HEMICELLULOSE EXTRACTION FROM AGRO-FOOD INDUSTRIAL WASTES AND ITS APPLICATION IN FOODS

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

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Hemicellulose is a valuable component of agro-food industrial wastes. Although there are some potential usage areas of hemicelluloses such as drug manufacture, encapsulation, and emulsification in food processing plants, they are not extensively utilized. In this study, effective extraction methods of the hemicelluloses from agro-food industrial wastes (corn peels and sugar beet pulp) and their application as coating material for banana fruits were investigated. Firstly, the effects of raw material type, particle size, and extraction conditions (alkaline concentration, temperature, time) and methods (direct alkaline extraction, alkaline extraction after component removal, and acidic extraction method) on hemicellulose yields were investigated. Sugar beet pulp and corn wastes were used as raw material. Alkali extraction resulted in 40.2% pure

extracts while acidic hydrolysis gave only 27.4% purity in the same extraction conditions. The optimal extraction conditions were found as 30°C temperature, 10% NaOH, and 24 h time (64.3%). It was also observed that removal of constituents such as fat, protein, starch, and soluble sugar increased the purity of hemicellulose from 40.2 to 58.2% at the same conditions.

After finding optimum hemicellulose yield for practicle use, it was used as edible coating material to prevent darkening of banana fruits and any quality losses. On the fourth day of storage at 4 ºC, there was no detected fungal decay of coated bananas; however, 20% of uncoated bananas (control) were infected. Moreover, the control group lost 5.1% of total weight but coated samples with 1%, 1.5%, and 2% hemicellulose (HC) lost 3.6%, 3.3%, and 3.1% of their total weight, respectively. Hemicellulose coating also protected firmness of bananas (701.1 gf for coated and 509.6 gf for uncoated samples at the end of 4th day). Color was another important quality parameter and it was showed that lightness and yellowness of coated bananas were preserved with very little losses at the end of storage period while control samples turned brown at an unacceptable level.

The results indicated that using both low alkaline concentration and low temperature allows to recover higher quality extracts. In addition, hemicellulose showed a characteristic of a good edible coating material for banana in order to preserve their visual acceptance and other quality parameters.

Keywords: Extraction, hemicellulose, optimization, purity, yield, edible coating, banana

ÖZ

ENDÜSTRİYEL TARIMSAL-GIDA ATIKLARINDAN HEMİSELÜLOZ ÖZÜTLENMESİ VE GIDALARDA KULLANILMASI

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Hemiselüloz tarımsal gıda atıklarının değerli bileşenlerinden biridir. Gıda işlemlerinde ilaç üretimi, kaplama ve emülsiyonlaştırma gibi kullanım alanları olmasına rağmen, hemiselüloz yaygın olarak kullanılmamaktadır. Bu çalışmada, hemiselülozun tarımsal gıda atıklarından (mısır kabuğu ve şeker pancarı küspesi) özütleme yöntemleri ve muzda koruyucu kaplama maddesi olarak kullanımı araştırıldı. İlk olarak, hammadde türünün, parçacık boyutunun, özütleme şartlarının (alkali yoğunluğu, sıcaklık, zaman) ve özütleme yöntemlerinin (doğrudan alkali ile özütleme, bileşenlerine ayırdıktan sonra alkali ile özütleme ve asit ile özütleme) hemiselüloz verimi üzerine etkileri araştırıldı. Şeker pancarı küspesi ve mısır atıkları hammadde olarak kullanıldı. Alkali ile özütleme %40.2 saf özüt verirken, aynı koşullarda asitle özütleme sadece %27.4 saflık verdi.

Optimum koşullar (%64.3), 30 ºC sıcaklık, %10 NaOH ve 24 saat süre olarak belirlendi. Ayrıca, Aynı koşullarda özütleme öncesi yağ, protein, nişasta ve çözünen şeker gibi bileşenlerin ayrılması hemiselüloz saflığını %40.2'den %58.2'ye artırdığı gözlendi.

Pratik bir kullanım için optimum hemiselüloz verimi bulunduktan sonra, hemiselüloz, muzda kararma ve diğer kalite kayıplarını önleyici, yenebilir kaplama maddesi olarak kullanıldı. Dört günlük 4 ºC'de saklama sonucunda kaplanan muzlarda hiç bozulma gözlenmezken kaplanmamış muzların %20'sinin bozulduğu görüldü. Ayrıca, kaplanmamış muzlarda (kontrol grubu) %5.1 toplam kütle kaybı olurken, bu kayıp %1, %1.5 ve %2 hemiselüloz (HS) ile kaplı örneklerde sırasıyla %3.6, %3.3 ve %3.1'dir. Hemiselüloz kaplama muzun dokusunu ve dayanıklılığını da korudu (4. gün sonunda ortalama kaplanmış örnekler 701.1 gf ve kaplanmamışlar 509.6 gf). Bir diğer önemli kalite parametresi olan renk ölçümlerine göre ise parlaklık ve sarılık kaplanmış muzlarda ufak kayıplar dışında korunurken, kaplanmamış muzlarda tamamen kayboldu.

Sonuçlar, düşük alkali yoğunluğunun ve düşük sıcaklığın daha yüksek verimde özütler elde etmeyi sağladığını gösterdi. Ayrıca, hemiselüloz, meyve ve sebzelerin görsel kabul ve diğer kalite parametrelerini korumada iyi bir yenebilir kaplama maddesi özelliği gösterdi.

Anahtar kelimeler: Özütleme, hemiselüloz, optimizasyon, saflık, verim, yenebilir kaplama, muz

Dedicated to my beloved husband

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INTRODUCTION

1.1 Agricultural Waste

Agricultural waste is named as by-products of agricultural activity because they are not the primary products. US Environmental Protection Agency describes the agricultural waste as a waste including harvest remains from grain, oilseed, vegetable, and orchard crops. It also includes manure and any output from poultry or other livestock operations in either solid or liquid form. Agricultural wastes are all a potential resource because they are widely available, virtually free, and renewable; thus, they have multiple uses, both on and off the farm. They can be converted into heat, steam, charcoal, methanol, ethanol, bio diesel. Moreover, they can be used as raw materials for animal feed, composting, energy and biogas generation and so on (Sabiiti, 2011).

Agricultural wastes, which are the residue of crops such as wheat, barley, corn, sugar beet, tobacco, cotton, rice, etc, have great impact on environment due to the amounts generated and the disposal methods used. Many of them are not still utilized and are usually left to rot or openly burned. These disposal practices cause environmental pollution. Although, uncontrolled burning of waste on farms became illegal on 15 May 2006 in UK and EU, agricultural waste burning is still a common practice in undeveloped countries. Agricultural waste burning is a source

of atmospheric pollution because it releases pollutants such as carbon monoxide, nitrous oxide, nitrogen dioxide and particles which are accompanied by the formation of ozone and nitric acid; hence, contributing to acid deposition thereby posing risk to human and ecological health. In order to prevent inappropriate waste treatment and its harmful effects, a greater awareness of the public and farmers about proper management and utilization of organic wastes in agriculture are strongly needed. Utilization of agricultural wastes should be the main goal because they have wide variety of usage areas. For example, they can be used as enhance of food security, bio-fertilizer, animal feed, and raw material for energy production. Due to their large amount of organic content, they can be directly added to soil without any risk (Sabiiti, 2011).

There are some present agricultural waste treatments. One of them is composting which reduces volume of waste and solves serious environmental problems such as wiping out large quantities of waste, killing pathogens, and reducing offensive odor. The other treatment is using both crop residues and animal waste as animal feed. After awareness of agricultural wastes having some valuable products, a great demand to find other end-uses for these wastes emerged especially in Europe and North America (Sabiiti, 2011).

There are two widespread agricultural wastes in Turkey. One of them is corn wastes. According to TUIK data, in 2011, 4.3 million tons of corn was produced. When corn composition is investigated, 48% of corn is observed as waste because only 52% of total plant is corn grain and the remaining ones are corn cobs, corn peel and, others. This means that 2 million tons of corn waste is produced every year. The other commonly produced agricultural waste is sugar beet pulp which produced 18 million tons in Turkey (TUIK, 2011). After processing, at least, 6 million tons of them were separated as sugar beet pulp.

1.1.1 Corn Wastes

Corn is a one seeded plant and corn grain is a caryopsis. Different species of corn grain have different composition, size and structure. Generally, the upper part of the corn is more digestible due to less lignin content (Inglett GE, 1970).

Corn wastes include stalks, peel (leaves and husks), and cobs which are the by-products of corn industry after harvesting and corn starch processing. Harvesting method, time, and genetic composition are the depending factor for the composition of corn by-products. These wastes are usually used as animal feed, bio-based products, and fuels. Recently, new technologies are experienced the usage of corn wastes as a lignocellulosic biomass and liquefaction; for example, resin was produced from corn bran with liquefying method using sulfuric acid at high pressure and temperature (Lee et al., 2000).

Corn fiber is another important and valuable by-product especially of corn wet-milling industry. It is a heterogeneous complex of carbohydrate polymers and lignin like the other lignocellulosic materials and composes of 10% of dry weight of the processed grain. Therefore, corn fiber is a renewable lignocellulosic biomass resource with sufficient quantities. Corn fiber and other lignocellulosic materials such as agricultural residues, herbaceous, woody plants, and municipal solid wastes gained importance after decreased fossil fuels and increased air pollution because they are renewable, abundant and inexpensive (Moniruzzaman et al., 1996).

Production of corn fiber is 4-5 million tons per year for wet milling alone in US. This abundant and low value by-products prices 60$ per ton; therefore, utilization of corn fiber is crucial for profit and sustainability (Rajagopalan et al., 2005).

Destarched corn fiber is a good source of soluble dietary fibers which controls blood cholesterol levels. They bind to cholesterol containing bile acids in gastrointestinal track and throw them away from the body as a waste. Corn fiber also contains valuable products such as cellulose and hemicellulose mainly composed of arabinoxylan as a raw material for corn fiber gum, emulsifiers, film formers, non-toxic adhesives, thickeners, paper additives, and stabilizers. Carbohydrates in corn fiber can be converted to fermentable sugars such as glucose and xylose. There are also some studies about production of soluble dietary fibers from corn fiber in order to use for human consumption as a functional food and functional food ingredient (Wang et al., 2008).

Corn production in North America is 42% of total corn production in the world. Asia and Europe follow them with 26% and 12% production. Corn wastes generally used as animal feed and remaining parts are lost in farm and during storage, handling, and transportation (FAOSTAT, 2010). FAOSTAT (2010) results also show that Turkey has the highest corn production capacity in Europe after France and Ukraine with a value of $4x10^5$ million $ of 4.3 million tons corn production annually. This means that Turkey has a high potential to use corn by-products in biotechnological fields. For instance, in USA, by-products of corn production are very valuable especially in bioethanol production and 400 L bioethanol/ton corn is produced (Shapouri et al., 2002). There are also some studies in Turkey about possible biomass resources. The most advantageous resources are selected according to agricultural properties, bioethanol producing properties, and financial properties. Under the light of these properties, Bulut (2006) records that wheat, corn, and sugar beet were more advantageous biomass.

1.1.2 Sugar Beet Wastes

The aim of sugar beet processing is to extract sucrose in the beet and change it into sugar crystals. There are five fundamental processing steps of sugar production from sugar beet. These are beet preparation, sugar extraction, juice purification, juice concentration, and crystallization (Barjol and Chavanes, 2003).

During traditional sugar beet processing practices, production of large amounts of wastes, by-product, and high consumption of energy, water, and lime cause environmental problems (Vaccari et al., 2005). Waste water, exhausted beet pulp, molasses, lime sludge, adhering soil, stones, and beet leaves are common by-products of sugar beet processing operation (Krajnc et al., 2007).

Large volume of water is needed for the manufacture of sugar from sugar beet. Waters used for cooling, cleaning, and pulp-pressing are sources of wastewaters in sugar beet industry. In addition, flume water representing 70% of the total waste volume is the main source of wastewaters (Dilek et al., 2003). Flume water contains high concentration of hydrocarbons and soluble organic matter because beet constituents are leached from cut and damaged surfaces into the flume water. Therefore, biological treatment after sedimentation is the most common treatment strategy for sugar beet industry (Shore et al., 1984). Because of presence of high concentration of hydrocarbons, anaerobic digestion is an effective biological treatment (Wang et al., 1986). Hence, scientists have numerous studies concentrated on anaerobic digestion of sugar beet processing wastewater (Shore et al., 1984, Wang et al., 1986, Iza et al., 1990, Farhadian et al., 2007).

One of the important by-products of sugar beet industry is beet-pulp remaining after sugar extraction. Approximately 250 kg of exhausted pressed pulp with 75-

80% water content is produced during 1 ton of beet processing. Sugar beet pulp contains 24-32% pectin, 24-32% hemicellulose, 22-30% cellulose and 3-4% lignin (Spagnuolo et al., 1997). The countries with an intensive cattle-raising industry put beet pulp to use for animal feed as a part of feed product compound or fed directly (Hutnan et al., 2000). On the other hand, some other countries do not utilize beet pulp and just dump it in landfills (Voragen et al., 1997). However, due to suitable composition of beet pulp for biological degradation, anaerobic digestion can be a possible alternative for the utilization of it (Weiland, 1993, Hutnan et al., 2001, Koppar and Pullammanappallil, 2007). After realizing the hemicellulose is a valuable component, extraction of hemicellulose from sugar beet pulp gains importance due its considerable high content of hemicellulose. One of the earliest study shows various procedures such as using a few different alkali concentration, treatment temperature, and particle size of raw material for extracting and isolating hemicellulose from sugar beet pulp. The yields of 8-16% hemicellulose can be obtained from sugar beet pulp and hemicellulose has a good potential for using as a food ingredient; for instance, it can be used as a bulking agent in food product formulation (Wen et al., 1988). Acidic hydrolysis of sugar beet by-products is also used to recover valuable monosaccharide such as xylose from hemicellulose because sugarcane hemicellulose is an important source of xylose which is utilized for production of ethanol, xylitol and single cell protein. Researches on utilizing polysaccharide components of sugar beet pulp still continue in order to create new functional food, non-food biomedical and industrial bioproducts and to establish sustainable and efficient isolation and conversion processes (Savary et al., 2004).

1.2 Hemicellulose

Hemicellulose is non-cellulosic cell wall polysaccharide of plants. It is a significant renewable resource of biopolymers comprising 50% of the biomass.

Hemicelluloses have an immense variety of structural types. Although scientist indicated their application potential many times, they have not processed extensively on an industrial scale. However, future shortage of energy sources, environmental problems due to petroleum based products, demands for healthy food, and alternative medicine increase the activities in research of hemicelluloses and other polysaccharides. It is expected that utilization and conversion of the hemicelluloses to useful products provide a fundamental solution to the problems mentioned above (Fischer and Heinze, 2006).

1.2.1 Chemical Structure

Hemicelluloses belong to the building components of the cell walls of higher plants together with cellulose and pectic polysaccharides (Waldron et al., 2003). Hemicellulose components may differ in content and structural features depending on the plant species and the present cell walls. Hemicelluloses have four general groups of structurally different polysaccharide types (Ebringerova et al., 2005):

(1) Xyloglycans (Xylans),

(2) Mannoglycans (Mannans),

(3) Xyloglucans, and

(4) Mixed-linkage β-glucans.

They have different structure with variation in side chain types, localization types, and distribution of glycoside linkages in main macromolecular chain (Ebringerova et al., 2005).

Xylans belong to the most abundant hemicellulose type in the plant kingdom. They can be grouped in to several structural sub-classes such as homoxylans, glucuronoxylans, arabinoxylans, and heteroxylans. Homoxylans are linear polysaccharides linked by β- (1→3) and β- (1→4) linkages. They are common in red and green algaes and rarely isolated from higher plants such as guar seed husks (Ebringerova et al., 2000). Glucuronoxylans are also represented as D-glucurono-D-xylan having single side chains of α-D-glucuronic acid. They can be isolated from fruits such as grape skins and hulls of Jojoba beans. Slightly branched types are water soluble due to acetylation of the xylan chains and highly branched types become slightly soluble by alkali solvents (Ebringerova et al., 2005). Arabinoxylans are typical hemicellulose components of starchy endosperm and outer layers of cereal grains. Arabinoxylans have (1→4) –xylopyranan backbone and attached α-Araf residues. The fine structure of them and different substituted frequencies cause large structural variety and affect the solubility. The last group of xylans is heteroxylans which have very complex structures and isolated from cereal bran, seeds, and gum exudates (Ebringerova et al., 2000).

Mannan-type hemicelluloses divided into two groups such as galactomannans and glucomannans & galactoglucomannans according to backbone structure. Galactomannans have backbone made up of β- (1→4) –linked mannopyranose units. Slightly branched ones are water insoluble whereas higher-branched types are water soluble and commercially used as guar gum and tara gum. Glucomannans and galactoglucomannans, on the other hand, possess both β-(1→4) –linked mannopyranose and glucopyranose residues in the main chain. They can be easily extracted by alkaline treatment and mainly found in soft woods, herbal plants, and grasses (Ebringerova, 2006).

Xyloglucans are major building material of higher plants and function as a storage polysaccharide. They have a cellulosic β- (1→4) –glucopyranan backbone with α-

D-Xylp residues. This hemicellulose type has structural diversity due to distribution of Xylp branches and additional galactose, arabinose, and xylose chains. Xyloglucans are extractible from seeds by hot water treatments. The last hemicellulose type is Mixed-linkage β-Glucans which have an unbranched backbone with the mixed linkage of (1→3) and (1→4) –β-D-glucans. Fine structure and different blocks arrangements cause different extractability by water and alkali (Ebringerova, 2006).

