ACETYLATION OF CORN STARCH FOR GELATIN SUBSTITUTION IN SOFT CANDY

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ABSTRACT

ACETYLATION OF CORN STARCH FOR GELATIN SUBSTITUTION IN SOFT CANDY

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In confectionery industry, hydrocolloid types are of great importance due to their vegan, Halal and Kosher suitability and their effects on product quality. Starches are plant based and economical alternatives. However, modification of starch is necessary to obtain the desired quality. The study is aimed to determine the effect of reagent amount and reaction time in acetylation of native, acid modified and oxidized corn starch on the reaction efficiency and physicochemical properties of starches. In addition, the texture properties of soft jelly with these starches have been analyzed. Characterization of starches was done by using chemical analysis (titration method) and instrumental analysis (DSC, XRD, RVA, transmittance). Premodification type of starch has great importance in terms of reaction efficiency. It was observed that acid modified starch had lower gelatinization temperature, higher enthalpy, and lower crystallinity level than native corn starch. In addition, acetylated acid modified starch had lower gelatinization temperature and more stable final viscosity than acetylated oxidized and native starches. These properties proved that acid modified starch had a higher tendency to acetylation reaction.

In soft jelly production, the usage of starch with low acetylation degree was more suitable in terms of hardness and stickiness values. The texture of the soft jelly made of gelatin and acetylated oxidized starch were close to each other. In addition, acetylated oxidized starch had higher transmittance value that differentiated it from the opaque acetylated acid modified soft jellies. On the other hand, soft jellies made with acetylated acid modified starch exhibited a gummy texture.

Keywords: Acetylation reaction, Acid modification, Oxidation, Corn starch, Starch-based soft jelly candy

ÖZ

YUMUŞAK ŞEKERLEMEDE JELATİN İKAMESİ OLARAK MISIR NİŞASTANIN ASETİLLENMESİ

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Şekerleme endüstrisinde, hidrokolloid türleri vegan, Helal ve Kosher uygunlukları ve ürün kalitesi üzerindeki etkileri nedeniyle büyük öneme sahiptir. Farklı kökenli nişastalar ise yaygın olarak tercih edilen, bitkisel bazlı ve ekonomik alternatiftir. Nişastalar, diğer hidrokolloidlerin yerine kullanılmak üzere modifiye edilebilir. Bu çalışmada, doğal, asit modifiye ve okside mısır nişastasının asetilasyonunda reaktant miktarı ve reaksiyon süresinin tepkime verimliliği ve nişastaların fizikokimyasal özellikleri üzerindeki etkilerinin belirlenmesi amaçlanmıştır. Ayrıca, bu nişastalarla üretilen yumuşak şekerleme ürününün tekstür özellikleri analiz edilmiştir. Nişastaların karakterizasyonu kimyasal analiz (titrasyon yöntemi) ve enstrümantal analiz (DSC, XRD, RVA, geçirgenlik) kullanılarak gerçekleştirilmiştir. Nişastanın ön modifikasyonu ve ön modifikasyon türü, tepkime verimliliği açısından büyük öneme sahiptir. Asitle modifiye edilen nişastanın, doğal mısır nişastasına göre daha düşük jelatinleşme sıcaklığına, daha yüksek entalpiye ve daha düşük kristallik seviyesine sahip olduğu görülmüştür. Ayrıca asetile asit modifiye nişasta, asetile okside ve doğal nişastalardan daha düşük jelatinleşme sıcaklığına ve daha kararlı

final viskoziteye sahip olmuştur. Bu özellikler asitle modifiye nişastanın asetilasyon reaksiyonuna eğiliminin daha yüksek olduğunu kanıtlamaktadır.

Yumuşak şekerleme uygulamasında, uygun sertlik ve yapışkanlığa sahip olması nedeniyle asetilasyon derecesi düşük nişastaların kullanılması uygun bulunmuştur. Jelatin içeren jöle şekerlemenin tekstürüne en yakın tekstür, asetile okside nişasta kullanılarak elde edilmiştir. Bu özelliklere ek olarak asetile okside nişasta daha yüksek geçirgenlik değerine sahip bulunmuştur ve bu özellik onu asetile asit modifiye edilmiş yumuşak şekerlemenin opak doğasından ayırmaktadır. Öte yandan, asetile asit modifiye nişasta jeli elastik bir tekstür sergilemiştir.

Anahtar Kelimeler: Asetilasyon reaksiyonu, Asit modifiye nişasta, Oksidasyon, Mısır nişastası, Nişasta bazlı yumuşak şekerleme

For those who want to be in change…

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CHAPTER 1

INTRODUCTION

1.1 Starch

Starch is a carbohydrate source of plants. It is composed of two polymer chains of glucose: amylose and amylopectin. Amylose (*Figure1.1*) is unbranched α[1→4] linked to D-glucose and amylopectin (*Figure 1.2*) has chains of α[1→4] D-glucose that is highly branched with α[1→6] branching links (Copeland, Blazek, et.al., 2009).

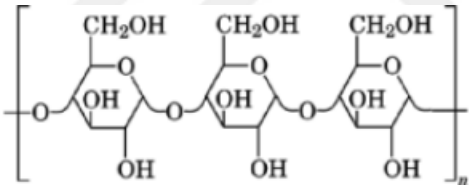


Figure 1.1 Structure of amylose molecule (Whistler, Daniel, 2000)

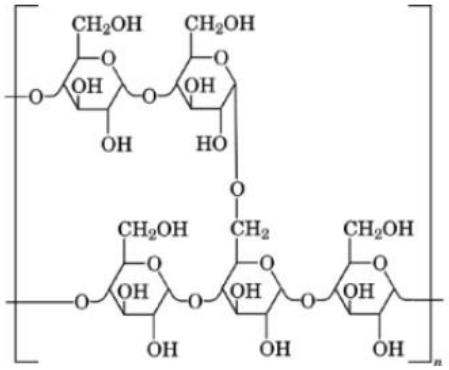


Figure 1.2 Structure of amylopectin molecule (Whistler, Daniel, 2000)

While amylose is a linear molecule, amylopectin is a branched molecule. There are so many starch sources such as potato, wheat, maize, tapioca, rice, waxy maize, etc. Their amylose/amylopectin ratios change with respect to origin. Waxy has the highest amylopectin ratio, while maize and wheat have the lowest amylopectin ratio (Ellis, Cochrane, et.al., 1998; Hoover, 1995). According to amylose/amylopectin ratio, starches paste temperature, peak viscosity, swelling power, paste viscosity, paste clarity and retrogradation rates differ *(Figure 1.3)*.

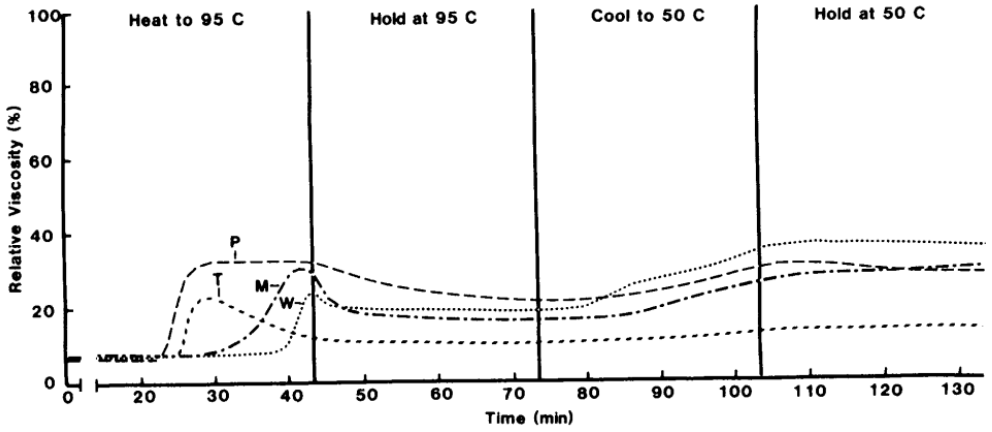


Figure 1.3 Viscosity profile of different types of native starches (Deffenbaugh, Walker, 1989)

Starch gelation is mainly about the heating of starch suspension in some amount of water. During heating of starch suspension, granules are broken down that is called as gelatinization (Ratnayake, Jackson, 2009). That is represented in *Figure 1.4.* The breakage starts above gelling temperature, the molecules absorb water, and the molecules are swollen. The molecular shape and order of starch are lost. The amylose crystallinity part of starch is solubilized while amylopectin part has only released. After the heat application, with the amylopectin skeleton, fluid and porous, gelatinized, swollen granules; the fluid is composed of hot amylose solution (Morris, 1990).

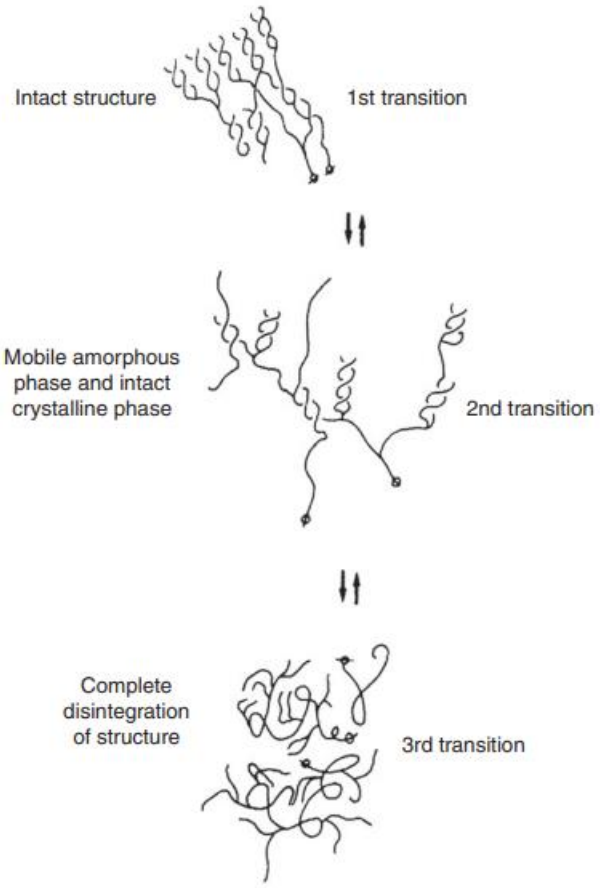


Figure 1.4 Phase transition of starch molecules during gelatinization (Ratnayake, Jackson, 2009)

After cooking, the temperature of starch pastes decreases, fluid becomes turbid viscoelastic paste. During cooling, retrogradation occurs; the disordered molecules become more ordered and increase crystallinity. Gel rheology changes, it is more rigid and firm. The water holding capacity is lost and the final texture is obtained (Gudmundsson, 1994; Morris, 1990). Thanks to having rigid gel, starch is preferred in many applications.

There are several limitations of native starches in many applications. Native starches have low shear resistance, low thermal stability, sensitive to enzymatic and acidic

conditions, high viscosity, degradation with heat, high retrogradation and syneresis level so some modifications are needed.

1.1.1 Acid Modified Starch

Acid modification is applied to degrade the starch, while keeping its granular form. Acid modification leads to reduced paste viscosity, enhanced gel forming and fluidity (Singh, Ali, 1987). Acid is added below gelatinization temperature. The granular shape and particle size of the acid modified starch are the same with the native starch. After acid modification, hot-paste viscosity decreases and its retrogradation level increases so the solid amount can be increased much more than the native starch in pastes (Rohwer, Klem, 1984).

Acid modification is the most preferred thinned starch modification type (Autio, Poutanen, 1994). It is done by using different types of acids, but hydrochloric acids and sulfuric acids are the most used ones. The modification level depends on different strength of acid, reaction temperature, reaction time and molecular characteristics of the starches (Singh, Ali, 2008). Acid reacts with the amorphous part of the molecules at first, then amylose molecules are hydrolyzed. Initially, crystallinity increases while amylose content and molecular mass decrease. High crystalline starch is used in food and textile industry (Atichokudomchai, Shobsngob & Varavinit, 2000).

In commercial production, 40-42 % of starch suspension is prepared. Determined amount of HCl or another acid like sulfuric acid is added and the temperature is kept in between 25-55°C for a certain period. Fluidity and viscosity are the main parameters of modification level control. Then by adding base, the pH is neutralized, and the reaction is finished (Rohwer, Klem, 1984).

After acid modification reaction, the gelatinization enthalpy and temperature increase with reduction of amylose content. Viscosity of starch gel decreases, swelling capacity, granule density and gel consistency increase due to

depolymerization (Kaur, et.al., 2022; Kaur, Oberoi, Sogi &Gill, 2011). After cooling of the gel, the hardness of the paste increases (Sun, Zhu & Xiol, 2015). Because of the irreversible crystallization of linear molecules, rigidity increases (Miles, et.al, 1985). Desired short textured gels that do not stretch when bitten are created for jelly applications.

The acid modified starch is used in jelly candy applications as a gelling agent. Acid modified starch gives a short and rigid texture with acceptable resilience (Lees, Jackson, 1971; Wurzburg, 1995; Chakraborty, et.al., 1999). In tableting applications, improved swelling and water binding properties makes acid modified starch advantageous. Acid modified starch is used as tablet binder, when bond strength and accelerated dissolution are needed (Okunlola, Akingbala, 2013). In mozzarella cheese, to decrease the amount of casein, acid modified starches can be used. Acid modified starch has low viscosity but provides rigid texture during cooling that is desirable for imitated cheese products (Carpenter, Finnie, & Olsen, 2016).

1.1.2 Oxidized Starch

Oxidation results in depolymerization and weakening of intermolecular bonds. Oxidation is applied to obtain low viscosity gel in high solid containing recipes. Commonly, sodium or calcium hypochlorite and hydrogen peroxide are used as reagents. The reagents attach to the glucose units at the reducing end (*Figure 1.5*), at first oxidizing agent degrades amylopectin molecules. Thus, the viscosity decreases. As a result of the oxidation reaction, starch molecules are oxidized to carbonyl and then to carboxyl groups. Hydroxyl groups of a starch molecule at C-2, C-3 and C-6 are transformed to carbonyl and carboxyl groups. The oxidation level is determined by the content of carbonyl and carboxyl groups (Vanier, et.al., 2017). Oxidation also stabilizes the viscosity while cooling of the gel. Thanks to carboxyl groups on the starch molecules, the linear amylose molecules cannot make hydrogen bond while cooling and the gel does not retrograde. So, the frozen stage stability is improved.

Moreover, the oxidized starch gel has higher clarity (Parovuori, et.al, 1995; Pietryzk, et.al., 2012).

Oxidation reaction is under mild to moderate alkaline conditions, and the presence of catalyst that is Cu (II) ion to accelerate reaction (Zhang, et.al, 2012).

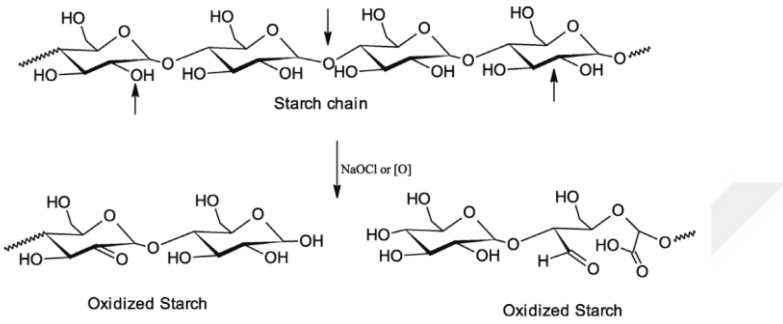


Figure 1.5 Oxidation reaction of a starch molecule (Egharevba, 2019)

Oxidized starch gels have intermediate/low viscosity, high paste clarity and soft texture since oxidation dissolves the granules, so the granules do not swell and thicken. Oxidized starch is preferred rather than acid thinned starch because of its less tendency to create gel structure. Oxidized starch has low temperature stability because of reduced retrogradation. Also, oxidation agents remove the pigments and increase the whiteness thanks to bleaching reaction (Egharevba, 2019; Sadowska, et.al.,2006).

Oxidized starches are used as stabilizer, gelling agent, and thickener in confectionary products such as cake fillings, powdered cake mix, pudding and cream. Due to having stable viscosity and less tendency to retrogradation, oxidized starch is used in sauces, soups, frozen dishes, baby foods as binder, thickener, and stabilizer (Tharanathan, 2007; Galkowska, Kapusniak, & Juszczak, 2023). Oxidized starch improves frozen food stability during storage (Chung & Seib, 1991). This starch acts

as an emulsifier by binding water and acts like a fat (Konoo, Ogawa, Mizuno, & Iso, 1996; Vanier, et.al., 2017).

1.1.3 Acetylated Starch

Acetylation is an esterification reaction in which hydroxyl groups of starch molecules are converted to acetyl groups (Betancur, Chel & Canizares, 1997; Chi, et.al, 2023) (*Figure 1.6*). Thus, gelatinization temperature decreases, translucency, viscosity, and freeze-thaw stability increase (Singh, Kaur, &Singh, 2004). Low Degree of Substitution (DS) level acetylated starches are used in food applications (Bello-Pérez, et.al., 2010; Tian, et.al., 2018).

