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Development of Pudding and Gravy with Rice Starch, Stearic Acid and Lysine

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DEVELOPMENT OF PUDDING AND GRAVY WITH RICE STARCH, STEARIC ACID AND LYSINE

A Thesis

Submitted to the Graduate faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The School of Nutrition and Food Sciences

by

Andrea Suazo Rosales
B.S. Escuela Agrícola Panamericana, 2018
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ABSTRACT

Starch is a common ingredient used in the food industry for many products. This study was focused on developing products (gravy and pudding) with a clean label starch. The clean label starch was prepared with rice starch, 1% stearic acid and 6% lysine, which was gelatinized, freeze-dried, and grinded. Both products were made with three different starch types: rice starch, commercial modified starch, and clean label starch. These starches were analyzed with DSC, SEM, Megazyme Amylose/Amylopectin kit, and RVA. With the results from the RVA, SEM and DCS it is inferred that the freeze-drying process has altered the structure of the clean label starch, causing significant damages. The gravies were prepared with starch, butter, beef broth, garlic powder, and black pepper. Then stored at -18°C±2 for three months, and samples were taken every two weeks. Before analyzing the samples, they were heated in the microwave for three minutes. The viscosity was measured with the RVA. Another analysis was performed in which the gravy was stored for three weeks, and the viscosity was measured using the Brookfield viscometer. Both studies showed that there is a significant difference in viscosity over time and between starches. The formulation for the pudding contained dry non-fat milk, water, sugar, vanilla, butter, salt, and starch. Pudding samples were prepared and placed hot into glass containers then refrigerated for three weeks. The pH was weekly monitored and after three weeks it remained constant at 6.4. The microbiology tests included Staphylococcus aureus, E. coli, coliforms, yeast, molds, Salmonella, Listeria, and aerobic bacteria; they were performed weekly for each product. There were no bacteria detected for both products, making them safe for consumption. A consumer study was conducted for both products in which the acceptability and the purchase intent of the product were evaluated. More than 70% of the consumers gave a score above 6 (like slightly) to both products, concluding that the products were accepted.
purchase intent is influenced after knowing that the product contained a clean label ingredient, having an increase of 36.4% for gravy and 10.3% for pudding.
1. INTRODUCTION

Starch is a common ingredient for desserts, soups, gravies, sauces, sausages, and many other foods. It is the main component used to thicken, hold together ingredients, and help with texture (Jobling, 2004). Starch is a polysaccharide carbohydrate found in many cereals and tubers. Starch is mainly composed of amylose and amylopectin. The main difference between these polymers of glucose is that amylose is unbranched, and amylopectin is branched (Damodaran et al., 2008). Modified starch is commonly used for food products. Since the natural form of the starch, native starch, is not suitable for food products due to its instability during storage, pasting properties, and retrogradation index (Kong et al., 2016). When starch is subjected to heat treatment and water, its granules absorb water and swell; the given name for this is gelatinization (McDonagh and Group, 2012). Gelatinization gives the texture to the product containing starch. As time passes, the granules lose water, making them form crystallites. This is known as retrogradation. Retrogradation causes stiffness in bread, stickiness, loss of crispness, and moisture migration to the surface (Kong et al., 2016).

There are many ways to modify starch; the most common is to modify them with chemicals. The most common starch modifications include acid hydrolysis, treatment with sodium or potassium hydroxide, hydrogen peroxide, oxidation with sodium hypochlorite, hydroxyl bonding, heat and acids, and alpha-amylase also (McDonagh, 2012). Chemically modified starches are not allowed to be placed in clean label products.

Clean label is a trend that is gaining strength each day in the food industry. It is considered a consumer's term, not a scientific term. According to The Institute of Food Technologists (IFT), a clean label is making products with natural, simple, non-synthetic
chemicals and easily recognized ingredients (Velissariou, 2018). This term also includes writing the ingredient's name as simple as possible, so consumers can understand what it means. Because of this significant trend, the food industry is investing in research to find substitutes for many of the ingredients commonly used. The food industry is constantly innovating and creating new products to fulfill the consumers’ demand. Due to the demand increase for clean label products, the industry has been investing in research to replace many additives, since all chemical modifications to the starch are excluded from the clean label trend.

The starch used for this study is a clean label starch, in which its functional properties and stability were changed by adding amino acids and fatty acids (Jiang, 2013). The clean label rice starch from this study is a response to the clean label demand. Simple and organic compounds, such as fatty acids and amino acids, are being added to make a physical change; without adding compounds that concern clean label consumers. It was tested in gravy to observe its behavior in frozen ready to eat foods. Freezing food is commonly used as a preservation method. This has gained popularity due to the ability not to lose its nutrients and have a long preservation time (Maity, 2012). Also, this starch was used for ready-to-eat refrigerated pudding. This study's primary objectives were (1) to develop a pudding and a gravy with clean label rice starch and test the products stability under frozen or refrigeration temperatures; (2) compare the clean label rice starch to a commercial modified starch; (3) characterize and analyze the clean label starch; and (4) determine consumer acceptability and purchase intent of the products.
2. LITERATURE REVIEW

This section reviews literature of starch, its components, and its food application. Also discussed are the different starches modifications and the changes in the structure.

2.1. Starch

History

Humans have been eating starch for a long time; starchy foods were traced to more than 6700 BC. The Egyptians used starch to adhere to its papyrus; to obtain the adherence characteristic, they modified starch by boiling water, wheat flour, and vinegar. The first extraction of starch is attributed to the Romans who used to soak seeds before pressing them to obtain a liquid; the obtained liquid was washed several times and then sun-dried. In Holland, the production of starch became popular around the year 1500. At that time, the use of starch was limited to the laundry industry. Since 1800, starch was used as a food ingredient; starch was heated with diluted acid to obtain a sweet substance. The starch production in America started with wheat as the main source of starch, but in a few years all the wheat was replaced by corn (Schwartz and Whistler, 2009).

Starch description

Starch is the primary source of carbon in plants, which is synthesized during the day in the chloroplast through photosynthesis (Kolbe et al., 2005). It is degraded at night by enzymes and then transported as sugar to different parts of the plant (McDonagh and Group, 2012). The degradation of starch is necessary to continue the leaf metabolism without photosynthesis. Plants
such as tubers have special parts designated for sugar storage; they store starch for more extended periods to use in future multiplication of the plant (Kolbe et al., 2005). Starch is a carbohydrate composed of amylose and amylopectin (Figure 1). In the process of starch synthesis, several enzymes are involved, such as starch synthase. Starch synthase has two major types, which are granule bound and soluble isoforms. Granule-bound isoform is associated with amylose synthesis (Denyer et al., 1995) by elongating malto-oligosaccharide (Tester et al., 2004). Soluble isoform in combination with starch-branching enzyme synthesizes amylopectin (Denyer et al., 1995). The soluble starch synthase creates the unit chains for amylopectin, and the starch branching enzyme links the linear chains to create the branches (Tester et al., 2004). Amylose is a linear chain with linkages in 1–4 α- D- glucopyranosyl units (Figure 2). The linkages give amylose a spiral or helical shape, in which the interior of that spiral contains many hydrogen atoms making it hydrophobic. The outside of the spiral contains the hydroxyl groups and is hydrophilic. Figure 1 shows detailed images of the structure of the granules.

Figure 1. Starch structure. “Starch structure (a) Cornstarch granules (30 µm), (b) semicrystalline and amorphous starch growth rings (120–500 nm), (c) crystalline and amorphous lamellae (9 nm), (d) growth rings and blocks internal structures (20–50 nm), (e) Double helices of amylopectin, (f) starch nanocrystals (crystalline lamellae), (g) the molecular structure of amylopectin (0.1–1 nm), (h) the molecular structure of amylose (0.1–1 nm) (Ogunsona et al., 2018)”.
Most starches contain about 25% amylose, except for high amylose (some corn starches) containing more than 50%. High amylose starches form strong gels and degrade faster, while high amylopectin content makes more stable and softer gels (Pérez and Bertoft, 2010). Amylopectin is a long molecule consisting of many branches; the branches are linked in 1-6 α-D- glucopyranosyl to a 1-4 α-D- glucopyranosyl backbone unit (Figure 2). Amylopectin branches are clustered and double helices. Starches contain about 75% of amylopectin, but there are starches containing 100% and are called waxy starches (Damodaran et al., 2008).

Figure 2. Graphic representation of amylose and amylopectin (Giri et al., 2018) (Permission approved, Appendix C).

The arrangement between amylose and amylopectin form the starch granules. Granules size varies from their plant source; for example, potato starch has big granules, and rice starch has small granules. They also have different shapes, such as spherical, angular, lenticular, oval, elongated, kidney, polygonal, and other shapes; one type of starch can have mixed shapes (Damodaran et al., 2008; Pérez and Bertoft, 2010). The Maltese cross is seen when the starch is
placed in a microscope with polarized light. Starch granules are composed of amylose and amylopectin layers, which are ordered radially. These layers make up semicrystalline units with different densities. The light ray passes through the granule’s layers, and due to the radial order in which they are, the light ray decomposes into two rays, forming the Maltese cross (Ratnayake and Jackson, 2008).