Hemicellulose is usually classified as Hemicellulose A and Hemicellulose B. Hemicellulose A (M_w < 25000 g mol^{-1}, water soluble at pH > 10) is less water soluble with linear chains while hemicellulose B (M_w > 500000 g mol^{-1}) is water-soluble and has branched yellowish precipitate (Kulkarni et al., 1999).

1.2.2 Isolation Methods

Lignocellulosic material consists of mainly three different types of polymers (lignocelluloses), namely cellulose, hemicellulose and lignin which compromise a large fraction of municipal solid waste, crop residues, animal manures, forest residues, and dedicated energy crops (Sims, 2003). The digestibility and enzymatic hydrolysis of hemicellulose and cellulose present in lignocellulosic biomass are limited by crystallinity of cellulose, lignin content, and accessible surface area (Chang and Holtzapple, 2000). Moreover, solubilization of lignocellulose components depends on temperature, moisture content and pH (Fengel and Wegener, 1984). Therefore, numerous studies have been conducted on pretreatment of lignocellulosic biomass in order to increase solubilization of lignocelluloses components, increase yields of fermentable sugars from cellulose and hemicellulose, and improve the rate of enzymatic hydrolysis.

There are various pretreatment methods used commonly for lignocellulosic biomass such as thermal (Ramos et al., 1992; Lawther et al., 1996; Ruiz et al., 2008), mechanical (Sidias and Koukios, 1989; Delgenes et al., 2002), chemical such as acid (Torget et al., 1991; Sanchez et al., 2004; Karimi et al., 2006; Silverstein et al., 2007), microwave (Park et al., 2004; Eskicioglu et al., 2007), ozone (Silverstein et al., 2007), and alkali (Playne, 1984; Vaccarino et al., 1987; Zhu et al., 2005 and 2006; Saha and Cotta, 2007; Silverstein, 2007; Zhao et al., 2007), and combination of thermal-acid (Emmel et al., 2003), thermal-alkali (Playne, 1984), microwave-alkali (Zhu et al., 2005 and 2006), microwave-acid-alkali (Zhu et al., 2006).

Hemicellulose in the plant cell wall is bound to cellulose and lignin. In order to isolate hemicellulose, separation of cellulose and lignin from plant raw material is needed. The composition of hemicellulose depends on sources, origin, and growth stage of feedstock and the composition of the extracted hemicellulose depends on isolation process. A number of methods including alkali, acid, dimethyl sulfoxide, water extraction as well as microwave and steam treatment are used to obtain hemicellulose from plant sources (Lindblad and Albertsson, 2005).

Alkali treatment of lignocellulosic substances of corn fiber disrupts the cell wall because alkali dissolves hemicellulose and lignin, hydrolyzes uronic and acidic acid esters, and lastly, causes swelling of cellulose. Thus, crystallinity of cellulose decreases and biodegradability of the cell wall increases due to cleavage of bonds between lignin and cellulose (Gaspar et al., 2007). For alkali treatment of hemicellulose, potassium hydroxide (KOH) (Eaks and Sinclair, 1980; Gaspar et al., 2007), sodium hydroxide (Wen et al., 1988; Gabrielii et al., 1999; Gaspar et al., 2007), alkaline hydrogen peroxide (Doner and Hicks, 1997; Maes and Delcour, 2001) can be used. Acid-hydrolysis is a water-based method that can also be used to solubilize hemicelluloses; for example, before the cooking stage,

hemicellulose can be extracted from wood chips. This method is usually carried out with organic acids at high temperatures and produces high yields of soluble sugars, mostly monosaccharides in a few seconds or minutes (Mendes et al., 2010). The other method is extraction of water-soluble hemicellulose with microwave-assisted heat treatment. Benkö et al. (2007) used corn fiber as a raw material for this extraction method. Microwave based heat extraction method can be a novel environmentally friendly means of hemicellulose isolation due to use of non-chemicals but it provides lower molecular weight and lower polymer recovery. Therefore, alkali method is more commonly researched for hemicellulose extraction.

1.2.3 Usage Areas

In the past, research was based on utilization of hemicellulose after converting into sugars, chemicals, fuel and as sources of energy. On the other hand, recent studies in the field of hemicellulose have been aimed to utilize it in their native or modified forms in various areas such as food and non-food applications (Ebringerova, 2006). For this aim, research has intensified because of gel-forming and film-forming properties of hemicellulose which have found in numerous applications in areas such as cellular therapy, drug delivery, emulsification, and encapsulation (Zhang et al., 2005). Xyloglucan from tamarind seed is an example of an extensively used hemicellulose utilized in drug manufacture (Coviello et al., 2007).

The pharmaceutical industry is an important application area of some hemicelluloses such as konjac glucomannan and guar gum which have been used commercially for many years. The hydrogels from xylan in combination with chitosan are used for biomedical purposes because ingested nanopaticles coated with xylan are capable of withstanding acidic media. Thus, it ensures passage to

the colon of a patient for the usage of nanoparticles as magnetic markers (Hansen and Plackett, 2008). In spite of the fact that there are some considerable research on hemicelluloses for biomedical purposes, the increasing need for specialized medicine and forms of customized drug delivery require new applications.

Food industry applications for hemicellulose include packaging films and food coating. Because of growing public awareness of environmental issues and the advantages in product marketing, food packaging material derived from sustainable resources is receiving interest. Low oxygen permeability and good mechanical properties in terms of flexibility and high strength are key requirements for food packaging materials. Hemicellulose-derived packaging materials have desirable oxygen permeability values when compare favorably with those of other biopolymers such as amylopectin, amylose, and chitosan. Recent patent applications on oxygen barriers from hemicellulose are also available (Hansen and Plackett, 2008). Furthermore, moisture barriers in the form of edible coatings are produced from hemicellulose with an only one obstacle which is the hygroscopic nature of the initial material. Increased hydrophobicity by either chemical modification or addition of hydrophobic compounds (emulsification) may be a solution for the problem of poor moisture barrier properties. As an example, Kittur et al. (2001) have used glycerol monostearate and Tween-80 to emulsify and improve wettability. In addition, there is a significant amount of research about modifying hemicellulose chemically to get stronger, more hydrophobic, and elastic products thereby finding a variety of potential application such as in selective membrane materials (Hansen and Plackett, 2008). For instance, esterification was used to generate hydrophobic coatings from beechwood xylan and maize bran heteroxylan (Moine et al., 2004) and to produce composite coating material of esters of arabinoxylan and cellulose from corn fiber (Bunchanan et al., 2003).

In the patent literature, many different application of hemicellulose can be seen. One of them is heteroxylan coatings for seeds and candy claimed to be superior to cellulose-based coatings. Hemicellulose coating can be applied to seeds of corn and soybean by spraying and do not hinder germination of the seeds. The coating may contain additives such as insecticides and herbicides as a barrier for detrimental species (Hansen and Plackett, 2008). Moreover, hemicellulose extracted from maize is used to form edible coating for encapsulation of flavoring and other components in foodstuffs. These coating may be an alternative to gum Arabic in terms of texture, stability, appearance, and texture in the sugar coating of almonds (Hansen and Plackett, 2008). Many different applications of hemicellulose have already been investigated; however, there is still ongoing work in this area and all the possibilities have certainly not been exploited.

1.3 Browning and Darkening Reactions on Fruits and Vegetables

Color has a direct effect on consumer decision to buy a product. Therefore, color is an important quality parameter of the food products. Maintaining the color of some foods such as fruits and vegetables is not easy because they are very susceptible to surrounding conditions. One of the important factor that cause browning of fruits and vegetables is polyphenoloxidase enzyme which is able to catalyze the aromatic compounds because of presence of phenolic groups on them. Phenolic compounds have very important functions in plants. For instance, they act as antioxidant and filters to protect the delicate cell structure from harmful effects of surrounding (Hamauzu, 2006). Indeed, they impart vital sensory attributes such as color, taste, and some nutritional properties in foods. When these phenolic compounds are oxidized to reactive oxygen molecules known as quinones, brown spots known as melanin on the fruits surface are formed. This browning cause the deterioration of taste and color of fruits and cause large economical losses (Robbins, 2003).

Oxidation of the phenolic compounds is occurred by polyphenoloxidase enzyme (PPO) which is widespread group of enzyme found in fungi, plants, animals, and bacteria. It has a copper containing active site and this metal ion enable it to oxidize phenolic groups of aromatic compounds. There are two types of PPOs: catecholoxidase and tyrosinase. Catecholoxidase catalyzes an aromatic group with two adjacent phenols (diphenol) to form two quinones while tyrosine can catalyze the two phenolic groups being adjacent to each other and change it to a diphenol (George, 2010).

Many polyphenolic compounds can commonly be found in fruits and vegetables and they act as substrates for PPOs. PPOs oxidize these polyphenols such as caffeic acid, chlorogenic acid, catechin, and epicatechin to quinones which are very reactive. Quinones react with each other and surrounding proteins to generate black melanin pigment which causes dark spots on the plant tissue and frequently makes the fruit and vegetable inedible. Thus, PPO activity causes substantial losses especially in ripening stage when fruits become susceptible. It is reported that PPO occur in all plants and exists in particularly high amounts in potato, mushroom, pear, peach, avocado, apple, and banana (George, 2010).

One of the world's most traded fruit is banana in both fresh and processed forms. The color of the bananas significantly influence market quality and consumer acceptability. Banana peel contains ethylene at the beginning of ripening. Natural acid containing ethylene causes ripening from green to yellow and makes banana sweet. Activity of these natural acids slows down in refrigerator condition (4ºC) so does ripening. However, some physical changes occur in the cell membrane of banana peel at cold temperatures. This increases enzyme contacts with oxygen in the air and yields dark color. That is, cold temperature causes rapid darkening of banana peel but it helps preserving firm and delicious content inside (Ahmad et al., 2007).

There are some simple methods to inhibit enzymatic browning (oxidation) that causes discolorization of fruits and vegetables. One of them is treating fruit and vegetables with ascorbic acid which prevents the reaction of the enzyme with oxygen. The other method is covering fruits and vegetables in order to prevent oxygen contact. For this aim, vacuum packaging, modified atmosphere packaging, and coating are suitable. Inactivation of enzyme by cooking, boiling or other heat treatments is also a way to prevent oxidation. Similarly, freezing can prevent oxidation by slows down the enzyme activity (George, 2010).

1.4 Edible Coating Methods, Formulations, and Applications

Edible films and coatings are covering material and have certain compositions. There is a little difference between a film and a coating because a film is a stand-alone covering material but a coating is performed directly on food surface itself (Ritala and Leskela, 2002). Business on this concept has gained importance in time and nowadays, films and coatings from renewable materials have various potential applications such as wound dressing, active food packaging, and drug capsules in the food and medicinal industry (Hansen and Plackett, 2008). In the past, foods were harvested and consumed immediately and there was no need for extra protection on foods. On the contrary, today, foods are not consumed just in time because of the increased transportation distribution system, storage needs, and supermarkets in the global economy. Therefore, some protection methods such as edible coatings, refrigeration, modified atmosphere packaging, and so on for foodstuffs become necessary to prolong shelf life and maintain texture, taste, and mouthfeel properties. Among these novel preservation methods, edible coatings step forward due to cost effective property and several mechanisms which include improving the natural layer of foods, decreasing moisture loss, controlling gas exchange (CO_2/O_2), reducing respiration rate, providing surface

sterility, and preventing loss of important volatile components (Munoz et al., 2008).

Edible coatings are categorized as hydrocolloids (proteins, cellulose derivatives, pectins, alginates, starches, and other polysaccharides), lipids (waxes, acylgycerols, and fatty acids), and composites (both lipids and hydrocolloids). Hemicellulose is in the group of hydrocolloids and the most investigated polysaccharide, recently. Hydrocolloids are also called gums generally composed of many hydroxyl groups or polyelectrolytes. These hydrophilic polymers are originated from vegetables, animal, or produced synthetically and microbially. They are already present in the food or added to control the function of properties of aqueous foods. There are some special applications of hydrocolloids such as suspension, adhesion, flocculation, foam stabilization, and film forming (Chaplin, 2012). For coating, uniformity is the key factor because non-uniformity decreases the success of application. Three types of possible defects on physical attributes of coatings are shown in Figure 1.1. In the region A, there is non-uniformity due different thickness on the sample; in the region B, non-uniformity occurs due to insertion of bubble; and the region C, mechanical damage of the food is observed.

Edible coatings can be applied to food products by dipping and spraying methods. In dipping method, food is dipped into the coating solution and air dried. Application of this method is not practical for fresh-cut fruits due to difficulty in collecting a good adhesion of the coating to the hydrophilic surface of the cut fruit. Hence, multilayer technique may be an alternative for the coating of minimally processed fruits (Skurtys, 2012).

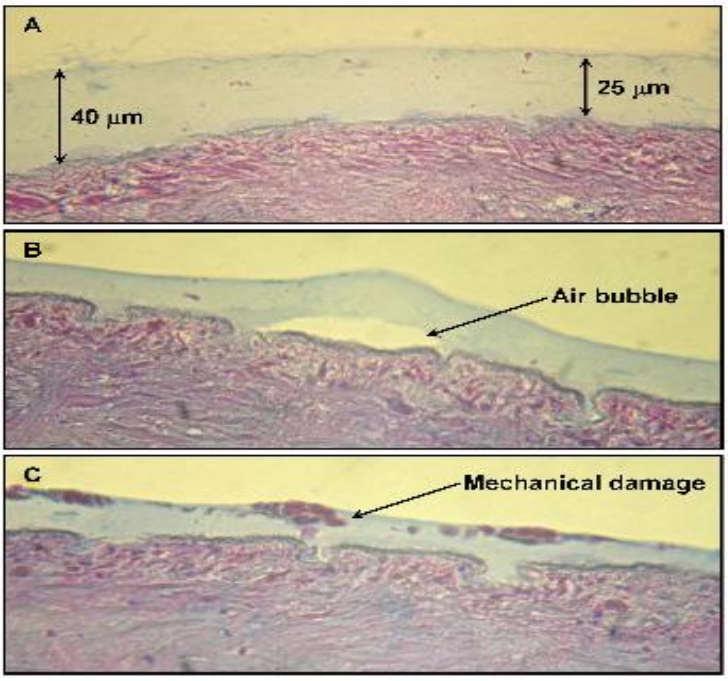

Figure 1.1 Possible defects on physical attributes of coating films

The other coating method is spraying which provides a thin uniform layer unlike dipping. In spraying method, edible coating material is just sprayed to the food surface and air dried. This method can be applied only on desired surfaces of the food and a thin second layer can also be performed by spraying (Skurtys, 2012). Edible coatings can be used for variety of manufactured foods such as fruits, vegetables, meats, poultry, seafood, nuts, cereals, cereal based products, bakery products, salt-type ingredients, and vitamins (Hansen and Plackett, 2008).

Skendi et al. (2003) produced water soluble β-glucans from different types of oats as a coating material. They isolated hemicelluloses by aqueous extraction and treated with acid. Corncob and grass xylans were isolated by alkaline extraction and birchwood xylan was obtained commercially. Peroval et al. (2004) extracted arabinoxylan from maize bran and plasticized with glycerol to use as a coating material. Some modifications were also tested including mixing the

hemicellulose/glycerol with oleic acid, palmitic acid, hydrogenated palm oil, or triolein. Glycerol monosterate was used for emulsification of the lipids and mixtures was treated with a homogenizer.

1.5 Aim of the Project

Hemicellulose is one of the most abundant polysaccharide in nature and various agricultural residues, such as corn peel, corn stover, rice straw, wheat straw, and sugar beet pulp. After realizing hemicellulose as a valuable product, researches about it have accelerated. Different raw materials and isolation methods have been investigated. One of the frequently used hemicellulose extraction method is alkali hydrolysis. However, researchers have generally focused on the following experiments after hemicellulose isolation. For example, Gaspar et al. (2007) investigated the effect of a few alkali treatments on the fermantability of differently pretreated corn fiber instead of their effects on yield and purity of hemicellulose.

Up to now, people have tried different raw materials and conditions for hemicellulose isolation but the effects of only one or two parameters can be observed in a single study where the aim of the researches was the use of hemicellulose as a valuable product. On the other hand, there is still a need for a work which involves different conditions and methods and mainly focuses on the optimal yield and purity of hemicellulose. Nowadays, usage of hemicellulose is a popular subject but good hemicellulose extract before using it is needed.

Therefore, the first aim of this thesis was to gather, combine and compare different conditions to extract hemicellulose, and to be able to analyze their interactions and effects. For this aim, different pretreatments of raw materials and

alkali and acidic methods were used. Then, for the best method, different extraction temperature (30 – 50 ºC), time (24 – 72 h), concentration (5 – 20 %), and particle size (0.8 and 1.1 mm) of raw materials were tried to find optimum conditions. Moreover, this work was not only a hemicellulose extraction but also an economical way to extract high amount of yield. Purity is another important factor. Lastly, in this work, two most commonly used raw materials are compared as a hemicellulose source (corn and sugar beet) and indicated the most effective one in the means of the easiest way and the highest yield.

