EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) IN PHYSICOCHEMICAL AND TEXTURAL PROPERTIES OF STARCH

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY
İLHAMİ OKUR

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

JULY 2018

Approval of the thesis:

EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) IN PHYSICOCHEMICAL AND TEXTURAL PROPERTIES OF STARCH

Submitted by **İLHAMİ OKUR** in partial fulfillment of the requirements for the degree of **Master of Science in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Serpil Şahin _____
Head of Department, **Food Engineering**

Prof. Dr. Hami Alpas _____
Supervisor, **Food Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Rahmi Ertan Anlı _____
Food Engineering Dept., Ankara University

Prof. Dr. Hami Alpas _____
Food Engineering Dept., METU

Assoc. Prof. Dr. Mecit Halil Öztop _____
Food Engineering Dept., METU

Asst. Prof. Dr. Emin Burçin Özvural _____
Food Engineering Dept.,
Çankırı Karatekin University

Asst. Prof. Dr. Cem Baltacıoğlu _____
Food Engineering Dept.,
Niğde Ömer Halisdemir University

Date: 30.07.2018

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

Name, Last Name: İlhami OKUR

Signature :

ABSTRACT

Effect of High Hydrostatic Pressure (HHP) in Physicochemical and Textural Properties of Starch

OKUR,İlhami

MS, Food Engineering Department, METU

Supervisor: Prof. Dr. Hami ALPAS

July 2018, 80 pages

Starch is the major polysaccharide consumed by human being. It is not classified as a dietary fiber as it is digestible by the enzymes present in the saliva and small intestines. However, it could become possible to modify the starch with thermal and non-thermal techniques. High Hydrostatic Pressure (HHP) is a cold pasteurization technique that has increased application in the food industry with minimum effect on the nutritional quality of the products. It is hypothesized that the use of HHP could be a modification strategy for starch. In this study, the effects of different HHP parameters (400 and 500 MPa) at different temperature (20˚C, 30˚C, and 40˚C) for 5, 15 and 30 min on in vitro digestibility and physicochemical properties of cornstarch were studied. The results showed that HHP treatment increased SDS (Slowly Digestible Starch) and RDS

(Rapid Digestible Starch) significantly ($p \leq 0.05$). In addition to this, it was shown that HHP treatment decreased the solubility and swelling power of the cornstarch and T_2 relaxation times increased with HHP treatment as measured by NMR (Nuclear Magnetic Resonance) Relaxometry experiments.

Keywords: Corn Starch, HHP, NMR, Digestibility

ÖZ

Yüksek Hidrostatik Basıncın (YHB) Nişastanın Fizikokimyasal ve Dokusal Özelliklerini Üzerine Etkisi

OKUR,İlhami

Yüksek Lisans, Gıda Mühendisliği Bölümü, ODTÜ

Tez Yöneticisi: Prof. Dr. Hami ALPAS

Temmuz 2018, 80 sayfa

Nişasta, insanlar tarafından tüketilen başlıca polisakkarittir. Tükürükte ve ince bağırsakta bulunan enzimler tarafından sindirilebildiği için diyet lifi olarak sınıflandırılmaz. Bununla birlikte, nişastayı termal ve termal olmayan tekniklerle değiştirmek mümkün olabilir. Yüksek Hidrostatik Basınç (YHB), gıda endüstrisinde ürünün besin kalitesine en az etkisi ile gıda uygulamalarında kullanımı artan soğuk bir pastörizasyon tekniğidir. YHB kullanımının nişastayı modiye etmek için yeni stratejisi olabileceği düşünülmektedir. Bu tez kapsamında, Farklı YHB parametrelerinin (400 ve 500 MPa) farklı sıcaklıklarda (20°C, 30°C ve 40°C) 5, 15 ve 30 dakika boyunca *in vitro* sindirilebilirlik üzerindeki etkileri, mısır nişastasının fiziko-kimyasal özellikleri incelenmiştir. Sonuçlara göre YHB işleminin YSN (Yavaşça Sindirilebilir Nişasta) ve HSN'yi (Hızlı Sindirilebilir Nişasta) önemli ölçüde arttırdığını göstermiştir ($p \leq 0.05$). Buna ek olarak YHB'nin, mısır nişastasının çözünürlüğünü ve şişme gücünü azalttığı

ancak NMR (Nükleer Manyetik Rezonans) Relaxometri ile ölçülen T_2 rahatlama sürelerini arttırtığı gözlenmiştir.

Anahtar Kelimeler: Mısır Nişastası, Yüksek Hidrostatik Basınç (YHB), NMR (Nükleer Manyetik Rezonans) Relaxometri, *in vitro* Sindirilebilirlik

To my family

ACKNOWLEDGEMENTS

I am grateful to my supervisor, Prof. Dr. Hami ALPAS, whose expertise, understanding, generous guidance and support made it possible for me to work on my thesis that was of great interest to me. It was a pleasure working with him.

I would like to express my gratitude to Assoc.Prof.Dr.Mecit ÖZTOP for his valuable suggestions and guidance through my thesis. His valuable suggestions were so important for my thesis and he helped me with all the problem that i faced.

I want to say thank to lab members especially for Eylül ÖZTÜRK, Bige TIRPANCI, Burcu KAYA, Ufuk YORGANCI. It was so nice and funny to work with such great lab friends. In addition to this, i would like to say thank to Assist. Prof. Dr. Sezen SEVDİN for her help. Thanks to her, I got used to working in this lab easier.

I also would like to express my gratitude to my all teacher at food engineering department who put their faith in me and urged me to do better and i am grateful to workers at our department especially for Mukaddes ÜNVER for solving all problems that i faced. I also would like to thank to Barış Özel for his help for my thesis and article

I am grateful to my friends, Arif Ozan KIZILDAĞ, Ömer Faruk ÇAPOĞLU, Misir MİSİRLİ and others who I do not write here. Furthermore, I would like to say thank to my workplace where is food engineering department at Niğde Ömer Halisdemir University for their help and support.

Lastly, i want to express my deepest gratitude to all members of my lovely family, Hüseyin OKUR, Sultan OKUR, İkbal SAKALLI, İlknur OKUR, Yağmur SAKALLI and Lütfi SAKALLI for their support me all the time, patience. I also want to express my deepest gratitude to my love, Pürlen SEZER, for her endless love, help me and patience while working in the laboratory. This study could not be finished without them.

Table of Contents

List of Figures

List of Tables

CHAPTER 1

INTRODUCTION

1.1 Starch

Starch has a major role in supplying metabolic energy in human nutrition and it is the reserve carbohydrate in plants found in many different plant organs such as seeds, fruits, tubers, and roots. Due to being a cheap material and easy to change its physicochemical properties with thermal treatment, starch has been widely used in the food industry (Jobling, 2004). Starch has been a widely available ingredient in the food industry as a gelling agent, thickener, bulking agent and water retention agent (Singh, Singh, Kaur, Singh Sodhi, & Singh Gill, 2003). Starch consists of essentially linear amylose and highly branched amylopectin molecules and it is digested in the gastrointestinal tract by α-amylases from pancreas and saliva. These enzymes belong to the family of α-glucosidases and after consuming cornstarch, it is firstly digested by α-amylase to α-limit dextrins. Then, oligosaccharides that are then cleaved by the brush border enzymes into glucose (Pencek et al., 2002).

The rate of glucose release and absorption from digesting starch plays an important role in human health (Zhang & Hamaker, 2009). Therefore, starch is classified into three groups according to the rate and extent of digestion *in vitro* which are rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Table 1). The starch fraction digested within 20 minutes of incubation corresponds to rapidly digestible starch (RDS) and, the starch fraction digested between 20 and 120 minutes is accepted as slowly digestible starch (SDS) and the remaining fraction corresponds to resistant starch (RS) (Figure 1) (Englyst, Kingman, & Cummings, 1992).

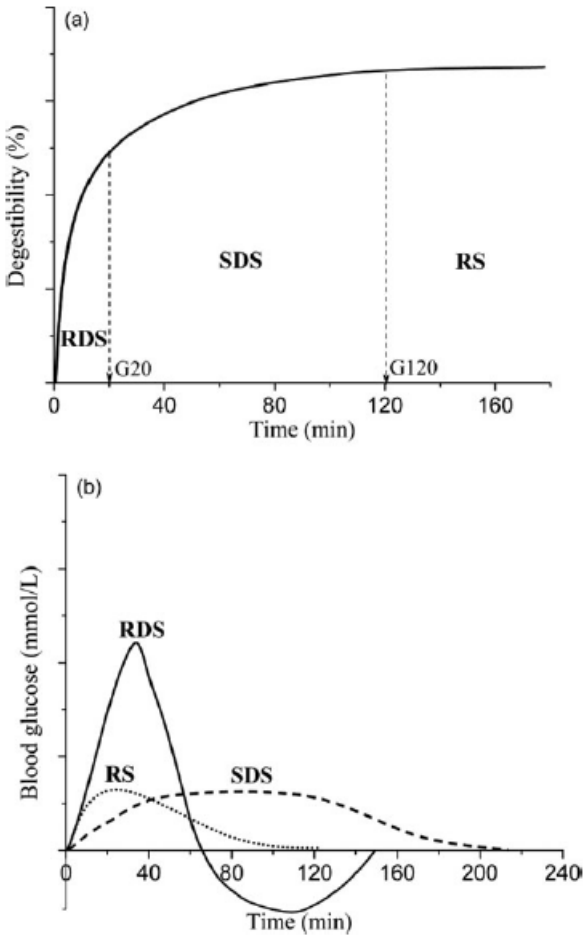

Figure 1. Classification of the starch. (a) *In vitro* digestion according to Englyst assay, and (b) *in vivo* glycemic response to RDS, SDS, and RS (Zhang & Hamaker, 2009).

RDS is rapidly digested since it is absorbed in the proximal and regions of the small intestine causing to a fast elevation of blood glucose. However, this rapid increases in blood glucose levels may further lead to some problems such as cell, tissue, and organ damage. On the other hand, SDS is digested slowly and this provides sustained glucose release and subsequently a slow and prolonged release of glucose, causing to prolonged energy availability compared to RDS. RS cannot be digested in the upper gastrointestinal tract but it is fermented by the colonic microflora, producing short-chain fatty acids that provide additional energy to the body along with butyrate that is beneficial to colonic health. RS is divided into three types which are physically indigestible starch, resistant starch granules and retrograded starch (Annison & Topping, 1994).

SDS is digested completely in the small intestine at a lower rate than RDS so SDS tends to provide a sustained supply of glucose with a low glycemic index (GI). This contributes to the control and prevention of various hyperglycaemia related diseases (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996). In addition, SDS is beneficial to maintain body weight when it is used as a raw material in the production of foodstuffs (Jenkins et al., 2002). As a result, foods having a high percent of SDS are accepted as functional foods with a low GI. Because of this, SDS has aroused interest in recent years (Zhang & Hamaker, 2009).

Table 1. Classification of starch according to digestion rate

Starch type	Examples	Digestion rate in the small intestine
Rapidly digestible starch (RDS)	White bread, Freshly cooked starch	~30 min peak blood glucose
Slowly digestible starch (SDS)	Most raw cereal starches	Complete digestion slowly
Resistant starch (RS)		
1. Physically inaccessible (RS1)	Partially milled grain	Resistant
2. Resistant starch granule (RS2)	Raw potato, Maize starch, banana starch	Resistant
3. Retrogradated starch (RS3)	Cooled and cooked potato	Resistant
4. Chemical modified starch (RS4)	Cross-linking starch	Slow to Resistant (depending on the modification types)

The gelatinization is a vital process for starch before use in the food industry because this process determines the proper conversion of starch in the processing of foods and emerging biodegradable starch-based materials (Luo, Li, & Lin, 2012). Normally, starch granules are insoluble in cold water. However, starch granules absorb water and swell when cornstarch is heated in water. This destabilizes starch' crystalline structure and this leads to loss of birefringence and this is called gelatinization (Donovan, 1979; Parker & Ring, 2001). During continuous heating, starch granules tend to swell to greater extents, and the crystallites melt. This leads to increase in molecular motion that causes to complete separation of amylose and amylopectin of the starch. The temperature when granules lose their birefringence is called as the gelatinization temperature. The swelling of the starch granules begins after the completion of this stage of gelatinization. This process is affected by several factors such as crystalline

order, structural changes in the amorphous region, amylose content (Levine & Slade, 1990).

Starch gelatinization is determined by many methods such Nuclear Magnetic Resonance (NMR), enzymatic digestibility, solubility and swelling power. All of these methods measure physicochemical properties and have advantages and disadvantages. As a result, it is important to use several different methods to characterize starch gelatinization properties (Lund, 1984).

