FORMULATION AND CHARACTERIZATION OF FOOD SIMULANTS FOR COOLING AND FREEZING APPLIANCES

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ABSTRACT

FORMULATION AND CHARACTERIZATION OF FOOD SIMULANTS FOR COOLING AND FREEZING APPLIANCES

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Electrical household and similar cooling and freezing appliance manufacturers are continuously seeking ways to improve properties of their products. To design such appliances and obtain generalized and standardized results, developing test methods using food simulants has been a new strategy. Drip loss is an important problem experienced by the consumers during thawing of meat and refrigerator manufacturers try to design appliances to minimize this problem. Food simulants are used to mimic the cooling and freezing responses of foods during thawing and quick freezing. In this study, different food simulants for 'drip loss' were developed by using different hydrocolloids such as corn starch (10%, 15%, 20%), curdlan (3%), agar (2%, 3% and 4%) and methylcellulose (5%). During freeze-thaw cycle, food simulants along with 'real foods' (sirloin and chicken breast) were stored at two different temperatures (-18^{o}C and -27^{o}C) for 20 h and thawed at room temperature for 4 h. For characterization of the food simulants, total drip loss, hardness, FT temperature cycle, water holding capacity (WHC), NMR and scanning electron microscopy (SEM) measurements were conducted. To observe how water distribution changed, all measurements were conducted before and after FT cycle. Total drip loss of real

food samples was also measured to compare with food simulants throughout thawing process. There were significant differences in drip loss rates for both curdlan-based food and methyl cellulose-based simulants frozen at two different temperatures ($p<0.05$). According to NMR analysis, curdlan-based samples with the lowest concentration had highest T_2 values before FT cycle. It was observed that these samples also had the highest total drip loss percentage after FT cycle. Moreover, total drip loss (%) was observed to be positively correlated with spin-spin (T_2) relaxation times before FT cycle ($r= 0.844$, $p<0.05$). After FT cycle, there was an increase in hardness values of curdlan-based food simulants while there was a decrease in methyl cellulose-based ones. SEM images showed that the addition of secondary polysaccharide with different concentration levels to methylcellulose-based and curdlan-based food simulants affected the microstructures of hydrogel.

Home appliances manufacturers should develop test methods with standard artificial materials, which are food simulants, to measure the freezing performance of their products. Food simulants formulated and characterized in this study can be used in the design of freezer systems.

Keywords: Refrigeration, Freezing, Food Simulants, Drip Loss, NMR Relaxometry

ÖZ

SOĞUTMA VE DONDURMA CİHAZLARI İÇİN GIDA SİMULANTLARININ FORMÜLASYONU VE KARATERİZASYONU

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Elektrikli ev aletleri ve benzeri soğutma ve dondurma cihaz üreticileri, ürünlerinin özelliklerini iyileştirmenin yollarını sürekli araştırmaktadır. Bu tür cihazları tasarlamak ve genelleştirilmiş ve standartlaştırılmış sonuçlar elde etmek için, simulant kullanarak test yöntemleri geliştirmek yeni bir strateji olmuştur. Damlama kaybı, tüketicilerin et çözdürme sırasında yaşadıkları önemli bir problemdir ve buzdolabı üreticileri bu sorunu en aza indirecek cihazlar tasarlamaya çalışmaktadır. Gıda simulantları, çözülme ve hızlı dondurma sırasında gıdaların soğutma ve dondurma tepkilerini taklit etmek için kullanılır. Bu çalışmada, mısır nişastası (%10, %15, %20), kurdlan (%3), agar (%2, %3 and %4) and metil selüloz (%5) gibi farklı hidrokolloidler kullanılarak ‘damlama kaybı’ için farklı gıda simulantları geliştirilmiştir. Dondurma-çözdürme döngüsü sırasında, gıda simulantları ile birlikte 'gerçek gıdalar' (bonfile ve tavuk göğsü) iki farklı sıcaklıkta (-18^{o}C ve -27^{o}C) 20 saat saklanmış ve oda sıcaklığında 4 saat çözülmüştür. Gıda simulantlarının karakterizasyonu için toplam damlama kaybı, sertlik, dondurma-çözdürme sıcaklık döngüsü, su tutma kapasitesi (STK), NMR ve taramalı elektron mikroskobu (TEM) ölçümleri yapılmıştır. Su dağılımının nasıl değiştiğini gözlemlemek için tüm

ölçümler dondurma-çözdürme (DÇ) döngüsünden önce ve sonra yapılmıştır. Gerçek gıda numunelerinin toplam damlama kaybı, çözdürme işlemi boyunca gıda simulantları ile karşılaştırmak için de ölçülmüştür. Hem kurdlan bazlı gıda hem de metil selüloz bazlı iki farklı sıcaklıkta dondurulan simulantlar için damla kaybı oranlarında önemli farklılıklar gözlenmiştir ($p<0.05$). NMR analizine göre, en düşük konsantrasyona sahip kurdlan bazlı numuneler DÇ döngüsünden önce en yüksek T_2 değerlerine sahiptir. Bu numunelerin de DÇ döngüsünden sonra en yüksek toplam damlama kaybı yüzdesine sahip olduğu gözlenmiştir. Ayrıca, toplam damlama kaybının (%) DÇ döngüsünden önceki spin-spin (T_2) gevşeme süreleri ile pozitif olarak ilişkili olduğu gözlenmiştir ($r= 0.844$, $p<0.05$). DÇ döngüsünden sonra, curdlan bazlı gıda simulantlarının sertlik değerlerinde artış olurken, metil selüloz bazlı olanlarda ise bir azalma olmuştur. TEM görüntüleri, metilselüloz bazlı ve curdlan bazlı gıda simulantlarına farklı konsantrasyon seviyelerine sahip sekonder polisakkarit ilavesinin hidrojelin mikro yapılarını etkilediğini göstermiştir.

Beyaz eşya üreticileri, ürünlerinin dondurma performansını ölçmek için gıda simulantları olan standart yapay malzemelerle test yöntemleri geliştirmelidir. Bu çalışmada formülize ve karakterize edilen gıda simulantları, dondurucu sistemlerinin tasarımında kullanılabilir.

Anahtar Kelimeler: Soğutma, Dondurma, Gıda simulantları, Damlama kaybı, NMR Relaksometri

To my beloved family...

To my parents Zeyneti & Özkan Baydemir who have given me my roots

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LIST OF ABBREVIATIONS

ABBREVIATIONS

A: Agar

ANOVA: Analysis of Variance

Car: Carragenan

C: Curdlan

CS: Corn Starch

FT: Freeze-Thaw

FTC: Freeze-Thaw Cycle

GA: Gum Arabic

GT: Gum Tragacanth

I: Inulin

κ- and ι-car: kappa and iota-carragenan

κ-car: kappa-carragenan

MC: Methyl Cellulose

MS: Modified Starch

NMR: Nuclear Magnetic Resonance

SA: Sodium Alginate

SEM: Scanning Electron Microscopy

T_1: Longitudinal (Spin-Lattice) Relaxation Time

T_2: Transverse (Spin-Spin) Relaxation Time

WHC: Water Holding Capacity

WPI: Whey Protein Isolate

XG: Xanthan Gum

CHAPTER 1

INTRODUCTION

1.1 Meat

Beef and Poultry

Meats and meat products are the most important part of balanced and healthy diets due to their protein, vitamin, mineral, and micronutrient content. Water and protein act as the main components of different types of meats, which are shown in Table 1.1.

Table 1. 1. Chemical composition (%) of different types of meat (FAO, 2007)

Meats	Water	Protein	Fat	Ash
Beef (lean)	75	22.3	1.8	1.2
Beef (carcass)	54.7	16.5	28	0.8
Chicken	75	22.8	0.9	1.2
Pork (lean)	75.1	22.8	1.2	1.0
Pork (carcass)	41.1	11.2	47	0.6

Meats are highly sensitive and perishable foods due to nearly neutral pH (around 5.6), containing more than 70% water and being rich in nutrients. Therefore, meat needs to be preserved if not consumed right away. Freezing is one of the most used and preferred preservation methods to prolong the shelf life of fresh foods. Thawing represents the final stage of food storage in which the quality of frozen products is affected significantly.

There are various parameters that affect the frozen meat quality in addition to the freezing and thawing process, which are summarized in Table 1.2 (Aidani et al., 2014; Berry et al., 2008; Gonçalves et al., 2017).

Table 1. 2. Summary of the most important factors affecting frozen meat quality

	Main factors	
Pre-freezing	• Species • Genetic livestock • Feed management • Microbial load • Packaging • Processing (cutting)	• Transportation • Slaughtering stress • Post-mortem processes • Temperature of pre-slaughtering
Freezing	• Freezing rate	• Type of the freezing technology
Frozen storage	• Temperature • Time	• Temperature fluctuation
Thawing	• Thawing rate • Tempering	• Type of the thawing technology

1.2 Freezing

Freezing is a complex process involving heat transfer as well as physical, microbiological, and chemical changes. Freezing (-18ºC or below) prevents the growth of microorganisms, reduces the rate of chemical reactions, and delays cellular metabolic reactions. Though freezing has less changes on food products than other preservation methods, it can induce '*freezer damages'* on foods. Especially perishable foods such as meat and meat products can be affected adversely and this may result in economic losses (Delgado & Sun, 2001).

Freezing process is described by three main stages: *pre-freezing* (pre-cooling to the freezing point), *freezing* (phase transition) and *sub-freezing* (reduction to the final storage temperature) (Barbosa-Cánovas et al., 2005) (Figure 1.1).

— *The pre-freezing stage is called as the period from the beginning of the freezing process to the appearance of the first ice crystal in the food.*
— *Phase transition from water to ice occurs at the second stage.*
— *When the product at the crystallization temperature drops to the target/desired storage temperature, the last stage is completed and recrystallization continues* (Kang et al., 2020; Persson & Löndahl, 1998).

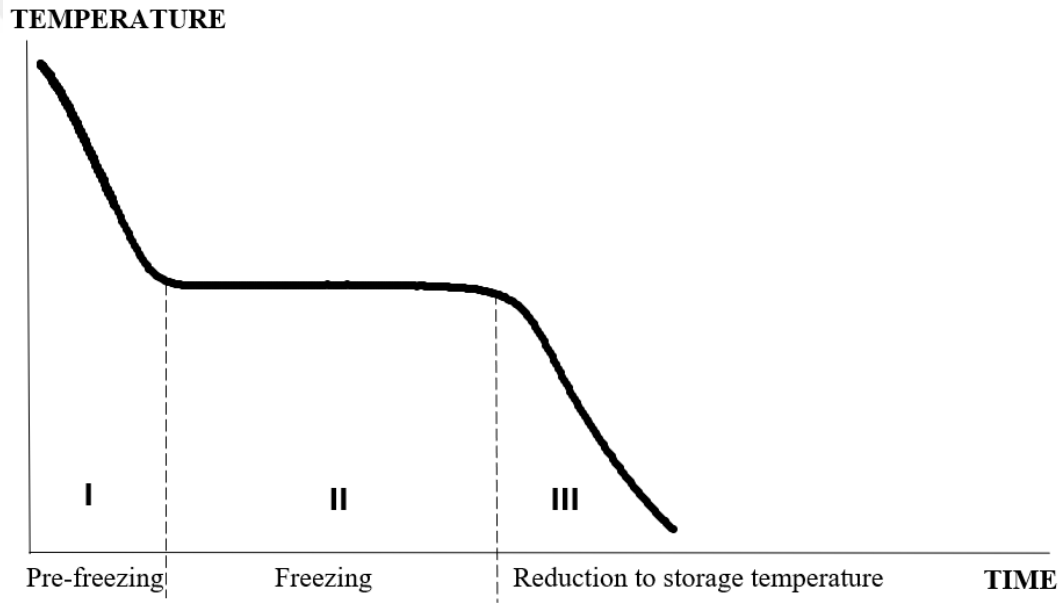


Figure 1. 1. The three stages of temperature change of foods throughout the freezing process (Barbosa-Cánovas et al., 2005)

In general, ice crystallization in foods is observed at a range of -1°C to -5°C, which is called as *the stage of ice crystal formation* (Giannakourou & Giannou, 2015).

In some sources, the freezing process is divided into four main stages. Apart from other stages, there is the super-cooling stage, which is defined as the region where temperature drops below the freezing point, but since it is not always observed it is

not common to define that stage.

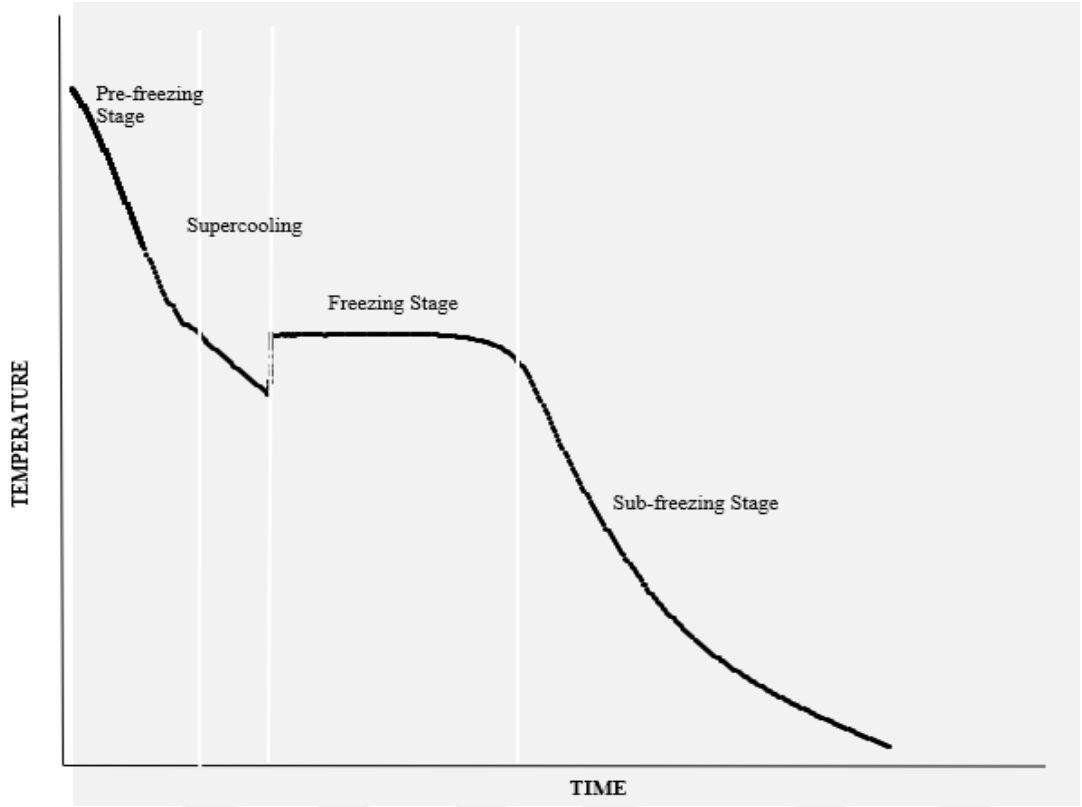


Figure 1. 2. The typical temperature-time curve of the freezing process (Alexandre et al., 2013)

During the freezing process, inevitable damages and deformations related to ice crystal formations cause food quality losses. Thus, *freezing rate, freezing time and duration of the frozen storage* should be carefully chosen.

Freezing Rate

Freezing rate is given as $^{o}C/h$ and simply defined as the temperature difference (from initial to final temperature) of a product divided by the freezing time taken until the final temperature is reached for the freezing. The quality of frozen foods is directly influenced by the freezing rate since the size and distribution of ice crystals that are formed during the freezing process depend on the freezing rate (Alam, 2007). During the freezing of food, water between the individual cells starts to freeze. Fast freezing of water is critical to prevent damages in cells since freezing causes irreversible

damages to the tissues. Otherwise, not only large ice crystals but also shrunk cells and destructed tissues are observed after slow freezing, which also directly affects the amount of liquid that splits out from the cells during thawing. Fast freezing allows to form a great number of small ice crystals. For slow and quick-freezing rates, the changes in the cells due to ice crystals is given in Figure 1.3.

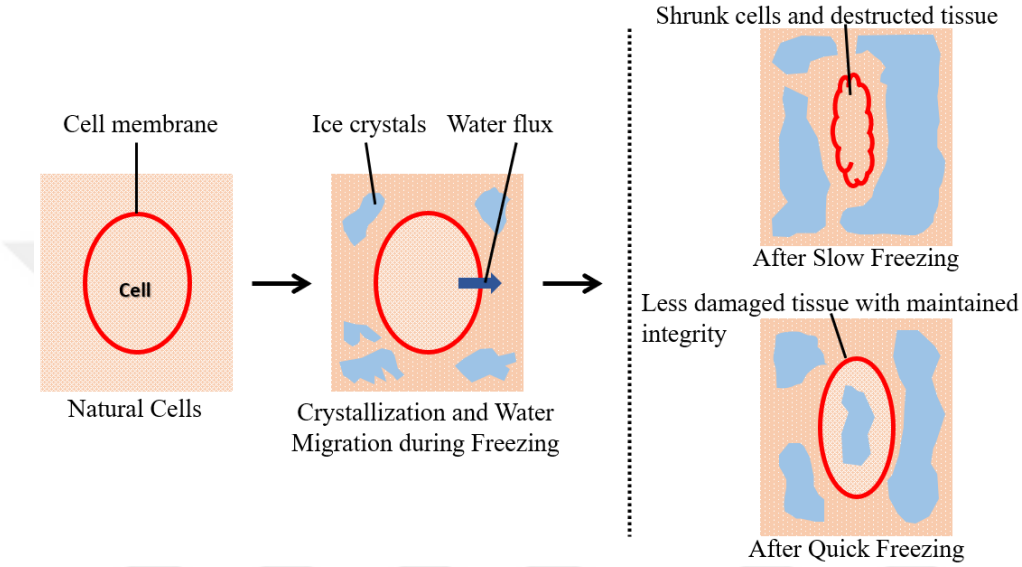


Figure 1. 3. The effect of freezing rate on the quality of frozen products (Fikiin, 2003)

Dalvi-Isfahan and Hamdami (2017) showed that accelerating freezing rates have an important effect on the quality of frozen meat samples. Although different freezing temperatures (-20, -30 and -40 ^{o}C) did not significantly change color, texture, and the shape of ice crystals of meat samples, it was shown to induce microstructural changes in the tissues. Furthermore, it was stated that when freezing temperature decreased, there was a reduction in drip loss in meat samples.

Freezing Time

Freezing time is the time required to decrease the food product temperature from its initial temperature to final storage temperature at its thermal center. Product size and shape, initial and final temperatures of the product, surface heat transfer coefficient;

enthalpy changes, thermal conductivity of the product all effect the freezing time (Fisher, 2019; Persson & Löndahl, 1998).

Frozen Storage

The sensorial quality of foods, such as texture, appearance, color, taste, and smell are negatively affected during frozen storage. Consumer acceptance of meats reduce due to the oxidation of oxymyoglobin on the surface, which remain brown after thawing (Henriott et al., 2020). In a study about the effect of frozen storage on the breast meat quality of broiler chickens, it was stated that the increase in drip loss, pH, darker coloration of chicken meat was caused by long frozen storage (Augustyńska-Prejsnar et al., 2019). The sub-freezing and subsequent storage can cause recrystallization, which is directly related to orientation, shape, size, number of the ice crystals in frozen foods. Much more stable and larger crystals may occur to form on the surface of ice crystals after initial crystal formations due to the poor storage conditions, which is called recrystallization (Zhu et al., 2019).

During the storage period of frozen products, the effect of temperature fluctuations is important since fluctuations cause the resizing and redistribution of ice crystals, which results in further microstructural damage, irreversible cell and tissue damage, the quality degradation during storage and significant drip loss in thawing (Gutiérrez et al., 2017; Sun, 2016; Wang et al., 2020).

1.3 Thawing Process

Thawing is a fundamental step for frozen food products before the consumption (Fadiji et al., 2021). Thawing is a longer process than freezing. The frozen part is at the center of foods; on the other hand, in freezing stage, the outer layer of food is in the frozen state. Thus, the time required for thawing process is higher than freezing time, which is expressed by the thermal conductivity. The thermal conductivity of ice is about four times greater than that of water (Nesvadba, 2008). The discrepancy between freezing and thawing times can be also expressed with the thermal

diffusivity. Thermal diffusivity of ice is nine times greater than that of water, which means ice crystals can respond to temperature change nine times as fast as water does. The removal or addition of heat through ice layers takes nine times longer than water layers in the freezing or thawing process (Ramaswamy & Marcotte, 2005).

Since the required time for thawing frozen foods is longer than freezing, thawing has more detrimental effects than freezing (Alarcón-Rojo & Janacua-Vidales, 2010). Thawing is completed when the center temperature of food reaches 0^{o}C. The thawing rate is basically influenced by dimensions, thermal conductivity, initial and final temperature of foods, enthalpy change, surface heat transfer coefficient, and environment temperature (Silliker et al., 1980). That is why, thawing systems should be designed by considering not only the process conditions that is needed to obtain a certain thawing time but also the effects on quality of foods such as drip loss, appearance, and microbial growth (James & James, 2010) should also be considered. It should be noted that that thawing process conditions should aim to decrease drip loss, microbiological growth, deterioration reactions and some evaporation losses. In addition, the most important temperature interval in thawing of meat is in the range of -10^{o}C and -2^{o}C, so it is an important need that meat must rapidly pass this range not to affect quality of the meats in a negative way (Calvelo, 1981). In the food industry, different thawing systems are used depending on the need, since all of them have different advantages and disadvantages.

1.4 Drip Loss

The water in foods cannot migrate back to the cells on thawing due to the cell wall damages inflicted from slow cooling or freezing and the inevitable result is defined as drip loss (Rahman & Velez-Ruiz, 2007).

Drip loss, loss of exudate, is the accumulation of liquids in the meat containers like storage box of pre-packaged meat or dishes of unwrapped meat due to exudation of liquid caused by several factors. Drip loss is also named as “purge loss”, “press loss”

and “thaw loss” depending upon the measurement method and when drip is measured (James, 2002). A significant amount (2-15%) of intracellular and intercellular fluids can come out from intact muscles of frozen products (James, 2008). There are many factors affecting the amount of drip, which are basically categorized into two parts: *internal and external factors*. The *internal* factors are listed as animal species, sex, age, weight of the animal, muscle regions, feeding management, transportation before pre-slaughter, slaughtering stress. *External* ones are divided into two categories: basic factors (size of ice crystal, protein denaturation) and direct factors (freezing rate, thawing rate, physical interference) (Devi et al., 2019; James & James, 2009). Different animal species tend to lose drip in descending order: beef, pork, lamb, and poultry.

Meat tissue can be also considered as a gel system since it is a polymeric network (proteins) where water is trapped. Drip loss is also defined as **syneresis** for the hydrogel systems. Syneresis, named as the release of the solvent in the gel network is a critical parameter for the stability of a gel system as it leads to a decrease in the volume of the gel (Panja et al., 2022).

1.5 Food Simulants for Refrigerators

The cooling responses of foods during thawing and quick freezing are affected by many factors such as nutrient composition, geometry, physical state of the food, packaging, thawing conditions, and temperature fluctuations during freezing. Since meat is an unstable and perishable food product, the quality parameters of meat are affected in a negative way. For example, drip loss observed during thawing of frozen meat is an important issue for most consumers because liquid loss decreases the meat weight, meat’s water binding ability, eating, and cooking quality which are main contributions for the meat value. Thus, the freezing of meats has been commonly investigated and enhanced by using different freezing technologies to provide less drip loss during the thawing process (Kadim & Mahgoub, 2007).

Food simulants are food substitutes that can simulate drying, cooling, freezing, thawing, heating, or reheating responses of foods based on certain criteria: stability, safety, cost, reproducibility, availability, freeze-thaw stability, meltable. Food simulants can mimic different types of foods such as alcoholic, aqueous, acidic foods or dry foodstuffs. Food simulant materials are the materials which reflect some properties (specific heat, thermal conductivity, mass transport, dielectric) of foods (Cai et al., 2014; James et al., 2002; James et al., 2017).

There are some material groups used as simulants in food applications for different purposes such as gelling agents, cellulose-based material, hydrogel, fat, and muscle phantom. There are summarized in Table 1.3 (Swain & James, 2005). Some of these materials (*marked with tick in the table*) have possible uses for further test studies on the cooling response of meat and meat products.

Table 1. 3. Some material groups used as the simulant

Material name	**Use**	**References**	**Possible usage for further testing on cooling response**
Gelatin	Gelling agent	(Djabourov et al., 1993; Panouillé & Larreta-Garde, 2009)	
Sodium alginate		(Fu et al., 2011; Larsen et al., 2015; Potter et al., 1994)	
Whey protein		(Bertrand & Turgeon, 2007; Fang & Guo, 2019; Hazrati & Madadlou, 2021; Lorenzen & Schrader, 2006)	

Table 1.3 (continued)

Bacto agar		(Arregui et al., 2003; Jaeger et al., 2015; Mohamed et al., 2021)	
Gellan Gum		(Nickerson et al., 2007)	
Starch solutions (soup analogues)	Soup simulant	(Sarker et al., 2013)	
Biogel (methyl methacrylate n-vinyl-2-pyrolidone)	Hydrophilic food simulant	(James et al., 2017)	
Tylose (hydroxyethyl methylcellulose)	Meat thermal simulant	(Anderson & Singh, 2005; Otero et al., 2006; Woolfe, 2000)	✓
SIK gel (carrageenan, sugar, glycerol)	Food simulant	(Nishinari & Watase, 1992; Stenner et al., 2016)	✓
SIK liquid (sugar, glycerol)	Liquid food simulant	(Nurgel & Pickering, 2005)	
Mashed potato	Fast food test simulant	(Canet et al., 2005; Chen et al., 2014)	
Polyacrylamide+ polycrylic acid	Hydrogel	(Bashir et al., 2020; Cheng et al., 2017; Li et al., 2002; Nesrinne & Djamel, 2017)	✓

To be able to mimic the cooling responses of meats with much more standardized artificial materials, hydrogel is a good option. Hydrogels have an important role in the food industry due to their high-water holding ability (Stojkov et al., 2021). Since hydrogels are basically macromolecular networks with cross linkage, they are able to absorb and retain large amount of water, as well as show syneresis (Mahdavinia et al., 2008). Furthermore, the different types of hydrocolloids are combined with the different concentrations to set the required viscous and textural properties. Thanks to their three most important functional properties such as *viscosity, gelation and solubility*, the combinations of hydrocolloids give many advantages such as innovative structural functionalities, reduction in levels of hydrocolloids and reduction in the unit cost of products (Goff & Guo, 2019).

