ability of fluid molecules to adhere to the surfaces of solids, a phenomenon known as adsorption.

3.1. Adsorption

Adsorption is a process which involves separation of a substance from one phase accompanied by its accumulation or concentration at the surface of another. The adsorbing phase is the adsorbent, and the material concentrated or adsorbed at the surface of that phase is the adsorbate. The adsorption takes place essentially in one of two ways. In the first, the adsorbate is bound to the surface by relatively weak forces that are Van der Waals or hydrogen bonds and the chemical nature of the adsorbed molecules remains unchanged in this adsoption known as physical adsorption. However in the second one called chemical adsorption, electrons are exchanged or shared between the adsorbate and the surface of adsorbent, so that chemical reaction occurs. The bond formed between the adsorbate and adsorbent is essentially a chemical

bond in chemical adsorption and therefore much stronger than that in physical adsorption.

Adsorption is a result of concentration gradient between the solution and that on the surface of the adsorbent together with intra-particle adsorption. The concentration difference between the solution and the adsorbent surface causes the adsorbate to adhere to the surface. Intra-particle diffusion occurs when the adsorbate flows along the pores inside the activated carbon. This is driven by concentration difference. It causes a reduction in concentration of adsorbate on the surface of activated carbon, thus allowing a greater amount of adsorbate to be removed from the solution. The adsoption isotherms are created from the equilibrium concentration of the liquid and the solid and therefore the individual effects of both intra-particle and surface adsorption are included.

Decolorization in glucose syrups is achieved by different systems in sugar refining as bone char; in United States, an import product of which supply can vary according to current political situation; activated carbons, and ion exchange resins which provide good ash and color removal and also can be regenerated chemically, however, the main obstacle for the widespread use ion exchange resins in sugar refineries is their relatively high cost (Ahmedna et al, 2000a). Therefore the usage of activated carbon is the prefable one.

Atiyeh and Duvnjak, (2004) reported that when fructose syrups were decolorized by 33% (w/v) activated carbon, more than 98% of the colorants were removed.

It has been reported by Al-Farsi (2003), that improved syrup quality was obtained with activated carbon which removed the color of date juice to give the lowest amount (60% in powder form and 57% in granular form), as well as reducing total ash.

3.2 Activated Carbon

Commercial activated carbons first appeared in the early part of the twentieth century, and their development was a large push in applications in sugar industry for decolorization.

Activated carbon is the amorphous form of carbon that has been specially treated to give high adsorption capacities. This material has a high capacity for adsorption, due primarily to the large surface area avaliable for adsorption, 500-1500 m^2/g, resulting from a large number of internal pores. Pore sizes generally range from 10-100Aº in radius. Most of the avaliable surface area is nonpolar in nature, but in interaction with oxygen (in production) does produce specific active sites giving the surface a lightly polar structure (Cheremisinoff and Ellerbusch, 1978). It is manufactured by carbonization and activation of carbonaceous materials such as wood, coal, peat (Smisek and Cerny, 1970) and agricultural by-products such as coconut shells, pulp mill residues, petroleum base residues and char from sewage sludge pyrolysis (Cheremisinoff and Ellerbusch, 1978). However for the manufacture of liquid–phase ones such as decolorizing carbons; lignite, coal, bones, wood, peat and paper mill waste, lignin are the most often used ones due to economic reasons (Kirk-Othmer, 2001).

Liquid–phase activated carbons are generally in powdered or granular form and approximately 60% of them are used in powdered form supplying large surface area. Liquid treatment with powder is a batch process usually which is followed by filtration and settling (Hutchins, 1997; Kirk-Othmer, 2001) while granular carbon can be used in a continuous process where the liquid is slowly percolated through fixed beds of carbon until the carbon becomes saturated with adsorbate (Kirk-Othmer, 2001).

Not only the properties of activated carbon but also the properties of the adsorbate have importance on adsorption. It has been reported that aromatic compounds are general more adsorbable than aliphatic compounds of similar molecular size and branched chains are usually more adsorbable than straight chains (Hassler, 1963). Little information is availible about the adsorption of a wide variety of specific materials by activated carbon (Hutchins, 1997). But it is known that dissimilar compounds have different adsorption affinity due to having different molecular weights and structures (Hassler, 1963). Table 3.1 gives the influence of substituent groups on adsorbability of organic compounds. It has been reported by Mantell (1946), that given the same carbon with different types and varietes of colors, the adsorption isotherms will be different.

An increase in size of molecule weight (size) of organics usually favors adsorption until particle becomes too large to penetrate into the carbon pores (Smisek and Cerny, 1970; Mattson and Mark, 1971; Hassler, 1963; Cheremisinoff and Ellerbusch, 1978; Hutchins, 1997), i.e. polysaccharides are better adsorbed than monosachharides (Hassler,

1963). This can be explained by the fact that as solubility of adsorbate decrease, the adsorptivity will increase as a result of increasing molecular weight (Hassler, 1963; Cheremisinoff and Ellerbusch, 1978; Hutchins, 1997) since a decrease in solubility reflects a reduced affinity between the solvent and adsorbate and also it has been reported that small molecules, which may become attached at a single point, can be desorbed as soon as this bond is broken. However larger molecules can be adsorbed initially by becoming attached through a single atom, the binding being strengthened when other points of contact are made. A larger molecule will not be released until all points of attachment are broken simultaneosly, which will not give in a desorption, and this will happen less often then breaking of a single bond holding a smaller molecule (Hassler, 1974).

Table 3.1 Influence of Substituent Groups on the Adsorbability of Organic Compounds (Hassler, 1963)

Substituent Group	***Nature of Influence***
Hydroxyl	Generally reduces adsorbability
Amino	Effect similar to that of hydroxyl but somewhat greater. Many aminoacids are not adsorbed to any appreciable extent
Carbonyl	Effect varies according to host molecule, glycoxylic acid more adsorbable to than acetic acid but similar increase does not occur when introduced into higher fatty acids
Double bonds	Variable effect as with carbonyl
Sulfonic	Usually decreases adsorbability
Nitro	Often increases adsorbability

Chou (1982), has reported that the diffusion of the colored compounds is a function of temperature and viscosity of a syrup, as well as the size of colorants. Thus it is more effective at higher temperatures and lower viscous syrups.

Contact time is very critical to the adsorption process. It should be sufficiently long to allow an approach to adsorption equilibrium and in order to obtain a meaningful adsorption isotherm, a range of carbon dosages should be applied (Cheremisinoff and Ellerbusch, 1978).

3.3 Properties of Activated Carbon for Sugar Decolorization

Physical and chemical properties of activated carbon determine its efficiency for removing sugar colorants. Physical properties include surface area, pore size distribution, particle size, bulk (apparent) density, hardness, while the most important chemical properties are surface chemistry, ash content and pH (Ahmedna, 2000b; Kirk-Othmer, 2001).

Surface Area

Large surface area is generally a requirement for a good adsorbent. For adsorption of compounds from liquid media such as sugar syrups, surface area is an important characteristic of an activated carbon. Since activated carbon has a large surface area due to having highest adsorptive porosity of any material known, it has a high capability of adsorbing organics such as colored materials from syrups. However, pore size distribution and surface chemistry should also be considered while selecting a good adsorbent. (Hutchins, 1997; Ahmedna, 2000a)

Pore Size Distribution

Activated carbon is a complex net work of pores of various shapes and sizes which include cylinders, rectangular cross sections as well as many irregular shapes. However pores are distributed into three groups with respect to their dimensions according to IUPAC definition (Cheremisinoff and Ellerbusch, 1978; Balcı, 1992).

Macropores : Pores with diameters larger than 50 nm (0.05 μm)
Mesopores : Pores with diameters between 2 nm and 50 nm (0.002 μm - 0.05 μm)
Micropores : Pores with diameters less than 2 nm (0.002 μm)

The macropores enable the molecules of adsorbate to pass rapidly to smaller pores situated deeper within the particle of the carbon. Since they have a specific area rarely exceeding 2 m^2/g, macropores have little or no effect on equilibrium values (Smisek and Cerny, 1970; Dubinin, 1987) but they effect the admission of adsorbates to micro and mesopores. Mesopores are the branches derived from macropores and serve as passages for molecules to reach to micropores which constitute the largest part of internal surface area. The macropore:mesopore:micropore ratio determines the suitability of an activated carbon for sugar decolorization. A carbon with substantial mesoporisity is generally recommended for adsorption of sugar colorants, which are made up of mixtures of compounds with varying molecular size (Ahmedna, 2000a).

Particle Size

The rate of adsorption depends inversely upon particle size. As particle size decreases, mass transfer zone shortens which results in reduced intraparticle diffusion and thus greater rate of diffusion and adsorption. That is why powdered activated carbon, providing a higher contact area than granular activated carbon, has a better decolorization power. However, very fine powdered carbon is undesirable and is discarded as dust because these particles have a negative effect on the filtration flow rate and promote channeling. An activated carbon having correct size and structure to act as good filter aids and to reduce pressure drop during filtration should be preferred (Ahmedna, 2000a).

Bulk Density

Bulk density is important when carbon is removed by filtration because it determines amount of carbon can be contained in a filter of a given capacity and how much treated liquid is retained by the filter cake. It is also used for granular activated carbons to determine bed capacity in the design of an adsoption system (Kirk-Othmer, 2001; Ahmedna, 2000a).

Hardness

Hardness or mechanical strength is important where pressure drop and carbon loses are a concern. A carbon should possess sufficient mechanical strength to withstand the abrasion resulting from continued use. In the course of carbon usage, due to the continuous mechanical friction between carbon particle and sugar liquor, particle breakdown and dust, which is undesirable, slowing down the filtration rate and

decreasing the amount of regenerated carbon formation occur. Therefore, carbons designed for sugar decolorization should have enough abrasion resistance to minimize attrition (Ahmedna, 2000a; Kirk-Othmer, 2001).

Surface Chemistry

The adsorption capacity of activated carbons is also strongly influenced by the presence of functional groups at the carbon surface. Activated carbons are known to contain a variety of heteroatoms such as oxygen, hydrogen, chlorine and sulfur. Heteroatoms are either derived from the starting material, and as a result of carbonization they become a part of chemical structure, or chemically bonded to the carbon during activation or during subsequent additional treatments, such as oxidation. These heteroatoms are bound to the edges of the carbon layers and form surface groups that greatly affect the adsorption behaviour of the activated carbon, with carbon-oxygen surface structures being by far the most important in influencing surface characteristics (Ahmedna, 2000a). The groups more frequently suggested to be present on the surface of activated carbon include; carboxyl groups, phenolic hydroxyl groups, normal lactones, carboxclic acid anhydrides and cylic peroxide. Carboxylic, lactone and phenolic are the functional groups generally considered as the ‘acidic surface oxides’ (Cheremisinoff and Ellerbusch, 1978).

The presence of surface oxygen complexes imparts a polar character to the activated carbon surface, which should affect preferential adsorption of polar organic solutes. The type and net charge of functional groups bonded to the carbon surface is important in understanding the

mechanism of adsorption between ionic adsorbates and the activated carbon. Although sugar colorants are made up of a complex mixture of polydispersed compounds of differing molecular size and net charges, sugar colorants are predominantly anionic. Therefore, the diversity of functional groups on the carbon surface affect the surface behaviour of carbon and thus enhances or reduces the affinity of carbon to the adsorbed raw sugar colorants via electrochemical mechanisms as; opposite charges of carbon surface and adsorbate enhance the adsorption (Ahmedna, 2000a).

Ash Content

The residue part when carbonaceous portion of a carbon is burned off is called as ash content which is composed of silica, aluminium, iron, magnesium and calcium. Ash in activated carbon is not desirable and is considered as an impurity. Since pH of the most carbons is produced by their inorganic components originating from the precursors or added during the manufacture, ash content may affect pH of the carbon (Ahmedna, 2000a).

pH

pH of an activated carbon influences the adsorption process indirectly since it affects the solubility of the adsorbate. An activated carbon having alkaline pH decreases solubility of the adsorbate, thus increases adsorbability, however a distintinctively alkaline carbon may also cause color development through alkaline degradation of organic impurities (Mudoga, 2002). On the other hand, acid carbons may be better sugar decolorizers but their efficiency is blocked by conversion of sucrose into non crystalline sugars (Hassler, 1963). When these factors are

considered, an activated carbon having a neutral pH range of 6-8 is recommended (Hassler, 1963).

These properties of activated carbon are determined by the manufacture way of it.

As a summary, activated carbons having larger total surface area, a well-developed macro- and mesoporosity, along with minimal surface charge (few carboxyl groups) and low ash content are desirable for a better sugar decolorization.