1.2 High Hydrostatic Pressure (HHP)

Nowadays, nearly all of the processed foods such as juice, milk and canned products are treated at a high temperature to kill microorganism. However, applying high temperature on foods results in decreasing nutritional quality of foods because many nutrients are heat sensitive. Several vitamins, enzymes, and proteins degrade under heat treatments. These changes do not only affect nutritional value but also they may affect color, texture, and flavor of processed foods (Khan et al., 2018; Rendueles et al., 2011). For example, vegetable tissues become soft and chemical compounds need to be added to regain firmness. In addition to these, consumers prefer foods that have longer shelf-life than fresh ones and have similar characteristics to the original product. This view brings a new challenge for the food industry so new processes need to be developed and adopted to satisfy consumer demands (Chawla, Patil, & Singh, 2011). As a result, novel technologies such as pulsed electric fields (PEF), irradiation, high-intensity light pulses, and high hydrostatic pressures (HHP) are currently under extensive research. Among novel technologies, HHP is one of the widest application in the food industry especially for extending the shelf-life of food products. By affecting the molecular structure of chemical compounds, necessary for metabolic metabolism, HHP inactivates molds, bacteria, viruses, and parasites (Alpas et al., 1999; Alpas, Kalchayanand, Bozoglu, & Ray, 2000; Alpas, Lee, Bozoglu, & Kaletunç, 2003). Also, bacterial spores partially destroyed by HHP. Furthermore, HHP does not destroy the food because it is applied all sides of food. Therefore, HHP is a powerful tool to improve food products of better nutritional and sensory quality, novel texture, and increased shelf-life (j. doona, Kustin, & Feeherry, 2010).

In the last decades, HHP become popular in the food industry (Dalai & Sahu, 2010). The first report about HHP treatment of food is about the effect of HHP on foodborne microorganisms in milk by applying 650 MPa pressure (Hite, 1899). The result of this report is that HHP provided a significant reduction in the number of viable microbes.

The typical HHP system consists of a high-pressure vessel and its closure, pressure generation device, temperature and pressure control device (Figure 2). Water is used mostly for pressure-transferring medium and pressure is applied uniformly on food and instantaneous manner throughout the whole biological sample regardless of direct contact with the pressure medium (Durance, 2002).

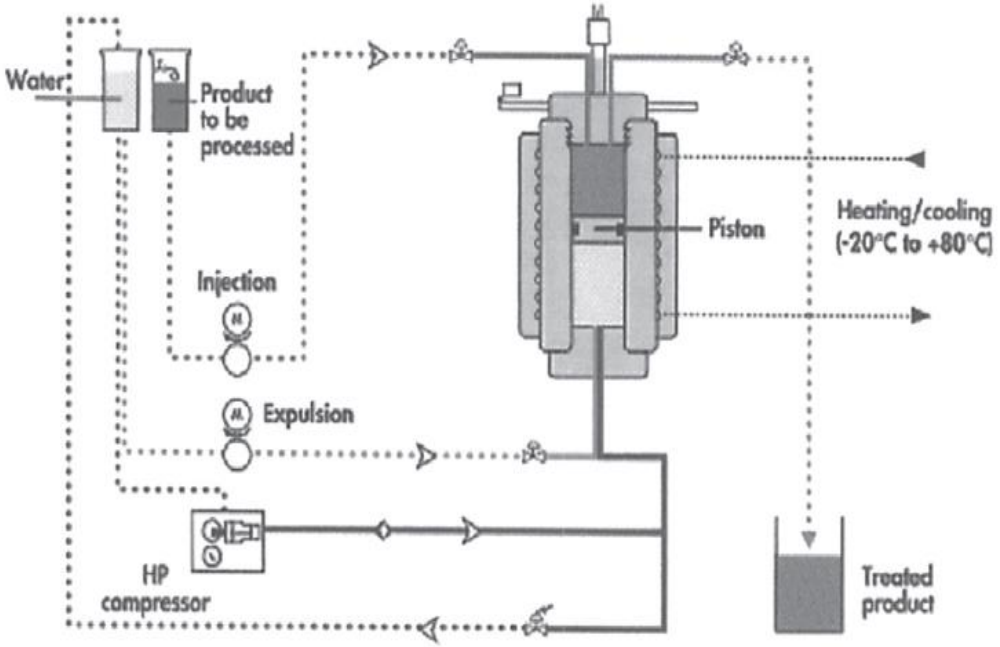

Figure 2. HHP processing

There are two operating principles which explains HHP technology. The first principle is Le Chatelier's principle. At this principle. equilibrium chemical reaction, phase transition and/or change in molecular configuration is performed by reducing the volume and this can be enhanced by pressure (j. doona et al., 2010). The second principle is an isostatic principle. At this principle, the transmittance of pressure is uniform and instantaneous and it is independent of the size and geometry of food (j. doona et al., 2010).

Because of the increasing popularity of HHP, food companies adopt HHP for production of microbiologically safe food products with better quality and taste. Nowadays, pressure-treated fresh fruit juices are available in commercial markets in the United States, Canada, Australia, Europe and East Asia countries like India, Taiwan, South Korea, and Japan. In 2016, the HHP food market was worth 11.03 billion USD and the HHP equipment market had a value of 0.47 billion USD. The market value of pressure-treated foods is expected to be worth 12 billion USD in 2018 (Grumezescu & Holban, 2018).

HHP was expended in the food industry and it is applied into a variety of food products (j. doona et al., 2010). Nowadays, HHP treated vegetable products, seafood, meat products, fresh fruits, and beverages are sold in the commercial markets on the world. HHP treated soups and sauces are also in the markets. Vegetables and meat take the lead with 27% each of the total, followed by juices and beverages with a 14% percentage. Seafood comes up with 12%, and other products completed the table with a percentage near to 20% (Elamin, Endan, Yosuf, Shamsudin, & Ahmedov, 2015).

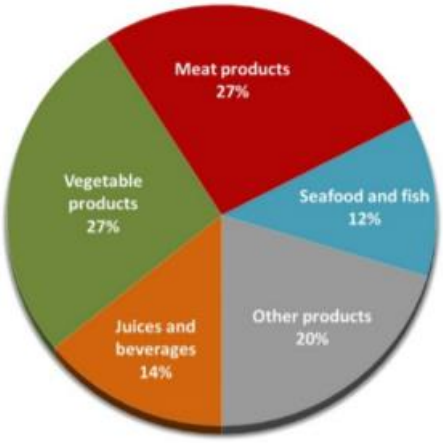

Figure 3. Total number of HHP industrial machines in production (Elamin et al., 2015)

Figure 4: Some examples of HHP treated food products

1.2.1. Effects of HHP treatment on Starch

In recent years, it is found that starch can be gelatinized and modified by using HHP (Yang, Chaib, Gu, & Hemar, 2017). Gelatinization by HHP treatment is similar to gelatinization by heating but pressure-induced gelatinization of starch much better preserves the granular structure of starch than the traditional heating process. However, there is a lack of knowledge on the use of HHP in starch chemical modification (Kim et al., 2012).

In literature, it is shown that HHP has an effect on the physicochemical properties of starches (Hu, Zhang, Jin, Xu, & Chen, 2017; W. Li et al., 2015; Oh, Pinder, Hemar, Anema, & Wong, 2008). Liu et al. (2017) stated that swelling power, hardness, and viscosity of tartary buckwheat starch (TBS) is decreased significantly with HHP treatment ($p \leq 0.05$). It is also shown that HHP is an important nonthermal modification method of TBS that can change the textural properties and *in vitro* digestibility of TBS and all of these changed were pressure-dependent. Another study related with starch indicated that HHP treatment causes to increase SDS content of waxy wheat starch (Hu et al., 2017). According to this study, SDS content reached a maximum (31.12%) at 600 MPa. Furthermore, HHP treatment leads to a structural change of wheat starch and this affects to the digestibility of wheat starch. Li & Zhu, (2018) showed that HHP causes to reduce solubility and swelling power of quinoa starch because HHP treament decreases the amylose leaching and increase the formation of amylose-lipid complexes.

1.3 Nuclear Magnetic Resonance (NMR) Relaxometry

NMR (Nuclear Magnetic Resonance) spectroscopy, which is the non-invasive and non-destructive method, is widely used for the analysis of physiological and biochemical changes in food samples in recent years because qualitative and quantitative data on physical and chemical properties of a wide range of samples can be gathered (Marcone et al., 2013; Spyros & Dais, 2012). NMR has the origins within the nucleus of atom types such as H, C, O and P and these individual atoms have the net nuclear spin. The effects of spin are noted in a magnetic field and this involves the energy exchange at least two levels (resonance) (Gidley, 2014). NMR experiments don't require to do separation of diverse food components but need relatively a small amount of efforts for sample pretreatment and preparation than traditional methods (Spyros & Dais, 2012). For the experiment, the food samples can be semi-solid, solid, and lipid. The obtained complex NMR spectra can be further treated with multivariate statistical analysis to get additional structural information of food systems (Flanagan, Gidley, & Warren, 2015) and this provides a characterization of these systems. Among nucleus of atom types, hydrogen is preferred mostly for the experiments because of hydrogen's abundance in food samples and high MR sensitivity (Kirtil & Oztop, 2016).

The working principle of NMR is the following: The sample has protons within itself which are randomly aligned without an external magnetic field. Then, it is put into magnets and this creates an external static magnetic field (B_0). The protons align themselves with the external magnetic field and they start to spin at the frequency that is proportional to magnetic field strength. Next, a radio frequency (RF) is applied and the protons come back the lowest energy state when RF is turned off. Finally, the relaxation signal is obtained (Figure 3).

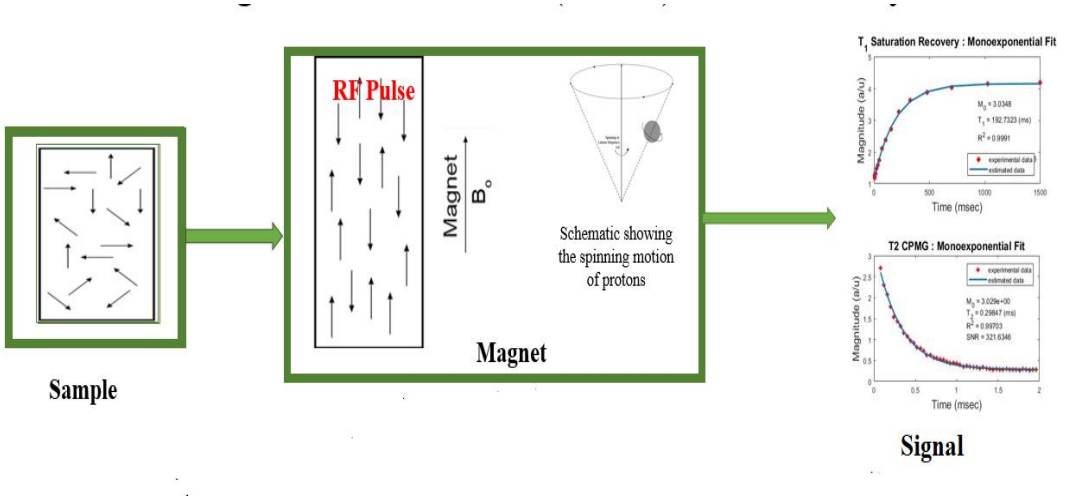

Figure 5. Schematic representation of NMR signal acquisition

Transverse relaxation time (T_2) is also called as spin-spin relaxation time that is the time constant for the transverse magnetization decay and the equilibrium value of zero (Figure 4).

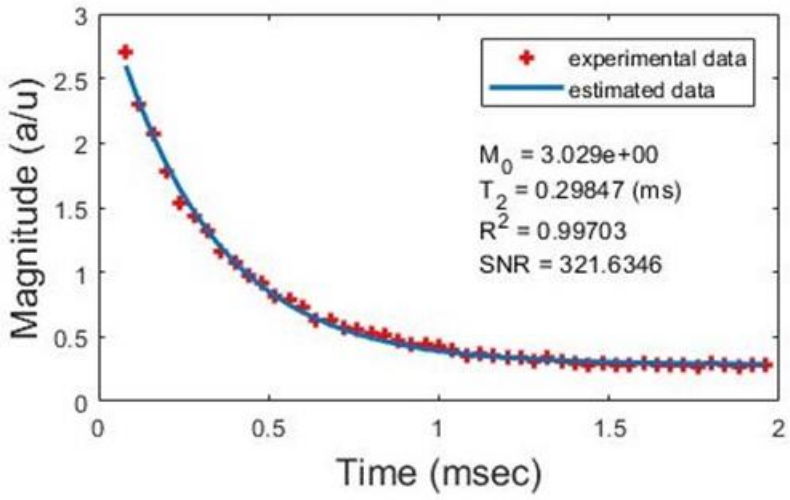

Figure 6. Representative T_2 relaxation Curve

1.3.1. Physicochemical properties of Starch by NMR Relaxometry

Gelatinization is related to starch interactions with water and it is fundamental for starch applications. Different NMR techniques such time-domain ^{1}H, as ^{13}C CP/MAS, ^{17}O, and ^{31}P NMR have been used to interpret the structural changes of starch (Zhu, 2017). 1H NMR relaxometry is a practical tool to analyze proton relaxation in starch gels because signals come from all protons in a sample so that distribution and mobility of protons could be well expressed (Hansen et al., 2009; W. Li et al., 2015). In literature, there are many research related with measuring starch gelatinization by NMR. According to Ritota, Gianferri, Bucci, & Brosio (2008), four water populations were observed in starch-water systems and one of them was related with the bulk of water. The others were related with chemical and diffusive exchanges of water with starch components and there was water uptake during gelatinization process. Another study showed that T_2 relaxation time was related with the nonexchangeable protons in CH of amylose and amylopectin (Rondeau-Mouro et al., 2015).