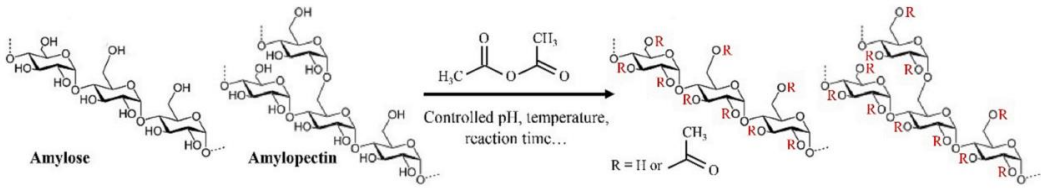


Figure 1.6 Illustration of starch acetylation reaction (Chi et.al., 2023)

Acetylation contributes to binding, thickening, adhesion, texturing and stabilizing of food materials thanks to resistance to retrogradation. Retrogradation is the recrystallization of starch during storage of starch paste or starch containing food products. This is an unfavorable property because it affects consumer approach (Zieba, Szumny & Kapelko, 2011). Reduction of retrogradation is due to prevention of alignment and association between linear starch molecules by acetyl groups (Singh, Sodhi, & Singh, 2012). Acetylated starch gels are more advantageous in terms of having decreased retrogradation level.

Acetylation reaction is carried out with an esterifying agent like acetic anhydride, acetic acid, and vinyl acetate monomer (Lin, et.al., 2019) in an aqueous medium in

the presence of sodium hydroxide (NaOH) as a catalyst (Lee Phillips, et.al., 1999; Sodhi, Singh, 2005; Bello-Pérez, Contreras-Ramos, & Jìmenez-Aparicio, 2000). The chemical reaction depends on reactant concentration, reaction time, pH, and the presence of catalyst (Betancur, Chel & Canizares, 1997).

As a result, acetylated starch gels clarity, freeze-thaw stability and viscosity increase and tendency to retrogradation decreases (Han, et.al., 2012) that makes acetylation reaction favorable.

Acetylated starches are used in noodles to increase transparency, hydrophilicity, to decrease digestive property, and to improve texture color and tensile properties (Lin, et.al., 2019). Also, acetylation of starch increases hardness in noodles (Choy, May, & Small, 2012). Acetylated starch replaces fat in processed meat, preserves the juiciness and thickness of the meat, thereby reducing the toughness of the meat, its loss during cooking and shrinkage of the meat. (Osman et.al., 2022). In sauces, acetylated starch is used as binder, thickener and stabilizer that improves smoothness and shiny look of soup and sauces (Egharevba, 2019). To stabilize texture in confectionary products, acetylation was applied to starch. Acetylation improves texture and transparency of confectionary products (Boursier, Quettier & Lesage, 2009).

1.2 Thickeners in Confectionary

In confectionary industry, soft jelly candies are highly preffered and have a high consumption rate. According to the Future Market Insights report (2023), Jellies and gummies have US$ 5 Billion in 2023 and its CAGR (Compound Annual Growth Rate) 5.1% from 2023 to 2033. In confectionary gels, there are high amount of sugar, gelling agents, food acid, flavouring and colouring. As sugar sources, glucose and sucrose are used, in common. Gelatin, pectin and starch are preffered as thickeners (Burey, Bhandari, Rutgers, Halley & Torley, 2009).

A gel creates an intermediate phase in between solid and liquid structures; gives elasticity like solid, and flow character like fluids. The several gelation agents are employed to get acceptable textural properties as a thickener and stabilizer. A gel has a resistance to flow under pressure and retains its mechanical rigidity as a high moisture 3D polimeric network (Banerjee & Bhattacharya, 2012).

1.2.1 Gelatin

Gelatin is the most commonly used gelling agent in soft jelly confectionaries. It is cheap and has readily been supplied from bovine, pig and marin fish skins, demineralized bones and hooves. Gelatin is a type of pure protein and is obtained by thermal degradation of collagens. Gelatin has 18-20 amino acids, glycide, proline and hydroxyproline present mostly. As seen in the *Figure 1.7*, the amino acids are bound by the hydrogen bonds (Yang, 1997). In food recipes, it is used as binder, thickener and stabilizer agent as water soluble polymer. Gelatin forms thermally reversible gels in water below body temperature 35°C, so gelatin has different organoleptic properties and flavour release (Baziwane & He, 2003). Gelatin carries both negative and positive electrostatic charges at the same time so, its gel mechanical strength differ according to pH level and ionic strength. Gelation with gelatin, which is close to the isoelectric points and has high ionic strength, creates a coarse and particulate gel network with high elasticity, thanks to the lowest level of electrostatic repulsion (Dille, Graget & Hattrem, 2015).

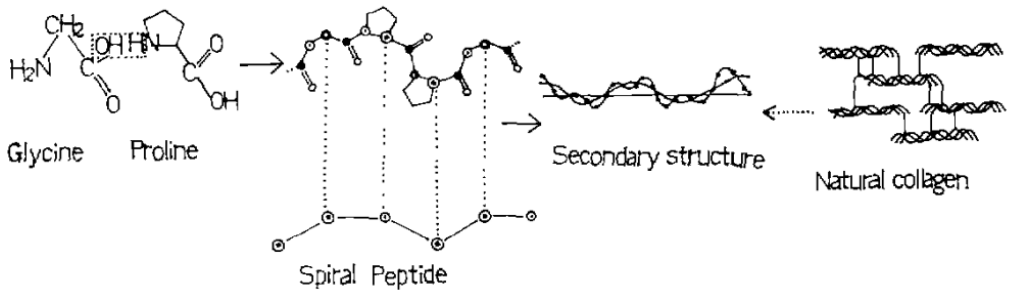


Figure 1.7 Gelatin configuration (Yang, 1997)

Structural changes in a gelatin chain affect specific properties of gelatin. In gelatin macromolecules, acidic and basic functional groups are present. Gelatin has ability to form a specific triple-stranded helix structure at low temperature solutions. Gelatin has a specific water interaction. These structural differences change stability of gelatin during heating and cooling (Kozlov, Burdygina, 1981).

In food applications, gelatin provides an important advantage thanks to its heat reversible gel formation properties. Gelatin swell in a cold water and absorbs 5-10 times more water than its volume. During heating, above melting point, gelatin starts to disorganize and form a gel when it is cooled. If gelatin is kept in hot water, the gelation power is lost (Congdon, 1914). The conversion between solution and gel formation is reversible and can be repeated. That is desirable property in food applications (Kumagai, et.al., 1993; Goodall et.al., 1988).

The bloom value of a gelatin is important. Bloom value express the gel strength and resilince of jelly under known strength. The strength affects the viscosity as seen in *Figure 1.8.* Higher amount of low bloom gelatin is needed to achieve the same texture as high bloom gelatin jelly production.

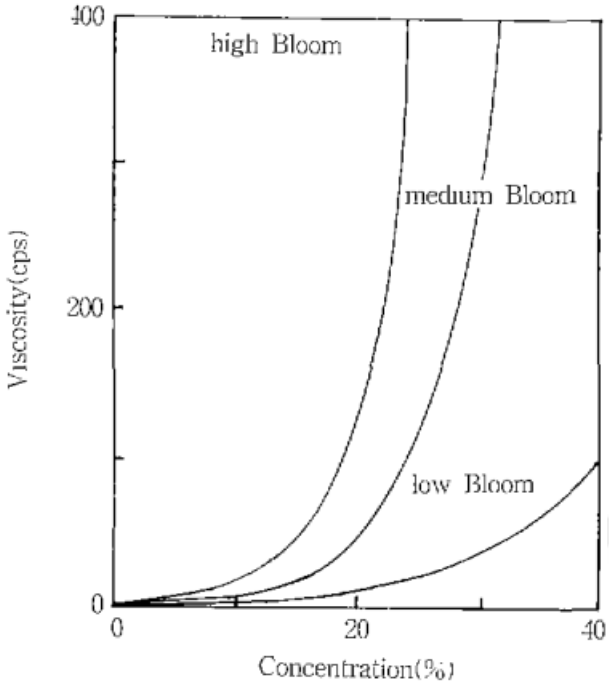


Figure 1.8 Viscosity characteristic of different concentrations of gelatin (Yang, 1997)

Gelatin is used in ready to eat dessert gels due to having clarity, elastic gel property and melting in mouth. In high solid confectionary, gelatin supplies low hot paste viscosity and elastic texture. Also, in foamed confectionary like marshmallow, gelatin is used as aeration agent and foam stabilizer. In low fat spreads, yogurt and creamy structures, gelatin is preferred because of fat like structure, emulsion stabilizer, prevention of syneresis and forming creamy texture ability (Morrison, et.al., 1999).

1.2.2 Pectin

Although gelatin is an advantageous thickener and stabilizer, there are some concerns about being animal based and being extracted from pigs which are not suitable for Vegans and Helal Food regulations. Therefore, pectin and starch are preferred in soft confectioneries. Pectin is a plant-based emulsifier, gelling agent,

glazing agent, stabilizer, and thickener. It is used in food, pharmaceutical, personal care, and polymer industries. There are two types of pectin; high methoxy pectin (HMP) and low methoxy pectin (LMP), which are different in terms of esterification level. Pectin shows Newtonian fluid behavior and forms gels at low concentrations. In jelly production, having high sugar concentration reduces water activity. So, presence of sugar promotes the interchain interaction, water comes out, hydrogen bonding is favorable and crosslinking increases. That occurs in HMP gels. In LMP gels, high amount of sugar causes dehydration and hydrogen bonding increases but, crosslinking decreases because of calcium ions on the LMP chains (Chan, Choo, Young, & Loh, 2016). In recipes with high sugar content like soft jelly, pectin forms a gel at low pH values. The form of gel making esterification increases with the decrease in pH below 3.0. When pectin is used in jelly production, there are some key points about the HMP and LMP usage. HMP degrades rapidly under high temperature and low pH conditions; and pectin does not show its gelling property, it affects jelly texture. Commercially, pectin that is obtained from apple is commonly preferred because of its high viscous gel capability although, citrus pectin gels have lighter color than apple pectin gels (May, 1990).

In food applications, especially in low calorie foods, pectin is used as a sugar and fat replacer (Hoefier, 1991). In the jams and jellies, pectin is preferred to increase the dry substance and to give a texture without adding sugar. Pectin decreases the calorie and sugar content that makes the jams and jellies suitable for diabetics. Pectin maintains texture stability in low pH jams and jellies (Peschardt, 1956). In jams that do not contain sweetener, to provide a stable texture and to keep the high moisture content of product during storage pectin is used. Pectin gives desired body and mouthfeel to jams (Towel, Christensen, 1959). Pectin is also used in instant jelly included dough mixtures. Being thermally stable and having high fiber content, pectin provides stable gel structure to jellies even in dough and batter. Pectin resists having different levels of acidity and soluble solids (Hoefier, 1991). In confectionary applications, pectin is used in neutral flavor candies. Pectin prevents settling of solids and lipid migration in edible coatings of confectionaries (Peschardt, 1956; Brake,

Fennema, 1993). In frozen food applications, pectin is used to retard crystal growth by preventing syrup loss and improvement of ice crystals in shape during thawing. Frozen fruits could be coated with pectin to supply improved texture and maintained quality in ice-creams (Decker, 1953; Thakur, et.al., 1997). For dietetic beverages, pectin is a sugar reduction alternative without affecting mouthfeel and texture. In some sauces, pectin can be used for desired flavor release and texture (Hoefier, 1991).

1.2.3 Starch

Starch is used as thickener and water binder providing desired texture and body to jelly confectioneries (Mauro, Kvasnica & Furcsik, 1991). Starch is preferred to gelatin by the consumers, who have concern about vegan and halal foods. There are different types of starches to give the best final structure. According to characteristics of starches during heat application and pasting, interaction with other ingredients, effects of rheological properties to pumping, depositing, and curing are changing (Habilla, Cheng, 2015).

1.2.3.1 Acid Modified Starch in Confectionary

Acid modified starch is commonly used in high solids containing food products. The gel characteristic of starch paste affects the production of jellies. For efficient jelly production, during cooking, pumping, depositing and storage the soft jelly melt must have appropriate fluidity (Habilla, Cheng, 2015). The acid modified starch is preferred because of low hygroscopicity, low sweetness and prevention of sugar crystallization (Murray & Luft, 1973). After heat application acid thinned starch starts to swell and form a low viscous paste thanks to that paste disperses uniformly without phase separation. If starch has a high amount of branched chain, the paste is going to be thick, and dispersion is affected adversely. The desired jelly like gels cannot be obtained (Autio, et.al., 1992; Leloup, Colonna, & Buleon 1991).

1.2.3.2 Acetylated Oxidized Starch in Confectionary

In food applications, oxidized starch is used due to having low viscosity and being tasteless. In oxidation reaction carbonyl and carboxyl groups are introduced thus, starch paste has lower tendency to retrogradation and because of reaction reagent oxidized starch gels have lighter color (Kuakpetoon and Wang, 2001). An acetylation reaction is needed if a food product needs a thickener, which has stable and resistance to retrogradation of pastes (Wurzburg, 1989). Oxidized acetylated starches enhance gummy like starch gels. Due to prevention of retrogradation and syneresis and having freeze-thaw stability acetylated- oxidized starch is an alternative thickener in jellies (Aini & Purwiyatno, 2010).

1.3 Techniques for Characterization of Starch

Modifications on starch molecules change the thermal properties, paste characteristics and crystallinity of starches. For analysis of variation in characteristics, according to reaction conditions and reaction types, there are some instrumental methods.

1.3.1 Rapid Visco Analyzer (RVA)

Rapid Visco Analyzer (RVA) is a viscosity measurement instrument by which the rheology of a starch gel during heating and cooling could be measured. RVA simulates a heating and cooling process in a small cup with an impeller under controlled temperature, shear conditions and determined periods. RVA could monitor changes in viscosity with specific time and temperature.

The RVA is a type of rotational viscosimeter, the viscosity of a fluid is determined by measurement of needed torque to turn a disc in a fluid at a determined speed (*Figure 1.9*). The impeller is rotated with a constant angular velocity (Ω) that is proportional to liquid viscosity (η). The impeller needs a torque G. The radius of

impeller is *a*, d is the distance of fixed plate from the impeller and ρ is the density of the product.

The viscosity is measured by using Equation (1) (Gupta, 2014);

$$\eta = \frac{Gd}{\pi a^4 \Omega} \quad (1)$$

During measurement, the starch suspension is heated, starch granules absorb water and start to swell. The inter crystalline structure breaks down and continuous starch gel is formed. The starch gel viscosity changes by heating and cooling. The test period is divided into 5 stages; preparation of starch suspension, heating, holding at maximum heating temperature, cooling, and holding final stage (*Figure 1.10*) (Balet, et.al., 2019). The torque changes are recorded in terms of viscosity in mPa.s and cP.

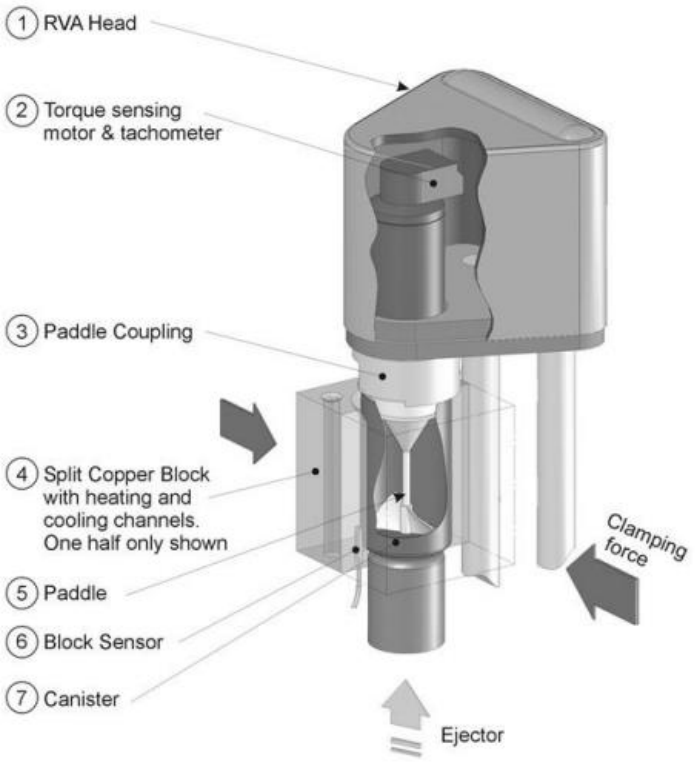


Figure 1.9 RVA Components (Perten Ins., n.d.)