3.4 Production of Activated Carbon

Activated carbon is obtained from a series of process which include (Cheremisinoff and Ellerbusch, 1978)

1. Removal of all water (dehydration)
2. Conversion of the organic matter to elemental carbon; driving off the noncarbon portion (carbonization); and
3. Burning off tars and pore enlargement (activation)

The activated carbon can be prepared in two ways:

1. By allowing the inactive carbonized product (prepared by the usual methods of carbonization) to react with suitable, usually gaseous, substances. This is known as *physical activation.*
2. By carbonizing material of vegetable origin with the addition of activating agents which influence the course of pyrolysis. The method is generally known as *chemical activation.*

3.4.1 Physical Activation

a. Carbonization

The main purpose of carbonization is to reduce the volatile content of source material in order to convert it to a suitable form of activation. During the phase of carbonization, by the pyrolytic decomposition of the precursor in the presence of an inert gas such as nitrogen, most of the non-carbon elements, particularly oxygen, nitrogen, and sulfur, are removed. The pyrolyzed product or char, with a poorly developed structure, consists of more or less disordered elementary graphitic crystallites. As a result of deposition and decomposition of tarry substances, free interstices between these crystalls are filled or at least blocked by disorganized (amorphous) carbon. (Smisek and Cerny, 1970; Balcı, 1992).

b. Steam or CO_2 Activation

Subsequent activation is required to increase surface area and create a highly developed pore structure for the selective adsorption of molecules of different sizes and polarities.

The resulting carbonized product, which has small adsorption capacity is subjected to a partial gasification at high temperature with steam, carbon dioxide, being endothermic and generally used, or at low temperature with air (Kirk-Othmer, 2001). This gasification selectively eliminates first more reactive atoms of the structure generating the porosity; further gasification will produce the final carbon with the pore structure sought

(Rodriguez-Reinoso and Molina-Sabio, 1992). During activation, first the disorganized carbon is removed, and by this, the surface of the elementary crystallites becomes exposed to the action of the activation agent to constitute new pore structure (Smisek and Cerny, 1970). Activation with CO_2 and widens the microporosity, with even a shift to meso- and macroporosity (Rodriguez-Reinoso and Molina-Sabio, 1992) however for the activation of many types of chars, steam is more prefable to carbon dioxide and much better than air (Hassler, 1963). This is because; the water molecule has a smaller dimension than carbon dioxide, which leads to faster diffusion into the porous structure and a faster reaction rate. Increasing activation temperature may produce better sugar decolorizing carbons (Pendyal et al, 1999). It has been reported by Márquez-Montesinos et al, (2001) that by activation of *Pinus caribaea* sawdust, more developed porous structure with a substantially higher contribution of mesoporosity compared to activation with CO_2 was obtained. The presence of a well-developed mesoporosity makes the resulting products good candidates for adsorbents having different molecular size ranges such as sugar colorants.

Bernardo et al, (1997) studied the decolorization of molasses' wastewater using activated carbon prepared from cane bagasse. The results were very promising and the steam activated carbons were effective as commercial activated ones for sugar decolorization since they had high adsorptive capacity, low ash content and surface ares greater than 400 m^2/g.

Starting material has also influence on the properties of activated carbon produced as activation method. For example Pendyal et al, (1999) has

reported that activated carbons consisting of sugarcane bagasse as precursor have better ability, closer to commercial reference carbons, compared to rice hulls and rice straw based ones for removing sugar colorants. It has been reported by Ahmedna et al (2000a,b), that binder also has influence on the properties of activated carbon properties as well as raw material. Physically (CO_2 and N_2 mixture) activated sugarcane bagasse derived carbon gave better adsorption results when combined with corn syrup compared to sugarcane molasses, beet molasses and coal tar as binders at the production.

Ahmedna et al, (1997) studied the performances of agricultural by-product-based activated carbons for raw sugar decolorization. The carbons having rice straw and rice hull as precursors had good adsorption capacities but unsatisfactory physical and chemical properties, while the activated carbons prepared from pecan shells had moderate adsorption, good physical and chemical characteristics and soybean hull derived activated carbons had poor adsorption properties and unsatisfactory physical and chemical properties.

It has been reported by Ahmedna (2000c), that granular activated carbons produced by steam activation of pecan shells had all desirable characteristics; larger surface area, a well developed macro- and mesoporosity and a minimal surface charge, for an efficient sugar decolorization. They could be lower cost alternative to existing coal-based commercial carbons since they were very similar to commercial activated carbons used in sugar industry.

3.4.2 Chemical Activation

Many decolorizing carbons are prepared by chemical activation (Kirk-Othmer, 2001). The starting material is treated at a high temperature, usually 400 to 1000 ºC with the activating agent which can be zinc chloride, potassium sulphide, potassium sulphide, potassium thiocyanate, phosphoric and sulphric acid; sometimes hydroxides of alkali metals, magnessium, and calcium chloride, in the form of a concentrated solution, usually by mixing or kneading. The impregnated material is heated in a rotary kiln from which air is excluded (calcination) and pyroytic decomposition, which create a porous structure and extented surface area, takes place. The carbonized material from kiln is cooled and activation agent is extracted from it (Smisek and Cerny, 1970).

It has been reported by Ahmedna et al, (2000b,c) that, chemical activation of pecan shell-derived carbons with phosphoric acid gave granular activated carbons, with high surface area and adequate pore size distribution, but with large surface charge which can be eliminated (especially carboxylic acids) by subjecting these activated carbons higher activation temperature treatment. The process conditions was 2 h soaking in 50% H_3PO_4 then pyrolysis, for ½ h at 170 ºC under N_2, which was followed by activation carried out for 1h at 450 ºC under N_2. Activated carbon was subsequently cooled overnight under nitrogen gas and washed in a soxhlet extractor until no residual phosphoric acid was present as determined by the absence of a precipitate when 10 drops of 0.01 M lead acetate were added to 100 mL of the final wash water. Before usage, the activated carbons were then dried overnight at 50 ºC.

Girgis et al, (1994) studied the production of activated carbon from sugar cane bagasse by carbonization in precense of H_3PO_4, H_2SO_4, HCl, HNO_3. The efficiency of activation was H_3PO_4> H_2SO_4 > HCl > HNO_3. Activated carbons obtained at low temperatures were essentially microporous with a low degree of mesoporosity. Raising temperature gave in products having higher surface area and total pore volume with developed mesoporosity and and low microporosity.

Activated carbons produced from lignocellulosic materials by chemical activation, using $ZnCl_2$, produces in only one step, a larger yield of activated carbon having microporosity as well as developed as in the activation CO_2, with the advantage of producing a much larger mesopore volume (Rodriguez-Reinoso and Molina-Sabio, 1992).

Mudoga, (2002) studied the decolorization of sugar syrups by sugar beet pulp based; activated by using phosphoric acid (Özer and Çam, 2002) or carbon dioxide under different conditions (time and temperature); and commercial activated carbons, one of which was NORIT. The results showed that beet pulp carbon, prepared at temperature of 750 ºC and activated for 5 h by carbon dioxide activation method, had high decolorization efficiency which was close to, the best of commercial activated carbons overall, DCL 320.

CHAPTER 4

MATERIALS AND METHODS

4.1. Materials

4.1.1. Substrate

Commercial wheat starch, PİYALE, which was purchased from market, was used as substrate. Properties of wheat starch are given in Table 4.1.

Table 4.1 Properties of the Wheat Starch

Starch	**Protein Content %**	**Lipid Content %**	**Dry Matter %**
PIYALE	1	0.71	92

4.1.2. Enzymes

The hydrolysis of starch into maltodextrins, liquefaction, was achieved by using thermostable pure α-amylase (*Bacillus licheniformis*, liberating 1 mg of maltose from starch in 3 min) obtained from Sigma Company. Maltodextrins were hydrolyzed to give glucose syrups, through saccharification process, by pure thermostable amyloglucosidase

(*Aspergillus niger*, liberating 1 mg of glucose from starch in 3 min) also obtained from Sigma. The properties of these enzymes are given in Table 4.2.

Table 4.2 Properties of the Enzymes

Enzyme	***Bacteria***	***Optimum Temperature, ^oC***	***Optimum pH***
α-amylase			
SIGMA	*B. licheniformis*	100	6.9
glucoamylase			
SIGMA	*A. niger*	60	4.5

4.1.3 Activated Carbons

NORIT PN 2 3730-1, which is a commercial peat based activated carbon produced by steam activation method, obtained from Norit Company (Mudoga, 2002) and agricultural waste based activated carbons that had been prepared in studies carried out in our department, under supervision of Prof. Dr. Hayrettin Yücel and Prof. Dr. Suzan Kıncal, were also used for color decolorization.

Hazelnut Shell Based Activated Carbons: (HS 50.4)

Hazelnut shell particles used as raw materials for activated carbon production, having sizes of 12-18 mesh, were obtained by crushing and sieving of hazelnut shells. The raw materials were treated with 50% (wt.) H_3PO_4 solution at 25 ºC for 24 hours. As a proportion, in the impregnation of hazelnutshells, 1 g shell was mixed with 2 ml of acid

solution which was followed by filtration and air drying at room temperature for 3 days. This procedure prepared the samples for carbonization which was carried for 2 hours at temperatures of 400 ºC under nitrogen flow (200 cm^3/min) at a heating rate of 20 ºC/min (Çuhadar, 2005).

Hazelnut Husk Based Activated Carbons: (HH 30.4)

Hazelnut husks, crushed and sieved to 20-50 mesh size, were treated with 30% (wt.) H_3PO_4 solution at 25 ºC for 24 hours. As a proportion, in the impregnation of hazelnutshells, 1 g shell was mixed with 5 ml of acid solution which was followed by filtration and air drying at room temperature for 3 days. After this process, carbonization was carried for 2 hours at the same operating conditions with hazelnut shell based activated carbons (Çuhadar, 2005).

Apricot Stone Based Activated Carbons (AS 3.1)

Same procedure was applied as in the impregnation of hazelnut shells and hazelnut husks but this time 1 g of apricot stones crushed and sieved to 10-18 mesh size, was treated with 2 ml of 50% (wt.) H_3PO_4 solution. Following, carbonization was carried out for 90 min at temperature of 300 ºC under nitrogen flow (180 cm^3/min) at a heating rate of 20 ºC/min (Yağşi, 2004).

The properties of these activated carbons are given in Table 4.3.

Table 4.3 Properties of the Activated Carbons

Activated Carbon Type	Total Pore Volume, cm^3/g	Mesopore Volume, cm^3/g	BET surface Area, m^2/g	Ash Content, %
NORIT	0.50	-	609.2	10.55
HS	0.27	0.03	426.0	1.60
HH[a]	1.05	0.58	1429.0	-
AS[a]	0.22	0.03	450.0	5.40

4.2 Methods

Liquefaction

The hydrolysis of wheat starch into maltodextrins was carried out in a batch system. 15 g samples which contained 27% (w/w) dry wheat starch, in buffer having a pH value of 6.5 (Appendix A) to which $CaCl_2$ was added to give 100 mg Ca^{++} per liter of solution to increase the thermostability of α-amylases were hydrolyzed for 45, 60, 75, 90 min with α-amylase (0.5% of dry matter), 20 μL, in a shaking water bath having a temperature of 97 ºC. Firstly, starches were weighted into erlenmeyer flasks and phosphate buffer solutions, pH 6.5, and $CaCl_2$ were put into tubes. Then covered flasks and tubes were placed into water bath for 5 min to stabilize tempeºC rature. Then the α-amylase was added to solution including, buffer and $CaCl_2$. Resulting solution was immediately mixed with starch and flasks were covered with flasks and at that moment, hydrolysis was started. The flasks were shaken at 80 oscillations per minute in shaking bath in order to obtain mixing. The experiments were carried out in triplicate. At the end of desired time, the hyrolysis was stopped decreasing temperature by pouring mixture into

[a] *mesopore + micropore volume= Total Pore Volume*

10 ml ice-water mixtures at –2 ºC and increasing pH to 12 by adding 1 ml 1 N NaOH to inactivate the enzyme. Before analysis, the samples were neutralized by 30 μL HCl. Finally the samples were diluted and analyzed immediately according to Nelson Somogyi Method (Somogyi, 1952) which is given in Appendix B.

Saccharification

Before saccharification process, the temperature of water bath was immediately reduced to 60 ºC and pH of the liquified starch was decreased to 4.5 by adding 0.3 ml acetic acid. Next 10 ml of liquified starch and 3.5 ml of acetate buffer solution, pH 4.5 (Appendix A), were placed into water bath in seperate flasks for 5 min to stabilize temperature. The enzyme amyloglucosidase, 20 μL at the level of 0.5% of dry matter, was added to acetate buffer solution and it was followed by addition of resulting mixture to the liquified starch flasks. At the end of 18 h, 1 ml of the hydrolyzate, glucose syrup, was withdrawn from solution and put into 8 ml of ice-water mixture at –2 ºC and analyzed according to Nelson Somogy Method (Somogyi, 1952) given in Appendix B.