For these reasons, hydrogels can be preferred to use as food simulants. Due to their water holding capacity and their syneresis effects on the freeze-thaw cycle, different food simulants, i.e. hydrogels, can be evaluated in terms of 'drip loss' by the characterization methods. The development of hydrogels with different concentrations is critical both to simulate and to cover different meat types and body parts of meats with different nutritional composition such as water, protein, fat, and ash content.

1.6 Hydrocolloids

Hydrocolloids are mostly polysaccharide and some protein substances that comprise of hydrophilic and linear or branched high molecular weight molecules with colloidal properties. Hydrocolloids are materials that hydrate in water so that they can produce low or high viscous solutions, pseudo-gels, or gels in water-based systems. Hydrocolloids are used to thicken and stabilize formulations and used for the purposes of emulsifying, thickening, suspending, whipping, and encapsulating in a variety of industrial sectors (Hoefler, 2004; Li & Nie, 2016).

Hydrocolloids come from various sources such as microorganisms, animals, plants with and without chemical modification. They are mainly classified according to their raw material origin and way of manufacturing and are listed in Table 1.4 (Goff & Hartel, 2013; Van Nieuwenhuyzen et al., 2006; Wüstenberg, 2014).

Table 1. 4. Sources of hydrocolloids used globally in the industry

Sources	***Hydrocolloids***
Plant Extracts	Agar, Carrageenan, Alginates, Starches, Cellulose, Pectins
Seeds	Locust Bean Gum, Guar Gum, Tara Gum, Tamarind Seed Gum
Plant Exudates	Gum Arabic, Karaya Gum, Tragacanth
Microbial Polysaccharides	Curdlan, Xanthan Gum, Gellan Gum, Dextran
Modified Polysaccharides	Cellulose Derivatives (Methylcellulose, Carboxymethylcellulose, Microcrystalline cellulose, Hydroxypropyl cellulose, Hydroxypropyl methylcellulose), Modified Starches
Proteins (Animal extracts)	Gelatine, Caseinates, Whey Protein, Soy Protein, Microparticulated Milk and Egg Proteins

1.6.1 Agar

Agars are produced from red seaweeds. They are linear polysaccharides consisting of agarose and agaropectin. Since agar has a high gel-forming ability, it is mostly used in the food industry such as dairy, confectionery, bakery, and meat products. Agar is a polysaccharide that has a thermoreversible gelation property. For the gelation of agar, it is needed to form an aqueous solution by heating up to 85^{o}C or higher temperatures and then cooled to much lower temperatures (35^{o}C).

Multicomponent gel systems including agar are preferred for various food applications since incorporation of sugars or other gums can affect the strength of agar gels (Stanley, 2006). Agar is used at levels of 0.5-2% to form a gel (Saha & Bhattacharya, 2010). Agar gels have brittle structures and can show syneresis (Glicksman, 1979).

Freeze-thaw stability of agar-methylcellulose hydrogel (1:1 ratio) was analyzed to observe the syneresis by freezing hydrogel samples at -18^{o}C for 24 hours and then thawing them 30^{o}C in a sealed container for one hour. After thawing, the weight of hydrogel samples reduced to 63±3% of its original weight since the ice crystals occurred during freezing physically disrupted the gel network and resulted in syneresis. It was also shown that the agar-methylcellulose hydrogels had a much more stable structure than the agar hydrogels when exposed to heating and melted (Thompson et al., 2017).

1.6.2 Alginate

Alginate is extracted from plant sources, which is found in cell walls and intercellular spaces of brown algae. By the addition of small amount of soluble calcium salts (calcium sulphate, calcium citrate), alginate viscosity can be increased at low alginate concentrations since ions react with alginates to cross-link the molecules and form a gel that is heat stable (Onsøyen, 1997). Alginate composite gels are formed when other biopolymers such as hydrocolloids (carrageenan, guar gum, pectin), starches (gelatinized, native, or resistant) and proteins (casein, whey protein) are added to alginate gels. In most recent studies, chitosan, gelatin, carrageenan, pectin and carboxymethyl cellulose have been combined with alginate to form composite gels. When compared to alginate gel, composite gels with chitosan, pectin, starch, or protein have harder and brittle gel structures (Ramdhan et al., 2020).

1.6.3 Carrageenan

Carrageenan, a naturally occurring polysaccharide, is obtained from red seaweeds like agar. Carrageenan has three types with different structures such as lambda, kappa, and iota. Carrageenan types have different functional properties, thickening and gelling properties (Imeson, 2009). Kappa carrageenan with potassium ions and iota carrageenan with calcium ions could form firm and soft elastic gels. Lambda carrageenan has a non-gelling property with cations. Lambda and iota carrageenan are freeze-thaw stable, but kappa carrageenan is not. Although kappa carrageenan has a higher syneresis effect, directly linked to freeze- thaw stability, iota does not show any syneresis. On the other hand, all carrageenan can dissolve in hot water at 80^{o}C. Hot solutions of kappa and iota carrageenans can form a gel structure after they are cooled to 30-70^{o}C (Blakemore & Harpell, 2010). Typical concentrations that are used to form a gel structure are 0.5-3% (Burey et al., 2008).

1.6.4 Curdlan

Curdlan, a microbial polysaccharide, is produced by *Alcaligenes faecalis var. myxogenes.* It is commonly used as a texture modifier, stabilizer, water binder, gelling agent in food industry. Curdlan is different from other gelling agents due to its stability against freezing and thawing. Thus, it is a unique gelling agent (Nishinari et al., 2009). Curdlan is insoluble in cold water but forms a firm, termo-irreversible and high-set gel when heated up to 80^{o}C. In jellies and jelly products, it has been used as gelling agent at levels of 1-5% (Miwa et al., 1994). Low set gels with a much lower gel strength and syneresis are obtained when curdlan aqueous suspension is heated between 55^{o}C and 60^{o}C and then cooled below 40^{o}C (Konno & Harada, 1991). When viscoelastic properties of curdlan gels at 2%, 4% and 8% were studied by creep tests and oscillatory dynamic experiments, it was shown that increase in heating temperature led to stronger and elastic gels (Funami et al., 1999).

Curdlan gels show syneresis but it can be repressed when starch is added to curdlan. Syneresis is not repressed by the addition of different sugar compounds at high concentrations (Ishida & Takeuchi, 1981).

Nakao et al. (1991) reported that curdlan gel had good freezing and thawing stability. When curdlan gel at a concentration of 4% was frozen at -40^{o}C for 2 h (then stored at -18^{o}C for 24 h) and thawed by holding under tap water for 30 min, drip loss was reported as 20.6% and it decreased to 2.1% and 8.9% after addition of 5% waxy corn starch and 20% sucrose. In the same study, it was mentioned that drip loss for 2% curdlan gels was about 35%, but curdlan gels made with 20% sucrose had a drip loss of 11.4%.

In another study, viscosity, syneresis, texture and heat stability of hydrogel complexes formed by curdlan and secondary biopolymers (xanthan gum, guar gum or locust bean gum, carrageenan) and exposed to five FT cycles between -16^{o}C for 18 h and 25^{o}C for 6 h had been studied (Williams et al., 2009). No syneresis was reported for curdlan-based hydrogels combined with guar and xanthan gum and they showed strong and stable water-holding capacity. It was observed that locust bean gum/curdlan formulation had a higher syneresis rate than κ-carrageenan/curdlan combination. At the end of the fifth freeze-thaw cycle, curdlan hydrogels combined with locust bean and κ-carrageenan had an average of 60% and 15% syneresis, respectively. Among all samples at the different combinations, xanthan gum/curdlan hydrogel had the highest freeze-thaw stability in terms of syneresis and heat stability although it showed the lowest viscosity.

1.6.5 Corn Starch

Starch is one of the most abundant polysaccharides in nature, consisting of amylose and amylopectin. Native starches are insoluble in cold water, depending on the insoluble or soluble amylose fraction (Zarski et al., 2021).

Starch-based hydrogels have been used for different applications such as agricultural uses, sorption dyes, drug delivery, food preservation, personal care, water, and tissue engineering (Ismail et al., 2013).

Freeze-thaw stability of corn starch gel has been shown to be affected by the presence of guar gum, xanthan gum, locust bean gum. The syneresis of corn starch gels with gums was generally less than the ones without gums at the first cycle. At the end of 5 cycles, it was found that the corn starch gel system with xanthan gum was the most effective in terms of reducing the syneresis (Yamazaki et al., 2013).

1.6.6 Modified Starch

Modified starch is obtained using physical, chemical, or enzymatic techniques to achieve specific desirable functional properties unlike native starch. When freeze-thaw stability of native and modified starch during multiple freeze-thaw cycles was examined in terms of syneresis, the percentage of syneresis of both starches showed a significant decrease with the repeated FT cycles (Ye et al., 2016). The water solubility of enzyme-modified starch was found to be higher than that of corn starch (Woo et al., 2021).

1.6.7 Gum Tragancth

It is a soluble polysaccharide of natural origin with high stability in a wide range of temperature and pH. It is used as an emulsifying agent with extremely long shelf life in the food industry (Otady et al., 2005). It is known as “Katira” in Iran and Turkey and has been recognized as *Generally Recognized as Safe* (GRAS) at the 0.2-1.3% (Kurt, 2018; Leimann et al., 2018). To investigate the effects of gum tragacanth on the stability and texture of a dairy product, gum tragacanth at different concentrations (0.1-0.5 %) was added to samples and syneresis rates were measured. The addition of 0.5% gum tragacanth resulted in less syneresis than the non-gum samples (Shiroodi et al., 2012).

1.6.8 Gum Arabic

Gum Arabic is a natural plant exudate polysaccharide obtained from the stems and branches of *Acacia Senegal or Acacia Seyal* trees. It is mostly used for marshmallows, pastilles, caramel-types products in the confectionery industry. When it is compared to other water-soluble polysaccharides, it has very low viscosity in water. If gum Arabic dissolves at high concentrations like 40-45%, high viscosity can be obtained. It is known that the viscosity of 30% gum Arabic solution is lower than 1% xanthan gum and sodium carboxymethylcellulose (Phillips & Williams, 2009). The addition of gum Arabic to whey protein isolate solutions was studied by Valim et al. (2009). For the analysis of two different WPI gelation processes such as heat-induced and cold-set gelation, 5% (w/w) of Arabic gum stock solution had been prepared and then maintained for 30 min under magnetic stirring. Then, the stock solutions were stored at 10 ^{o}C for 48 h. It was noted that if gum Arabic concentration increased, the water holding capacity of the cold-set gels systems increased due to the strong hydrophilic character and structure of gum Arabic. In another study conducted by Li et al. (2021), 0.0-5.0% (w/v) gum arabic and 1.0% (w/v) konjac glucomannan was dissolved in distilled water heated to 50^{o}C, and then stirred. The gels were obtained after the mixtures were heated at 90^{o}C for 30 min with continuous mixing and stored in a refrigerator for 1 day. It was shown that the hardness values of 0-2.0% gum Arabic decreased as the concentration increased whereas the hardness increased with the increase from 2.5% to 4.0%. It was indicated that it was probably due to the crosslinking between polymer chains or lessening of the water.

1.6.9 Inulin

Inulin is known as a storage carbohydrate. Since inulin is found naturally in various plants, it is frequently preferred in daily human diets and used in food industry as a gelling agent, texture improver, fat replacer, foam maker, and emulsion stabilizer (Wouters, 2010). According to a study (Kim et al., 2001), inulin was almost insoluble

at 25-50^{o}C, but higher temperature increased solubility. Inulin-water solution started to form gel after 30 % inulin concentration at 90^{o}C for 5 min and cooled to 25^{o}C for 1 day. Moreover, it was indicated that the low inulin concentrations (5%, 10% (w/v)) were not enough to form gel network after both heating and cooling process. Inulin can be used as a good fat replacer, especially in dairy products such as fermented milk, kefir, yogurt, custard, mousse, cheese products, fresh kashar cheese, ice cream. Inulin has the ability not only to modify textural behavior like thickness or hardness but also to mimic mouthfeel attributes as creaminess or smoothness (Meyer et al., 2011).

1.6.10 Methyl Cellulose

It is one of water-soluble cellulose derivatives produced from cellulose. It is soluble in cold water, not in hot water. After the methyl cellulose (MC) solution is heated, gel structure starts to form at the gelation temperature (50-90^{o}C). Required temperature for low concentration solutions (0.5%) of MC to obtain a gel is between 50 and 75^{o}C (Nussinovitch, 1997). Methyl cellulose has a different thermo gelation property than other hydrocolloids. If temperature of methyl cellulose solutions increases, viscosity of MC gels decreases at first. Then, at the point of thermal gel temperature (50-60^{o}C), a sharp increase in viscosity occurs due to the onset of hydrophobic gelation, i.e. intermolecular and intramolecular association. At very low temperatures, hard and brittle methyl cellulose-based gels form (Haque & Morris, 1993; Joseph, 2020). In Figure 1.4, change in complex modulus of MC gels with respect to temperature are given.

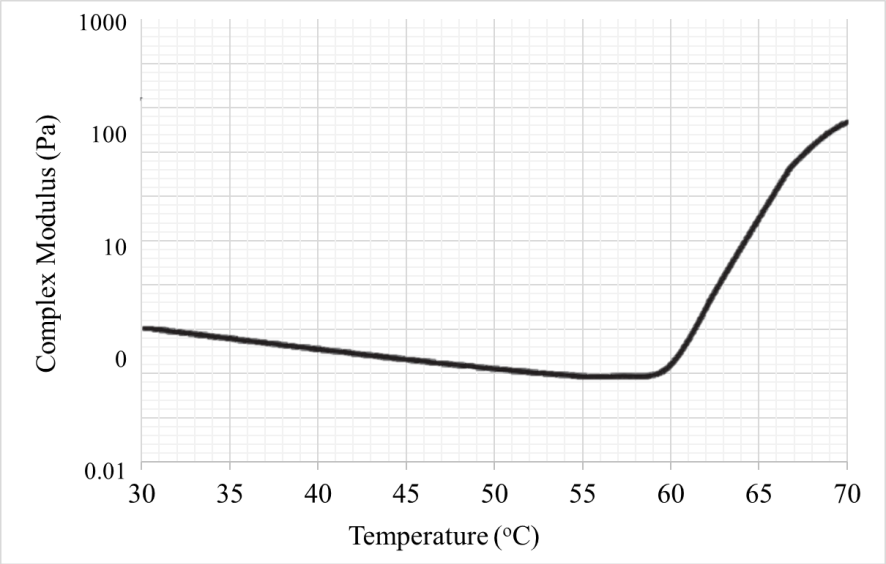


Figure 1. 4. The complex modulus curve depending on gelling temperature for a methyl cellulose solution (Cash & Caputo, 2010)

The study conducted by Martin et al. (2008) showed that methyl cellulose combined with other polysaccharides like agar has a much faster gelling time and higher maximum force applied than agar or MC alone.

Tylose gel, commercially known as M-package, consists of methyl cellulose, water, and others. It is used as the test package for cooling performance tests on Household Cooling Appliances, according to standards ISO 5155, ISO 7371, ISO 8187, ISO 8561, EN 441-4, EN 441-5. The composition of gels varies slightly depending on the freezing point (Srl, 2009). It is very similar to a food system with the same amount of water, which is meat. To measure some mechanical properties of food systems during freezing, especially at different levels of freezing, chocolate and Tylose gel were selected as a food and model foods, respectively (Tremeac et al., 2008).

1.6.11 Xanthan Gum

Xanthan gum is the most well-known bacterial polysaccharide and its source is *Xanthomonas campestris.* It is widely preferred to be used as thickening, stabilizing and gelling agent in both food and nonfood applications. It is soluble in cold or hot water. It forms thermo-irreversible gels with thermal transition temperatures at very low concentrations (0.1-0.3%) (Urlacher & Noble, 1997). In an investigation of the effects of xanthan gum on the freeze-thaw properties of starch gels studied by Lo and Ramsden (2000), it is shown that xanthan gum has increased the freeze-thaw stability of starch gels. In another study, it was investigated that whether the freeze-thaw cycle stability of hydrogel complexes formed by 1% (w/v) curdlan and a secondary biopolymer such as 1% (w/v) of κ-carrageenan, xanthan, locust bean, and guar gum (Williams et al., 2011) increased.

1.6.12 Whey Protein Isolate

Whey protein isolate (WPI) is used as a gel-forming agent in food industry. Its thermal gelation behavior is based on the initially denaturation-unfolding step and, later aggregation into protein particles. Basically, a macroscopic network is formed by hydrophobic interactions, hydrogen bonds and disulfide bridges throughout the gelation step (McSwiney et al., 1994). WPI gels can be formed by the preparation of 20% w/v protein solutions. The protein solutions are heated in an 80^{o}C water bath for 30 min and then cooled in a refrigerator overnight (Barbut, 1995).

There are numerous studies to understand the interactions between whey protein and the polysaccharides such as pectin, xanthan gum, carboxymethyl cellulose, kappa carrageenan. The effect of κ-carrageenan on the textural properties of WPI gels that were formed by 3% and 10% w/w WPI protein suspensions followed by heating at 80^{o}C 30 min at various pH values was investigated. The addition of κ-carrageenan decreased the shear stress of 10% WPI gels in acidic conditions while it leads to high shear stress values over the pH range of 5-11. The highest stress results of 10% WPI-

κ-carrageenan and 3% WPI-κ-carrageenan mixture gels were observed at pH 6 and pH 6-7, respectively (Mleko et al., 1997). Ren and Wang (2019) analyzed the effect of modified starches on gelling properties of whey protein isolate understand whey protein-starch interactions.

In a research conducted by Shiroodi et al. (2015), it was stated that there was an improvement on freeze-thaw stability and water holding capacity of whey protein isolate gel mixed with the xanthan-curdlan hydrogel complex. Shiroodi and his colleagues also investigated the effect of adding xanthan-curdlan hydrogel complex at 0.25 and 0.50% w/w concentrations on the freeze-thaw stability of WPI gel. After mixtures of WPI and xanthan gum-curdlan hydrogel complex were frozen at -18^{o}C for 18 h, mixture samples were thawed at room temperature for 6 h. It was observed that the syneresis amount of WPI gel with addition of xanthan-curdlan hydrogel complex decreased at a significant level after the 5th freeze-thaw cycle. Similarly, Shiroodi and Lo (2015) examined the effects of various pH values on the rheology of mixed gels consisting of WPI and xanthan gum-curdlan hydrogel complex to characterize the gelation temperature changes of WPI on heating and cooling.

1.7 Objective of the Study

There are numerous studies that investigated the relationship between drip loss and freezing rate, thawing rate, thawing methods, and storage period. While studying real foods, different test results can be obtained due to variabilities from foods and environmental factors. To evaluate performance of cooling and freezing appliances, generalized and standardized test results are very important for '*white goods'* industry. Since food simulants can be also used to mimic the cooling responses of foods, developing test methods using food simulants is a good idea to improve properties of cooling and freezing appliance manufacturers' products and design such appliances. To our knowledge, there has not been any research related with refrigeration food simulants which mimic freeze-thaw behavior of different meat types.

The objectives of this study are to design food simulants by combining possible hydrocolloids to simulate freeze-thaw behavior of real foods and to compare the thawing behavior of these simulants with beef and poultry especially in terms of drip loss.

The specific objectives of the study are listed as follows.

- To formulate potential food simulants by combining different possible hydrocolloids such as curdlan, corn starch, methylcellulose, and agar
- To compare the effects of different freezing temperatures (-18^{o}C and -27^{o}C) on the thawing behavior of food simulants in terms of drip loss
- To characterize the hydrogel-based food simulants with time domain NMR Relaxometry, Scanning Electron Microscope (SEM) and other physical measurements

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

The materials used to prepare hydrocolloid gels are given in Table 2.1.

Table 2. 1. The raw materials that are provided by different suppliers

Material names	Suppliers
Methyl Cellulose (MC), Gum Arabic (GA), Alginate (Alg), Gum Tragacanth (GT), Carrageenan (Car) and Sodium Alginate (SA)	Rotel İç ve Dış Ticaret A.Ş. (Istanbul, Turkey)
Xanthan Gum (XG) and Inulin (I)	Smart Kimya Tic. Ve Dan. Ltd. Şti (Izmir, Turkey)
Agar (A)	Düzey Laboratuvar Kimyasalları ve Cihazları San. ve Tic. Ltd. Şti. (Istanbul, Turkey)
Curdlan (C)	DiaGen Biyoteknolojik Sistemler Sağlık Hizmetleri ve Otomasyonu San. Tic. A.Ş. (Ankara, Turkey)
Corn starch (CS)	Dr. Oetker Gıda San. ve Tic. A.Ş. (Izmir, Turkey)
Whey Protein Isolate (WPI)	Nutricost (Utah, USA)

Beef and chicken meats were purchased from a local butcher in Eskişehir, Turkey. Sirloin steak and chicken breast sections that are composed of low fats were selected as meat samples.

2.2 Methods

2.2.1 Food Simulant Production and Characterization

2.2.1.1 Preparation of food simulants

For simulant design, hydrogels were formulated using different hydrocolloids. Many different hydrocolloids and their combinations were tested to be used as a simulant. Gelation of the individual and mixtures of the hydrocolloid solution was the main criteria. After some preliminary studies listed in Table D.1-D.4, it was deduced that some hydrocolloid combinations were not designed to form the hydrogel complexes. The hydrogels were prepared according to some methods mentioned in the Table D.1-D.4, with some minor revisions. Physical properties of the hydrogels; *color; hardness; form (whether solid or liquid); ability to retain their shapes* were evaluated. Hydrogels to be tested were selected not only based on their 'gel' behaviour but also whether they showed syneresis or not was another selection criterion. Preparation of the hydrogels that were selected to be in this study will be explained in detail in the latter section.

Formulations of the polymer composition of food simulants studied in this thesis were given in Table 2.2.

Table 2. 2. Composition of the simulants used in the study

Food Simulant Name	Curdlan (%)	Corn Starch (%)	Methyl Cellulose (%)	Agar (%)
C3_CS10	3	10	0	0
C3_CS15	3	15	0	0
C3_CS20	3	20	0	0
MC5_A2	0	0	5	2
MC5_A3	0	0	5	3
MC5_A4	0	0	5	4

Predetermined amounts of all hydrocolloid types that were determined by the preliminary studies were weighed and added to beakers with the ultrapure water. The stock solutions were stirred with magnetic stirrer (CMAG HS 7, IKA, Staufen, Germany) until polymers were well hydrated and afterwards homogenized at 3000 rpm for 5 minutes by using a high-speed homogenizer (T18 Digital UltraTurrax, IKA, Staufen, Germany). The polymer solution was poured into rectangular silicon molds with 11*7*2 cm dimensions (L*W*H) in Figure 2.1 to obtain almost same geometries with meat samples. The polymer solution was aged at room temperature for 4 hours to allow gel formation and obtain more uniform and rigid gel structures. After gel formation, the samples were placed into freezer bag for the freeze-thaw cycle. Preparation of each hydrogel was described separately.



Figure 2. 1. Food simulants after molding to silicone molds

The detailed preparation of food simulants is given in Figure 2.2 and Figure 2.3.

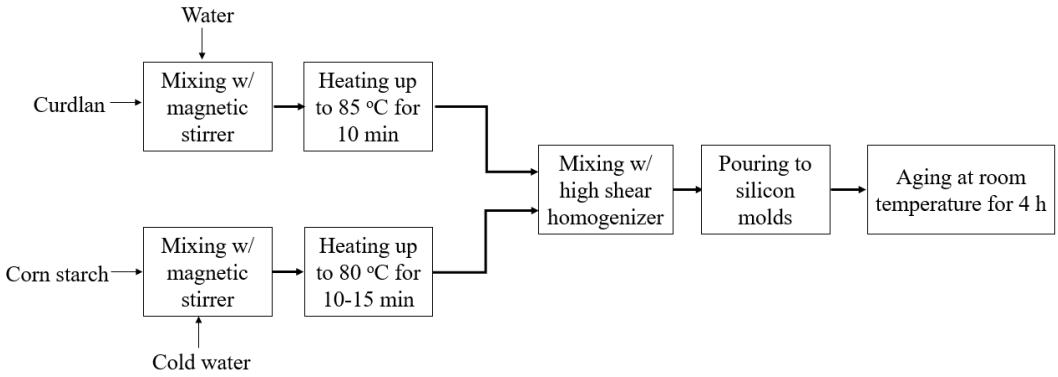


Figure 2. 2. Production flow chart of curdlan-based food simulants

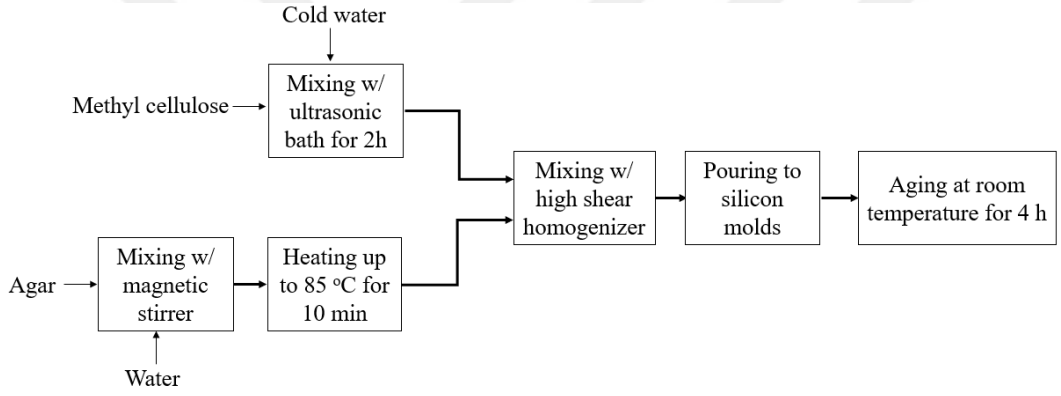


Figure 2. 3. Production flow chart of methyl cellulose-based food simulants

2.2.1.2 Sample preparation for freezing-thawing cycle

Following the preparation of hydrogels, before the freezing thermocouples were inserted into the geometric center of each food simulant, along the horizontal axis of the rectangular shape. Simulants were put into freezer bags and placed to home-type upright freezers (Arçelik, Eskişehir, Turkey) at two different temperatures (-18°C and -27°C) for 20 hours.

Three thermocouples were also placed on the three shelves of the upright freezer, top, middle, and bottom. These thermocouples were connected to the channels of the

data acquisition system that had a 20-channel multiplexer (Agilent 34901A, LXI Data Acquisition/Switch Unit, Farnell, UK) with an accuracy of ±0.01°C. Time-temperature plots were acquired continously during the process.

Temperature data were collected during the freezing-thawing cycles. The initial temperature of food simulants was adjusted to 10°C as in the method of Islam et al. (2014). In the calculation of freezing rate, the change of temperature was recorded from 10°C to -18°C and from 10°C to -27°C.