For starch granules to form a gel it is necessary for them to be exposed to heat and water, this process is called gelatinization. Granules mixed with water and no heat can entrap small amounts of water, but when they are subjected to heat and water, they can entrap larger amounts of water. Heat breaks layer structure and birefringence, making the granules interact with water and swell, ultimately losing their structure. After undergoing this process and cooling, gels and pastes are obtained (Ratnayake and Jackson, 2008). A gel's strength will depend on several factors, such as temperature, water/starch ratio, source of starch, shear force, amylose/amylopectin ratio, degree of heterogeneity in granules (Damodaran et al., 2008). Gelatinization is irreversible damage to the granules; this occurs at different temperatures depending on each starch type. With gelatinization occurring, there is a disruption of the molecular order of the granules. The first thing to lose is the Maltese cross; then, the granule structure is transformed into a formless sack (Muñoz et al., 2015).

2.2. Native starch

Starch in its native form is when it has been isolated from its botanical source with minimal treatment without modifying any of its physicochemical properties (Builders and Arhewoh, 2016). This minimal treatment involves pulverizing the botanical source; the powder of which the starch is mixed with water. The mixture is filtered and then is left aside to let it sit the
sediment. Once there is sediment, the remaining liquid is decanted. The sediment is washed several times and dried afterward (Benesi et al., 2004). As it has been mentioned, native starch is characterized by the Maltese cross. Native starch is commonly used in pharmaceutical applications (pharmaceutical excipient), since it has desirable attributes such as its white color and soft powder; and when is a hydrated and heated it forms a smooth viscous gel (Builders and Arhewoh, 2016). It is also used as an adhesive in paper binders, textiles, and chemical production (Ogunsona et al., 2018).

**Limitations**

After a gel is cooled, starch molecules reassociate; this phenomenon is called retrogradation. Amylose content is associated with a faster retrogradation. Retrogradation is one of the most important aspects to take into consideration in research. A study made by Yu et al. (2009) was to determine if there was a relationship between amylose content and retrogradation. This study used rice starch with different amylose percentages (Yu et al., 2009). They concluded that the higher the amount of amylose, the faster it will retrograde. Retrogradation affects food properties by changing its texture, such as firmness of bread, stickiness, loss of crispness, and moisture migration to the surface (Kong et al., 2016). The consumption of retrograded food is typical; many scientists focus on the digestibility of retrograded starch. Digestibility is an essential property of food products due to its resistance to enzymatic hydrolysis (Sasaki et al., 2009). Most food industries that work with starch try to avoid retrogradation, which is one of the main reasons to modify starches. Native starches are also insoluble in cold water, limiting their application in food products (Builders and Arhewoh, 2016). There are many ways to modify a starch; the most common is to modify them with chemicals.
2.3. Modified starch

Description

Egyptians were the first to produce modified starch by boiling wheat starch with vinegar; this produced a smooth adhesive used in their papyrus. It is in the middle ages of Europe when the first dated use of modified starch for the textile industry was discovered. Starch used to be hydrolyzed with vinegar and used to stiffen fabrics. The modification of starch became famous in America when it started to be used as a food ingredient (sweeteners) and to produce ethanol (Schwartz and Whistler, 2009). It was years later when the use of starch was mainly for the food industry. Due to the limitations already mentioned of native starch, the need emerges for modified starch to improve products' quality. Starch is considered modified when its physical and chemical characteristics have been altered to improve its functional characteristics; this can be done by the addition of chemicals, enzymes, genetic modification, the addition of additives, or the combinations of treatments (Shah et al., 2016). Modified starches have different applications such as edible coatings/films (Shah et al., 2016), breadmaking (Miyazaki et al., 2006), canned foods (Singh et al., 2006), biodegradable foams for packaging (Pornsuksomboon et al., 2016), fat replacer in snacks (Sajilata and Singhal, 2005), fruit fillings, candy, and baking goods (Agudelo et al., 2014). The concern with modification is the use of chemicals added to the starch; the starch requires several washes to remove the chemicals, then this water with chemical needs treatment to be discarded. There is an increase in the use of natural resources and process for these starches (Grgić et al., 2019).
Types of modified starches

The most common starch modifications include acid hydrolysis, treatment with sodium or potassium hydroxide, hydrogen peroxide, oxidation with sodium hypochlorite, hydroxyl bonding, heat, acids, and alpha-amylase (McDonagh, 2012). Modified starches can be mainly categorized into derivatized, cross-linked, and dextrinized starch, according to their process and the chemicals added to them (Laurentin and Edwards, 2013). Figures 3 (graphic) and 4 (reactions) summarize the different modifications of starch.

![Starch modifications diagram](image)

Figure 3. Starch modifications (Guarás, et al., 2017) (Permission approved, Appendix C).
Derivatized starch

The derivatization adds functional groups to starch; an example is the addition of lipophilic groups (Korma et al., 2016). Corn starch is commonly derivatized with monostarch and distarch ester groups (Damodaran et al., 2008). The derivatization can be done using esterification with propylene oxide, acetic anhydride, vinyl acetate, or octenyl succinic anhydride. Figure 5 shows examples of some esterification with acetic acid, maleic anhydride and octanoyl chloride; these modifications were used to substitute thermo plastic polymers. The use of these chemicals in potato starch altered the chain structure of the starch by adding bulky and hydrophobic groups. This caused a reduction of amylose content that results in lower moisture absorption, reduced the swelling capacity of the starch, and increase solubility (Morán et al., 2012). Derivatized starches are mainly used for frozen foods since they provide thaw stability to the product (Singh et al., 2007). The use of these starches is not limited to the food
industry; derivatized starch with octenyl succinic anhydride is used as an emulsion stabilizer and other for uses (Sweedman et al., 2013).

Figure 5. Representation of chemical reactions of potato starch with acetic acid (acetylation), maleic anhydride (maleinization), octanoyl chloride (octanoylation) (Morán et al., 2012) (Permission approved, Appendix C).

Cross-linked starch

A cross-linked starch is starch in which difunctional groups are added between the starch molecules (Damodaran et al., 2008). Cross-linked starches use acetate, phosphates, and hydroxypropyl ether. This starch provides shear stability, acid, heat, and cold stability (Sajilata and Singhal, 2005). This starch can be used as a fat replacer (Sajilata and Singhal, 2005); it can also improve the firmness of breadcrumbs (Miyazaki et al., 2006), meat sauce, and dressings (Laurentin and Edwards, 2013).
A crosslinking with corn starch and phosphorous chloride was evaluated in a study (Shah et al., 2016). The results showed a stable viscosity in acidic conditions, slow gelatinization rate and low initial viscosity. It was recommended to be used in combination with methods such as, esterification, hydrolysis, and oxidation to make it suitable for canned foods (Shah et al., 2016). The crosslink of the starch with phosphorous oxychloride shown in figure 6, was made to restrict granules swelling this prevented the gelatinization of the starch. After this step it was oxidized to complete the modification of the starch, this resulted in a modified starch with low enthalpy gelatinization and retrogradation (Korma et al., 2016).

**Dextrinized starch**

Dextrinized starches are produced by the depolymerization of glucose polymers with acids, oxidizing agents, or amylolytic enzymes. The uses of these starches vary. They can be used for fat replacers, microencapsulation, candies, pan-coating, coated nuts, jellies, syrups, and dairy products. These starches are characterized by their adhesion, film formation, and solubility in cold water (Singh et al., 2007; Damodaran et al. 2008; Abbas, Khalil, Hussin, 2010; Laurentin
and Edwards 2013; Shah et al., 2016). Oxidized starches have a lower gelatinization temperature, lower set back paste, and decreased hot-paste viscosity (Whistler and Daniel, 2000). The modification with enzymes has been used mainly to produce fructose and glucose syrups and maltodextrin (Miguel et al., 2013).

2.4. Clean label starch

A clean label is a trend that is gaining strength each day in the food industry. It is considered a consumer's term, not a scientific term. According to The Institute of Food Technologists (IFT), clean labels make products with natural, simple, non-synthetic chemicals and easily recognized ingredients (Velissariou, 2018). This term also includes writing the ingredient's name as simple as possible so that consumers can understand its meaning. Due to this significant trend, the food industry is investing in research to find substitutes for many of the ingredients commonly used. The food industry is continuously innovating and creating new products to fulfill the consumers' demand.

Types of clean label starches

Heat-moisture treatment modification

Starch can be subjected to a heat-moisture treatment, in which a physical alteration occurs, and the starch is modified. This physical modification involves water addition at a low level, below 35%, then the starch is heated at high temperatures. Jirannuntakul et al. (2013) explained how this process is made by soaking the starch overnight, then heating it with enough water to have a 25% moisture. Then the starch cake was heated in a hot-air oven at 100°C for 16
hours. The moisture after the heating dropped to 10%. They studied the potato starch structure with an atomic force microscope (AFM), after and before the treatment. They concluded that there were morphological changes in the starch granules; there was a partial melting of the surface and a compression of amylopectin double helix chains. These changes affected the gelatinization temperature, swelling, and granule size (Jiranuntakul et al., 2013). Puncha-arnon and Uttapap (2013) applied the same methodology for rice starch and rice flour to study their properties. They concluded that the heat-moisture treatment had more significant effects on rice flour than in rice starch, resulting in higher peak viscosity for the flour, lower gel hardness, and a higher pasting temperature (Puncha-arnon and Uttapap, 2013). In 2010, another study tested rice starch with different amylose content, low, medium, and high. Their moisture was adjusted at different percentages (15%, 20%, and 25%). These samples were kept at 4°C for four days. After the determined time, they were heated at 100°C for one hour. They concluded that heat and moisture affected the starch reducing its swelling power and solubility, which also affected the pasting properties of the high amylose starch by reducing the amylose content (Zavareze et al., 2010).