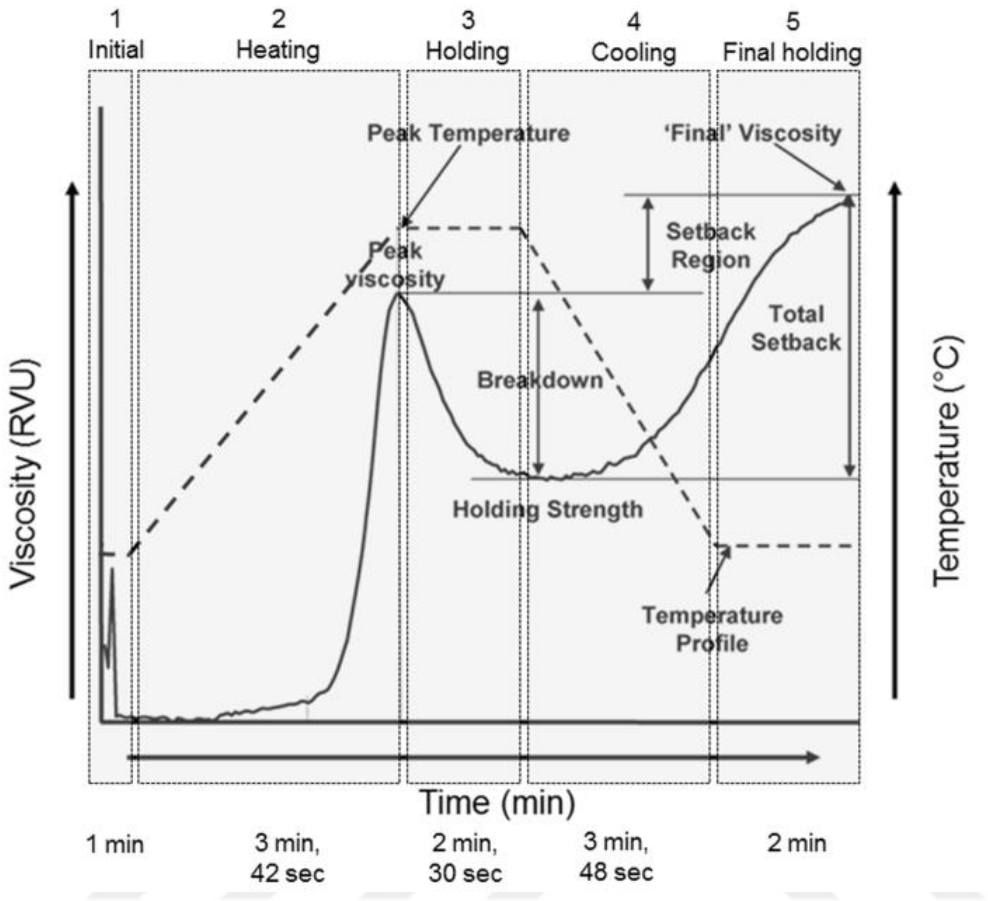


Figure 1.10 RVA pasting profile and method stages (Balet, et.al., 2019)

Pasting in the second heating stage displays a sudden increase in viscosity, that point express gelatinization temperature. The highest viscosity during heating is peak viscosity, the maximum swollen granules are obtained at that point. In the holding stage, the granules are melting/broken and viscosity decreases. In the cooling stage, retrogradation occurs and viscosity increases. The differences between peak viscosity and final viscosity are called setback viscosity. That indicates retrogradation level of starch molecules. The final viscosity shows the viscosity of sample after a period and under a certain condition (Batey, 2007).

1.3.2 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a thermal analysis technique. In principle, the required energy to change temperature of sample and the references (as shown in *Figure 1.11*) are measured in a controlled period of heating procedure. The heating continues until the differences is zero between sample and reference and the energy is recorded. This method is primarily employed for both qualitative and quantitative assessment of temperature changes, including exothermic and endothermic reactions, as well as heat capacity. Calorimetry is measurement of heat released or absorbed while heating by calorimeter (Gill, et.al., 2010).

DSC is used to analyze the effect of heating on crystalline polymer, glass transition and crystallizations.

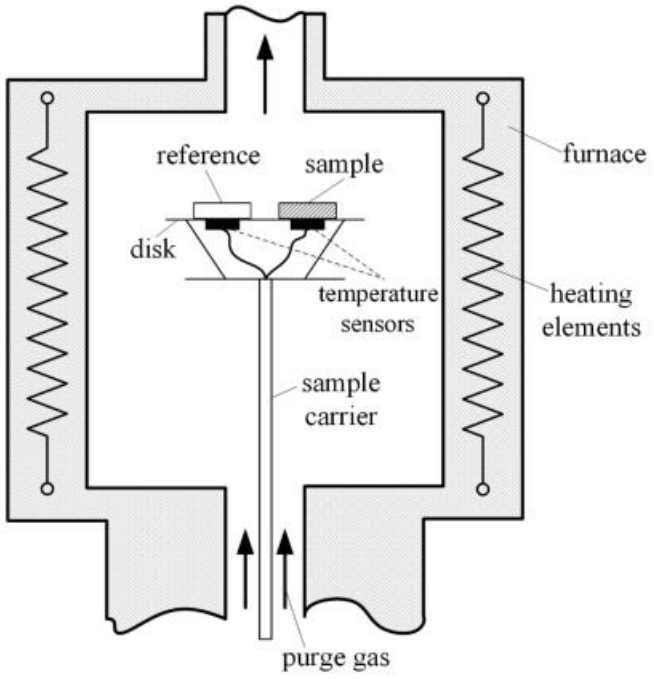


Figure 1.11 Heat flow of DSC measurement cells (Zheng, et.al., 2019)

There are two pans placed on a heater underneath each pan. The heaters are operated, and two pans (sample and reference pans) are heated with a specific rate, 10℃ /min in usual. The heating rates of sample and reference must be constant. The sample pan cannot be heated at the same rate due to the presence of a sample in it, unlike the reference pan, which remains empty. Sample pan needs more heat so, sample pan heater has higher rate of heating.

In DSC aluminum pan can be used if the product does not react with aluminum and does not need more heat than 600℃. The sample weighed 3-20 mg. The reference pan and sample pan should be the same. The pans are closed with a cover and hermetically sealed not let to leak. To prevent the reaction of samples with air, oxygen or burning, an inert gas is used such as nitrogen, helium, argon etc. Nitrogen is used in sensitive analysis, commonly with 50mL/min flow rate.

DSC represents the result on a heat flux vs temperature or time graph. According to reaction, exothermic or endothermic, peaks could be positive or negative. The transition enthalpy is measured by taking integral of peak corresponding to transition (Akash & Rehman, 2020).

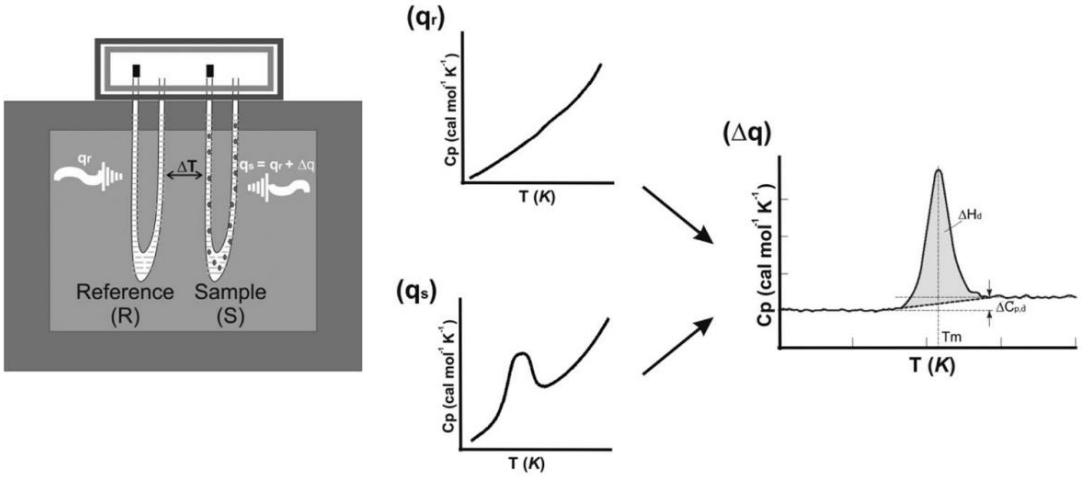


Figure 1.12 DSC reaction setup (Gill, et.al., 2010)

As shown in the *Figure 1.12*, for the same temperature increase (ΔT) of sample and reference, the needed heat for sample (q_s) is more than needed heating energy for reference (q_r). The given excess heat is absorbed by sample (Δq). DSC plots the temperature change because of this excess energy (q_s). The integral under the peak gives enthalpy of the sample (Gill, et.al., 2010). The onset temperature (T_o) is a certain point during heating where gelation and endothermic events begin (Chiotelli, Meste, 2002).

The DSC of a starch suspension gives the gelatinization character of granules, gelatinization temperature (onset temperature), enthalpy, etc. *(Figure 1.13*).

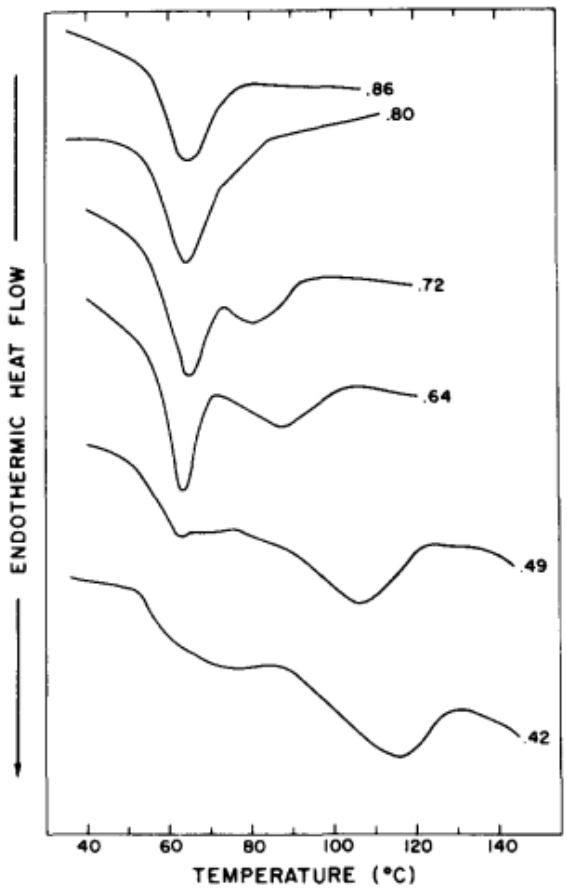


Figure 1.13 An example of pea starch DSC profile in different concentrations of water suspension (Biliaderis, 1983)

1.3.3 X- Ray Diffraction

X-ray diffraction (XRD) is a useful non-destructive method used to analyze and characterize crystalline materials. It gives data about structures, phases, preferred crystal orientations (texture), and various structural parameters including average grain size, crystallinity, strain, and crystal defects. X-rays are generated by a cathode tube, then created X-rays are filtered and monochromatic rays are produced. The collimated X-rays are for concentrated and directed to sample.

According to Bragg's Law (Equation 2):

$$n\lambda = 2d \sin\theta \quad (2)$$

Where, n = integer, λ = X-rays wavelength, d is interplanar spacing to generate diffraction, and u = diffraction angle as shown in *Figure 1.14*.

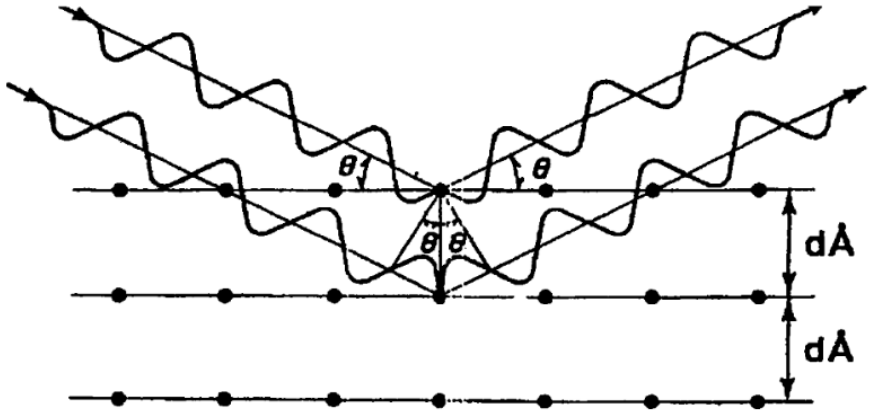


Figure 1.14 Bragg's Law representation of X-Ray Diffraction (Chatterjee, 2000).

The diffracted X-Rays are detected and counted. The sample is scanned at an angle range of 2 θ to scan the entire random orientation of the powdered sample in possible diffraction directions. The obtained diffraction peaks are converted to d spacing that

are identical for each compound. There is an archive for d spacing of compounds (Bunaciu, Udriştioiu, & Aboul-Enein, 2015).

X-ray diffractometers comprise three fundamental components: an X-ray tube, a sample holder, and an X-ray detector (Connolly, 2007). In the tube, electrons accelerated to a target, when the electrons have enough energy to remove from inner shell electrons of target material; the X-ray spectra, K_α components are produced. The wavelengths are specific to target material (Cu, Fe, Mo, Cr). Cu is commonly used target material with CuK_α radiation. The obtained radiation is concentrated and directed onto the sample. The detector rotates and the intensity of the reflected X-rays are recorded (*Figure 1.15*).

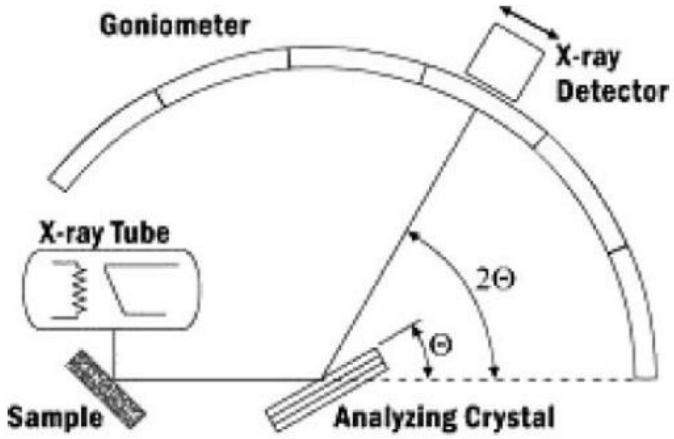


Figure 1.15 X-ray diffractometer operation system (Bunaciu, et.al., 2015)

For modified starches, X-ray diffraction is used to characterize the starch type, modification level and potential modification type. The level of crystallinity gives the intramolecular and intermolecular interactions of amylose or amylopectin molecules. Increase in crystallinity proves creation of double helices by making hydrogen bonding in between amylopectin molecules. Or destruction of a crystal structure indicates that the hydroxyl groups of a starch chain have already made

another bond with reagent. Therefore, hydrogen bonding or recrystallization is not possible. X-ray diffraction angles and the crystallinity intensity give some information about the starch molecule, reaction efficiency of modification, etc. (Amaya- Liano, et.al., 2011; Ma, et.al., 2021; de Oliveria, et.al., 2018; El Halal, et.al., 2015; Haq, et.al., 2019; Bertoft, 2004; Chi, et. al., 2023).

1.4 Physical Properties of Soft Jelly

In soft jelly, there are many quality attributes that must be controlled. Color, opacity, taste, morphologic and structural parameters are important. However, the most important parameter that determines the type of jelly according to the purpose of application is texture. Texture primarily refers to the tactile sensations elicited by physical stimuli arising from the contact between a part of the body and the food (Bourne, 2002). The texture is expressed by springiness, cohesiveness, firmness, smoothness, roughness, slipperiness, creaminess. The analytical expression of sensory evaluations needs a certain judgement. Conventional sensory analysis is not stable and repeatable with human evaluation.

The working principle of texture analyzer is based on rate or scale of deformation in the mouth or instrument. The test is done by controlling force and measuring deformation or controlling deformation rate and measuring needed force for determined deformation (Zhang, Xiong, 2017).

The parts of texture analyzer can be seen in *Figure 1.16.*

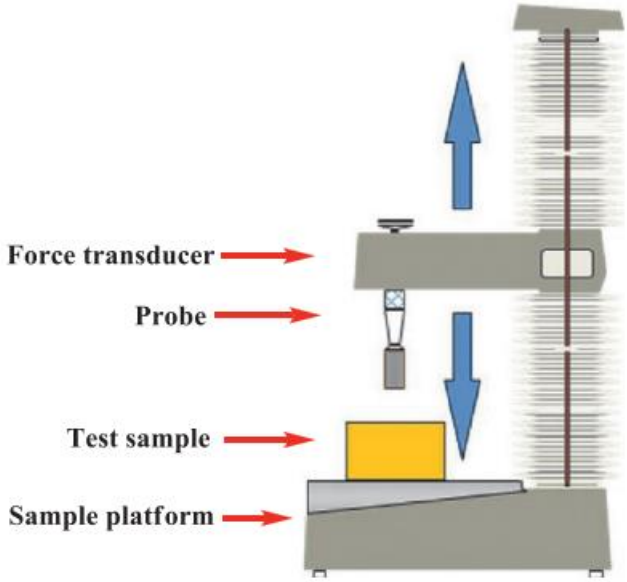


Figure 1.16 Texture analyzer (Liu, Cao, Liu, 2019)

The arm of force moves up and down that applies a strength on the sample causes changes due to deformation force and pressure. The probe transmits the changes of deformation to the force transducer. Then, hardness, fractures, springiness, chewiness, firmness, toughness, fibrousness, gumminess, stickiness/tackiness, cohesiveness, yield, extension, resilience, and gel strength parameters are expressed (Liu, Cao, Liu, 2019).