Freezing rate of each sample was calculated using the following formula:

$$\text{Freezing rate } (\Delta T^{o}C)/h) = \frac{T_{initial}\,(^{o}C) \; - \; T_{final}\,(^{o}C)}{\text{time (h) taken from } T_{initial} \text{ to } T_{final}} \qquad (1)$$

Food simulants were frozen (-18°C, 20 h and -27°C, 20 h) and thawed at the room temperature (25°C) for 4 hours as in the freezing-thawing method of Lee et al. (2002).

2.2.1.3 Drip loss measurements for food simulants

Drip loss of food simulants were measured by a special setup. Food simulants that were placed on a wire rack were weighed and recorded.



Figure 2. 4. Schematic representation of special setup used to thaw food simulants

Drip loss of the samples during thawing was calculated by measuring the weights of the samples with a precision balance (MS6002SDR Mettler Toledo, Giessen, Germany). Following freezing, the samples were taken out of the refrigerators at -18 and -27°C and weights of the samples (A) were recorded. The samples were removed from the wire rack immediately after thawing. The surface of the samples was wiped with a dry absorbent paper to remove the water that was released and then the weight of them (B) was recorded. Measurements were conducted hourly for 4 hours throughout the thawing stage. Drip loss was calculated according to the following formula:

$$\text{Drip Loss (\%)} = \frac{A_{\text{(weight of frozen sample)}} - B_{\text{(weight of thawed sample)}}}{A_{\text{(weight of frozen sample)}}} \times 100 \quad (2)$$

2.2.1.4 Hardness of food simulants

TA Plus Texture Analyzer (LLOYD Instruments, TA Plus Ametek, UK) was used to measure the hardness of the food simulants. Simulant samples were cut into 2 cm cubes. Cylinder probes with 6 mm and 8 mm diameter were used for curdlan-based and methyl cellulose-based simulants, respectively. Test mode was selected as the compression and the instrument was set to a speed of 100 mm/min. Measurements were conducted immediately before freezing (i.e. after aging of hydrogels) and after the completion of the freeze-thaw cycle. All measurements were performed in replicates and the hardness values were recorded. After freeze-thaw cycle, the percentage change in hardness of food simulants were also calculated as following:

$$\text{\% Change} = \frac{\text{Hardness}_{\text{after FT cycle}}\,(\text{N}) - \text{Hardness}_{\text{before FT cycle}}\,(\text{N})}{\text{Hardness}_{\text{before FT cycle}}\,(\text{N})} \times 100 \quad (3)$$

2.2.1.5 Water holding capacity (WHC) of food simulants

Water holding capacity of the food simulants was measured according to the method of Chen et al. (2020), with minor revisions. The gel samples (1.5 g) were weighed accurately and then put into a 15 mL centrifuge tube (W_1). After centrifuging at 3000×g at 4°C for 20 min, water in the top of centrifuge tube was wiped with filter paper and then the weight of gel sample and centrifuge tube was recorded (W_2). Experiments were done before and after freeze-thaw process of the samples. For the calculation of water holding capacity, the following equation was used:

$$\text{WHC (\%)} = \frac{W_{2(\text{weight of gel sample + tube after centrifugation})}}{W_{1(\text{weight of gel sample + tube before centrifugation})}} \times 100 \quad (4)$$

The percentage change between WHC values before and after freeze-thaw cycle was calculated as following:

$$\text{\% Change} = \frac{\text{\% WHC}_{\text{before FT cycle}} - \text{\% WHC}_{\text{after FT cycle}}}{\text{\% WHC}_{\text{before FT cycle}}} \times 100 \quad (5)$$

2.2.1.6 Nuclear Magnetic Resonance (NMR) Relaxometry experiments for food simulants

A benchtop NMR system operating at a ^1H frequency of 20.35 MHz and equipped with a 10 mm diameter radio frequency coil (Resonance Systems GmBH, Kircheim unter Teck/Germany) was used. A Carr-Purcell-Meiboom-Gill (CPMG) sequence was used to find T_2 relaxation times with 2 ms echo time. Acquisition parameters used for CPMG sequence changed for different hydrogel formulations as given in Table 2.3. NMR experiments were done for all food simulants both just before freeze-thaw cycle and after freeze-thaw cycle at room temperature. T_2 relaxation

times for each sample were conducted in triplicates and analyzed by using MATLAB (Mathworks Inc, U.S.A) with mono and biexponential fitting.

Table 2. 3. Parameters used for CPMG Pulse Sequence throughout the experiments

Food simulants	Repetition time (ms)	Number of scans	Echo time (μs)	Number of echoes
C3_CS10	8000	4	2000	1000-1200
C3_CS15	8000	4	2000	900-1500
C3_CS20	8000	4	2000	800-900
MC5_A2	8000	4	2000	600-950
MC5_A3	8000	4	2000	600-700
MC5_A4	8000	4	2000	450-600

After freeze-thaw cycle, the percentage changes in T_2 relaxation times of food simulants were calculated with the equation below.

$$\% \text{ Change} = \frac{T_{2 \text{ after FT cycle}}(\text{ms}) - T_{2 \text{ before FT cycle}}(\text{ms})}{T_{2 \text{ before FT cycle}}(\text{ms})} \times 100 \quad (6)$$

2.2.1.7 Scanning Electron Microscopy (SEM) experiments for food simulants

Images of the food simulants were obtained by a scanning electron microscope (JEOL, JSM 6400, Tokyo, Japan) at METU Central Laboratory. Before the measurements, all samples were freeze-dried. Freeze-dried samples were sticked to the metal stubs and then covered with gold-palladium alloy. The experiments were conducted at 100× and 500× magnification for all samples with an accelerating voltage of 30 kV.

2.2.2 Preparation of '*Real Food*' samples to compare with the simulants

Sirloin and chicken breast meats that were purchased as meat samples were cut into rectangular shape. Meats with same mass (160±5) and geometry with 11*7*2 cm dimensions (L*W*H) were prepared and obtained. All samples were put into freezer bags shortly before freezing.

2.2.2.1 Drip loss measurement of '*Real Food*' samples

Drip losses of sirloin and chicken breast samples were measured as simulants.

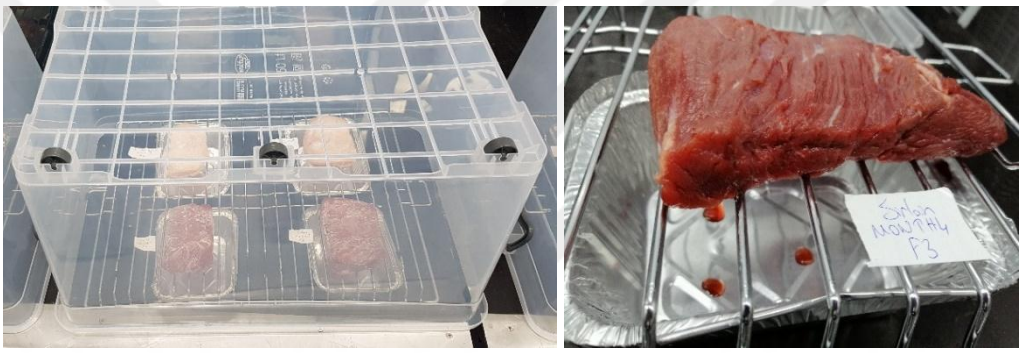


Figure 2. 5. Schematic representation of special setup used to thaw meat samples

Some preliminary experiments were done to determine the storage time during freezing for real food samples. Real food samples, sirloin and chicken breast, were stored in 3 household appliances (2 different models) with three different temperature settings (-18^{o}C, -20^{o}C and -27^{o}C) during 10 various storage periods such as 24 h, 1 week, 2 weeks, 3 weeks, 4 weeks, 8 weeks, 12 weeks, 16 weeks, 20 weeks and 24 weeks. Real food samples were thawed for up to 6 hours. However, since samples were already thawed according to the temperature-time graph and no drip in the aluminum cup under meat samples was observed after 4 hours, the thawing process was kept as 4 hours. Also, real food samples were stored at -18^{o}C and -27^{o}C for both 20 h and 24 h to determine overall freeze-thaw cycle time. In the light of these preliminary studies, total freeze-thaw cycle time was determined as 24 hours.

2.2.2.2 Temperature measurements of '*Real Food*' samples

Temperature data were collected from temperature sensors placed in the center of each meat sample used as done for food simulants. Meat samples were stored in the same freezers that were used in the experiments of food simulants.

2.2.3 Statistical analysis

For statistical analysis, Minitab V18 (Minitab Inc., UK) was used. All measurements were repeated in at least triplicate. Analysis of variance (ANOVA) and Tukey's comparison test at 95% confidence interval were run to determine whether there was significant difference measured experimental parameters. The statistical analysis results are given in the Appendix section.

2.3 Experimental Design

The experimental designs of food simulants and 'real food' samples are summarized in Table 2.4, Table 2.5, and Table 2.6.

Table 2. 4. Experimental design for food simulants

Factors	**Levels**	**Responses**	
		Experiments	Measurement Time (h) *(during FT cycle)*
Food Simulant Type	Curdlan 3%+CS 10% Curdlan 3%+CS 15% Curdlan 3%+CS 20% MC 5%+Agar 2% MC 5%+Agar 3% MC 5%+Agar 4%	Hardness	0, 24
		Drip Loss	20, 21, 22, 23, 24
		WHC	0, 24
Freezing Temperature (^oC)	-18, -27	NMR	0, 24
		SEM	0, 24
Thawing Temperature (^oC)	25	Center Temperature	0-20, 20-24

Table 2. 5. Experimental design for '*Real Food*' samples (Part 1)

Sample	**Storage Period**	**Freezing Temperature (ºC)**	**Thawing Temperature (ºC)**	**Experiments**
Sirloin Chicken Breast	24h, Week 1, 2, 3, 4, 8, 12, 16, 20, 24	-18 -20 -27	25	Drip Loss Temperature

Table 2. 6. Experimental design for *'Real Food'* samples (Part 2)

Sample	**Freezing Temperature (ºC)**	**Thawing Temperature (ºC)**	**Experiments**	**Measurement Time (h) (during freeze-thaw cycle)**
Sirloin	-18	25	Drip Loss	20, 21, 22, 23, 24
Chicken Breast	-27		Temperature	0-20, 20-24

CHAPTER 3

RESULTS AND DISCUSSION

The experimental results were investigated for both the real food samples, i.e. sirloin and chicken breast, and food simulants prepared with different hydrocolloids at different concentrations (Table 2.2).

3.1 Drip Loss

One of the most important quality indicators for foods exposed to freeze-thaw cycle is the drip loss. Drip loss is also equivalent to the syneresis of a hydrogel. In this study, syneresis of the hydrogels and drip loss of 'real food' samples have been evaluated.

'Real Food' samples

Preliminary studies were conducted with the real food samples to determine the test conditions for freezing and subsequent thawing processes. For this data set, thawing conditions were kept constant as 25 ^{o}C for 4 hours. As shown in the Table 3.1, the percentage drip loss of 'real food' samples increased with increase in storage time.

Effects of freezing and thawing on drip loss were studied by Ngapo et al. (1999). It was shown in the study that high freezing rates resulted in low drip losses. They investigated the effects of six different freezing rates, two storage period and three different thawing rates on the drip loss of pork samples. Their results showed that there was no significant difference between the drip losses of the samples after 4 weeks storage period.

On the contrary, many researchers illustrated that there was a significant effect of storage period throughout the freezing on drip loss (Augustyńska-Prejsnar et al., 2018; Farouk & Swan, 1998). Also, it was observed that freezer temperature and the

storage duration played a vital role in drip loss of meat samples. When the storage of meat samples was increased from 24 h to 72 h, there was not a significant difference in drip loss of meats stored for 72 h across different freezer temperatures. Furthermore, it was found that drip loss of meats stored at -18^{o}C and -40^{o}C for 24 h or 72 h was very close to each other and did not differ significantly compared to those stored at 4^{o}C and -10^{o}C (Ab Aziz et al., 2020).

In this study, initially 3 different freezing temperatures were tested for the real food samples. Like the above-mentioned studies, no significant difference between different freezer temperatures was mostly observed ($p>0.05$) for the drip loss of both meat types that were stored at a long period of time, i.e. greater than 24 h. In addition, no significant difference was observed between the freezing times of chicken breast and sirloin samples at -18 and -20 ^{o}C ($p>0.05$) after 24 hours and 3 weeks of storage periods, respectively. That is why, for the freezing temperatures to be tested for the simulants; -18 ^{o}C and -27 ^{o}C were selected for the rest of the study to compare the drip loss results for the simulants.

On the other hand, storage time was significant on the drip loss ($p<0.05$). The drip loss for sirloin samples (at all temperatures) and chicken breast samples (at -20 and -27 ^{o}C) stored for 24 weeks was almost 2 times higher than those stored for 24 hours.

In this study, the goal is to design a food simulant to mimick the drip loss of meat samples. Using a longer freezing time would definitely yield higher drip losses which was confirmed with the real food samples. However, even at short freezing times, reasonable amount of drip loss was observed (~2-2.5%) for both meat types. Furthermore, there was no significant difference between 20 and 24 hours of storage times on total drip loss (%) of both 'real food' samples stored at -18 and -27 ^{o}C ($p>0.05$). That is why for the simulants that will be designed afterwards freezing time was kept shorter at 20 hrs.

For the experiments performed in Table 3.1 thawing time was kept as 4 hrs as stated.

Table 3. 1. Drip (weight) loss (%) of 'real food' samples following freezing at different temperatures for different storage periods and subsequent thawing (associated with *Experimental Design Table 2.5*)

	Storage period									
Freezer temperature (ºC)	24 h	Week 1	Week 2	Week 3	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Sirloin										
-18 (Freezer A)	2.02±0.12 ^{ab,B}	1.99±0.22 ^{ab,B}	2.12±0.28 ^{ab,B}	2.53±0.23 ^{a,B}	2.08±0.25 ^{a,B}	2.13±0.36 ^{a,B}	4.25±0.28 ^{ab,A}	4.44±0.79 ^{a,A}	3.95±0.62 ^{a,A}	4.57±0.23 ^{a,A}
-27 (Freezer A)	1.67±0.24 ^{b,C}	1.45±0.26 ^{b,C}	1.69±0.00 ^{b,C}	1.72±0.21 ^{b,C}	2.09±0.07 ^{a,C}	2.08±0.43 ^{a,C}	5.06±0.71 ^{a,AB}	5.24±0.72 ^{a,A}	3.78±0.74 ^{a,B}	4.04±0.42 ^{a,AB}
-20 (Freezer B)	2.15±0.19 ^{a,B}	2.53±0.17 ^{a,B}	2.91±0.51 ^{a,B}	3.10±0.04 ^{a,B}	2.47±0.04 ^{a,B}	2.91±0.55 ^{a,B}	3.12±0.23 ^{b,B}	5.01±0.59 ^{a,A}	4.61±0.28 ^{a,A}	4.54±0.55 ^{a,A}
Chicken breast										
-18 (Freezer A)	2.36±0.23 ^{ab,D}	3.14±0.31 ^{a,CD}	2.89±0.35 ^{a,CD}	2.93±0.27 ^{a,CD}	3.90±0.03 ^{a,BC}	3.54±0.43 ^{b,BCD}	4.66±0.80 ^{a,B}	6.72±0.71 ^{a,A}	3.63±0.47 ^{a,BCD}	2.54±0.08 ^{b,CD}
-27 (Freezer A)	1.72±0.25 ^{b,F}	2.09±0.40 ^{b,EF}	3.09±0.32 ^{a,CDE}	2.51±0.37 ^{a,DEF}	4.59±0.30 ^{a,AB}	3.45±0.56 ^{b,BCDE}	3.45±0.47 ^{a,BCDE}	5.28±0.24 ^{a,A}	3.76±0.13 ^{a,BCD}	4.18±0.65 ^{a,ABC}
-20 (Freezer B)	2.77±0.32 ^{a,C}	3.28±0.26 ^{a,C}	2.85±0.26 ^{a,C}	2.70±0.40 ^{a,C}	3.72±0.51 ^{a,BC}	5.17±0.36 ^{a,AB}	3.42±0.59 ^{a,BC}	6.01±1.14 ^{a,A}	4.33±0.90 ^{a,ABC}	4.47±0.30 ^{a,ABC}

Small letters (a-b) mean that they differ significantly ($p<0.05$) for each food sample between three different freezer temperatures in the same column. Capital ones (A-F) mean that they differ significantly between different storage periods in the same row. Each value was given as the mean ±standard deviation (SD), n=3.

Food simulants

As stated before, drip loss equivalence in a hydrogel is known as the syneresis (*exclusion of water from a gel system*). Gravimetric methods to measure the syneresis are good guidances on the evaluation freeze-thaw stability of starch gels which has also been used as an indicator of the tendency of starch to retrograde. Retrogradation is speeded up from the exposure of starch gels to freeze-thaw cycles (Yuan & Thompson, 1998). At the freezing stage of the starch gel, a phase separation as starch-rich and bulk water phase begins with the formation of ice crystals. Upon thawing process, water is released from the starch gel network and syneresis occurs. With the repeated freeze-thaw cycles, in starch-rich region, phase separation continuously increases due to the increase in amylopectin retrogradation (Karim et al., 2000).

In this study, corn starch was used as one of the gelling agents for the design of food simulants as a co-polymer in curdlan based hydrogels (Table 2.2) and drip loss values were calculated at different freezing temperatures followed by 4hrs of thawing time (Table 3.2).

It was observed that the addition of corn starch at different concentration levels to the formulations considerably influenced the syneresis of curdlan-based gels containing curdlan and corn starch ($p<0.05$). As the added corn starch concentration increased, the syneresis of curdlan-based food simulants frozen at -18^{o}C and -27^{o}C decreased significantly during thawing process ($p<0.05$).

The effect of waxy corn starch at different concentrations (2.5%, 5%, 10%) on the syneresis of curdlan gel exposed to freeze-thaw cycle has also been studied by Nakao et al. (1991). After freezing and thawing, syneresis rates of 2%, 4% and 6% curdlan gels alone formed by heating at 100-130^{o}C were measured as 35.0%, 20.6% and 10.3%, respectively. The syneresis values of 2 and 4% curdlan gels decreased from 35.0% and 20.6% to approximately 2% with the addition of 5% waxy corn starch. It was reported that the syneresis of these curdlan gels mixed with 10% starch concentration was the lowest, even less than 2%.

Table 3. 2. Comparison of total drip loss (%) of 'real food' samples and curdlan-based food simulants

Freezer temperature (ºC)	Samples	Total drip loss (%)
-18ºC	Sirloin	1.79±0.09 ^{cd}
	Chicken breast	1.90±0.18 ^{cd}
	C3_CS10	6.97±0.88 ^{a}
	C3_CS15	3.86±0.41 ^{b}
	C3_CS20	2.86±0.64 ^{bcd}
-27ºC	Sirloin	1.73±0.12 ^{d}
	Chicken breast	1.61±0.07 ^{d}
	C3_CS10	6.24±0.71 ^{a}
	C3_CS15	3.46±0.58 ^{bc}
	C3_CS20	1.65±0.32 ^{d}

All data were given as the mean ± standard deviation (n=3). Small letters (a-d) show that they are significantly different for each sample ($p<0.05$).

The effect of different sugars on syneresis of curdlan gels was investigated by Ishida and Takeuchi (1981). Gels containing 2% of curdlan combined with starch types such as potato, high amylose, corn, soluble, and waxy corn at different concentrations (0%, 2%, 5%, 10%) had lower syneresis values compared to the curdlan gels without starch. It was explained that starch repressed the syneresis of curdlan gels. When the syneresis of curdlan gel with 2% concentration alone was 12%, it was shown that the syneresis values of gels combined with 2%, 5% and 10% of corn starch were 6.8%, 1.6%, and 0.3%, respectively. Curdlan gel mixed with 10% of corn starch had the lowest syneresis value when compared to the others. In fact, that was also the reason why curdlan was not used alone as food simulant. It showed high syneresis.

It is also important to mention the synergestic effect of the two polymers. According to a research done by Yamazaki et al. (2013), corn starch gels had a higher syneresis rate compared to corn starch gels combined with different gums. The addition of different gum types to corn starch gel system resulted in significant reductions in syneresis. It was mentioned this declining trend in syneresis was not only related to starch retrogradation, but also to the thickening ability of hydrocolloid.

Likewise, a recent study discussed that the addition of gum Cordia (GC) to corn starch gels reduced the percentage syneresis. It was stated that the gum Cordia interaction with amylose either caused limitation of the amylose-amylose interaction or binding extra water from the system, resulted in decreased syneresis rates. Also, if higher gum concentration was added to the corn starch gel systems, there could be a reduction in syneresis due to the formation of gum-gum regions. Furthermore, other researchers have also reported that addition of hydrocolloid gums (xanthan gum, xyloglucan, locust bean gum) enhanced the freeze-thaw stability of different starch systems (Arocas et al., 2009; Hussain et al., 2020; Pongsawatmanit et al., 2006; Sikora et al., 2008).

In another research, the effects of freezing rate on retrogradation rate, i.e. syneresis of starch gels both for with and without hydrocolloids, were examined during the five freeze-thaw (FT) cycles. Effect of different freezing rates (0.06, 0.09 and 2.30^{o}C/min) was shown to have a significant effect in the 1st FT cycle. High syneresis rate was observed in starch gels with or without hydrocolloids at a low freezing rate (Muadklay & Charoenrein, 2008).

In our case, as shown in Table 3.2, when the effect of both two different freezer temperatures (-18 and -27^{o}C) and corn starch at different concentrations (10%, 15%, 20%) on the syneresis of curdlan-corn starch food simulants was examined, significant differences were detected ($p<0.05$). Significant differences in the percentage drip loss were noticed among C3_CS15 samples and C3_CS20 samples frozen at -18^{o}C and -27^{o}C. There was no significant difference between the percentage drip loss results of C3_CS10 samples frozen at -18 and -27^{o}C ($p>0.05$).

The results demonstrated an interaction between curdlan and corn starch, which was consistent with the literature. The addition of corn starch to curdlan gels resulted in reduction in drip loss percentage since corn starch suppressed the syneresis of curdlan gels. C3_CS20 samples had lower drip loss percentage than C3_CS10 and C3_CS15 samples. Also, low drip loss percentage was observed in curdlan-corn starch gels frozen at a high freezing rate.

The 2nd set of hydrogel-based food simulants were formulated by using agar and methyl cellulose combinations. The concentrations of the hydrocolloids were determined from preliminary experiments as stated in Section 2.

Table 3. 3. Comparison of total drip loss (%) of 'real food' samples and methylcellulose-based food simulants

Freezer temperature (ºC)	**Samples**	**Total drip loss (%)**
-18ºC	Sirloin	1.79±0.09 ^{ab}
	Chicken breast	1.90±0.18 ^{ab}
	MC5_A2	2.48±0.31 ^{a}
	MC5_A3	1.78±0.56 ^{ab}
	MC5_A4	1.38±0.06 ^{b}
-27ºC	Sirloin	1.73±0.12 ^{ab}
	Chicken breast	1.61±0.07 ^{ab}
	MC5_A2	1.49±0.13 ^{b}
	MC5_A3	1.74±0.20 ^{ab}
	MC5_A4	1.26±0.06 ^{b}

The results were given as the mean ± standard deviation (n=3). Small letters (a-b) mean that they are not significantly different ($p<0.05$).

As seen in Table 3.3, methyl cellulose-based food simulants formulated with 3% and 4% concentrations of agar solutions have a lower syneresis (*drip loss*) than that of 2%, except the ones stored -27^{o}C. The effects of both two different freezer temperatures (-18 and -27^{o}C) and agar concentrations (2%, 3%, 4%) for methyl cellulose-agar food simulants in terms of the syneresis were significant ($p<0.05$).

In a research, the freeze-thaw stability of the 1.0% w/v agar and 1.0% w/v MC combination hydrogels was studied. After freeze-thaw cycle, it was analyzed that the weight of hydrogels decreased to 63±3% of its initial mass. It was stated that it was most probably related to the formation of ice crystals at freezing stage. It has been underlined that these ice crystals caused physical disruption of the gel network and then a weak gel structure to be obtained during the thawing, which lead to syneresis (Thompson et al., 2017). A similar effect was also observed in our study, but in our case syneresis was less since higher concentrations were used in the formulations.

Also, from Table 3.3, it was concluded that there was no significant difference between MC5_A3 and 'real food' samples frozen at -18^{o}C and -27^{o}C in terms of total drip loss results ($p>0.05$).

3.2 Hardness Analysis

Textural properties of hydrogels are important for evaluating the freeze-thaw stability of hydrogels. Hardness value (N) is the maximum force required to compress the sample (Marfil et al., 2012). Hardness is an indication of the gel strength and structure under compression (Calvarro et al., 2016).

Table 3.4 shows the hardness results of the curdlan-based and methyl cellulose-based food simulants before (after aging of the hydrogels) and after freeze-thaw cycle. The effects of freezer temperature and food simulant type on the hardness values of food simulants were examined. For each sample stored at a given freezer temperature, there was a significant difference between the hardness values before and after the freeze-thaw cycle ($p<0.05$).

For curdlan-corn starch hydrogels, as starch concentration increased, hardness values before FT cycle increased ($p<0.05$). Moreover, for these hydrogels, at both freezing temperatures, hardness values increased after thawing.

Effect of freezer temperature on the final hardness values of curdlan- corn starch hydrogels was also found not to be significant ($p>0.05$) whereas the effect of starch concentration was significantly different ($p<0.05$).

It is known that hardness for corn starch gels is mainly influenced by retrogradation since retrogradation is related to the syneresis of water and the crystallization of amylopectin in the starch systems (Miles et al., 1985). In starch gel system, starch would have more consistent gelatinization and harder gels would be obtained after the retrogradation of amylose and amylopectin (Zhang et al., 2017).

For methyl cellulose-based samples, there was no significant difference between the hardness values of MC5_A3 and MC5_A4 before FT cycle ($p>0.05$). Effects of different freezer temperatures (-18 and -27^{o}C) and agar concentrations (2, 3 and 4%) in hardness values after FT cycle were found to be significant ($p<0.05$). The hardness values of methyl cellulose-agar samples at higher concentrations were found to be higher than the others ($p<0.05$). However, the hardness values of MC5_A2 samples frozen at -18 and -27^{o}C and subsequently thawed were not significantly different ($p>0.05$). The hardness values for all methyl cellulose-agar samples decreased significantly ($p<0.05$) upon freeze-thawing in agreement with Harnkarnsujarit et al. (2016). Their study, which investigated the effect of freezing temperature (-20 ^{o}C, -50 ^{o}C, -90 ^{o}C) on mechanical (hardness) changes of freeze-thawed maltodextrin-agar gels, showed that fresh agar gels containing high dextrose equivalents of maltodextrin had harder structures. After freeze-thawing process, the hardness values for all different combinations reduced drastically since freezing process induces ice-solute phase separation and irreversible changes in junction networks.