**Plasma modification**

Modification of starch with plasma is an innovation created as an alternative for chemically modified starches. Plasma is the fourth state of matter in which electrons, molecules, atoms, free radicals, and ions are excited. Having a large amount of energy can be canalized and used for decontamination, modification of food attributes, decreased cooking time, and improved seed germination, for example (Zhu, 2017). Cold plasma was used in rice starch to modify its properties. A bell apparatus was used to treat the starch with cold plasma; between two
electrodes, a thin layer of rice starch was placed on a petri dish. The samples were exposed to a power level of 40W and 60W, with different intervals of time. Results showed that this treatment decreased the amylose content, pasting temperature and peak gelatinization temperature (Thirumdas et al., 2017).

Other non-chemical modifications

Some of the other technologies used to modify starch without chemicals include high pressure, ultrasound, high hydrostatic pressure, electric pulse field, and different plasma exposures. These technologies were tried in different starch types, all of them gave different results. On starches that were exposed to high pressure, their shape was affected, the gelatinization behavior (temperature) and their water-binding (swelling) changed. The starches exposed to ultrasound had damage to their structure, affecting their crystalline structure; this affected their pasting property. Pulse electric field was used on potato, corn, and tapioca starch; they all had some surface damage, and their peak viscosity decreased. All these different treatments have effects on the properties of starch, but none significantly affect the taste, odor, or nutritional value (Grgić et al., 2019).

Ozonation and amino acids have been used to modify the properties of rice starch. Pure oxygen was added to rice starch samples for 30 minutes, the results showed a reduction of peak, minimum and final viscosity. This modification altered the pasting time by reducing it; this produced a faster swelling of the granules and a less rigid gel after cooling. To this sample lysine was added, the addition of this amino acid improved the cooking stability and showed a lower retrogradation tendency. They concluded that starch ozonated alone is suitable for thickening and
that ozonated starch with lysine resulted an alternative for chemically oxidized starch (An and King, 2009).

2.5. Rice starch

Oryza sativa best known as rice, is a cereal that has been consumed for more than 5,000 years. In some Asian countries is their main caloric source, having the highest digestible energy compared to other cereal grains (Zhou et al., 2002). It is an essential cereal in Southeast Asia (Setyaningsih et al., 2015). Rice consumption is not limited to Asia, it is consumed worldwide. In 2019 to 2020; 493,126 thousand metric tons were consumed globally (Shahbandeh, 2021).

Corn and wheat starches are the most used for food products, but due to the allergic reaction that people may have, rice starch has become a great substitute. It is mainly used in gluten-free products.

Rice has been studied due to its content for phenolic compounds and melatonin. A study showed that there are antioxidant compounds in rice; and that its level is positive correlated to its amount of amylose (Setyaningsih et al., 2015). Starch is the main component of rice (Sasaki et al., 2009) but is not commonly used due to its high demand for consumption as milled rice (Zavareze, et al., 2010). Rice starch is used in many products because it has minimal allergic reactions, is white has neutral odor, and a weak flavor (Song et al., 2006). It is also characterized by its small granule size (1-9 μm). Rice is commonly used in baby foods due to its opaque gel (Damodaran et al., 2008). The granules' small size helps with the mouthfeel, so it is frequently used as a fat-replacer in products such as soups, gravies, salad dressings, dairy, bakery, and meat products (Amagliani et al., 2016). As a native starch, it has the same problems already mentioned, which results in the need to modify it.
3. MATERIALS AND METHODS

3.1. Clean label rice starch preparation

To prepare the clean label starch the following ingredients were used; food grade rice starch (HerbaMYL H200, Fairfield, NJ), 6% lysine (NVS Labs, Lake Forest, CA), 1% of stearic acid (Sigma-Aldrich, St. Louis, MO), and water. This formulation was obtained from Jiang’s dissertation (2013). The percentage of water used was 89.3% and for solids 10.7%. These were used first at a small scale in the RVA to obtain the temperatures and scale it to bigger batches (Jiang, 2013). To scale up 100g of clean label starch was used with 6g of lysine (NVS Labs, Lake Forest, CA), 1g of stearic acid (Sigma-Aldrich, St. Louis, MO), 93g of food grade rice starch (HerbaMYL H200, Fairfield, NJ) and 833.3 ml of water were used. The percentages of liquid-solid were used the same. These percentages were used for all batches, around 20 to 25 batches were prepared to be used in all the studies. The solids were added in a beaker with water and placed on a hot plate and mixed with a stirring bar. When the starch started to thicken the stirring was done by hand until it reached a temperature close to the one in the RVA (92°C).

After preparing the gel, the Pilot Freeze Dryer (SP Scientific) was used. The gel was spread into freeze dryer trays. These trays were closed with their lids that contained a filter; this filter allowed the water to come out during freeze drying. The samples were placed in the freezer at -20°C for 24 hours. When the samples were completely frozen, they were transferred to the freeze dryer to dry them for 3 days. Once they were dry, they were ground in a blender to reduce the particle size. Then they were placed in a Cyclone Sample Mill (UDY Corporation model 3010-030) with a 0.5 mm screen; to have a homogenous powder.
3.2. Pudding preparation

Different formulations were tested for pudding. The ingredients used were dry non-fat milk (Great value), sugar (Great value), starch, unsalted butter (Land O Lakes), salt (Great Value), and vanilla (Great value). The pudding was made with three different types of starch, native rice starch (HerbaMYL H200, Fairfield, NJ), clean label starch (rice starch with stearic acid and lysine), and corn modified starch (PRECISA® Cream 20, Ingredion, USA). One of the formulations was adjusted to obtain the most similar consistency in each pudding, increasing the percentage of modified starch used. Table 1 shows the percentages used for each pudding. To prepare the pudding starch, milk, water, sugar and salt were added at the same time; they were heated and stir continuously. When they started to boil the butter and vanilla were added. The flavor of vanilla is enhanced when it is added at the end. Three batches for each starch were prepared. The samples (40 grams) were poured into two-ounce glass containers that were previously autoclaved and closed immediately, then they were stored in the refrigerated room at 5°C.

Table 1. Formulations of puddings with different starches.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Clean label starch§</th>
<th>Modified starchε</th>
<th>Rice starch</th>
<th>Modified starch¥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>73.89%</td>
<td>73.89%</td>
<td>73.89%</td>
<td>69.43%</td>
</tr>
<tr>
<td>Milk</td>
<td>8.21%</td>
<td>8.21%</td>
<td>8.21%</td>
<td>7.72%</td>
</tr>
<tr>
<td>Sugar</td>
<td>5.47%</td>
<td>5.47%</td>
<td>5.47%</td>
<td>5.14%</td>
</tr>
<tr>
<td>Starch</td>
<td>5.47%</td>
<td>5.47%</td>
<td>5.47%</td>
<td>11.17%</td>
</tr>
<tr>
<td>Butter</td>
<td>5.47%</td>
<td>5.47%</td>
<td>5.47%</td>
<td>5.14%</td>
</tr>
<tr>
<td>Vanilla</td>
<td>1.37%</td>
<td>1.37%</td>
<td>1.37%</td>
<td>1.28%</td>
</tr>
<tr>
<td>Salt</td>
<td>0.109%</td>
<td>0.109%</td>
<td>0.109%</td>
<td>0.102%</td>
</tr>
</tbody>
</table>

§ Clean label starch= This starch was made with rice starch, lysine and stearic acid.
¥ This formulation was adjusted to improve consistency (more starch was added).
ε Modified starch= Commercial modified corn starch.
3.3. Gravy preparation

Different formulations were tested for the gravy. The ingredients used were beef broth, starch, butter (Land O lakes), salt (Great value), pepper (McCormick, Hunt Valley, MD), garlic powder (McCormick), and water. The percentages used are in table 2. All ingredients were heated in a non-stick pan with continuous stirring until they boiled at 90°C. They were held at boiling temperature for 1 minute, then (100g) were placed in sanitized containers (heat resistant 8 oz plastic containers). They were stored at -20°C.

Table 2. Formulation of gravies with different starches.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Clean label starch§</th>
<th>Modified starch$\times$</th>
<th>Rice starch</th>
<th>Modified starch\¥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth</td>
<td>47.77%</td>
<td>47.77%</td>
<td>47.77%</td>
<td>47.17%</td>
</tr>
<tr>
<td>Water</td>
<td>42.46%</td>
<td>42.46%</td>
<td>42.46%</td>
<td>41.92%</td>
</tr>
<tr>
<td>Butter</td>
<td>5.31%</td>
<td>5.31%</td>
<td>5.31%</td>
<td>5.24%</td>
</tr>
<tr>
<td>Starch</td>
<td>3.72%</td>
<td>3.72%</td>
<td>3.72%</td>
<td>4.93%</td>
</tr>
<tr>
<td>Salt</td>
<td>0.42%</td>
<td>0.42%</td>
<td>0.42%</td>
<td>0.41%</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.26%</td>
<td>0.26%</td>
<td>0.26%</td>
<td>0.25%</td>
</tr>
<tr>
<td>Black pepper</td>
<td>0.053%</td>
<td>0.053%</td>
<td>0.053%</td>
<td>0.052%</td>
</tr>
</tbody>
</table>

§ Clean label starch= This starch was made with rice starch, lysine and stearic acid.
$\¥$ This formulation was adjusted to improve consistency (more starch was added).
$\times$ Modified starch= Commercial modified corn starch.

3.4. Microbiology methods

All the pudding and gravy samples were tested for the following bacteria *Listeria*, aerobic bacteria, *Salmonella, E. coli*, coliforms, *Staphylococcus aureus*, yeast and mold. These tests were performed in order to assure the safety of the product. The products samples were evaluated after production and weekly during the storage studies (three weeks). Microbiology evaluations were made in triplicates and they were diluted from $10^0$ to $10^{-3}$. All culture media agars and broths
were purchased from Neogen Corporation (Lansing, Michigan USA), and the petrifilms were purchased from 3M (3M™ St. Paul, Minnesota, USA).