The second aim of this thesis was to use hemicellulose from renewable sources as an edible coating material on selected fruits. In order to prolong shelf life and maintain important properties such as color, texture, taste, and mouthfeel, edible coatings from natural sources for foodstuffs are often necessary as a air and moisture barrier. Therefore, hemicelluloses have gained interest but there are limited sources especially for formation of edible coating from hemicellulose. Hence, our work showed hemicellulose usage as a coating material to prevent darkening of banana peel. Hemicellulose solution was applied to surface of whole bananas by spraying method. Because banana is the most traded fruit in the world and susceptible to environmental conditions due to high PPO activity, this fruit was used for the experiments. Bananas were stored in the refrigerator (4ºC) and quality analysis such as color, texture, weight loss, fungal decay, and sensory analysis were fulfilled during five days.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Corn Wastes and Sugar Beet Pulp as a Raw Material

Corn peels and corn cobs were collected from local market in Ankara. Sugar beet pulp was purchased from Sugar Factory in Etimesgut, Ankara. All raw materials were dried at 70°C in oven for 24 h. The dried raw materials were ground and stored at 4°C until use.

2.1.2 Fruit Material

Bananas (*Musa acuminata*) were purchased from local markets in Ankara. Before use, they were washed with distilled water and air dried.

2.1.3 Enzyme and Supplier

In order to seperate starch from dried and ground corn wastes, *α-amylase* from *Aspergillus oryzae* was used as an enzyme which was puchased from SIGMA-

Aldrich and stored at refrigerator condition (4ºC). The supplier report for enzyme activity was considered during this study.

2.1.4 Edible Coating Material

To prepare a coating solution, hemicellulose as a polysaccharide derivative and Tween-80 as an emulsifier were used. Hemicelluloses were extracted from corn peel by 10% alkali solution (NaOH) at 30ºC for 24 hours. Then, pH was adjusted to 5. After waiting 1 hour, 2-volume ethanol was added and kept at refrigerator condition (4ºC) for 24 hours. Finally, hemicelluloses were separated by filtration. Isolated hemicelluloses were dried in oven at 40ºC for 24 hours and pounded in mortar to get small and uniform particle size. By this method, hemicelluloses A and B were not separated from each other because for coating application there is no need to use them separately. Moreover, application of this method is not time consuming and more practical when total mass was considered.

Tween-80 was purchased from MERCK and used to emulsify and improve wettability of solution.

2.2 Methods

2.2.1 Chemical Analysis of Raw Materials

Raw materials were tested for moisture, ash, protein, fat, crude fiber, lignin and total carbohydrate.

2.2.1.1 Moisture Content

For moisture content determination, gravimetric method was used (AOAC, 1984). One gram of ground sample was weighed and dried at 105°C until constant weight was reached. Dried samples were placed in desiccators for cooling before recording the final weight. Difference between the final and the initial weight gave the total moisture content of the sample. Analysis was done in duplicate.

Calculation of the moisture content was done by the following formula:

$$\% \text{ Moisture} = \frac{A-B}{A} \times 100 \qquad \textbf{(Equation 2.1)}$$

Where;

A: Initial weight (g) (Weight of wet sample)

B: Final weight (g) (Weight of dry sample)

2.2.1.2 Ash Content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating. Therefore, for ash content determination, firstly, 1 gram sample was dried at 105°C until constant weight was reached. Then, dried samples were burned in an ash oven for 24 h at 550°C (AOAC, 1984). Results of two replicates were considered.

The ash content of the sample was calculated using the following formula:

$$\% \text{ Ash} = [(W_1 - W_2) / W_1] \times 100 \quad \textbf{(Equation 2.2)}$$

Where;

W_1: Initial weight (g) (weight after drying)

W_2: Final weight (g) (weight after burning)

2.2.1.3 Protein Content

Nitrogen content was estimated by Kjeldahl method and multiplied by 6.25 to predict crude protein content (Esteban et al., 2007). According to the method, 1 gram sample was added into the burning tube together with 5 gram K_2SO_4, 0.15 gram $CuSO_4$ and 15 ml H_2SO_4. Ready-to-burn tubes were bedded into burning unit and process continued at 400ºC until blue-green color was observed. After burning, 50 ml distilled water was added into each tubes and tubes were located in the Kjeldahl apparatus. Thus, distillation took place and samples became ready for titration in order to determine not only the amount of NaOH used up but also the amount of nitrogen in the samples. After all, crude protein amount was calculated as follow;

$$\text{Crude Protein \%} = \frac{(inital\ HCl - consumed\ NaOH)\ x\ F\ x\ 0.0014008}{1 gram\ sample\ x\ 100\ /1000} \quad \textbf{(Equation 2.3)}$$

Where;

F: Correction factor (6.25 for foods)

Initial HCl: 25 ml

2.2.1.4 Fat Content

The total fat content was determined by Soxhlet-extraction method (Hara and Radin, 1978).

Five gram of dried and ground sample was wrapped with filter paper and placed in Soxhlet apparatus. Balloon connected to Soxhlet apparatus was filled with 300 ml of hexane. System was heated and vaporized hexane reached the sample and during its precipitation, it extracted the fat from the sample. Process continued for 4 hours, at the end Soxhlet apparatus was disconnected, and hexane was distilled. Thus, only fat remained in the balloon and after cooling, weight of the fat and balloon was recorded. Actual fat content was calculated as follows:

$$\% \text{ Fat} = \frac{a-b}{c} \times 100 \qquad \textbf{(Equation 2.4)}$$

Where;

a: weight of fat and balloon

b: weight of balloon

c: weight of sample

2.2.1.5 Crude Fiber Content

For crude fiber determination, samples were first hydrolyzed by boiling in sulfuric acid solution (0.255 N) for 30 min, and subsequently filtered and washed. For calcinations, samples were boiled in potassium hydroxide solution (0.313 N) for 30 min, then filtered, and washed, respectively. The obtained residue was dried at 105^{o}C for 24 h and burned at 550°C until constant weight. This procedure was also repeated for blank. The difference between the dry organic/inorganic residue weight and the ash residue weight was used as fibrous material and the fibre content was calculated as a percentage by weight, C_{fibre}, by the formula:

$$C_{fibre} = [(b-c) \times 100] / a \qquad \textbf{(Equation 2.5)}$$

Where;

a : the mass (g) of the sample,

b: the loss of mass after ashing

c: the loss of mass after ashing for the blank

2.2.1.6 Total Carbohydrate Content

The total carbohydrate content was estimated from the following difference formula (Sahin and Sumnu, 2006):

Total carbohydrate content (%) = dry sample % - fat % - protein % -

ash % - fiber % **(Equation 2.6)**

2.2.2 Extraction Methods

2.2.2.1 Alkaline Methods for Hemicellulose Extraction

Crude hemicellulose (HC) was extracted from raw materials using NaOH for 24 h. Subsequently, samples were filtered and their pH was adjusted to 5.0 using 37% HCl solution. After 1 h, samples were centrifuged at 4°C and 9000 rpm to obtain hemicellulose A (HCA). The supernatant was mixed with double volume of 98% ethanol and kept at 4°C for another 24 h. The resulting precipitate, known as hemicellulose B (HCB) was separated by filtration using cheesecloth (Gaspar et al., 2007). The results were reported as yield (g total crude HC / g dry matter). This procedure is so called Method 1 and summarized in Figure 2.1. Other methods compare hemicellulose extraction with NaOH after fat, soluble sugar, protein and starch were separated one at a time to assess effect of individual component on yield and purity of hemicellulose extract. All methods are presented in Figure 2.1.

Method 1: **Direct Alkali Extraction** NaOH extraction , 24 h ↓ Filtration ↓ pH=5 adjustment (with 37% HCl) ↓ Centrifuge, 4ºC, 9000 rpm ↓ ↓ Supernatant residue ↓ (HCA) 2 volume ethanol ↓ Filtration ↓ Residue (HCB)	**Method 3:** **Removal of sugar and protein** %80 ethanol, 24h, 27-30 ºC ↓ Washing 3 times ↓ 2% NaCl, 24h, 27-30 ºC ↓ Washing 3 times ↓ Continue with Method 1
Method 2: **Lipid removal** Hexane extraction, 4 h ↓ Lipid free residue ↓ Continue with Method 1	**Method 4:** **Removal of starch** Amylase enzyme in acetate buffer pH= 4.8, 120 ºC, 1 h ↓ Filtration ↓ Starch free solid residue ↓ Continue with Method 1

Figure 2. 1 Alkali methods for hemicellulose extraction

2.2.2.1.1 Separation of Fat, Protein, Soluble Sugar, and Starch

Fat was separated by hexane extraction, which is same with the method indicated in section *2.2.1.4 Fat Content* (method 2).

In order to separate soluble sugar and protein, the procedure (method 3) was as follows; 5 g of dried and ground sample was suspended in 80% ethanol at room temperature for 24 h. After the ethanol solution was removed by filtration using vacuum filter, the solid was washed with 2% NaCl solution at room temperature for 24 h to remove crude protein. Then, solid was washed with distilled water and filtered again. Remaining solid was free from soluble sugar and protein (Reddy et al., 1983).

For starch separation (method 4), 1 L heat-resistant flask was used and slurry was prepared in it. For slurry preparation, 0.05 M acetate buffer at pH 4.8, 5 g corn sample, and 0.0025 g alpha-amylase enzyme (from Aspergillus oryzae with an activity of 48 U/ mg dry solid) was used. Slurry was hydrolyzed at 120ºC for 1 h in autoclave. Then, same amount of enzyme was added and stirred for 1 h at 90ºC. Finally, slurry was filtered and starch-free solid residue was obtained (Gaspar et al., 2007).

2.2.2.2 Acidic Method for Hemicellulose Sugars Extraction

To 1 g of dried and ground raw material, 10 ml of 72% H_2SO_4 solution was added and kept for 1 h at room temperature. Then, samples were diluted with 140 ml of distilled water and autoclaved at 121°C for 15 min to achieve dilute acidic hydrolysis. After hydrolysis, pH was adjusted to 6.5 using NaOH solution. Settled

particles were separated by centrifugation and the hemicellulose sugars containing supernatant was concentrated under vacuum dryer at 40°C before HPLC analysis (Mendes et al., 2010).

2.2.3 HPLC Analysis for Purity Determination

Glucose, arabinose, and xylose contents of crude hemicellulose extract were determined by chromatography using a Shimadzu HPLC system. Concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 g/L for glucose, arabinose, and xylose were used as standards.

First of all, 1 g of dry hemicellulose sample was hydrolyzed with concentrate acid. For this, 10 ml 72% (v/v) H_2SO_4 was added to raw material and kept at room temperature for 1 h. Then, 140 ml of distilled water was added and sample was autoclaved at 120ºC for 1 h. After filtration, 1.5 g $Ba(OH)_2$ 8.H_2O was added to 30 ml of supernatant. The solution was stirred for 1-2 min and centrifuged. Separated supernatant was diluted 1:3 with 4 mM H_2SO_4. For dilution, 0.5 ml sample and 1.0 ml mobile phase was mixed in Eppendorf tube and centrifuged to be sure for clear solution. Finally, the sample was ready for injection to HPLC column. The column used in analysis was Aminex HPX 87H (300 x 7.8 mm); (Bio-Rad, USA) and the eluent was 4 mM H_2SO_4 at a flow rate of 0.6 ml/min. Each run was carried out at 54°C for 25 min (Gaspar et al., 2007; Varga et al., 2005).

Peaks that define glucose, arabinose, and xylose at certain time intervals (glucose at 9-10^{th} min, xylose at 10-11^{th} min, and arabinose at 11-12^{th} min) were monitored after each run. The Shimadzu HPLC system calculated the areas under peaks using a function of height and weight of peaks after determined the starts and ends

of the peaks. According to these peak areas, concentration of glucose, arabinose, and xylose were quantified and the results were used for yield calculation.

2.2.4 Optimisation of Alkaline Extraction Method

2.2.4.1 Experimental Design

Effects of time, temperature and alkaline concentration on extraction of crude hemicellulose were individually investigated using classical one-factor at a time approach. Alkaline concentrations of 5, 10, 15, 20 %, temperature of 30, 35, 40, 45, 50 °C, and extraction period of 24, 48, 72, 96 h were tested. Firstly, at constant concentration (10%) and time (24 h), temperature was changed and optimum temperature was found. The results obtained were expressed as % yield (g total crude HC/g dry material). Then, optimum temperature was kept constant and optimum concentration was found for 24 hours. Finally, different times were investigated to find optimum time at the recorded optimum temperature and concentration. All results were furthermore used as a basis in optimization study for constructing the statistical plan using Response Surface Method (RSM).

2.2.4.2 Statistical Design and RSM

The individual and combined effects of alkaline concentration, time, and temperature on extraction yield were studied by Box-Behnken response surface design method (Box and Behnken, 1960) using the statistical software MINITAB® 15.1 (Minitab Inc. State College, PA, USA). Independent variables which were concentration, temperature and time are shown as coded and uncoded form in Table 2.1. Coded levels using integers (-1, 0, +1) represented minimum, middle, and maximum levels of effective variables such as alkaline concentration,

time, and temperature. Combination of points of (±1, 0, 0), (0, ±1, 0), (0, 0, ±1) and center point (0, 0, 0) were used to measure means, standard deviations, and lack of fit (Khuri and Cornell, 1987).

Table 2. 1 Coded and uncoded variables of independent factors in RSM

Variables		Coded levels		
		-1	0	1
		Uncoded levels		
Alkali concentration	%(w/v)	10	15	20
Extraction Temperature	°C	30	40	50
Extraction time	h	24	48	72

The predictive model was written as a second order polynomial equation as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad \textbf{(Equation 2.7)}$$

where Y is the response (crude hemicellulose) , X_1, X_2, X_3 represent independent variables of alkaline concentration, temperature and time, respectively and β's are regression coefficients.

The analysis of variance (ANOVA) and regression analysis were performed to define the coefficients of the predictive model and significant terms using MINITAB® 15.1 (Minitab Inc. State College, PA, USA). The optimum conditions for maximizing the extraction of crude hemicellulose were determined using Response Optimizer tool in MINITAB® 15.1.

2.2.5 Coating Formulations and Application

To prepare 100 ml of coating solution (1-2% total solids for banana) 1.0, 1.5, and 2.0 g of hemicelluloses were weighed and dissolved in 100 ml distilled water. Then, Tween-80 (0.2 ml) was added to emulsify and improve wettability. The solution was beat for 10 min by blender and stirred for 30 min by laboratory stirrer for homogenization. If any insoluble still remained, they were separated by filtration using vacuum filter (Kittur et al., 2001).

Fruits were randomly distributed into four groups and each group was assigned 10 samples. Every fruit sample was washed and dried. First group was control and none of the samples was coated. The other groups consisted of coated fruits by spraying (a) 1%; (b) 1.5%; and (c) 2% hemicellulose solutions. Fruits were air-dried after coating application. Fruits were subsequently stored at 4ºC in refrigerator.

2.2.6 Quality Attributes

2.2.6.1 Fungal Decay

Fungal decay was visually inspected daily during storage. Results were expressed as the percentage of fruits infected.

2.2.6.2 Weight Loss

Bananas were weighed at the beginning of the experiment just after coating and air-drying, and thereafter each day during the storage period. Weight loss was expressed as the percentage loss of the initial total weight. Results were the average of 10 samples for each group.

Simple calculation of the percent weight loss was done by the following formula:

$$\% \text{ Weight loss} = \frac{A-B}{A} \times 100 \qquad \textbf{(Equation 2.8)}$$

Where;

A= Initial weight (g)

B= Final weight (g)

2.2.6.3 Texture Analysis

For texture analysis TAX-T2 texturometer was used. Before the experiment, the computer and the machine were turned on and a 5 mm diameter flat probe was placed. Secondly, calibration was done at the page of T.A Calibrate. At first, force

was calibrated by 2 kg and then, height was calibrated. After calibration, a new project page was opened and TA settings were entered. Finally, it was ready to run a test. For a test, one of the whole bananas was placed under the probe and during the tissue breakage force was applied. This gave the firmness of the fruit samples as the maximum penetration force (gram force). Results were an average of 10 samples in each group which were control group and three different coated group (1%, 1.5%, and 2% of total coating material). Tests were applied to the fresh bananas and results were recorded as firmness of 0th day. Then, tests were repeated every day for 4 days.

2.2.6.4 Color

Banana's external color was evaluated with Datacolor 110^{TM} (HITEX Services, Inc. Easton, PA, USA). Initially, program of Datacolor Tools was opened in the computer and calibration was done. For calibration, white, black and green colors were defined respectively. To start experiment, in the program, Screen Form defined as CIE OUTPUT and Std Instrument defined as Lab. This meaned that external color would be evaluated according to CIE L*a*b* coordinates where L*is lightness, a* is a chromacity coordinate defining greenness(-) to redness(+), and b* is another chromacity coordinate defining blueness(-) to yellowness (+). Ten readings were taken for each groups and 40 bananas were used in total.

2.2.6.5 Sensory Analysis

Sensory attributes consisted of colour, taste, flavor, and texture. Because of this work focused on the darkening of bananas at refrigerator condition, only visual acceptance was evaluated as a sensory analysis by 20 member of an untrained

panelists. 7-point hedonic scale was used to conduct this sensory evaluation. The scores were:

(7) like extremely

(6) like very much

(5) like moderately

(4) neither like nor dislike

(3) dislike moderately

(2) dislike very much

(1) dislike extremely

Acceptability level was determined as 4 because huge color difference started just after score 4 as seen in Appendix D. Fruit score below 4 was considered unacceptable. Ten samples of each group without fungal decay were evaluated on the initial day (0th day) and every day for 4 days.