Table 3. 4. Hardness (N) values of food simulants before and after freeze-thaw cycle

Freezer temperature (°C)	**Food Simulants**	**Hardness (N)**	
		Before FT cycle	After FT cycle
Curdlan-based			
-18°C	C3_CS10	0.13±0.02 ^{c,B}	1.19±0.12 ^{de,A}
	C3_CS15	0.31±0.08 ^{b,B}	1.33±0.06 ^{d,A}
	C3_CS20	0.49±0.09 ^{a,B}	2.50±0.11 ^{a,A}
-27°C	C3_CS10	0.13±0.02 ^{c,B}	1.07±0.11 ^{e,A}
	C3_CS15	0.31±0.08 ^{b,B}	1.73±0.02 ^{c,A}
	C3_CS20	0.49±0.09 ^{a,B}	2.01±0.09 ^{b,A}
Methylcellulose-based			
-18°C	MC5_A2	0.86±0.08 ^{b,A}	0.34±0.03 ^{e,B}
	MC5_A3	1.83±0.05 ^{a,A}	0.81±0.09 ^{c,B}
	MC5_A4	1.68±0.43 ^{a,A}	1.23±0.06 ^{a,A}
-27°C	MC5_A2	0.86±0.08 ^{b,A}	0.33±0.02 ^{e,B}
	MC5_A3	1.83±0.05 ^{a,A}	0.57±0.02 ^{d,B}
	MC5_A4	1.68±0.43 ^{a,A}	0.95±0.03 ^{b,B}

The results were given as the mean ± standard deviation (n=6). Small letters (a-e) indicate that they are significantly different for each sample frozen at different freezer temperatures in the same column. Capital letters (A-B) indicate that they are significantly different for each sample and freezer temperature in the same row ($p<0.05$).

After freeze-thaw cycle, the percentage changes in hardness values of food simulants are given in the Figure 3.1. The textural changes for curdlan-based and methylcellulose-based samples in terms of hardness (N) did not follow the same trend after they were frozen and subsequently thawed. There was an increase in hardness values of curdlan-based simulants after freeze-thaw cycle whereas there

was a decrease in that of methylcellulose-based simulants for both freezer temperatures.

The highest percentage change among the curdlan-based samples was seen in the C3_CS10 stored at -18^{o}C and -27^{o}C samples. Different than the samples stored -27^{o}C, the percentage change in hardness results of C3_CS20 frozen at -18^{o}C was higher than that of C3_CS15, which was probably based on the starch dilution effect.

In a current study done by Hussain et al. (2020), it was reported that corn starch gel solutions that were mixed with different replacement levels of the gum Cordia (GC) (0%, 3%, 6%, 9%, 12%), a non-conventional hydrocolloid, the hardness values of the samples were higher compared to control sample containing no gum, but the samples containing 9% and 12% gum had less hardness values than 6%. It was mentioned that the reason for this discrepancy would be the starch dilution effect because increase in gum concentration reduced the amylose content and weakened the its network.

For the methylcellulose-based samples, the lowest percentage change in hardness values occurred in samples of MC5_A4 stored at -18^{o}C, followed by the ones stored at -27^{o}C. The effects of freezer temperature and different agar concentration levels in percentage hardness changes for methyl cellulose-based samples were significantly noticed ($p<0.05$).

The percentage changes in hardness values of curdlan-based and methyl cellulose-based samples with the lowest concentration stored at both -18 and -27^{o}C were not significantly different ($p>0.05$).

The study conducted by Shang et al. (2021) indicated that the addition of different starches (amylose and amylopectin) at different concentration levels of 0-5% to konjac glucomannan gels reduced the syneresis rate but increased the hardness before and after FT cycle. The percentage changes of syneresis rate of 1% and 4% of konjac glucomannan-starch samples were approximately 32% and 15%. The

hardness values of unfrozen samples with amylose of 1% and 4% increased from ~5.3N to 27.53 N and ~7N to 53.38N.

Consistent with the literature, there was a relationship between the percentage changes in hardness values and total drip loss (%). The percentage change (%) in hardness values of food simulants had a positive correlation with total drip loss percentage ($r= 0.866$, $p<0.05$). It can be said that as curdlan-based or methyl cellulose-based samples with the lowest corn starch or agar concentration had the highest drip loss results, these samples had the highest percentage change in hardness values. The results of our study are consistent with the findings of the recent study mentioned above.

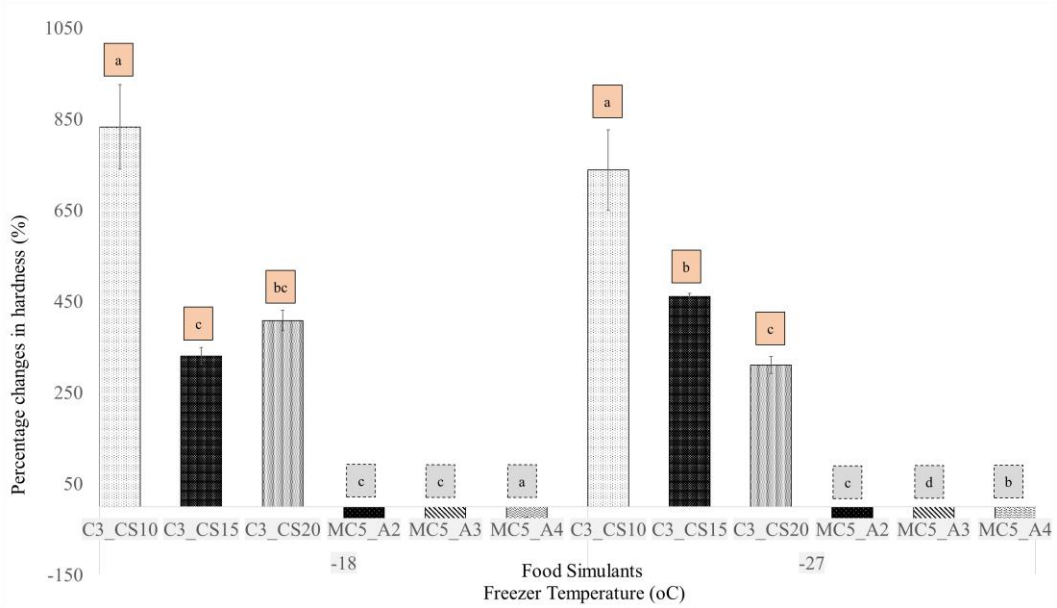


Figure 3. 1. The percentage changes in hardness values of the food simulants frozen at -18^{o}C and -27^{o}C temperatures. Means ± standard deviation values (n=6) followed by small letters (a-d) within the graph are significantly different for samples, separately ($p<0.05$)

3.3 Water Holding Capacity (WHC)

Water holding capacity is one of the most critical quality parameters for the meats and food gels because it is relevant to its stability, functional properties, texture, and microbial safety (Kruif et al., 2015). It is directly influenced from muscle structure, location of water in muscle, some physical or biochemical factors in muscle like net charge affect, genetic factors and steric effects and postmortem proteolysis like protein oxidation. All these factors have a key role in explaining the mechanism of water retention capacity. This mechanism is centered in the water binding structures and the proteins, especially the myofibrillar protein (Huff-Lonergan & Lonergan, 2005).

The water holding capacity in meats changes due to the changes occurred in the cells and extracellular components, which is mostly caused by freeze storage period, freezing and thawing process conditions (Geiges, 1996; Leygonie et al., 2012). Low freezer temperatures and longer storage times resulting in higher drip loss and decrease in water holding capacity of frozen beef and chicken breast have been confirmed by some studies (Añón & Calvelo, 1980; Vieira et al., 2009).

The combination of decrease in storage temperature and increase in storage period caused an increase in the formation of ice crystals and ruptured the tissue membrane. As a result of these, the water retention ability of muscles was affected (Ab Aziz et al., 2020).

In this study, effects of freezer temperature on the water holding capacity values of different hydrogels were also investigated. Results are given in Table 3.5.

Before curdlan-based and methylcellulose-based samples were frozen, the water holding capacity values of all samples were not significantly affected by concentration of second hydrocolloid (corn starch or agar) ($p>0.05$). On the contrary, there was a significant difference between the water holding capacity values of all samples after freeze-thaw cycle ($p<0.05$).

Table 3. 5. Water holding capacity (%) values of food simulants before and after freeze-thaw cycle

Freezer temperature (ºC)	**Food Simulants**	**WHC (%)**	
		Before FT cycle	After FT cycle
Curdlan-based			
-18ºC	C3_CS10	99.31±0.52 [a]	94.86±1.50 [abc]
	C3_CS15	99.22±0.58 [a]	92.83±1.34 [c]
	C3_CS20	99.76±0.18 [a]	95.40±0.92 [ab]
-27ºC	C3_CS10	99.31±0.52 [a]	93.79±0.67 [bc]
	C3_CS15	99.22±0.58 [a]	94.50±1.28 [abc]
	C3_CS20	99.76±0.18 [a]	96.59±1.31 [a]
Methylcellulose-based			
-18ºC	MC5_A2	99.88±0.11 [a]	99.83±0.14 [a]
	MC5_A3	99.95±0.07 [a]	99.90±0.12 [a]
	MC5_A4	99.83±0.17 [a]	99.95±0.08 [a]
-27ºC	MC5_A2	99.88±0.11 [a]	99.52±0.32 [b]
	MC5_A3	99.95±0.07 [a]	99.83±0.14 [a]
	MC5_A4	99.83±0.17 [a]	99.74±0.06 [ab]

Small letters (a-c) mean that they are significantly different for separately each curdlan-based and methylcellulose-based sample in the same column ($p<0.05$). All data were given as the mean ± standard deviation (n=6).

Considering the relationship between drip loss and water holding capacity, it can be said that this relation will be inverse for these samples since high drip loss, i.e. high-water release from the samples, will lead to less water retention at the end of the freeze-thaw cycle. There was a negative relationship between the percentage drip

loss results and water holding capacity values after FT cycle for food simulant samples ($r = -0.704$, $p<0.05$).

For the combinations of curdlan and corn starch frozen at -18°C and -27°C, the one with the highest concentration had the highest water holding capacity after freeze-thaw cycle and the lowest drip loss during thawing, which was not surprising. The addition of corn starch at different concentration levels to curdlan gel significantly influenced the syneresis and water holding capacity of curdlan-based hydrogel exposed to freeze-thaw cycle. C3_CS20 samples frozen at -18°C and -27°C had the lowest rate of syneresis (approximately 2.86% and 1.65%, respectively) throughout the thawing and had the highest water holding capacity values among the curdlan-based samples after freeze-thaw cycle.

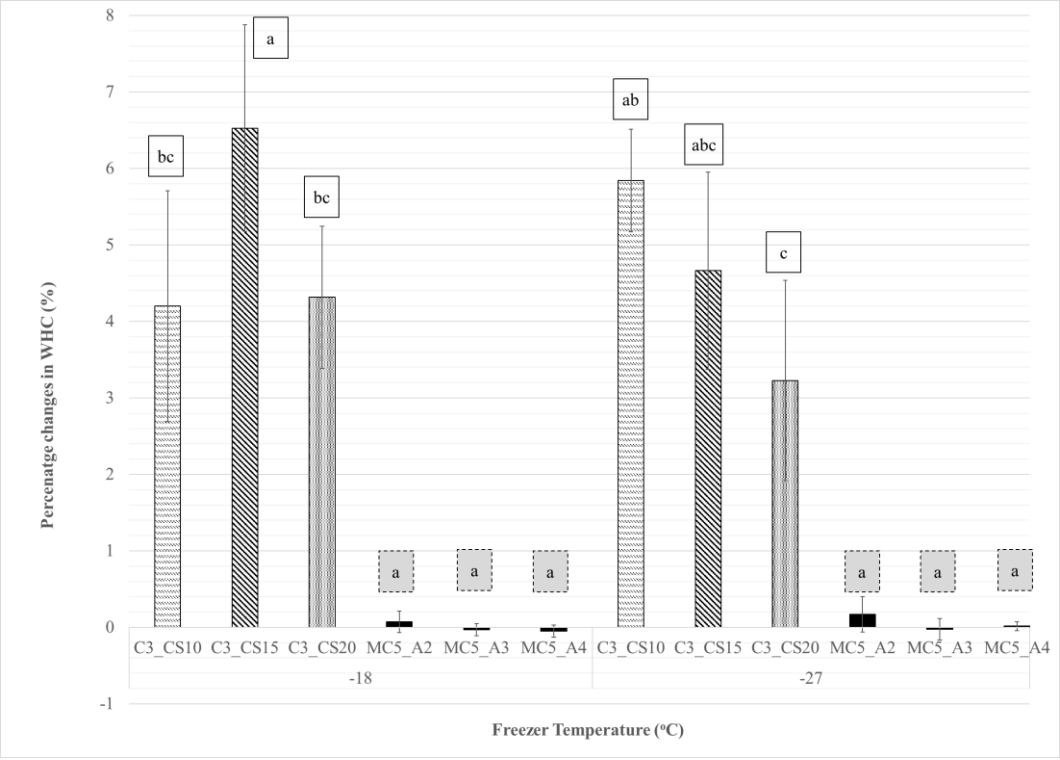


Figure 3. 2. Changes in WHC values of the food simulants frozen at -18°C and -27°C temperatures. Small letters (a-d) mean that they differ significantly for each sample frozen at different freezer temperatures. Error bars represent ± standard deviation.

Thus, the percentage change in the water retention capacity of curdlan-based samples with high concentrations was lower than other samples as shown in Figure 3.2. Among the samples stored at -18ºC, C3_CS15 had the highest percentage change in water holding capacity results. The reason could be curdlan-amylose interactions. In the study conducted by Hussain et al. (2020), it was stated that addition of gum Cordia (GC) at different concentrations induced less syneresis rates (i.e. high water retention capacity) and that was probably caused by GC-amylose interaction that limited amylose-amylose interactions or binding of extra water in the system.

On the other hand, it was seen that the percentage change in water holding capacity of both curdlan-based and methylcellulose-based samples frozen at two different freezer temperatures did not differ significantly ($p>0.05$).

It was concluded that there was a strong relation between total drip loss (%) and the percentage changes in water holding capacity (%) of the food simulants. The drip loss (%) of curdlan-based food simulants frozen at -18ºC in ascending order were as follows C3_CS10 > C3_CS15 > C3_CS20 and the percentage change in water holding capacity (%) of the same samples were as follows C3_CS15 > C3_CS10 = C3_CS20. Likewise, the drip loss (%) of curdlan-based food simulants frozen at -27ºC in ascending order were as follows C3_CS10 > C3_CS15 > C3_CS20 and the percentage change in water holding capacity (%) of the same samples were as follows C3_CS10 > C3_CS15 > C3_CS20.

Pearson correlation analysis indicated that there was a positive correlation between the total drip loss (%) and the percentage changes in water holding capacity (%) of food simulant samples ($r= 0.684$, $p<0.05$). Besides, there was a negative correlation between the percentage total drip loss and water holding capacity results of food simulant samples after FT cycle ($r=-0.704$, $p<0.05$).

The effect of the different starches (amylose and amylopectin) and freezing conditions on water holding capacity, syneresis rate and the hardness of konjac glucomannan gels exposed to freeze-thaw (FT) cycle was investigated by Shang et al. (2021). The water holding capacity results of frozen konjac glucomannan-stach

gels with different starch concentrations (0, 1, 2, 3, 4 and 5%) increased with the increased starches content whereas the syneresis rate after FT cycle decreased significantly ($p<0.05$). Also, the hardness of unfrozen gels with amylose increased when starch concentration increased from 0% to 4%. However, the hardness values of gels with amylopectin gels reduced when starch concentration exceeded 3%. It was also stated that the hardness of gel samples with amylose were higher than those of samples with amylopectin.

Similarly, relationship between change in water holding capacity of the food simulants exposed to freeze-thaw cycle and change in hardness values of these samples was observed. There was positive correlation between these properties ($r= 0.827$, $p<0.05$).

3.4 Freezing-Thawing Curves

Investigation of the freeze-thaw curve is a good pathfinder for simulating the real food samples in terms of drip loss behavior during thawing process with an artificial material.

As meat is highly composed of water, the quality of frozen meats is directly influenced by the consecutive freeze-thaw processes prior to consumer consumption. Freezing rate is an important parameter for the formation of small ice crystals and minimization of texture damage and drip loss upon the thawing. Also, thawing process and its time should be taken into consideration to reduce and even prevent the physical and chemical changes in the food and food-like systems (Akhtar et al., 2013).

In our study, the freezing process of the samples was completed in 20 hours followed by a thawing process that lasted for 4 hours. The temperature-time curves for both 'real food' and food simulant samples stored at -18^{o}C and -27^{o}C are given in Figure 3.3 and Figure 3.4, respectively.

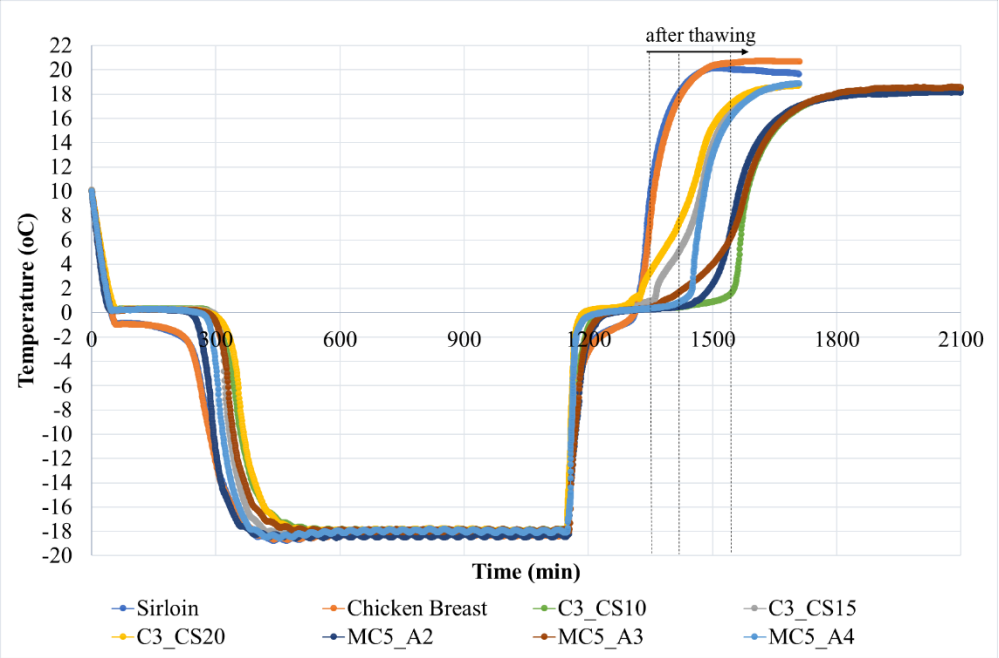


Figure 3. 3. Representative freeze-thaw curves of food simulants and 'real food' samples stored at -18^{o}C

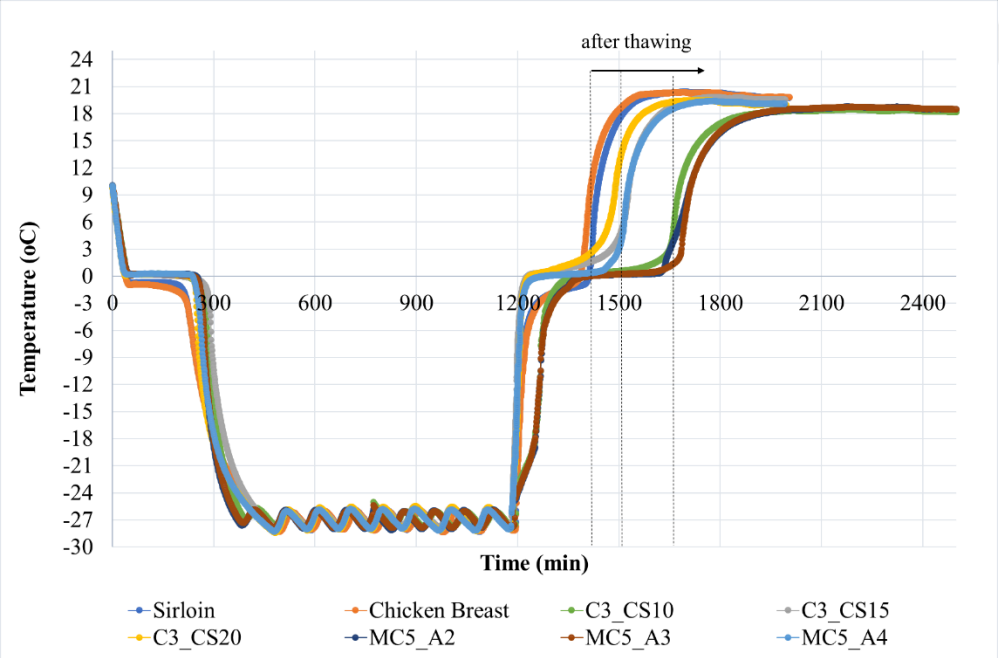


Figure 3. 4. Representative freeze-thaw curves of food simulants and 'real food' samples stored at -27^{o}C

Table 3. 6. The average freezing rates of curdlan-based food simulants and 'real food' samples

Freezer Temperature (ºC)	Samples	Freezing rate (ΔT°C/h)
-18	Sirloin	3.86 ±0.67 [ab]
	Chicken Breast	3.85 ±0.62 [ab]
	C3_CS10	2.96 0.16 [b]
	C3_CS15	4.17 ±0.26 [ab]
	C3_CS20	3.03 ±0.10 [b]
-27	Sirloin	5.48 ±0.78 [a]
	Chicken Breast	5.53 ±0.88 [a]
	C3_CS10	4.62 ±0.20 [ab]
	C3_CS15	5.56 ±0.93 [a]
	C3_CS20	4.78 ±0.43 [ab]

Small letters (a-b) mean that they differ significantly for each sample ($p<0.05$). Data are given mean ± standard deviation.

Table 3. 7. The average freezing rates of methyl cellulose-based food simulants and 'real food' samples

Freezer Temperature (ºC)	Samples	Freezing rate (ΔT°C/h)
-18	Sirloin	3.86 ±0.67 [bc]
	Chicken Breast	3.85 ±0.62 [bc]
	MC5_A2	4.40 ±0.05 [abc]
	MC5_A3	3.05 ±0.25 [c]
	MC5_A4	3.68 ±0.70 [bc]
-27	Sirloin	5.48 ±0.78 [ab]
	Chicken Breast	5.53 ±0.88 [ab]
	MC5_A2	6.14 ±0.20 [a]
	MC5_A3	5.71 ±0.26 [ab]
	MC5_A4	5.34 ±0.36 [ab]

Small letters (a-c) mean that they differ significantly for each sample ($p<0.05$). Data are given mean ± standard deviation.

For each sample stored at two different freezer temperatures, the average freezing rate values were given in Table 3.6 and Table 3.7. The freezing rates were calculated as the ratio of change in temperature to the total freezing time using equation (1).

The freezing rate results of 'real food' samples; sirloin and chicken breast, were significantly different for two different freezer temperatures ($p<0.05$) whereas there was no difference between the freezing rates of sirloin and chicken breast samples stored at -18°C or -27°C ($p>0.05$). The freezing rates of sirloin and chicken breast samples were the same at these freezing temperatures. Sirloin and chicken breast samples stored at -27°C had higher freezing rates and lower percentage drip loss than the ones at -18 °C.

The effect of freezing rates on drip loss from samples of pork was examined by Ngapo et al. (1999). The average drip losses (%) of the samples with the slower freezing rates were significantly different from those of the control (fresh) samples. However, there was no significant difference between drip (weep) loss (occurring within 30 min of removal from the muscle) of fresh samples and drip loss of frozen thawed samples with higher freezing rates. Pork samples frozen at lower rates had higher drip loss rates than fresh samples and samples frozen at higher rates.

Similarly, curdlan-based and methyl cellulose-based food simulants stored at -18°C had lower freezing rate values than those stored at -27°C. As expected, very low freezing temperature (lower than -18°C) had higher freezing rate throughout freezing. C3_CS10 samples frozen at -18°C had the lowest freezing rate between curdlan-based samples at two different freezer temperatures. As a result of this, C3_CS10 samples had the highest total drip loss (%) during thawing process.

An investigation about the effects of different hydrocolloids (XG, LBG, KGM and GG) and different freezing rates (2.30, 0.90 and 0.06 °C/min) on the freeze-thaw stability of tapioca starch gels was carried out (Muadklay & Charoenrein, 2008a). After the first freeze-thaw cycle, it was shown that fast freezing rate reduced percentage syneresis of starch gels, i.e. the starch retrogradation. In this study, 'real food' samples and food simulants with higher freezing rates had lower syneresis.

Methyl cellulose-based samples combined with the lowest agar concentration (MC5_A2) frozen at -27^{o}C had the fastest freezing rate and lowest percentage drip loss. The average freezing rates of methyl cellulose-agar samples stored -18^{o}C were significantly different ($p<0.05$) and lower than those frozen at -27^{o}C. Thus, the percentage drip loss of the samples frozen at -18^{o}C was higher. Since different freezing conditions influence ice crystal sizes in freeze-thawed polysaccharide gels, the syneresis of these gels is directly affected. Quick freezing can lead to obtain gel samples with small ice crytals compared to normal freezing and less drip loss in gels (Harnkarnsujarit et al., 2016).

Depending on the average freezing rate results of C3_CS15, MC5_A4 at -18^{o}C and C3_CS15, MC5_A3, MC5_A4 at -27^{o}C samples were not significantly different compared to 'real food' samples ($p>0.05$).

3.5 Nuclear Magnetic Resonance Relaxometry (NMR)

NMR Relaxometry is a very effective, non-destructive, easy-to-use, and time-saving technique to characterize properties of materials and foods for the evaluation of the quality. It enables non-invasive detection of protons in liquids or solids (Windt et al., 2021). Benchtop TD-NMR has been mostly used for various applications such as to assess solid content in fats, to determine shelf-life stability of foods, to measure oil and moisture content in foods (van Duynhoven et al., 2010). In meat industry, NMR is frequently preferred to be used for understanding the relationship between water mobility, water distribution, water binding ability and microstructure of the meat (Bertram & Aaslyng, 2007; Straadt et al., 2007). Bertram et al. (2002) has reported that there is a strong correlation between T_2 relaxation times and water holding capacity in pork meat. Following up this correlation, it was stated that that the drip loss from meat could be relevant with a single water population detected by NMR T_2 parameters.