### 3.4.1. Listeria

For the determination of *Listeria spp.* in the sample, Oxford agar (Neogen Corporation; Lansing, Michigan, USA) was needed and was prepared following manufacturing instructions. The samples were enriched with UVM (University of Vermont modified) broth (Neogen Corporation; Lansing, Michigan, USA). The broth was prepared the same day that it was going to be used. UVM broth and sample were added to a sterile filter bag (Whirl-Pak, Nasco LLC, Wisconsin, USA) in a ratio of 1:10 (10 grams of sample and 90 ml of broth), then homogenized for 120 seconds using a stomacher (Easy Mix Biomerieux SA, France), and incubated at 32°C for 48 hours. After the incubation period it was homogenized for 60 seconds using a stomacher (Easy Mix Biomerieux SA, France), then with an inoculation loop is was streaked on the Oxford agar plates. The plates were incubated for 48 hours, expecting to get full color (black halo) if bacteria were present.

### 3.4.2. Aerobic bacteria

For determination of aerobic bacteria, an Aerobic Plate Count (APC) (Neogen Corporation; Lansing, Michigan, USA) media was used. To prepare the media manufacturing instructions were followed. Peptone water (used at 1%) and sample were placed in a sterile bag (Whirl-Pak, Nasco LLC, Wisconsin, USA) in a 1:10 ratio (1 gram of sample with 9 ml of peptone water), and homogenized for 120 seconds using a stomacher (Easy Mix Biomerieux
SA, France). Serial dilutions ($10^0$ to $10^{-3}$) were made with peptone water. The inoculum was spread with a hockey stick on plates for Aerobic Plate Count (APC) (Neogen Corporation; Lansing, Michigan, USA). They were incubated for 24 hours at 35°C.

3.4.3. *Salmonella*

For the determination of *Salmonella* it was necessary to enrich the sample before plating. Xylose Lysine Deoxycholate (XLD) (Neogen Corporation; Lansing, Michigan, USA) agar was used for the plates, it was prepared using manufacturing instructions. The sample was enriched with Tetrathionate Broth Base (TT broth). To prepare the broth 91.5 grams of media were added to 960 ml of distilled water. The broth was heated and stirred until it boiled. An Iodine/potassium solution needed to be added into the broth before using it. To prepare this solution 8 grams of KI and 5 grams of iodine were added to 40ml of distilled water. The enriched broth was mixed in a 1:10 ratio with the sample (10 grams of sample and 90ml of enrichment) and added to a sterile filter bag (Whirl-Pak, Nasco LLC, Wisconsin, USA) in a ratio 1:10 (10 grams of sample and 90 ml of broth), then homogenized for 120 seconds using a stomacher (Easy Mix Biomerieux SA, France). Finally, it was incubated at 42°C for 24 hours. After the incubation period it was homogenized for 60 seconds using a stomacher (Easy Mix Biomerieux SA, France), then with an inoculation loop was streaked on XLD plates (Xylose Lysine Deoxycholate; Neogen Corporation; Lansing, Michigan, USA).
3.4.4. *E. coli* and coliforms

The initial inoculum was prepared with 1 gram of sample and 9 ml of peptone water (prepared at 1%) placed into a sterile filter bag (Whirl-Pak, Nasco LLC, Wisconsin, USA). It was homogenized for 120 seconds using a stomacher (Easy Mix Biomerieux SA, France). Serial dilutions (10⁰ to 10⁻³) were made with peptone water. Then 1ml of the inoculum was placed on *E. coli* and coliforms petrifilms (3M™ St. Paul, Minnesota, USA). These petrifilms were incubated at 32°C for 24 and 48 hours. At 24 hours they were observed for the presence of blue dyed bacteria and at 48 hours they were observed for the presence of bubbles around the purple/blue bacteria.

3.4.5. *Staphylococcus aureus*

The initial inoculum was prepared with 1 gram of sample and 9 ml of peptone water (prepared at 1%) placed into a sterile filter bag (Whirl-Pak, Nasco LLC, Wisconsin, USA). It was homogenized for 120 seconds using a stomacher (Easy Mix Biomerieux SA, France). Serial dilutions (10⁰ to 10⁻³) were made with peptone water. Then 1ml of the solution was placed on Staph Express Count Plates. These petrifilms (3M™ St. Paul, Minnesota, USA) were incubated at 35°C for 24hrs.

3.4.6. Yeast and mold

The initial inoculum was prepared with 1 gram of sample and 9 ml of peptone water (prepared at 1%) placed into a sterile filter bag (Whirl-Pak, Nasco LLC, Wisconsin, USA). It was homogenized for 120 seconds using a stomacher (Easy Mix Biomerieux SA, France). Serial
dilutions (10^0 to 10^{-3}) were made with peptone water. Then 1ml of the solution was placed on Yeast and Mold petrifilms (3M™ St. Paul, Minnesota, USA). They were incubated at room temperature in a dark cabinet for 3-5 days.

3.5. Storage studies

3.5.1. Long term storage study for gravy

The storage study of gravy was made with 3 different types of starch (rice starch, commercial modified starch and our clean label starch), each one with triplicates. They were prepared with starch, butter, beef broth, garlic powder, and black pepper following table 2 formulations. The gravy was stored at -18°C for 3 months, in which samples were taken every 2 weeks. Before analyzing the samples, they were heated in the microwave (GE® Countertop Turntable Microwave Oven, at 2450 MHz and 900 W) for 3 minutes this time was based on the time in which most microwaved foods are heated. These samples were directly taken form the freezer. For this part of the study the viscosity was measured with the RVA, setting the RVA at room temperature and 160 rpm. Other parameters measured were syneresis and freeze-thaw stability.

3.5.2. Short term storage study for gravy

The gravy was made with 3 different types of starch (rice starch, commercial modified starch and our clean label starch), each one with duplicates. They were prepared with starch, butter, beef broth, garlic powder, and black pepper following table 2 formulations. The gravy was stored at -18°C for 4 weeks, in which samples were taken every week. Before analyzing the
samples, they samples need to be place in a 100 ml beaker to measure its viscosity (this was the best container to measure it). It was necessary to defrost the sample before placing them in the beaker (they were not completely defrost), then the beaker with the gravy was heated for 1 minutes in the microwave (GE® Countertop Turntable Microwave Oven, at 2450 MHz and 900 W). This time was determined by trials, a study suggested 1.5 minutes for freeze thaw stability cycles (Varavinit et al., 2000), but gravy was being overheated; so, the time was reduced at 1 minute per beaker sample. Then viscosity, freeze-thaw stability and syneresis were measured. For this part of the study the viscosity was measured with the Brookfield viscometer, using a RV3 spindle at 50. The viscosity was measured at different temperatures to observe the differences in viscosity due to temperature. Other parameters measured were syneresis and freeze-thaw stability.

3.5.3. Accelerated storage study for pudding

The pudding was made with 3 different types of starch (rice starch, commercial modified starch and our clean label starch), each one with triplicates. The formulation contained dry non-fat milk, water, sugar, vanilla, butter, salt and starch; following table 1 formulation. Pudding samples were prepared and hot filled into 4 oz glass containers with plastic lids, which were previously autoclaved. Pudding samples were placed in an accelerated shelf-life chamber in which the light was 5-LS, temperature 39°C, and humidity of 75%. Samples were kept in the chamber for a week. Then their syneresis and pH were analyzed.
3.5.4. Room temperature storage study of pudding

The same formulations were followed for this section. Pudding samples were prepared and placed hot into glass containers with plastic lids, which were previously autoclaved. Samples were kept at room temperature (~25°C) for 1 week, then their syneresis and pH were analyzed.

3.5.5. Storage study of pudding at refrigerated temperature

The formulation for all puddings is in table 1. For this study the adjusted formulation for modified starch was used. Pudding samples were prepared and placed hot filled into glass containers with metal lids, which were previously autoclaved. Samples were kept at 5°C for 4 weeks. The viscosity, syneresis, pH and microbiology were analyzed weekly. For this study three samples of each starch were analyzed.

3.6. Viscosity

3.6.1. Viscosity measurement for gravy

Gravy samples (two replicate of each starch) were weekly taken out of the freezer to measure viscosity. The samples were left at room temperature for 30 min, then they were transferred to 100ml beakers. The samples in the beaker were heated in the microwave for 1 minute. The gravy was evaluated in the Brookfield DV-II viscometer (Brookfield Engineering Lab Inc., Stoughton, MA) with a RV-3 spindle at 50 rpm; this method was determined by the percentage of the torque from each spindle tested in gravy samples. The samples were measured
in the viscometer at 60°C, 70°C and 55°C; to determine the differences in viscosity according to
time and temperature. This process was followed for four weeks.

3.6.2. Viscosity measurement for pudding

Pudding samples (three replicates for each starch) were taken out of the refrigerator to
measure its viscosity (in the same container), and were kept in a cooler to assure that the
viscosity was measured between 5 - 7°C. Their viscosity was measure with the Brookfield DV-II
viscometer (Brookfield Engineering Lab Inc., Stoughton, MA) using a T-D spindle and 50 rpms;
this method was determined by the percentage of the torque from each spindle tested in pudding
samples. The viscosity was recorded for four weeks. All samples were performed in triplicates.