2.2.6.7 Statistical Analysis of Quality Tests

One-way analysis of variance (ANOVA) was used to perform statistical analysis of results and Tukey Test was used for pair wise comparison using the statistical software MINITAB® 15.1 (Minitab Inc. State College, PA, USA). Analyzed data and plotted graphs were also taken from MINITAB® 15.1.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Composition of Raw Materials

The results of the chemical analysis for corn peel, corn cob and sugar beet pulp are given in Table 3.1 as averages of two replicates on a wet basis. The total hemicellulose content was found as 41.27% in corn peel, 55% in corn cob, and 14.14% in sugar beet pulp. Total carbohydrate was 65.06% in corn peel, 60.28% in corn cob and 65.76% in sugar beet pulp. Total carbohydrate here contains the hemicellulose fraction. Thus, for corn peel 63.4% of total carbohydrate and 44% of total dry matter was hemicellulose. For corn cob this portion was changed as 91.2% of total carbohydrate and 54.0% of total dry matter, however, for sugar beet pulp, hemicellulose content was only 21.5% of total carbohydrate and 14.8% of total dry matter. That is, hemicellulose was obtained at higher levels from corn wastes compared to sugar beet pulp. Thus, the following studies continued with corn wastes, especially with corn peel due to easy preparation.

Table 3. 1 Constituents of raw materials

Constituent	Content (%w/w) [a]		
	Corn peel	Corn cob	Sugar beet pulp
Dry solid	94.10	93.00	98.20
Protein	3.29	3.44	10.18
Hexane/ethanol extract	5.50	7.40	2.40
Fat	1.20	0.88	0.86
Crude fiber	22.00	26.40	17.30
Hemicellulose	41.27	55.00	14.14
Ash	2.55	2.00	4.10
Total carbohydrate	65.06	60.28	65.76

[a] Results belong to two replicates.

3.2 Effects of Extraction Methods on Hemicellulose Yield

3.2.1 Alkaline Method

Hemicellulose yields, obtained with different alkaline extraction methods, were compared (Table 3.2). The highest yield of crude hemicellulose was 38.4% from direct alkaline method (Method 1) in comparison to fat-free alkaline extraction (22.4%, Method 2) and protein and sugar-free alkaline extraction (17.9%, Method 3). This result implied that during the separation of constituents, some hemicelluloses were lost. Our main goal was to understand the effects of other constituents on hemicellulose extraction and the results showed that there was no significant effect of individual constituents on crude hemicellulose yield. This

idea supported the study of Gaspar at al. (2007), which also compared untreated and destarched corn fiber and reported no significant difference between the hemicellulose levels obtained after different pretreatments. Thus, direct alkaline extraction method was used for the following experiments.

Table 3. 2 Effects of different hemicellulose extraction procedures

Method	Total Hemicellulose Yield (g/g)
Direct alkaline extraction (Method 1)	0.384
Alkaline extraction after fat was separated (Method 2)	0.224
Alkaline extraction after protein and soluble sugar was separated (Method 3)	0.179

3.2.2 Acidic Method

HPLC analysis was performed to determine the purity of hemicellulose extracts obtained by acidic method. The results showed low purity levels (27%) compared to alkaline extraction method was achieved. Thus, acidic method was not further used.

3.3 Effective Factors on Hemicellulose Extraction

3.3.1 Alkaline Concentration

Effects of alkaline concentration, time, temperature, and particle size on extraction of crude hemicelluloses from corn peel were examined. Different alkaline (NaOH) concentrations (5, 10, 15, 20 %) were used at room temperature for 24h. Average results are shown in Figure 3.1. One-way ANOVA indicated that concentration had significant effect on hemicellulose yields ($p<0.05$). For 10 – 20 % NaOH concentration, however, statistically there was no significant difference ($p>0.05$). In other words, increasing the alkaline concentration from 10% to 20% increased the hemicellulose amount from 38.4% to 42.5% but this difference was not found significant. The quantities of hemicelluloses were almost the same for 15% and 20% NaOH concentration (42.7 and 42.5% respectively) and both of them were higher than the hemicelluloses extracted at 10% NaOH concentration (38.4%). In order to optimize reagent cost and waste, 15% alkaline concentration was favored as an ideal level for hemicellulose extraction in further experiments.

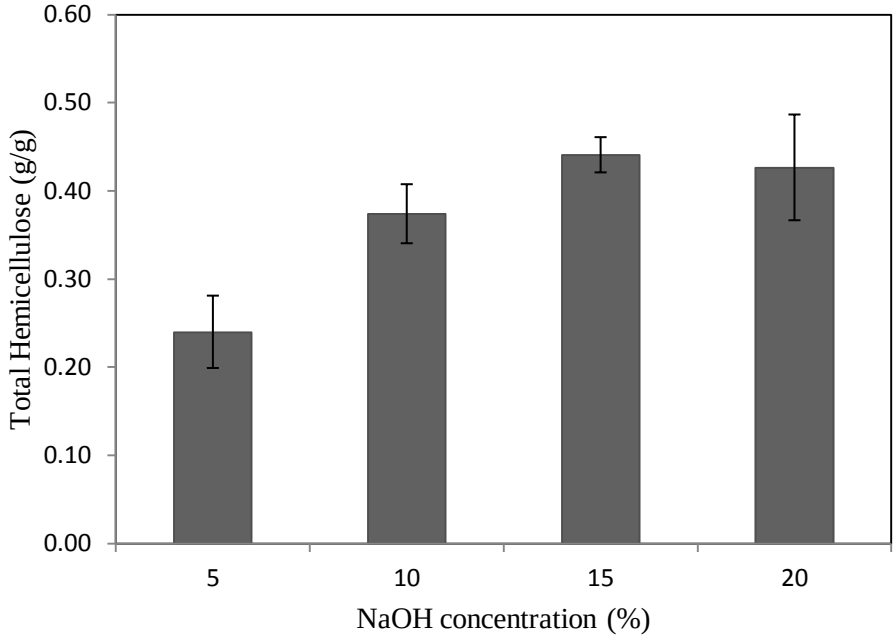

Figure 3. 1 Hemicellulose extraction yields at various alkaline concentrations (30°C, 24h)

3.3.2 Extraction Temperature

In order to understand the temperature effect on extraction, different temperatures (30, 35, 40, 45, 50°C) were used at 15% alkaline concentration for 24 h. As temperature increased, hemicellulose slightly increased (Figure 3.2). Statistically for temperatures of 35 – 50°C, the amounts of hemicellulose were not significantly different ($p>0.05$). Furthermore, the hemicellulose at 50°C was higher than the ones at 30°C ($p<0.05$). According to these results, the best hemicellulose extraction temperature was chosen as 40°C (44.2%). Higher temperature was not considered for cost and energy savings during extraction. There are varying alkaline concentrations and temperatures for hemicellulose extraction in literature for different materials. However, periods of extraction are not indicated. Studies focus on only one factor and interactions are not mentioned.

For instance, Wen et al. (1988) treated sugar beet pulp for 5.0, 7.5, 10.0, and 12.5 % alkaline concentrations at 4°C, 25°, and 40°C temperatures. The optimum hemicellulose (14.6% of total dry matter) was obtained for 10% alkali concentration at 25°C. This condition and total crude hemicellulose were very close to results of our present studies in which total crude hemicellulose was 14.8% of total dry matter for 10% alkali concentration and 30°C temperature.

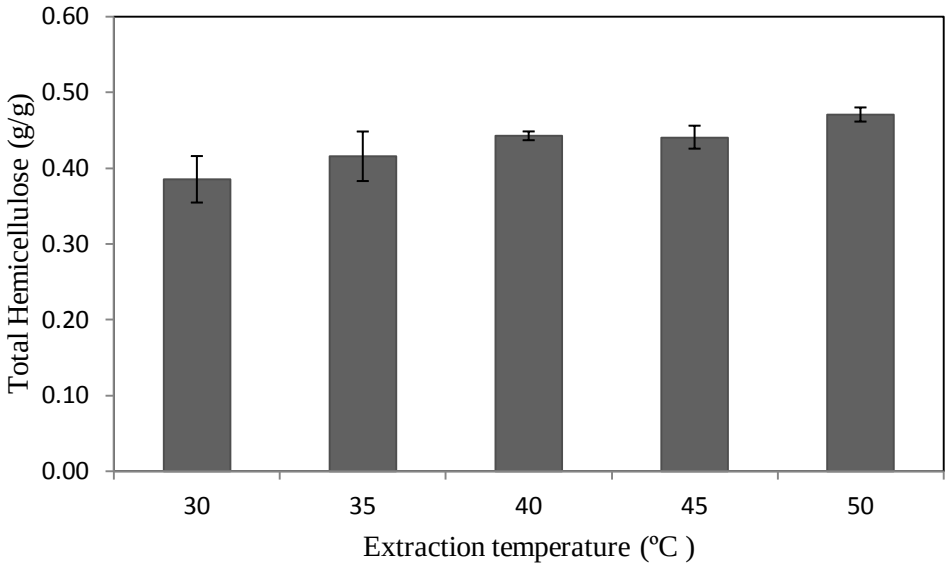

Figure 3. 2 Hemicellulose extraction yields vs temperatures (15% NaOH, 24h)

3.3.3 Time

The time effect on extraction was examined after ideal concentration and temperature were determined for extraction. Periods of 24, 48, 72, 96 h were used at 40°C and 15% NaOH concentration. Statistically, time had significant effect on hemicelluloses yield ($p<0.05$). The results are shown in Figure 3.3. Twenty-four hours and 96 h showed similar results. Therefore, the period of 24 h was chosen as ideal for the following experiments to save time and energy.

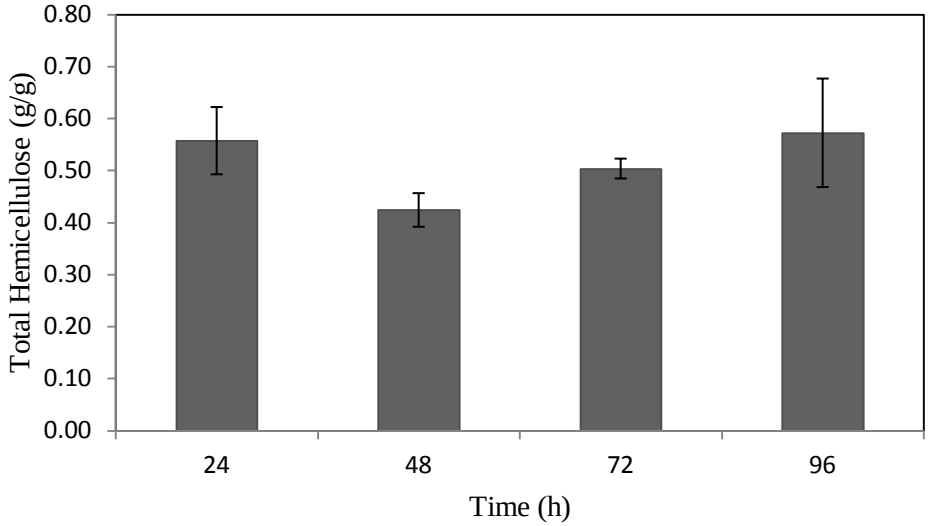

Figure 3. 3 Hemicellulose extraction yields vs extraction times

(40°C, 15% NaOH)

3.3.4 Particle size

Two different particle sizes (0.8 and 1.1 mm) of corn peel and sugar beet pulp were used and their effects on hemicellulose extraction were tested. Larger particles (1.1 mm) were obtained using laboratory miller (Thomas-WILEY Laboratory Mill, Model 4) and finer particles (0.8 mm) were obtained using ball-miller (Retsch Planetary Ball Mill PM 100). According to Figure 3.4, smaller particles gave higher levels for sugar beet pulp. As expected, increasing surface area between solute and solvent caused increasing amount of product. On the other hand, it was unexpected that the larger particles produced higher hemicelluloses levels for corn peel. The following studies continued using laboratory miller since corn peel was used as a raw material in RSM study in the view of previous results.

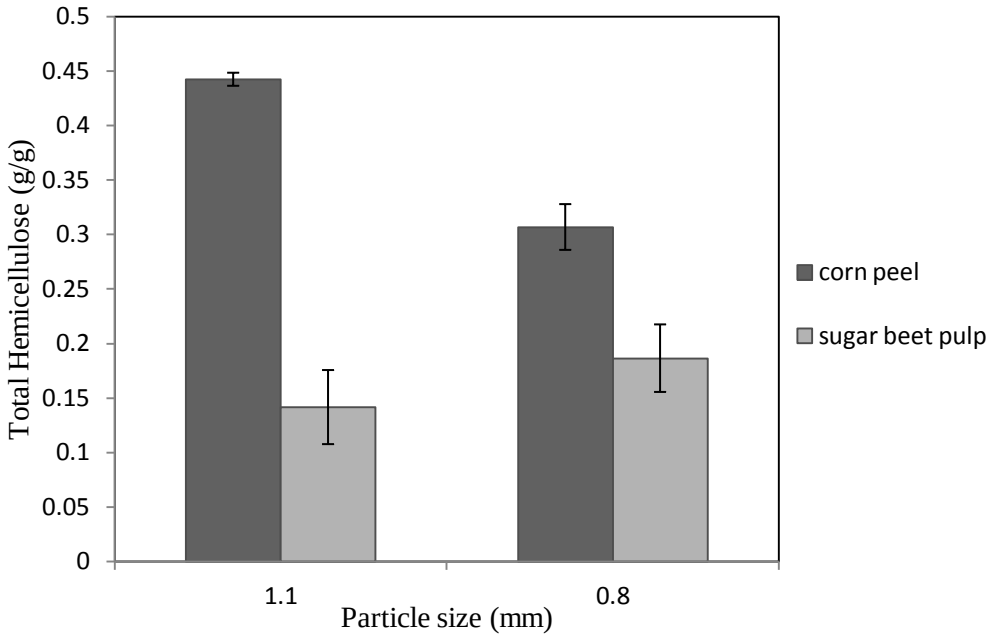

Figure 3. 4 Hemicellulose extraction with different particle sizes of raw materials

3.4 RSM and Optimization

The experimental design of Box-Behnken response surface model and its results are presented in Table 3.3. Run-6 was cancelled because the HPLC analysis gave almost zero yield of extract under Run-6 conditions (50°C, 20% and 48h). A second order polynomial equation was found as a suitable equation to represent the data. According to Table 3.4, insignificant terms were excluded and the equation became as follows:

$$Y = -4.747 + 0.160X_1 + 0.283X_2 - 0.001X_1^2 - 0.004X_2^2 - 0.004X_1X_2$$

(Equation 3.1)

where Y is crude hemicellulose, X_1, X_2 and X_3 are values of temperature, concentration, and time, respectively.

Table 3. 3 Experimental design for hemicellulose extraction using RSM*

No	Temp. (°C)	Conc. (%)	Time (h)	HC** (ave) (g/g)
1	50	15	24	0.51±0.03
2	30	20	48	0.69±0.01
3	50	10	48	0.35±0.01
4	40	10	24	0.33±0.01
5	30	10	48	0.33±0.03
6	50	20	48	Cancelled
7	40	15	48	0.58±0.06
8	50	15	72	0.34±0.02
9	30	15	24	0.50±0.06
10	40	10	72	0.34±0.06
11	40	20	72	0.58±0.02
12	40	20	24	0.71±0.02
13	30	15	72	0.43±0.03
14	40	15	48	0.57±0.01
15	40	15	48	0.58±0.03

* -1, 0, and +1 are the coded levels for 30, 40 and 50^{o}C; for 10, 15, and 20%; and for 24, 48, and 72 h, respectively in RSM, ** HC: hemicellulose

Table 3. 4 ANOVA results* and estimated regression coefficients for the uncoded hemicellulose model

Term	Coef	p
Regression		0.000
Linear		0.001
Square		0.003
Interaction		0.001
Lack-of-fit		0.140
Constant	-4.747	0.000
Temp (X_1)	0.160	0.000
Conc (X_2)	0.283	0.000
Time (X_3)	0.005	0.633
Temp*Temp	-0.001	0.002
Conc*Conc	-0.004	0.024
Time*Time	0.000	0.826
Temp*Conc	-0.004	0.000
Temp*Time	-0.000	0.524
Conc*Time	-0.000	0.367

* result is significant when $P<0.05$

ANOVA results (Table 3.4) indicated that the quadratic model was found statistically significant ($p<0.05$) at the 95% confidence interval. Due to very low p values, linear ($p=0.001$), quadratic ($p=0.003$), and interaction effects ($p=0.001$) were highly significant. The fitting of experimental data to the regression model was checked by the value of determination coefficient (R^2). ANOVA results indicated that $R^2=0.79$ when Run 6 was taken as '0 g/g' and $R^2=0.91$ when Run 6

was used as is (0.92 g/g), which was cancelled due to zero purity as stated before. Thus, it was concluded that the model was adequate since in both case R^2>0.75 (Chauhan and Gupta, 2004). The insignificant lack of fit (p=0.140>0.05) also proved that the model fitted well to the experimental data. Among the factors, temperature and concentration showed significant effects (p<0.05) but time had insignificant effect (p=0.633) on hemicellulose yield. Interactions between temperature-temperature, concentration-concentration and temperature-concentration showed significant effects; whereas time interactions were insignificant.

The response surface plots for the effects of concentration, temperature and time and their interactions based on the quadratic model are presented in Figure 3.5, 3.6, and 3.7, respectively. In Figure 3.5 and 3.6, it can be seen that time indicated insignificant effect and hemicellulose yield increased nonlinearly with increasing concentration. The increase of extraction temperature from 30°C to 40°C slightly increased the hemicellulose yield, but above 40°C a sharp decrease on hemicellulose yield was observed. In Figure 3.7, the hemicellulose yield showed high dependency on changing temperature and concentration. In other words, while for 20% (w/v) concentration, hemicellulose yield decreased with increasing temperature, for 10% (w/v) concentration, up to 40°C hemicellulose extract increased and above 40°C it decreased. It was estimated that further increase of temperature above 40°C can not extract more hemicellulose and can cause degradation.