For the hydrogel systems, NMR relaxometry gives very detailed information about the structure and physicochemical properties of hydrogels. NMR helps not only to give information about the mobility and relaxation of protons within the hydrogels and food systems but also to identify water distribution and molecular interactions between hydrocolloids and water (Ersus et al., 2010; Williams et al., 2011). NMR measurements helped to understand the structure, to assess water holding capacity and to analyze the porous structure of hydrogels (Hinrichs et al., 2003). Evaluation of the swelling and water uptake behaviors of composite hydrogel systems formulated using different hydrocolloids has been studied (Ozel et al., 2017). According to the results, it was found that water uptake into the gel matrix had a relationship between T_2 relaxation times since the relaxation times showed the proton population in the entrapped water of the composite hydrogel matrix.

The moisture content and molecular mobility of starch suspensions has also been determined by using NMR techniques and was interpreted with T_2 relaxation time (Choi & Kerr, 2003). Karim et al. (2000) reported that there were various NMR studies in the analysis of water in starch-based products and of starch retrogradation. It was mentioned that since the spin-spin relaxation time was sensitive to changes in molecular mobility, T_2 time enabled starch molecules to be distinguished from the more mobile liquid state or the more stationary solid state.

According to a research about retrogradation of corn starches by molecular analysis, when corn starches with different amylose and amylopectin content were mixed with different hydrocolloids such as gum arabic, guar gum or xanthan gum, it was shown that T_2 times were the highest for corn starches with the lowest amylose content. The mean spin-spin (T_2) values of normal corn starch with Arabic gum (AG) and guar gum (GG) stored for 1, 2 and 10 days at 5^{o}C were between 419-470 ms whereas these values for binary pastes of waxy corn starches with AG and GG were in the range of 811-904 ms. Since corn starches with high amylose content had lower water availability (*mobility of water molecules was more limited*), T_2 value of waxy corn starch was nearly twice as high as normal corn starch. T_2 results for waxy corn starch binary pastes showed bound water mobility (Sikora et al., 2019).

The impact of bound water and water mobility in agar gels at different concentration levels (1%, 2%, 3%, 4%, 5%) was also investigated by NMR in another study (Bertasa et al., 2018). There was an inverse linear correlation between the spin-spin relaxation time (T_2) and agar concentrations. Agar gels with the higher concentration had the shorter relaxation times. Also, these hydrogels showed slower water loss because of more polymer network which prevent water release from the gel structure.

Table 3. 8. T_2 (spin-spin) relaxation time values of food simulants before and after freeze-thaw cycle

Freezer temperature (ºC)	**Food Simulants**	**T_2 (ms)**	
		Before FT cycle	After FT cycle
Curdlan-based			
-18ºC	C3_CS10	0.305±0.011 [a]	0.277±0.023 [b]
	C3_CS15	0.233±0.023 [b]	0.382±0.023 [a]
	C3_CS20	0.188±0.014 [c]	0.242±0.011 [bc]
-27ºC	C3_CS10	0.305±0.011 [a]	0.241±0.020 [bc]
	C3_CS15	0.233±0.023 [b]	0.210±0.003 [c]
	C3_CS20	0.188±0.014 [c]	0.251±0.011 [bc]
Methylcellulose-based			
-18ºC	MC5_A2	0.184±0.021 [a]	0.160±0.007 [b]
	MC5_A3	0.121±0.007 [b]	0.135±0.006 [c]
	MC5_A4	0.089±0.005 [c]	0.096±0.004 [e]
-27ºC	MC5_A2	0.184±0.021 [a]	0.246±0.005 [a]
	MC5_A3	0.121±0.007 [b]	0.132±0.001 [c]
	MC5_A4	0.089±0.005 [c]	0.112±0.002 [d]

Small letters (a-e) represent that they are significantly different for separately each sample frozen at -18ºC and -27ºC in the same column ($p<0.05$). All data were given as the mean ± standard deviation (n=3).

Before freeze-thaw cycle, T_2 relaxation times of both curdlan-based and methyl-cellulose-based food simulants were significantly different for different corn starch and agar concentrations ($p<0.05$). Curdlan-based samples with the lowest concentration of corn starch had the highest T_2 times. The same trend was observed in methyl cellulose-based samples.

Effects of freezer temperature and agar concentration on final T_2 values (after FT cycle) for methylcellulose-based food simulants were found to be significant ($p<0.05$). For curdlan-based samples, T_2 values after FT cycle were significantly affected from the freezer temperature ($0<0.05$) whereas different corn starch concentrations had no significant effect on these values ($p>0.05$).

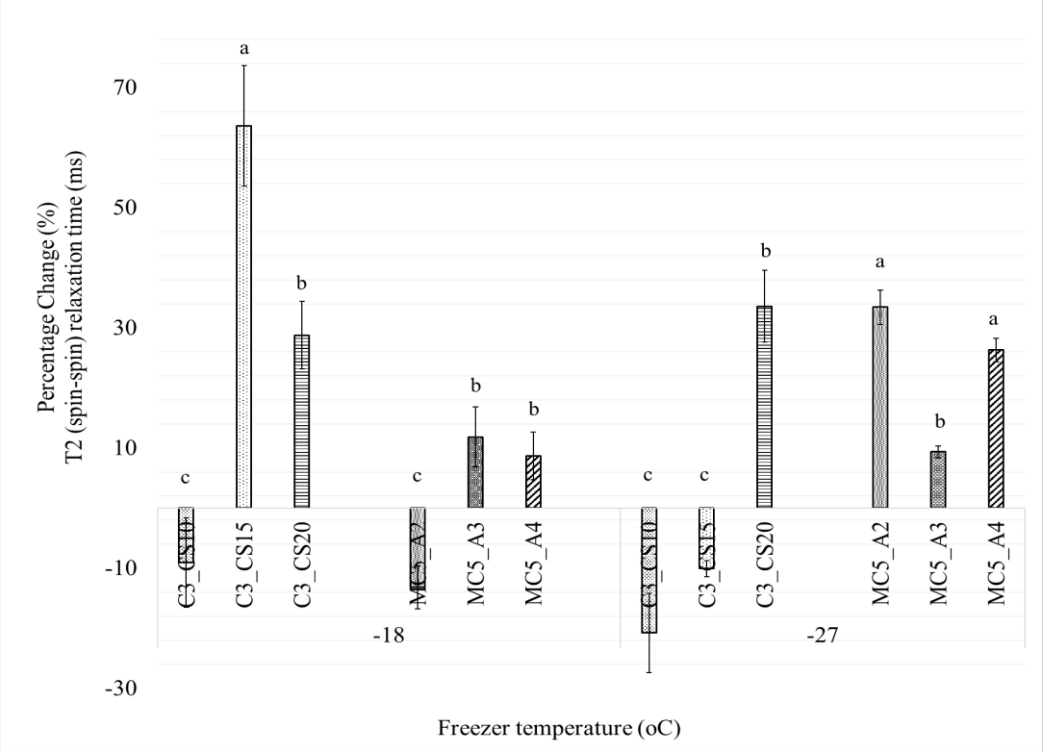


Figure 3. 5. Percentage changes in the mean T_2 relaxation time values of food simulants stored at -18^{o}C and -27^{o}C

The percentage changes of T_2 relaxation times for both curdlan-based and methyl cellulose-based samples stored at two different temperatures (-18 and -27 ^{o}C) showed significant differences ($p<0.05$). The highest percentage change in T_2 times among curdlan-based samples was observed in C3_CS15 samples stored at -18 ^{o}C. For methyl cellulose-based samples, there was no significant difference between the percentage change in T_2 results of MC5_A3 samples stored at -18 and -27 ^{o}C. The effect of different agar concentrations in the percentage change of T_2 times was not significant for MC5_A3, MC5_A4 stored at -18 ^{o}C and MC5_A2 and MC5_A4 stored at -27 ^{o}C the samples.

A significant positive correlation between T_2 relaxation time values of the samples before freeze-thaw cycle and total drip loss (%) was observed ($r= 0.844$, $p<0.05$). T_2 values are critical to express the interactions between water and hydrogels since T_2 relaxation times of hydrogels were found to be highly correlated with water retention (Williams et al., 2011). Luo et al. (2022) investigated the improvement in freeze-thaw stability of rice starch (RS) gels with the addition of xanthan gum (XG), soybean protein hydrolysates (SPHs) and the mixture of XG-SPHs by syneresis measurement through NMR experiments. T_2 values (*water mobility*) for fresh gel samples before FTC in ascending order were as RS>RS/XG>RS/SPHs>RS/XG+SPHs. There was a positive correlation between spin-spin relaxation time and syneresis. The syneresis rates of all gel samples exposed to FT cycle were in the same order (RS>RS/XG>RS/SPHs>RS/XG+SPHs).

When viewed from this aspect, curdlan-based samples with the lowest concentration of corn starch, i.e. high-water content, had the longest T_2 times; as a result, these samples had the highest percentage of drip loss after freezing followed by thawing. Similarly, methyl cellulose-based samples with the lowest concentration of agar had long T_2 values and high drip loss rates.

3.6 Scanning Electron Microscopy (SEM)

SEM analysis was used to observe the effect of freezer temperature and food simulant type on the morphological structures of hydrogels. It was basically done to examine microstructure, morphology, and pore size in the curdlan-based and methylcellulose-based food simulants after both aging and freeze-thaw cycle.

SEM images of frozen-thawed curdlan-based and methylcellulose-based food simulants are presented in Figure 3.6, Figure 3.7, Figure 3.8, and Figure 3.9 (d-f) and compared with the structure of fresh (aged) curdlan-based and methylcellulose-based (a-c) samples.

The diameter of the pores observed in the curdlan-based samples with high corn starch concentrations was observed to be larger than those prepared at low concentrations. Also, the pores of these samples were heavily dispersed. The microstructure of samples frozen at -27^{o}C were more compact with the finest pores when compared to the samples frozen at -18^{o}C. That could be because the high freezing rate could provide the fast phase transition for freezable moisture into the ice crystals and the formation of smallest ice crsytals in gel samples (Zhu et al., 2020).

Wang and his colleagues (2013) investigated the effect of multiple freeze-thaw cycles on the microstructure and physicochemical properties of four different starch gels by SEM and texture analyzer. It was shown that starch gels mostly showed honeycomb-like network structure. That was explained by the formation of the starch-rich regions in the gel matrix and the partially unfrozen ice crystals during the freezing process.

In a research conducted by Williams et al. (2011), it was mentioned that a “honeycomb” like structure existed in the micrograph of curdlan. It was stated that the elasticity of curdlan samples could be the reason. Since curdlan had a high syneresis tendency, it was explained that this elasticity could give the mobility to

free water in and around gel matrix. Likewise, Figure 3.6 and 3.7 showed that curdlan-based food simulants had “honeycomb” like structures before and after freeze-thaw cycle.

Figure 3.8 and Figure 3.9 have illustrated that the pore size of the methylcellulose-based samples with the lowest agar concentrations was much smaller and denser than that of the samples with the highest agar concentrations.

According to a research done by Martin et al. (2008), the pore size and morphology of methylcellulose-agarose hydrogels have been investigated. When the microstructure of hydrogels was characterized, the addition of different agarose types at different concentrations to 7% methylcellulose hydrogel resulted in the formation of more pores with larger diameter compared to the methylcellulose control samples. It was also stated that the highest percentage of pore size distribution on the base methylcellulose hydrogel was smaller than 10 μm in diameter.

The microstructure of agar-methylcellulose (1%-1% w/v) hydrogels had also been investigated by SEM analysis. The agar hydrogel alone had a pentagonal pore structure whereas MC hydrogel alone had a very well-ordered layered sheet-like structure on a scale which was a larger than 1 mm. The agar-MC hydrogels had both agar-like and MC-like structures in agar-rich and MC-rich regions. It was shown that these regions were randomly distributed in the three-dimensional hydrogel structure of mixed gels due to heterogeneity (Thompson et al., 2017). Similarly, Figure 3.8 and 3.9 displayed that methylcellulose-based food simulants had similar structures and were consistent with the above-mentioned study in the literature.

The large pore structures observed in Figure 3.6, Figure 3.7, Figure 3.8, and Figure 3.9 may be due to the stability of the gel structure since thick string networks in the microstructure of the hydrogel samples provides the gel strength. On the other hand, the small pore-size may be liable for the high water holding capacity of gel samples and the stability of texture properties during the storage period (Mao et al., 2001). That is, water holding capacity of food gels is directly related with the

microstructures because of the gel shrinkage and textural changes of these gels. It was reported that there was a negative correlation between WHC and pore sizes of food gels. As an example, for WPI-xanthan gum gels, small pore sizes may cause high swell ratios since these small pores could more water had higher WHC (Wang et al., 2020).

For curdlan-based and methyl cellulose-based samples frozen at -27 ^{o}C and subsequently thawed, as shown in Figure 3.7 and Figure 3.9, C3_CS10 samples had more smaller pore sizes when compared with the samples that had higher concentrations, i.e. C3_CS15 and C3_CS20 samples.

SEM images demonstrated that gel matrices were affected by the addition of secondary polysaccharide with different concentrations.

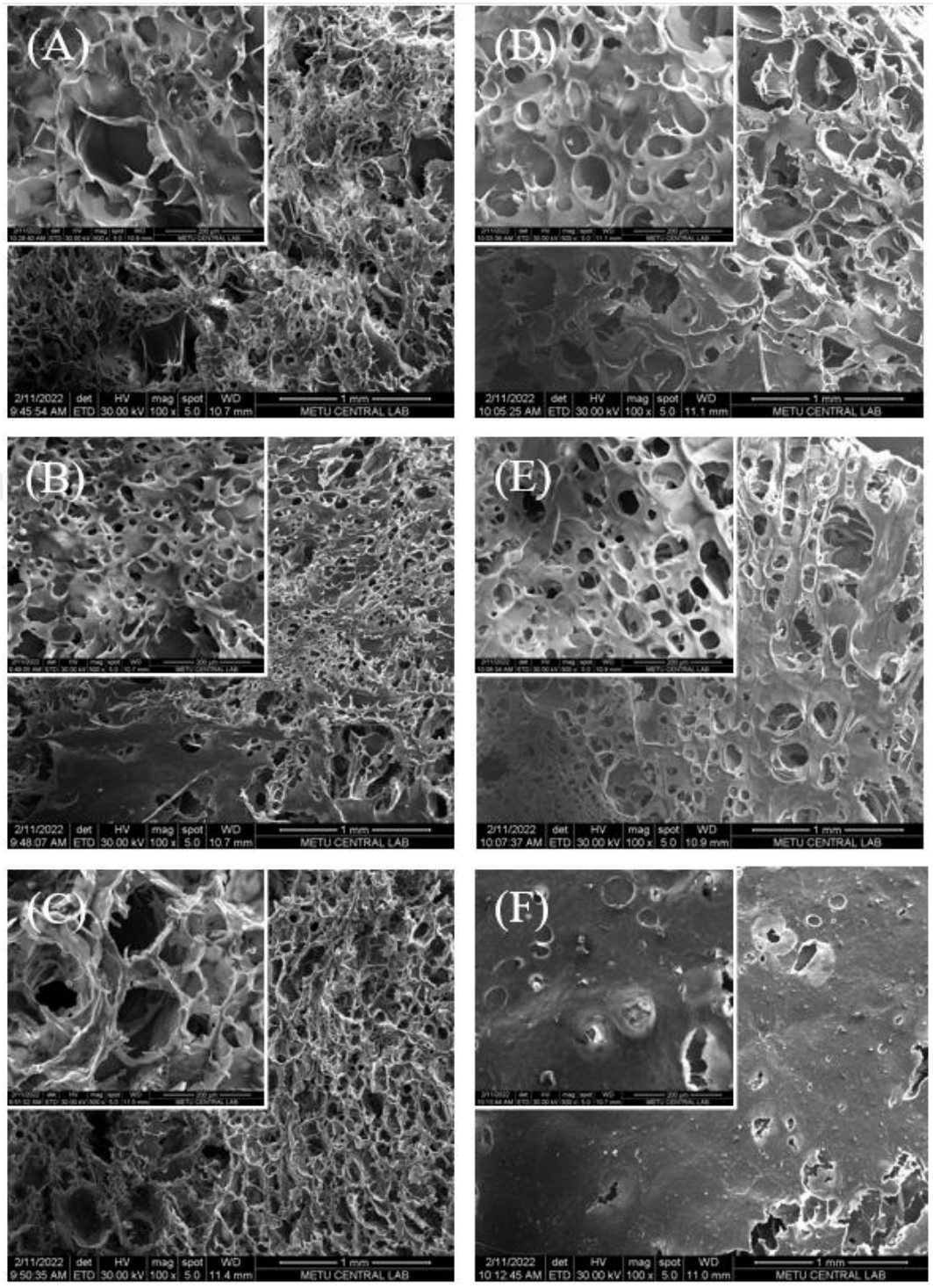


Figure 3. 6. SEM micrographs of curdlan-based food simulants (A) C3_CS10, (B) C3_CS15, (C) C3_CS20 after aging, (D)-(F) after frozen at -18ºC and thawed, respectively

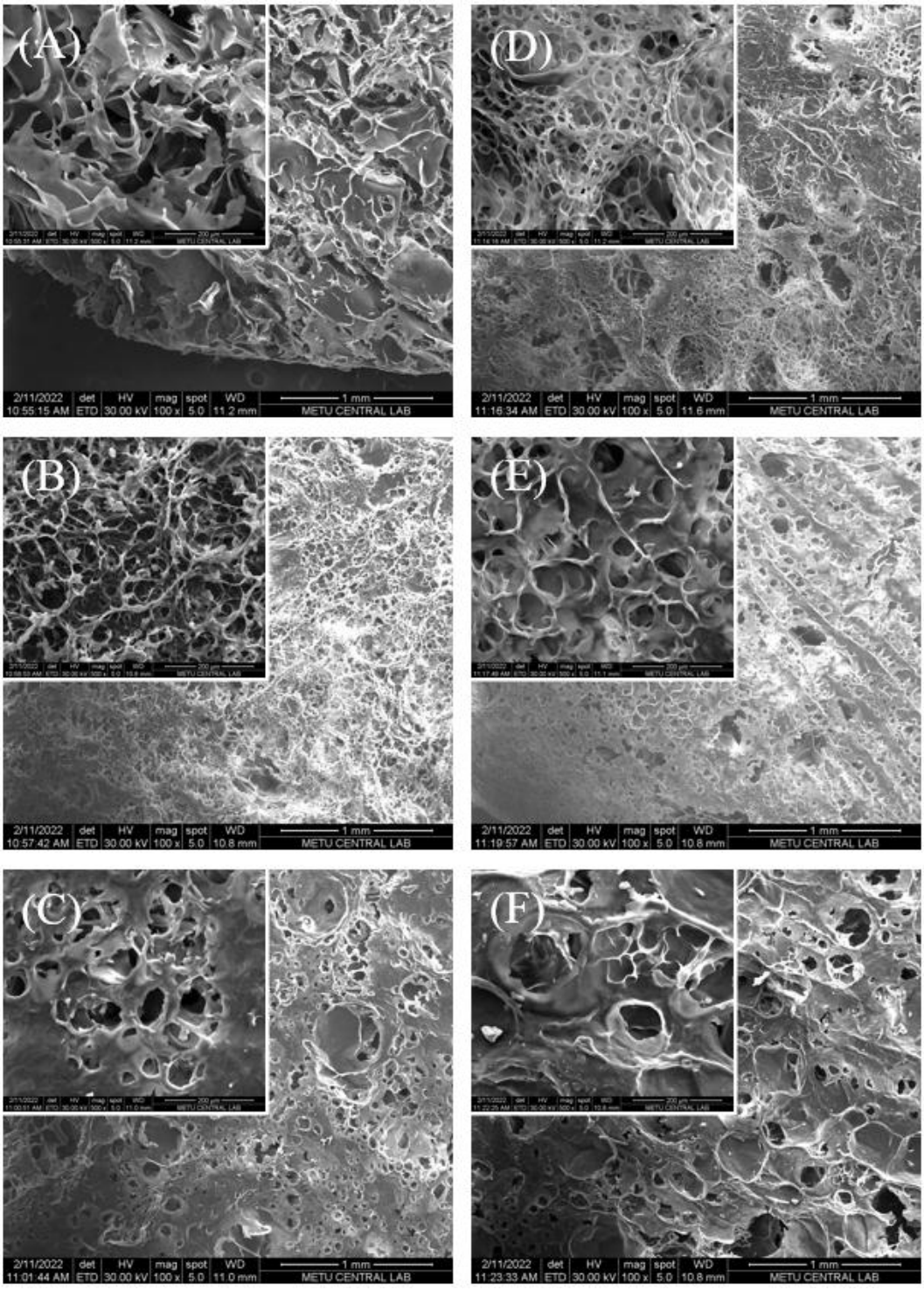


Figure 3. 7. SEM micrographs of curdlan-based food simulants (A) C3_CS10, (B) C3_CS15, (C) C3_CS20 after aging, (D)-(F) after frozen at -27^{o}C and thawed, respectively

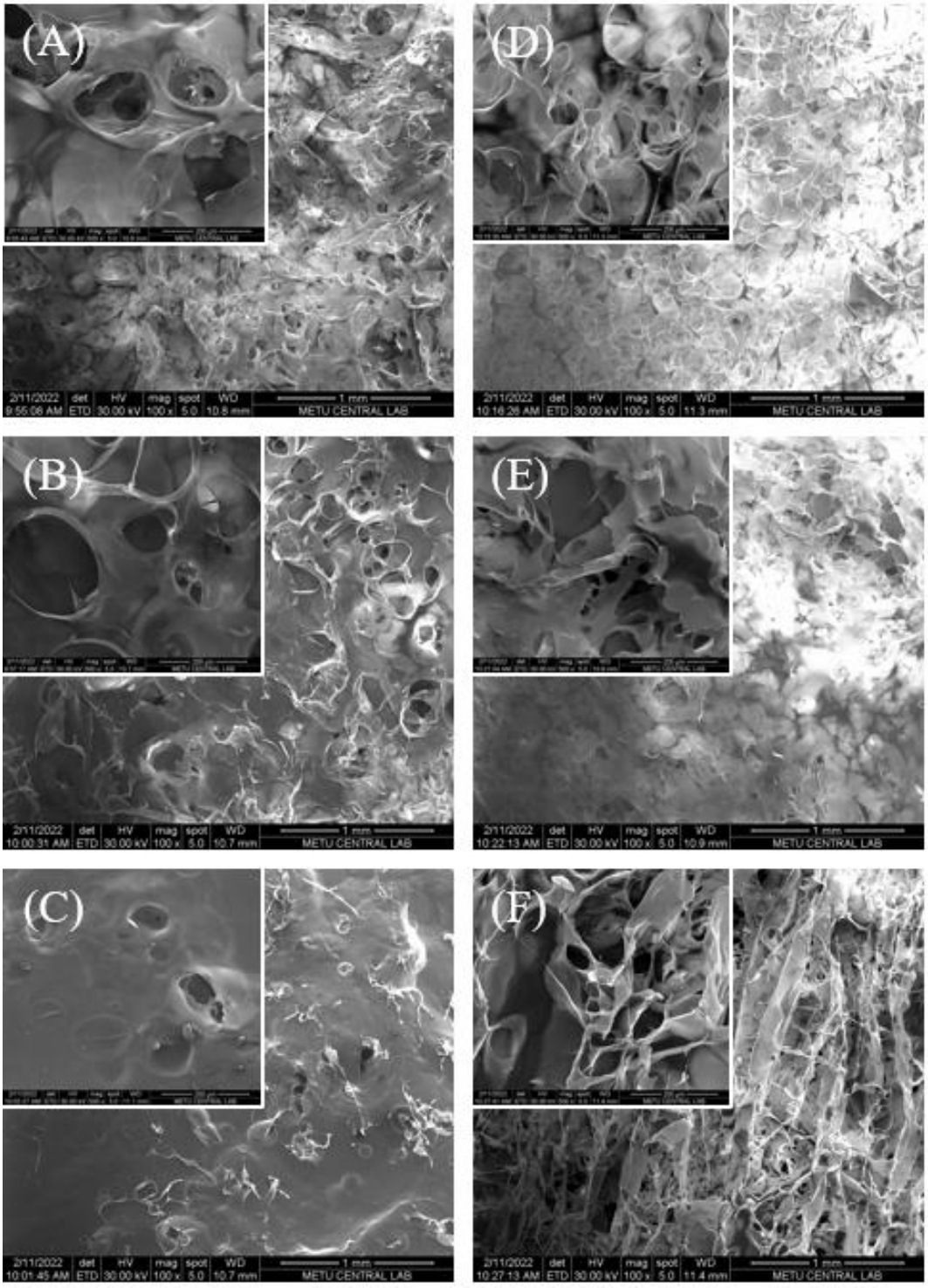


Figure 3. 8. SEM micrographs of methylcellulose-based food simulants (A) MC5_A2, (B) MC5_A3, (C) MC5_A4 after aging, (D)-(F) after frozen at -18ºC and thawed, respectively

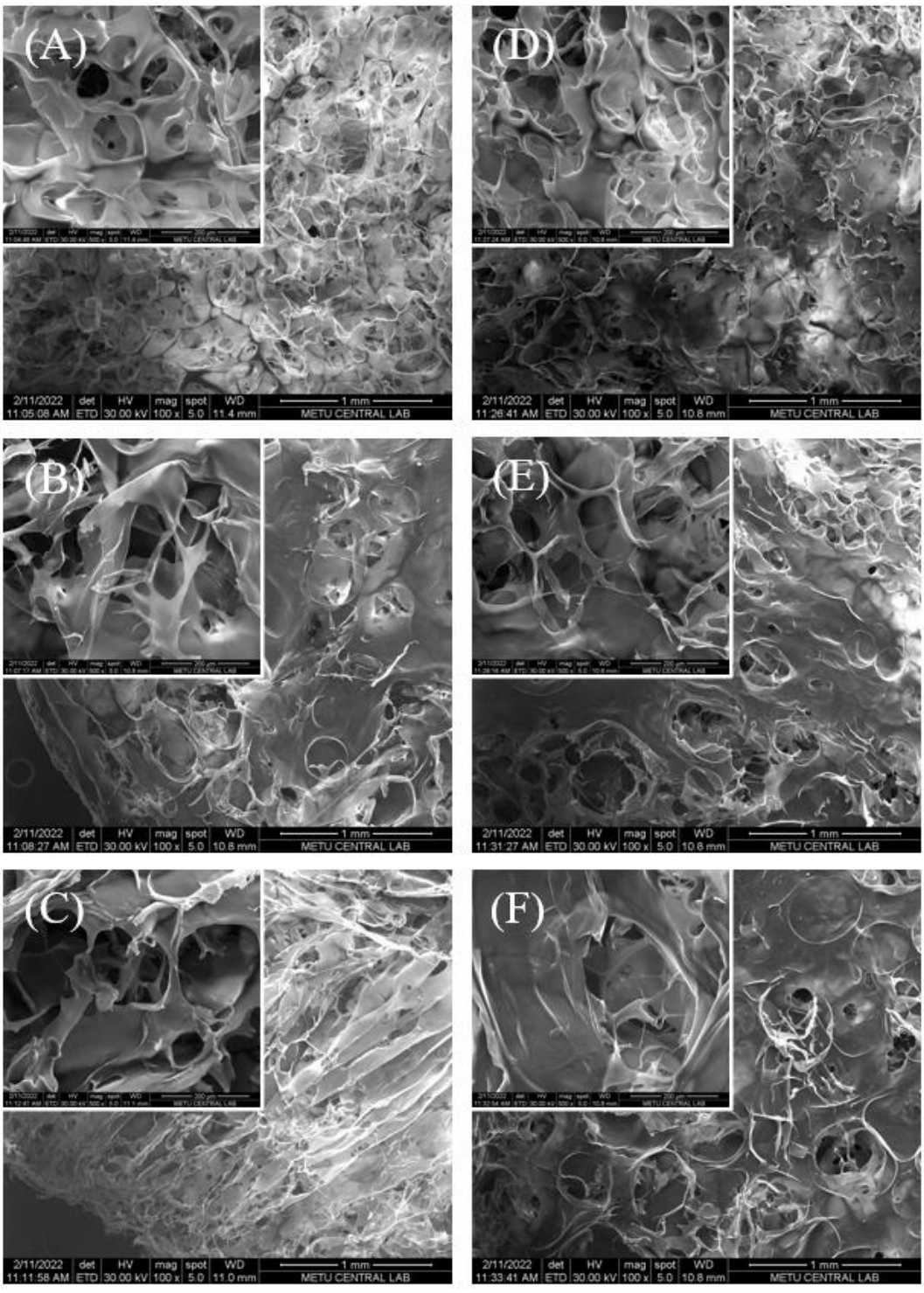


Figure 3. 9. SEM micrographs of methylcellulose-based food simulants (A) MC5_A2, (B) MC5_A3, (C) MC5_A4 after aging, (D)-(F) after frozen at -27^{o}C and thawed, respectively

CHAPTER 4

CONCLUSION AND RECOMMENDATION

In this study, different food simulants to simulate drip loss in meat were developed and characterized for cooling and freezing appliances. These food simulants consisted of two parts: curdlan-based and methylcellulose-based. Curdlan-based food simulants were formulated by mixing 3% of curdlan solution with corn starch at concentrations of 10%, 15%, 20% in a 1:1 ratio. Similarly, for methylcellulose-based samples, different agar concentrations (2%, 3% and 4%) were added to 5% of methylcellulose solution.