3.7. Syneresis

To measure syneresis 10 grams of sample (pudding and heated gravy) were placed in
15ml centrifuge tubes. The tubes were centrifuged in Compact II Centrifuge (Sparks, MD) at
1200 rpm for 20 minutes. The supernatant was removed with a pipettor. The tubes were
weighted before centrifuging and after removing the supernatant. To obtain the percentage of
syneresis the following formula was used.

\[
\% \text{Syneresis} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial Weight}} \times 100
\]

Formula 1. Syneresis formula (Deetae, 2008).
3.8. Freeze-thaw stability

To measure the freeze-thaw stability of gravy, samples were subjected to freezing-heating cycles, in which the samples were frozen at -20°C and then heated in the microwave for 3 minutes. Each cycle was repeated for three times on the same sample, and it was performed weekly. Syneresis was measured in each cycle.

3.9. Color analysis

The color of gravy and pudding samples were analyzed using a Minolta Baking Meter BC-10 (Japan), this instrument evaluates color, lightness and darkness. The parameters evaluated were lightness (*L), redness-greenness (*a), and yellowness-blueness (*b) values. The colorimeter was calibrated using a white tile before using it. Then enough sample (~100ml) to cover a wide glass container was poured in, and the instrument (covered with a clear bag) was immersed in the sample, three replicates were made for each product.

3.10. Amylose content

The amylose contents for food grade native rice starch (HerbaMYL H200, Fairfield, NJ), clean label starch (rice starch with stearic acid and lysine), modified corn starch (PRECISA® Cream 20, Ingredieon, USA), and native rice starch (Sigma Aldrich, St, Louis, MO), modified starch and clean label rice starch was determine using the Megazyme Amylose/Amylopectin Assay Kit (Megazyme International Ireland Limited, Bray, Ireland). This method is approved by AOAC method 996.11 and AACC method 76-13.01 and is a standard method (ICC Standard Method No. 168 and RACI Standard Method). The detection method is through absorbance
using a spectrophotometer at a wavelength of 510 nm. Amylose content was calculated with formula 2.

\[
\text{Amylose, } \% \left( \frac{w}{w} \right) = \frac{\text{Absorbance (Con A Supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times \frac{6.15 \times 100}{9.2} \times 1\]

Formula 2. Amylose content formula.

### 3.11. Rapid visco-analysis

A Rapid Visco-Analyzer (RVA-4, Newport Scientific) was used to obtain heating temperatures, shearing and apparent viscosity, which were used for scaling up the batch of starch. The method used was AACCI Method 61-02.01. The sample was held for 10 seconds at 50°C, stirring continuously at 960 rpm. The temperature increased gradually at 12°C/min until it reached 95°C, during this part of the process the sample was mixed 160rpm. Then the sample was cooled to 50°C at a rate of -12°C/min. For the RVA analysis 3 grams of solids were used, corresponding to lysine 6% (Sigma Chemical, St. Louis, MO), stearic acid 1% (Sigma Chemical, St. Louis, MO), and the rest was starch (93%) (Sigma Chemical, St. Louis, MO); these were made weight basis. These 3 grams were added to a cannister that contained 25ml of distilled water; having a total weight of 28 grams. Three replicates were performed all the starches (modified starch (MS), food grade rice starch (FGRS), Sigma native starch (SNS), clean label modified starch (CLS)).
3.12. Differential scanning calorimeter (DSC)

The DSC (DSC Q100, TA Instruments-Waters LLC) starch samples were prepared 24 hours before running the equipment. To prepare the reference sample 20 μl of distilled water were placed in a steel high volume pan. In another pan 10 mg of starch were mixed with 20 μl of distilled water. Pans were hermetically sealed by covering them with a lid that had a rubber ring on the inside. The samples were left at room temperature for 24 hours. The temperature in the DSC was set at 25°C to avoid freezing. The estimated running time was 30 min and temperature was raised from 25°C to 150°C. The starch samples analyzed were commercial modified corn starch, food grade rice starch and clean label starch.

3.13. Scanning electro-microscope (SEM)

Dry starch samples were analyzed by Scanning Electro- Microscope (SEM) (FEI Quanta 3D FEG FIB/SEM Sputter Coater), 1 cm² of starch was sputter coated with platinum (EMS550X Sputter Coater) before placing them on the microscope. Images of sigma native rice starch, food grade native rice starch, modified starch and our clean label starch were taken at 5.00 kV. The images were taken at different magnifications from 200x to 20,000x.

3.14. Consumer evaluations

A consumer sensory study was conducted at the LSU AgCenter Sensory Laboratory (Baton Rouge LA, USA). All participants were at least 18 years old, with ages between 18 to 60 years old. They had an education level completed from high school degree to PhD degree. The aspects of study included demographics; of gender, ethnicity, age and education level. Both
samples were served in a two-ounce plastic cups with lids, which contained a code that represented the type of starch used. Unsalted crackers and water were used to clean the palate before and after each sample. Everything was served in white plastic tray. Both products had this clean label statement, “This is a clean label product. According to The Institute of Food Technologists (IFT) "Clean Label" is making products with natural, simple, non-synthetic chemicals and easy to recognize ingredients”.

3.15. Sensory study of pudding

Pudding was prepared the day before following table 1 formulation for clean label starch and modified starch, and stored at 5°C. For this study 87 people participated. Two different samples were presented to the consumer, one of them was prepared with modified starch and the other one with the clean label starch. They were served in two-ounce cups. A 9-point Hedonic scale was used to evaluate appearance, color, flavor, and overall liking. Purchase intent was also evaluated by asking their purchase intent before reading the claim that stated that one of the products was made with a clean label starch, the question was repeated after the claim.

3.16. Sensory study of gravy

Pudding was prepared following the formula from table 2, using the adjusted modified starch gravy. The samples were prepared a day before the consumer study. For this study 75 people participated. The samples presented to the consumers were the clean label gravy and the modified starch gravy. This samples were presented in white plastic trays. In the trays the two samples were provided in plastic cups (2 oz) with coded lids, a bag with napkins and unsalted
crackers, and a cup with water. Also, a 9-point Hedonic scale was used to evaluate appearance, color, flavor, and overall liking. Purchase intent was also evaluated by asking their purchase intent before reading the claim that stated that one of the products was made with a clean label starch, the question was repeated after the claim.

3.17. Statistical analysis

Statistical software SAS (v.9.4) was used for data analysis. The following data was analyzed by ANOVA with a post-ANOVA Tukey test; the RVA data, color, syneresis and thaw stability cycles. The viscosities through the weeks were analyzed with a Completely Randomized Design with repeated measures over time, followed by a Duncan test. For the sensory study RStudio (2009, v.1.3.1056) was the statistical software used. The data was analyzed with an independent T-test was used to analyze the attributes of appearance, color, texture, overall liking, and flavor (score with a hedonic scale).
4. RESULTS AND DISCUSSION

4.1. Amylose content

The amylose content of the four starches was evaluated with the Megazyme Amylose/Amylopectin Assay Kit. The amylose content results were 21% for the food-grade rice starch, 19.1% for the clean label starch, 13.5% sigma native rice starch, and for the commercial modified corn starch was 62.6%.

4.2. Rapid visco-analyzer

The Rapid Visco-Analyzer (RVA) is an instrument used to imitate the physical and chemical changes in which starch undergoes during processing. The aspects measured in the RVA are pasting temperature, peak viscosity, trough viscosity, breakdown viscosity, final viscosity, and setback. The pasting temperature is the temperature at which the starch starts pasting. At this point, hydrogen bonds have been weakened enough by the temperature, making granules absorb water faster and gelatinize (Bahnassey and Breene, 1994). The peak's viscosity exhibits the swelling ability of granules at their maximum viscosity. The trough viscosity is the minimum viscosity that the starch will have after swelling and holding the maximum temperature. The difference between trough viscosity and peak viscosity is the breakdown value (Juhász and Salgó, 2008). The breakdown indicates the starch's stability in the process and is correlated with the content of amylopectin. The breakdown represents the disruption of gelatinized granules (Han and Hamaker, 2001).

In table 3, we see the comparison of different starches and different conditions in the RVA. The clean label (CLS) prepared with rice starch, stearic acid, and lysine had the lowest pasting temperature. All pasting temperatures were significantly different. The results of
commercial modified starch (MS) peak viscosity in the RVA are like those reported by Lin et al. (2013), in which they were testing waxy rice starch and waxy corn starch. The clean label starch (CLS) was exposed to a freeze-drying process, the gel was prepared then frozen at -20 °C, and freeze-dried for three days. This starch presented a greater breakdown value with a mean of 1375cP. It has been proven that freeze-drying conditions affect B-type polymorphic starches (tubers starches) more than A-type polymorphic starches (cereal starches), disrupting molecular order, crystallinity and damaging the surface of the granules (Zhang et al., 2014; Bao 2019). It is inevitable that changes occur in starch during freeze-drying, such as local fracture and structure disorganization, resulting from the removal of water with pressure and low temperatures (Zhang et al., 2014).

Table 3. Rapid Visco-Analyzer results for different starches.