Decolorization of Glucose Syrups

After saccharification, glucose syrups were centrifuged for 20 min at 1669 g to remove insoluble solids. Before decolorization, the absorbance of centrifuged glucose syrups was determined using Hitachi U-3200 spectrophotometer at 420 nm against water using a 10 mm cell (Anon, 1994) as measure of the color, and the color level was expressed in

ICUMSA units. Glucose syrups were placed in a shaking water bath at 80 ºC (Mudoga, 2002) for 5 min to stabilize the temperature. At this point, different type and amount of activated carbons (at a ratio of 0.25, 0.50, 0.75, 1.0% (w/v)) to glucose syrups and for some syrups also in addition to these values 0.6 and 0.83% (w/v) to be studied was added. At the end of 30 min, the decolorized syrup was centrifuged for 20 min at 1669 g and filtered using Schleicher & Schuell Blue Band 589/3 filter paper to completely remove the activated carbon, and the color was measured again (Anon, 1994). Glucose syrup production and decolorization is given in Fig. 4.1.

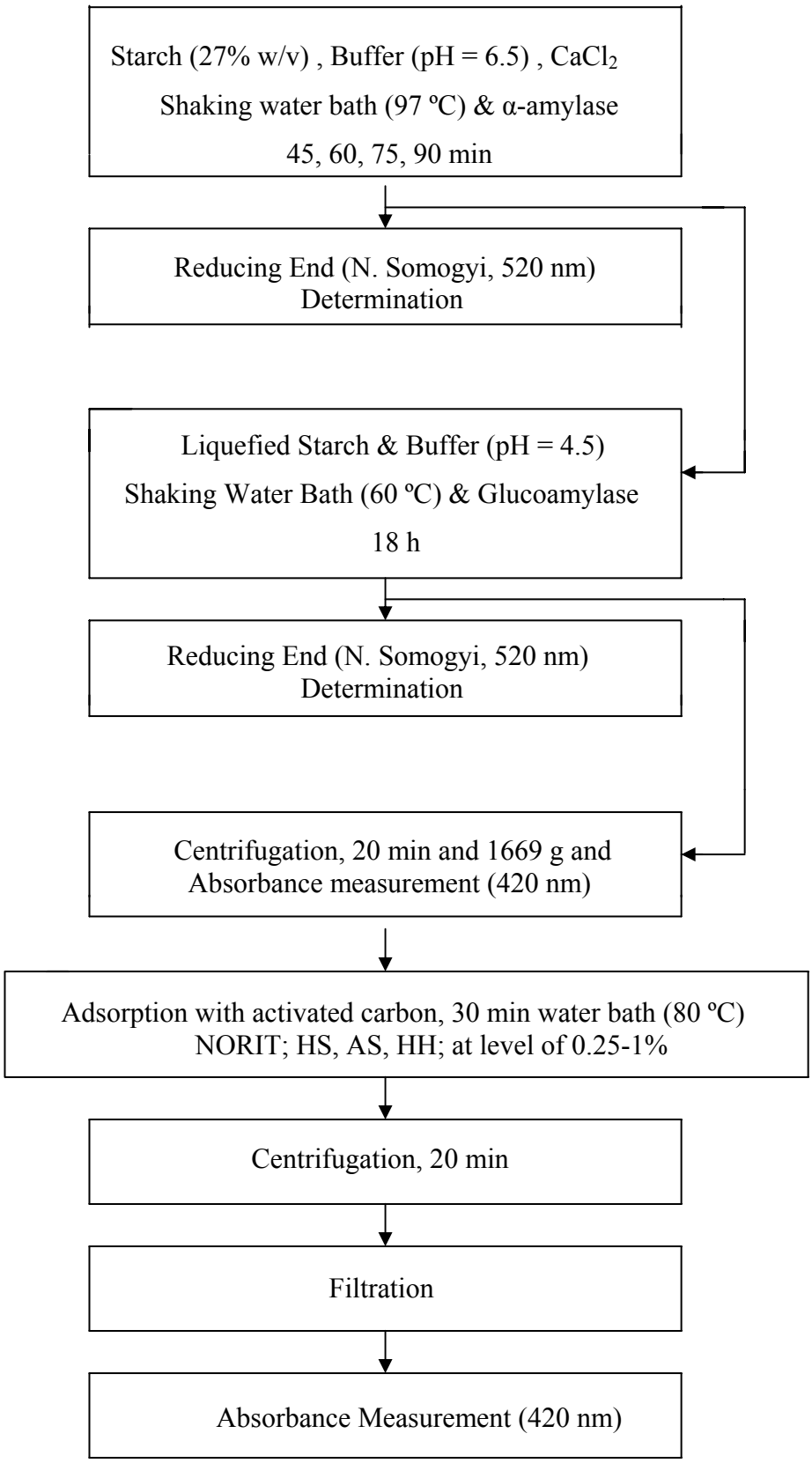

Fig. 4.1 Glucose Syrup Production and Decolorization

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Percentage Conversion in Maltodextrins and Glucose Syrups

The percentage conversion is defined as the moles of reducing ends produced divided by the total moles of glucose units that would have been obtained from complete hydrolysis of initial starch.

Commercial wheat starch, 27% (w/w), was liquified for four different periods as 45, 60, 75 and 90 min at 97 ºC in shaking bath with Sigma amylase (0.1% of dry matter). Reproducibilities of maltodextrins obtained by 45 min and 60 min liquefaction time were checked by 3 and 5 experiments respectively and standard deviation was found as 0.9 (10%); for maltodextrins obtained by 45 min and 0.7 (5.4%); for maltodextrins obtained by 60 min (Fig. 5.1).

The results on conversion values during hyrolysis are given in Fig. 5.2 along with the results of previous study by Sakintuna, (2001) for comparison. As time increased, conversion increased due to increased split of starch chain by α-amylase resulting in increased reducing ends. For maltodextrins, the highest conversion was 21%, obtained at 90 min, while lowest was 9%, for the one obtained at 45 min. 13% and 19% were the conversions of maltodextrins obtained at 60 and 75 min respectively.

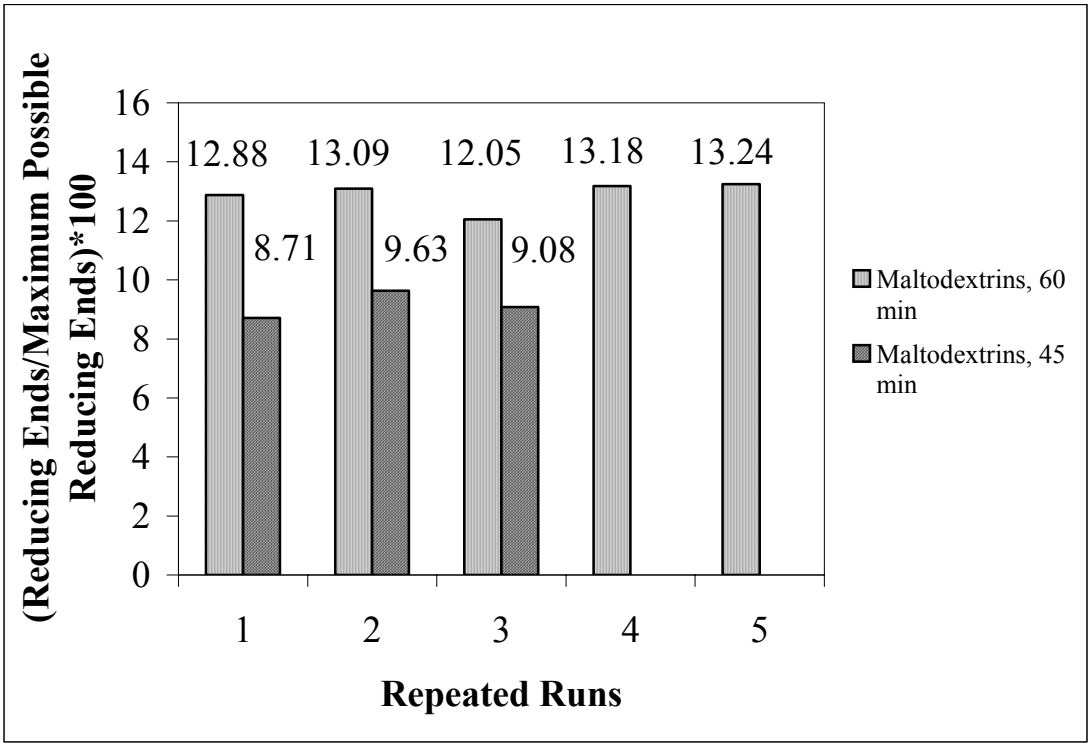

Fig. 5.1 Reproducibilities of Maltodextrins

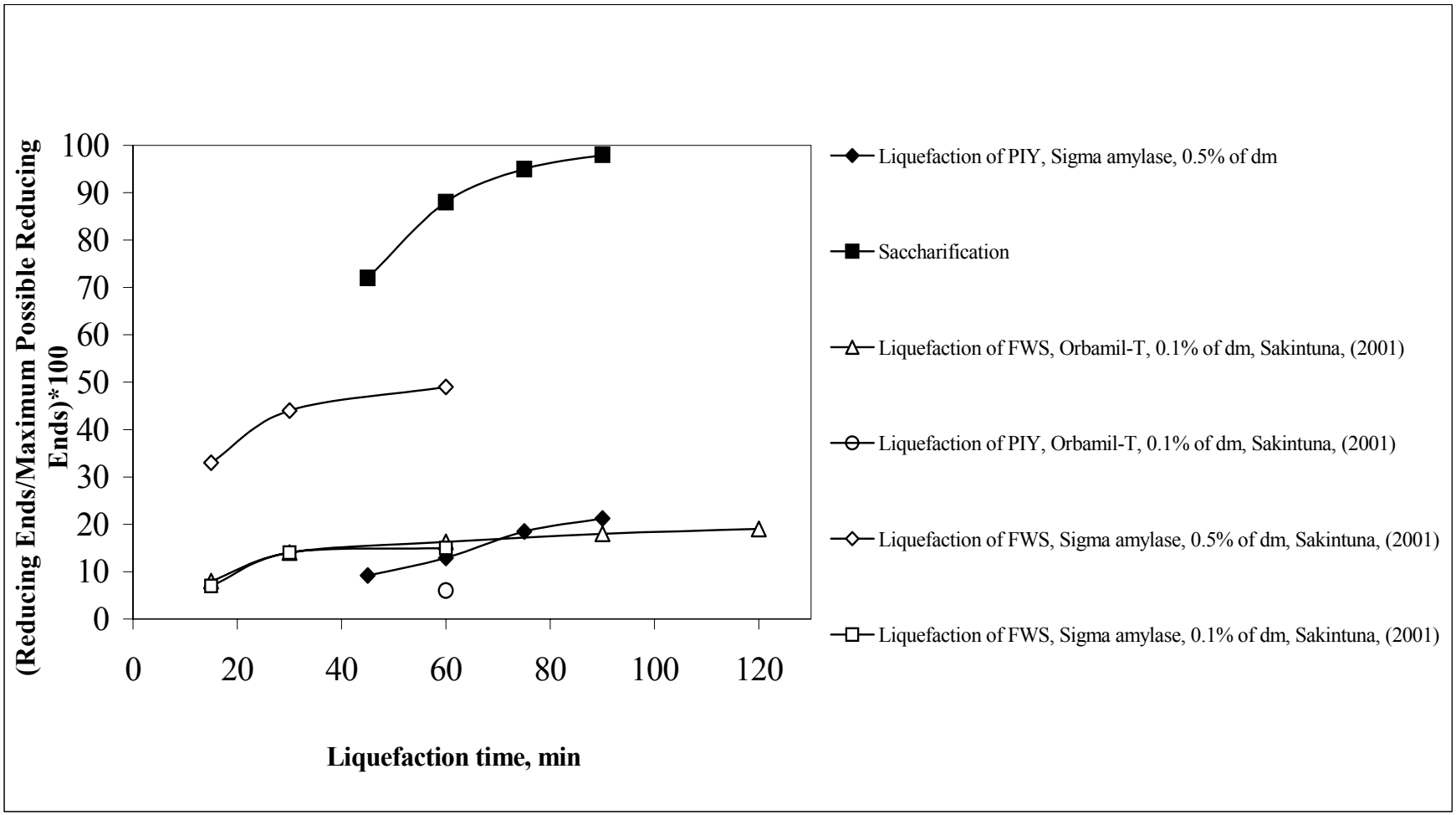

Fig. 5.2 Percentage Conversion in the Production of Maltodextrins and Glucose Syrups at 27% (w/w) Wheat Starch

These results are in agreement with those of Sakintuna, (2001) when the differences in the hydrolysis behaviour of freshly prepared wheat starch (FWS) and the commercial wheat starch (PIY) are taken into account. As indicated by their results at the enzyme dosage, 0.1% of dry matter, the catalytic effects of Sigma and Orbamil-T α-amylases are the same, and the conversion in FWS is about 3.5 times higher than that in PIY. The results of the present work on PIY compared to those by Sakintuna, (2001) on FWS have the same ratio at all liquefaction times.

Saccharification of maltodextrins to obtain glucose syrups were carried out in shaking water bath at 60 ºC for 18 h with Sigma glucoamylase amylase (0.5% of dry matter).