A comprehensive investigation of drip loss behavior of curdlan-based and methylcellulose-based food simulants under freeze-thaw cycle was obtained by using some characterization methods to mimic the cooling responses of meats after freezing and subsequent thawing. These methods were total drip loss, hardness analysis, water holding capacity (WHC), freezing-thawing temperature measurements, NMR T_2 relaxation measurements and SEM image analyses.

NMR analysis demonstrated that T_2 relaxation times of the food simulants at low concentration levels were higher than the others for both curdlan-based and methylcellulose-based food simulants before FT cycle. Curdlan-based and methyl cellulose-based food simulants frozen at -18^{o}C and -27^{o}C showed significant differences in the percentage drip loss ($p<0.05$).

According to SEM images, the addition of secondary polysaccharide type with different concentrations to methylcellulose-based and curdlan-based food simulants influenced the microstructure of these hydrogels.

The curdlan-based and methyl cellulose-based food simulants with high concentrations in terms of corn starch and agar had high hardness values before and after freeze-thaw process. There was a positive correlation between percentage

changes in hardness and total drip loss (%) of the frozen-thawed food simulants ($r=0.866$, $p<0.05$).

Since curdlan hydrogel is known to be more susceptible to syneresis after freeze-thaw cycle, the curdlan-based samples were used. It was observed that they had a good freeze thaw stability. The addition of corn starch at different concentrations to curdlan solutions suppressed the syneresis of curdlan-based food simulants, so the samples with highest corn starch concentration had higher water holding capacity and hardness results. There was no significant difference in the percent change of water holding capacity for methyl cellulose-based food simulants frozen at -18^{o}C and -27^{o}C.

The effect of two different freezer temperatures on the mean freezing rate of samples was significantly different ($p<0.05$). In contrast, there was no significant difference in the mean freezing rates of methyl cellulose-based samples together with the real foods ($p>0.05$), but the results of curdlan-based samples differed significantly ($p<0.05$).

In conclusion, it was possible to engineer food simulants by mixing the two hydrocolloids at different concentrations. It was found out that C3_CS20, MC5_A3 and MC5_A4 food simulants would be a good choice to mimic cooling responses of meats such as sirloin and chicken breast after freeze-thaw cycle in terms of drip loss. These food simulants could be recommended to be used as an artificial material instead of real meats to obtain more generalized and standardized results in the measurement of freezing performance of electrical household and similar freezing appliances. To better understand the effect of freezer temperature on drip loss of meats, lower or higher freezing rates than those used in this study could be performed in comparison with these simulants. The stability of food simulants could be also evaluated after multiple freeze-thaw cycles.

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APPENDICES

A. Analysis of variance for 'Real Food' samples and food simulants

Table A. 1. Analysis of variance of frozen-thawed 'real food' samples_part 1. Effect of freezer temperature and storage period on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples

(1) Drip (weight) loss (%) of sirloin after thawing

General Linear Model: Weight Loss (%) after thawing versus Freezer temperature (ºC); Storage period

Factor Information

Factor	Type	Levels	Values
Freezer temperature (ºC)	Fixed	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)
Storage period	Fixed	10	24 h; Week 1; Week 12; Week 16; Week 2; Week 20; Week 24; Week 3; Week 4; Week 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage period	9	99,965	11,1072	39,15	0,000
Freezer temperature (ºC)	2	3,190	1,5948	5,62	0,006
Error	67	19,006	0,2837		
Lack-of-Fit	18	10,564	0,5869	3,41	0,000
Pure Error	49	8,443	0,1723		
Total	78	121,793			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,532614	84,39%	81,83%	78,04%

(2) Drip (weight) loss (%) of chicken breast after thawing

General Linear Model: Weight Loss (%) after thawing versus Freezer temperature (ºC); Storage period

Factor Information

Factor	Type	Levels	Values
Freezer temperature (ºC)	Fixed	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)
Storage period	Fixed	10	24 h; Week 1; Week 12; Week 16; Week 2; Week 20; Week 24; Week 3; Week 4; Week 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage period	9	81,796	9,0885	20,78	0,000
Freezer temperature (ºC)	2	3,092	1,5458	3,53	0,035
Error	65	28,426	0,4373		
Lack-of-Fit	18	16,902	0,9390	3,83	0,000
Pure Error	47	11,523	0,2452		
Total	76	113,876			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,661301	75,04%	70,81%	64,63%

Table A. 2. Analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on total drip (weight) loss (%) for food simulants (1) curdlan-based and (2) methylcellulose-based samples compared to 'real food' samples

(1) General Linear Model: Total weight loss (%) versus Freezer temperature (ºC); Samples

Factor Information

Factor	Type	Levels	Values
Freezer temperature (ºC)	Fixed	2	-27; -18
Samples	Fixed	5	C3_CS10; C3_CS15; C3_CS20; Chicken Breast; Sirloin

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	1	2,132	2,1318	8,23	0,010
Samples	4	80,187	20,0467	77,40	0,000
Error	19	4,921	0,2590		
Lack-of-Fit	4	1,041	0,2602	1,01	0,435
Pure Error	15	3,881	0,2587		
Total	24	85,757			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,508938	94,26%	92,75%	90,37%

(2) General Linear Model: Total weight loss (%) versus Freezer temperature (ºC); Samples

Factor Information

Factor	Type	Levels	Values
Freezer temperature (ºC)	Fixed	2	-27; -18
Samples	Fixed	5	Chicken Breast; MC5_A2; MC5_A3; MC5_A4; Sirloin

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	1	0,5133	0,51329	5,26	0,034
Samples	4	1,2811	0,32027	3,28	0,035
Error	18	1,7549	0,09750		
Lack-of-Fit	4	0,7619	0,19048	2,69	0,075
Pure Error	14	0,9930	0,07093		
Total	23	3,7903			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,312244	53,70%	40,84%	21,47%

Table A. 3. Analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on final hardness values for both curdlan-based (1) and methylcellulose-based food simulants (2)

(1) General Linear Model: Hardness_final (N) versus Freezing temperature (ºC); Food Simulants

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants_1	Fixed	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	0,1241	0,12409	3,02	0,093
Food Simulants_1	2	8,0382	4,01910	97,91	0,000
Error	29	1,1905	0,04105		
Lack-of-Fit	2	0,9366	0,46832	49,81	0,000
Pure Error	27	0,2538	0,00940		
Total	32	9,2972			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,202610	87,20%	85,87%	82,94%

(2) General Linear Model: Hardness_final (N) versus Freezing temperature (ºC); Food Simulants

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants_1	Fixed	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	0,17867	0,17867	27,71	0,000
Food Simulants_1	2	3,03714	1,51857	235,50	0,000

Error	26	0,16766	0,00645		
Lack-of-Fit	2	0,11050	0,05525	23,20	0,000
Pure Error	24	0,05716	0,00238		
Total	29	3,65486			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0803018	95,41%	94,88%	93,88%

Table A. 4. Analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on percentage changes of hardness values for both curdlan-based (1) and methylcellulose-based food simulants (2)

(1) General Linear Model: Change (%) versus Freezing temperature (ºC); Food Simulants

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants_1	Fixed	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	12473	12473	2,19	0,149
Food Simulants_1	2	1359564	679782	119,50	0,000
Error	29	164973	5689		
Lack-of-Fit	2	77075	38537	11,84	0,000
Pure Error	27	87898	3255		
Total	32	1525747			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
75,4237	89,19%	88,07%	85,75%

(2) General Linear Model: Change (%) versus Freezing temperature (ºC); Food Simulants

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants_1	Fixed	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	614,6	614,58	27,56	0,000
Food Simulants_1	2	4771,5	2385,75	106,98	0,000
Error	26	579,8	22,30		
Lack-of-Fit	2	331,3	165,67	16,00	0,000
Pure Error	24	248,5	10,35		
Total	29	6506,5			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4,72248	91,09%	90,06%	88,14%

Table A. 5. Analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on final water holding capacity values for both curdlan-based (1) and methylcellulose-based food simulants (2)

(1) General Linear Model: WHC_final (%) versus Freezing temperature (ºC); Food Simulants_1

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18

Food Simulants_1	Fixed	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	3,180	3,180	1,81	0,188
Food Simulants_1	2	34,644	17,322	9,86	0,000
Error	32	56,224	1,757		
Lack-of-Fit	2	12,762	6,381	4,40	0,021
Pure Error	30	43,462	1,449		
Total	35	94,047			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,32552	40,22%	34,61%	24,34%

(2) General Linear Model: WHC_final (%) versus Freezing temperature (ºC); Food Simulants_1

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants_1	Fixed	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	0,35133	0,35133	12,31	0,001
Food Simulants_1	2	0,26574	0,13287	4,65	0,017
Error	32	0,91366	0,02855		
Lack-of-Fit	2	0,08228	0,04114	1,48	0,243
Pure Error	30	0,83138	0,02771		
Total	35	1,53073			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,168973	40,31%	34,72%	24,46%

Table A. 6. Analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on changes of water holding capacity values for both curdlan-based (1) and methylcellulose-based food simulants (2)

(1) General Linear Model: WHC_change (%) versus Freezing temperature (ºC); Food Simulants_1

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants_1	Fixed	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	1,719	1,719	0,85	0,362
Food Simulants_1	2	20,868	10,434	5,19	0,011
Error	32	64,340	2,011		
Lack-of-Fit	2	20,308	10,154	6,92	0,003
Pure Error	30	44,033	1,468		
Total	35	86,927			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,41797	25,98%	19,04%	6,32%

(2) General Linear Model: WHC_change (%)_1 versus Freezing temperature (ºC); Food Simulants_1

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants_1	Fixed	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	0,02275	0,022748	1,46	0,237

Food Simulants_1	2	0,13667	0,068334	4,38	0,022
Error	29	0,45247	0,015602		
Lack-of-Fit	2	0,01029	0,005143	0,31	0,733
Pure Error	27	0,44218	0,016377		
Total	32	0,60103			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,124909	24,72%	16,93%	1,02%

Table A. 7. Analysis of variance of all samples. Effect of freezer temperature and samples on freezing rate values for curdlan-based (1) and methylcellulose-based (2) food simulants compared to'real food' samples and 'real food' samples alone (3) frozen at -18ºC and -27ºC

(1) General Linear Model: Freezing rate (ΔT°C/h) versus Freezer Temperature (ºC); Samples

Factor Information

Factor	Type	Levels	Values
Freezer Temperature (ºC)	Fixed	2	-27; -18
Sample	Fixed	5	C3_CS10; C3_CS15; C3_CS20; Chicken Breast; Sirloin

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer Temperature (ºC)	1	13,1198	13,1198	52,91	0,000
Sample	4	3,9578	0,9895	3,99	0,023
Error	14	3,4713	0,2480		
Lack-of-Fit	4	0,0764	0,0191	0,06	0,993
Pure Error	10	3,3949	0,3395		
Total	19	20,5489			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,497947	83,11%	77,07%	65,52%

(2) General Linear Model: Freezing rate (ΔT°C/h) versus Freezer Temperature (ºC); Samples

Factor Information

Factor	Type	Levels	Values
Freezer Temperature (ºC)	Fixed	2	-27; -18
Sample	Fixed	5	Chicken Breast; MC5_A2; MC5_A3; MC5_A4; Sirloin

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer Temperature (ºC)	1	17,4955	17,4955	64,86	0,000
Sample	4	1,8678	0,4669	1,73	0,199
Error	14	3,7766	0,2698		
Lack-of-Fit	4	0,7776	0,1944	0,65	0,641
Pure Error	10	2,9990	0,2999		
Total	19	23,1399			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,519384	83,68%	77,85%	66,69%

(3) General Linear Model: Freezing rate (ΔT°C/h) versus Freezer Temperature (ºC); Samples

Factor Information

Factor	Type	Levels	Values
Freezer Temperature (ºC)	Fixed	2	-27; -18
Samples_1	Fixed	2	Chicken Breast; Sirloin

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer Temperature (ºC)	1	5,44253	5,44253	12,33	0,017
Samples_1	1	0,00057	0,00057	0,00	0,973
Error	5	2,20695	0,44139		
Lack-of-Fit	1	0,00189	0,00189	0,00	0,956
Pure Error	4	2,20506	0,55127		
Total	7	7,65004			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,664372	71,15%	59,61%	26,15%

Table A. 8. Analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on final T_2 values for curdlan-based (1) and methylcellulose-based (2) food simulants

(1) General Linear Model: T2(ms)_day 1 versus Freezing Temperature (ºC); Food Simulants

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants	Fixed	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	0,014221	0,014221	6,77	0,023
Food Simulants	2	0,003850	0,001925	0,92	0,426
Error	12	0,025221	0,002102		
Lack-of-Fit	2	0,022859	0,011430	48,41	0,000

Pure Error		10	0,002361	0,000236
Total		15	0,042266	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0458445	40,33%	25,41%	0,00%

(2) General Linear Model: T2(ms)_day 1 versus Freezing Temperature (ºC); Food Simulants

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants	Fixed	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	0,004868	0,004868	9,94	0,007
Food Simulants	2	0,030864	0,015432	31,51	0,000
Error	14	0,006856	0,000490		
Lack-of-Fit	2	0,006608	0,003304	159,87	0,000
Pure Error	12	0,000248	0,000021		
Total	17	0,042588			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0221297	83,90%	80,45%	73,39%

Table A. 9. Analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on percentage change of T_2 values for curdlan-based (1) and methylcellulose-based (2) food simulants

(1) General Linear Model:* (%) Change** T_2 ***versus Freezing Temperature (ºC); Food Simulants

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18

Food Simulants	Fixed	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	2329,1	2329,14	5,86	0,032
Food Simulants	2	5876,6	2938,31	7,40	0,008
Error	12	4767,5	397,30		
Lack-of-Fit	2	4386,9	2193,43	57,62	0,000
Pure Error	10	380,7	38,07		
Total	15	13518,4			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
19,9323	64,73%	55,92%	35,36%

(2) General Linear Model:* (%) Change T_2 *versus Freezing Temperature (ºC); Food Simulants

Factor Information

Factor	Type	Levels	Values
Freezing temperature (ºC)	Fixed	2	-27; -18
Food Simulants	Fixed	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezing temperature (ºC)	1	1912,4	1912,37	13,64	0,002
Food Simulants	2	203,2	101,59	0,72	0,502
Error	14	1962,4	140,17		
Lack-of-Fit	2	1830,1	915,07	83,03	0,000
Pure Error	12	132,3	11,02		
Total	17	4077,9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
11,8394	51,88%	41,57%	20,45%

B. One-way analysis of variance for 'Real Food' samples and food simulants

Table B. 1. One way analysis of variance of frozen-thawed 'real food' samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; 24 hours

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Type	Levels	Values
Freezer temperature (ºC)	Fixed	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,3720	0,18599	5,21	0,049
Error	6	0,2143	0,03572		
Total	8	0,5863			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,188987	63,45%	51,26%	17,76%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping	
-20 (Freezer B)	3	2,147	A	
-18 (Freezer A)	3	2,0198	A	B
-27 (Freezer A)	3	1,667		B

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Type	Levels	Values

Freezer temperature (ºC)	Fixed	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	1,6631	0,83154	11,62	0,009
Error	6	0,4292	0,07153		
Total	8	2,0923			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,267459	79,49%	72,65%	53,84%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping	
-20 (Freezer B)	3	2,768	A	
-18 (Freezer A)	3	2,364	A	B
-27 (Freezer A)	3	1,724		B

Means that do not share a letter are significantly different.

Table B. 2. One way analysis of variance of frozen-thawed 'real food' samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 1

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	1,7420	0,87100	18,22	0,003

Error		6	0,2868	0,04780
Total		8	2,0288	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,218625	85,86%	81,15%	68,19%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping	
-20 (Freezer B)	3	2,5264	A	
-18 (Freezer A)	3	1,987	A	B
-27 (Freezer A)	3	1,449		B

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	1,9098	0,95488	9,91	0,018
Error	5	0,4816	0,09631		
Total	7	2,3913			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,310341	79,86%	71,81%	43,18%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-20 (Freezer B)	3	3,279	A
-18 (Freezer A)	3	3,145	A

-27 (Freezer A)	2	2,092	B

Means that do not share a letter are significantly different.

Table B. 3. One way analysis of variance of frozen-thawed 'real food' samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 2

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	1,9424	0,9712	7,24	0,033
Error	5	0,6705	0,1341		
Total	7	2,6129			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,366187	74,34%	64,08%	42,26%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping	
-20 (Freezer B)	3	2,905	A	
-18 (Freezer A)	3	2,124	A	B
-27 (Freezer A)	2	1,68736		B

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,08963	0,04481	0,43	0,671
Error	5	0,51756	0,10351		
Total	7	0,60719			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,321734	14,76%	0,00%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-27 (Freezer A)	3	3,093	A
-18 (Freezer A)	3	2,892	A
-20 (Freezer B)	2	2,854	A

Means that do not share a letter are significantly different.

Table B. 4. One way analysis of variance of frozen-thawed 'real food' samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 3

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	2,4191	1,20954	32,10	0,001
Error	5	0,1884	0,03768		
Total	7	2,6075			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,194124	92,77%	89,88%	83,62%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping	
-20 (Freezer B)	2	3,0997	A	
-18 (Freezer A)	3	2,532	A	
-27 (Freezer A)	3	1,718		B

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,1690	0,08452	0,63	0,580
Error	4	0,5405	0,13513		
Total	6	0,7096			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,367596	23,82%	0,00%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-18 (Freezer A)	2	2,925	A
-20 (Freezer B)	3	2,703	A
-27 (Freezer A)	2	2,515	A

Means that do not share a letter are significantly different.

Table B. 5. One way analysis of variance of frozen-thawed ‘real food’ samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 4

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,2152	0,10762	3,32	0,142
Error	4	0,1298	0,03246		
Total	6	0,3451			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,180169	62,37%	43,56%	11,92%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-20 (Freezer B)	2	2,4701	A
-27 (Freezer A)	2	2,0931	A
-18 (Freezer A)	3	2,075	A

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,8443	0,4222	3,62	0,159

Error		3	0,3501	0,1167
Total		5	1,1945	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,341628	70,69%	51,15%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-27 (Freezer A)	2	4,591	A
-18 (Freezer A)	2	3,9000	A
-20 (Freezer B)	2	3,721	A

Means that do not share a letter are significantly different.

Table B. 6. One way analysis of variance of frozen-thawed 'real food' samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 8

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,8535	0,4267	2,07	0,272
Error	3	0,6175	0,2058		
Total	5	1,4710			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,453704	58,02%	30,03%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-20 (Freezer B)	2	2,905	A
-18 (Freezer A)	2	2,134	A
-27 (Freezer A)	2	2,079	A

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	5,333	2,6667	12,60	0,011
Error	5	1,059	0,2117		
Total	7	6,392			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,460138	83,44%	76,81%	57,63%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping	
-20 (Freezer B)	3	5,173	A	
-18 (Freezer A)	2	3,537		B
-27 (Freezer A)	3	3,455		B

Means that do not share a letter are significantly different.

Table B. 7. One way analysis of variance of frozen-thawed ‘real food’ samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 12

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	3,7846	1,8923	10,61	0,025
Error	4	0,7135	0,1784		
Total	6	4,4981			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,422339	84,14%	76,21%	42,55%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping	
-27 (Freezer A)	2	5,059	A	
-18 (Freezer A)	3	4,249	A	B
-20 (Freezer B)	2	3,125		B

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	2,122	1,0611	2,97	0,162
Error	4	1,430	0,3575		
Total	6	3,552			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,597917	59,74%	39,61%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-18 (Freezer A)	2	4,655	A
-27 (Freezer A)	3	3,452	A
-20 (Freezer B)	2	3,415	A

Means that do not share a letter are significantly different.

Table B. 8. One way analysis of variance of frozen-thawed 'real food' samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 16

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Type	Levels	Values
Freezer temperature (ºC)	Fixed	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	1,010	0,5049	1,02	0,416
Error	6	2,977	0,4962		
Total	8	3,987			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,704436	25,33%	0,44%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-27 (Freezer A)	3	5,238	A
-20 (Freezer B)	3	5,009	A
-18 (Freezer A)	3	4,441	A

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	2,538	1,2689	1,73	0,269
Error	5	3,667	0,7335		
Total	7	6,205			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,856429	40,90%	17,26%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-18 (Freezer A)	3	6,722	A
-20 (Freezer B)	3	6,014	A
-27 (Freezer A)	2	5,276	A

Means that do not share a letter are significantly different.

Table B. 9. One way analysis of variance of frozen-thawed ‘real food’ samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 20

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,8768	0,4384	1,12	0,411
Error	4	1,5677	0,3919		
Total	6	2,4444			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,626031	35,87%	3,80%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-20 (Freezer B)	2	4,614	A
-18 (Freezer A)	2	3,952	A
-27 (Freezer A)	3	3,777	A

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Levels	Values
Freezer temperature (ºC)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,8348	0,4174	1,19	0,367
Error	6	2,1049	0,3508		
Total	8	2,9396			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,592296	28,40%	4,53%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (ºC)	N	Mean	Grouping
-20 (Freezer B)	3	4,328	A
-27 (Freezer A)	3	3,7555	A
-18 (Freezer A)	3	3,627	A

Means that do not share a letter are significantly different.

Table B. 10. One way analysis of variance of frozen-thawed 'real food' samples_part 1. Effect of freezer temperature on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; week 24

(1) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (ºC)

Factor Information

Factor	Type	Levels	Values
Freezer temperature (ºC)	Fixed	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (ºC)	2	0,5377	0,2689	1,50	0,297
Error	6	1,0769	0,1795		
Total	8	1,6146			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,423652	33,30%	11,07%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (°C)	N	Mean	Grouping
-18 (Freezer A)	3	4,568	A
-20 (Freezer B)	3	4,543	A
-27 (Freezer A)	3	4,037	A

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Freezer temperature (°C)

Factor Information

Factor	Levels	Values
Freezer temperature (°C)	3	-18 (Freezer A); -20 (Freezer B); -27 (Freezer A)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Freezer temperature (°C)	2	4,4900	2,2450	9,52	0,030
Error	4	0,9436	0,2359		
Total	6	5,4336			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,485700	82,63%	73,95%	57,79%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Freezer temperature (°C)	N	Mean	Grouping
-20 (Freezer B)	2	4,466	A
-27 (Freezer A)	3	4,180	A
-18 (Freezer A)	2	2,5410	B

Means that do not share a letter are significantly different.

Table B. 11. One way analysis of variance of frozen-thawed ‘real food’ samples_part 1. Effect of storage period on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; at -18ºC

(1) One-way ANOVA: Weight Loss (%) after thawing versus Storage period

Factor Information

Factor	Levels	Values
Storage period	10	24 h; Week 1; Week 12; Week 16; Week 2; Week 20; Week 24; Week 3; Week 4; Week 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage period	9	33,090	3,6766	26,27	0,000
Error	18	2,519	0,1399		
Total	27	35,609			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,374096	92,93%	89,39%	81,53%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage period	N	Mean	Grouping	
Week 24	3	4,568	A	
Week 16	3	4,441	A	
Week 12	3	4,249	A	
Week 20	2	3,952	A	
Week 3	3	2,532		B
Week 8	2	2,134		B
Week 2	3	2,124		B
Week 4	3	2,075		B
24 h	3	2,0198		B
Week 1	3	1,987		B

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Storage period

Factor Information

Factor	Levels	Values
Storage period	10	24 h; Week 1; Week 12; Week 16; Week 2; Week 20; Week 24; Week 3; Week 4; Week 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage period	9	41,437	4,6041	23,88	0,000
Error	15	2,893	0,1928		
Total	24	44,330			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,439135	93,47%	89,56%	81,71%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage period	N	Mean	Grouping		
Week 16	3	6,722	A		
Week 12	2	4,655		B	
Week 4	2	3,9000		B	C
Week 20	3	3,627		B	C D
Week 8	2	3,537		B	C D
Week 1	3	3,145			C D
Week 3	2	2,925			C D
Week 2	3	2,892			C D
Week 24	2	2,5410			C D
24 h	3	2,364			D

Means that do not share a letter are significantly different.