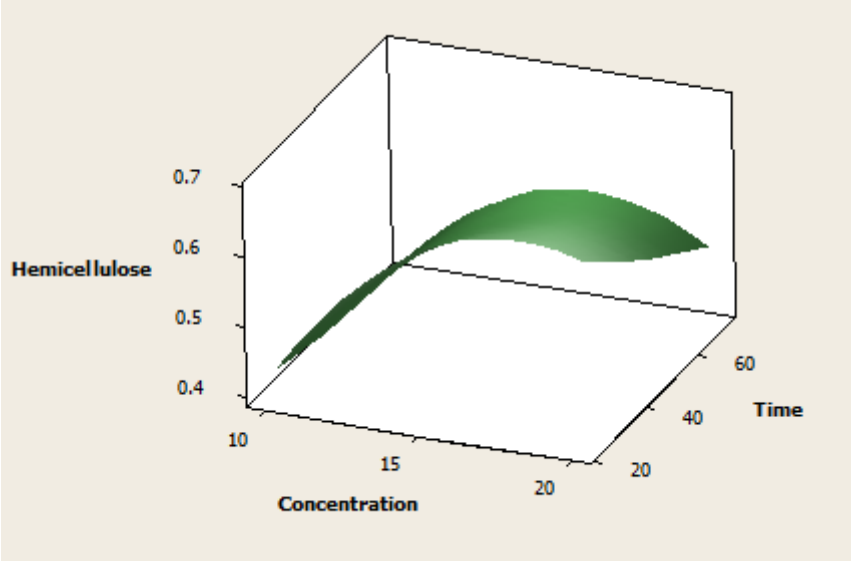

Figure 3. 5 Surface plot showing the effect of concentration (%NaOH) and time (h) on hemicellulose extract (g/g) (Constant value: extraction temperature: 40°C)

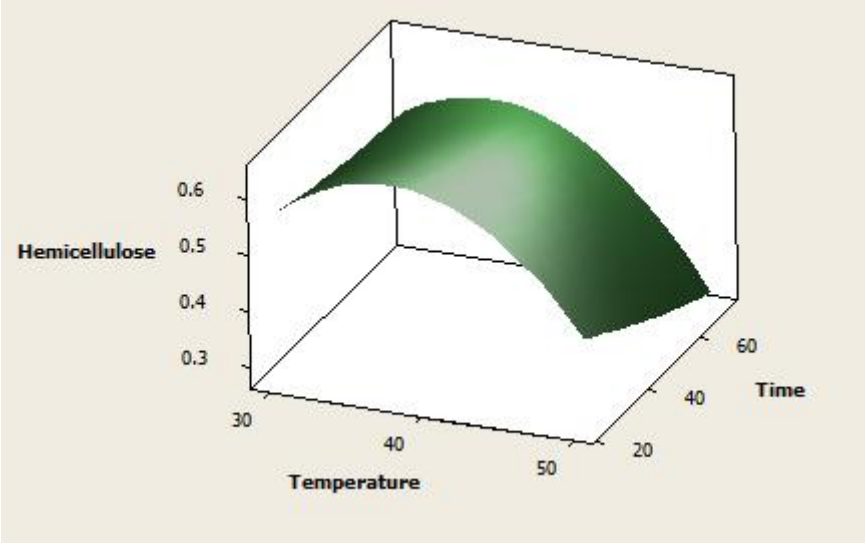

Figure 3. 6 Surface plot showing the effect of temperature (°C) and time (h) on hemicellulose extract (g/g) (Constant value: alkaline concentration: 15%)

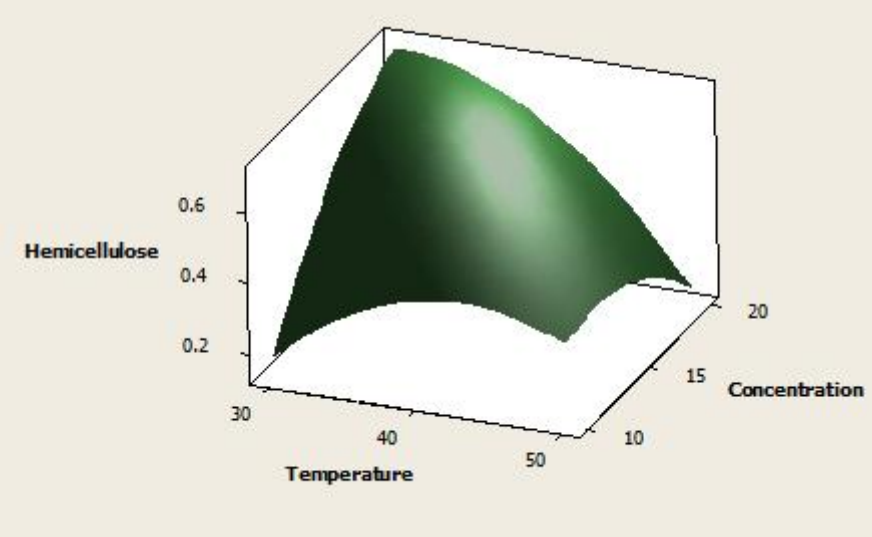

Figure 3. 7 Surface plot showing the effect of temperature (°C) and concentration (%NaOH) on hemicellulose extract (g/g) (Constant value: time: 48 h)

The contour plots to show clearly the effects of process variables (two varied while the third was kept at the mid value) are given in Figure 3.8, 3.9, and 3.10. A high yield of 0.60 g/g was observed in all combinations of temperature, concentration, and time. High dependency nature of yields on changing temperature and concentration is also clear in contour plots. At 20% (w/v) NaOH concentration, the hemicellulose levels decrease with increasing temperature, whereas for 10% (w/v) concentration, the hemicellulose yield increases up to 40^{o}C and above 40°C it decreases.

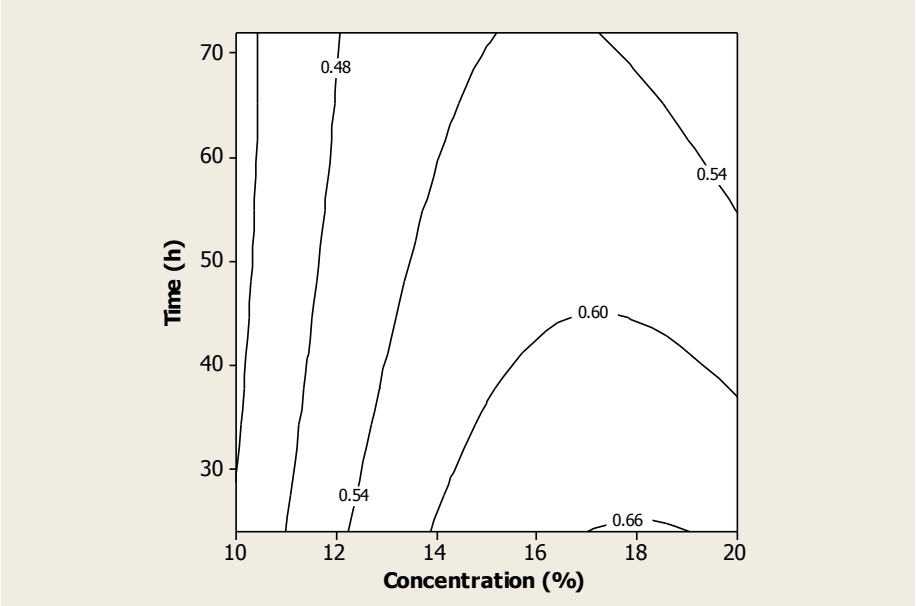

Figure 3. 8 Contour plot showing the effect of concentration (%NaOH) and time (h) on hemicellulose extract (g/g) (Constant value: extraction temperature: 40°C)

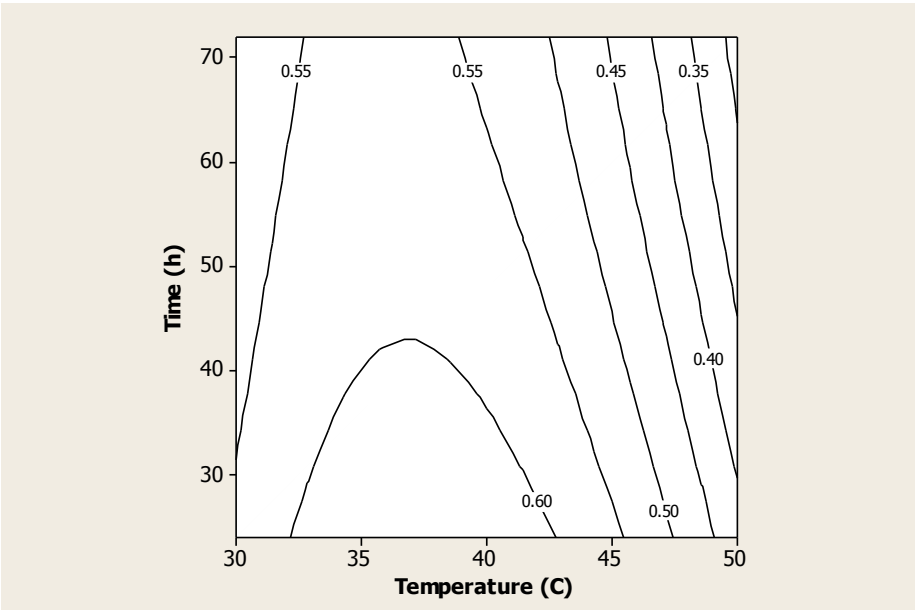

Figure 3. 9 Contour plot showing the effect of temperature (°C) and time (h) on hemicellulose extract (g/g) (Constant value: alkaline concentration: 15%)

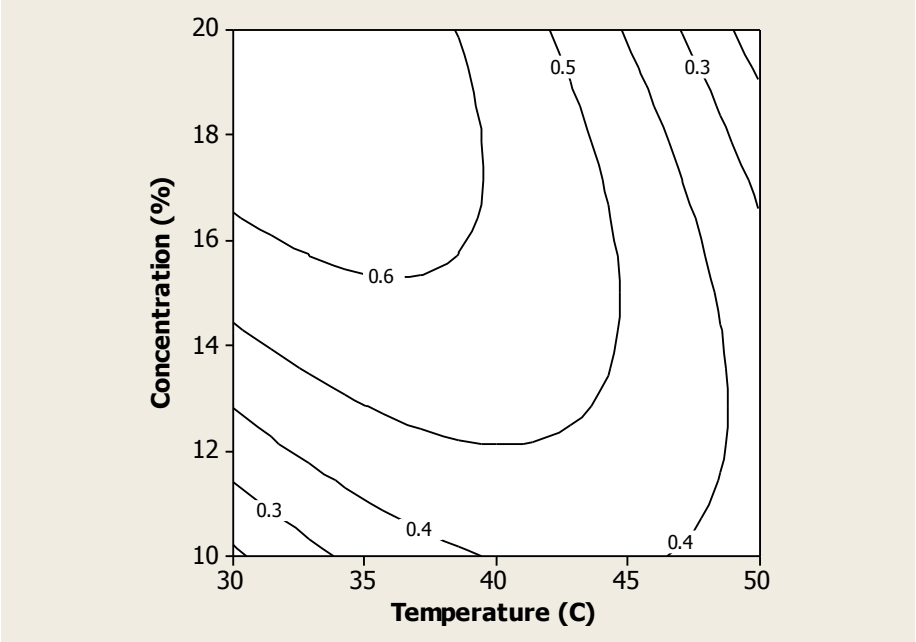

Figure 3. 10 Contour plot showing the effect of temperature (°C) and concentration (%NaOH) on hemicellulose extract (g/g)

(Constant value: time: 48h)

Therefore, in order to determine the optimal extraction conditions, response optimizer tool in MINITAB® 15.1 (Minitab Inc. State College, PA, USA) was used. According to Table 3.5, optimum conditions for hemicellulose extraction were found as 31°C of temperature, 20% of alkaline concentration and 24 h of time. Response optimizations results are close to optimum conditions, which were found by one-factor at a time approach, discussed previously.

To confirm the validity of experimental strategy and the optimum conditions, three additional runs were conducted. The results indicated that the predicted hemicellulose yield (0.76 g/g) was close to experimental yield (0.64), having very low standard deviation (0.09) and noting that the standard deviation of three

repeated runs was also 0.09. This showed that the model was useful to predict the hemicellulose yield at a specified condition.

Table 3. 5 Response optimization

	Goal	Lower	Target	Upper
HC	Maximum	0.3	0.76	0.76

Global Solution

Temp = 31.0000

Conc = 20.0000

Time = 24.0000

Predicted Responses = 0.7602

Composite Desirability = 1.00000

3.5 Purity of Hemicellulose Extracts

A sample of HPLC chromatogram from purity analysis is given in Figure 3.11 to show sugar standards against an extract obtained at 15% NaOH, 40^{o}C, and 24 h. In the sample curve, the peaks were not sharply separated and shoulders appeared due to very close retention times of the sugars (glucose, xylose, and arabinose) under analysis conditions. Because our aim was to define the total hemicellulose content instead of individual sugar content, further improvements in HPLC

analysis were not considered. The calculated area under the curve gave the total sugar content of the sample. Thus, the percentage of the total sugar in the sample was used as the purity level.

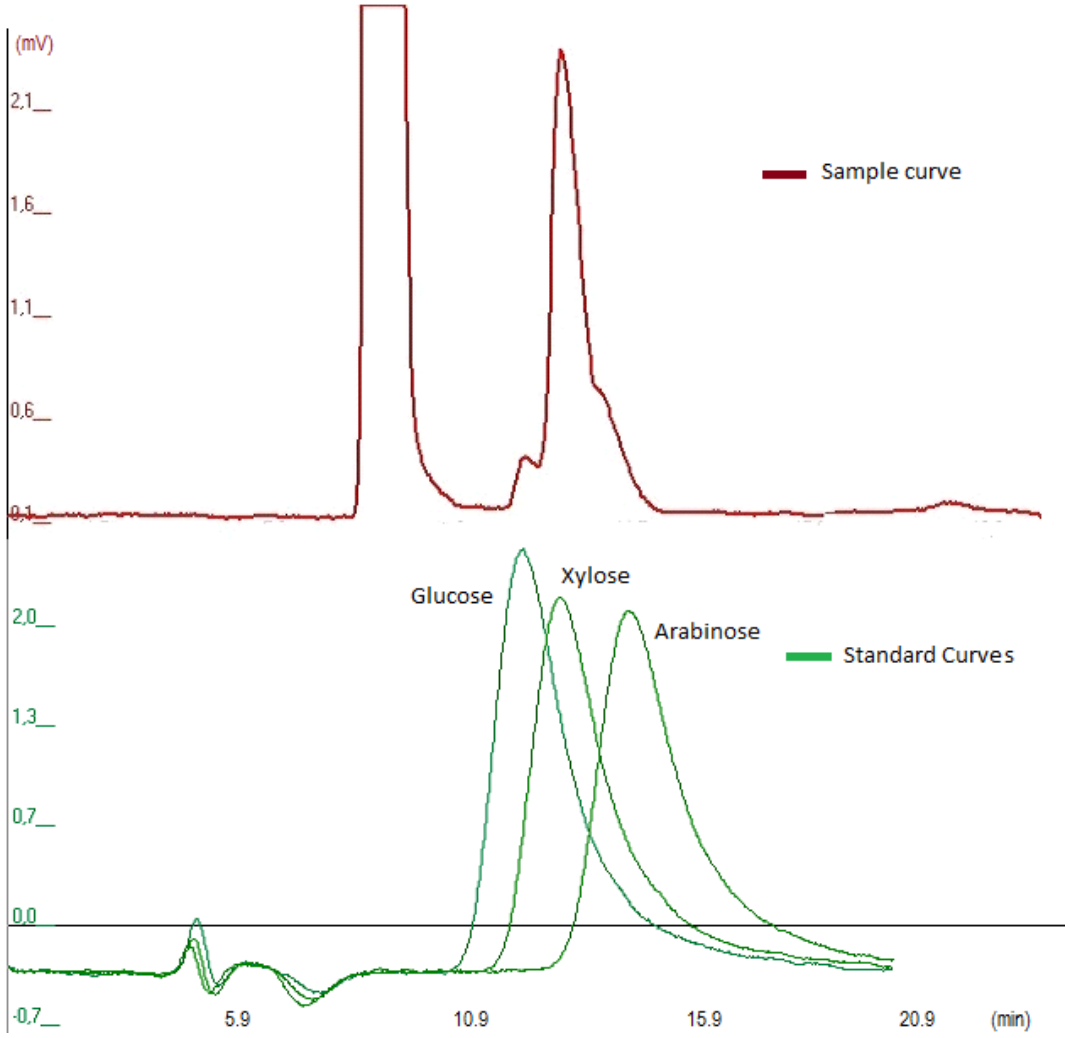

Figure 3. 11 A sample of chromatographic view for hemicelluloses extract vs. sugar standards

A constant extraction temperature (30°C) and time (24 h) were used to analyze the effect of alkaline concentration on the purity of hemicellulose extracts. Alkaline concentrations of 10%, 15% and 20% were used and analyzed by HPLC. According to Figure 3.12, extraction with 10% NaOH resulted in 64% purity of hemicellulose while 15% and 20% NaOH concentrations provided only 40% and 18% pure extracts, respectively. Thus, high alkaline concentrations caused low hemicellulose purity. This was associated with intermediate components formed during pH treatment using HCl solution.