The percentage conversion of glucose syrups had values between 72-98 for 45-90 min liquefaction times (Fig. 5.2). Table of Appendix C1 gives numerical values of percentage conversion in maltodextrins and glucose syrups. Sakintuna, (2001) studied the saccharification of maltodextrins obtained from flow system, batchwise at 60 ºC. Two glucoamylases (*A. Niger*) (0.1% of dry matter), obtained from Orba and Sigma were used, and 70-90% conversions were obtained at 18-72 h saccharification time for PIY. This can be considered reasonable aggreement considering the different liquefaction processes and enzyme dosages.

5.2 Color of Glucose Syrups

The color of glucose syrups increased with increasing liquefaction times (Coca et al, 2004; Mauron, 1981) as has been expected. This was due to, Maillard reaction was started with the reaction of reducing ends and aminoacids and as reducing end amount increased, browning increased as in the situation of glucose syrups produced at increasing liquefaction times. The highest level of color was obtained in glucose syrups produced at 90 min liquefaction time as 1424 ICUMSA units and least level was obtained as 657 ICUMSA units in glucose syrups produced at 45 min liquefaction time (Fig. 5.3). Numerical values are given Table of Appendix C2.

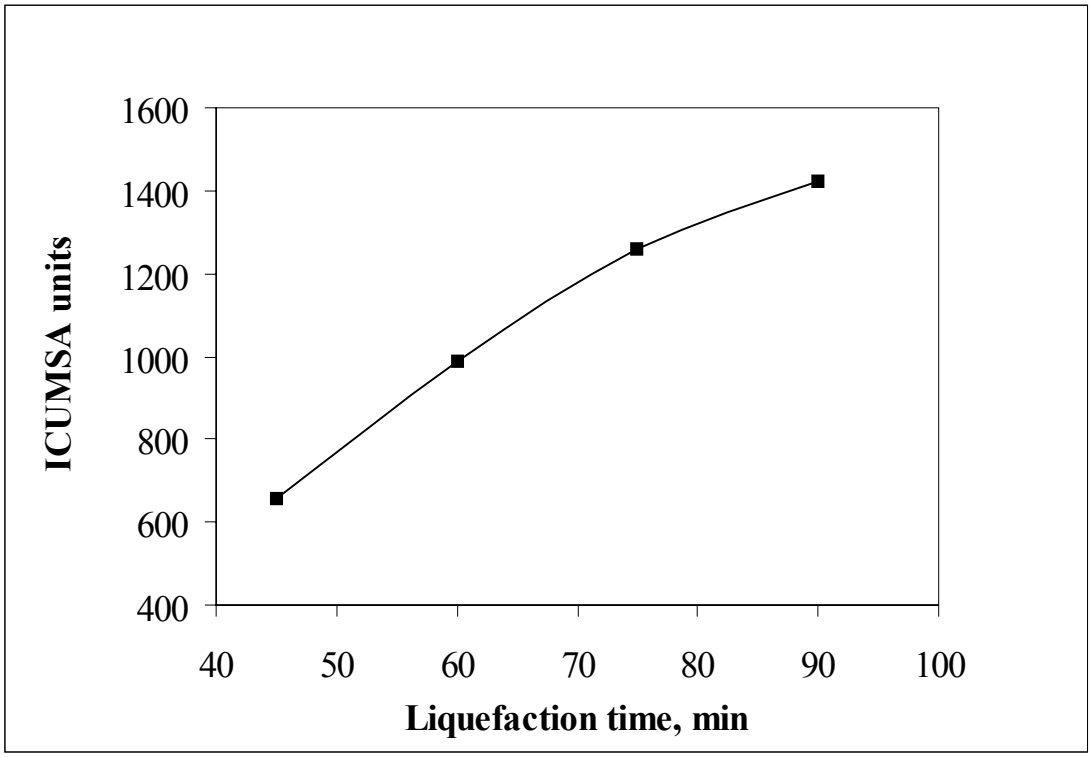

Fig. 5.3 Color of Glucose Syrups

5.3 Decolorization of Glucose Syrups

5.3.1 Decolorization with NORIT

Glucose syrups that were produced at different liquefaction times were decolorized by different amounts of NORIT, hazelnut husk based activated carbon, HH, the hazelnut shell, HS, and apricot stone, AS, based activated carbons.

In order to determine correct decolorization time, glucose syrups obtained at 90 min liq. time were decolorized by 0.3% (w/v) of NORIT. 30 min in whichequilibrium was established, was chosen as the decolorization time (Fig 5.4) (Table of Appendix D1).

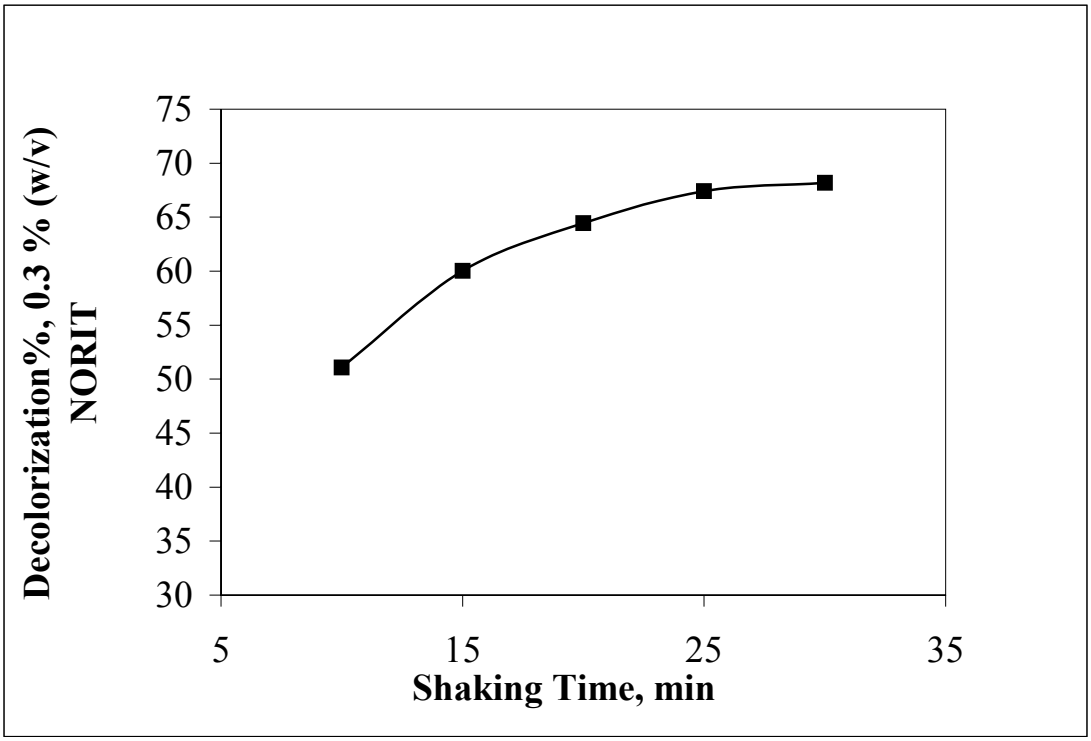

Fig.5.4 Optimum Shaking Time for Decolorization of Glucose Syrups

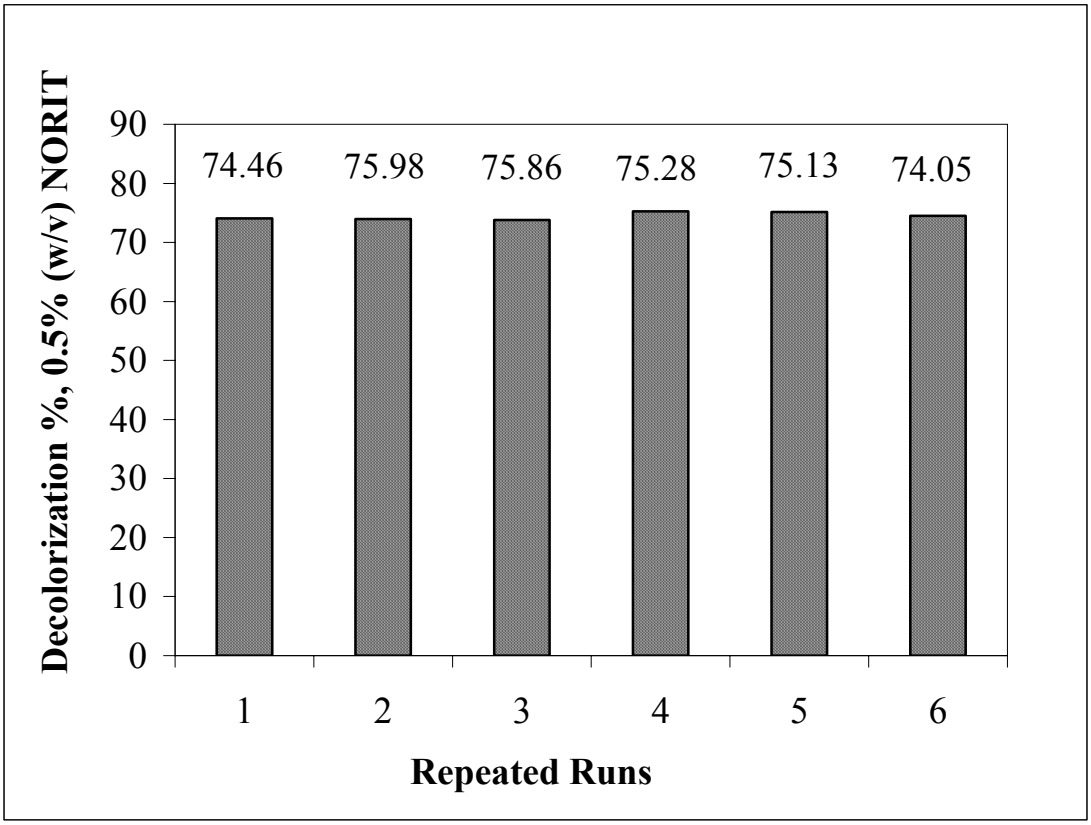

Fig. 5.5 Reproducibility of Decolorization of Glucose Syrups

Reproducibility of decolorization of glucose syrups, obtained by 60 min liquefaction time, at 0.5% (w/v) NORIT was checked for 6 experiments and standard deviation was found as 0.58 (0.77%) (Fig. 5.5).

When glucose syrups that were produced at different liquefaction times were decolorized by different amounts of NORIT, the results given in Figures 5.6, 5.7, 5.8, 5.9, and 5.10 were obtained.

Figure 5.6 is the plot of carbon dosage, NORIT% (w/v), against percent decolorization. The carbon dosage is important since it determines the extent the adsorptivity that will increase of decolorization and may be

used to predict the cost of carbon per ton of glucose syrup in industrial manufacturing.

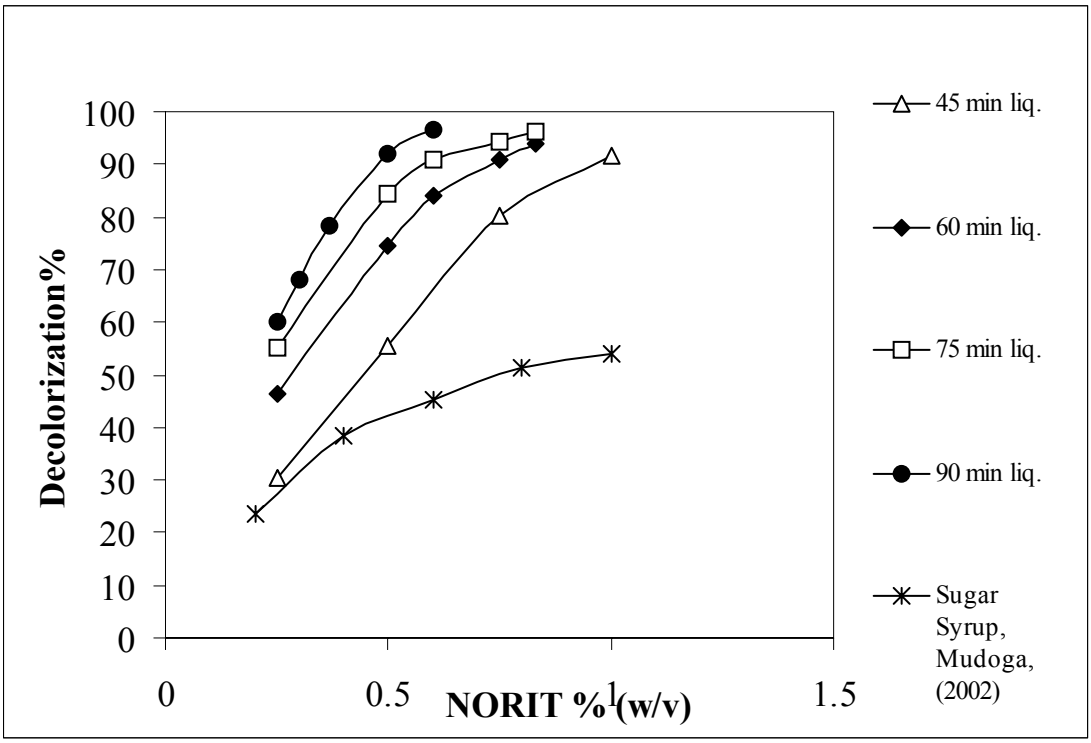

Fig. 5.6 Decolorization Percentage by NORIT

The increase in the amount of NORIT resulted in increased adsorption effect as expected. Interestingly, the fractional removal for any given dosage was highest for the syrup with the longest liquefaction time, or the darkest color. For example with 0.5% of NORIT; 55.68%, 74.46%, 84.20%, 91.4% decolorization values were obtained for the glucose syrups produced at liquefaction times as 45, 60, 75 and 90 min respectively. The numerical values are given in Table of Appendix D2. Mudoga (2002), obtained 23.7%, 38.3%, 45.2%, 51.4%, 54.0%

decolorization values, respectively with 0.2%, 0.4%, 0.6%, 0.8%, 1.0% NORIT dosages, which are lower than the ones obtained for the glucose syrups in the present study (Fig. 5.6). This might be probably due to different characteristics of sugar syrups and enzymatically produced glucose syrups. Since sugar syrups contain caramels, melanins and alkaline degradation products in addition to melanoidins of which glucose syrups contain.