The starch gels have different viscosity and paste character with respect to the amylose/amylopectin ratio, modification type with different reagent, modification level under different conditions. According to different conditions, starch could be analyzed from different perspectives. Starch gel retrogradation level could be represented desired hardness, springiness, stickiness, or starch paste stability in viscosity could be shown with smoothness, stickiness in numerical values (Sodhi, Singh, 2005; Sandhu, Singh, 2006; Aini& Purwiyatno, 2010).

1.5 Objectives of the Study

Starch-based additives are unable to fully substitute animal-based gelatin. In commercial applications, only up to 30% of gelatin can be effectively replaced by starch-based alternatives. The difficulty in achieving a complete replacement of gelatin with single modified starch, such as acid modified or oxidized starch, lies in the occurrence of retrogradation reactions. During the maturation process, retrogradation reactions take place between hydroxyl groups on the starch molecules in the jelly, making it challenging to achieve 100% gelatin replacement with a single starch modification. For this reason, it was decided to study the possibility of usage of dual modified starch in soft jelly production.

An acetylation reaction could be done to improve stability and retrogradation resistance properties of starch. If degree of substitution (DS) level of acetylated starch is between 0.01-0.20, acetylated starch has film forming ability, good binding, adhesion, thickening, stabilizing and texturizing properties (Chi, et.al., 2008). In addition, acetylation reaction increases peak viscosity, breakdown, final viscosity, retrogradation and pasting temperature and reduce gel hardness, adhesiveness, and gumminess of starch in jelly production (Colussi, et.al., 2015).

High amylose starch serves as an alternative for pre-modification, particularly in guiding the selection of acid modification as a suitable pre-modification method. This is attributed to the presence of a significant quantity of short chains in the molecular structures of high amylose starch like acid modified starch. Thermal and viscosity stability of high amylose corn starch increased with acetylation. Freeze thaw stability is gained and paste aggregation is decreased by acetylation.

As stated in the previous studies, acetylation reaction can be applied to improve paste properties of acid modified and oxidized starches. Acetylation reaction efficiencies and the effects of pre-modification on paste properties have been studied. However, the comparison between different types of pre-modifications has not been studied for low acetylation degree starches, which can be used in food systems as a thickener.

Also, the reaction efficiency to decrease the usage of chemicals has not been studied before for acetylation reactions of acid modified and oxidized starches. Moreover, the paste characteristics of acetylated starches have not been analyzed. In the context of jelly applications, the textural properties of jellies containing acetylated acid-modified and oxidized starches have not been compared.

Therefore, the main objective of this thesis is to determine the effects of reagent amount and reaction time in acetylation of native, acid modified and oxidized corn starch on the reaction efficiency and the physicochemical properties of starches. Characterization of starches was done by acetylation percent, rapid visco analyzer (RVA), X-Ray Diffraction (XRD), differential scanning calorimeter (DSC) and transmittance per cent analysis. Another objective of the study is to observe the effects of acetylated starches on the texture profile of soft jelly candies.

CHAPTER 2

MATERIALS AND METHODS

2.1 MATERIALS

Native corn starch was obtained from Sunar Mısır (Adana, Turkey). Sodium Hydroxide (having 100 % concentration NaOH), Hydrochloric Acid (HCl with 37 % concentration) and Vinyl Acetate Monomer 100 % (VAM) were purchased (Merck KGaA, Germany) for pH adjustment and as a reagent. Hydrogen Peroxide (having 50 % H_2O_2 concentration) and citric acid were provided from Sunar Mısır (Adana,Turkey). Corn syrup (DE=42, 80Bx) and molding starch (high compressibility, oil content max 0.1 %) were used from Sunar Mısır Company (Adana, Turkey). Sucrose (Bal Küpü, Aksaray, Turkey) was purchased from a local market in Adana, Turkey. Gelatin (having 250 bloom) was provided by Halavet Gıda (İstanbul, Turkey).

2.2 METHODS

2.2.1 Reaction Methodology

2.2.1.1 Acid Modification

The acid modification reaction was done according to Wurzburg O.B. (1987) with some modifications. Reaction was done by treatment of 36-40% native corn starch slurry (Rohwer, Klem, 1984) with 5 % diluted hydrochloric acid at pH 4.4-4.5 in 1 L reactor (Çalışkan Lab, Ankara, Turkey) with heating jacket (Refrigerating/heating

circulator, Cole- Parmer, Vernon Hills,United States). The reaction was performed at 52℃ by mixing at 250 rpm (Mechanic mixer, IKA, Works GmbH & Co. KG, Staufen, Germany) for 8 hours (*Figure A.1*). Then the pH (pH meter, WTW 3110, Weilheim, Germany) was increased to 6.5-7.0 to neutralize and finish the reaction. The starch slurry was filtered under vacuum (Value 1 Stage Vacuum Pump, Warsaw, Poland). The cake was dried in convection oven at 40℃ to 10 % moisture content. Then, it was milled with grinder (Retsch ZM 200, Germany) to obtain particles less than 75 microns in size.

2.2.1.2 Oxidation Reaction

The oxidation reaction was done according to Wurzburg O.B. (1987) and Dournel P., (2015) with some modifications. Reaction was conducted in jacketed reactor with 40% native corn starch slurry. The pH of starch slurry was brought to 9.5 by using 3% NaOH. Then, H_2O_2 with a concentration of 50%, 10% by mass of starch, was added into the suspension. The reaction temperature was set to 45˚C (Refrigerating/heating circulator, Cole- Parmer, United States) which is below gelatinization temperature of starch; the suspension was mixed with mechanical mixer (IKA, Germany) at 250 rpm for 4.5 hours (*Figure A.2*). Then, suspension was cooled to 25-30℃ and the pH was decreased to 6.5-7.0 with 4% HCl. The starch slurry was filtered under vacuum (Value 1 Stage Vacuum Pump, Warsaw, Poland). The cake was dried in convective drying oven at 40℃ to 10% moisture content. Then, it was milled with grinder (Retsch ZM 200, Haan, Germany) to obtain particles less than 75 microns in size.

2.2.1.3 Acetylation Reaction

Acetylation reaction conditions were determined according to Wurzburg O.B. (1987) with some modifications. As a reagent Acetic Anhydride is used usually but, there are some challenges about its purchase so, Vinyl Acetate Monomer (VAM) was

preferred. VAM also has permission from Food and Drug Administration (FDA) which is up to 2.5 % Acetyl content (Part C.R.F., 2022). For starches with a low degree of acetylation, the starch reacts with Vinyl Acetate reagent in water suspension at pH 9-10 and temperature 25-46˚C for 45-60 minutes.

The starch slurry was prepared in 42 % concentration by native corn starch, acid modified starch (*Figure A.3*) and oxidized starch (*Figure A.4*). In the preliminary experiments, acetyl content at pH of 9.0 and 9.5 and temperature of 40 and 45℃ were determined. A determined starch suspension containing 42 % solid was heated in jacketed reactor. The temperature was kept constant in the jacketed reactor by water circulation. During reaction, because of the acetylation the pH always decreases to continue the reaction in maximum efficiency, the pH was kept in between 9.0-9.5 by the addition of 2 % NaOH. The effects of reagent amount (1.33 % and 5 % of starch amount in suspension) and reaction time (30 min and 75 min) in native, acid modified and oxidized starch on starch properties were studied. Acetylation reaction conditions and the abbreviations for them were given in Table 2.1.

Table 2.1 *Acetylation reaction conditions of different types of starches*

Name	Starch Type	Reagent Amount (%)	Reaction Time (min)
N_Ref	Native	0	0
N_1.33_30	Native	1.33	30
N_1.33_75	Native	1.33	75
N_5_30	Native	5	30
N_5_75	Native	5	75
A_Ref	Acid Modified	0	0
A_1.33_30	Acid Modified	1.33	30
A_1.33_75	Acid Modified	1.33	75
A_5_30	Acid Modified	5	30
A_5_75	Acid Modified	5	75
O_Ref	Oxidized	0	0
O_1.33_30	Oxidized	1.33	30
O_1.33_75	Oxidized	1.33	75
O_5_30	Oxidized	5	30
O_5_75	Oxidized	5	75

2.2.2 Soft Jelly Production

In commercial production of soft jelly candy, 6 % of total recipe is animal-based gelatin. In this study, 50 % of gelatin was replaced by starch.

To prepare soft jelly candy, 200 g of glucose syrup with soluble solid content of 80 Bx, 200 g sucrose and 200 g distilled water was mixed in a metal pan and heated. The solution temperature was kept in between 110 - 120°C by using a heater (*Figure A.5*) until the final soluble solid content is 80-82 Bx (*Figure A.6*). Then, the solution was cooled to 75-80°C. On another side, 15 g gelatin was dissolved in 27.5 g distilled water in water bath at 65°C. Also, 15 g starch and 30 g distilled water was mixed, and the starch suspension was heated to 90-95°C for 20 min, the starch was gelatinized. After that, the gelatin and starch were added into sugar and glucose syrup

solution which was at 75-80 ℃. That mixture was mixed well, then 5 g of citric acid was added to adjust pH to 3.5. The molding starch was preconditioned, it was dried to 5.5-6.0 % moisture content. Then, the jelly candies were shaped by using cylindrical molds with 1.5 cm diameter and 2.0 cm height.

The obtained jelly melt was poured into the molds (*Figure A.7*). They were incubated under 55 % RH and 25 ℃ conditions (*Figure A.8*).

2.2.3 Characterization of Starches

2.2.3.1 Acetylation Percent

The method approved by FAO for % Acetyl group (%Ac) was used (JEFCA, 2001). A sample of 5 g was weighted in 4 digits (w) and added to a 250mL conical flask. Distilled water of 50mL and 2-3 drops of phenolphthalein were added onto starch sample. The suspension was titrated with 0.1N NaOH to get permanent pink color to neutralize the suspension. Then, 25 mL of 0.45N NaOH was added into the flask, a stopper was put on flask. Then, the suspension was shaken vigorously on a mechanical shaker for 30min. The temperature should not exceed 30℃, to prevent starch gelation. After 30 min, the stopper was removed and the excess alkali was titrated with 0.2N HCl (N) until colorless/ white suspension was obtained. The volume of 0.2N HCl titrant (S) was recorded. Blank (B) titration on 25mL of 0.45N NaOH was performed with 0.2N HCl. Acetyl content (%) was calculated by using Equation (3).

$$\%Ac = \frac{(B-S)\times 0.043\times 100}{w} \quad (3)$$

2.2.3.2 Rapid Viscosity Analyzer (RVA)

For native starch, 8 % (dry basis), for acid modified and oxidized starch, 20 % (dry basis) suspension was prepared for Rapid Visco Analyzer (RVA) (RVA 4500, Perkin

Elmer Inc., USA) was used to determine retrogradation. The suspension was stirred at 900rpm for 20s, then stirrer speed was fixed to 100 rpm until the end. The temperature started from 50°C and was held for 2 min, then it is increased to 95˚C in 10 min and kept at that temperature for 4 min, decreased to 40°C in 12 min and kept at 40°C for 22 min. Corn Refining Association council (1994) method was taken as reference but, there were some modifications on method to analyze cold paste viscosity (Table 2.2).

Table 2.2 *Methods of Rapid Visco Analyzer for Starch*

Time (hh:mm:ss)	Function Type	Value
00:00:00	Temperature	50˚C
00:00:00	Speed	960 rpm
00:00:20	Speed	100rpm
00:02:00	Temperature	50˚C
00:12:00	Temperature	95˚C
00:16:00	Temperature	95˚C
00:28:00	Temperature	40˚C
00:50:00	End	

2.2.3.3 Differential Scanning Calorimeter (DSC)

Thermal Analysis System DSC2 (Mettler Toledo, Germany) was operated by flow of nitrogen gas through the system at a rate of 50 mL/min. For the measurement 8-10 mg of starch was used and starch suspension with starch: water ratio of 1:3 was prepared in a 30 µl aluminum pan. The sample pan was hermetically sealed to prevent evaporation. The sample was heated from 25°C to 180°C with 10 °C/min. Analyzes were done for each sample of modified starches to observe gelatinization temperature of starches. StarE software was used to calculate gelatinization temperature (T_m), peak temperature (T_p), onset temperature (T_O) and enthalpy.

2.2.3.4 X-Ray Diffraction (XRD)

X-Ray Diffraction experiments were performed by using D2 Phaser (Bruker, Germany). The X-Ray diffractometer was using CuK_{α} radiation and operation tube at 30kV and 10mA. In the methods, diffractograms were obtained from 2θ = 2.5˚ - 60˚ in continuous scanning mode to get data. Scanning speed was 2.75˚/min. Dıffrac.Sutte.Eva was used as a software; the data were analyzed by StripKα method to discriminate the other radiations other than CuK_{α}. The crystallinity and their intensity at the significant 2θ radiation were determined (Equation 4). Analyzes were done for each sample of modified starches to observe their crystalline type and crystal intensity.

$$\%Relative\ Crystallinity = \frac{Crystalline\ Area}{Total\ Area} \times 100 \quad \text{(Amaya-Llano, et.al., 2011)(4)}$$

2.2.3.5 Transmittance Percent

Transmittance (%) of a starch gel was measured by preparation of 2% (in weight) starch suspension with distilled water and different types of starches with different levels of acetylation (Lin et.al., 2019). The starch suspensions heated to 90-95 ℃ for 30 min. The gels were cooled to room temperature then, the transmittance of gels was measured at 620 nm, in spectrophotometer (Schmadzu UVmini-1240 Spectrophotometer, Japan) and distilled water was used as a blank. By using Equation 5, transmittance values were calculated.

$$Absorbance = \log_{10}\left(\frac{100\%}{Transmittance\%}\right) \quad \text{(Larsen, 1971)} \qquad (5)$$

2.2.4 Texture of Soft Jelly

The hardness and stickiness of soft jelly candies were measured by using Stable Micro System TA-XT PLUS texture analyzer (Stable Micro System, England). A compression test with 36 mm cylindrical probe was used for hardness and stickiness

measurement. The pre-test speed, test speed and post-test speed of the instrument were 0.5mm/sec; trigger force was determined as 5g; due to having 2cm height soft jelly the distance was determined as 4 mm. The force required for specified deformation was measured.

2.2.5 Statistical Analysis

Statistical analysis of all experimental results was performed by using Analysis of Variance (ANOVA) with general linear model tool in Minitab (Minitab Inc., UK). The comparison of the results was done with Tukey's comparison test at 95 % confidence level.

CHAPTER 3

RESULT AND DISCUSSION

There are many parameters in acetylation reactions of starch such as reaction temperature, pH, time, and reagent amount. The effect of pH and temperature on percent of acetyl content was studied with %42 native starch suspension that had 3 % reagent in the preliminary experiments. The results are given in Table 3.1.

The increase in pH and temperature affected the reaction efficiency adversely. Similar results were observed in the study of Han et.al., (2012). Esterification reactions are exothermic, so the temperature increase is unfavorable for binding of reagent by starch and formation of reaction centers. Thus, Degrees of Substitution (DS) decreases with the increase in temperature. Up to a certain concentration, the addition of NaOH promotes permeation to amorphous and crystalline regions; then, disrupts the intra structure hydrogen bonds and increases the reaction efficiency. Furthermore, the increase in pH and the addition of NaOH cause starch gelation so the contact area of reagent and starch is inhibited. Furthermore, at higher pH, the reagent hydrolyzes, and the amount of esterifying agent reduces (Han et.al., 2012; Rahim et.al., 2022). According to the result of this preliminary experiments, the reaction temperature and pH were kept constant at 35℃ and 9.0, respectively and by using native, acid modified and oxidized starches, the effects of reagent amount and reaction time on percentage of acetyl content have been studied by keeping the temperature and pH constant at these values.