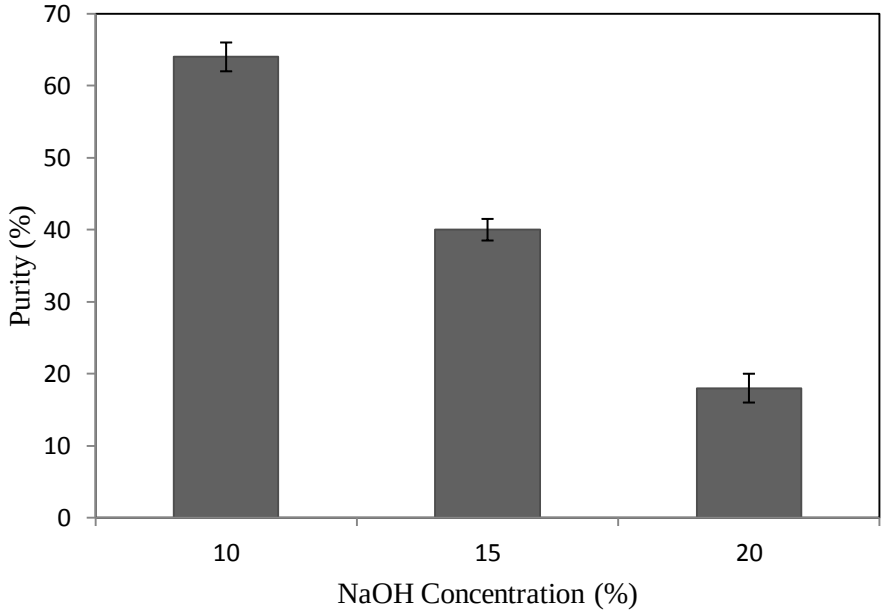

Figure 3. 12 Effects of NaOH concentration on purity of extracts

To understand the temperature effect on the purity of hemicellulose, 10% NaOH concentration and 24 h of time were used and the results are presented in Figure 3.13. Increasing the temperature decreased the purity of hemicellulose approximately to zero at 50°C. Purity levels higher than 60% were achieved at

30°C, whereas only 45% purity was observed at 40°C. Therefore, high temperature caused important loss of purity in the hemicellulose extracts. As a result, the optimum hemicellulose extraction conditions were revised as 30°C of temperature, 10% of NaOH concentration, and 24 h of time.

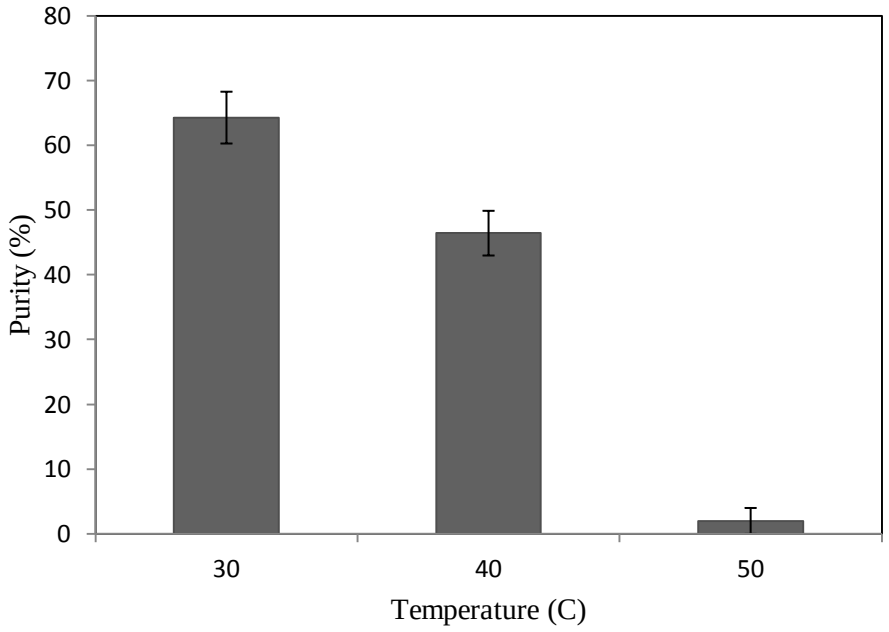

Figure 3. 13 Effects of temperature on purity of extracts

Hemicellulose extraction methods were then compared based on the purity of extracts. In Figure 3.14, the purity of extracts was 40.18% for direct alkaline extraction (Method 1) and 27.36% for acidic hydrolysis (Method 3). However, extraction with alkaline after separation of fat, starch, protein and soluble sugar (Method 2) gave 58.2% purity. It was observed that separation of components other than hemicellulose produced higher purity levels.

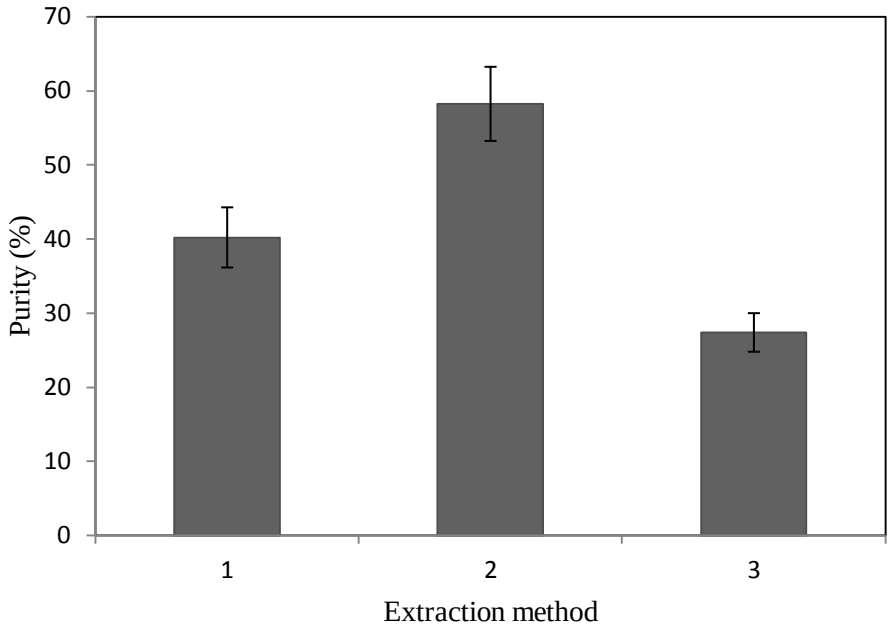

Figure 3. 14 Effects of extraction method on purity of extracts (1: Direct alkaline method, 2: Alkaline method after fat, protein, and starch removal, 3: Acidic hydrolysis method)

HPLC results also showed that acidic hydrolysis was not an effective method for hemicellulose sugars extraction due to low purity level. To get the hemicellulose in solid form with higher purity level, alkaline method is practical for industrial application. After all, we suggested that hemicellulose should be extracted with alkaline solution and with low concentration and temperature, and shorter period of time. For better results in yield, components such as lipids, starch, protein, and soluble sugar should be separated before alkaline extraction.

3.6 Quality Attributes of Fruits Coated with Hemicellulose

3.6.1 Loss of Fruit Due to Visible Fungal Growth

Signs of fungal decay were observed on uncoated bananas after fourth day of storage at 4 ºC. On the fourth day, there was no detected fungal decay of bananas coated with 1%, 1.5%, and 2% hemicellulose by visual inspection. However, 20% of control bananas (uncoated) was infected by molds. After eight days of storage, 60% of fungal decay was detected on the uncoated fruits while only 20% -30% of fruits was infected on the coated fruits. There was no significant difference between the infection percents of bananas coated with 1%, 1.5%, and 2% hemicellulose ($p>0.05$).

3.6.2 Weight Loss

In order to prolong shelf life and maintain significant sensory properties such as texture, taste and mouthfeel, edible coating materials became necessary for foodstuffs. The moisture content of foods is one of the important factor to preserve such quality properties. Therefore, coatings as a moisture barrier, which seals small wounds, limits water transfer and delays dehydration gained interest in the food industry (Hansen et al., 2008). Because moisture evaporation and respiration through the fruit skin were associated with weight loss, it was controlled for 4 days as a measurable quality attributes of coating. Results given in Figure 3.15 showed that all samples lost weight during storage; however, the loss of weight of uncoated fruits was significantly greater than that of coated fruits ($p<0.05$).

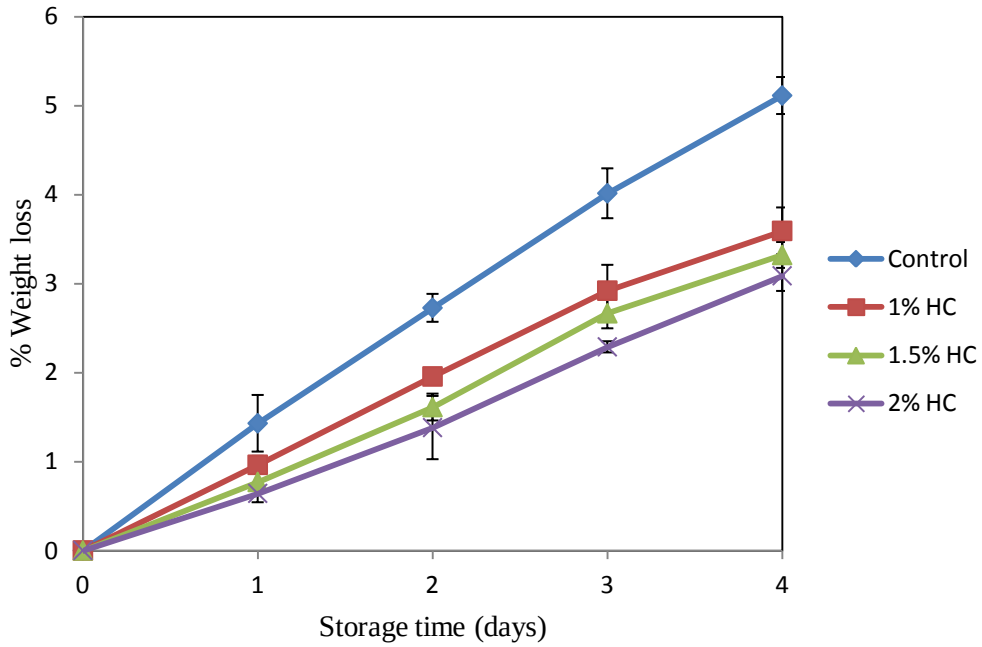

Figure 3. 15 Loss of weight of bananas as a function of storage time at 4 ºC

At the end of the fourth day, uncoated samples (control group) lost 5.1% of total weight but coated samples with 1%, 1.5%, and 2% hemicellulose (HC) lost 3.6%, 3.3%, and 3.1% of their total weight, respectively. There is slight decrease in the percentage loss of weight between coated samples and the least weight loss observed for 2% hemicellulose coated bananas; however, these differences were not significant according to one-way ANOVA ($p>0.05$).

Because of using unpeeled bananas for coating experiment, they were not very susceptible to rapid water loss but prevention of water loss was still important for quality of samples.

3.6.4 Firmness

For fresh fruit and vegetables, texture is a critical quality parameter in the consumer perspective because textural changes directly affect shelf life of fruits and their quality. During ripening of bananas, textural changes take place rapidly and this causes excessive tissue softening and subsequent spoilage. Thus, heavy loss of crop is observed every year. Some technologies were applied to preserve textural properties of fruits but most of them were expensive and not widely available (Sanwal and Payasi, 2007). Therefore, coating is an emerging technology to preserve quality of fresh fruits and vegetables.

In Figure 3.16, firmness of control (uncoated samples) and coated bananas with 1%, 1.5%, and 2% of hemicellulose during the storage period of 4 days at 4 ºC were represented. Similar initial firmness value was observed for all samples ($p>0.05$). Higher hemicellulose content of coating solution than 1% exerted a beneficial effect on fruit firmness. It means that coating material with 1.5% and 2% hemicellulose (HC) had significantly higher firmness value than 1% hemicellulose coated fruits and uncoated fruits ($p<0.05$). Moreover, firmness value of coated samples with 1.5% and 2% hemicellulose slightly decreases to 825.5 gf & 866.3 gf from 893 gf & 895 gf, respectively during four days of storage period. Although there was some decrease on firmness values of 1% HC-coated samples to 701.1 gf from 896 gf, at the end of fourth day, they were still in a good condition when compared to uncoated samples because firmness of uncoated samples (control) decreased continuously to 509.6 gf from 894.2 gf. Increasing hemicellulose content in coating solution raised the preservation degree because of higher barrier potential but after a while (between 1.5% and 2% HC), no significant difference was observed ($p>0.05$).

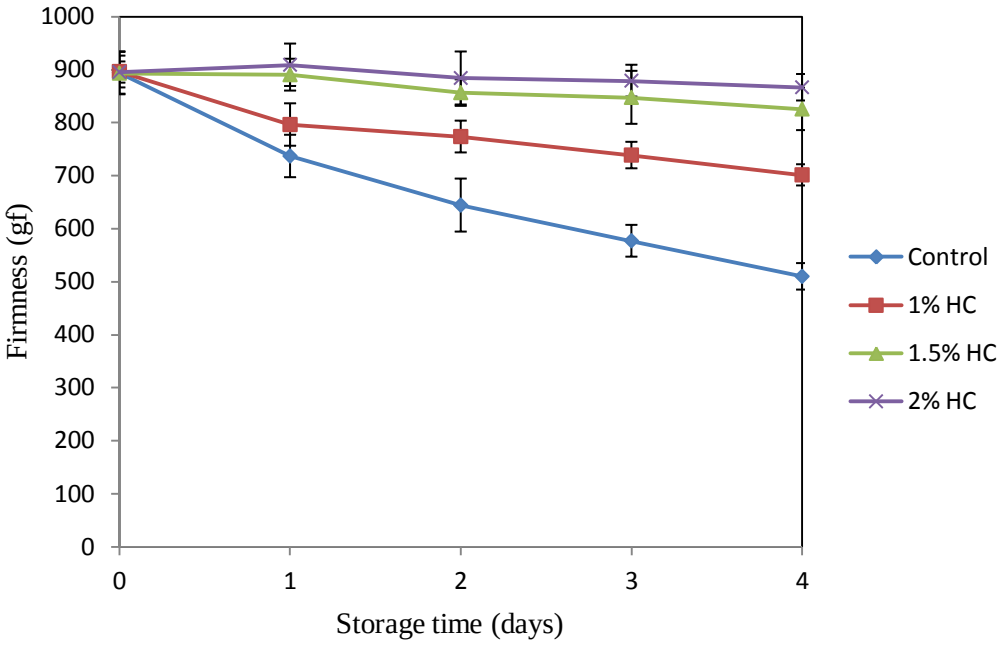

Figure 3. 16 Effect of hemicellulose coatings on the firmness of bananas stored at refrigerator condition (4 ºC)

Beneficial effect of surface coating of bananas were also observed by Kolekar et al. (1988) using sucrose stearate emulsion (1.0%) and delayed bananas ripening for 4 days. The other used pollysaccharide derivatives such as carboxymethyl cellulose, starch, and *N,O*-carboxymethyl chitosan, etc with addition of lipid component such as glycerol monosterate or palmitic acid as a coating material for banana and they got higher firmness (150-177 kgf) value than uncoated samples (112 kgf) during storage period at room temperature (Kittur et al., 2001). On the other hand, hemicellulose coating without addition of any substance showed some advantages with its more natural, edible, and water-soluble characteristics in this study.

3.6.5 External Colour

Color is an important factor that directly affects consumer decision whether buy the product or not. At the beginning of ripening, banana peel contains ethylene which causes ripening from green to yellow and it also contain some natural acids which makes banana sweet during the ripening process. In the refrigerator condition (4 ºC), activity of these natural acids slows down so does ripening. However, cold temperatures cause changes at the physical state of cell membrane in the banana peel and increase enzyme (PPO) contacts with oxygen in the air, so it produces dark color. Although cold temperature causes darkening of banana peel, it helps preserving firm and delicious content inside (Ahmad et al., 2007).

In order to slow down darkening of banana peel, hemicellulose coating was applied to decrease air contact and polyphenoloxidase activity. Results are shown in Figure 3.17, 3.18, and 3.19 as changes of L*, a*, and b* values during storage time, respectively. Coating application did not affect initial color coordinates of fruits. The L* parameter represents lightness and it is an indicator of fruit darkening. In Figure 3.17, it can be observed that L* values of all samples decreased with storage time. One-way ANOVA results showed that uncoated bananas were significantly darker than coated bananas throughout the storage period; however, no significant differences were found among bananas treated with different concentration of hemicellulose. By the end of the fourth day, control samples lost 30% of their lightness while decreases in L* values of coated samples with 1%, 1.5%, and 2% of hemicellulose were around 14.1, 13.4, and 11.1, respectively. Results explained that application of coating delayed bananas darkening at 4ºC. Experiment was continued up to 4^{th} day because after that sensory evaluation did not gave acceptable results.