In an adsorption process, adsorption continues until equilibrium is established with the concentration in the solution. The plot of C; color residual against X/M, color removal per amount of carbon, on a rectangular coordinate yields in curvilinear graph; adsorption isotherm, as shown in Fig. 5.7. The data for this isotherm is given in Table of Appendix D.3.

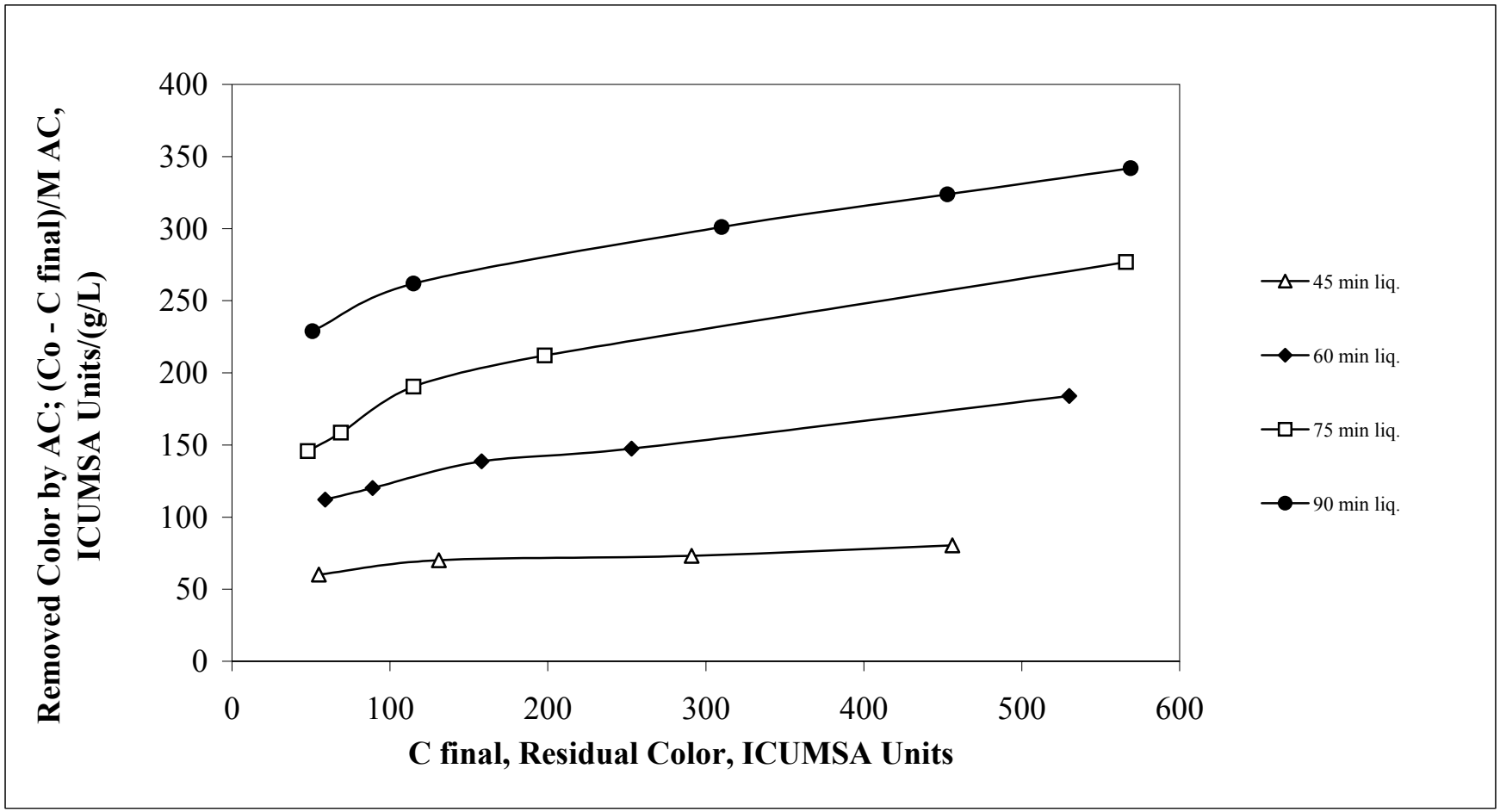

Fig. 5.7 Glucose Syrups Decolorization Isotherms for NORIT

5.3.1.1 Langmuir and Freundlich Isotherms

The Langmuir isotherm has a theoretical basis and is given by the equation (3.0), where q_0 and K are empirical constants.

$$X/M = (q_0C)/(K+C) \quad \text{.....(3.0)}$$

In this equation, X is the units of impurity held by M (weight) units of carbon. It is the difference between C_0; the concentration of impurity remaining unadsorbed at equilibrium; and C_f; concentration of final impurity. Plotting the data 1/C against 1/(X/M), fit of data to Langmuir isotherm, to give a straight line (Geankoplis, 1983), was checked and isotherm parameters, q_0 and K were determined (Fig. 5.8 and Fig. 5.14).

The Freundlich isotherm, which is emprical, is very useful in calculations involving activated carbon. That is described by the equation (4.0)

$$X/M = KC^{1/n} \quad \text{.....(4.0)}$$

The relationship may be linearized by the logarithmic form of equation (5.0)

$$\log X/M = \log K + 1/n \log C \quad \text{.....(5.0)}$$

K and 1/n are constants of which values are changible with respect to used carbon and nature of impurity (Mudoga, 2002). Plotting C against X/M on a logarithmic scale yielded the graphs shown in Fig. 5.9 and Fig. 5.15.

The examination of the shapes of the adsorption isotherms in Fig. 5.7 shows a shift from Langmuir type to Freundlich type as the liquefaction time changes from 45-90 min. This is possibly due to changing molecular sizes and shapes of colored compounds. This was also reflected in the isotherms being quite separated from each other. For example, those of glucose syrups produced at 75 and 90 min liquefaction time which had similar percentage conversion values, were distinct from each other. This is probabaly due to longer times resulting in colored substances having increased molecular weight, were effective on the color formation and decolorization of it.

Fit to Langmuir equation (Fig. 5.8), of which numerical values are given in Table of Appendix D4, was tried, and resulting q_0, K values and regression coefficients for are given in Table 5.1

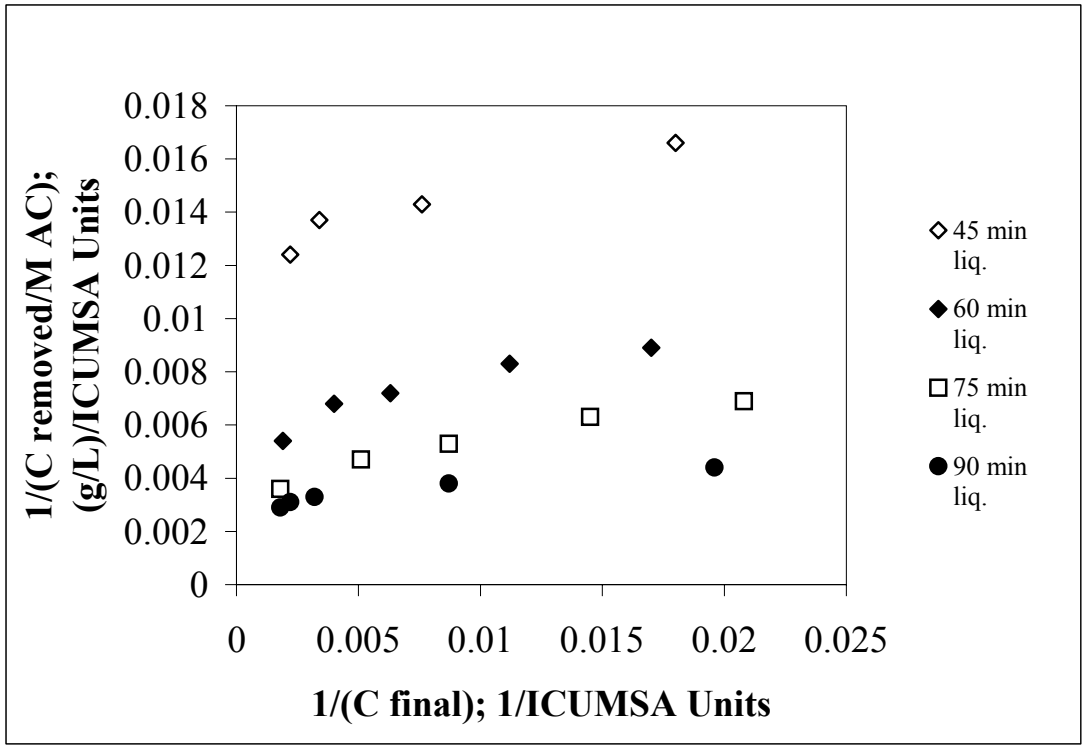

Fig. 5.8 Langmuir Plots for NORIT

Table 5.1 The Langmuir Isotherm Parameters for NORIT; q_0, K and R^2 Values

Liquefaction Time, min	q_0	K	R^2
45	80.65	18.87	0.94
60	178.57	37.99	0.90
75	277.78	46.69	0.96
90	344.83	26.93	0.94

When the data, obtained for NORIT in decolorization of sugar syrups by Mudoga, (2002); is fitted to Langmuir equation, q_0 and K are found as –144.93 and –163.23 respectively which are quite lower than the ones obtained in the present study, while regression coefficient is found as 0.96 which is close to regression coefficients obtained in the present study. The differences between these two studies are probably due the different characteristics of sugar and enzymaticly produced glucose syrups.

Fit to Freundlich equation (Fig. 5.9) was also tried and resulting K, 1/n and R^2 values are given in Table 5.2

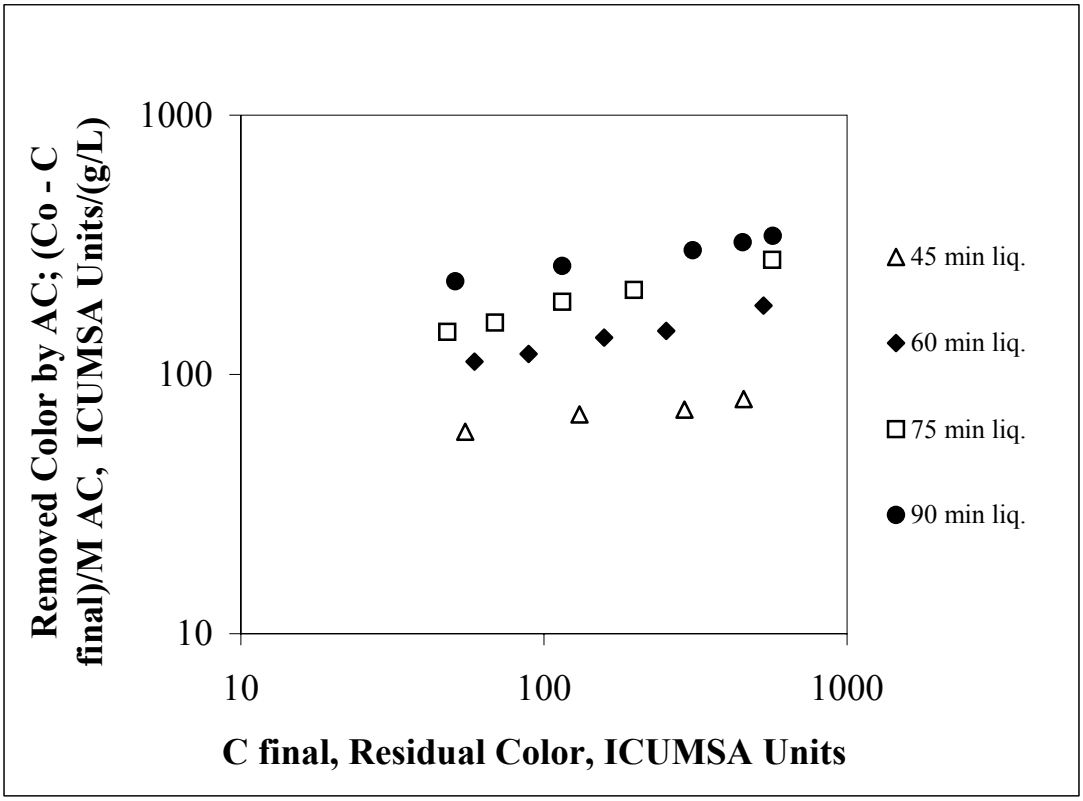

Fig. 5.9 Freundlich Plot Isotherms for NORIT

Table 5.2 The Freundlich Isotherm Parameters for NORIT; K, 1/n and R^2 values

Liquefaction Time, min	K	1/n	R^2
45	36.14	0.13	0.96
60	41.82	0.23	0.98
75	53.58	0.26	0.99
90	120.23	0.16	0.99

When Freundlich isotherm parameters are compared with the ones obtained by decolorization of sugar syrups by NORIT, by Mudoga, (2002); K is found as –39.43 which is quite lower than the values obtained in the present study; and 1/n is as 2.11, which is higher than the values obtained in present study, and regression coefficient is found as

0.97, which is similar regression coefficient of glucose syrups. The differences between these two studies are probably due the different characteristics of sugar and enzymaticly produced glucose syrups as mentioned before.