<table>
<thead>
<tr>
<th>TRT</th>
<th>Pasting Temp (°C)</th>
<th>Peak Viscosity</th>
<th>Trough Viscosity</th>
<th>Breakdown</th>
<th>Final Viscosity</th>
<th>Total Setback</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLS</td>
<td>62.41 ± 0.04E</td>
<td>4662 ± 110.87A</td>
<td>3287 ± 59.86A</td>
<td>1375 ± 98.53A</td>
<td>4219 ± 51B</td>
<td>932 ± 32.92D</td>
</tr>
<tr>
<td>MS</td>
<td>68.83 ± 0.03D</td>
<td>2749 ± 125.7C</td>
<td>2530 ± 108.7B</td>
<td>225.7 ± 22.9C</td>
<td>4301 ± 154.6B</td>
<td>1771 ± 46.8A</td>
</tr>
<tr>
<td>RS</td>
<td>79.93 ± 0.03C</td>
<td>3782.7 ± 49.2B</td>
<td>3296 ± 67.09A</td>
<td>486.7 ± 29.9B</td>
<td>4658.3 ± 98.4A</td>
<td>1362 ± 35.3B</td>
</tr>
<tr>
<td>RS+L+St</td>
<td>92.20 ± 0.03A</td>
<td>2215 ± 97.34D</td>
<td>1999.67 ± 61.85C</td>
<td>216± 37.64C</td>
<td>2874.67 ± 93.88D</td>
<td>875 ± 82.87D</td>
</tr>
<tr>
<td>SRS</td>
<td>82.42 ± 0.88B</td>
<td>2757 ± 103.4C</td>
<td>2177.7 ± 74.2C</td>
<td>579.3 ± 55.7B</td>
<td>3337.7 ± 43.02C</td>
<td>1160 ± 32.4C</td>
</tr>
</tbody>
</table>

a. CLS, clean label starch (already freeze dried); MS, commercial modified starch; RS, food grade rice starch; RS+L+St, rice starch, 6% lysine, 1% stearic acid (not freeze dried and they were added directly to the canister); SRS, sigma native rice starch (not food grade).  
b. All values are expressed in centipoise cP (1 cP=1 mPa s).  
c. Values followed by the same letter in the column are not significantly different (P>0.05).  
d. Breakdown= peak viscosity – trough viscosity; Total Setback= final viscosity – trough viscosity.  
e. Data expressed as means ± standard deviation.
The total setback value is the difference between the trough viscosity (minimum viscosity) and the final viscosity. This is considered to represent the retrogradation tendency (An and King, 2009). The lower the value, the less retrogradation tendency the starch has. There was no significant difference in the total setback values of RS+L+St and CLS. Both starches contained the same percentages of lysine (6%) and stearic acid (1%), the main difference is that CLS was gelatinized then freeze dried, while all ingredients of RS+L+St were added directly to the canister. These starches had the lowest total setback values (there was no significant difference between them), having the lowest retrogradation tendency. This low retrogradation tendency can be attributed to the addition of the amino acid (lysine) (An and King, 2009). Low retrogradation tendency starch maintains quality stability for a longer time, than starch that has a fast retrogradation tendency. MS has the highest retrogradation tendency which is represented by its high total set back value, this may be due to its greater amylose content.

4.3. Differential scanning calorimeter

The DSC determines the thermal behavior of starch. The starch subjected to freeze-drying (CLS) had a significantly lower gelatinization temperature and a lower ΔH (Table 4). The ΔH is a representation of the structural organization and stability of crystalline areas. The lower this is value, the lower the percentage of the organized structure and a decrease in the stability of crystalline areas (Colussi et al., 2017). These results can be compared to a study by Zhang et al. (2014), they compared different drying treatments on maize and potato starch; they concluded that the freeze-dried process makes significant changes in the starch structure. There is a change in the molecular order and crystallinity during freeze-drying, making $T_o$ and ΔH lower than the other treatments (Zhang et al., 2014).
Table 4. Differential scanning calorimeter results for different starches used in both products.

<table>
<thead>
<tr>
<th></th>
<th>First peak</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Second peak</th>
<th></th>
<th></th>
<th></th>
<th>Third peak</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₀</td>
<td>Tₚ</td>
<td>ΔH(J/g)</td>
<td>T₀</td>
<td>Tₚ</td>
<td>ΔH(J/g)</td>
<td>T₀</td>
<td>Tₚ</td>
<td>ΔH(J/g)</td>
<td>T₀</td>
<td>Tₚ</td>
<td>ΔH(J/g)</td>
</tr>
<tr>
<td>CLS</td>
<td>46.6±1.4C</td>
<td>56.2±1.4C</td>
<td>0.35±0.05B</td>
<td>74±3.6B</td>
<td>94.1±0.96A</td>
<td>0.31±0.14A</td>
<td>106±0.14A</td>
<td>0.31±0.04A</td>
<td>117±2.84A</td>
<td>120±0.32A</td>
<td>125±0.82A</td>
<td>0.08±0.05A</td>
</tr>
<tr>
<td></td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
<td>125±120±</td>
</tr>
<tr>
<td>RS</td>
<td>71±0.02A</td>
<td>76.5±0.09A</td>
<td>1.33±0.04A</td>
<td>90±1.11A</td>
<td>93.9±0.4A</td>
<td>0.11±0.09A</td>
<td>101±0.9B</td>
<td>0.11±0.02B</td>
<td>110±1.6B</td>
<td>116±0.6B</td>
<td>120±0.6B</td>
<td>0.03±0.01A</td>
</tr>
<tr>
<td></td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
<td>120±120±</td>
</tr>
<tr>
<td>MS</td>
<td>56.5±0.3B</td>
<td>64.4±0.14B</td>
<td>1.67±0.32A</td>
<td>103.9±7.2A</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

a. CLS, clean label starch (already freeze dried); MS, commercial modified starch; RS, food grade rice starch; T₀, onset temperature; Tₚ, peak temperature; T_c, conclusion temperature; ΔH, change of enthalpy.
b. Temperatures are expressed in degree Celsius units; ΔH is expressed in Joule per gram (J/g).
c. Values followed by the same letter in the column are not significantly different (P>0.05).
d. Data expressed as means ± standard deviation
The first peak in the DCS curves (figure 7 and figure 8) is caused by the melting of amylopectin, changing its structure. The second peak is caused by the melting of the amylose-lipid complex, and this one occurs in between 90-120 °C (Shahbazi et al., 2018). In this case, it occurred at 105 °C for the clean label starch and at 110°C for the food-grade rice starch. The third peak has been related to the order-disorder transition of amylose-lipid complexes (Fukuoka et al., 2002; Primo-Martin et al., 2007). The third peak is observed in clean-label starch and rice starch at a temperature higher than 120°C (Table 4, Figure 7, and Figure 8). Sievert and Pomeranz (1990) suggested that a peak between 120°C and 177°C can be due to the presence of resistant starch. They inferred that retrograded amylose (due to heat treatment) is essential for the
formation of resistant starch (Sievert and Pomeranz, 1990). Modified starch (Figure 9) presented one peak with two maximum temperatures.

Figure 8. DSC graph of food grade rice starch.
4.4. Scanning electron microscopy (SEM)

The scanning electron (SEM) was used to observe the surface of starch granules. Electron micrographs are presented in Figure 10. Different magnifications were used to appreciate better the granules. Food grade rice starch and Sigma rice starch are both native starches, but rice starch (A) appears to have been heated, as it does not show intact granules like the Sigma rice starch (B). The clean label starch has visible damage on the surface due to the heating, gelatinization, and then the freeze-drying process. Rice starch granules are more susceptible to damage by heat (Tester, 1997). A study with potato starch showed that the starch dried with freeze-drying methods had more wrinkles and scratches compared to the potato starch that was dried with an oven and with ethanol drying (Zhang et al., 2014). The rough surface and scratches are mainly due to the water as vapor trying to come out of the inside of the solid structure. Zhang et al.
believe that micropores can be formed during this process, but they cannot be observed with a SEM. Figure 11 shows a group pieces of granules from the clean label starch, and it is evident that they have damage on the surface. The data from the DSC showed that the gelatinization temperature for clean label starch was the lowest (Table 4), indicating that it takes a lower temperature to reach gelatinization and desired viscosity. The damage of the granules can make more soluble a starch. This is beneficial for food applications in which it is desired to hydrate the granules and form gels at a lower temperature (Tester, 1997).

Figure 10. Electron micrographs of starches. Food grade rice starch (A) magnification 800x; sigma native rice starch (B) magnification 20,000; clean label rice starch (C) magnification 2,000x; commercial modified corn starch (D) magnification 6,500x.

Figure 10, image D is commercial waxy corn modified starch (PRECISA® Cream 20, Ingredieon, USA); this starch has passed a modification process. It can be inferred that this starch was modified with acid due to its shape and its exo-erosion damage on the surface. This
image was compared to Ulbrich et al. (2019) SEM images in their study. They studied different HCl acid concentrations (0.3, 0.6, and 0.9M) applied to waxy corn starch and high amylose starch during specific periods (4, 10, and 20 hours) (Ulbrich et al., 2019).

Figure 11. Clean label starch image. Clean label starch with 1000X magnification.

4.5. Storage study

4.5.1. Viscosity of gravies

Gravy viscosities were measured with the RVA, creating a setting to measure only viscosity at room temperature (25°C) at 160 rpm of mixing. The values obtained from this setting were peak and final viscosity. The gravy was defrosted by microwaving it for 3 minutes; when its temperature cooled to room temperature, it was poured into a canister. The gravy was analyzed over six-weeks with a two-week interval. Table 5 is a summary of the apparent
viscosity. Initially there were no changes in formulations; all percentages were kept constant, even though the consistency of the gravy with modified starch was more liquid than the others. That is why the amount of modified starch was increased, but no more than 5% to be considered gravy (Zeng et al., 1996). The modified starch gravy had a liquid consistency during the study. The peak viscosity of this gravy increased significantly in week 2 and week 4, but there was no significant difference in week 6 from those mentioned. In contrast, it is observed that the gravy rice starch gravy was significantly different during the six weeks. Th clean label starch significantly increased its viscosity after the first week but decreased in week 6.