Table B. 12. One way analysis of variance of frozen-thawed ‘real food’ samples_part 1. Effect of storage period on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; at -20^{o}C

(1) One-way ANOVA: Weight Loss (%) after thawing versus Storage period

Factor Information

Factor	Levels	Values
Storage period	10	24 h; Week 1; Week 12; Week 16; Week 2; Week 20; Week 24; Week 3; Week 4; Week 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage period	9	24,858	2,7620	17,31	0,000
Error	15	2,394	0,1596		
Total	24	27,252			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,399477	91,22%	85,95%	77,46%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage period	N	Mean	Grouping	
Week 16	3	5,009	A	
Week 20	2	4,614	A	
Week 24	3	4,543	A	
Week 12	2	3,125		B
Week 3	2	3,0997		B
Week 2	3	2,905		B
Week 8	2	2,905		B
Week 1	3	2,5264		B
Week 4	2	2,4701		B
24 h	3	2,147		B

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Storage period

Factor Information

Factor	Levels	Values
Storage period	10	24 h; Week 1; Week 12; Week 16; Week 2; Week 20; Week 24; Week 3; Week 4; Week 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage period	9	31,468	3,4965	9,43	0,000
Error	16	5,934	0,3708		
Total	25	37,402			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,608971	84,14%	75,21%	60,71%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage period	N	Mean	Grouping		
Week 16	3	6,014	A		
Week 8	3	5,173	A	B	
Week 24	2	4,466	A	B	C
Week 20	3	4,328	A	B	C
Week 4	2	3,721		B	C
Week 12	2	3,415		B	C
Week 1	3	3,279			C
Week 2	2	2,854			C
24 h	3	2,768			C
Week 3	3	2,703			C

Means that do not share a letter are significantly different.

Table B. 13. One way analysis of variance of frozen-thawed ‘real food’ samples_part 1. Effect of storage period on drip (weight) loss (%) for sirloin (1) and chicken breast (2) samples; at -27ºC

(1) One-way ANOVA: Weight Loss (%) after thawing versus Storage period

Factor Information

Factor	Levels	Values
Storage period	10	24 h; Week 1; Week 12; Week 16; Week 2; Week 20; Week 24; Week 3; Week 4; Week 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage period	9	52,581	5,8423	26,48	0,000
Error	16	3,530	0,2206		
Total	25	56,111			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,469705	93,71%	90,17%	83,66%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage period	N	Mean	Grouping		
Week 16	3	5,238	A		
Week 12	2	5,059	A	B	
Week 24	3	4,037	A	B	
Week 20	3	3,777		B	
Week 4	2	2,0931			C
Week 8	2	2,079			C
Week 3	3	1,718			C
Week 2	2	1,68736			C
24 h	3	1,667			C
Week 1	3	1,449			C

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Weight Loss (%) after thawing versus Storage period

Factor Information

Factor	Levels	Values
Storage period	10	24 h; Week 1; Week 12; Week 16; Week 2; Week 20; Week 24; Week 3; Week 4; Week 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage period	9	25,793	2,8659	17,00	0,000
Error	16	2,697	0,1686		
Total	25	28,491			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,410591	90,53%	85,21%	75,98%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage period	N	Mean	Grouping					
Week 16	2	5,276	A					
Week 4	2	4,591	A	B				
Week 24	3	4,180	A	B	C			
Week 20	3	3,7555		B	C	D		
Week 8	3	3,455		B	C	D	E	
Week 12	3	3,452		B	C	D	E	
Week 2	3	3,093			C	D	E	
Week 3	2	2,515				D	E	F
Week 1	2	2,092					E	F
24 h	3	1,724						F

Means that do not share a letter are significantly different.

Table B. 14. One way analysis of variance of frozen-thawed 'real food' samples frozen for 20 and 24 hours. Effect of storage period on drip (weight) loss (%) for sirloin and chicken breast samples stored at -18ºC (1) and at -27ºC (2)

(1) One-way ANOVA: Total weight loss (%) versus Samples_18ºC

Factor Information

Factor	Levels	Values
Samples	4	Chicken Breast_-18ºC_20h; Chicken Breast_-18ºC_24h; Sirloin_-18ºC_20h; Sirloin_-18ºC_24h

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	3	0,21575	0,07192	5,91	0,059
Error	4	0,04864	0,01216		
Total	7	0,26440			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,110276	81,60%	67,80%	26,41%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping
Chicken Breast_-18ºC_24h	2	2,2349	A
Sirloin_-18ºC_24h	2	1,9492	A
Chicken Breast_-18ºC_20h	2	1,904	A
Sirloin_-18ºC_20h	2	1,7886	A

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Total weight loss (%) versus Samples_27ºC

Factor Information

Factor	Levels	Values
Samples_1	4	Chicken Breast_-27ºC_20h; Chicken Breast_-27ºC_24h; Sirloin_-27ºC_20h; Sirloin_-27ºC_24h

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples_27oC	3	0,04011	0,01337	0,48	0,714
Error	4	0,11148	0,02787		
Total	7	0,15159			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,166945	26,46%	0,00%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples_1	N	Mean	Grouping
Sirloin_-27ºC_24h	2	1,774	A
Sirloin_-27ºC_20h	2	1,7338	A
Chicken Breast_-27ºC_24h	2	1,616	A
Chicken Breast_-27ºC_20h	2	1,6136	A

Means that do not share a letter are significantly different.

Table B. 15. One-way analysis of variance of 'real food' samples_part 2. Effect of freezer temperature and meat type on total drip (weight) loss (%) for 'real food' samples

One-way ANOVA: Total weight loss (%) versus* 'real food' *samples

Factor Information

Factor	Levels	Values
Samples	4	Chicken Breast_-18ºC; Chicken Breast_-27ºC; Sirloin_-18ºC; Sirloin_-27ºC

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	3	0,08735	0,02912	1,86	0,277

Error	4	0,06250	0,01562
Total	7	0,14984	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,124998	58,29%	27,01%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping
Chicken Breast_-18ºC	2	1,904	A
Sirloin_-18ºC	2	1,7886	A
Sirloin_-27ºC	2	1,7338	A
Chicken Breast_-27ºC	2	1,6136	A

Means that do not share a letter are significantly different.

Table B. 16. One-way analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and sample type on total drip (weight) loss (%) for control samples vs food simulant samples such as curdlan-based (1) and methylcellulose-based (2)

(1) One-way ANOVA: Total weight loss (%) versus Samples

Factor Information

Factor	Levels	Values
Samples	10	C3_CS10_-18; C3_CS10_-27; C3_CS15_-18; C3_CS15_-27; C3_CS20_-18;C3_CS20_-27; Chicken Breast_-18; Chicken Breast_-27; Sirloin_-18; Sirloin_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

Samples	9	81,876	9,0974	35,16	0,000
Error	15	3,881	0,2587		
Total	24	85,757			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,508635	95,47%	92,76%	88,10%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping		
C3_CS10_-18	2	6,975	A		
C3_CS10_-27	3	6,243	A		
C3_CS15_-18	3	3,860		B	
C3_CS15_-27	3	3,465		B	C
C3_CS20_-18	3	2,859		B	C	D
Chicken Breast_-18	2	1,904			C	D
Sirloin_-18	2	1,7886			C	D
Sirloin_-27	2	1,7338				D
C3_CS20_-27	3	1,645				D
Chicken Breast_-27	2	1,6136				D

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Total weight loss (%) versus Samples

Factor Information

Factor	Levels	Values
Samples	10	Chicken Breast_-18; Chicken Breast_-27; MC5_A2_-18; MC5_A2_-27; MC5_A3_-18; MC5_A3_-27; MC5_A4_-18; MC5_A4_-27; Sirloin_-18; Sirloin_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

Samples	9	2,7973	0,31081	4,38	0,007
Error	14	0,9930	0,07093		
Total	23	3,7903			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,266328	73,80%	56,96%	37,28%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping	
MC5_A2_-18	3	2,477	A	
Chicken Breast_-18	2	1,904	A	B
Sirloin_-18	2	1,7886	A	B
MC5_A3_-18	3	1,780	A	B
MC5_A3_-27	3	1,736	A	B
Sirloin_-27	2	1,7338	A	B
Chicken Breast_-27	2	1,6136	A	B
MC5_A2_-27	2	1,4908		B
MC5_A4_-18	2	1,3812		B
MC5_A4_-27	3	1,2598		B

Means that do not share a letter are significantly different.

Table B. 17. One-way analysis of variance of aged food simulants. Effect of concentration of second hydrocolloid on initial hardness values (N) for curdlan-based (1) and methylcellulose-based (2) food simulant samples

(1) One-way ANOVA: Hardness_initial (N) versus Samples

Factor Information

Factor	Levels	Values
Samples	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	2	0,7940	0,397018	78,37	0,000
Error	33	0,1672	0,005066		
Total	35	0,9612			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0711751	82,61%	81,55%	79,30%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping		
C3_CS20	12	0,4909	A		
C3_CS15	12	0,3087		B	
C3_CS10	12	0,12714			C

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Hardness_initial (N) versus Samples

Factor Information

Factor	Levels	Values
Samples	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	2	5,955	2,97740	40,19	0,000

Error	29	2,148	0,07408
Total	31	8,103	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,272180	73,49%	71,66%	68,43%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping	
MC5_A3	8	1,8261	A	
MC5_A4	12	1,681	A	
MC5_A2	12	0,8555		B

Means that do not share a letter are significantly different.

Table B. 18. One-way analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on final hardness values (N) for curdlan-based (1) and methylcellulose-based (2) food simulant samples

(1) One-way ANOVA: Hardness (N) (after freezing+thawing) versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	6	C3_CS10_-18; C3_CS10_-27; C3_CS15_-18; C3_CS15_-27; C3_CS20_-18;C3_CS20_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	5	9,0433	1,80866	192,38	0,000
Error	27	0,2538	0,00940		

Total	32	9,2972	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0969626	97,27%	96,76%	96,06%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping			
C3_CS20_-18	6	2,4952	A			
C3_CS20_-27	6	2,0145		B		
C3_CS15_-27	3	1,7318			C	
C3_CS15_-18	6	1,3290				D
C3_CS10_-18	6	1,1861				D E
C3_CS10_-27	6	1,0661				E

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Hardness (N) (after freezing+thawing) versus Food Simulants**Factor Information**

Factor	Levels	Values
Food Simulants	6	MC5_A2_-18; MC5_A2_-27; MC5_A3_-18; MC5_A3_-27; MC5_A4_-18; MC5_A4_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	5	3,59770	0,719540	302,10	0,000
Error	24	0,05716	0,002382		
Total	29	3,65486			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0488034	98,44%	98,11%	97,59%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping
MC5_A4_-18	6	1,2348	A
MC5_A4_-27	3	0,9499	B
MC5_A3_-18	5	0,8104	C
MC5_A3_-27	4	0,57196	D
MC5_A2_-18	6	0,3442	E
MC5_A2_-27	6	0,33214	E

Means that do not share a letter are significantly different.

Table B. 19. One-way analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on difference between initial and final hardness values of curdlan-based food simulants; C3_CS10 frozen at -18^{o}C *(1.1)* and -27^{o}C *(1.2)*, C3_CS15 frozen at -18^{o}C *(2.1)* and -27^{o}C *(2.2)*, C3_CS20 frozen at -18^{o}C *(3.1)* and -27^{o}C *(3.2)*

(1.1) One-way ANOVA: Hardness (N) versus C3_CS10_-18

Factor Information

Factor	Levels	Values
C3_CS10_-18	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C3_CS10_-18	1	3,48477	3,48477	495,86	0,000
Error	10	0,07028	0,00703		
Total	11	3,55505			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)

0,0838319	98,02%	97,83%	97,15%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C3_CS10_-18	N	Mean	Grouping
Hardness_final (N)	6	1,1861	A
Hardness_initial (N)	6	0,10831	B

Means that do not share a letter are significantly different.

(1.2) One-way ANOVA: Hardness (N)_1 versus C3_CS10_-27

Factor Information

Factor	Levels	Values
C3_CS10_-27	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C3_CS10_-27	1	2,53967	2,53967	399,16	0,000
Error	10	0,06363	0,00636		
Total	11	2,60330			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0797660	97,56%	97,31%	96,48%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C3_CS10_-27	N	Mean	Grouping
Hardness_final (N)	6	1,0661	A
Hardness_initial (N)	6	0,14598	B

Means that do not share a letter are significantly different.

(2.1) One-way ANOVA: Hardness (N) versus C3_CS15_-18

Factor Information

Factor	Levels	Values
C3_CS15_-18	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C3_CS15_-18	1	2,70461	2,70461	1586,86	0,000
Error	10	0,01704	0,00170		
Total	11	2,72165			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0412841	99,37%	99,31%	99,10%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C3_CS15_-18	N	Mean	Grouping
Hardness_final (N)	6	1,3290	A
Hardness_initial (N)	6	0,37955	B

Means that do not share a letter are significantly different.

(2.2) One-way ANOVA: Hardness (N)_1 versus C3_CS15_-27

Factor Information

Factor	Levels	Values
C3_CS15_-27	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C3_CS15_-27	1	4,46463	4,46463	8664,30	0,000
Error	7	0,00361	0,00052		
Total	8	4,46824			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0227000	99,92%	99,91%	99,87%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C3_CS15_-27	N	Mean	Grouping

Hardness_final (N)	3	1,7318	A	
Hardness_initial (N)	6	0,23775		B

Means that do not share a letter are significantly different.

(3.1) One-way ANOVA: Hardness (N) versus C3_CS20_-18

Factor Information

Factor	Levels	Values
C3_CS20_-18	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C3_CS20_-18	1	13,1160	13,1160	2022,70	0,000
Error	10	0,0648	0,0065		
Total	11	13,1808			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0805256	99,51%	99,46%	99,29%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C3_CS20_-18	N	Mean	Grouping
Hardness_final (N)	6	2,4952	A
Hardness_initial (N)	6	0,40424	B

Means that do not share a letter are significantly different.

(3.2) One-way ANOVA: Hardness (N)_1 versus C3_CS20_-27

Factor Information

Factor	Levels	Values
C3_CS20_-27	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C3_CS20_-27	1	6,19420	6,19420	1321,92	0,000
Error	10	0,04686	0,00469		

Total	11	6,24105

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0684526	99,25%	99,17%	98,92%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C3_CS20_-27	N	Mean	Grouping
Hardness_final (N)	6	2,0145	A
Hardness_initial (N)	6	0,5776	B

Means that do not share a letter are significantly different.

Table B. 20. One-way analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid on difference between initial and final hardness values of methylcellulose-based food simulants; MC5_A2 frozen at -18ºC *(1.1)* and -27ºC *(1.2)* MC5_A3 frozen at -18ºC *(2.1)* and -27ºC *(2.2)* MC5_A4 frozen at -18ºC *(3.1)* and -27ºC *(3.2)*

(1.1) One-way ANOVA: Hardness (N) versus MC5_A2_-18

Factor Information

Factor	Levels	Values
MC5_A2_-18	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MC5_A2_-18	1	1,01678	1,01678	1460,10	0,000
Error	10	0,00696	0,00070		
Total	11	1,02375			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0263890	99,32%	99,25%	99,02%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MC5_A2_-18	N	Mean	Grouping
Hardness_initial (N)	6	0,92638	A
Hardness_final (N)	6	0,3442	B

Means that do not share a letter are significantly different.

(1.2) One-way ANOVA: Hardness (N)_1 versus MC5_A2_-27

Factor Information

Factor	Levels	Values
MC5_A2_-27	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MC5_A2_-27	1	0,614005	0,614005	2089,43	0,000
Error	10	0,002939	0,000294		
Total	11	0,616944			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0171424	99,52%	99,48%	99,31%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MC5_A2_-27	N	Mean	Grouping
Hardness_initial (N)	6	0,78454	A
Hardness_final (N)	6	0,33214	B

Means that do not share a letter are significantly different.

(2.1) One-way ANOVA: Hardness (N) versus MC5_A3_-18

Factor Information

Factor	Levels	Values
MC5_A3_-18	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

MC5_A3_-18	1	2,78274	2,78274	587,96	0,000
Error	9	0,04260	0,00473		
Total	10	2,82534			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0687956	98,49%	98,32%	97,70%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MC5_A3_-18	N	Mean	Grouping
Hardness_initial (N)	6	1,8205	A
Hardness_final (N)	5	0,8104	B

Means that do not share a letter are significantly different.

(2.2) One-way ANOVA: Hardness (N)_1 versus MC5_A3_-27

Factor Information

Factor	Levels	Values
MC5_A3_-27	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MC5_A3_-27	1	2,15335	2,15335	3921,52	0,000
Error	4	0,00220	0,00055		
Total	5	2,15554			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0234331	99,90%	99,87%	99,68%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MC5_A3_-27	N	Mean	Grouping
Hardness_initial (N)	2	1,8428	A
Hardness_final (N)	4	0,57196	B

Means that do not share a letter are significantly different.

(3.1) One-way ANOVA: Hardness (N) versus MC5_A4_-18

Factor Information

Factor	Levels	Values
MC5_A4_-18	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MC5_A4_-18	1	0,003008	0,003008	1,13	0,312
Error	10	0,026561	0,002656		
Total	11	0,029569			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0515374	10,17%	1,19%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MC5_A4_-18	N	Mean	Grouping
Hardness_initial (N)	6	1,2665	A
Hardness_final (N)	6	1,2348	A

Means that do not share a letter are significantly different.

(3.2) One-way ANOVA: Hardness (N)_1 versus MC5_A4_-27

Factor Information

Factor	Levels	Values
MC5_A4_-27	2	Hardness_final (N); Hardness_initial (N)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
MC5_A4_-27	1	2,62267	2,62267	4783,13	0,000
Error	7	0,00384	0,00055		
Total	8	2,62650			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0234161	99,85%	99,83%	99,72%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MC5_A4_-27	N	Mean	Grouping
Hardness_initial (N)	6	2,09502	A
Hardness_final (N)	3	0,9499	B

Means that do not share a letter are significantly different.

Table B. 21. One-way analysis of variance of frozen-thaw food simulants. Effect of freezer temperature and concentration of second hydrocolloid for change of hardness values (%) of food simulants for curdlan-based (1) and for methylcellulose-based (2)

(1) One-way ANOVA: Change (%) versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	6	C3_CS10_-18; C3_CS10_-27; C3_CS15_-18; C3_CS15_-27; C3_CS20_-18;C3_CS20_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	5	1437848	287570	88,33	0,000
Error	27	87898	3255		
Total	32	1525747			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
57,0569	94,24%	93,17%	91,70%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping		
C3_CS10_-18	6	832,9	A		
C3_CS10_-27	6	738,5	A		
C3_CS15_-27	3	461,10		B	
C3_CS20_-18	6	408,25		B	C
C3_CS15_-18	6	330,60			C
C3_CS20_-27	6	310,35			C

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Change (%) versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	6	MC5_A2_-18; MC5_A2_-27; MC5_A3_-18; MC5_A3_-27; MC5_A4_-18; MC5_A4_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	5	6258,0	1251,60	120,88	0,000
Error	24	248,5	10,35		
Total	29	6506,5			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3,21780	96,18%	95,39%	94,23%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping

MC5_A4_-18	6	-26,53	A			
MC5_A4_-27	3	-43,48		B		
MC5_A3_-18	5	-56,43			C	
MC5_A2_-18	6	-59,76			C	
MC5_A2_-27	6	-61,175			C	
MC5_A3_-27	4	-69,252				D

Means that do not share a letter are significantly different.

Table B. 22. One-way analysis of variance of aged food simulants. Effect of concentration of second hydrocolloid for initial water holding capacity (%) of food simulants for curdlan-based (1) and methylcellulose-based (2) food simulants

(1) One-way ANOVA: WHC_initial (%) versus Food Simulants_1

Factor Information

Factor	Levels	Values
Food Simulants_1	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants_1	2	0,9981	0,4991	2,36	0,129
Error	15	3,1761	0,2117		
Total	17	4,1743			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,460154	23,91%	13,77%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants_1	N	Mean	Grouping
C3_CS20	6	99,7567	A

C3_CS10	6	99,312	A
C3_CS15	6	99,216	A

Means that do not share a letter are significantly different.

(2) One-way ANOVA: WHC_initial (%) versus Food Simulants_1

Factor Information

Factor	Levels	Values
Food Simulants_1	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants_1	2	0,03883	0,01941	1,18	0,337
Error	14	0,23065	0,01648		
Total	16	0,26948			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,128355	14,41%	2,18%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants_1	N	Mean	Grouping
MC5_A3	5	99,9476	A
MC5_A2	6	99,8770	A
MC5_A4	6	99,8284	A

Means that do not share a letter are significantly different.

Table B. 23. One-way analysis of variance of frozen-thaw food simulants. Effect of freezer temperature and concentration of second hydrocolloid for final water holding capacity (%) of food simulants for curdlan-based (1) and methylcellulose-based (2) food simulants

(1) One-way ANOVA: WHC_final (%) versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	6	C3_CS10_-18; C3_CS10_-27; C3_CS15_-18; C3_CS15_-27; C3_CS20_-18; C3_CS20_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	5	50,59	10,117	6,98	0,000
Error	30	43,46	1,449		
Total	35	94,05			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,20363	53,79%	46,09%	33,45%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping		
C3_CS20_-27	6	96,59	A		
C3_CS20_-18	6	95,40	A	B	
C3_CS10_-18	6	94,86	A	B	C
C3_CS15_-27	6	94,50	A	B	C
C3_CS10_-27	6	93,79		B	C
C3_CS15_-18	6	92,83			C

Means that do not share a letter are significantly different.

(2) One-way ANOVA: WHC_final (%) versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	6	MC5_A2_-18; MC5_A2_-27; MC5_A3_-18; MC5_A3_-27; MC5_A4_-18; MC5_A4_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	5	0,6994	0,13987	5,05	0,002
Error	30	0,8314	0,02771		

Total	35	1,5307	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,166471	45,69%	36,64%	21,79%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping	
MC5_A4_-18	6	99,95	A	
MC5_A3_-18	6	99,90	A	
MC5_A3_-27	6	99,83	A	
MC5_A2_-18	6	99,83	A	
MC5_A4_-27	6	99,74	A	B
MC5_A2_-27	6	99,52		B

Means that do not share a letter are significantly different.

Table B. 24. One-way analysis of variance of frozen-thaw food simulants. Effect of freezer temperature and concentration of second hydrocolloid for percentage changes on WHC values (%) of food simulants for curdlan-based (1) and methylcellulose-based (2) food simulants

(1) One-way ANOVA: WHC_change (%) versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	6	C3_CS10_-18; C3_CS10_-27; C3_CS15_-18; C3_CS15_-27; C3_CS20_-18; C3_CS20_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	5	42,89	8,579	5,84	0,001
Error	30	44,03	1,468		
Total	35	86,93			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,21151	49,35%	40,90%	27,06%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping		
C3_CS15_-18	6	6,53	A		
C3_CS10_-27	6	5,84	A	B	
C3_CS15_-27	6	4,66	A	B	C
C3_CS20_-18	6	4,32		B	C
C3_CS10_-18	6	4,20		B	C
C3_CS20_-27	6	3,23			C

Means that do not share a letter are significantly different.

(2) One-way ANOVA: WHC_change (%)_1 versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	6	MC5_A2_-18; MC5_A2_-27; MC5_A3_-18; MC5_A3_-27; MC5_A4_-18; MC5_A4_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	5	0,1578	0,03157	1,93	0,122
Error	27	0,4408	0,01633		
Total	32	0,5986			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,127772	26,37%	12,73%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping
MC5_A2_-27	4	0,17	A

MC5_A2_-18	6	0,07	A
MC5_A4_-27	6	0,02	A
MC5_A3_-27	6	-0,03	A
MC5_A3_-18	5	-0,03	A
MC5_A4_-18	6	-0,05	A

Means that do not share a letter are significantly different.

Table B. 25. One-way analysis of variance of all samples. Effect of curdlan-based (1) and methylcellulose-based (2) food simulants and 'real food' samples frozen at -18ºC and -27ºC for freezing rate (ΔT°C/h) values

(1) One-way ANOVA: Freezing rate (ΔT°C/h) versus Samples

Factor Information

Factor	Levels	Values
Samples	10	C3_CS10_-18C; C3_CS10_-27C; C3_CS15_-18C; C3_CS15_-27C; C3_CS20_-18C; C3_CS20_-27C; Chicken Breast_-18C; Chicken Breast_-27C; Sirloin_-18C; Sirloin_-27C

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	9	17,154	1,9060	5,61	0,006
Error	10	3,395	0,3395		
Total	19	20,549			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,582656	83,48%	68,61%	33,92%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping
C3_CS15_-27C	2	5,560	A

Chicken Breast_-27C	2	5,531	A	
Sirloin_-27C	2	5,483	A	
C3_CS20_-27C	2	4,778	A	B
C3_CS10_-27C	2	4,624	A	B
C3_CS15_-18C	2	4,171	A	B
Sirloin_-18C	2	3,864	A	B
Chicken Breast_-18C	2	3,850	A	B
C3_CS20_-18C	2	3,0258		B
C3_CS10_-18C	2	2,965		B

Means that do not share a letter are significantly different.

(2) One-way ANOVA: Freezing rate (ΔT°C/h) versus Samples

Factor Information

Factor	Levels	Values
Samples	10	Chicken Breast_-18C; Chicken Breast_-27C; MC5_A2_-18C; MC5_A2_-27C;MC5_A3_-18C; MC5_A3_-27C; MC5_A4_-18C; MC5_A4_-27C; Sirloin_-18C; Sirloin_-27C

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	9	20,141	2,2379	7,46	0,002
Error	10	2,999	0,2999		
Total	19	23,140			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,547634	87,04%	75,38%	48,16%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping
MC5_A2_-27C	2	6,144	A
MC5_A3_-27C	2	5,705	A B

Chicken Breast_-27C	2	5,531	A	B	
Sirloin_-27C	2	5,483	A	B	
MC5_A4_-27C	2	5,336	A	B	
MC5_A2_-18C	2	4,3982	A	B	C
Sirloin_-18C	2	3,864		B	C
Chicken Breast_-18C	2	3,850		B	C
MC5_A4_-18C	2	3,683		B	C
MC5_A3_-18C	2	3,051			C

Means that do not share a letter are significantly different.

Table B. 26. One-way analysis of variance of aged food simulants. Effect of concentration of second hydrocolloid for initial T_2 values (ms) of curdlan-based (1) and methylcellulose-based (2) food simulants frozen at -18ºC and -27ºC

(1) One-way ANOVA: T2(ms)_day 0 versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	3	C3_CS10; C3_CS15; C3_CS20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	2	0,041419	0,020709	76,23	0,000
Error	15	0,004075	0,000272		
Total	17	0,045494			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0164823	91,04%	89,85%	87,10%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping		
C3_CS10	6	0,30467	A		
C3_CS15	6	0,23317		B	
C3_CS20	6	0,18817			C

Means that do not share a letter are significantly different.