The changes in T_2 depend on water content and the degree of gelatinization (Cheetham & Tao, 1998). At heat set gelling, starch granules disintegrate during heating process and this leads to decrease T_2 values (Tananuwong & Reid, 2004). On the other hand, at HHP treatment, less broken-intact starch granules cannot interact with the continuous liquid phase intensely so the free portion of the water molecules is increased and this causes to increase the T_2 value.

1.4 Objectives of The Study

The aim of the study is to investigate the effects of different HHP parameters on the solubility, swelling power and *in vitro* digestibility of starch. In addition to this, it is also aimed to interpret the effects of different HHP parameters on physicochemical properties of starch by Nuclear Magnetic Resonance (NMR) Relaxometry.

CHAPTER 2

MATERIALS AND METHODS

2.1 Material

Cornstarch (Kenton, Turkey) was supplied from the local market (Ankara, Turkey). Before HHP treatment, cornstarch slurries (10%, w/v) was prepared with water and equilibrated at room temperature for a day.

2.2 High Hydrostatic Pressure (HHP) Treatment

High Hydrostatic Pressure (HHP) treatment was performed with 760.0118 type pressure equipment supplied by SITEC-Sieber Engineering AG, Zurich, Switzerland (Figure 5). Pressure equipment has 100 ml volume and a heating-cooling system whose internal diameter is 24 mm and length is 153 mm. The rate of pressure increase and pressure release was approximately 5-10 s for the designed system and pressurization time reported in this study exclude the pressure increase and release times. Control group was prepared with cornstarch slurries (10%, w/v) without any heat and pressure treatment. Prepared cornstarch slurries (10%, w/v) were pressured in 25 ml sterile polyethylene cryotubes (Biosigma Sri, CLEARLINE®, CryoGen®Tubes) at two different pressure (400 and 500 MPa) at three different temperature (20,30 and 40 ˚C) for 5,15 and 30 min.

Figure 7. HHP equipment

2.2 Experimental Design

HHP treatment conditions were determined according to primary studies in literature (Hu, Xie, Jin, Xu, & Chen, 2014; Hu et al., 2017; H Liu et al., 2016; Tian, Li, Zhao, Xu, & Jin, 2014). After HHP treatment, samples were lyophilized (LGJ-10, China) for 48 hours to obtain powder form. For each sample, scanning electron microscope (SEM), *in vitro* digestibility, NMR, solubility, and swelling power analysis were done in triplicate. Experimental design is shown at Table 2.

Table 2. Independent Variables

Pressure (MPa)	**Temperature (°C)**	**Time (Min)**
400	20	5
400	30	5
400	40	5
400	20	15
400	30	15
400	40	15
400	20	30
400	30	30
400	40	30
500	20	5
500	30	5
500	40	5
500	20	15
500	30	15
500	40	15
500	20	30
500	30	30
500	40	30

2.4 *in vitro* Digestibility

200 mg cornstarch sample was dissolved into 15 ml phosphate buffer (Sigma, Germany) at pH 5.2 by using seven glass balls having 10 mm diameter. After this, the mixture was equilibrated at 37 ˚C for 5 min. Next, the mixture was hydrolyzed by 5 ml mixed enzyme mixture which included porcine pancreatic α-amylase (Sigma, Germany), 290 U/ml; amyloglucosidase (Sigma, Germany), 15 U/ml where U is defined as the amount of enzyme that liberates 1.0 mg glucose from starch in 1 min at pH 5.2 and in a water bath shaker at 37 ˚C. At time intervals of 20 and 120 min, 500 µl aliquots of the hydrolyzed solution were taken from the sample. Then, samples mixed with 4 ml of absolute ethanol (Sigma, Germany) to deactivate the enzyme and they were centrifuged at 385 *g* for 10 min (Hettich EBA 20, Germany).

The reducing sugar content in the supernatant was measured by 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959). 20 µl of sample mixed with 980 µl of distilled water. Next, 15 ml DNS solution was put into samples and vortex the mixtures. After this, all prepared mixtures was put into a water bath at 90-100˚C until a color change at mixtures was observed. Then, all mixtures were measured at 540 nm by using spectrophotometer (Shimadzu UV-1700, Japan). Absorbance value was converted into reducing sugar concentration by using a glucose standard curve. The percentage of the hydrolyzed sample was calculated by multiplying the reducing sugar content by a conversion factor from glucose to starch of 0.9, which considering the removal of one water molecule per glucose unit.

The percentages of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistance (RS) fractions in starch samples were calculated by using the following formula:

$$\text{RDS (\%)}=\frac{(\text{G20}-\text{FG})}{TS}x0.9x100$$

$$\text{SDS (\%)}=\frac{(\text{G120}-\text{G20})}{TS}x0.9x100$$

$$\text{RDS (\%)}=\frac{(\text{TS}-\text{SDS}-\text{RDS})}{TS}x0.9x100$$

G_{20} and G_{120} denoted the amounts of glucose released at 20 and 120 min hydrolyzation; respectively. Furthermore, FG denoted the amount of free glucose in starch and TS means total starch weight (G. Li & Zhu, 2018).

2.5 Solubility (SI)

At 60,70,80 and 90°C, solubility (SI) was calculated in triplicate. In brief, 200 mg cornstarch sample and 10 ml of distilled water were mixed in 15 ml centrifuge tubes and kept in a water bath for 30 min at the solubility temperature. While samples were in a water bath, the sample-water mixture was vortexed every 5 minutes. After 30 min, the mixture was centrifuged at 2408 g for 15 min and the supernatant was removed and incubated at 110 °C for 8 h. Then, the sample was cooled at room temperature at desiccator. The solubility of the starch was measured according to the following formula (Hang Liu, Guo, et al., 2016):

$$\textbf{Solubility}=\frac{Weight\ of\ dried\ supernatant}{weight\ of\ starch}$$

2.6 Swelling Power (SP)

At 60,70,80 and 90°C, the swelling power (SP) was measured in triplicate. 200 mg cornstarch sample and 10 ml of distilled water were put into 15 ml centrifuge tubes and kept in a water bath for 30 min at swelling power temperature. While samples were in a water bath, the sample-water mixture was vortexed every 5 minutes. After 30 min, the mixture was centrifuged at 2408 g for 15 min and the supernatant was

removed and incubated at 110 ˚C for 8 h. Then, the sample was cooled at room temperature at desiccator. SP of the starch was measured according to following formula:

Swelling Power (SP) = $\frac{W2-W1}{weight\ of\ starch}$

where W_2 means the weight of starch in the tube with precipitate after cooling in a water bath (after decanting supernatant) and W_1 means the weight of the tube with starch sample after cooling desiccator (Liu et al., 2016).

2.7 Nuclear Magnetic Resonance (NMR) Relaxometry Measurements

Spin-spin relaxation time experiments (T_2) were carried out a 0.5 T NMR spectrometer operating at a Larmor frequency of 20.34 MHz, equipped with a 10-mm diameter radio frequency coil (Spin Track SB, Russia) (Figure 6). Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to record relaxation data with 50 ms echo time, 50 echoes, 4 scans and 3s repetition time.

Figure 8. NMR relaxometry

2.8 Scanning Electron Microscopy (SEM)

To interpret the morphological analysis of samples after HHP treatment, scanning electron microscopy (SEM) was used. The analysis was performed with a scanning electron microscope (SEM, Quanta SC7620, England) and samples were coated with a thin layer of Au-Pd (6–11 nm; 10 mA; 40 s) at room temperature before imaging.

2.9 Statistical Analysis

Sigma Plot software package (SigmaPlot Ver.12, Chicago, IL, USA) was used to analyze the results and using p-values less than 0.05 was considered as statistically significant. Three-way ANOVA was used to determine those parameters (pressure, time and temperature) significantly effecting the physicochemical properties of cornstarch. Tukey's multiple range test was implemented to assess significant differences among the experimental mean values ($\alpha < 0.05$).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Solubility (SI) and Swelling Power (SP)

The effect of HHP on the solubility (SI) and swelling power (SP**)** of cornstarch is shown in Fig.7 and Fig.8. According to these figures, the SI and SP of all samples reduced with HHP treatment and the reduction of SI and SP were correlated with pressure, temperature and time parameters. Furthermore, the highest value for the SI and SP of all samples was found at 90 ˚C. Pressure, temperature and time were statistically significant at 60 ˚C, 70 ˚C, 80 ˚C ($p \leq 0.05$). On the other hand, pressure, temperature and time were not statistically significant at 90˚C ($p > 0.05$). The previous studies also showed that the SI and SP of the starch decrease by HHP treatment and this is in agreement with our findings (Kim, Choi, Kim, & Baik, 2010; G. Li & Zhu, 2018; W. Li et al., 2015).

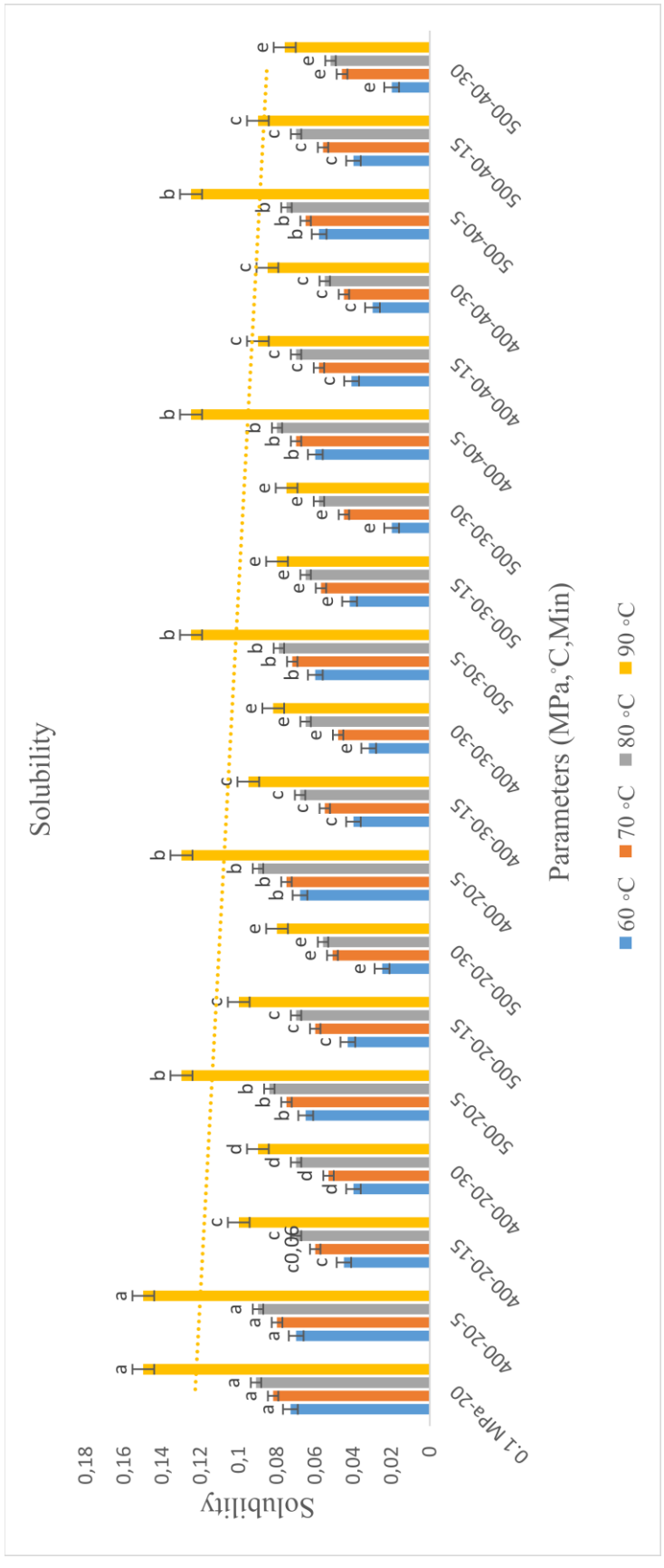

Figure 9. The solubility (SI) of Cornstarch as affected by HHP.