Table 5. Six-week study of gravy’s apparent viscosity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PV</td>
<td>FV</td>
<td>PV</td>
<td>FV</td>
</tr>
<tr>
<td>RS</td>
<td>540.3±</td>
<td>373±</td>
<td>2182.3±</td>
<td>1210.7±</td>
</tr>
<tr>
<td></td>
<td>85D</td>
<td>59C</td>
<td>226.6A</td>
<td>110.2A</td>
</tr>
<tr>
<td>MS</td>
<td>66.7±</td>
<td>27.7±</td>
<td>107.3±</td>
<td>74.0±</td>
</tr>
<tr>
<td></td>
<td>0C</td>
<td>9.1B</td>
<td>12B</td>
<td>11B</td>
</tr>
<tr>
<td>CLS</td>
<td>664.5±</td>
<td>431.7±</td>
<td>1924.7±</td>
<td>1236±</td>
</tr>
<tr>
<td></td>
<td>17.7B</td>
<td>17.6C</td>
<td>255A</td>
<td>141.6A</td>
</tr>
</tbody>
</table>

a. CLS, clean label starch (already freeze dried); MS, commercial modified starch; RS, food grade rice starch.
b. PV, peak viscosity; FV final viscosity.
c. All values are expressed in centipoise (cp).
d. Values followed by the same letter in the row for each parameter are not significantly different (P>0.05).
e. These apparent viscosities were measured with the RVA at room temperature.
f. Data expressed as means ± standard deviation.

A trend can be followed in all starches on their peak viscosity through the six weeks. After two weeks, all products' viscosities increased, but after that point, all viscosities started to decrease. This can be compared to a study made with gravy stored for six months at -18 ±2°C; they recorded a decrease of the gravy's viscosity after the first month of storage. It was suggested that this could be due to emulsion stability at freezing temperatures (Modi et al., 2006).
4.5.2. Gravy viscosity comparison

In this study, the gravy formulation with modified starch was adjusted as in table 2. The small particle size of rice starch increases the swelling power over the waxy corn starch (Lin et al., 2013). This allows the starch to absorb more water, which may be the reason why it was necessary to add more modified corn starch to the formation to get a similar consistency as the other gravies with rice starch. In the following table (Table 6), the gravies are compared from week 0 to week 3, which showed an increase in viscosity over time. The freezing temperature is an essential factor for the amylose to be dissolved. The lower the freezing temperature is, the more amylose will be dissolved, and amylopectin increases by rearranging (retrogradation), this causes an increase of the viscosity of the starch (Su et al., 2020).

Table 6. Comparison table of gravy’s viscosity in the three-week study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Week 0 (cp)</th>
<th>Temperature (°C)</th>
<th>Week 3 (cp)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>264±25.6B</td>
<td>56</td>
<td>423±2.63A</td>
<td>56</td>
</tr>
<tr>
<td>MS¥</td>
<td>383±39.6B</td>
<td>55</td>
<td>770±83.7A</td>
<td>57</td>
</tr>
<tr>
<td>CLS</td>
<td>222±40B</td>
<td>57</td>
<td>374±38.7A</td>
<td>56</td>
</tr>
</tbody>
</table>

a. CLS, clean label starch (already freeze dried); MS, commercial modified starch; RS, food grade rice starch.
b. The apparent viscosity was measure in the Brookfield at 50 RPM with a RV3 spindle.
c. Values followed by the same letter in the row are not significantly different (P>0.05)
d. Data expressed as means ± standard deviation.
¥ This gravy contained modified starch and its formula was adjusted following table 2.

4.5.3. Thaw stability cycles and syneresis

In this study, the gravy was stored frozen, and samples were reheated in the microwave in each cycle. There was a study where native potato starch gels were microwaved reheated after several days of refrigeration (Colussi et al., 2017). Colussi et al. (2017) indicated that reheating a
gel in a microwave destroyed the gel structure since it appeared to be loosened after reheating twice (Colussi et al., 2017). Their results also showed that the microstructural characteristics changed compared to fresh gel; the gels that were retrograded and reheated had a more compact network. Repeated freeze-thaw cycles create an extensive retrogradation due to the association of amylose and amylopectin, resulting in cloudiness and syneresis (Hoover et al., 1988), which were observed in this study.

In table 7, the same gravy was heated in each cycle (three times the same sample); 10 grams of each sample were taken and placed in a centrifuge tube. The differences in the cycles are due to the dehydration of the gravy in the microwave for sample CLS. MS and RS remained constant; MS was liquid during all three cycles. The appearance of MS changed during the three cycles, the separation of liquid and the clumps of gravy were evident. To get a more representative sample of the gravy, all samples were stirred before placing it in the centrifuge tubes.

Table 7. Reheated gravy’s freeze thaw stability cycles (syneresis percentage).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>12% ± 1B</td>
<td>18% ±0A</td>
<td>16% ± 4AB</td>
</tr>
<tr>
<td>MS</td>
<td>60% ± 0A</td>
<td>58% ±1AB</td>
<td>54% ± 2B</td>
</tr>
<tr>
<td>CLS</td>
<td>32% ± 4A</td>
<td>18% ± 1B</td>
<td>9% ± 3C</td>
</tr>
</tbody>
</table>

a. CLS, clean label starch (already freeze dried); MS, commercial modified starch; RS, food grade rice starch.
b. Data expressed as means ± standard deviation.
c. Values followed by the same letter in the row are not significantly different (P>0.05)
d. Data was calculated using formula 1.

The syneresis of gravy was measured for three weeks. In this study, the gravy with modified starch followed the formulation from table 2. This gravy contained a higher percentage
of starch to make it similar to the consistency of the other gravies. These gravies were defrosted at room temperature for 30 min and heated for 1 minute in the microwave. The samples were heated once each week. Syneresis, was measured at room temperature. The rice starch tended to increase syneresis over the weeks (Table 8). CLS maintained its syneresis constant over three weeks. The syneresis of modified starch decreased after the first week, then stayed constant in week two and three.

Table 8. Gravy’s syneresis during three-week study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>4%±1C</td>
<td>16%±0B</td>
<td>26%±0A</td>
<td>21%±3AB</td>
</tr>
<tr>
<td>MS*</td>
<td>35%±2A</td>
<td>26%±1B</td>
<td>29%±0B</td>
<td>25%±1B</td>
</tr>
<tr>
<td>CLS</td>
<td>18%±0A</td>
<td>21%±3A</td>
<td>17%±1A</td>
<td>20%±2A</td>
</tr>
</tbody>
</table>

a. CLS, clean label starch (already freeze dried); MS, commercial modified starch; RS, food grade rice starch.
b. Data expressed as means ± standard deviation.
c. Values followed by the same letter in the row are not significantly different (P>0.05)
   ¥ This gravy contained modified starch and its formula was adjusted following table 2.

4.5.4. Pudding viscosity and syneresis

Puddings’ apparent viscosities were analyzed with the Brookfield viscometer. The comparison between weeks was made with measures repeated in time and Duncan’s post-test. The results are expressed in table 9. Rice starch pudding had no significant changes in its viscosity from week one to week three. While measuring the viscosity, it was observed that after one week at 5℃, a firm gel was formed. Over the weeks, the gel formed was not changing, but its syneresis was increasing (showed in table 10), the gel was shrinking. The increase of syneresis in native potato starch gels after seven days of refrigeration was reported previously (Colussi et al., 2017). In clean label starch, it was observed that there was no significant difference between week zero and week one, then there was no significant difference between
There was a significant difference on week three compared to the past weeks’ viscosities. This can be related to the increase of syneresis showed in table 10, in which the syneresis of CLS was increased; by increasing the syneresis, the gel becomes firmer. For this study, it was necessary to increase the amount of modified starch in the formulation in comparison to the other starches since its consistency at the same percentage level was liquid. This pudding needed a higher amount of modified starch to make its consistency look like the others. The modified starch presented similar viscosity results to the clean label starch in differences between weeks.

Table 9. Apparent viscosity of pudding stored at 5°C for three weeks.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>22823.3 ± 861.3A</td>
<td>19483.2 ± 1438.5B</td>
<td>18408.6 ± 1149.5B</td>
<td>17783.96 ± 383.1B</td>
</tr>
<tr>
<td>MS¥</td>
<td>23079.5 ± 899BA</td>
<td>21427.1 ± 622.6BC</td>
<td>19296.3 ± 1334.3C</td>
<td>25146.3 ± 734.2A</td>
</tr>
<tr>
<td>CLS</td>
<td>13019.6 ± 300.5B</td>
<td>12124.3 ± 405.2CB</td>
<td>10237.7 ± 2040.8C</td>
<td>15814.9 ± 1285A</td>
</tr>
</tbody>
</table>

a. CLS, clean label starch (already freeze dried); MS, commercial modified starch; RS, food grade rice starch.
b. All values are expressed in centipoise (cp).
c. Data expressed as means ± standard deviation.
d. Values followed by the same letter in the row are not significantly different (P>0.05).
e. These apparent viscosities were measure by the Brookfield at 50 RMP with a T-D spindle.
¥ This pudding contained modified starch and its formula was adjusted following table 1.