(2) One-way ANOVA: T2(ms)_day 0 versus Food Simulants

Factor Information

Factor	Levels	Values
Food Simulants	3	MC5_A2; MC5_A3; MC5_A4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Food Simulants	2	0,028417	0,014209	81,26	0,000
Error	15	0,002623	0,000175		
Total	17	0,031040			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0132229	91,55%	90,42%	87,83%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Food Simulants	N	Mean	Grouping		
MC5_A2	6	0,18433	A		
MC5_A3	6	0,12100		B	
MC5_A4	6	0,08867			C

Means that do not share a letter are significantly different.

Table B. 27. One-way analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid for final T_2 values (ms) of curdlan-based (1) and methylcellulose-based (2) food simulants frozen at -18ºC and -27ºC

(1) One-way ANOVA: T2(ms)_day 1 versus Samples

Factor Information

Factor	Levels	Values
Samples	6	C3_CS10_-18; C3_CS10_-27; C3_CS15_-18; C3_CS15_-27; C3_CS20_-18; C3_CS20_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	5	0,039905	0,007981	33,80	0,000
Error	10	0,002361	0,000236		
Total	15	0,042266			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0153661	94,41%	91,62%	83,06%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping		
C3_CS15_-18	2	0,3815	A		
C3_CS10_-18	2	0,2770		B	
C3_CS20_-27	3	0,25133		B	C
C3_CS20_-18	3	0,24233		B	C
C3_CS10_-27	3	0,2413		B	C
C3_CS15_-27	3	0,20967			C

Means that do not share a letter are significantly different.

(2) One-way ANOVA: T2(ms)_day 1 versus Samples

Factor Information

Factor	Levels	Values
Samples	6	MC5_A2_-18; MC5_A2_-27; MC5_A3_-18; MC5_A3_-27; MC5_A4_-18; MC5_A4_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	5	0,042340	0,008468	409,74	0,000
Error	12	0,000248	0,000021		
Total	17	0,042588			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0045461	99,42%	99,18%	98,69%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping
MC5_A2_-27	3	0,246	A
MC5_A2_-18	3	0,160	B
MC5_A3_-18	3	0,135	C
MC5_A3_-27	3	0,132	C
MC5_A4_-27	3	0,112	D
MC5_A4_-18	3	0,096	E

Means that do not share a letter are significantly different.

Table B. 28. One-way analysis of variance of frozen-thawed food simulants. Effect of freezer temperature and concentration of second hydrocolloid for percentage changes on T2 values (ms) of curdlan-based (1) and methylcellulose-based (2) food simulants frozen at -18ºC and -27ºC

(1) One-way ANOVA: (%) Change versus Samples

Factor Information

Factor	Levels	Values
Samples	6	C3_CS10_-18; C3_CS10_-27; C3_CS15_-18; C3_CS15_-27; C3_CS20_-18; C3_CS20_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	5	13137,7	2627,54	69,02	0,000
Error	10	380,7	38,07		
Total	15	13518,4			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6,16993	97,18%	95,78%	91,65%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping		
C3_CS15_-18	2	63,62	A		
C3_CS20_-27	3	33,57		B	
C3_CS20_-18	3	28,79		B	
C3_CS10_-18	2	-9,08			C
C3_CS15_-27	3	-10,079			C
C3_CS10_-27	3	-20,79			C

Means that do not share a letter are significantly different.

(2) One-way ANOVA: (%) Change versus Samples

Factor Information

Factor	Levels	Values
Samples	6	MC5_A2_-18; MC5_A2_-27; MC5_A3_-18; MC5_A3_-27; MC5_A4_-18; MC5_A4_-27

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

Samples	5	3945,7	789,14	71,60	0,000
Error	12	132,3	11,02		
Total	17	4077,9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3,31979	96,76%	95,41%	92,70%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping		
MC5_A2_-27	3	33,45	A		
MC5_A4_-27	3	26,32	A		
MC5_A3_-18	3	11,85		B	
MC5_A3_-27	3	9,366		B	
MC5_A4_-18	3	8,65		B	
MC5_A2_-18	3	-13,20			C

Means that do not share a letter are significantly different.

C. Pearson correlation analysis

Table C. 1. Correlation between total weight (drip) loss and percentage changes in hardness of food simulant samples frozen at -18ºC and -27ºC

Correlation: Total weight loss (%); % Change in hardness

Pearson correlation	0,866
P-value	0,000

Table C. 2. Correlation between total weight (drip) loss and WHC (%) after FT cycle of food simulant samples frozen at -18ºC and -27ºC

Correlation: Total weight loss (%); WHC_final (%)

Pearson correlation	-0,704
P-value	0,000

Table C. 3. Correlation between total weight (drip) loss and percentage changes in WHC of food simulant samples frozen at -18ºC and -27ºC

Correlation: Total weight loss (%); WHC_change (%)

Pearson correlation	0,684
P-value	0,000

Table C. 4. Correlation between percentage changes in hardness and percentage changes in WHC of food simulant samples frozen at -18ºC and -27ºC

Correlation: % Change in hardness; WHC_change (%)

Pearson correlation	0,827
P-value	0,000

Table C. 5. Correlation between total weight (drip) loss and T_2 values before FT cycle of food simulant samples frozen at -18ºC and -27ºC

Correlation: Total weight loss (%); T_2(ms)_day 0

Pearson correlation	0,844
P-value	0,000

D. Hydrogels

Table D. 1. Hydrogels with single hydrocolloid type

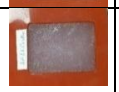
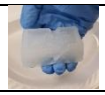
Hydrocolloids	Gel preparation	Reference(s)	Physical properties of hydrogels				Photo		Total drip loss (%)
			Color	Hardness	Form	Ability to retain rigid shape	After aging	After thawing	
C, 1%	dissolved in water, heated up to 85-90°C for 10-20 min, and cooled to 25°C	(Khan et al., 2007)	Semi-transparent	Very soft	Liquid	×			92.25% *(after 4h)*
C, 2%			Semi-transparent	Very soft	Liquid	×			74.78% *(after 4h)*
C, 3%			Semi-transparent	Very soft	Solid	✓			36.38% *(after 4h)*
GT, 0.5%	swell rapidly in cold water for 15 min	(Binsi et al., 2017; Saha & Bhattacharya, 2010)	Transparent	Very soft	Liquid	×		-	-
GT, 1%			Transparent	Very soft	Liquid	×		-	-
GT, 1.5%			Transparent	Very soft	Liquid	×		-	-
GT, 2%			Semi-transparent	Very soft	Solid-liquid	×			68.14% *(after 3h)*

Table D. 1. (continued)

κ- and ι-car, 4%	dissolved in water, heated to 80°C for 15 min, and cooled to room temperature	(Gulrez et al., 2010)	Transparent	Hard	Solid	✓			26.83% *(after 5h)*
κ-car, 1%			Semi-transparent	Soft	Solid	✓			-
κ-car, 2%			Semi-transparent	Hard	Solid	✓			47.79% *(after 3h)*
κ-car, 3%			Semi-transparent	Hard	Solid	✓			40.44% *(after 3h)*
I, 35%	dissolved in water, heated up to 90°C for 15 min by mixing w/ a magnetic stirrer, and cooled to room temperature	(Kim et al., 2001; Kirtania et al., 2021a)	Milky	Soft	Solid	×			7.14% *(after 3h)*
GA, 1%	dissolved in water, stirred using magnetic stir, heated in a water bath at 60°C for 30 min, cooled to room temperature and aged at 4°C for 1 day	(Binsi et al., 2017; Li et al., 2021)	Transparent	Very soft	Liquid	×		-	-
GA, 2%			Transparent	Very soft	Liquid	×		-	-
GA, 3%			Transparent	Very soft	Liquid	×		-	-

Table D. 1. (continued)

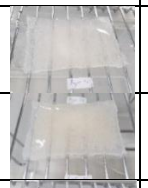
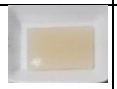
MC, 15%	dissolved in water, heated up to ˜90°C for 15 min, and cooled to room temperature	(Thirumala et al., 2013)	Milky	Hard	Solid	✓			0.06% *(after 5h)*
A, 1%	dissolved in water, heated up to 90°C for 10-15 min, and cooling to room temperature	(Stanley, 2006)	Transparent	Soft	Solid	✓			49.32% *(after 4h)*
A, 2%			Transparent	Hard	Solid	✓			34.05% *(after 4h)*
A, 3%			Semi-transparent	Hard	Solid	✓			19.64% *(after 4h)*
WPI, 15%	dissolved in water, dissolved w/ a magnetic stirrer overnight, waited in water bath at 75°C for 45 min, and stored in refrigerator for 90 min	(Barbut, 1995; Shiroodi & Lo, 2015)	Milky	Soft	Solid	✓		-	-
WPI, 17%			Milky	Soft	Solid	✓		-	-
WPI, 20%			Milky	Hard, brittle	Solid	✓		-	-
XG, 0.5%	dissolved in water, heated up to ˜85°C for 30 min, and cooled to room temperature	(Ghebremedhin et al., 2020)	Transparent	Very soft	Liquid	✗		-	-

Table D. 1. (continued)

XG, 1%			Semi-transparent	Very soft	Liquid	×		-	-
XG, 1.5%			Milky	Very soft	Liquid	×		-	-
XG, 2%			Milky	Soft	Solid	×			1.10% *(after 3h)*
CS, 2.5%	dissolved in water, heated to 80°C for 10-15 min and cooled to room temperature	(Do et al., 2012; Shen et al., 2015)	Transparent	Very soft	Liquid	×		-	-
CS, 5%			Semi-transparent	Very soft	Solid	✓		-	-
CS, 7.5%			Milky	Very soft	Solid	✓			3.62% *(after 4h)*
CS, 20%			Milky	Hard, brittle	Solid	✓		-	1.97% *(after 4h)*
CS, 25%			Milky	Hard, brittle	Solid	✓		-	2.46% *(after 4h)*
CS, 30%			Milky	Hard, brittle	Solid	✓		-	2.76% *(after 4h)*

Table D. 2. Combinations of hydrogels with two different hydrocolloid types

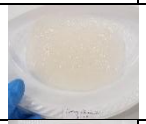
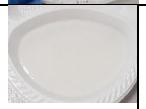
Hydrocolloids	Gel preparation	Reference(s)	Physical properties of hydrogels				Photo		Total drip loss (%)
			Color	Hardness	Form	Ability to retain rigid shape	After aging	After thawing	
C, 2%+XG, 2%	dissolved in water, heated to ~85-90 °C for 15-20 min w/ magnetic stirrer and cooled to room temperature	(Ghebremedhin et al., 2020; Khan et al., 2007)	Semi-transparent	Very soft	Liquid	×	-		-
C, 2%+GT, 2%		(Binsi et al., 2017; Khan et al., 2007)	Transparent	Very soft	Liquid	×	-		-
C, 2%+A, 2%		(Khan et al., 2007; Stanley, 2006)	Semi-transparent	Soft	Solid	✓	-		44.22% *(after 3h)*
C, 2%+GA, 6%		(Khan et al., 2007; Li et al., 2021)	Semi-transparent	Very soft	Liquid	×	-		-
C, 2%+ Alg, 3%		(Khan et al., 2007)	Transparent	Very soft	Liquid	×	-		-
MC, 20%+ GT, 4%	dissolved in water, heated to ~85-90°C for 15 min cooled to 25°C	(Binsi et al., 2017; Saha & Bhattacharya, 2010; Thirumala et al., 2013)	Milky	Very soft	Liquid	×		-	-

Table D. 2. (continued)

MC, 20%+ SA, 2%	dissolved in cold water, heated up to ~90°C for 20-30 min, and cooled to room temperature	(Babu et al., 2007; Thirumala et al., 2013)	Milky	Very soft	Solid	×		-	-
MC, 20%+ SA, 4%			Milky	Very soft	Solid	×		-	-
MC, 20%+ MS, 8%		(Autio & Poutanen, 1994; Thirumala et al., 2013)	Milky	Hard, brittle	Solid	×		-	-
MC, 20%+ MS, 10%			Milky	Soft	Solid	×		-	-
MC, 20%+ A, 6%	heated up to ~85-90°C for 10-15 min, homogenized w/ a ultra-turrax homogenizer and cooled to room temperature	(Stanley, 2006; Thirumala et al., 2013; Thompson et al., 2017)	Semi-transparent	Hard	Solid	✓			0.05% *(after 6h)*
MC, 20%+ κ- and ι-car, 6%		(Gulrez et al., 2010; Thirumala et al., 2013)	Semi-transparent	Hard	Solid	✓			0.52% *(after 6h)*
MC, 20%+ XG, 4%		(Ghebremedhin et al., 2020; Thirumala et al., 2013)	Milky	Very soft	Solid	×		-	-
MC, 20%+ I, 8%		(Kim et al., 2001; Kirtania et al., 2021a; Thirumala et al., 2013)	Milky	Soft	Solid	✓			1.94% *(after 6h)*
I, 6%+SA, 6%	dissolved in		Transparent	Very soft	Liquid	×	-	-	-

Table D. 2. (continued)

I, 4%+ SA, 2%	deionized water, heated up to 90°C for 15 min cooled to room temperature	(Kim et al., 2001; Kirtania et al., 2021a)	Transparent	Very soft	Liquid	×		-	-
I, 10%+ SA, 4%			Transparent	Very soft	Liquid	×		-	-
I, 10%+ SA, 6%			Transparent	Very soft	Liquid	×		-	-
I, 4%+ XG, 2%	I: heated up to 90°C for 10 min and cooled to 45°C, XG: blended in cold water w/ a magnetic stirrer, Mixed w/ ultra-turrax and cooled to 25°C	(Ghebremedhin et al., 2020; Kim et al., 2001; Kirtania et al., 2021a; Mandata & Palogou, 2003)	Semi-transparent	Very soft	Liquid	×		-	-
I, 10%+ GT, 6%	dissolved in deionized water, heated up to 90°C for 15 min cooled to 25°C	(Binsi et al., 2017; Kim et al., 2001; Kirtania et al., 2021a)	Milky	Soft	Solid	×		-	-
SA, 6%+ κ-car, 2%	dissolved in deionized water,		Semi-transparent	Very soft	Solid	×		-	-

Table D. 2. (continued)

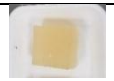
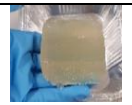
SA, 4%+ κ- and ι-car, 4%	heated up to 80°C for 15 min, and cooled to room temperature	(Gulrez et al., 2010; Yu et al., 2019)	Semi-transparent	Soft	Solid	✓			0.07% *(after 5h)*
SA, 2%+ κ- and ι-car, 2%			Transparent	Soft	Solid	✓			-
SA, 6%+ κ- and ι-car, 2%			Semi-transparent	Very soft	Solid	×		-	-
SA, 4%+ κ-car, 4%			Semi-transparent	Very soft	Solid	✓		-	-
MC, 5%+ κ-car, 1%	MC: mixed w/ cold water in the ultrasonic bath until undissolved particles, κ-car: heating up to 80°C for 15 min, Mixed and cooled to room temperature	(Thompson et al., 2017; Tomšič et al., 2008)	Transparent	Very soft	Solid	×		-	-
MC, 5%+ κ-car, 2%			Semi-transparent	Soft	Solid	✓			0.02% *(after 2h)*
MC, 5%+ κ-car, 3%			Semi-transparent	Hard	Solid	✓			0.52% *(after 2h)*
MC, 15%+ GA, 4%	MC: mixed w/ cold water in ultrasonic bath until undissolved particles,	(Binsi et al., 2017; Li et al., 2021; Thompson et al., 2017)	Semi-transparent	Hard	Solid	✓	-	-	-
MC, 5%+ GA, 1%			Transparent	Very soft	Solid	×		-	-

Table D. 2. (continued)

MC, 5%+ GA, 2%	GA: dispersed and aged at 4ºC for 24h, Mixture was heated at 60ºC for 30 min in water bath and aged at 4ºC for 24h		Transparent	Very soft	Solid	×		-	-
MC, 5%+ GA, 3%			Transparent	Very soft	Solid	×		-	-
MC, 10%+ GA, 2%			Semi-transparent	Hard	Solid	✓			1.76% *(after 6h)*
MC, 5%+ A, 1%	MC: disperse w/ cold water in ultrasonic bath until undissolved particles, A: heated to 85ºC for 10 min, Homogenized w/ a ultra-turrax homogenizer and cooled to 25ºC for 1 day	(Stanley, 2006; Thompson et al., 2017)	Semi-transparent	Soft	Solid	✓			13.06% *(after 3h)*
MC, 5%+ A, 2%			Semi-transparent	Hard	Solid	✓			0.49% *(after 3h)*
MC, 5%+ A, 3%			Milky	Hard	Solid	✓			1.03% *(after 3h)*
MC, 5%+ GT, 0.5%	MC: disperse w/ cold water in ultrasonic bath until undissolved particles, GT: swelled rapidly in cold water for 15 min, Mixed w/ magnetic stirrer and stored in refrigerator for 24h	(Binsi et al., 2017; Thompson et al., 2017)	Transparent	Very soft	Solid	×		-	-
MC, 5%+ GT, 1%			Semi-transparent	Very soft	Solid	×		-	-
MC, 5%+ GT, 1.5%			Semi-transparent	Soft	Solid	×		-	-

Table D. 2. (continued)

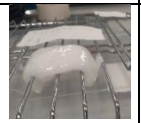
MC, 5%+ WPI, 15%	MC: dispersed in cold water w/ ultrasonic bath til undissolved particles, WPI: dissolved in a magnetic stirrer one night in water bath at 75°C for 45 min, Mixed, stored in refrigerator for 90 min	(Barbut, 1995; Shiroodi & Lo, 2015; Thompson et al., 2017)	Milky	Very soft	Solid	×		-	-
MC, 5%+ WPI, 17%			Milky	Very soft	Solid	×		-	-
MC, 5%+ WPI, 20%			Milky	Very soft	Solid	×		-	-
MC, 5%+ I, 15%	MC: mixed w/ cold water in the ultrasonic bath until undissolved particles, I: heated up to 90°C for 10 min and cooled to 45°C, Mixed and cooled at 8°C for 24h	(Kim et al., 2001; Kirtania et al., 2021a; Thompson et al., 2017)	Transparent	Very soft	Solid	×		-	-
MC, 5%+ I, 25%			Semi-transparent	Very soft	Solid	×		-	-
MC, 5%+ I, 35%			Milky	Soft	Solid	✓			1.47% *(after 2h)*

Table D. 2. (continued)

MC, 5%+ XG, 0.25%	MC: mixed w/ cold water in the ultrasonic bath until undissolved particles, XG: blended in cold water w/ a magnetic strirrer, Mixed w/ a ultra-turrax homoganiser, and stored for 6 h at 25°C	(Parisa et al., 2021; Thompson et al., 2017)	Semi-transparent	Very soft	Solid	×		-	-
MC, 5%+ XG, 0.5%			Milky	Very soft	Solid	×		-	-
MC, 5%+ XG, 0.75%			Milky	Very soft	Solid	×		-	-
C, 3%+ GA, 1%	C: heated up to 85°C for 10 min, GA: dissolve in water, heated in a water bath at 60°C for 30 min, cooled to room temperature, Mixed w/ a magnetic stirrer, and cooled to 25°C	(Binsi et al., 2017; Khan et al., 2007; Li et al., 2021)	Semi-transparent	Very soft	Liquid	×		-	-
C, 3%+ GA, 2%			Transparent	Very soft	Liquid	×		-	-
C, 3%+ GA, 3%			Transparent	Very soft	Liquid	×		-	-

Table D. 2. (continued)

C,3%+XG,0.5%	C: heated up to 85ºC for 10 min, XG: blended in cold water w/ a magnetic stirrer, Mixed w/ a magnetic stirrer, and cooled to 25ºC	(Khan et al., 2007; Parisa et al., 2021)	Transparent	Very soft	Solid	×		-	-
C,3%+XG,1%			Transparent	Very soft	Solid	×		-	-
C,3%+XG,1.5%			Milky	Very soft	Solid	×		-	-
C,3%+CS,5%	C: heated up to 85ºC for 10 min, CS: heated up to 80ºC for 10-15 min, Mixed w/ a high shear homogenizer and cooled to room temperature	(Khan et al., 2007; Shen et al., 2015)	Semi-transparent	Very soft	Solid	×		-	-
C,3%+CS,10%			Milky	Very soft	Solid	✓			5.76% *(after 4h)*
C,3%+CS,15%			Milky	Soft	Solid	✓			2.66% *(after 4h)*
C,3%+CS,20%			Milky	Soft	Solid	✓			0.33% *(after 4h)*
C,3%+CS,25%			Milky	Soft	Solid	✓			0.11% *(after 4h)*
C,3%+CS,30%			Milky	Soft	Solid	✓			-0.78% *(after 4h)*

Table D. 2. (continued)

C,3%+A,2%	C: heating up to 85°C for 10 min, A: heating up to 85°C for 10 min, Mixed w/ a magnetic stirrer and cooled to room temperature	(Khan et al., 2007; Stanley, 2006)	Milky	Soft	Solid	✓			2.13% *(after 4h)*
C,3%+A,3%			Milky	Soft	Solid	✓			1.17% *(after 4h)*
C,3%+A,4%			Semi-transparent	Hard, brittle	Solid	✓			1.04% *(after 4h)*
C,3%+ κ-car,2%	C: heating up to 85°C for 10 min, κ-car: heating up to 80°C for 15 min, Mixed and cooled to room temperature	(Gulrez et al., 2010; Khan et al., 2007)	Semi-transparent	Soft	Solid	✓			5.50% *(after 4h)*
C,3%+ κ-car,3%			Semi-transparent	Hard, brittle	Solid	✓			1.06% *(after 4h)*
C,3%+ κ-car,4%			Semi-transparent	Hard, brittle	Solid	✓			1.23% *(after 4h)*
C,3%+WPI,26%	C: heated up to 85°C for 10 min, WPI: dissolved w/ a magnetic stirrer overnight and waited water bath at 75°C for 45 min, Mixed and stored in refrigerator for 90 min	(Khan et al., 2007; Shiroodi & Lo, 2015)	Milky	Soft	Solid	✓			3.49% *(after 4h)*
C,3%+WPI,30%			Milky	Very soft	Solid-liquid	×		-	-

Table D. 3. Combinations of hydrogels with three different hydrocolloid types

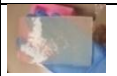
The amount of hydrocolloids (g)	Gel preparation	Reference(s)	Physical properties of hydrogels				Photo		Total drip loss (%)
			Color	Hardness	Form	Ability to retain rigid shape	After aging	After thawing	
C+MS+A (1, 3, 1)	dissolved in 100 ml of water, heated to ~90-95 °C for 10-15 min and cooled to room temperature	(Autio & Poutanen, 1994; Khan et al., 2007; Stanley, 2006)	Semi-transparent	Soft	Solid	✓	-		15.98% *(after 3h)* 26.30% *(after 5h)*
C+MS+A (0.5, 1, 1)			Transparent	Soft	Solid	✓			32.35% *(after 4h)*
C+GA+A (0.5, 0.5, 2)		(Binsi et al., 2017; Khan et al., 2007; Li et al., 2021; Stanley, 2006)	Transparent	Soft	Solid	✓			15.88% *(after 4h)*
C+XG+A (1, 1, 1)		(Ghebremedhin et al., 2020; Khan et al., 2007; Stanley, 2006)	Semi-transparent	Soft	Solid	✓	-		0.02% *(after 3h)* 1.98% *(after 5h)*
C+XG+A (0.5, 0.5, 1)			Transparent	Soft	Solid	✓			7.30% *(after 4h)*
C+Alg+A (1, 2, 3)		(Khan et al., 2007; Oliver-Ferrándiz et al., 2021; Stanley, 2006)	Semi-transparent	Hard	Solid	✓	-		2.38% *(after 3h)*

Table D. 3. (continued)

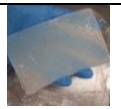
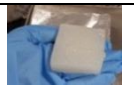
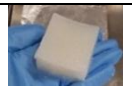
C+GT+A (1, 1, 1)		(Binsi et al., 2017; Khan et al., 2007; Stanley, 2006)	Transparent	Soft	Solid	✓	-		5.72% *(after 3h)* 8.83% *(after 5h)*
I+MS+A (10, 6, 3)		(Autio & Poutanen, 1994; Guo et al., 2021; Kim et al., 2001; Kirtania et al., 2021b; Stanley, 2006)	Milky	Hard	Solid	✓			0.92% *(after 4h)*
C+XG+Alg (1, 0.5, 1)		(Ghebremedhin et al., 2020; Khan et al., 2007; Tan & Yeong, 2015)	Semi-transparent	Very soft	Liquid	×	-		-
C+XG+Alg (1, 1, 1)			Semi-transparent	Soft	Solid	✓	-		1.32% *(after 3h)*
C+XG+Alg (1, 1.5, 1)			Semi-transparent	Soft	Solid	✓	-		1.45% *(after 5h)*
MC+SA+κ-and ι-car (6, 2, 2)		(Babu et al., 2007b; Gulrez et al., 2010; Thirumala et al., 2013)	Milky	Very soft	Solid	×		-	-
MC+SA+κ-and ι-car (8, 1, 1)			Semi-transparent	Hard	Solid	✓			0.36% *(after 5h)*

Table D. 4. Combinations of hydrogels with four different hydrocolloid types

The amount of hydrocolloids (g)	Gel preparation	Reference(s)	Physical properties of hydrogels				Photo		Total drip loss (%) *(after 4h)*
			Color	Hardness	Form	Ability to retain rigid shape	After aging	After thawing	
C+XG+A+Alg (0.5, 0.5, 0.5, 0.5)	dissolved in 100 ml of water, heated to ˜85-90 ºC for 15-20 min and cooled to room temperature	(Ghebremedhin et al., 2020; Khan et al., 2007; Oliver-Ferrándiz et al., 2021; Stanley, 2006; Tan & Yeong, 2015)	Semi-transparent	Very soft	Liquid	×	-		15.58%
C+XG+A+GT (0.5, 0.5, 0.5, 0.5)		(Binsi et al., 2017; Ghebremedhin et al., 2020; Khan et al., 2007; Stanley, 2006)	Semi-transparent	Soft	Solid	✓			5.14%
C+XG+A+MS (0.5, 0.5, 0.5, 0.5)		(Autio & Poutanen, 1994; Ghebremedhin et al., 2020; Khan et al., 2007; Mandata & Palogou, 2003; Stanley, 2006)	Semi-transparent	Soft	Solid	✓			39.43%
MC+SA+XG+MS (2, 1, 0.2, 1)		(Babu et al., 2007b; Ghebremedhin et al., 2020; Mandata & Palogou, 2003; Suliwarno, 2014; Thirumala et al., 2013)	Semi-transparent	Very soft	Liquid	×		-	-
MC+SA+XG+MS (2, 2, 0.2, 1)			Semi-transparent	Soft	Solid	×		-	-