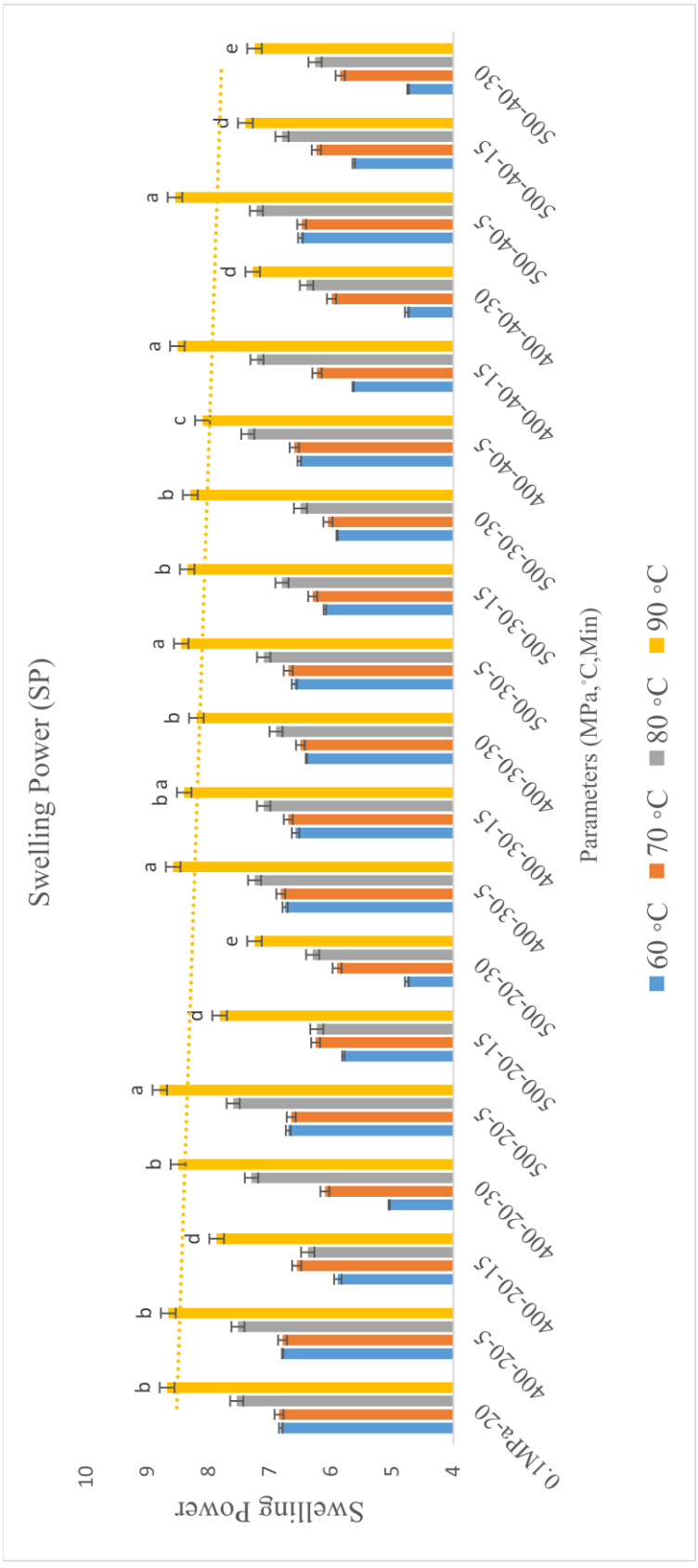

Figure 10. The swelling power (SP) of Cornstarch as affected by HHP.

The SI and SP analysis of the starch show an evidence about structural changes of the starch granules after HHP treatment (Singh & Kaur, 2004) and this analysis is affected by different factors like amylose content, amylose-amylopectin ratio and length of branching (Hoover, 2001). Rearrangement of starch molecules may occur due to HHP treatment and this further inhibits swelling of cornstarch and disintegrated starch granules limited the solubilization of amylose (Oh et al., 2008). Although the reduction of the SI and SP of the starch occurred because of HHP treatment, more studies are required to explain the mechanism of HHP treatment inhibiting SI and SP of starch which is beyond the main aim of this study.

3.2 *in vitro* Digestibility

Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) content of the cornstarch at different HHP treatment were shown in Fig. 9. According to the results, RDS and SDS were raised but RS decreased by HHP treatment. HHP treatment destroyed the structure of starch granules and disrupted the double helix. This caused a decrease in the percentage of RS fraction. Highest RS content was observed at native starch (62.5%). On the other hand, the highest RDS and SDS content (30% and 45% respectively) were observed at 500 MPa-40 ˚C- 30 min. Pressure and temperature were statistically significant ($p \leq 0.05$). However, time was not statistically significant ($p > 0.05$).

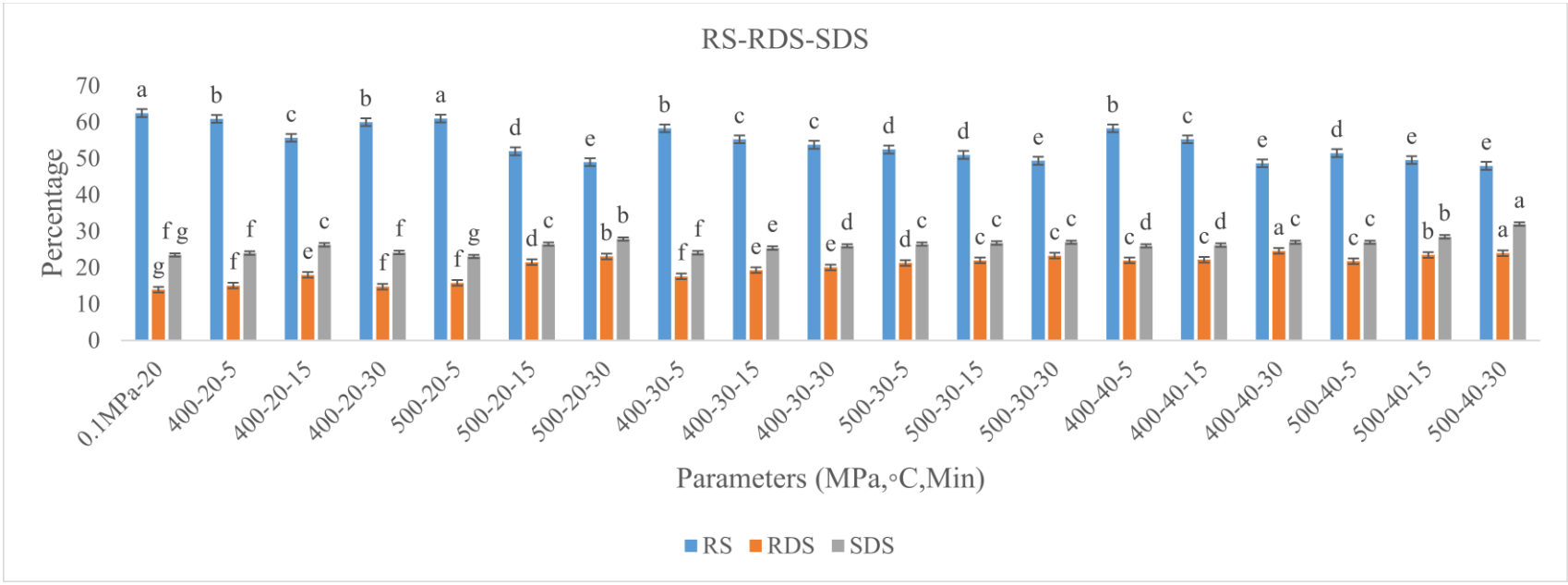

Figure 11. The content of RDS, SDS, and RS fractions in HHP-treated cornstarch samples. 0.1 MPa-20˚C shows native starch. RDS, SDS, RS show rapidly digestible starch, Slowly digestible starch and resistant starch respectively.

The results showed that HHP treatment could change the structure of starch (Hu et al., 2017). Also, a high enough level of pressure is reported to destroy the helical form of the amylopectin chains and these reasons may lead to a decrease in the percentage of RS content in starch and raise the percentage of SDS and RDS content as our pressure range was within these reported limits (Hu et al., 2017; W. Li, Bai, Mousaa, Zhang, & Shen, 2012).

3.3 Nuclear magnetic resonance (NMR) relaxometry

Spin-spin relaxation time experiments (T_2) result at different HHP parameters were shown in Fig. 10. T_2 values by HHP treatment raised with respect to T_2 values of control according to results. Pressure, temperature and time were not statistically significant on T_2 ($p>0.05$) except for the HHP treatment of 500 MPa-40°C-30 min. At 500 MPa-40°C-30 min, T_2 of the samples also increased significantly with respect to other HHP treated samples ($p<0.05$). Despite the T_2 increasing effect of HHP treatment on starch-water systems, several studies indicated a decrease in T_2 values during heat-induced starch gelatinization without HHP treatment (Gonera & Cornillon, 2002; Ozel, Dag, Kilercioglu, Sumnu, & Oztop, 2017; Tananuwong & Reid, 2004). The possible reasons behind this phenomenon are mainly related to the effect of HHP on the crystalline and supramolecular structures (lamellae characteristics, fractal structures etc.) of starch granules (Yang, Gu, et al., 2016). Also, differences in the nature of heat and HHP induced starch gelling also contribute to this reverse T_2 correlation between conventional and HHP methods regarding starch gelatinization (Yang et al., 2017). One of the differences in the shear forces (i.e. stirring) applied during conventional heating of a starch suspension. Stirring provokes granule disintegration which is an absent incident in starch gelatinization by HHP (BeMiller & Huber, 2015).

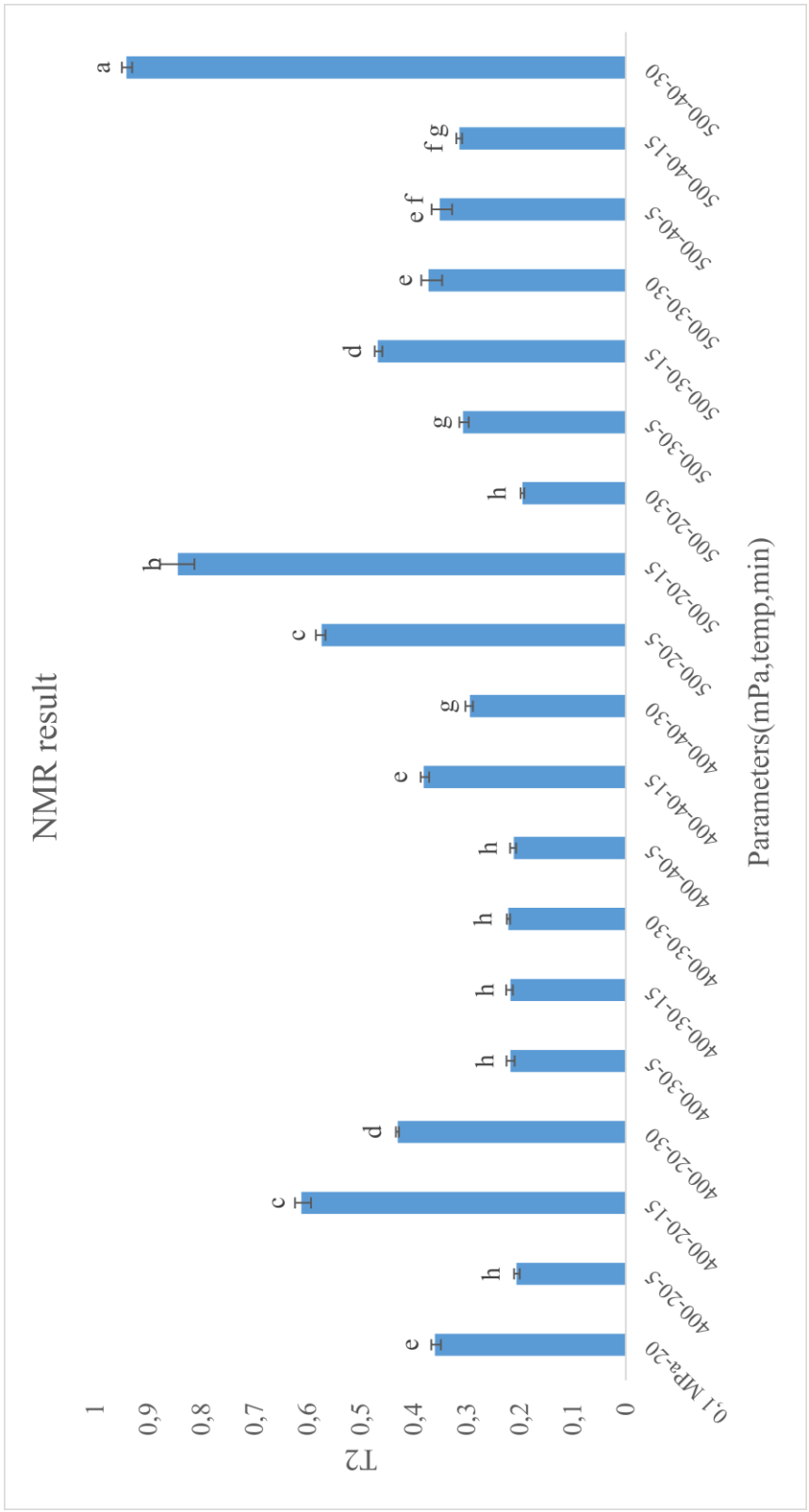

Figure 12. T_2 result of HHP treated cornstarch samples. 0.1 MPa-20˚C shows native starch.