Isotherms well fitted to Langmuir and Freundlich equations due to regression coefficients having values higher than 0.9. But fit to Freundlich equation was better for all types of syrups, as indicated in Fig. 5.8 and Fig. 5.9, and also by the higher R^2 values.

The carbon dosages were plotted against the residual color units to obtain Fig. 5.10. The residual color remained higher for syrups liquefied for longer times up to about 0.3% dosage, at which all syrups had about 400 ICUMSA units of color. At higher carbon dosages, the level of residual color decreased as liquefaction time increased. The residual color values are given in Table of Appendix D5.

From this result it is understood that colored substances are different from each other in structure and molecular weight (Coca et al., 2004), due to the complex mechanism of Maillard reaction which is beyond of the scope of the present study. But it has been reported by several authors that (Mauron, 1981; Martins et al, 2001; Coca et al, 2004) that during Maillard reaction, the molecular weight of colored substances increase due to polymerization reactions. As mentioned before; little information is availible about the adsorption of a wide variety of specific materials by activated carbon (Hutchins, 1997) and it is known that dissimilar compounds have different adsorption affinity due to having different molecular weights and structures (Hassler, 1963). And it has

been reported that higher molecular weight organics are known (Hassler, 1963; Smisek and Cerny, 1970; Mattson and Mark, 1971; Cheremisinoff and Ellerbusch, 1978; Hutchins, 1997) to have higher affinity for activated carbon surfaces, as a result of increasing molecular weight and present results; less residual color level with decolorization of more densely colored syrups by same amount of activated carbon is in agreement with this.

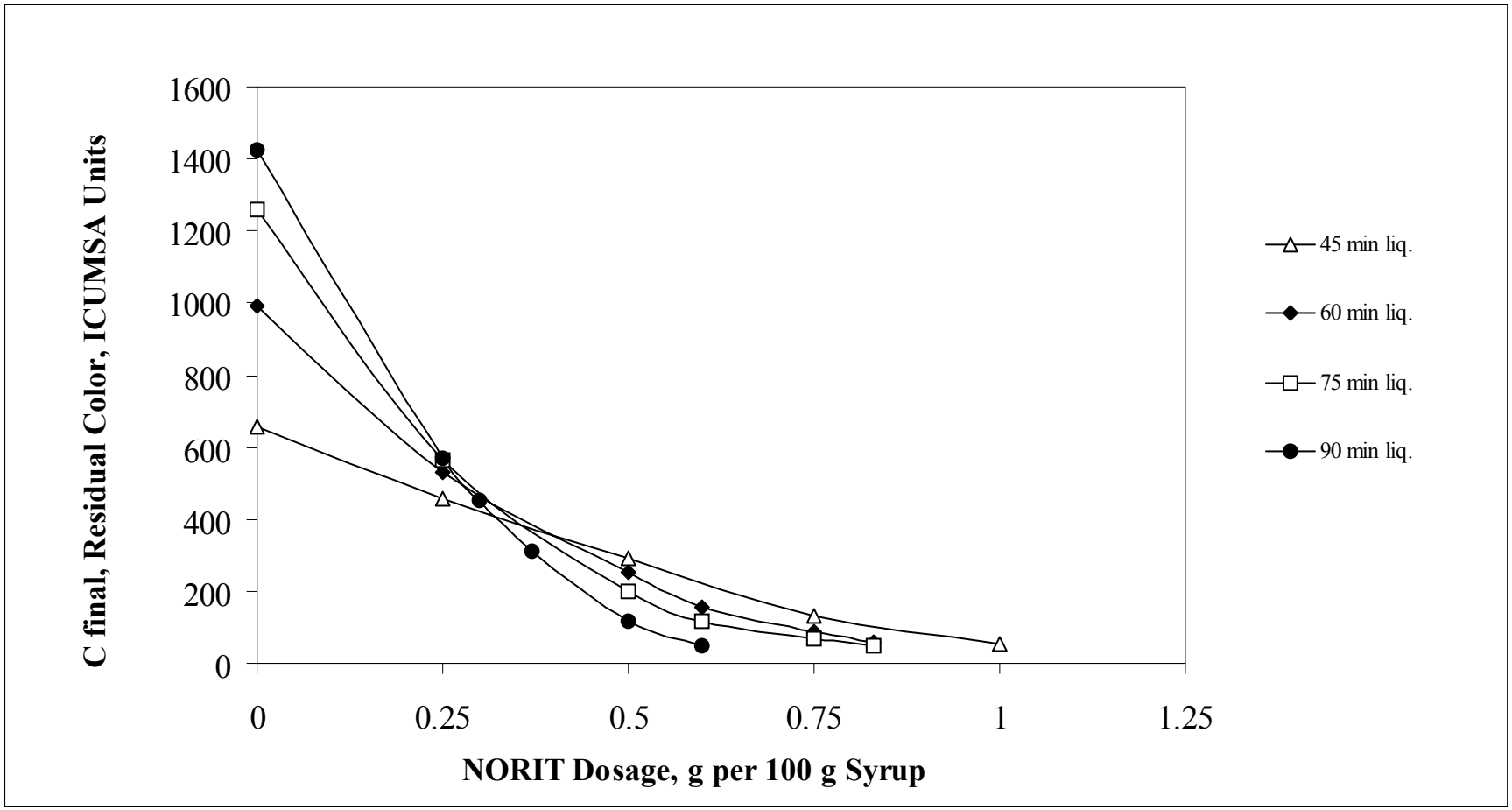

Fig.5.10 Residual Color of Glucose Syrups after Decolorization by NORIT

5.3.2 Decolorization with Agricultural Waste Based Activated Carbons

As mentioned before, commercial glucose syrups should have color not exceeding 200 ICUMSA units, and typical production process target lower values such as 100 to be on the safe side. The NORIT dosages required to reduce the color of syrups liquefied for 45, 60, 75 and 90 min to 100 ICUMSA units were, 0.82%, 0.73%, 0.65% and 0.53% respectively.

The decolorization performances of the agricultural waste based activated carbons were compared with NORIT on this basis. The dosage of activated carbon was adjusted to be the same as that of NORIT required for 100 ICUMSA final color and the final color values were determined (Fig. 5.11). Among the samples studied with syrups liquefied for 90 min the hazelnut husk based activated carbon, HH, was the best, giving a final color of 101, practically the same as NORIT. Although HH based activated carbon has large BET surface area and high mesopore volume, its ash content and surface charge might have resulted in lower performance. The hazelnut shell, HS, and apricot stone, AS, based activated carbons, both of which have a considerably smaller mesopore volume than HH based ones, had very similar performances, reducing the color to less than 180 ICUMSA units for syrups liquefied both for 45 min and 90 min. A target of 100 units, of which NORIT achieved, would require somewhat higher amounts. This is due to lower BET surface areas and total pore volumes of AS and HS activated carbons than NORIT. Table of Appendix E1 gives the numerical values of this comparison.

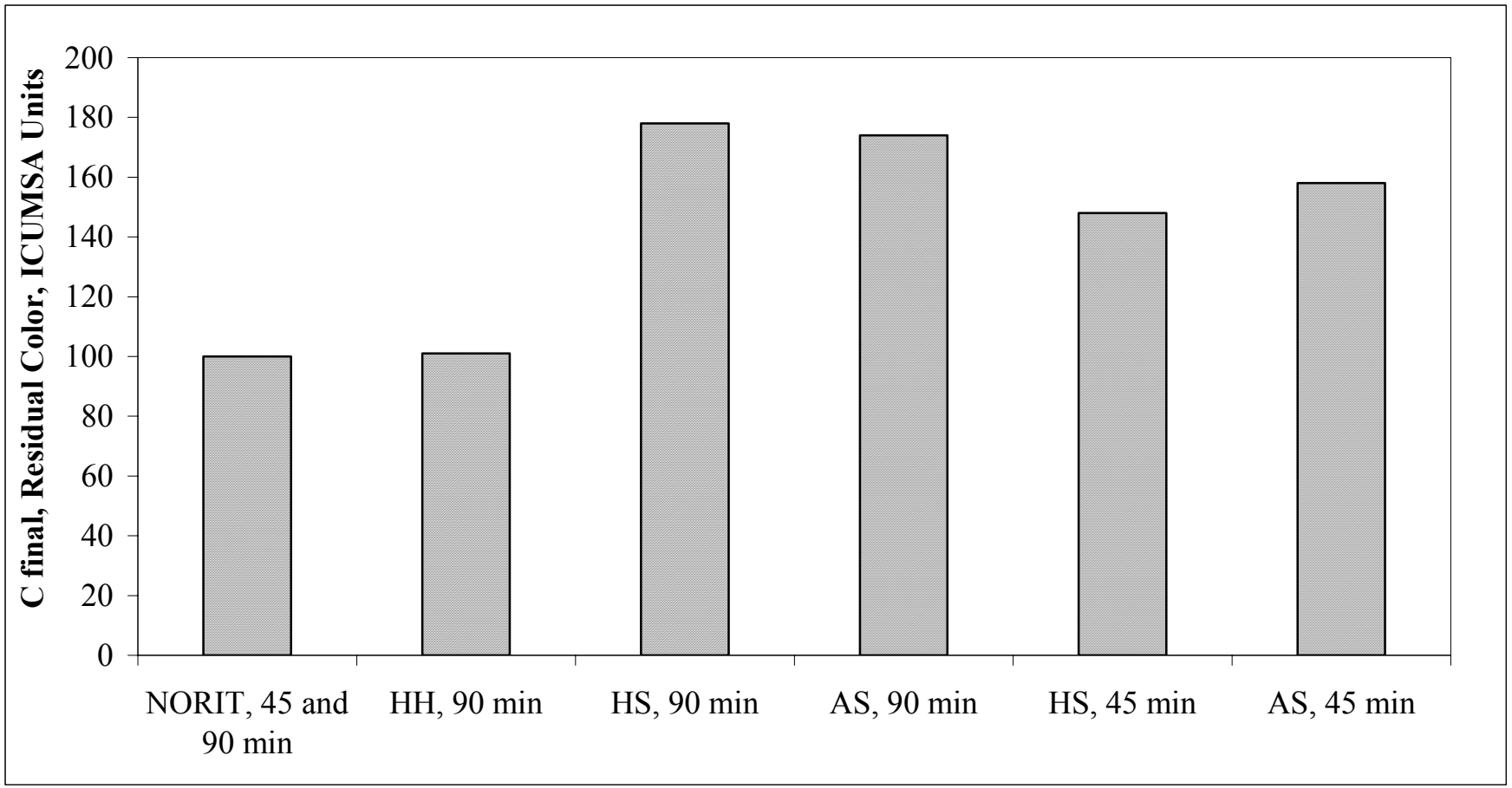

Fig 5.11 Comparison of Performance of Activated Carbons with That of NORIT at the Dosage Required by NORIT to Reduce the Final Color to 100 ICUMSA Units

The decolorization of 45 min and 90 min liquefied syrups with different amounts of AS dosage was also carried out (Fig. 5.12, Fig. 5.13). Langmuir (Fig. 5.14, Table 5.3) and Freundlich isotherm (Fig. 5.15, Table 5.4) parameters were determined. Fit to both isotherms were well due to having regression coefficients higher than 0.9 (Table 5.3 and Table 5.4). But since AS has pores in micro size mainly, isotherms fitted better to Langmuir equation to which most of the microporous adsorbents follow. The behaviours of AS and NORIT were found to be quite similar in Fig. 5.12, Fig. 5.13, and Fig. 5.16. The data for decolorization of glucose syrups by AS are tabulated in Tables of Appendix E2 to E5.