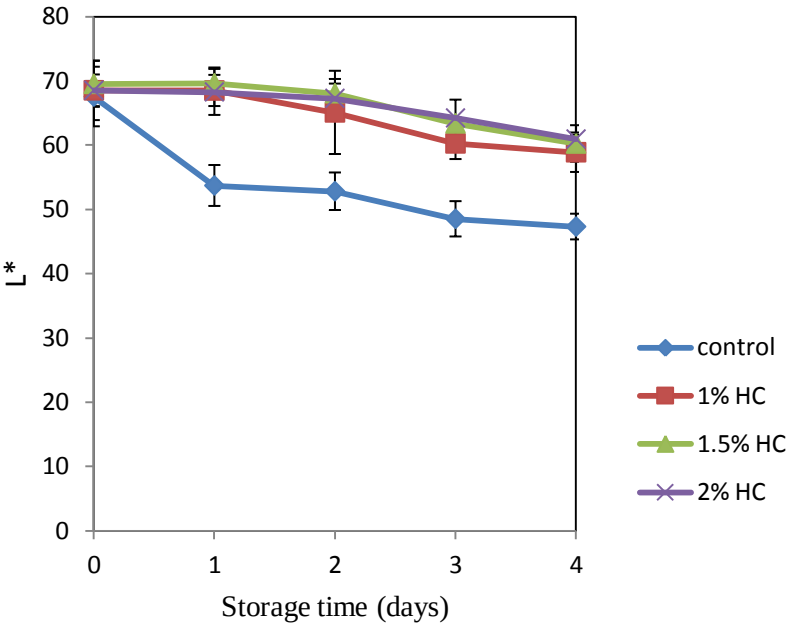

Figure 3. 17 Effect of hemicellulose coatings on the L* values of bananas stored at refrigerator condition (4 ºC)

Changing in a* value of banana peel during storage were presented in Figure 3.18. a* is a chromacity coordinate and positive values indicate redness and negatives indicate greenness. For bananas, decrease in L* and b* values and increase in a* values indicate browning. Even though, slower increase was observed in a* values of coated fruits (Figure 3.18), significant difference was not observed among the coated and uncoated fruits ($p>0.05$). It means that only a* value is not enough for interpretation of external color of banana.

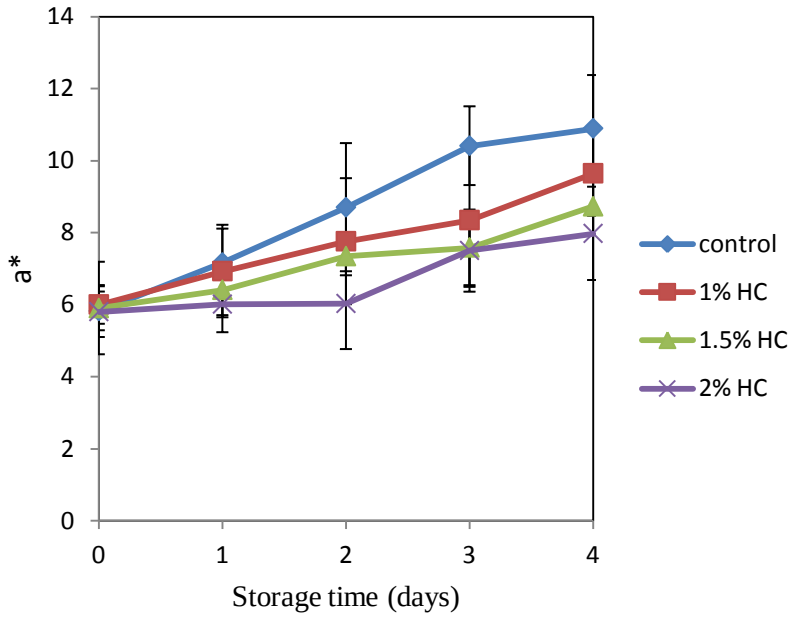

Figure 3. 18 Effect of hemicellulose coatings on the a* values of bananas stored at refrigerator condition (4 ºC)

The other chromacity coordinate is b* of which positive values represent yellowness and negative values represent blueness. As expected, external color analysis of banana gave all positive values of b* (Figure 3.19). Yellowness of all bananas decreased during storage period. However, decrease in yellowness of control (uncoated) bananas was significantly higher than coated ones among which there was no significant difference in b* values. For coated samples, no decrease in b* value was observed at the end of the first day and after that slight decrease was started while rapid and continuous decrease in b* values of control samples was observed.

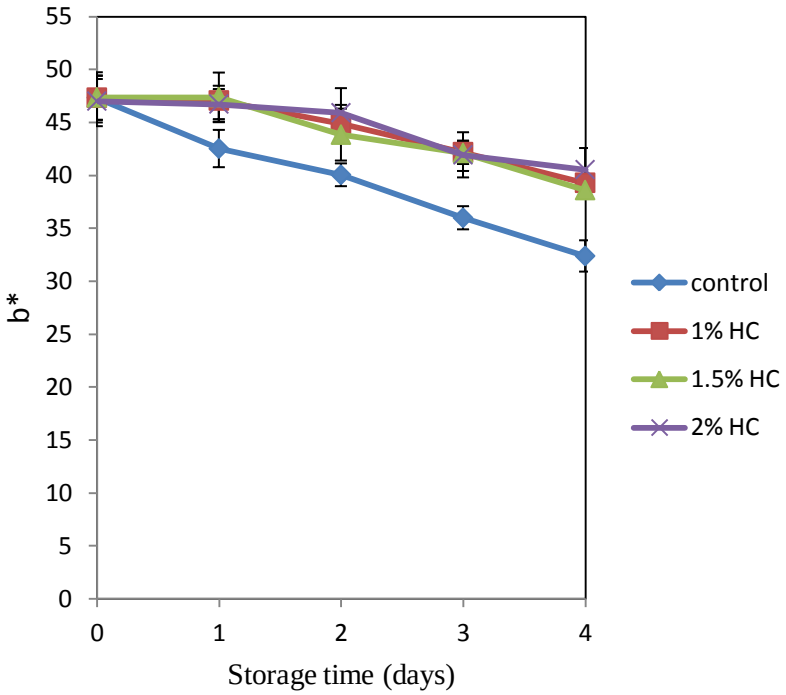

Figure 3. 19 Effect of hemicellulose coatings on the b* values of bananas stored at refrigerator condition (4 ºC)

Moreover, b* values of coated samples at the end of the fourth day were almost equal to the b* values of uncoated samples at the second day of storage time. For example, after four days of storage at 4 ºC, b* values of coated bananas with 1%, 1.5%, and 2% hemicellulose were 39.3, 39, and 40.5, respectively. These results were very similar to b* value of uncoated bananas which was 40 at the end of the second day. This means that coating bananas with 1-2% hemicellulose delayed browning up to two times of non-treated samples.

3.6.6 Visual Acceptance

Each day of storage time, coated and uncoated bananas were evaluated visually by 20 untrained panelists as sensory analysis just before the analytical tests. Results were recorded according to 7-point hedonic scale and given at Table 3.6. Initially (day 0), consumers showed similar preference for coated and uncoated fruits as a proof that coating application did not alter bananas color and appearance. Throughout the storage time, all bananas lost their visual acceptance. Hemicellulose coating concentration did not significantly affect visual acceptance of bananas ($p>0.05$). Every coated sample had acceptable scores with sensory test up to the fifth day of storage period. After the fourth day, neither coated nor uncoated samples got acceptable score (< 4).

Table 3. 6 Overall visual acceptance of bananas during storage period at 4 ºC

	Overall visual acceptance			
Treatments / Storage time	Control	1% HC	1.5% HC	2% HC
Day 0	6.4 ± 0.5	6.5 ± 0.5	6.6 ± 0.6	6.7 ± 0.5
Day 1	4.4 ± 0.7	6.0 ± 0.7	6.1 ± 0.7	6.4 ± 0.6
Day 2	3.5 ± 0.8	5.8 ± 0.7	5.6 ± 0.6	5.6 ± 0.8
Day 3	2.7 ± 0.8	5.0 ± 0.7	5.2 ± 0.7	5.1 ± 0.7
Day 4	2.3 ± 0.7	3.9 ± 0.8	4.0 ± 0.8	4.1 ± 0.7
Day 5	2.1 ± 0.6	3.2 ± 0.7	3.1 ± 0.7	3.3 ± 0.6

Consumer showed significant preference for coated ($p<0.05$) after 1, 2, 3, and 4 days and coated bananas obtained scores between the categories “like extremely” and “neither like nor dislike” on the hedonic scale. On the other hand, uncoated bananas fell below the limit of acceptability by the second day of storage. Lower levels of darkening and browning directly correlated with the greater visual acceptance of fruits by consumers. By experiments, hemicellulose coating showed successful effect on banana peel; thus, darkening was delayed while acceptability of banana by consumer was prolonged.

CHAPTER 4

CONCLUSION

Hemicellulose is a valuable product; therefore, extraction and usage of it is important. This project gathered, combined and compared many different conditions to extract hemicellulose, and analyzed their interactions and effects before showing a usage area.

Firstly, this study presented extraction of hemicellulose from corn peel, corn cob, and sugar beet pulp with various conditions and methods. The highest yield of hemicellulose were achieved from corn residues (1.1mm), which were ground in laboratory miller, compared to the sugar beet pulp. Alkaline extraction method gave higher purity levels of hemicellulose than the acidic method. The alkaline extraction conditions were also improved after contaminant materials (fat, protein, and carbohydrate) were selectively removed. The effects of extraction temperature, time and alkaline concentration were assessed and optimisation was performed. Conventional one-factor at a time approach showed that the optimum conditions were 15% NaOH at 40°C for 24 h. However, response surface method predicted the optimum condition as 20% concentration, 31°C temperature and 24 h time. Furthermore, 65% yield was obtained at 30°C with 10% of alkaline concentration and 24 h of time. The results revealed that low alkaline concentration and temperature could give high hemicellulose yields and purity and the separation of raw material components before extraction increases the yield and purity of hemicellulose extracts.

Secondly, the study presented the usage of extracted hemicellulose from renewable sources as an edible coating material. In order to prolong shelf life and maintain important properties such as color, texture, taste, and mouthfeel, edible coatings from natural sources for foodstuffs are often necessary as a air and moisture barrier. Banana was used for the experiments in this study because it is the most traded fruit in the world and susceptible to environmental conditions due to high PPO activity. During storage of four days at 4ºC, the results indicated that hemicellulose can be a good coating material to prevent darkening of banana peel. It was showed that lightness and yellowness of coated bananas were preserved with very little losses at the end of storage period while uncoated samples turned to unacceptable appearance. Moreover, hemicellulose coating preserved texture, moisture content, and acceptable appearance of bananas by preventing or retarding fungal decay, loss of weight, firmness and color.

REFERENCES

Ahmad, S.H., Razak, A.R., Shaari, N., and Mohamed, M.T.M., 2007, "Plastid ultrastructure, chlorophyll contents, and colour expression during ripening of Cavendish banana at 17°C and 27°C", *New Zealand Journal of Crop and Horticulutral Science,* Vol: 35, pp. 201–210

AOAC, 1984, "Official methods of analysis", *Association of Official Analytical Chemists,* Washington, DC.

Barjol, J., and Chavanes, H., 2003, "Environmental report: Beet growing and sugar production in Europe." International Confederation of European Beet Growers (CIBE) and the European Committee of Sugar Manufacturers (CEFS), *JCB Offset –Wavre.*

Benkö, Zs., Andersson, A., Szengyel, Zs., Gaspar, M., Reczey, K., and Stalbrand, H., 2007, "Heat extraction of corn fiber hemicellulose", *Appl. Biochem. Biotechnol.,*Vol: 136-140, pp. 253-265

Box, G.E.P. and Behnken, D.W., 1960, "Some new three level designs for study of quantitative variables", *Technometrics,* Vol: 2, pp. 455-475

Buchanan, C. M.; Buchanan, N. L.; Debenham, J. S.; Gatenholm, P.; Jacobsson, M.; Shelton, M. C.; Watterson, T. L.; Wood, M. D., 2003, *Carbohydr. Polym.*, Vol: 52, pp. 345–357

Bulut B., 2006, "Tarıma dayalı alternatif yakıt kaynaklarından biyoetanol üretimi ve Türkiye için en uygun hammadde seçimi", Yüksek Lisans tezi, Yıldız Teknik Üniversitesi Fen Bilimleri Enstitüsü, Endüstri Mühendisliği, Danışman: Y. Doç. Dr. Hayri Baraçlı

Chang, V.S., Holtzapple, M.T., 2000, ‘’Fundamental factors affecting enzymatic reactivity’’, *Appl. Biochem. Biotechnol.*, Vol: 84, pp. 5–37

Chaplin, M., 2012, Hydrocolloids and gums. Available from: http://www.lsbu.ac.uk/water/hydro.html. Accessed June 25, 2012

Chauhan, B. and Gupta, R., 2004, "Application of statistical experimental design for optimization of alkaline protease production from Bacillus sp. RGR-14", *Process Biochemistry*, Vol: 12, pp. 2115-2122

Coviello, T., Matricardi, P., Marianecci, C., and Alhaique, F. J., 2007, "Polysaccharide hydrogels for modified release formulations", *Journal of Controlled Release*, Vol: 119, pp. 5–24

Delgenés, J.P., Penaud, V., Moletta, R., 2002, ‘’Pretreatments for the enhancement of anaerobic digestion of solid wastes’’, *Biomethanization of the Organic Fraction of Municipal Solid Wastes,* IWA Publishing, pp. 201–228

Dilek, F. B., Yetis, U., and Gökçay, C. F., 2003, "Water savings and sludge minimization in a beet-sugar factory through re-design of the wastewater treatment facility", *J. Clean. Prod.*, Vol: 11, pp. 327–331

Doner, L.W. and Hicks, K.B., 1997, "Isolation of hemicellulose from corn fiber by alkaline hydrogen peroxide extraction", *Cereal Chemistry Journal,* Vol: 74(2), pp. 176-181

Eaks, I.L., Sinclair, W.B., 1980, "Cellulose-hemicellulose fractions in the alcohol-insoluble solids of Valencia orange peel", *Journal of Food Science,* Vol: 45, p. 985

Ebringerova, A., 2005, “Structural Diversity and Application Potential of Hemicelluloses”, *Macromol. Symp.*, Vol: 232, pp. 1–12

Ebringerova, A., Hromadkova, Z., and Heinze, T., 2000, *Macromol. Rapid Commun.*, Vol: 21, p. 542

Ebringerova, A., Hromadkova, Z., and Heinze, T., 2005, “Hemicellulose”, *Adv. Polym. Sci*, Vol: 186, pp. 1–67

Emmel, A., Mathias, A.L., Wypych, F., Ramos, L.P., 2003, “Fractionation of Eucalyptus grandis chips by dilute acid- catalysed steam explosion’’, *Bioresource Technol.*, Vol: 86, pp. 105– 115

Eskicioglu, C., Terzian, N., Kennedy, K.J., Droste, R.L., Hamoda, M., 2007, “A thermal microwave effects for enhancing digestibility of waste activated sludge’’, *Water Res.*, Vol: 41, pp. 2457–2466

Esteban, M.B.,Garcia, A.J., Ramos, P., and Marquez, M.C., 2007, “Evaluation of fruit-vegetable and fish wastes as alternative feedstuffs in pig diets”, *Waste Management*, Vol: 27, pp. 193-200

Farhadian, M., Borghei, M., and Umrania, V. V., 2007, “Treatment of beet sugar wastewater by UAFB bioprocess”, *Bioresour. Technol.*, Vol:98, pp. 3080–3083

Fengel, D., Wegener, G., 1984, ‘’Wood: Chemistry, Ultrastructure, Reactions’’, De Gruyter, Berlin

Fischer, K. and Heinze, T., 2005, “Hemicelluloses”, Macromolecular Symposia-232: Selected contributions from the conference in Wiesbaden (Germany), June 27-30

Food and Agriculture Organization of the United Nations, FAOSTAT, available online at http://faostat.fao.org/default.aspx, last accessed date: June 10, 2010

Gabrielii, I., Gatenholm, P., Glasser, W.G., Jain, R.K., and Kenne, L., 2000, "Seperation, characterization and hydrogel-formation of hemicellulose from aspen wood", *Carbohydrate Polymer*, Vol: 43(4), pp. 367-374

Gaspar, M., Kalman, G., & Reczey, K., 2007, "Corn fiber as a raw material for hemicellulose and ethanol production", *Process Biochemistry,* Vol: 42, pp. 1135-1139

George, H., 2010, "Polyphenol Oxidase Causes Browning of Fruits and Vegetables", available from: Suite101.com. Accessed June 25, 2012

Hamauzu, Y., 2006, "Role and evolution of fruit phenolic compounds during ripening and storage", *Stewart Postharvest Review,* Vol: 2(2), pp. 1-7

Hansen, N.M.L. and Plackett, D., 2008, "Sustainable films and coatings from hemicelluloses: A Review", *Biomacromolecules,* Vol: 9(6), pp. 1493-1505

Hara, A. and Radin, N.S., 1978, "Lipid extraction of tissues with a low toxicity solvent", *Analytical Biochemistry*, Vol: 90, pp. 420-426

Hutnan, M., Drtil, M., and Mrafkova, L., 2000, "Anaerobic biodegradation of sugar beet pulp." *Biodegradation*, Vol: 11, pp. 203–211

Hutnan, M., Drtil, M., Derco, J., Mrafkova, L., Hornak, M., and Mico, S., 2001, "Two-step pilot-scale anaerobic treatment of sugar beet pulp." *Polish J. Environ. Studies*, Vol: 10(4), pp. 237–243

Inglett, G.E., 1970, “Kernel structure, composition, and quality‖ In: Inglett G.E. (ed), “Corn: culture, processing, products”, Westport, Conn., Avi Pub. Co.