Starch granules include amorphous rings having disordered amylose and amylopectin conformations and semi-crystalline rings having a lamellar structure with alternating crystalline and amorphous regions. The semicrystalline structure of starches plays an important role in starch gelatinization by HHP (Yang, Gu, et al., 2016). HHP is effective both on the lamellar and crystal structures. Firstly, HHP treatment of starch-water slurries can result in a transition from A-type to B-type crystalline structures (Katopo, Song, & Jane, 2002). Native corn starch mainly consists of an A-type crystalline structure (Tananuwong & Reid, 2004). Because of its staggered lattice unit, A-type crystalline structures contain less water molecules per unit cell with respect to B-type crystallines having a more open packing helices inducing a more linear structure. B-type crystalline starches possess a larger amount of interhelical water leading to better hydrogen bonding networks. The helix structure is stabilized by a high number of associated water molecules via van der Waals forces (Yang, Gu, & Hemar, 2013). As a result, B-type crystalline starches are more resistant to pressure. By HHP treatment, the A-type crystalline structure of corn starch granules starts to partially convert into B-type structures (Yang, Swedlund, et al., 2016). The emergence of B-type crystals favors less double helix dissociation contrary to intense double helix dissociation promoted by conventional heat induced starch gelatinization (Figure 11) (Pei-Ling, Xiao-Song, & Qun, 2010). This distinct impact of HHP treatment on the starch crystalline structure during gelatinization causes to less swelling of starch granules due to poor amylose leaching and granules remain intact (Yang et al., 2017). Restricted granule swelling is also induced by the presence of minor amount of lipids within the starch molecules since starch gelatinization under HHP could form amylose-fatty acid complexes (Katopo et al., 2002). During HHP treatment, most of the amylose were retained within the granules so that the formation of these complexes limited the gelatinization of starch granules. These effects of HHP treatment on starch granules supported the decrease in SI and SP of our samples. HHP caused limited granule swelling thus lower SP and SI were enhanced by granule disintegration because of the increased hydrogen bonding capability between the exposed starch amorphous regions and water which could not be achieved by HHP, completely (Yang et al., 2017). Although swelling and solubilization properties of HHP treated starch granules depend on the type of the starch, similar trends for SI and SP of different types of HHP treated starches were also observed by several studies (Guo et al., 2015; W. Li et al., 2012; Oh et al., 2008). In heat set gelling, on the other hand, starch

granules swell and disintegrate. This promotes more interaction between the starch and water molecules resulting in decreasing T_2 values (Tananuwong & Reid, 2004). However, less broken-intact HHP treated starch granules cannot interact with the continuous liquid phase intensely and the free portion of the water molecules increases the T_2 values.

heat

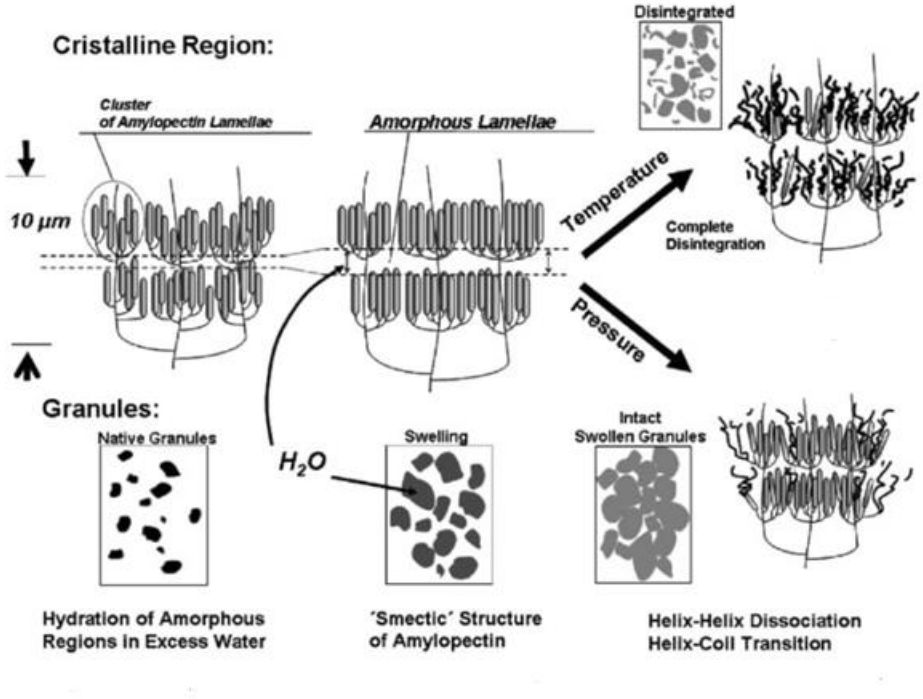

Figure 13: Schematic diagrams of starch granule gelatinization induced by heat treatment or HHP treatment (Pei-Ling et al., 2010).

In our study, at 500 MPa pressure, water was forced into starch granules and increased their degree of hydration (Buckow, Jankowiak, Knorr, & Versteeg, 2009). This was mainly achieved by the effect of HHP on the lamellar and lattice structure of starch. At first, pressure induced compression decreased both the lamellar distance and lattice space but, at the onset of gelatinization, water penetrated into the lamellar blocks. After water migration, both the lamellar distance and lattice space increased (Yang et al., 2017). Diffused water was entrapped within the starch crystalline structure by high pressure and this entrapped water was another reason for the increased T_2 value of starch powders that were obtained from starch-water slurries exposed to 500 MPa-40 °C-30 min HHP treatment. Therefore, we could possibly propose that 30 min HHP

treatment at 500 MPa and 40 ˚C is the onset for cornstarch gelatinization. Shen et al. (2018) reported that HHP treatment on high amylose maize starch under 400 MPa did not change the fractal dimension significantly indicating that the 400 MPa and lower pressures had no significant influence on starch structure. In contrast, they reported a reduction in fractal dimension when the pressure was increased beyond 400 MPa, suggesting a minimum pressure value around 500 MPa for the beginning of starch gelatinization (Shen et al., 2018). Furthermore, complete gelatinization of starch granules under HHP treatment through strong interactions between the amylose and amylopectin chains leading to the formation of cavities and fractures on the granule surface was observed starting from 600 MPa pressure (Hang Liu, Wang, Cao, Fan, & Wang, 2016; Shen et al., 2018). All of these reported findings are in agreement with the proposed cornstarch gelatinization onset claim of this study. The distinct increase in T_2 value for 500 MPa-40 ˚C-30 min HHP treatment revealed that the starch gelatinization under HHP could be monitored by NMR Relaxometry via transverse relaxation parameters.

3.4 Morphological Changes

Morphological changes of cornstarch granules were performed by Scanning Electron Microscopy (SEM). Native cornstarch granules were smooth and had irregularly oval, spherical, and polygonal shapes with no cavities or fissures on their surfaces (Fig. 12A). When compared with native cornstarch, morphological changes started to form slowly at HHP treated samples especially at 500 MPa-40 ˚C-30 min (Fig. 12B,12C, and 12D). These samples' granules collapsed and gained an erythrocyte shape which is the typical granular structure upon pressure gelatinization as reported in literature (Douzals, Perrier Cornet, Gervais, & Coquille, 1998).

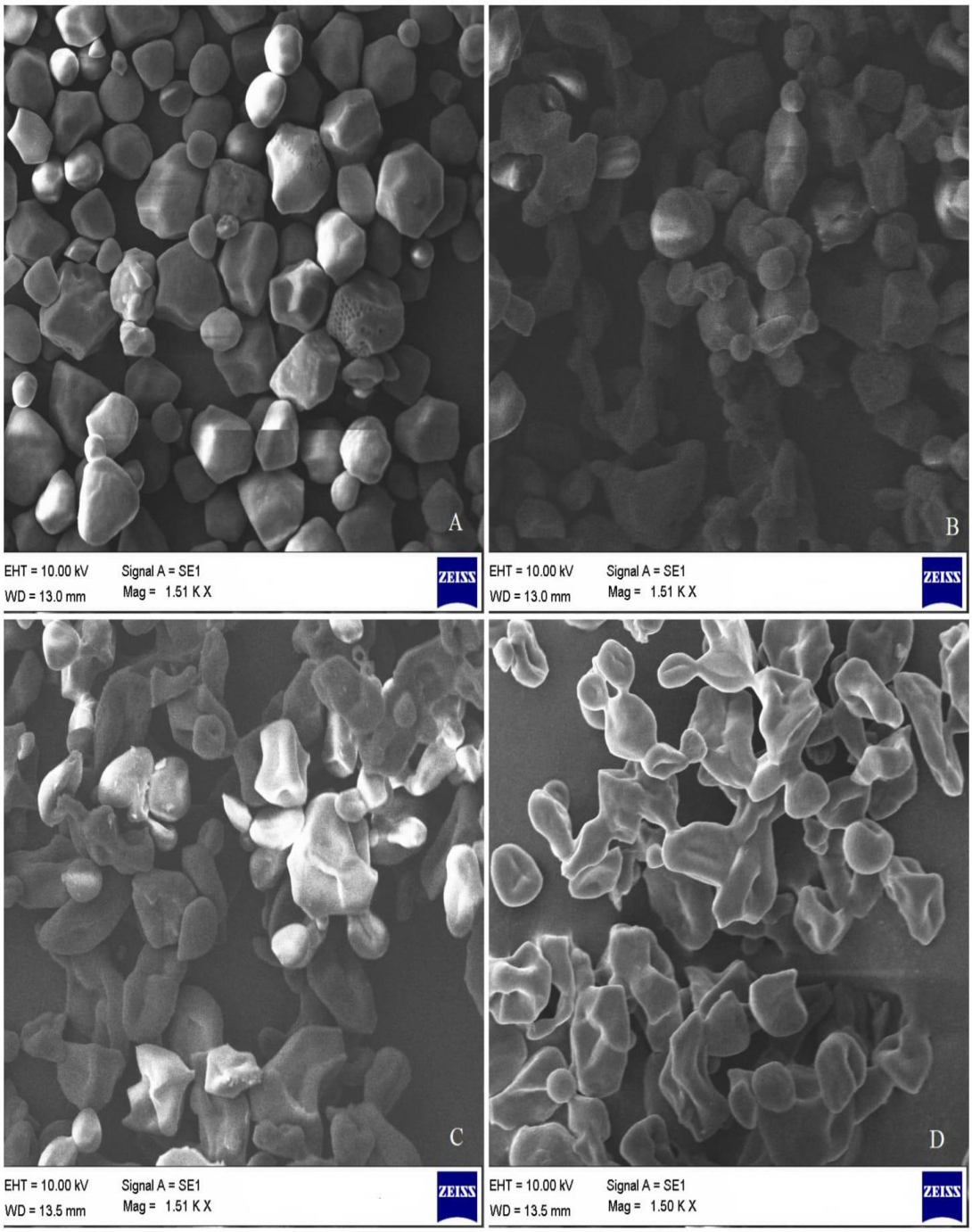

Figure 14. SEM images of HHP-treated cornstarch samples, Panel (A) 0.1 MPa-20˚C (Native Starch) Panel (B) HHP treatment (500 MPa-40˚C-5 min). Panel (C) HHP treatment (500 MPa-40˚C-15 min). Panel (D) HHP treatment (500 MPa-40˚C-30 min)

The results indicated that cornstarch morphological properties was affected by HHP treatment time. HHP treatment caused strong interactions between amylose and amylopectin chains and this is related with a compact structure with cavities, fissures, and holes on the surface (Błaszczak, Valverde, & Fornal, 2005). These observations were also in good agreement with previous studies (Katopo et al., 2002; W. Li et al., 2012).