Table 3.1 *Acetylation reaction conditions at different temperatures and pH values*

Starch Type	Temp. (℃)	pH	Time (min)	Acetylation (%)
Native	35	9	90	0.45
Native	40	9	90	0.15
Native	35	9.5	90	0.23

3.1 Physicochemical Properties of Starch

3.1.1 Percentage of Acetyl Content

The abbreviations used for native corn, acid modified and oxidized corn starch reacted under different conditions are shown in Table 2.1. The hydroxyl groups of starches are substituted by acetyl groups in acetylation reaction. It was observed that the percentage of acetyl content increased with an increase in reagent amount in the reaction for all types of starches (Table 3.2). The probability of interaction between vinyl acetate monomer and starch increases with an increase in reagent amount (Betancur, Chel & Canizares, 1997; Tupa et.al.,2015; Trela, Romallo & Albani, 2020; Babic, et.al., 2007; Colussi et.al., 2015 and Han et.al., 2012). Also, having high amount of anhydrous vinyl acetate is getting wider surface and contact area to react with starch hydroxyl groups that increase rate of reactions (Rahim et.al., 2022). Reaction efficiency is also related to the positions of hydroxyl groups on inlet starches. In chemical modification, the most reactive group on a starch molecule is the hydroxyl group of C6 atom. That is followed by carbon 2 and 3 (Wurzburg, 1986).

As shown in Table 3.2, as the reaction time increased from 30 min to 75 min, the percentage of acetyl content increased when 1.33 % reagent was used. Similarly, according to the studies of Ackar et.al., (2015) and Subroto et.al., (2023) the percentage of acetyl content increased with the reaction time. On the other hand, only for the acetylated native corn starch, the percentage of acetyl content decreased with

time when 5% reagent was used as seen in Table 3.2. The decrease in the percentage of acetyl content can be explained by the completion of reagent and acetylated ends are started to hydrolyze (Han, et.al., 2012). In the reaction medium, when reaction time is getting longer, esterification reaction is affected by porous structure, swelling characteristic, reagent thermal motion and side reactions of starch molecules.

Table 3.2 *Percentage of acetyl content of starches*

Reagent amount (%)	Reaction time (min)	Acetyl Content (%)		
		Acetylated native corn starch (N)	Acetylated acid modified corn starch (A)	Acetylated oxidized corn starch (O)
1.33	30	0.24^{e}	0.46^{d}	0.32^{e}
1.33	75	0.30^{e}	0.50^{d}	0.44^{d}
5	30	1.35^{b}	1.73^{a}	1.47^{b}
5	75	1.19^{c}	1.63^{a}	1.36^{b}

Samples with different letters (a, b ie) are significantly different in terms of acetyl content ($p<0.05$)

In acetylation reaction, starch type is an important parameter. In this study, it was observed that acid modified starch (A) had the highest reaction efficiency, that was followed by oxide starch (O) and native starch (N). Under the same conditions, acetylation reaction of oxidized starch was more efficient than native starch. This is since oxidized starch contains partially depolymerized starch chains that weaken the starch molecules. Thus, oxidized starch is more susceptible to penetration of reagents (Pietrzyk, et.al., 2014). In another study, it was observed that in dual modifications the decrease in hydroxyl groups decreased the acetylation reaction substitution (Wojiecchowski, et.al., 2018). That claims that dual modification decreases hydroxyl groups on the starch molecule that causes reduction in reaction efficiency. Also, in the study of Pietrzyk et.al., (2014), it was observed that when carboxyl group of premodified starch increased, acetylation reaction was affected

adversely, and the substitution efficiency decreased. As also seen in this study, in oxidized starch (O) and native starch (N), acetylation reaction substitution efficiency increased, while the hydroxyl group decreased.

For acid thinned acetylated starch (A), the percentage of acetyl content was the highest when compared to acetylated native (N) and oxidized starches (O). This may be due to having more distracted amorphous structures. There is no study on acetylation reaction efficiency of acid modified starch in literature, but the reaction efficiency of acid modified starch can be supported with several technical evidence. During acid modification, acid can attract amylopectin at first, so linear molecules and hydroxyl groups increase. Thus, acetylation reaction efficiency increased with the hydroxyl groups and amylose chains. As can be seen in the study of Hoover and Sosulski (1985), the acetylation reaction efficiency of starches varied with different degrees of amylose content and acetyl content increased with amylose content. Luo and Shi (2012) observed that high amylose starch showed the highest reaction efficiency when compared with regular and waxy corn starches under the same reaction conditions. Min, Hongbo, &Yanping, (2017) also observed that high amylose corn starch had higher acetylation esterification efficiency than regular corn starch thanks to having linear starch chains and hydroxyl groups on the surface of that linear chains. This supported the result of obtaining the highest reaction efficiency in acid thinned starch due to having shorter starch chains.

3.1.2 Rheological Properties of Starches

Rheological properties of starch were determined by RVA. Native corn starch can form viscous paste when heated in water suspension. The obtained paste is followed by cooling. While heating and cooling, the starch paste viscosities are changing with temperature and shear; also, the native starch tends to decompose and retrograde. For that reason, modifications are needed.

Gelling or pasting temperature of native, acid thinned and oxidized starches decrease with acetylation reaction as seen in Table 3.3, Table 3.4, and Table 3.5, respectively. The increase in percentage of acetyl content with reagent amount resulted in a decrease in pasting temperature.

Table 3.3 *RVA parameters of native and acetylated native corn starches*

Parameters	*N_Ref*	*N_1.33_30*	*N_1.33_75*	*N_5_30*	*N_5_75*
Pasting Temperature (℃)	72.1	71.78	73.25	69.33	68.48
Peak Viscosity (cP)	2664	3055	2396	3094	3084
Hold Viscosity (cP)	1802	1756	1565	1529	1518
Final Viscosity (cP)	4571	4727	3599	3957	3567
Setback Viscosity (cP)	2769	2971	2034	2428	2049

The initial paste temperature of acid thinned, oxidized, and acetylated starches decreased with acetylation reaction. It was also shown by other researchers that pasting temperature decreased with increased acetylation degree (Betancur, Chel & Canizares, 1997; Jeong, Bae, Oh, 1993).

Because of modification of starch, the bonds on the starch molecules are weakened and D-glycosidic bonds are scission during modification. Addition of carbonyl and carboxyl groups in oxidation reaction and acetyl groups in acetylation reaction disorganize the intra-granular structure so associative forces are reduced in amorphous regions. Also, intra/inter molecular hydrogen bonds disorganize the stabilized starch structure (Akintayo & Akintayo, 2009; Gonzalez, Perez, 2002; Aini & Purwiyatno, 2010; Ma, et.al., 2021).

Table 3.4 *RVA parameters of acid thinned and acetylated acid thinned starches*

Parameters	*A_Ref*	*A_1.33_30*	*A_1.33_75*	*A_5_30*	*A_5_75*
Pasting Temperature (℃)	71.2	70.33	69.85	66	66.8
Peak Viscosity (cP)	9064	11519	9641	13874	11521
Hold Viscosity (cP)	1619	1239	1009	452	519
Final Viscosity (cP)	27733	23506	18178	2458	2385
Setback Viscosity (cP)	26114	22267	17168	2005	1866.3

Table 3.5 *RVA parameters of oxidized and acetylated oxidized starches*

Parameters	*O_Ref*	*O_1.33_30*	*O_1.33_75*	*O_5_30*	*O_5_75*
Pasting Temperature (℃)	69.95	68.5	69.1	65.75	66.3
Peak Viscosity (cP)	15521	14078	8138	10162	10211
Hold Viscosity (cP)	2134	1821	1163	423.5	419.5
Final Viscosity (cP)	12314	10318	24433	2361	2360
Setback Viscosity (cP)	10180	8489	23269	1937.5	1940.5

While heating, the starch granules absorb water and reach the highest viscosity in the heating section; after that point starch granules release and amylose/amylopectin chains leach because of osmotic pressure in the starch granules. During heating to 95℃ , the starch paste viscosity increases because of swelling and leaching of granules (Gonzalez, Perez, 2002). Oxidized starch was found to have higher peak viscosity than acid thinned starch, since oxidized starch had higher swelling power, that increased hydration capacity, and the interaction between starch chains within amorphous and crystalline regions (Oderinde, Omoyeni, 2019; Bello-Perez et.al., 2000). Peak viscosity increased with acetylation when 1.33 % reagent amount and 30 min reaction time were used for all starches as shown in Table 3.3-3.5. peak viscosity increased with acetylation degree. The reason is the increase in the water absorption, solubility, and swelling power of starch granules after acetylation (Gonzalez, Perez, 2002; Jeong, Bae, Oh, 1993; Babic et.al., 2007). Acetylation affects hydration of molecules by inhibiting close association of chains to form hydrogen bonds and hydrophilicity which leads to gelatinization at lower

temperatures and greater swelling of granules. On the other hand, gelatinization temperature decreases, starch molecules absorb more water and peak viscosity increases (Aini & Purwiyatno, 2010; Ma et.al., 2021).

Peak viscosity decreases after a point of acetylation (when the reaction time increased to 75 min in the case of using 1.33% reagent amount), so water holding capacity decreases. In addition, during modification with acid, oxidation and acetylation, there is a breaking of some glucoside bonds, that increases the decrease in molecular weight of starch molecules (Aini & Purwiyatno, 2010; Ma et.al., 2021) and consequently decrease in peak viscosity. The extension of reaction time might cause hydrophobicity with more acetyl group introduction and decrease hydrogen bonding thus, paste viscosity could decrease (Luo, Shi, 2012).

After releasing of starch granules, amylose and amylopectin molecules leach out and the viscosity of the paste at the lowest point that is called as hold viscosity decreases with acetylation reaction. At the hold point, the starch gel viscosity depends on the amylose leakage, amylose-lipid complex formation, swelling of granules and the competition for free water of amylose and granules (Singh, Sodhi, & Singh, 2012). If heat is continued to be applied after gelatinization temperature for a while, the starch continues to weaken, and thermal breakdown occurs. The starch paste turns into a gel-like structure containing swollen granules, and granule fragments (Olkku, Rha, 1978; Bechtel et. al., 1964). The linearity of the molecules is at the top point, so viscosity is at the lowest point.

Setback viscosity, which shows the retrogradation tendency of starches, is the highest for native, acid thinned and oxidized starches before acetylation. That is, the retrogradation level decreases with acetyl content. Acetyl groups prevent alignment and association of macromolecules, so retrogradation tendency decreases (Singh, Kaur, & Singh, 2004). Oxidation and acetylation suppress the increase of the viscosity sharply and stabilize the final viscosity (*Figure 3.1*) (Aini & Purwiyatno, 2010). The final viscosity stability means showing low tendency to the retrogradation while cooling; when acetylation is applied the increase in the final viscosity and its

angle decreases as seen in Figure *3.1*. In the figure, native starch viscosity increases drastically but, acetylated starches increased until a lower point than they kept the viscosity during cooling period. The stearic effect of radical groups that were bound on the starch molecules decreases the recrystallization of the starch molecules (Ambigaipalan, et.al., 2013; Compart, et.al., 2023) so, retrogradation tendency was decreased.

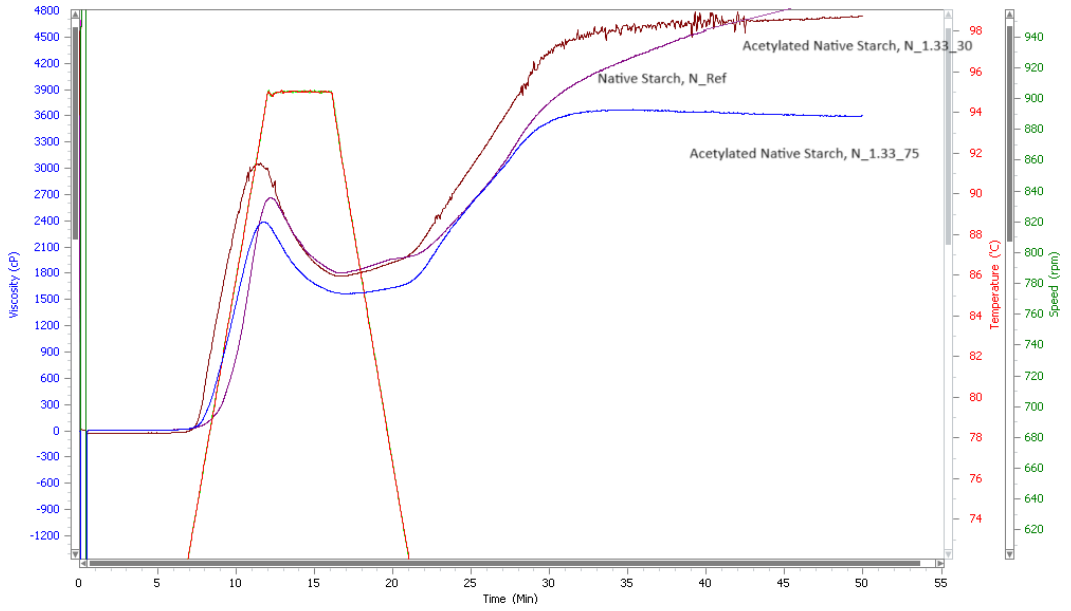


Figure 3.1 RVA profile of Native starch and acetylated native starches

When reaction time is kept longer, past a certain point acetyl content decreases and retrogradation increases (O_1.33_30 and O_1.33_75). Acetylation reaction depends on reaction time; with the addition of caustic; alkali degradation begins at high pH and high temperature. Thus, the stability of starch pastes decreases because of the breakdown on the starch chains (Oderinde, Omoyeni, 2019). That causes higher viscosity profile and higher retrogradation levels.

As shown in *Figure 3.2*, acetylated oxidized and acid thinned starches had different viscosity profiles even though they underwent the same reactions. Retrogradation

tendency of acetylated acid modified starch decreased more than acetylated oxidized starch. That is, oxidized and acid thinned starches had 0.32 and 0.46 % acetyl content, respectively, which showed that acid modified starch had higher reaction efficiency than oxidized starch so, its retrogradation level was lower.

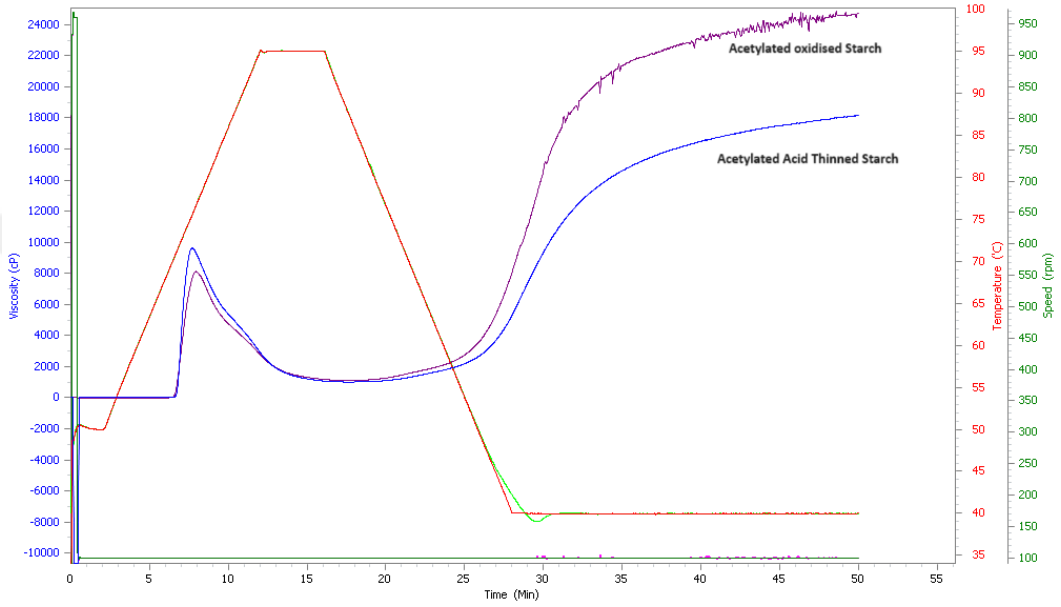


Figure 3.2 RVA profile of low acetyl containing acetylated oxidized and acid thinned starches

Starches containing high acetyl amount (all starches having 5% reagent amount) had the lowest retrogradation level. Their final viscosities values almost did not change. As also seen in the study of Colussi et.al., (2015), viscosity profile did not change too much with the increase in reagent amount. When the reagent amount was high (5 %), retrogradation tendency did not change with reaction time (Table 3.5). That was caused by having high amount of acetylation, acetyl ends which created high resistance to shear and heat. For that reason, viscosity of paste did not change as seen in Figure *3.3* (Trela, Ramallo, &Albani, 2020).