Iza, J., Palencia, J. I., and Fdz-Polanco, F., 1990, “Waste water management in a sugar beet factory: a case study of comparison between anaerobic technologies.” *Water Sci. Technol.,* Vol: 22(9), pp. 123–130

Karimi, K., Kheradmandinia, S., and Taherzadeh, M.J., 2006, ‘’Conversion of rice straw to sugars by diluteacid hydrolysis’’, *Biomass Bioenerg.*, Vol: 30, pp. 247–253

Khuri, A.I. and Cornell, J.A., 1987, “Response Surfaces: Designs and analysis”, Marcel Dekker Inc. New York

Kittur, F.S., Saroja, N., and Tharanathan, H.R.N., 2001, “Polysaccharide-based composite coating formulation for shelf life extension of fresh banana and mango”, *Eur Food Res Technol,* Vol: 213, pp. 306-311

Kolekar, T.G., Modak, H.M. & Jadhav, S.J., 1988, “Shelf-life extension of banana by use of sucrose ester formation”, *Indian Journal of Plant Physiology,* Vol: 31, pp. 16–20

Koppar, A., and Pullammanappallil, P., 2007, “Single-stage, batch, leach-bed, thermophilic anaerobic digestion of spent sugar beet pulp.” *Bioresour. Technol.,* Available online, doi:10.1016/j.biortech.200706.051.

Krajnc, D., Mele, M., and Glavic, P., 2007, “Improving the economic and environmental performances of the beet sugar industry in Slovenia: increasing fuel efficiency and using by-products for ethanol.” *J. Clean. Prod.,* Vol: 15, pp. 1240–1252

Kulkarni, N., Shendye, A., & Rao, M., 1999, "Molecular and biotechnological aspects of xylanases", *FEMS Microbiology Reviews,* Vol: 23, pp. 411-456

Lawther, J.M., Sun, R., and Banks, W.B., 1996, ''Effect of steam treatment on the chemical composition of wheat straw'', *Holzforschung,* Vol: 50, pp. 365–371

Lee S-H., Yoshioka M., Shiraishi N., 2000, "Preparation and Properties of Phenolated Corn Bran (CB)/ Phenol/ Formaldehyde Cocondensed Resin", *Journal of Applied Polymer Science,* Vol: 77, pp. 2901-2907

Lindblad, M. S., Albertsson, A. C., 2005, "Oxygen barrier materials from renewable sources. In S. Dumitriu (Eds.)", *Polysaccharides: Structural diversity and functional versatility* (pp. 491–508). Marcel Dekker, New York.

Maes, C. and Delcour, J.A., 2001, "Alkaline hydrogen peroxide extraction of wheat bran non-starch polysaccharides", Journal of Cereal Science, Vol: 34(1), pp. 29-35

Mendes, C.V.T., Rocha, J.M.S., Sousa, G.D.A., & Carvalho, M.G.V.S., 2010, "Extraction of hemicelluloses prior to kraft cooking: A step for an integrated biorefinery in the pulp mill", *In XXI TECNICELPA Conference and Exhibition,* Lisbon, Portugal.

Moine, C., Gloaguen, V., Gloaguen, J. M., Granet, R., and Krausz, P., 2004, *J. En*V*iron. Sci. Health, Part B*, Vol: 39, pp. 627–640

Moniruzzaman, M., Dien, B.S., Ferrer, B., Hespell, R.B., Dale, B.E., Ingram, L.O., and Bothast, R.J., 1996, "Ethanol Production from AFEX Pretreated Corn Fiber by Recombinant Bacteria", *Biotechnology Letters,* Vol: 18, pp. 985-990

Munoz, P.H., Almenar, E., Valle, V.D., Velez, D., and Gavara, R., 2008, "Effect of chitosan coating combined with postharvest calcium treatment on strawberry

(Fragaria _ ananassa) quality during refrigerated storage", *Food Chemistry,* Vol: 110, pp. 428-435

Park, B., Ahn, J.H., Kim, J., Hwang, S., 2004, "Use of microwave pretreatment for enhanced anaerobiosis of secondary sludge'', *Water Sci. Technol.*, Vol: 50, pp. 17–23

Peroval, C., Debeaufort, F., Despre, D., and Voilley, A., 2002, "Edible Arabinoxylan-Based Films. 1. Effects of Lipid Type on Water Vapor Permeability, Film Structure, and Other Physical Characteristics", *J. Agric. Food Chem.,* Vol: 50, pp. 3977–3983

Playne, M. J., 1984, "Increased Digestibility of Bagasse by Pretreatment with Alkalis and Steam Explosion'', *Biotechnol.and Bioeng.*, Vol: 26, pp. 426– 433

Rajagopalan, S., Ponnampalam, E., McCalla, D., and Stowers, M., 2005, "Enhancing profitability of dry mill ethanol plants: Process modeling and economics of conversion of degermed defibred corn to ethanol", *Applied Biochemistry and Biotechnology,* Vol: 120(1), pp. 37-50

Ramos, L.P., Breuil, C., and Saddler, J.N., 1992, ''Comparison of steam pretreatment of eucalyptus, aspen, and spruce wood chips and their enzymic hydrolysis.'' *Appl. Biochem. Biotechnol.*, pp. 37–48

Reddy, N.R., Palmer, J.K., Pierson, M.D., and Bothast, R.J., 1983, "Wheat straw hemicelluloses: Composition and fermentation by human colon bacteroides", *J.Agric.Food. Chem.,* Vol: 31, pp. 1308-1313

Ritala, M., Leskelä, M., 2002, *In Handbook of Thin Film Materials*. San Diego, CA: Academic Press.

Robbins, R.J., 2003, “Phenolic Acids in Foods: An Overview of Analytical Methodology”, *Journal of Agriculture and Food Chemistry,* Vol: 51(10), pp. 2866-2887

Ruiz, E., Cara, C., Manzanares, P., Ballesteros, M., Castro, E., 2008, ‘’Evaluation of steam explosion pretreatment for enzymatic hydrolysis of sunflower stalks’’, *Enzyme Microb. Tech.*, Vol: 42, pp. 160-166

Sabiiti, E., 2011, “Utilising Agricultural Waste to Enhance Food Security and Conserve The Enviroment”, *African Journal of Food, Agriculture, Nutrition and Development,* Vol: 11, pp. 1-9

Saha, B.C. and Cotta, M.A., 2007, “Enzymatic saccharification and fermentation of alkaline peroxide pretreated rice hulls to ethanol’’, *Enzyme Microb. Tech.*, Vol: 41, pp. 528–532

Sahin, S. and Sumnu, G.S., 2006, Physical properties of foods, New York, Springer

Sanchez, G., Pilcher, L., Roslander, C., Modig, T., Galbe, M., and Liden, G., 2004, ‘’Dilute-acid hydrolysis for fermentation of the Bolivian straw material Paja Brava’’, *Bioresource Technol.*, Vol: 93, pp. 249–256

Sanwal, G.G., Payasi, A., 2007, “Garlic extract plus sodium metabisulphite enhances shelf life of ripe banana fruit”, *International Journal of Food Science and Technology,* Vol: 42(3), pp. 303-311

Savary, B.J., Hicks, K.B., Fishman, M.L., Hotchkiss, A.T., and Liu, L., 2004, “Sustainable technologies and valuable newpolysaccharide-basedproducts from sugar beet pulp”, SPRI Conference on Sugar Processing Research

Shapouri, H., Duffield, J.A. and Wang, M., 2002, “The Energy Balance of Corn Ethanol: An Update”, Agricultural Economic Report No: 814, U.S. Department of Agriculture, Economic Research Service, Office of the Chief Economist, Office of Energy Policy and New Uses , Washington, D.C.

Shore, M., Broughton, N. W., and Bumstead, N., 1984, “Anaerobic treatment of waste waters in the beet sugar industry.” *Water Pollut. Control,* Vol: 83(4), pp. 499–506

Sidiras, D.K. and Koukios, E.G., 1989, ‘’Acid saccharification of ball-milled straw.’’ *Biomass*, Vol: 19, pp. 289–306

Silverstein, R.A., Chen, Y., Sharma-Shivappa, R.R., Boyette, M.D., and Osborne, J., 2007, “A comparison of chemical pretreatment methods for improving saccharification of cotton stalks’’, *Bioresource Technol.*, Vol: *98*, pp. 3000–3011

Sims, R., 2003, ‘’Biomass and resources bioenergy options for a cleaner environment in developed and developing countries’’, *Elsevier Science*, London, UK.

Skendi, A., Biliaderis, C. G., Lazaridou, A., and Izydorczyk, M. S., 2003, “Structure and rheological properties of water soluble β-glucans from oat cultivars of *Avena sativa* and *Avena bysantina*”, *J. Cereal Sci.*, Vol: 38, pp. 15–31

Skurtys, O., 2012, Food Hydrocolloid Edible Films and Coatings. Available from: http://intrawww.ing.puc.cl/siding/datos/public_files/profes/fpedreschi_GTSNWECWJOGDA/Food%20Hydrocolloid%20Edible%20Films%20and%20Coatings.pdf. Accessed June 25, 2012

Spagnuolo, M., Crecchio, C., Pizzigallo, M. D. R., Ruggiero, P., 1997, "Synergistic effects of cellulolytic and pectinolytic enzymes in degrading sugar beet pulp." *Bioresour. Technol.,* Vol: 60, pp. 215–222

Torget, R., Himmel, M.E., and Grohmann, K., 1991, ''Dilute sulfuric acid pretreatment of hardwood bark.'' *Bioresource Technol.,* Vol: 35, pp. 239– 246

TUIK: Turkish State Institute of Statistics. 2011. Crop Production Statistics. Available from: www.tuik.gov.tr. Accessed November 2, 2011

Vaccari, G., Tamburini, E., Sgualdino, G., Urbaniec, K., and Klemes, J., 2005, "Overview of the environmental problems in beet sugar processing: possible solutions." *J. Clean. Prod.*, Vol: 13, pp. 499–507

Vaccarino, C., Lo Curto, R.B., Tripodo, M.M., Bellocco, E., Laganfi, G., and Patan, R., 1987, "Effect of SO2, NaOH and Na2CO3 pretreatments on the degradability and cellulase digestibility of grape marc'', *Biol. Waste.*, Vol: 20, pp. 79– 88

Varga, E., Thomsen, A.B., and Feng, L., 2005, Ethanol production from corn, corn stover and corncob from the Jilin province of China, in: Petersen, L.S., Larsen, H. (Eds), Technologies for Sustainable Energy Development in the Long Term. Proceedings, Riso International Energy Conference, May 23-25, 2005, Riso National Laboratory, Denmark, pp. 369-378

Voragen, A. G. J., Oosterveld, A., Schols, H. A., and Beldman, G., 1997, "Pectic substances from sugar beet pulp: Extraction and fractionation, structural features, functional properties and enzymic modification", In: *Transformation and Modification of Carbohydrates,* Proc. of 4th Int. Workshop on Carbohydrates as Organic Raw Materials, Vienna, Austria, March 20–21: 29

Waldron, K.W., Parker, M.L., and Smith, A.C., 2003, *Compreh. Rev. Food Sci. Food Safety*, Vol:2, p. 105

Wang, B. Z., Sui, J., Liu, R. F., Yang, G., and Qi, P. S., 1986, "Anaerobic reactors treating beet sugar effluents." *Effluent Water Treat.*, Vol: 26(5), pp. 150–162

Wang, B., Cheng, B., and Feng, H., 2008, "Enriched Arabinoxylan in Corn Fiber for Value-Added Products", *Biotechnology Letters*, Vol: 30, pp. 275-279

Weiland, P., 1993, "One- and two-step anaerobic digestion of solid agroindustrial residues." *Water Sci. Technol.*, Vol: 27, pp. 145–151

Wen, L.F., Chang, K.C., Brown, G., & Gallaher, D.D., 1988, "Isolation and characterization of hemicellulose and cellulose from sugar beet pulp", *Journal of Food Science*, Vol: 53, pp. 826

Zhang, Y. Q., Xie, B. J., and Gan, X., 2005, "Bibliography of carbohydrate polymers", *Carbohydrate Polymers*, Vol: 60, pp. 27–31

Zhao, X., Zhang, L., and Liu, D., 2007, "Comparative study on chemical pretreatment methods for improving enzymatic digestibility of crofton weed stem'', *Bioresource Technol.*, Vol: 99, pp. 3729-3736

Zhu, S., Wu, Y., Yu, Z., Liao, J., Zhang, Y., 2005, "Pretreatment by microwave/alkali of rice straw and its enzymatic hydrolysis'', *Process Biochem.*, Vol: 40, pp. 3082–3086

Zhu, S., Wu, Y.,Yu, Z., Wang, C., Yu, F., Jin, S., Ding, Y.,Chi, R., Liao, J., and Zhang, Y., 2006, "Comparison of three microwave/chemical pretreatment processes for enzymatic hydrolysis of rice straw'', *Biosyst. Eng.*, Vol: 93, pp. 279-283

APPENDIX A

PREPARATION OF ACETATE BUFFER

1. To prepare 0.05 M acetic acid solition, 2.9 ml acetic acid was made to 1000 ml.

2. To prepare 0.05 M sodium acetate solution, 4.1 g sodium acetate was dissolved in 1000 ml distilled water.

3. To get 100 ml pH 4.8 acetate buffer, 20 ml acetic acid and 30 ml sodium acetate solutions were mixed and final volume was adjusted to 100 ml with distilled water.

4. The final pH was adjusted using a sensitive pH meter.

APPENDIX B

CONSTITUENTS OF RAW MATERIAL

Table B. 1 Analysis of corn fiber

Constituent	Replicate 1 (g/g)	Replicate 2 (g/g)
Moisture content	0.060	0.065
Fat content	0.012	0.008
Fat and extractives	0.046	0.064
Crude fiber	0.201	0.239
Protein	0.031	0.035
Hemicellulose	0.438	0.446
Ash	0.026	0.025

Table B. 2 Analysis of corn stalk

Constituent	Replicate 1 (g/g)	Replicate 2 (g/g)
Moisture content	0.073	0.07
Fat content	0.009	0.009
Fat and extractives	0.076	0.070
Crude fiber	0.278	0.250
Protein	0.037	0.032
Hemicellulose	0.507	0.591
Ash	0.017	0.020

Table B. 3 Analysis of sugar beet pulp

Constituent	Replicate 1 (g/g)	Replicate 2 (g/g)
Moisture content	0.017	0.018
Fat content	0.009	0.008
Fat and extractives	0.026	0.023
Crude fiber	0.185	0.160
Protein	0.110	0.094
Hemicellulose	0.166	0.117
Ash	0.039	0.042

APPENDIX C

RESULTS OF ANOVA AND TUKEY‘S MEAN COMPARISON TEST FOR HEMICELLULOSE YIELD

Table C. 1 Results of one-way ANOVA and Tukey‘s mean comparison test for the effect of temperature on hemicellulose yield

```
Source  DF        SS        MS     F      P
Temp     4  0,010180  0,002545  4,63  0,048
Error    6  0,003297  0,000550
Total   10  0,013477

S = 0,02344   R-Sq = 75,53%   R-Sq(adj) = 59,22%

                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level  N     Mean    StDev  --+---------+---------+---------+-------
30     3  0,38493  0,03066  (-------*--------)
35     2  0,41530  0,03267        (---------*---------)
40     2  0,44230  0,00580              (----------*---------)
45     2  0,44050  0,01513              (---------*---------)
50     2  0,47040  0,00933                      (----------*---------)
                                --+---------+---------+---------+-------
                                0,360     0,400     0,440     0,480

Pooled StDev = 0,02344
```

Table C. 2 Results of one-way ANOVA and Tukey‘s mean comparison test for the effect of time on hemicellulose yield

```
Source  DF       SS       MS     F      P
time     3  0,05034  0,01678  4,78  0,023
Error   11  0,03858  0,00351
Total   14  0,08893

S = 0,05923   R-Sq = 56,61%   R-Sq(adj) = 44,78%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  -----+---------+---------+---------+----
24     4  0,55715  0,06471                (--------*-------)
48     4  0,42390  0,03238  (-------*-------)
72     4  0,50355  0,01912           (-------*-------)
96     3  0,57227  0,10436                  (---------*--------)
                           -----+---------+---------+---------+----
                              0,400     0,480     0,560     0,640

Pooled StDev = 0,05923
```

Table C. 3 Results of one-way ANOVA and Tukey‘s mean comparison test for the effect of concentration on hemicellulose yield

```
Source  DF       SS       MS     F      P
conc     3  0,06898  0,02299  5,86  0,012
Error   11  0,04317  0,00392
Total   14  0,11216

S = 0,06265   R-Sq = 61,51%   R-Sq(adj) = 51,01%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
 5     3  0,23980  0,04105  (-------*-------)
10     4  0,37380  0,03351              (------*------)
15     3  0,39693  0,07590                (-------*-------)
20     5  0,42640  0,07892                    (------*-----)
                           ----+---------+---------+---------+-----
                             0,20      0,30      0,40      0,50

Pooled StDev = 0,06265
```

APPENDIX D

APPEARANCE OF HEMICELLULOSE COATED AND UNCOATED BANANAS

Figure D. 1 Appearance of hemicellulose coated bananas at the 0th day

Figure D. 2 Appearance of uncoated bananas at the 0th day

Figure D. 3 Appearance of hemicellulose coated bananas at the 1st day

Figure D. 4 Appearance of uncoated bananas at the 1st day

Figure D. 5 Appearance of hemicellulose coated bananas at the 4th day

Figure D. 6 Appearance of uncoated bananas at the 4th day