CHAPTER 4

Conclusion

As far as we know that there is no report on the effect of High Hydrostatic Pressure (HHP) on physicochemical properties of cornstarch by Nuclear Magnetic Resonance (NMR) Relaxometry. HHP treatment caused an increase in SDS and RDS content of cornstarch. On the other hand, RS content of cornstarch decreased by HHP treatment. The SI and SP of cornstarch decreased. According to NMR results, the T_2 values increased by HHP treatment since less broken-intact HHP treated starch granules cannot interact with the continuous liquid phase intensely and the free portion of the water molecules. In addition to these, HHP treatment affected the morphological properties of cornstarch. These results indicated that HHP treatment causes structural changes of cornstarch and this had an effect upon physicochemical properties of cornstarch. Because of starch gelatinization occurs during HHP treatment, HHP treatment can be applied on some food products like pudding without applying any heat treatment during production.

Acknowledgements

This study was funded by Middle East Technical University Research Fund Project [grant number BAP-03-14-2017-001]. In addition, COST Action CA 15209 European Network on Relaxometry is acknowledged as some of the findings are discussed in the action's network meetings and suggestions were taken into consideration in the final text. Finally, a part of the funding also came from Dr. Oztop's award of Science Academy's Young Scientist Awards Program (BAGEP).

References

Alpas, H., Kalchayanand, N., Bozoglu, F., & Ray, B. (2000). Interactions of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. *International Journal of Food Microbiology*, *60*(1), 33–42. https://doi.org/10.1016/S0168-1605(00)00324-X

Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunne, C. P., & Ray, B. (1999). Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. *Applied and Environmental Microbiology*, *65*(9), 4248–51. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=99771&tool=pmcentrez&rendertype=abstract

Alpas, H., Lee, J., Bozoglu, F., & Kaletunç, G. (2003). Evaluation of high hydrostatic pressure sensitivity of Staphylococcus aureus and Escherichia coli O157:H7 by differential scanning calorimetry. *International Journal of Food Microbiology*, *87*(3), 229–237. https://doi.org/10.1016/S0168-1605(03)00066-7

Annison, G., & Topping, D. L. (1994). Nutritional Role of Resistant Starch: Chemical Structure vs Physiological Function. *Annual Review of Nutrition*, *14*(1), 297–320. https://doi.org/10.1146/annurev.nu.14.070194.001501

BeMiller, J. N., & Huber, K. C. (2015). Physical Modification of Food Starch Functionalities. *Annual Review of Food Science and Technology*, *6*(1), 19–69. https://doi.org/10.1146/annurev-food-022814-015552

Błaszczak, W., Valverde, S., & Fornal, J. (2005). Effect of high pressure on the structure of potato starch. *Carbohydrate Polymers*, *59*(3), 377–383. https://doi.org/10.1016/j.carbpol.2004.10.008

Buckow, R., Jankowiak, L., Knorr, D., & Versteeg, C. (2009). Pressure-temperature phase diagrams of maize starches with different amylose contents. *Journal of Agricultural and Food Chemistry*, *57*(24), 11510–11516. https://doi.org/10.1021/jf902246t

Chawla, R., Patil, G. R., & Singh, A. K. (2011). High hydrostatic pressure technology in dairy processing: A review. *Journal of Food Science and Technology*, *48*(3), 260–268. https://doi.org/10.1007/s13197-010-0180-4

Cheetham, N. W. H., & Tao, L. (1998). Solid state NMR studies on the structural and conformational properties of natural maize starches. *Carbohydrate Polymers*, *36*(4), 285–292. https://doi.org/10.1016/S0144-8617(98)00004-6

Cummings, J. H., Beatty, E. R., Kingman, S. M., Bingham, S. A., & Englyst, H. N. (1996). Digestion and physiological properties of resistant starch in the human large bowel. *British Journal of Nutrition*, *75*(05), 733. https://doi.org/10.1079/BJN19960177

Dalai, S. R., & Sahu, J. K. (2010). High hydrostatic pressure (HPP) in food processing: Design aspects and applications. *Assam University Journal of Science & Technology : Physical Sciences and Technology*, *6*(2), 70–87.

Donovan, J. W. (1979). Phase transitions of the starch–water system. *Biopolymers*, *18*(2), 263–275. https://doi.org/10.1002/bip.1979.360180204

Douzals, J. P., Perrier Cornet, J. M., Gervais, P., & Coquille, J. C. (1998). High-Pressure Gelatinization of Wheat Starch and Properties of Pressure-Induced Gels. *Journal of Agricultural and Food Chemistry*, *46*(12), 4824–4829. https://doi.org/10.1021/jf971106p

Durance, T. (2002). *Handbook of Food Preservation*. *Food Research International* (Vol. 35). https://doi.org/10.1016/S0963-9969(00)00143-5

Elamin, W. M., Endan, J. B., Yosuf, Y. A., Shamsudin, R., & Ahmedov, A. (2015). High Pressure Processing Technology and Equipment Evolution: A review. *Journal of Engineering Science and Technology Review*, *8*(5), 75–83.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46 Suppl 2*(July), S33-50. https://doi.org/10.1111/j.1750-3841.2010.01627.x

Flanagan, B. M., Gidley, M. J., & Warren, F. J. (2015). Rapid quantification of starch molecular order through multivariate modelling of ^{13} C CP/MAS NMR spectra. *Chemical Communications*, *51*(80), 14856–14858. https://doi.org/10.1039/C5CC06144J

Gidley, M. J. (2014). Starch NMR. *Starch Polymers*, 243–253. https://doi.org/10.1016/B978-0-444-53730-0.00029-4

Gonera, A., & Cornillon, P. (2002). Gelatinization of Starch / Gum / Sugar Systems. *Starch/Stärke*, *54*, 508–516.

Grumezescu, A. M., & Holban, A. M. (2018). *Food Processing For Increased Quality and Consumption* (18th ed.). Elsevier.

Guo, Z., Zeng, S., Lu, X., Zhou, M., Zheng, M., & Zheng, B. (2015). Structural and physicochemical properties of lotus seed starch treated with ultra-high pressure. *Food Chemistry*, *186*, 223–230. https://doi.org/10.1016/j.foodchem.2015.03.069

Hansen, M. R., Blennow, A., Farhat, I., Nørgaard, L., Pedersen, S., & Engelsen, S. B. (2009). Comparative NMR relaxometry of gels of amylomaltase-modified starch and gelatin. *Food Hydrocolloids*, *23*(8), 2038–2048. https://doi.org/10.1016/j.foodhyd.2009.05.008

Hite, B. H. (1899). The effects of pressure in the preservation of milk. *Bulletin of the West Virginia University Agricultural Experimental Station Morgantown*, (58), 15–35.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, *45*(3), 253–267. https://doi.org/10.1016/S0144-8617(00)00260-5

Hu, X. P., Xie, Y. Y., Jin, Z. Y., Xu, X. M., & Chen, H. Q. (2014). Effect of single-, dual-, and triple-retrogradation treatments on in vitro digestibility and structural characteristics of waxy wheat starch. *Food Chemistry*, *157*, 373–379. https://doi.org/10.1016/j.foodchem.2014.02.065

Hu, X. P., Zhang, B., Jin, Z. Y., Xu, X. M., & Chen, H. Q. (2017). Effect of high hydrostatic pressure and retrogradation treatments on structural and physicochemical properties of waxy wheat starch. *Food Chemistry*, *232*, 560–565. https://doi.org/10.1016/j.foodchem.2017.04.040

j. doona, C., Kustin, K., & Feeherry, F. (2010). *Case Studies in Novel Food Processing Technologies*. *Woodhead Publishing Limited*. https://doi.org/10.1017/CBO9781107415324.004

Jenkins, D. J. A., Kendall, C. W. C., Augustin, L. S. A., Franceschi, S., Hamidi, M., & Marchie, A. (2002). Glycemic index : overview of implications in health and disease 1 – 4, *76*(May), 266–273.

Jobling, S. (2004). Improving starch for food and industrial applications. *Current Opinion in Plant Biology*, *7*(2), 210–218. https://doi.org/10.1016/j.pbi.2003.12.001

Katopo, H., Song, Y., & Jane, J. L. (2002). Effect and mechanism of ultrahigh hydrostatic pressure on the structure and properties of starches. *Carbohydr. Polym.*, *47*, 233–244.

Khan, M. K., Ahmad, K., Hassan, S., Imran, M., Ahmad, N., & Xu, C. (2018). Effect of novel technologies on polyphenols during food processing. *Innovative Food Science and Emerging Technologies*, *45*(December 2017), 361–381. https://doi.org/10.1016/j.ifset.2017.12.006

Kim, H. S., Choi, H. S., Kim, B. Y., & Baik, M. Y. (2010). Characterization of acetylated corn starch prepared under ultrahigh pressure (UHP). *Journal of Agricultural and Food Chemistry*, *58*(6), 3573–3579. https://doi.org/10.1021/jf903939y

Kim, H. S., Kim, B. Y., & Baik, M. Y. (2012). Application of ultra high pressure (UHP) in starch chemistry. *Critical Reviews in Food Science and Nutrition*, *52*(2), 123–141. https://doi.org/10.1080/10408398.2010.498065

Kirtil, E., & Oztop, M. H. (2016). 1H Nuclear Magnetic Resonance Relaxometry and Magnetic Resonance Imaging and Applications in Food Science and Processing. *Food Engineering Reviews*, *8*(1), 1–22. https://doi.org/10.1007/s12393-015-9118-y

Levine, H., & Slade, L. (1990). Influences of the glassy and rubbery states on the thermal, mechanical, and structural properties of doughs amd baked products. *Dough Rheology and Baked Product Texture*, 157–330. https://doi.org/10.1007/978-1-4613-0861-4_5

Li, G., & Zhu, F. (2018). Effect of high pressure on rheological and thermal properties of quinoa and maize starches. *Food Chemistry*, *241*(August 2017), 380–386. https://doi.org/10.1016/j.foodchem.2017.08.088

Li, W., Bai, Y., Mousaa, S. A. S., Zhang, Q., & Shen, Q. (2012). Effect of High Hydrostatic Pressure on Physicochemical and Structural Properties of Rice Starch. *Food and Bioprocess Technology*, *5*(6), 2233–2241. https://doi.org/10.1007/s11947-011-0542-6

Li, W., Tian, X., Liu, L., Wang, P., Wu, G., Zheng, J., … Zhang, G. (2015). High pressure induced gelatinization of red adzuki bean starch and its effects on starch physicochemical and structural properties. *Food Hydrocolloids*, *45*, 132–139. https://doi.org/10.1016/j.foodhyd.2014.11.013

Liu, H., Guo, X., Li, Y., Li, H., Fan, H., & Wang, M. (2016). In vitro digestibility and changes in physicochemical and textural properties of tartary buckwheat starch under high hydrostatic pressure. *Journal of Food Engineering*, *189*, 64–71. https://doi.org/10.1016/j.jfoodeng.2016.05.015

Liu, H., Guo, X., Li, Y., Li, H., Fan, H., & Wang, M. (2016). In vitro digestibility and changes in physicochemical and textural properties of tartary buckwheat starch under high hydrostatic pressure. *Journal of Food Engineering*, *189*, 64–71. https://doi.org/10.1016/j.jfoodeng.2016.05.015

Liu, H., Wang, L., Cao, R., Fan, H., & Wang, M. (2016). In vitro digestibility and changes in physicochemical and structural properties of common buckwheat starch affected by high hydrostatic pressure. *Carbohydrate Polymers*, *144*, 1–8. https://doi.org/10.1016/j.carbpol.2016.02.028

Lund, D. (1984). Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization. *C R C Critical Reviews in Food Science and Nutrition*, *20*(4), 249–273. https://doi.org/10.1080/10408398409527391

Luo, X., Li, J., & Lin, X. (2012). Effect of gelatinization and additives on morphology and thermal behavior of corn starch/PVA blend films. *Carbohydrate Polymers*, *90*(4), 1595–1600. https://doi.org/10.1016/J.CARBPOL.2012.07.036

Marcone, M. F., Wang, S., Albabish, W., Nie, S., Somnarain, D., & Hill, A. (2013). Diverse food-based applications of nuclear magnetic resonance (NMR) technology. *Food Research International*, *51*(2), 729–747. https://doi.org/10.1016/J.FOODRES.2012.12.046

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/ac60147a030

Oh, H. E., Pinder, D. N., Hemar, Y., Anema, S. G., & Wong, M. (2008). Effect of high-pressure treatment on various starch-in-water suspensions. *Food Hydrocolloids*, *22*(1), 150–155. https://doi.org/10.1016/J.FOODHYD.2007.01.028

Ozel, B., Dag, D., Kilercioglu, M., Sumnu, S. G., & Oztop, M. H. (2017). NMR relaxometry as a tool to understand the effect of microwave heating on starch-water interactions and gelatinization behavior. *LWT - Food Science and Technology*, *83*, 10–17. https://doi.org/10.1016/j.lwt.2017.04.077