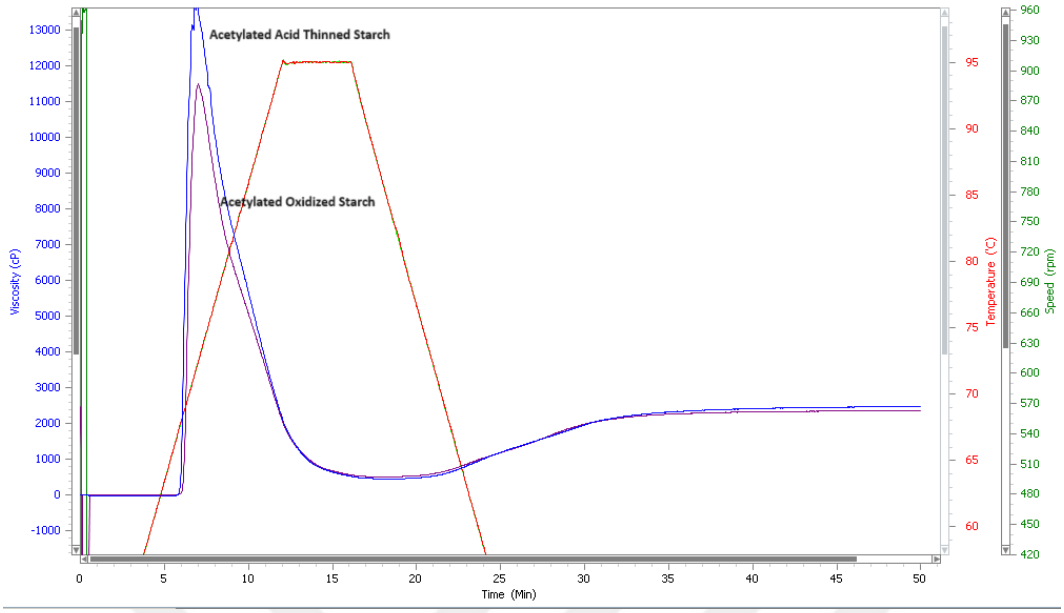


Figure 3.3 RVA profile of high acetyl containing acetylated oxidized and acid thinned starches

3.1.3 Thermal Properties of starches

Differential Scanning Calorimeter (DSC) was used to determine the thermal properties of starches. Acid and oxidation modifications did not affect the thermal properties of starches. As seen in Table 3.6-3.8), native corn, acid thinned and oxidized starches had similar T_o, T_p, T_c and ΔH values.

As can be seen in the enthalpy results of reference and acetylated starches (Table 3.6-3.8), acetylation increased the gelatinization enthalpy of starches. Acid thinned or oxidized starches had lower ΔH than native forms because of the granular disruption (Wotton, Bamunuarachchi, 1979). Acids could attack the amorphous regions first that could cause the loss of granule ordered structure before gelatinization and could decrease enthalpy (Muhr et al.,1984; Amaya-Llano, et.al., 2011).

Table 3.6 *Thermal properties of native and acetylated corn starches*

Parameters	*N_Ref*	*N_1.33_30*	*N_1.33_75*	*N_5_30*	*N_5_75*
Onset Temperature (℃)	126.9	137.9	118.4	131.8	111.2
Peak Temperature (℃)	123	144.7	115.7	133.3	120.2
End set Temperature (℃)	139	158	123.5	144.9	155.2
Enthalpy (J/g)	1296	1421.9	404.9	1480.7	1202.1

It was shown that acetylation reaction increased the onset, peak and end set temperatures of native corn starch. The same situation was observed in oxidized starches. The lowest thermal property values were observed before acetylation reaction.

Table 3.7 *Thermal properties of acid modified and acetylated acid thinned starches*

Parameters	*A_Ref*	*A_1.33_30*	*A_1.33_75*	*A_5_30*	*A_5_75*
Onset Temperature (℃)	120.3	94.9	128.8	115	117.3
Peak Temperature (℃)	123.2	98.5	131.1	127.7	129.5
End set Temperature (℃)	128.7	104.7	142.3	142.1	147.1
Enthalpy (J/g)	1251	1249.5	1195.3	1257.7	1099.1

Table 3.8 *Thermal properties of oxidized and acetylated oxidized starches*

Parameters	*O_Ref*	*O_1.33_30*	*O_1.33_75*	*O_5_30*	*O_5_75*
Onset Temperature (℃)	119.2	118.6	136.5	128.5	122.3
Peak Temperature (℃)	122.8	127.7	138.8	130.5	129.5
End set Temperature (℃)	125.5	143.9	150.9	144.2	139.9
Enthalpy (J/g)	1117.7	1364.9	1020.3	979.5	1311.6

There are some studies in which acid modification, oxidation, acetylation could increase gelatinization temperature (Sanchez-Rivera et.al., 2005).

Enthalpy values were shown to increase with acetylation degrees as proven with N_Ref, N_1.33, N_5, A_Ref, A_1.33, A_5, O_Ref, O_1.33 and O_5 values (Table 3.9). ΔH represents the sequence loss of double helices. If there is a reduction in ΔH value, some double helices in crystal and amorphous regions are distracted during acetylation. Lower ΔH shows lower percentage of organized or stable crystals. ΔH of the native starch increases with acetylation reaction at first (Tran, Piyachomkwan & Sriroth, 2007); during acetylation ΔH decreases with increase in acetylation degree and time (Lou, Shi 2012; Chiotelli, Meste, 2002). Also, enthalpy decreases when reaction period extends because of molecular alkaline distraction.

Table 3.9 *Gelatinization enthalpy of starches*

Reagent amount (%)	Reaction time (min)	Gelatinization enthalpy (J/g)		
		Acetylated native corn starch (N)	Acetylated acid modified corn starch (A)	Acetylated oxidized corn starch (O)
0	0	1296	1251	1117.7
1.33	30	1421.9[a]	1249.5[a]	1364.9[a]
1.33	75	404.9[b]	1195.3[a]	1020.3[a]
5	30	1480.7[a]	1257.7[a]	979.5[a]
5	75	1202.1[a]	1099.1[a]	1311.6[a]

Samples with different letters (a, b ie) are significantly different in terms of gelatinization enthalpy ($p<0.05$)

3.1.4 X-Ray Diffraction of Starches

The diffusion peaks of 2Theta radiation diffractions were in 10, 15, 17, 22 in native, acid thinned and oxidized starch diffractions, as shown in *Figure 3.4*. However, their crystalline intensities were changing. There are three types of crystallinities in a starch molecule. If the diffraction sharp peaks located at t 15°, 17°, 18°, 23° (2θ), that indicates type A starch molecules (Zobel, 1988). Acetylated, acid, oxidized and

native corn starches have distinct diffraction at these points. The type of crystals is A. Having 10° diffraction indicates that, there is a degree of polymerization of hydroxyl groups by acetyl groups (Colussi et al., 2014; Wojeicchowski, et.al., 2018).

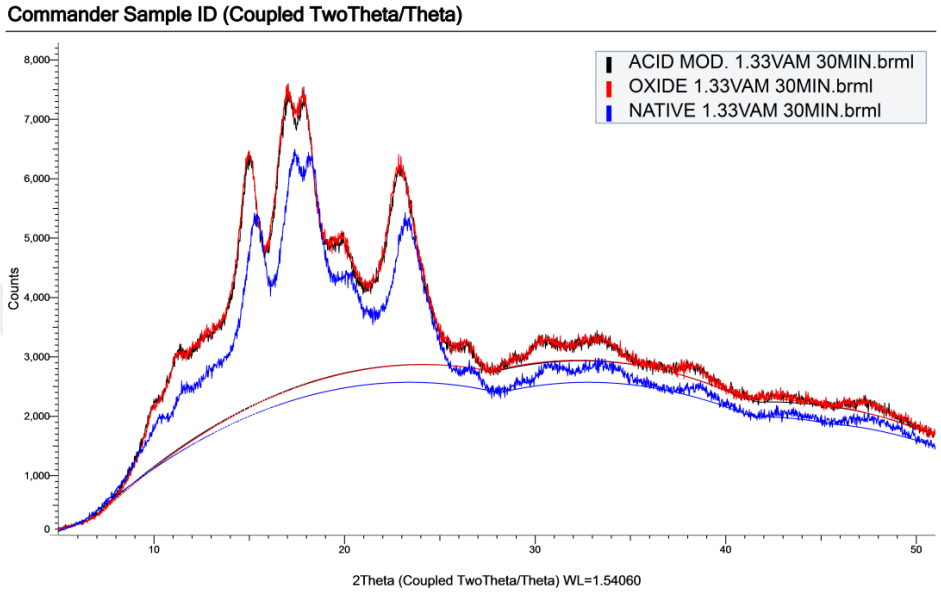


Figure 3.4 Diffraction of different types of acetylated starches

Table 3.10 *Percentage of crystallinity of acetylated starches*

Reagent amount (%)	Reaction time (min)	Crystallinity (%)		
		Acetylated native corn starch (N)	Acetylated acid modified corn starch (A)	Acetylated oxidized corn starch (O)
0	0	37	38.1	37.4
1.33	30	35.6^{c}	$39.2^{a,b,c}$	$37.6^{a,b,c}$
1.33	75	$38.9^{a,b,c}$	$36.1^{b,c}$	40.8^{a}
5	30	$38.7^{a,b,c}$	$36.9^{a,b,c}$	$39.5^{a,b}$
5	75	$38.55^{a,b,c}$	40.7^{a}	$39.3^{a,b,c}$

Samples with different letters (a, b ie) are significantly different in terms of crystallinity ($p<0.05$)

X-Ray diffraction gives an idea about the crystallinity of a starch granule. The main part of crystallinity is provided by amylopectin content (Hoover, Sosulski, 1985). Reactive amylose molecules are present in amorphous regions (Mbougueng et al., 2012; Cheetham & Tao, 1998). Acetylation did not have a direct effect on X-ray diffraction because acetylation was known to occur in amorphous regions (Hoover, Sosulski, 1985; Sodhi, Singh, 2005). Acetyl groups could penetrate only amorphous regions. As also seen in Table 3.10, , there were no significant differences in the change in the crystallinity.

For high acetylation degrees, acetylation could disturb the intra and inter molecular hydrogen bonds that are responsible for highly ordered crystalline structures. The loss of ordered crystalline structure increased with acetylation degree (Luo, Shi, 2012). Because of that, structures could lose their crystallinity with longer reaction times, A_1.33_30 and A_1.33_75.

The highest reduction in crystallinity was seen in acid thinned starches since acid modification disorganizes and degrades starch molecules. Also, the acetylated acid thinned starch presented the highest reduction in crystallinity with acetylation; A_1.33_30, A_1.33_75, A_5_30. This showed that acid modified starch had the smallest starch chains, higher amorphous structure destruction as a premodification. According to the studies of Kainuma & French (1971) and Amaya-Llano et.al. (2011), cleavage of molecules in amorphous region while acid modification lets reordered starch chains and gives more crystalline structure that is observed with sharper crystalline peaks. That proves having highest crystalline value of acetylated acid modified starch.

3.1.5 Percentage of Transmittance

Transmittance values of starch gels increased respectively in native, acid thinned and oxidized starches. The native starches have the lowest transmittance values, as

seen in the table below (Table 3.11). That increases with acetylation reaction; N_1.33 and N_5.

Table 3.11 *Transmittance values of starches*

Reagent amount (%)	Reaction time (min)	Transmittance		
		Acetylated native corn starch (N)	Acetylated acid modified corn starch (A)	Acetylated oxidized corn starch (O)
0	0	2.518	31.989	71.285
1.33	30	2.603^{g}	43.514^{f}	$59.382^{c,d}$
1.33	75	1.998^{g}	50.536^{e}	71.451^{a}
5	30	4.004^{g}	56.241^{d}	66.760^{b}
5	75	2.882^{g}	57.482^{d}	$63.169^{b,c}$

Samples with different letters (a, b ie) are significantly different in terms of transmittance values ($p<0.05$)

Light transmittance is related to electrostatic repulsion of radical groups that are attended after acetylation reaction (Oderinde, Omoyeni, 2019). As seen in (Table 3.11) the acetylated oxidized starch gels absorbance values; oxidized starches have the lowest absorbance values. Chemically substituted hydroxyl groups retard retrogradation and ordered crystalline gel formation thus, transmittance increases (Lawal, 2004; Singh et.al., 2004). Moreover, Bello-Perez et.al. (2000) studies, acetylated starch gels do not lose clarity due to low level of retrogradation.

Also, absorbance values decrease with %acetyl content; O_1.33_30 and O_5_30 have 0.238 and 0.179 absorbance values respectively. That reason is, as it is mentioned before, radical groups on starch chains; transmittance increases with radical groups. When the disorganization increases the light transmittance increases (Lui, Ramsden, & Corke, 1997). Table 3.4, A_5_30 and A_5_75 show when %acetyl content decreases, the light transmittance increases due to alkali degradation occurring with long reaction time. For high acetyl contented starch gels,

transmittance value is lower than native starches (Trela, Ramallo, &Albani, 2020); however, there was not any experience about that. The transmittance value changes with different acetylation degrees.

Acetylated oxidized starch is more advantageous due to having a high transmittance value than acetylated acid thinned starch in food application (Oderinde, Omoyeni, 2019). As also seen in *Figure 3.5*, the lighter color was observed in acetylated oxidized starch gels, that is followed by acetylated acid thinned starch and acetylated native corn starch gels.



Figure 3.5 Pastes of acetylated starches

(a) acetylated acid thinned starch; (b) acetylated oxidized starch; (c) acetylated native starch

3.2 Texture of Soft Jelly

Having low acetylation degree, acid modified and oxidized starches were used in jelly applications. In confectionary gels, as mentioned before fluidity must be high to make application the controlled and easy so acetylated native starch was not

preferred. Also, the acetylation degrees of starch should be low to be used in jellies to get the desired amount of retrogradation while cooling/ maturing. As seen in RVA graphs (*Figure 3.2*), the paste viscosity of starches with high DS did not change during cooling so the prepared jellies could not be shaped and kept in the proper form. A low level of retrogradation is necessary in the jellies.

Low DS acetylated starches are more suitable for food application to get stable viscosity, water absorption, resistance to retrogradation and jelly like textures. In confectionary recipes with high solid content, low hot viscosity starches are used. For this reason, acid modified and oxidized starches were preferred to observe the effect of the acetylation reaction on starches.

The acid thinned and oxidized starches with different acetylation degree were used as a thickener in soft jelly candy recipes (*Figure A.9*). The amount of gelatin replacement was determined as 50% to observe the effects of starch on texture. That is, the hydrogel of jellies contained 50% starch and 50% gelatin. In industrial applications, 30-40% of gelatin is substituted by acid thinned starches. Samples containing 100% gelatin (Gel. 100) was chosen as a control.

Table 3.12 *Texture of soft jellies with different thickeners*

Reagent Amount (%)	Premodification Type	Samples	Hardness (g)	Adhesiveness (g)
0	-	Gel._100	912.4	-0.18
1.33	Acid Mod.	A_1.33-50_Gel.-50	454.9^{b}	-0.45^{b}
5	Acid Mod.	A_5-50_Gel.-50	456.69^{b}	-0.19^{b}
1.33	Oxidation	O_1.33-50_Gel.-50	778.32^{a}	-0.15^{b}
5	Oxidation	O_5-50_Gel.-50	273.3^{c}	-7.52^{a}

Reaction time of all starches was 30 minutes.

Different letters (a, b ie) in the same column indicate the significant difference between the samples ($p<0.05$)

As seen in Table 3.12, acetylation of starch decreased the hardness of the gels. Also, the adhesiveness value increased with acetylation degree. The reason for lower hardness is the deformation of starch granules, retrogradation in gel and crystallization of amylopectin. The acetylated starches with low DS can lead to soft textured starch gels thanks to slight depolymerization; although more amylose molecules can reassociate and weaken the starch gels. This fact is known to increase the gumminess and adhesiveness values (Colussi, et.al., 2015; Sodhi, Singh, 2005; Singh, Sodhi, &Singh, 2012).

Soft jellies made of acetylated acid thinned and oxidized starches with the same amount of reagent showed different hardness values. Acetylated acid thinned starches had higher acetyl content than acetylated oxidized starch. Hardness values of soft jellies produced using these starches, that is demonstrated by A_1.33-50_Gel-50 and O_1.33-50_Gel. -50, were 455 g and 778 g, respectively. The gels with oxidized starch undergo retrogradation more than the ones with acid modified starch. This was proven before in part 3.1.2. and *Figure 3.1*. In the RVA graph acetylated oxidized starch moved above acetylated acid modified starch gel profile. The amylose chains and linear chains of acid modified starch made bonds with acetyl ends thanks to that retrogradation tendency decreased. So, having a higher amount of linear chain in a premodified starch is main parameter to determine viscosity profile after acetylation. Rigidity of gelatinized starch, interactions of dispersed and continuous phases depend on amylose matrixes (Biliaderis, 1998). Starch with higher substitution levels inhibits amylose interaction and leads to reduced hardness values of gels. This weakening of the gels is also confirmed by the lower cohesiveness, chewiness, and gumminess values of starch gels (Liu et.al,1997; Sodhi, Singh, 2005).

As presented in Table 3.12, O_5-50_Gel.-50 had higher adhesive character than O_1.33-50_Gel.-50. The adhesiveness increased with acetylation degree. Water holding capacity was shown to increase with acetyl content (Gonzalez, Perez, 2002). That supported the increase in adhesiveness value.