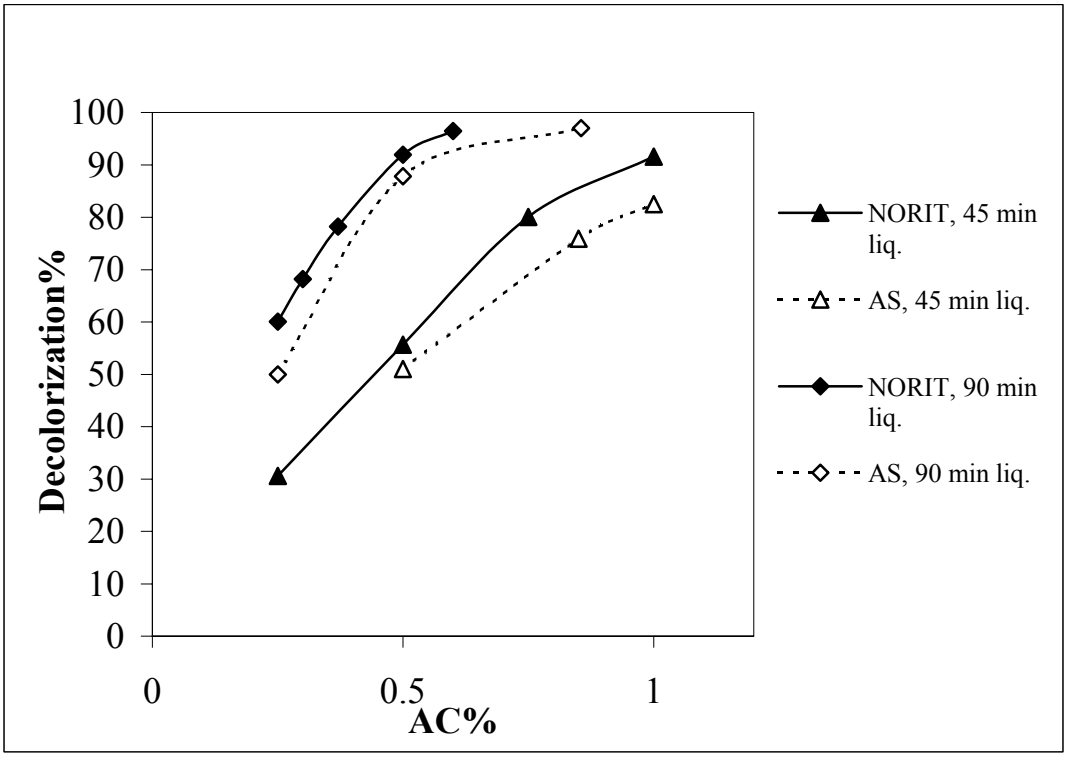

Fig. 5.12 Decolorization Percentage of Glucose Syrups by NORIT and AS

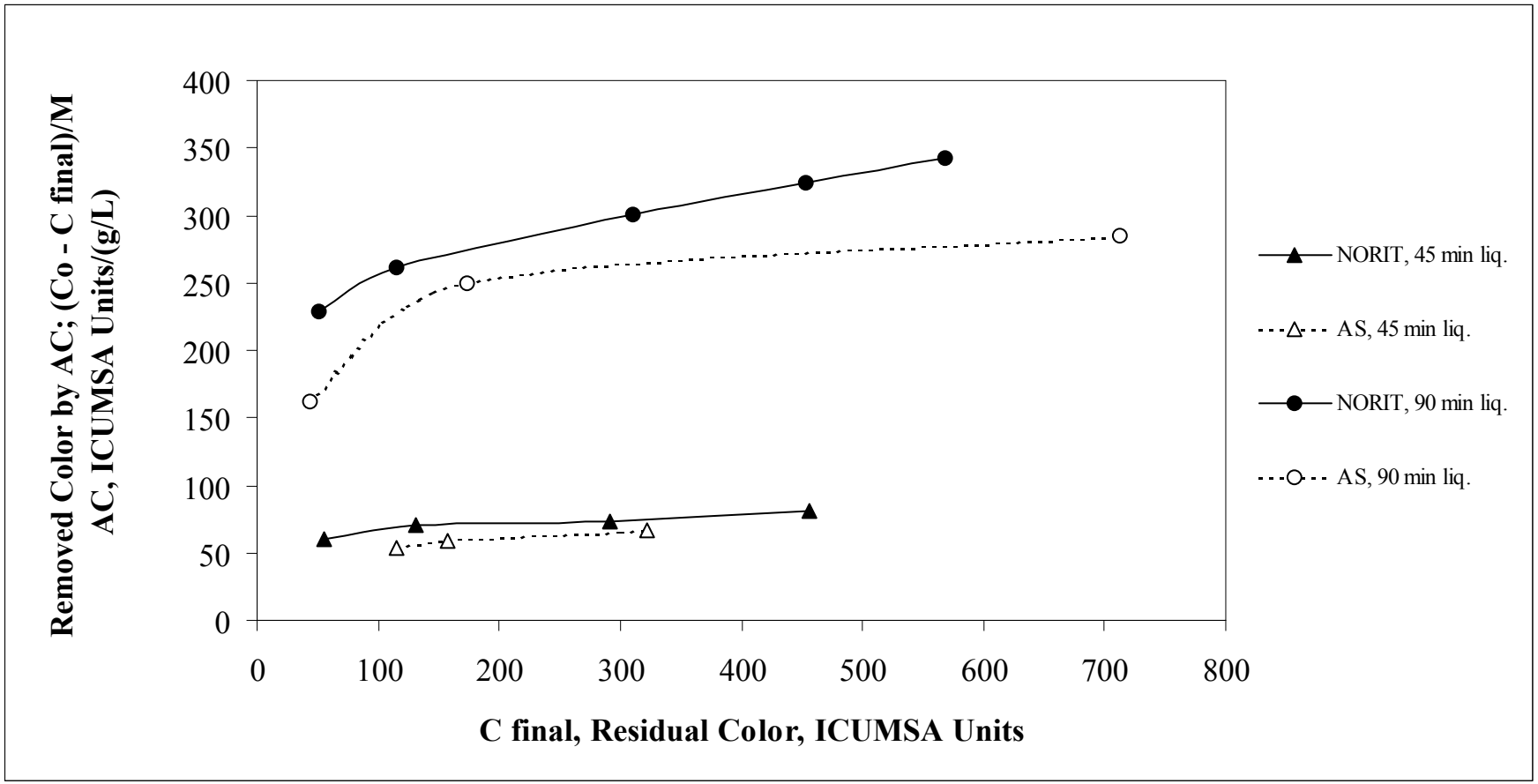

Fig. 5.13 Glucose Syrups Decolorization Isotherms for NORIT and AS

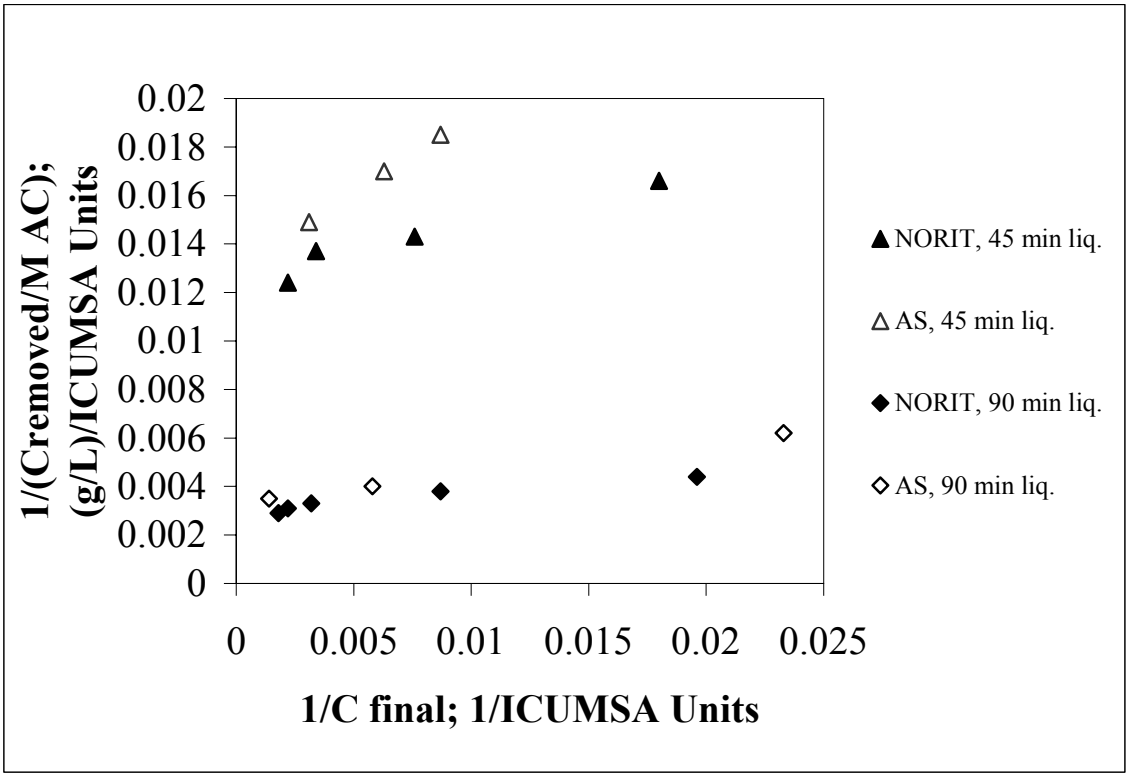

Fig. 5.14 Langmuir Plots for NORIT and AS

Table 5.3 The Langmuir Isotherm Parameters for AS; q_0, K and R^2 Values

Liquefaction Time, min	q_0	K	R^2
45	77.52	49.89	0.99
60	-	-	-
75	-	-	-
90	303.30	37.58	0.99

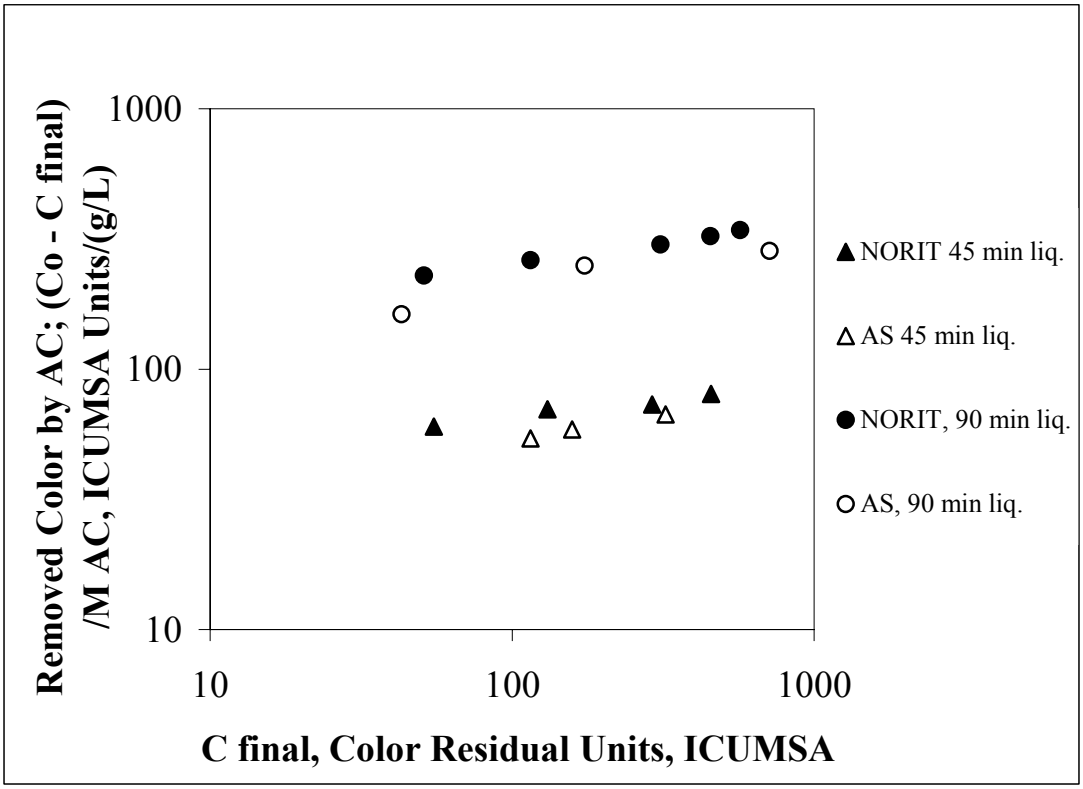

Fig. 5.15 Freundlich Plot Isotherms for NORIT and AS

Table 5.4 The Freundlich Isotherm Parameters for AS; K, 1/n and R^2 Values

Liquefaction Time, min	K	1/n	R^2
45	21.69	0.20	0.98
60	-	-	-
75	-	-	-
90	81.28	0.20	0.91