Parker, R., & Ring, S. G. (2001). Aspects of the Physical Chemistry of Starch. *Journal of Cereal Science*, *34*(1), 1–17. https://doi.org/10.1006/JCRS.2000.0402

Pei-Ling, L., Xiao-Song, H., & Qun, S. (2010). Effect of high hydrostatic pressure on starches: A review. *Starch - Stärke*, *62*(12), 615–628. https://doi.org/10.1002/star.201000001

Pencek, R. R., Koyama, Y., Lacy, D. B., James, F. D., Fueger, P. T., Jabbour, K., … Wasserman, D. H. (2002). Transporter-mediated absorption is the primary route of entry and is required for passive absorption of intestinal glucose into the blood of conscious dogs. *The Journal of Nutrition*, *132*(7), 1929–34. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12097672

Rendueles, E., Omer, M. K., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT - Food Science and Technology*, *44*(5), 1251–1260. https://doi.org/10.1016/j.lwt.2010.11.001

Ritota, M., Gianferri, R., Bucci, R., & Brosio, E. (2008). Proton NMR relaxation study of swelling and gelatinisation process in rice starch-water samples. *Food Chemistry*, *110*(1), 14–22. https://doi.org/10.1016/j.foodchem.2008.01.048

Rondeau-Mouro, C., Cambert, M., Kovrlija, R., Musse, M., Lucas, T., & Mariette, F. (2015). Temperature-Associated Proton Dynamics in Wheat Starch-Based Model Systems and Wheat Flour Dough Evaluated by NMR. *Food and Bioprocess Technology*, *8*(4), 777–790. https://doi.org/10.1007/s11947-014-1445-0

Shen, X., Shang, W., Strappe, P., Chen, L., Li, X., Zhou, Z., & Blanchard, C. (2018). Manipulation of the internal structure of high amylose maize starch by high pressure treatment and its diverse influence on digestion. *Food Hydrocolloids*, *77*, 40–48. https://doi.org/10.1016/j.foodhyd.2017.09.015

Singh, N., & Kaur, L. (2004). Morphological, thermal, rheological and retrogradation properties of potato starch fractions varying in granule size. *Journal of the Science of Food and Agriculture*, *84*(10), 1241–1252. https://doi.org/10.1002/jsfa.1746

Singh, N., Singh, J., Kaur, L., Singh Sodhi, N., & Singh Gill, B. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry*, *81*(2), 219–231. https://doi.org/10.1016/S0308-8146(02)00416-8

Spyros, A., & Dais, P. (2012). *NMR spectroscopy in food analysis*. *RSC Food Analysis Monographs*. https://doi.org/10.1016/j.pnmrs.2008.09.002

Tananuwong, K., & Reid, D. S. (2004). DSC and NMR relaxation studies of starch-water interactions during gelatinization. *Carbohydrate Polymers*, *58*(3), 345–358. https://doi.org/10.1016/j.carbpol.2004.08.003

Tian, Y., Li, D., Zhao, J., Xu, X., & Jin, Z. (2014). Effect of high hydrostatic pressure (HHP) on slowly digestible properties of rice starches. *Food Chemistry*, *152*, 225–229. https://doi.org/10.1016/J.FOODCHEM.2013.11.162

Yang, Z., Chaib, S., Gu, Q., & Hemar, Y. (2017). Impact of pressure on physicochemical properties of starch dispersions. *Food Hydrocolloids*, *68*, 164–177. https://doi.org/10.1016/J.FOODHYD.2016.08.032

Yang, Z., Gu, Q., & Hemar, Y. (2013). In situ study of maize starch gelatinization under ultra-high hydrostatic pressure using X-ray diffraction. *Carbohydrate Polymers*, *97*(1), 235–238. https://doi.org/10.1016/j.carbpol.2013.04.075

Yang, Z., Gu, Q., Lam, E., Tian, F., Chaieb, S., & Hemar, Y. (2016). In situ study starch gelatinization under ultra-high hydrostatic pressure using synchrotron SAXS. *Food Hydrocolloids*, *56*, 58–61. https://doi.org/10.1016/j.foodhyd.2015.12.007

Yang, Z., Swedlund, P., Hemar, Y., Mo, G., Wei, Y., Li, Z., & Wu, Z. (2016). Effect of high hydrostatic pressure on the supramolecular structure of corn starch with different amylose contents. *International Journal of Biological Macromolecules*, *85*, 604–614. https://doi.org/10.1016/J.IJBIOMAC.2016.01.018

Zhang, G., & Hamaker, B. R. (2009). Slowly digestible starch: Concept, mechanism, and proposed extended glycemic index. *Critical Reviews in Food Science and Nutrition*, *49*(10), 852–867. https://doi.org/10.1080/10408390903372466

Zhu, F. (2017). NMR spectroscopy of starch systems. *Food Hydrocolloids*, *63*, 611–624. https://doi.org/10.1016/j.foodhyd.2016.10.015

APPENDICES

APPENDIX A. ANOVA Results of General Full Factorial Regressions

General Full Factorial Regressions: Solubility of Cornstarch (60°C);Pressure,Temperature,Time

Three Way Analysis of Variance

Data source: Data 1 in Notebook1

Balanced Design (No Interactions)

Dependent Variable: Col 4

Normality Test (Shapiro-Wilk) Passed (P = 0.356)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure	1	0.000156	0.000156	48.431	0.002
Temperature	2	0.000131	0.0000657	20.397	0.008
Time	2	0.00388	0.00194	601.310	<0.001
Residual	4	0.0000129	0.00000322		
Total	17	0.00431	0.000254		

The difference in the mean values among the different levels of Pressure are greater than would be expected by chance after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.002). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature are greater than would be expected by chance after allowing for the effects of differences

in Pressure and Time. There is a statistically significant difference (P = 0.008). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Time are greater than would be expected by chance after allowing for the effects of differences in Pressure and Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
400.000 vs. 500.000	0.00589		29.842	0.002	Yes

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
20.000 vs. 40.000	0.00650		38.870	0.007	Yes
20.000 vs. 30.000	0.00433		35.913	0.030	Yes
30.000 vs. 40.000	0.00217		32.957	0.207	No

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
5.000 vs. 30.000	0.0357		348.670	<0.001	Yes
5.000 vs. 15.000	0.0217		329.566	<0.001	Yes
15.000 vs. 30.000	0.0140		319.104	<0.001	Yes

Power of performed test with alpha = 0.0500: for Pressure : 0.999

Power of performed test with alpha = 0.0500: for Temperature : 0.955

Power of performed test with alpha = 0.0500: for Time : 1.000

Least square means for Pressure :

Group Mean

400.0000.0473

500.0000.0414

Std Err of LS Mean = 0.000598

Least square means for Temperature :

Group Mean

20.0000.0480

30.0000.0437

40.0000.0415

Std Err of LS Mean = 0.000733

Least square means for Time :

Group Mean

5.0000.0635

15.0000.0418

30.0000.0278

Std Err of LS Mean = 0.000733

General Full Factorial Regressions: Solubility of Cornstarch (70°C);Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in 70-solubility.JNB

Balanced Design (No Interactions)

Dependent Variable: Col 4

Normality Test (Shapiro-Wilk) Passed (P = 0.520)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure	1	0.0000161	0.0000161	7.225	0.050
Temperature	2	0.000133	0.0000665	29.925	0.004
Time	2	0.00188	0.000940	423.075	<0.001
Residual	4	0.00000889	0.00000222		
Total	17	0.00209	0.000123		

The difference in the mean values among the different levels of Pressure are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.050).

The difference in the mean values among the different levels of Temperature are greater than would be expected by chance after allowing for the effects of differences in Pressure and Time. There is a statistically significant difference (P = 0.004). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Time are greater than would be expected by chance after allowing for the effects of differences in Pressure and Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
20.000 vs. 40.000	0.00650		310.681	0.004	Yes
20.000 vs. 30.000	0.00450		37.394	0.014	Yes
30.000 vs. 40.000	0.00200		33.286	0.163	No

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
5.000 vs. 30.000	0.0248		340.805	<0.001	Yes
5.000 vs. 15.000	0.0152		324.921	<0.001	Yes
15.000 vs. 30.000	0.00967		315.884	<0.001	Yes

Power of performed test with alpha = 0.0500: for Pressure : 0.474

Power of performed test with alpha = 0.0500: for Temperature : 0.993

Power of performed test with alpha = 0.0500: for Time : 1.000

Least square means for Pressure :

Group	**Mean**
400.000	0.0604
500.000	0.0586

Std Err of LS Mean = 0.000497

Least square means for Temperature :

Group Mean

20.0000.0632

30.0000.0587

40.0000.0567

Std Err of LS Mean = 0.000609

Least square means for Time :

Group Mean

5.0000.0728

15.0000.0577

30.0000.0480

Std Err of LS Mean = 0.000609

General Full Factorial Regressions: Solubility of Cornstarch (80°C);Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in 80-solubility.JNB

Balanced Design (No Interactions)

Dependent Variable: Col 4

Normality Test (Shapiro-Wilk) Passed (P = 0.437)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure	1	0.000133	0.000133	19.843	0.011
Temperature	2	0.000122	0.0000611	9.083	0.033
Time	2	0.00170	0.000851	126.603	<0.001
Residual	4	0.0000269	0.00000672		
Total	17	0.00214	0.000126		

The difference in the mean values among the different levels of Pressure are greater than would be expected by chance after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.011). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature are greater than would be expected by chance after allowing for the effects of differences in Pressure and Time. There is a statistically significant difference (P = 0.033). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Time are greater than would be expected by chance after allowing for the effects of differences in Pressure and Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
400.000 vs. 500.000	0.00544		26.300	0.011	Yes

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
20.000 vs. 40.000	0.00633		35.983	0.029	Yes
20.000 vs. 30.000	0.00250		32.362	0.321	No
30.000 vs. 40.000	0.00383		33.622	0.128	No

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
5.000 vs. 30.000	0.0237		322.359	<0.001	Yes
5.000 vs. 15.000	0.0142		313.384	0.002	Yes
15.000 vs. 30.000	0.00950		38.975	0.007	Yes

Power of performed test with alpha = 0.0500: for Pressure : 0.899

Power of performed test with alpha = 0.0500: for Temperature : 0.666

Power of performed test with alpha = 0.0500: for Time : 1.000

Least square means for Pressure :

Group	**Mean**
400.000	0.0731

500.0000.0677

Std Err of LS Mean = 0.000864

Least square means for Temperature :

Group Mean

20.0000.0733

30.0000.0708

40.0000.0670

Std Err of LS Mean = 0.00106

Least square means for Time :

Group Mean

5.0000.0830

15.0000.0688

30.0000.0593

Std Err of LS Mean = 0.00106

General Full Factorial Regressions: Solubility of Cornstarch (90°C);Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in 80-solubility.JNB

Balanced Design (No Interactions)

Dependent Variable: Col 4

Normality Test (Shapiro-Wilk) Passed (P = 0.841)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure	1	0.000242	0.000242	6.785	0.060
Temperature	2	0.000415	0.000207	5.815	0.046
Time	2	0.00809	0.00404	113.394	0.066
Residual	4	0.000143	0.0000357		
Total	17	0.00901	0.000530		

The difference in the mean values among the different levels of Pressure are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Temperature and Time. There is not a statistically significant difference (P = 0.060).

The difference in the mean values among the different levels of Temperature are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Pressure and Time. There is a statistically significant difference (P = 0.046).