The oxidation reaction increases retrogradation resistance and stickiness due to the presence of carbonyl and carboxyl groups in the starch chain, which reduces the possibility of hydrogen bonding (Dias et.al., 2011). In the study of Wojiecchowski et.al. (2018), dual modification by oxidation and acetylation reduces the hardness and adhesiveness values. Functional groups create a steric effect that prevent starch chains from approaching each other thus, gel loose texture (Ali, Hasnain, 2013). Also, amino groups of gelatin and carboxyl group of oxidized starch participate in a bounding reaction so, stiffness and resistance of the gel decreases (Moreno, et.al., 2017). The polymerization reaction in between gelatin and oxidized starch radical groups increases softness of the gelatin gel structure (Dong, et.al., 2019). This explains the reason for obtaining too soft gels with the acetylated oxidized starch.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this study, acetylation reactions were designed for different native and premodified starches. Acetylation reaction was performed with different reagent amounts and for different reaction times. Also, the effects of different starches on the texture of soft jelly were evaluated.

Acid modified starch had higher acetylation reaction efficiency than oxidized starch and native corn starch. The presence of hydroxyl groups and shorter starch chains are favorable to react. Acetylated acid modified starches had lower gelatinization temperature than the others. In addition, the required energy for gelatinization of these starches was lower. X-ray diffraction results showed that acid modified starch had more amorphous structure, which meant that most of the hydroxyl groups present in the amorphous side and crystallinity was related to the amylopectin content of starch molecule. This showed that acid modified starch had shorter chains than oxidized starch with higher amount of hydroxyl groups. Thus, acid modified starches were more susceptible to acetylation reaction. This result can also be observed by the acetyl content in acetylation reaction of acid modified starch, which had the highest value. Oxidized starch also had short starch chain, however, there were carbonyl and carboxyl groups on the chain. This case might have prevented the acetylation reaction efficiently. According to DSC and XRD analysis results, acetylated oxidized starches had higher onset temperatures; despite acid and oxidized starches having similar initial thermal properties. Also, acetylated oxidized starches had higher crystallinity. This was the evidence of more stabilized structure, carbonyl/carboxyl groups stabilized granules, and high amylopectin content of oxidized starch. Because of these reasons acetylated oxidized starches had a lower

degree of acetylation than acid modified starches. By the same reasons of having high number of amylopectin/long chains, fewer hydroxyl groups, native corn starch acetylation reaction had lower acetylation efficiency than acid and oxidized starches. In food applications to have a low viscous thickener, acetylation reaction with acid modified starch is more advantageous than oxidized starch. For the same rate of acetylation, acid modified starch consumes lower reagent, reaction time is shorter and because of that NaOH consumption will be less. Its production will be more economical and energy saving.

Acetylated acid modified and oxidized starches had different paste properties. Because of reaction efficiency, acetylated acid modified starches had less retrogradation tendency than acetylated oxidized starches in low DS starches. The retrogradation tendency and final viscosity stability while cooling decrease with degree of acetylation. When the degree of acetylation increased, there were no distinct differences between premodification types. The retrogradation level decreased with acetylation degree. However, there could be alkaline degradation with increase in reaction time. Starches undergoing alkaline degradation have lower acetylation level, despite starch being reacted with high amount of reagent in proper conditions.

In confectionary recipes, to substitute gelatin as a low viscosity thickener, acetylated starches were used by replacing 50% of gelatin amount. The obtained soft jellies with low DS acetylated acid modified and oxidized starches had similar texture properties in terms of hardness and adhesiveness parameters. High DS acetylated oxidized starch provided jellies with softer and sticky texture that could be resembled to Turkish Delight.

To conclude, the reaction of acetylated acid modified starch was more sufficient than acetylated oxidized starch due to reaction efficiency. The acetylated acid modified starch had a more stable paste viscosity profile. The thermal and rheological properties increased with acetylation degree. Gelatin substitution with acetylated acid modified and oxidized starches with low DS acetylation were the best options.

Otherwise, gummy like structure was lost with acetylation and premodification type. For 100% replacement of gelatin, starch viscosity should be increased, and acetylation degree should be kept at low levels.

In future studies, starch degradation can be kept at a lower level to increase viscosity of premodified starches. Additionally, low levels of acetylation should be performed to reduce retrogradation. To obtain a harder texture, viscosity stability must be reduced. As a type of pre-modification, acid-modified starch should be preferred to reduce the chemical amount and reaction time.

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APPENDICES

ANOVA TABLES

A. Analysis of variance for effects of parameters on acetylation reaction

Table A.1 *Analysis of variance of acetylated starches. Effect of starch type, reagent amount and reaction time on Percentage of acetyl content*

General Linear Model: Percentage of Acetyl Content versus Starch Type, Reagent Amount, Reaction Time

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	3	Native, Acid Mod., Oxidized
Reaction Time	Fixed	2	30, 75
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	2	0,0748	0,03741	31,48	0,000
Reaction Time	1	0,0024	0,00240	2,02	0,171
Reagent Amount	1	2,4067	2,40667	2025,25	0,000
Starch Type*Reaction Time	2	0,0052	0,00260	2,19	0,138
Starch Type*Reagent Amount	2	0,0183	0,00917	7,71	0,003
Reaction Time*Reagent Amount	1	0,0147	0,01470	12,37	0,002
Starch Type*Reaction Time*Reagent Amount	2	0,0030	0,00148	1,24	0,310
Error	20	0,0238	0,00119		
Total	31	10,3666			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0344722	99,77%	99,64%	99,37%

Comparisons for %Acetyl Content

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Grouping
Acid Mod.	12	1.07833	A
Oxidized	10	0.89583	B
Native	10	0.77000	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time	N	Mean	Grouping
30	16	0.926389	A
75	16	0.903056	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent Amount	N	Mean	Grouping

5.00	14	1.45389	A
1.33	18	0.37556	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time	N	Mean	Grouping		
Acid Mod. 30	6	1.09333	A		
Acid Mod. 75	6	1.06333	A		
Oxidized 75	5	0.90083		B	
Oxidized 30	5	0.89083		B	
Native 30	5	0.79500			C
Native 75	5	0.74500			C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reagent Amount	N	Mean	Grouping				
Acid Mod. 5.00	6	1.67667	A				
Oxidized 5.00	4	1.41500		B			
Native 5.00	4	1.27000			C		
Acid Mod. 1.33	6	0.48000				D	
Oxidized 1.33	6	0.37667					E
Native 1.33	6	0.27000					F

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time*Reagent Amount	N	Mean	Grouping
30 5.00	7	1.51389	A
75 5.00	7	1.39389	B
75 1.33	9	0.41222	C
30 1.33	9	0.33889	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time*Reagent Amount	N	Mean	Grouping
Acid Mod. 30 5.00	3	1.72667	A
Acid Mod. 75 5.00	3	1.62667	A
Oxidized 30 5.00	2	1.46500	B
Oxidized 75 5.00	2	1.36500	B
Native 30 5.00	2	1.35000	B
Native 75 5.00	2	1.19000	C
Acid Mod. 75 1.33	3	0.50000	D
Acid Mod. 30 1.33	3	0.46000	D
Oxidized 75 1.33	3	0.43667	D
Oxidized 30 1.33	3	0.31667	E
Native 75 1.33	3	0.30000	E

Native 30 1.33	3	0.24000	E	

Means that do not share a letter are significantly different.

Table A.2 *Analysis of variance of acetylated starches. Effect of starch type, reagent amount and reaction time on gelatinization temperature*

General Linear Model: Gelatinization Temp. versus Starch Type, Reaction Time, Reagent Amount

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	3	Native, Acid Mod., Oxidized
Reaction Time	Fixed	2	30, 75
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	2	16,244	8,1219	50,06	0,000
Reaction Time	1	0,350	0,3504	2,16	0,157
Reagent Amount	1	25,462	25,4616	156,93	0,000
Starch Type*Reaction Time	2	2,864	1,4318	8,82	0,002
Starch Type*Reagent Amount	2	2,172	1,0860	6,69	0,006
Reaction Time*Reagent Amount	1	0,859	0,8587	5,29	0,032
Starch Type*Reaction Time*Reagent Amount	2	3,885	1,9424	11,97	0,000
Error	20	3,245	0,1622		
Total	31	163,218			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,402796	98,01%	96,92%	95,28%

Comparisons for Gelatinization Temp.

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Groupin g
Native	10	70.7083	A
Acid Mod.	12	68.2992	B
Oxidized	10	67.4125	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time	N	Mean	Grouping
75	16	68.9625	A
30	16	68.6508	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent Amount	N	Mean	Grouping
1.33	18	70.4694	A
5.00	14	67.1439	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time	N	Mean	Grouping		
Native 75	5	70.8625	A		
Native 30	5	70.5542	A		
Acid Mod. 75	6	68.3250		B	
Acid Mod. 30	6	68.2733		B	
Oxidized 75	5	67.7000		B	C
Oxidized 30	5	67.1250			C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reagent Amount	N	Mean	Grouping			
Native 1.33	6	72.5167	A			
Acid Mod. 1.33	6	70.0917		B		
Native 5.00	4	68.9000			C	
Oxidized 1.33	6	68.8000			C	
Acid Mod. 5.00	6	66.5067				D

Oxidized 5.00	4	66.0250	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time*Reagent Amount	N	Mean	Grouping
75 1.33	9	70.7333	A
30 1.33	9	70.2056	A
75 5.00	7	67.1917	B
30 5.00	7	67.0961	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time*Reagent Amount	N	Mean	Grouping		
Native 75 1.33	3	73.2500	A		
Native 30 1.33	3	71.7833	B		
Acid Mod. 30 1.33	3	70.3333		C	
Acid Mod. 75 1.33	3	69.8500		C	D
Native 30 5.00	2	69.3250		C	D E
Oxidized 75 1.33	3	69.1000			D E
Oxidized 30 1.33	3	68.5000			E

Native 75 5.00	2	68.4750	E	
Acid Mod. 75 5.00	3	66.8000		F
Oxidized 75 5.00	2	66.3000		F
Acid Mod. 30 5.00	3	66.2133		F
Oxidized 30 5.00	2	65.7500		F

Means that do not share a letter are significantly different.

Table A.3 *Analysis of variance of acetylated starches. Effect of starch type, reagent amount and reaction time on setback viscosity*

General Linear Model: Setback Visco. versus Starch Type, Reaction Time, Reagent Amount

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	3	Native, Acid Mod., Oxidized
Reaction Time	Fixed	2	30, 75
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	2	592611718	296305859	270352,06	0,000
Reaction Time	1	38989504	38989504	35574,37	0,000
Reagent Amount	1	615782443	615782443	561845,29	0,000
Starch Type*Reaction Time	2	329752473	164876237	150434,52	0,000
Starch Type*Reagent Amount	2	279528776	139764388	127522,25	0,000

Reaction Time*Reagent Amount	1	18446240	18446240	16830,51	0,000
Starch Type*Reaction Time*Reagent Amount	2	137778568	68889284	62855,19	0,000
Error	20	21920	1096		
Total	31	2246183813			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
33.1059	100.00%	100.00%	100.00%

Comparisons for Setback Visco.

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Grouping
Acid Mod.	12	10826.7	A
Oxidized	10	8909.1	B
Native	10	2370.2	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time	N	Mean	Grouping
75	16	8054.50	A
30	16	6682.78	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent Amount	N	Mean	Grouping
1.33	18	12699.7	A
5.00	14	2037.6	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time	N	Mean	Grouping
Oxidized 75	5	12604.9	A
Acid Mod. 30	6	12236.0	B
Acid Mod. 75	6	9517.3	C
Oxidized 30	5	5213.3	D
Native 30	5	2699.1	E
Native 75	5	2041.2	F

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reagent Amount	N	Mean	Grouping

Acid Mod. 1.33	6	19717.5	A			
Oxidized 1.33	6	15879.2		B		
Native 1.33	6	2502.3			C	
Native 5.00	4	2238.0				D
Acid Mod. 5.00	6	1935.8				E
Oxidized 5.00	4	1939.0				E

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time*Reagent Amount	N	Mean	Grouping		
75 1.33	9	14157.2	A		
30 1.33	9	11242.1		B	
30 5.00	7	2123.4			C
75 5.00	7	1951.8			D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time*Reagent Amount	N	Mean	Grouping
Oxidized 75 1.33	3	23269.3	A

Acid Mod. 30 1.33	3	22266.7		B				
Acid Mod. 75 1.33	3	17168.3			C			
Oxidized 30 1.33	3	8489.0				D		
Native 30 1.33	3	2970.7					E	
Native 30 5.00	2	2427.5						F
Native 75 1.33	3	2034.0						G
Native 75 5.00	2	2048.5						G
Acid Mod. 30 5.00	3	2005.3						H
Acid Mod. 75 5.00	3	1866.3						H
Oxidized 30 5.00	2	1937.5						G H
Oxidized 75 5.00	2	1940.5						G H

Means that do not share a letter are significantly different.

Table A.4 *Analysis of variance of acetylated starches. Effect of starch type, reagent amount and reaction time on onset temperature*

General Linear Model: Onset Temp. versus Starch Type, Reaction Time, Reagent Amount

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	3	Native, Acid Mod., Oxidized
Reaction Time	Fixed	2	30, 75
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	2	1849,35	924,68	149,52	0,000
Reaction Time	1	1144,47	1144,47	185,06	0,000
Reagent Amount	1	404,41	404,41	65,39	0,000
Starch Type*Reaction Time	2	1497,04	748,52	121,03	0,000
Starch Type*Reagent Amount	2	346,39	173,20	28,01	0,000
Reaction Time*Reagent Amount	1	499,60	499,60	80,78	0,000
Starch Type*Reaction Time*Reagent Amount	2	250,36	125,18	20,24	0,000
Error	12	74,21	6,18		
Total	23	3260,55			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.48684	97.72%	95.64%	90.90%

Comparisons for Onset Temp.

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Grouping
Oxidized	8	126,484	A
Native	8	124,814	A
Acid Mod.	8	113,993	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Reaction

Time	N	Mean	Grouping
75	12	122,394	A
30	12	121,133	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent

Amount	N	Mean	Grouping
1.33	12	122,499	A
5	12	121,027	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Starch

Type*Reaction

Time	N	Mean	Grouping
Native 30	4	134,857	A
Oxidized 75	4	129,407	A B
Oxidized 30	4	123,560	B C
Acid Mod. 75	4	123,005	C
Native 75	4	114,770	D
Acid Mod. 30	4	104,980	E

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reagent Amount | N | Mean | Grouping

Native 1.33	4	128,122	A			
Oxidized 1.33	4	127,535	A			
Oxidized 5	4	125,432	A	B		
Native 5	4	121,505		B	C	
Acid Mod. 5	4	116,145			C	D
Acid Mod. 1.33	4	111,840				D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time*Reagent Amount | N | Mean | Grouping

75 1.33	6	127,880	A	
30 5	6	125,147	A	
30 1.33	6	117,118		B
75 5	6	116,908		B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time*Reagent Amount | N | Mean | Grouping

Native 30 1.33	2	137,855	A

Oxidized 75 1.33	2	136,495	A		
Native 30 5	2	131,860	A	B	
Acid Mod. 75 1.33	2	128,755	A	B	
Oxidized 30 5	2	128,545	A	B	
Oxidized 75 5	2	122,320		B	C
Oxidized 30 1.33	2	118,575			C D
Native 75 1.33	2	118,390			C D
Acid Mod. 75 5	2	117,255			C D
Acid Mod. 30 5	2	115,035			C D
Native 75 5	2	111,150			D
Acid Mod. 30 1.33	2	94,925			E

Means that do not share a letter are significantly different.

Table A.5 *Analysis of variance of acetylated starches. Effect of starch type, reagent amount and reaction time on enthalpy*

General Linear Model: Enthalpy versus Starch Type, Reaction Time, Reagent Amount

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	3	Native, Acid Mod., Oxidized
Reaction Time	Fixed	2	30, 75
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	2	30873	15436	0,79	0,474
Reaction Time	1	2938	2938	0,15	0,704
Reagent Amount	1	67	67	0,00	0,954

Starch Type*Reaction Time	2	487914	243957	12,56	0,001
Starch Type*Reagent Amount	2	118233	59116	3,04	0,085
Reaction Time*Reagent Amount	1	5453	5453	0,28	0,606
Starch Type*Reaction Time*Reagent Amount	2	220798	110399	5,68	0,018
Error	12	233077	19423		
Total	23	1998429			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
139,367	88,34%	77,65%	53,35%

Comparisons for Enthalpy

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Grouping
Acid Mod.	8	1200,43	A
Oxidized	8	1169,10	A
Native	8	1127,41	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time	N	Mean	Grouping
30	12	1292,40	A

75	12	1038,89	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent Amount	N	Mean	Grouping
5	12	1221,80	A
1.33	12	1109,49	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time	N	Mean	Grouping
Native 30	4	1451,31	A
Acid Mod. 30	4	1253,64	A
Oxidized 30	4	1172,23	A
Oxidized 75	4	1165,96	A
Acid Mod. 75	4	1147,22	A
Native 75	4	803,50	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reagent Amount	N	Mean	Grouping
Native 5	4	1341,39	A

Acid Mod. 1.33	4	1222,43	A B
Oxidized 1.33	4	1192,61	A B
Acid Mod. 5	4	1178,42	A B
Oxidized 5	4	1145,58	A B
Native 1.33	4	913,43	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time*Reagent Amount	N	Mean	Grouping
30 1.33	6	1345,48	A
30 5	6	1239,31	A
75 5	6	1204,29	A
75 1.33	6	873,49	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time*Reagent Amount	N	Mean	Grouping
Native 30 5	2	1480,65	A
Native 30 1.33	2	1421,98	A
Oxidized 30 1.33	2	1364,94	A
Oxidized 75 5	2	1311,63	A
Acid Mod. 30 5	2	1257,74	A

Acid Mod. 30 1.33	2	1249,54	A
Native 75 5	2	1202,13	A
Acid Mod. 75 1.33	2	1195,33	A
Acid Mod. 75 5	2	1099,10	A
Oxidized 75 1.33	2	1020,28	A
Oxidized 30 5	2	979,53	A
Native 75 1.33	2	404,87	B

Means that do not share a letter are significantly different.