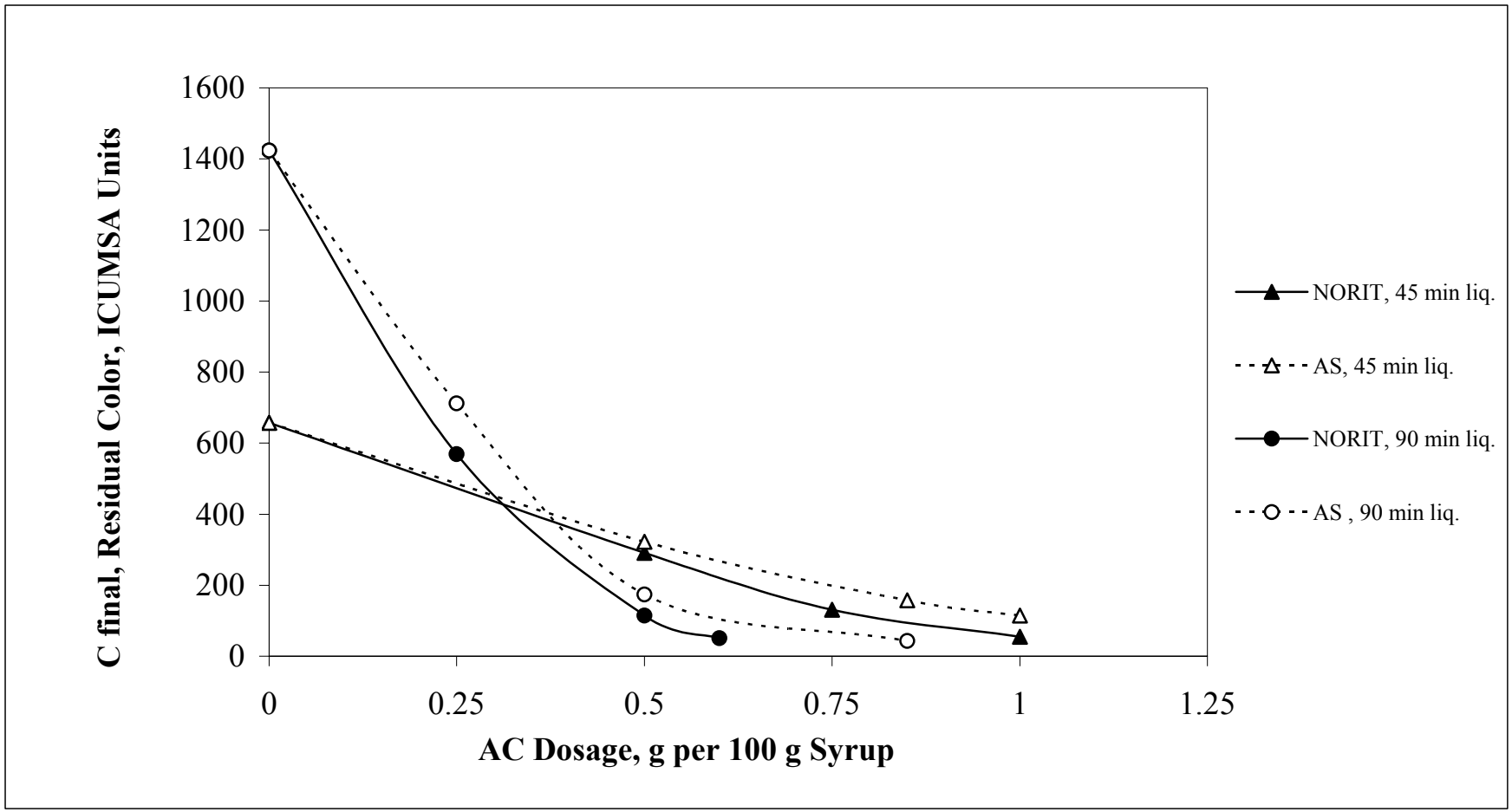

Fig. 5.16 Residual Color of Glucose Syrups after Decolorization by NORIT and AS

CHAPTER 6

CONCLUSIONS

When starch was hydrolysed at different liquefaction times to give maltodextrin syrups and then for 18 h to give glucose syrups, it was seen that increase in liquefaction time resulted in higher % conversion values of both products. As expected, color levels of glucose syrups increased when liquefaction time was increased. Glucose syrups that were produced at 90 min liquefaction time which had the highest amount of color, however, required the smallest amount of activated carbon for the same final color with all types of activated carbons. This was due to the increased molecular weight of colored substances during Maillard reaction**,** for which the activated carbons were expected have increased affinity. When the adsorption capacities of the commercial product, NORIT, and the hazelnut or apricot stone based ones were compared, it was seen that hazelnut husk based carbons were as good as NORIT, while adsorption capacities of apricot stone and hazelnut shell based carbons were similar to each other and somewhat less than those of NORIT and hazelnut husk based carbons. This result showed that agricultural waste based activated carbons could be good alternative to commercial ones for the decolorization of glucose syrups.

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APPENDIX A

BUFFER SOLUTIONS

Preparation of buffers:

pH: 6.5 42 ml 0.2 M KH_2PO4, 58 ml 0.2 M Na_2HPO_4

pH: 4.5 23.3 ml 0.04 M H_3PO_4, 23.3 ml 0.04 M HAc,
23.3 ml 0.04 M H_3BO_3, 30 ml 0.2 M NaOH

APPENDIX B

NELSON-SOMOGYI METHOD

The samples were diluted about 0.002. 2 ml of solution which was prepared by mixing 1 volume of solution B and 4 volumes of solution A, was mixed with the 2 ml of hydrolysis sample. A blank solution was prepared by 2 ml of distilled water. All samples were run in three parallels. The blank and solutions were heated at the bath at 97 ºC for 20 minutes, then solutions allowed for cooling. After cooling, 1 ml of solution C was added to each of them. Blank solution was used for calibration of the spectrophotometer. Then the absorbance values of samples were recorded at 520 nm.

SOLUTION A: 12 g Na-K Tartarate, 24 g Na_2CO_3, 16 g $NaHCO_3$,
144 g Na_2SO_4, Complete this solution to 800 ml

SOLUTION B: 10% $CuSO_4$, 4 g $CuSO_4$, 36 g Na_2SO_4
Complete this solution to 200 ml

SOLUTION C: 5 g Ammonium Molibdate, 0.6 g $Na_2HAsO_4.7H_2O$,
90 ml of distilled water, 5 ml of distilled water,
4.2 ml H_2SO_4, Wait 25 minutes, at 55 ºC water bath

The data obtained from spectrophotometer was in absorbance values which were converted to concentration values by the calibration graph given in Fig. B.1

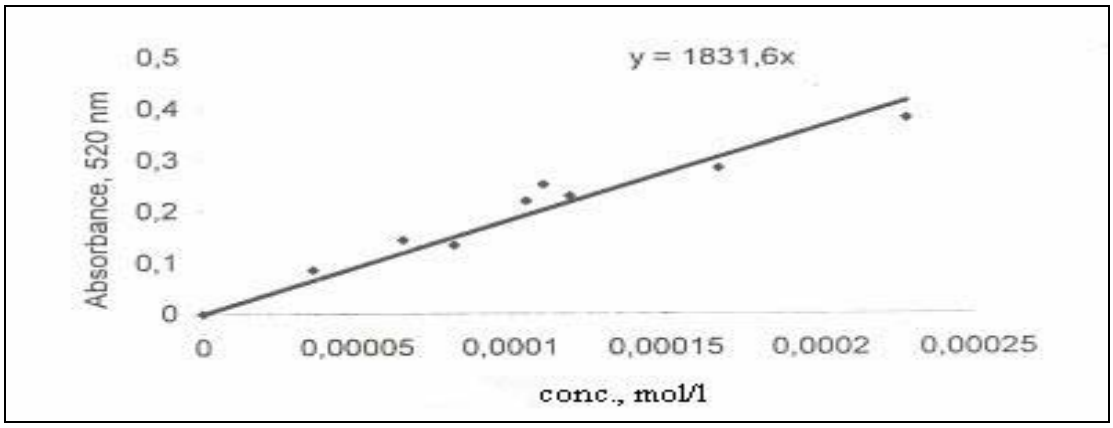

Fig. B.1 Calibration Graph of Glucose

Conversion values were expressed as the ratio of reducing ends (total oligosaccharide amount) to maximum possible reducing ends (glucose initially present in starch).

Glucose Initially Present in Starch (gmol): $\frac{StarchAmount(g)}{0.9} * \frac{1}{180g/gmol}$

$$\%\ Conversion : \frac{Total\ Oligosaccharide\ Amount\ (gmol)}{Glucose\ Initially\ Present\ in\ Starch\ (gmol)}$$

APPENDIX C

Table C.1 Percentage Conversion in Maltodextrins and Glucose Syrups

	Percentage Conversion; %	
Liq. time, min	Maltodextrins	Glucose Syrups
45	9	72
60	13	88
75	19	95
90	21	98

Table C.2 Color of Glucose Syrups

Liq. time, min	Color, ICUMSA Units
45	657
60	990
75	1258
90	1424

APPENDIX D

Table D.1 Optimum Shaking Time for Decolorization of Glucose Syrups

Decolorization time, min	Decolorization%
10	51.07
15	60.03
20	64.46
25	67.41
30	68.19

Table D.2 Decolorization Percentage of Glucose Syrups by NORIT

Decolorization Percentage				
	Liquefaction Time, min			
NORIT%	45	60	75	90
0.25	30.59	46.48	55.02	60.04
0.30	-	-	-	68.19
0.37	-	-	-	78.25
0.50	55.68	74.46	84.20	91.94
0.60	-	84.07	90.83	96.43
0.75	80.09	91.06	94.48	-
0.83	-	94.00	96.17	-
1.00	91.58	-	-	-

Table D.3 Glucose Syrups Decolorization Isotherms for NORIT

Glucose Syrups			
Liq. time, min	M AC, g/L	C_f, ICUMSA Units	$(C_o-C_f)/AC$, ICUMSA Units/g/L
45	2.5	456	80.4
	5.0	291	73.2
	7.5	131	70.1
	10.0	55	60.2
60	2.5	530	184.0
	5.0	253	147.4
	6.0	158	138.7
	7.5	89	120.1
	8.3	59	112.2
75	2.5	566	276.8
	5.0	198	212.0
	6.0	115	190.5
	7.5	69	158.5
	8.3	48	145.8
90	2.5	569	342.0
	3.0	453	323.7
	3.7	310	301.1
	5.0	115	261.8
	6.0	51	228.8

Table D.4 Langmuir Plots for NORIT

Liq. time, min	1/Cfinal; 1/ICUMSA Units	1/(C removed)/M AC; (g/L)/ICUMSA Units
45	0.0022	0.0124
	0.0034	0.0137
	0.0076	0.0143
	0.0182	0.0166
60	0.0019	0.0054
	0.0040	0.0068
	0.0063	0.0072
	0.0112	0.0083
	0.0170	0.0089
75	0.0018	0.0036
	0.0051	0.0047
	0.0087	0.0053
	0.0145	0.0063
	0.0208	0.0069
90	0.0018	0.0029
	0.0022	0.0031
	0.0032	0.0033
	0.0087	0.0038
	0.0196	0.0044

Table D.5 Residual Color of Glucose Syrups after Decolorization by NORIT

Liq. time, min	AC%	C_f, ICUMSA Units
45	0.00	657
	0.25	456
	0.50	291
	0.75	131
	1.00	55
60	0.0	990
	0.25	530
	0.50	253
	0.60	158
	0.75	89
	0.83	59
75	0.00	1258
	0.25	566
	0.50	198
	0.60	115
	0.75	69
	0.83	48
90	0.00	1424
	0.25	569
	0.30	453
	0.37	310
	0.50	115
	0.60	51

APPENDIX E

Table E.1 Comparison of Performance of Activated Carbons with That of NORIT at the Dosage Required by NORIT to Reduce the Final Color to 100 ICUMSA Units

	Cf, ICUMSA	
	Liquefaction time, min	
Type of AC	45	90
NORIT	100	100
HH	-	101
HS	148	178
AS	158	174

Table E.2. Decolorization Percentage of Glucose Syrups by AS

	Decolorization Percentage, %	
	Liquefaction time, min	
AC%	45	90
0.25	-	49.99
0.50	51.02	87.80
0.85	75.93	96.98
1.00	82.50	-

Table E.3 Glucose Syrups Decolorization Isotherms for AS

Glucose Syrups			
Liq. time, min	M AC, g/L	C_f, ICUMSA Units	(C_o-C_f)/MAC, ICUMSA Units/g/L
45	5.0	322	67.00
	8.5	158	58.71
	10.0	115	54.20
90	2.5	712	284.80
	5.0	174	250.00
	8.5	43	162.47

Table E.4 Langmuir Plots for AS

Liq. time, min	1/Cfinal; 1/ICUMSA Units	1/(C removed)/M AC; (g/L)/ICUMSA Units
45	0.0031	0.0149
	0.0063	0.0170
	0.0067	0.0185
90	0.0014	0.0035
	0.0058	0.0040
	0.0233	0.0062

Table E.5. Residual Color of Glucose Syrups after Decolorization by AS

Liq. time, min	AC%	C_f, ICUMSA
45	0.00	657
	0.50	322
	0.85	158
	1.00	115
90	0.00	990
	0.25	712
	0.50	174
	0.85	43