The difference in the mean values among the different levels of Time are greater than would be expected by chance after allowing for the effects of differences in Pressure and Temperature. There is a not statistically significant difference (P = 0.066). To isolate which group(s) differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
5.000 vs. 30.000	0.0495		320.303	<0.001	Yes
5.000 vs. 15.000	0.0383		315.722	0.001	Yes
15.000 vs. 30.000	0.0112		34.580	0.067	No

Power of performed test with alpha = 0.0500: for Pressure : 0.447
Power of performed test with alpha = 0.0500: for Temperature : 0.453
Power of performed test with alpha = 0.0500: for Time : 1.000

Least square means for Pressure :

Group	**Mean**
400.000	0.105
500.000	0.0979

Std Err of LS Mean = 0.00199

Least square means for Temperature :

Group	**Mean**
20.000	0.108
30.000	0.0978
40.000	0.0985

Std Err of LS Mean = 0.00244

Least square means for Time :

Group	**Mean**

5.0000.131

15.0000.0925

30.0000.0813

Std Err of LS Mean = 0.00244

General Full Factorial Regressions: The Swelling Power of Cornstarch (60°C);Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in 60-Swelling.JNB

Balanced Design (No Interactions)

Dependent Variable: 70

Normality Test (Shapiro-Wilk) Passed (P = 0.498)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure		10.194	0.194	25.712	0.007
Temperature		20.235	0.118	15.568	0.013
Time		21.111	0.555	73.501	<0.001
Residual		40.030	20.00756		
Total		171.660	0.0976		

The difference in the mean values among the different levels of Pressure are greater than would be expected by chance after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.007). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature are greater than would be expected by chance after allowing for the effects of differences in Pressure and Time. There is a statistically significant difference (P = 0.013). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Time are greater than would be expected by chance after allowing for the effects of differences in Pressure

and Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
400.000 vs. 500.000	0.183	4.772	0.009	Yes

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
30.000 vs. 40.000	0.757	16.082	<0.001	Yes
30.000 vs. 20.000	0.557	11.831	<0.001	Yes
20.000 vs. 40.000	0.200	4.251	0.013	Yes

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
5.000 vs. 30.000	1.375	29.223	<0.001	Yes
5.000 vs. 15.000	0.703	14.948	<0.001	Yes
15.000 vs. 30.000	0.672	14.275	<0.001	Yes

Power of performed test with alpha = 0.0500: for Pressure : 0.934

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Power of performed test with alpha = 0.0500: for Time : 1.000

Least square means for Pressure :

Group	**Mean**
400.000	6.042
500.000	5.859

Std Err of LS Mean = 0.0272

Least square means for Temperature :

Group Mean

20.0005.832

30.0006.388

40.0005.632

Std Err of LS Mean = 0.0333

Least square means for Time :

Group Mean

5.0006.643

15.0005.940

30.0005.268

Std Err of LS Mean = 0.0333

General Full Factorial Regressions: The Swelling Power of Cornstarch (70°C);Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in 70-Swelling.JNB

Balanced Design (No Interactions)

Dependent Variable: 60

Normality Test (Shapiro-Wilk) Passed (P = 0.770)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure	1	0.151	0.151	22.773	0.009
Temperature	1	0.922	0.922	138.884	<0.001
Time	1	2.836	2.836	427.067	<0.001
Residual	4	0.0266	0.00664		
Total	7	0.913	0.524		

The difference in the mean values among the different levels of Pressure are greater than would be expected by chance after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.009). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature are greater than would be expected by chance after allowing for the effects of differences in Pressure and Time. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Time are greater than would be expected by chance after allowing for the effects of differences in Pressure and Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.
All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
400.000 vs. 500.000	0.183		26.749	0.009	Yes

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
30.000 vs. 40.000	0.757		322.743	<0.001	Yes
30.000 vs. 20.000	0.557		316.731	<0.001	Yes
20.000 vs. 40.000	0.200		36.011	0.029	Yes

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
5.000 vs. 30.000	1.375		341.328	<0.001	Yes
5.000 vs. 15.000	0.703		321.140	<0.001	Yes
15.000 vs. 30.000	0.672		320.188	<0.001	Yes

Power of performed test with alpha = 0.0500: for Pressure : 0.934
Power of performed test with alpha = 0.0500: for Temperature : 1.000
Power of performed test with alpha = 0.0500: for Time : 1.000

Least square means for Pressure :

Group	**Mean**
400.000	6.042
500.000	5.859

Std Err of LS Mean = 0.0272

Least square means for Temperature :

Group Mean

20.0005.832

30.0006.388

40.0005.632

Std Err of LS Mean = 0.0333

Least square means for Time :

Group Mean

5.0006.643

15.0005.940

30.0005.268

Std Err of LS Mean = 0.0333

General Full Factorial Regressions: The Swelling Power of Cornstarch (80°C);Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in Notebook1

Balanced Design (No Interactions)

Dependent Variable: 60

Normality Test (Shapiro-Wilk) Passed (P = 0.770)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	0.151	0.151	22.773	0.009
Temperature	1	0.922	0.922	138.884	<0.001
Time	1	2.836	2.836	427.067	<0.001
Residual	4	0.0266	0.00664		
Total	7	0.913	0.524		

The difference in the mean values among the different levels of Pressure are greater than would be expected by chance after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.009). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature are greater than would be expected by chance after allowing for the effects of differences in Pressure and Time. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Time are greater than would be expected by chance after allowing for the effects of differences in Pressure

and Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
5.000 vs. 30.000	0.732	37.706	0.012		Yes
5.000 vs. 15.000	0.588	36.197	0.026		Yes
15.000 vs. 30.000	0.143	31.510	0.580		No

Power of performed test with alpha = 0.0500: for Pressure : 0.460

Power of performed test with alpha = 0.0500: for Temperature : 0.0514

Power of performed test with alpha = 0.0500: for Time : 0.911

Least square means for Pressure :

Group	**Mean**
400.000	7.047
500.000	6.757

Std Err of LS Mean = 0.0775

Least square means for Temperature :

Group	**Mean**
20.000	6.888
30.000	6.942
40.000	6.875

Std Err of LS Mean = 0.0949

Least square means for Time :

Group Mean

5.0007.342

15.0006.753

30.0006.610

Std Err of LS Mean = 0.0949

General Full Factorial Regressions: The Swelling Power of Cornstarch (90°C);Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in 90-Swelling.JNB

Balanced Design (No Interactions)

Dependent Variable: 90

Normality Test (Shapiro-Wilk) Passed (P = 0.114)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure	10.207	0.207	0.904	0.396	
Temperature	20.853	0.427	1.863	0.268	
Time	21.625	0.813	3.550	0.130	
Residual	40.916	0.229			
Total	174.616	0.272			

The difference in the mean values among the different levels of Pressure are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Temperature and Time. There is not a statistically significant difference (P = 0.396).

The difference in the mean values among the different levels of Temperature are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Pressure and Time. There is not a statistically significant difference (P = 0.268).

The difference in the mean values among the different levels of Time are not great enough to exclude the possibility that the difference is just due to random sampling

variability after allowing for the effects of differences in Pressure and Temperature. There is not a statistically significant difference (P = 0.130).

Power of performed test with alpha = 0.0500: for Pressure : 0.0503
Power of performed test with alpha = 0.0500: for Temperature : 0.120
Power of performed test with alpha = 0.0500: for Time : 0.266

Least square means for Pressure :

Group Mean

400.0008.233

500.0008.019

Std Err of LS Mean = 0.159

Least square means for Temperature :

Group Mean

20.0008.150

30.0008.380

40.0007.848

Std Err of LS Mean = 0.195

Least square means for Time :

Group Mean

5.0008.523

15.0008.058

30.0007.797

Std Err of LS Mean = 0.195

General Full Factorial Regressions:Spin-spin relaxation time experiments (T2) result;Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in Notebook1

Balanced Design (No Interactions)

Dependent Variable: Col 7

Normality Test (Shapiro-Wilk) Passed (P = 0.168)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	10.138	0.138	2.308	0.203	
Temperature	20.0960	0.0480	0.805	0.509	
Time	20.0796	0.0398	0.667	0.562	
Residual	40.239	0.0597			
Total	170.815	0.0480			

The difference in the mean values among the different levels of Pressure are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Temperature and Time. There is not a statistically significant difference (P = 0.203).

The difference in the mean values among the different levels of Temperature are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Pressure and Time. There is not a statistically significant difference (P = 0.509).

The difference in the mean values among the different levels of Time are not great enough to exclude the possibility that the difference is just due to random sampling

variability after allowing for the effects of differences in Pressure and Temperature. There is not a statistically significant difference (P = 0.562).

Power of performed test with alpha = 0.0500: for Pressure : 0.142
Power of performed test with alpha = 0.0500: for Temperature : 0.0514
Power of performed test with alpha = 0.0500: for Time : 0.0514

Least square means for Pressure :

Group Mean

400.0000.310

500.0000.485

Std Err of LS Mean = 0.0814

Least square means for Temperature :

Group Mean

20.0000.477

30.0000.301

40.0000.415

Std Err of LS Mean = 0.0997

Least square means for Time :

Group Mean

5.0000.311

15.0000.473

30.0000.409

Std Err of LS Mean = 0.0997

General Full Factorial Regressions: RDS; Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in RS.JNB

Balanced Design (No Interactions)

Dependent Variable: SDS

Normality Test (Shapiro-Wilk) Passed (P = 0.972)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure	1	28.376	28.376	9.239	0.038
Temperature	2	74.021	37.011	12.050	0.020
Time	2	24.648	12.324	4.012	0.111
Residual	4	12.286	3.071		
Total	17	163.324	9.607		

The difference in the mean values among the different levels of Pressure are greater than would be expected by chance after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.038). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature are greater than would be expected by chance after allowing for the effects of differences in Pressure and Time. There is a statistically significant difference (P = 0.020). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Time are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Pressure and Temperature. There is not a statistically significant difference (P = 0.111).

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
500.000 vs. 400.000	2.511		24.299	0.039	Yes

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
40.000 vs. 20.000	4.967		36.942	0.018	Yes
40.000 vs. 30.000	2.417		33.378	0.152	No
30.000 vs. 20.000	2.550		33.564	0.133	No

Power of performed test with alpha = 0.0500: for Pressure : 0.584
Power of performed test with alpha = 0.0500: for Temperature : 0.796
Power of performed test with alpha = 0.0500: for Time : 0.305

Least square means for Pressure :

Group	**Mean**
400.000	19.300
500.000	21.811

Std Err of LS Mean = 0.584

Least square means for Temperature :

Group	**Mean**
20.000	18.050
30.000	20.600

40.00023.017

Std Err of LS Mean = 0.715

Least square means for Time :

Group Mean

5.00018.933

15.00021.083

30.00021.650

Std Err of LS Mean = 0.715

General Full Factorial Regressions: SDS;Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in rds.JNB

Balanced Design (No Interactions)

Dependent Variable: SDS

Normality Test (Shapiro-Wilk) Passed (P = 0.960)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure		114.401	14.401	10.294	0.033
Temperature		219.408	9.704	6.937	0.050
Time		215.551	7.776	5.558	0.070
Residual		4 5.596	1.399		
Total		1767.403	3.965		

The difference in the mean values among the different levels of Pressure are greater than would be expected by chance after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.033). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Pressure and Time. There is not a statistically significant difference (P = 0.050).

The difference in the mean values among the different levels of Time are not great enough to exclude the possibility that the difference is just due to random sampling

variability after allowing for the effects of differences in Pressure and Temperature. There is not a statistically significant difference (P = 0.070).

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
500.000 vs. 400.000	1.789		24.537	0.033	Yes

Power of performed test with alpha = 0.0500: for Pressure : 0.634

Power of performed test with alpha = 0.0500: for Temperature : 0.535

Power of performed test with alpha = 0.0500: for Time : 0.433

Least square means for Pressure :

Group	**Mean**
400.000	25.467
500.000	27.256

Std Err of LS Mean = 0.394

Least square means for Temperature :

Group	**Mean**
20.000	25.333
30.000	25.967
40.000	27.783

Std Err of LS Mean = 0.483

Least square means for Time :

Group	**Mean**
5.000	25.117
15.000	26.617
30.000	27.350

Std Err of LS Mean = 0.483

General Full Factorial Regressions: RS;Pressure.Temperature.Time

Three Way Analysis of Variance

Data source: Data 1 in Notebook1

Balanced Design (No Interactions)

Dependent Variable: Col 4

Normality Test (Shapiro-Wilk) Passed (P = 0.407)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Pressure	1	99.405	99.405	9.472	0.037
Temperature	2	64.108	32.054	3.054	0.157
Time	2	99.218	49.609	4.727	0.088
Residual	4	41.977	10.494		
Total		17326.849	19.226		

The difference in the mean values among the different levels of Pressure are greater than would be expected by chance after allowing for the effects of differences in Temperature and Time. There is a statistically significant difference (P = 0.037). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature are not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Pressure and Time. There is not a statistically significant difference (P = 0.157).

The difference in the mean values among the different levels of Time are not great enough to exclude the possibility that the difference is just due to random sampling

variability after allowing for the effects of differences in Pressure and Temperature. There is not a statistically significant difference (P = 0.088).

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
400.000 vs. 500.000	4.700		24.353	0.037	Yes

Power of performed test with alpha = 0.0500: for Pressure : 0.596

Power of performed test with alpha = 0.0500: for Temperature : 0.222

Power of performed test with alpha = 0.0500: for Time : 0.366

Least square means for Pressure :

Group	**Mean**
400.000	56.256
500.000	51.556

Std Err of LS Mean = 1.080

Least square means for Temperature :

Group	**Mean**
20.000	56.433
30.000	53.383
40.000	51.900

Std Err of LS Mean = 1.323

Least square means for Time :

Group	**Mean**
5.000	57.083
15.000	53.150
30.000	51.483

Std Err of LS Mean = 1.323

APPENDIX B: Glucose Standard Curve