Table A.6 *Analysis of variance of acetylated starches. Effect of starch type, reagent amount and reaction time on crystallinity*

General Linear Model: Crystallization versus Starch Type, Reaction Time, Reagent Amount

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	3	Native, Acid Mod., Oxidized
Reaction Time	Fixed	2	30, 75
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	2	12,670	6,3350	6,79	0,011
Reaction Time	1	9,610	9,6100	10,31	0,007
Reagent Amount	1	4,840	4,8400	5,19	0,042
Starch Type*Reaction Time	2	26,682	13,3408	14,31	0,001
Starch Type*Reagent Amount	2	15,352	7,6758	8,23	0,006

Reaction Time*Reagent Amount	1	23,461	23,4613	25,16	0,000
Starch Type*Reaction Time*Reagent Amount	2	34,856	17,4279	18,69	0,000
Error	12	11,190	0,9325		
Total	23	70,565			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,965660	84,14%	69,61%	36,57%

Comparisons for Crystallization

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Grouping
Oxidized	8	39,2750	A
Acid Mod.	8	38,2125	A B
Native	8	37,9375	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time	N	Mean	Grouping
75	12	39,0417	A
30	12	37,9083	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent Amount	N	Mean	Grouping
5	12	38,9417	A
1.33	12	38,0083	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time	N	Mean	Grouping
Oxidized 75	4	40,025	A
Native 75	4	38,725	A B
Oxidized 30	4	38,525	A B
Acid Mod. 75	4	38,375	A B
Acid Mod. 30	4	38,050	A B
Native 30	4	37,150	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reagent Amount	N	Mean	Grouping
Oxidized 5	4	39,375	A
Oxidized 1.33	4	39,175	A
Acid Mod. 5	4	38,825	A
Native 5	4	38,625	A

Acid Mod. 1.33	4	37,600	A
Native 1.33	4	37,250	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time*Reagent Amount

Amount	N	Mean	Grouping
75 5	6	39,5167	A
75 1.33	6	38,5667	A B
30 5	6	38,3667	A B
30 1.33	6	37,4500	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time*Reagent Amount

Amount	N	Mean	Grouping		
Oxidized 75 1.33	2	40,75	A		
Acid Mod. 75 5	2	40,70	A		
Oxidized 30 5	2	39,45	A	B	
Oxidized 75 5	2	39,30	A	B	C
Acid Mod. 30 1.33	2	39,15	A	B	C
Native 75 1.33	2	38,90	A	B	C
Native 30 5	2	38,70	A	B	C
Native 75 5	2	38,55	A	B	C

Oxidized 30 1.33	2	37,60	A	B	C
Acid Mod. 30 5	2	36,95	A	B	C
Acid Mod. 75 1.33	2	36,05		B	C
Native 30 1.33	2	35,60			C

Means that do not share a letter are significantly different.

Table A.7 *Analysis of variance of acetylated starches. Effect of starch type, reagent amount and reaction time on transmittance*

General Linear Model: Transmittance versus Starch Type, Reaction Time, Reagent Amount

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	3	Native, Acid Mod., Oxidized
Reaction Time	Fixed	2	30, 75
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	2	3432,8	1716,41	1442,47	0,000
Reaction Time	1	49,3	49,32	41,45	0,000
Reagent Amount	1	162,0	161,99	136,14	0,000
Starch Type*Reaction Time	2	81,4	40,72	34,22	0,000
Starch Type*Reagent Amount	2	64,2	32,11	26,98	0,000
Reaction Time*Reagent Amount	1	16,7	16,71	14,05	0,003
Starch Type*Reaction Time*Reagent Amount	2	59,1	29,55	24,84	0,000
Error	12	14,3	1,19		
Total	23	17667,3			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,09083	99,92%	99,85%	99,68%

Comparisons for Transmittance

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Grouping
Oxidized	8	65,1904	A
Acid Mod.	8	51,9435	B
Native	8	2,8721	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time	N	Mean	Grouping
75	12	41,2533	A
30	12	38,7507	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent Amount	N	Mean	Grouping
5	12	41,7565	A
1.33	12	38,2475	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time | N | Mean | Grouping

Oxidized 75	4	67,3101	A		
Oxidized 30	4	63,0707		B	
Acid Mod. 75	4	54,0094			C
Acid Mod. 30	4	49,8776			D
Native 30	4	3,3039			E
Native 75	4	2,4404			E

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reagent Amount | N | Mean | Grouping

Oxidized 1.33	4	65,4165	A			
Oxidized 5	4	64,9642	A			
Acid Mod. 5	4	56,8619		B		
Acid Mod. 1.33	4	47,0251			C	
Native 5	4	3,4434				D
Native 1.33	4	2,3009				D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reaction Time*Reagent Amount | N | Mean | Grouping

30 5	6	42,3352	A
75 1.33	6	41,3287	A
75 5	6	41,1778	A
30 1.33	6	35,1663	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reaction Time*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reaction Time*Reagent Amount	N	Mean	Grouping
Oxidized 75 1.33	2	71,4513	A
Oxidized 30 5	2	66,7597	B
Oxidized 75 5	2	63,1688	B C
Oxidized 30 1.33	2	59,3816	C D
Acid Mod. 75 5	2	57,4824	D
Acid Mod. 30 5	2	56,2414	D
Acid Mod. 75 1.33	2	50,5364	E
Acid Mod. 30 1.33	2	43,5138	F
Native 30 5	2	4,0044	G
Native 75 5	2	2,8823	G
Native 30 1.33	2	2,6034	G
Native 75 1.33	2	1,9984	G

Means that do not share a letter are significantly different.

B. Analysis of variance for effects of starches on soft jelly

Table B.1 *Analysis of variance of soft jellies. Effect of starch type and reagent amount on hardness*

General Linear Model: Hardness versus Starch Type, Reagent Amount

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	2	Acid Mod., Oxidized
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	1	209256	209256	36,81	0,000
Reagent Amount	1	7	7	0,00	0,973
Starch Type*Reagent Amount	1	256907	256907	45,20	0,000
Error	12	68210	5684		
Total	15	597921			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
75,3937	88,59%	85,74%	79,72%

Comparisons for Hardness

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Grouping
Oxidized	8	525,809	A

Acid	8	455,776	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent Amount	N	Mean	Grouping
1.33	8	616,586	A
5	8	364,999	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type*Reagent Amount	N	Mean	Groupin g
Oxidized 1.33	4	778.317	A
Acid Mod. 5.00	4	456.697	B
Acid Mod. 1.33	4	454.855	B
Oxidized 5	4	273.300	C

Means that do not share a letter are significantly different.

Table B.2 *Analysis of variance of soft jellies. Effect of starch type and reagent amount on adhesiveness*

General Linear Model: Adhesiveness versus Starch Type, Reagent Amount

Factor Information

Factor	Type	Levels	Values
Starch Type	Fixed	2	Acid Mod., Oxidized
Reagent Amount	Fixed	2	1.33, 5.00

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Starch Type	1	0,177	0,1770	0,02	0,888
Reagent Amount	1	0,133	0,1326	0,02	0,903
Starch Type*Reagent Amount	1	58,255	58,2551	6,87	0,022
Error	12	101,764	8,4803		
Total	15	260,204			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2,91209	60,89%	51,11%	30,47%

Comparisons for Adhesiveness

Tukey Pairwise Comparisons: Starch Type

Grouping Information Using the Tukey Method and 95% Confidence

Starch Type	N	Mean	Grouping
Oxidized	8	3,83500	A
Acid	8	0,31625	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Reagent

Amount	N	Mean	Grouping	
5	8	3,85500	A	
1.33	8	0,29625		B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Starch Type*Reagent Amount

Grouping Information Using the Tukey Method and 95% Confidence

Starch

Type*Reagent

Amount	N	Mean	Grouping	
Oxidized 5	4	7,5225	A	
Acid 1.33	4	0,4450		B
Acid 5	4	0,1875		B
Oxidized 1.33	4	0,1475		B

Means that do not share a letter are significantly different.



Figure A.1 Suspension reaction reactor



Figure A.2 Experimental set up for the oxidation reaction

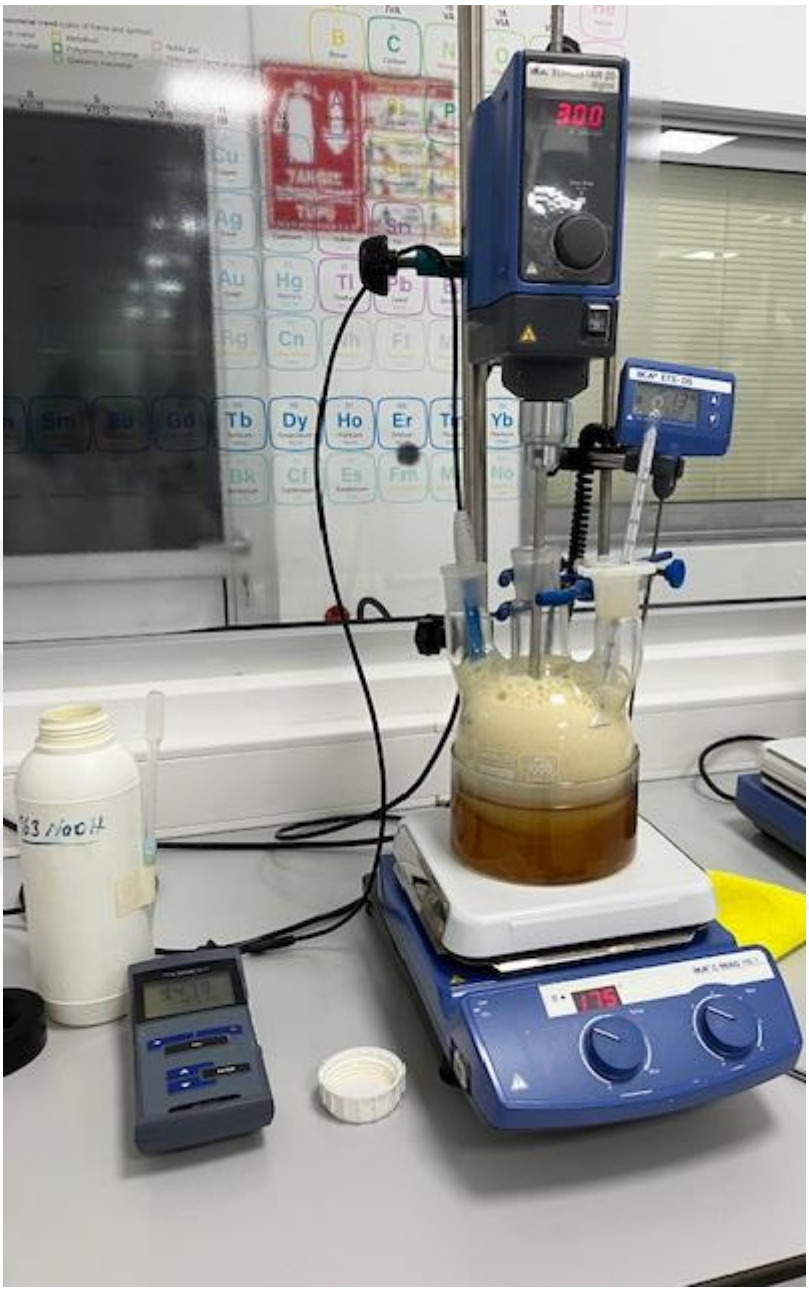


Figure A.3 Experimental set up for the acetylation reaction of acid modified starch

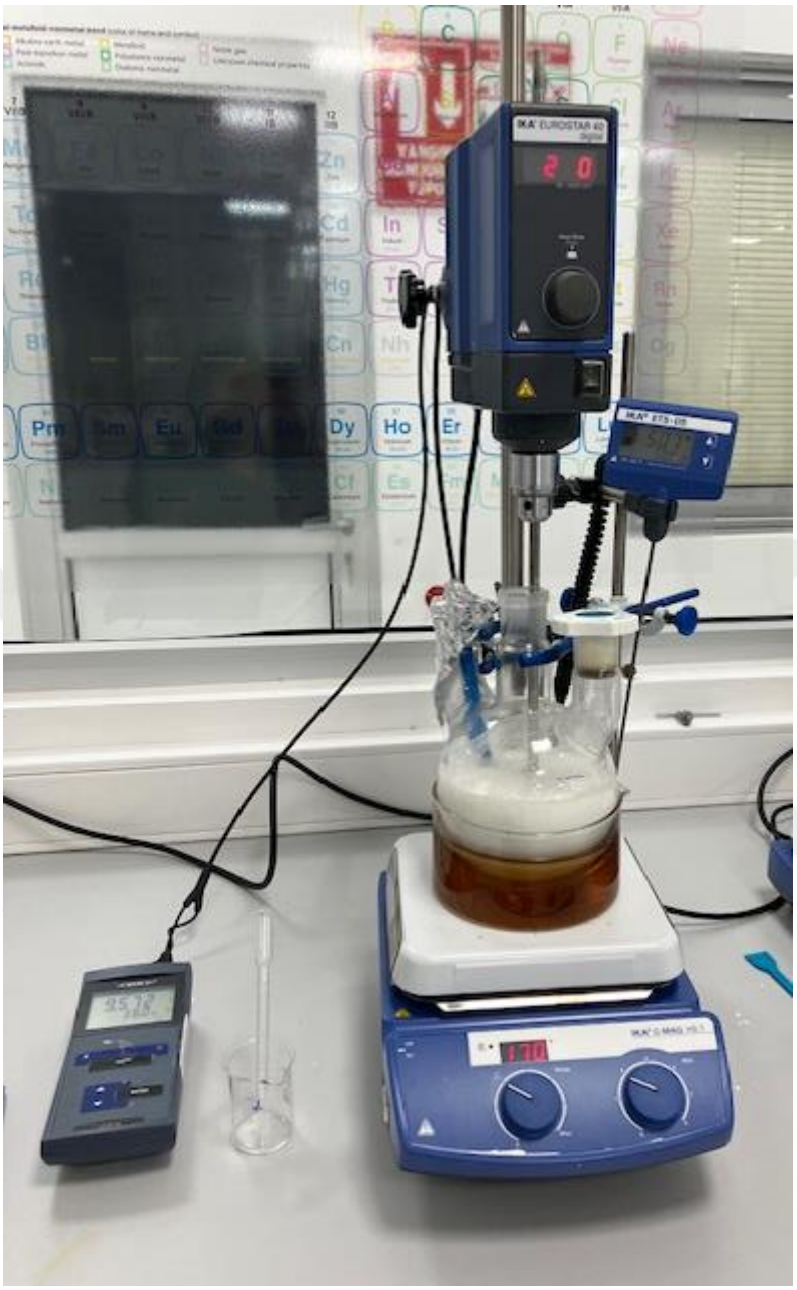


Figure A.4 Experimental set up for the acetylation reaction of oxidized starch



Figure A.5 Mixing in soft Jelly candy production



Figure A.6 Cooking in soft jelly candy production



Figure A.7 Melt deposition in soft jelly candy production

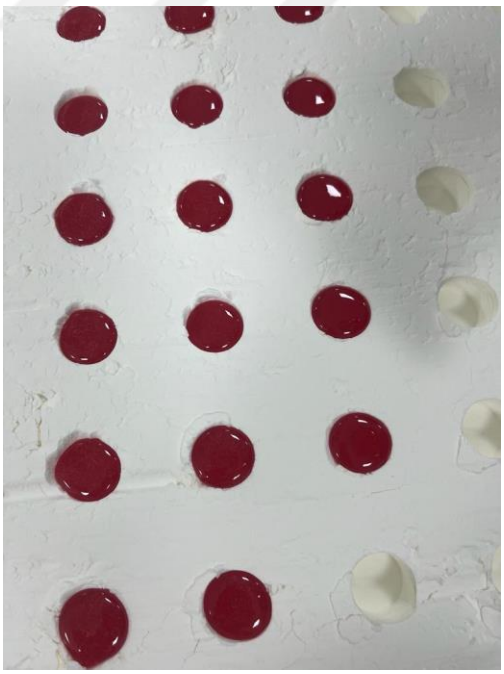


Figure A.8 Maturation of soft jelly candies



Figure A.9 Soft jelly candies containing different starches