Table 10. Percentage of pudding syneresis during three weeks at 5°C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>0%±0D</td>
<td>7%±1.3C</td>
<td>16%±1.6B</td>
<td>20%±1A</td>
</tr>
<tr>
<td>MS¥</td>
<td>0%±0B</td>
<td>0.4%±0.1A</td>
<td>0%±0B</td>
<td>0%±0B</td>
</tr>
<tr>
<td>CLS</td>
<td>0%±0D</td>
<td>8%±3C</td>
<td>14%±0.6B</td>
<td>24%±1A</td>
</tr>
</tbody>
</table>

a. CLS, clean label starch (already freeze dried); MS, commercial modified starch; RS, food grade rice starch.
b. Data expressed as means ± standard deviation.
c. Values followed by the same letter in the row are not significantly different (P>0.05).
¥ This pudding contained modified starch and its formula was adjusted following table 1.
4.5.5. Pudding’s pH

The potential of hydrogen (pH) is a measurement to indicate if a substance is acid or basic, based on its hydrogen ion concentration. The measurement is based on a scale from 0 to 14. Bacteria growth can be inhibited by the addition of weak acid; the acid enters the membrane in its undissociated form, and then it dissociates with the internal pH. This causes the bacteria to try to regulate its intercellular pH, but it is too much that it kills the bacteria (Salmond et al., 1984). When pH is combined with other factors such as water activity and temperature, it can limit the growth of bacteria (Koutsoumanis and Sofos, 2005). Bacteria have specific ranges of growth according to the pH in which they are surrounded. There are acidophile bacteria, neutrophile, and alkalophiles (Booth, 1985). There is only one group of bacteria capable of growing in an acid medium, these are the acidophile bacteria, and they grow at less than pH 3 (Baker-Austin and Dopson, 2007). The pH is also an indicator of microbiological parameters. The pH of a medium with bacteria can be an indicator of which bacteria it is. In a food matrix, if there is a change of pH in a certain period, it is an indicator of the presence of bacteria. Bacteria can produce by-products such as isopropanol, acetic acid, and butyric acid (Kim et al., 2011). The production of by-products depends on conditions such as temperature, media, and aerobic or anaerobic conditions.

Table 11. Puddings’ pH average for 3 weeks.

<table>
<thead>
<tr>
<th>Pudding pH</th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.41±0.6^A</td>
<td>6.42±0.3^A</td>
<td>6.37±0.5^A</td>
<td>6.38±0.2^A</td>
</tr>
</tbody>
</table>

a. Data expressed as means ± standard deviation.
b. Values followed by the same letter in the row are not significantly different (P>0.05).
In this study, the pH of the pudding was monitored for three weeks to determine if its pH will become more acidic due to the presence of bacteria. There was no significant difference in pH during the three weeks (Table 11).

The storage study at accelerated conditions and at room temperature resulted in the pudding's spoilage in less than a week. This happened due to the previous conditions mentioned; the pH of these samples dropped from 6.4 to 4.3 (data not shown). The samples had syneresis, and a characteristic smell of fermented milk was perceived.

4.6. Microbiology of gravy and pudding

Microbiological tests were performed during the storage studies to assure that the product was safe for consumption and that the procedure followed food safety practices. The tests were performed in gravy and pudding weekly. There was no bacterial growth detected in either one. These results allow us to conclude that the products are safe for consumption for three weeks. There was no growth of bacteria due to the control of temperature during cooking (Gill et al., 2009) and then refrigeration (Evans et al., 2004) after putting it in a closed container.

4.7. Color of pudding and gravy

Color is an important attribute for food products, and consumers are attracted to their food colors. They base their purchase decision on the visual aspects of the product. If the products do not meet their expectations in color and appearance, they will not purchase it. Processing may influence the visual color of products, degree of light exposure, and chemical interaction between ingredients (Modi et al., 2006). The color values are represented by L*
representing lightness, a* representing redness-greenness, and b* representing yellowness-bluneness (Gordon, 2021).

L* values in the pudding samples (Table 12), were above 80, with the rice starch puddings being the most white, which is a characteristic of the rice starch (Song et al., 2006). The negative a* value represents a slightly green color; the samples were in a range between -2.5 to -3.6. The b* value represents a yellowish color and the samples were in arrange of 6.2 to 7.8. The yellowish color can be attributed to the vanilla and the butter.

<table>
<thead>
<tr>
<th>Axis</th>
<th>Clean label starch</th>
<th>Modified starch (^{\text{¥}})</th>
<th>Rice starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>84±1(^{\text{A}})</td>
<td>80.6±0.4(^{\text{B}})</td>
<td>83.8±0.8(^{\text{A}})</td>
</tr>
<tr>
<td>-a*</td>
<td>-2.9±0.1(^{\text{AB}})</td>
<td>-3.6±0.5(^{\text{A}})</td>
<td>-2.5±0.5(^{\text{B}})</td>
</tr>
<tr>
<td>+b*</td>
<td>7.8±0.1(^{\text{A}})</td>
<td>6.2±0.4(^{\text{B}})</td>
<td>7.8±0.1(^{\text{A}})</td>
</tr>
</tbody>
</table>

a. Data expressed as means ± standard deviation.
b. Values followed by the same letter in the row are not significantly different (P>0.05).
¥ This pudding contained modified starch and its formula was adjusted following table 1.

The gravy values for color are presented in table 13. The lightness in these products is above 50% (mid-gray), the a+ value that is less than 1 provides the redness, and b+ yellow color is related to the brownish color that is characterized by the gravy. The beef broth and starch are the providers of the color in the gravy. There was no significant difference between the clean label starch and the modified starch in color.
Table 13. Gravy color values.

<table>
<thead>
<tr>
<th>Axis</th>
<th>Clean label starch</th>
<th>Modified starch(^¥)</th>
<th>Rice starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>54.3±1.5(^A)</td>
<td>51.8±1.5(^A)</td>
<td>52±0.9(^A)</td>
</tr>
<tr>
<td>+a*</td>
<td>0.7±0.1(^B)</td>
<td>0.8±0.1(^B)</td>
<td>1±0.1(^A)</td>
</tr>
<tr>
<td>+b*</td>
<td>6.1±0.1(^A)</td>
<td>5.5±0.6(^A)</td>
<td>5.2±0.4(^A)</td>
</tr>
</tbody>
</table>

a. Data expressed as means ± standard deviation.
b. Values followed by the same letter in the row are not significantly different (P>0.05).
\(^¥\) This pudding contained modified starch and its formula was adjusted following table 2.

4.8. Consumer study

4.8.1. Pudding consumer study

The consumer study was held in the LSU AgCenter School of Nutrition and Food Sciences sensory laboratory and 87 people participated in the study. The gender distribution indicated (Figure 12) that 53% of the population were female and 47% were male. There was no significant difference between men and women likings between samples (data not shown). Neither had any influence in the evaluation of the pudding.

Figure 12. Pudding gender distribution.
A study in which color and perception were tested indicated that people related color to flavor when the only variance was color (Garber et al., 2000). The consumers of that study argued that the food had off-flavors and several people reported themselves as ill; all food had the same ingredients. The only thing that was changed was the color. Therefore, for the food that had the right color, the comments were that the food was tasty and had good texture and smell (Garber et al., 2000).

The attribute of color was evaluated in a consumer study of pudding. Color had a score of 6.4 based on a 9-point score (table 14). This score indicated that the color was acceptable for the consumers. There was no significant difference between the two samples (clean label and modified starch). In another study, children tended to prefer vanilla pudding that was more white than yellow (Kristanti and Herminiati, 2019). A study conducted in 2016 revealed that people tend to consume light-colored puddings than dark-colored puddings (Madzharov et al., 2016).

Table 14. Clean label pudding and modified starch pudding comparative hedonic scale table.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Clean label pudding</th>
<th>Modified starch pudding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Median</td>
</tr>
<tr>
<td>Appearance</td>
<td>6.38 ±1.83A</td>
<td>7</td>
</tr>
<tr>
<td>Color</td>
<td>6.44 ±1.84A</td>
<td>7</td>
</tr>
<tr>
<td>Overall texture</td>
<td>7.05 ±1.63A</td>
<td>7</td>
</tr>
<tr>
<td>Flavor</td>
<td>7.31 ±1.50A</td>
<td>8</td>
</tr>
<tr>
<td>Overall liking</td>
<td>7.25 ±1.47A</td>
<td>8</td>
</tr>
</tbody>
</table>

- Values followed by the same letter in the row are not significantly different (P>0.05)
- N=87
- Data expressed as means ± standard deviation.

Results showed that there was no significant difference in the attributes of color and appearance, and both had a score of 6, which represents that the product was acceptable for the
consumers (Table 14). There was a significant difference between samples for overall texture (which can also be referred to as consistency), flavor and overall liking. The pudding with clean label starch was more liked over the modified starch. The clean label pudding contained rice starch which is characterized by its white color and bland flavor (Song et al., 2006), allowing the vanilla flavor to be better perceived by the consumer. While the pudding with modified starch contained corn starch in a higher amount (due to consistency), this pudding obtained a lower score in flavor and overall liking. It can be inferred that it is due to the corn starch. It has been reported that corn starch affects the sweetness of a product (Kälviäinen et al., 2007). Corn starch in companion with other ingredients has been used to mask or reduce the bitter flavor of pharmaceuticals (Sohi et al., 2004). An informal interview was performed after the sensory to a significant amount of the consumers. They commented that pudding with modified starch had a starchy flavor or raw flour flavor. The difference in the liking of overall texture is due to the difference in apparent viscosity showed in table 9. People liked a less viscous pudding.

The purchase intent of panelists is presented in the following table (Table 15). The overall liking and purchase intent were asked of the consumers before showing the clean label statement. The clean label statement claimed that the pudding contained natural, simple, non-synthetic chemical ingredients easy to recognize. Their purchase intent was increased by 10.3% on the yes answers after the statement. The highest purchase motive is healthy products, but a large population is influenced by the clean label trend (Asioli et al., 2017).
Table 15. Purchase intent of pudding.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Overall Liking A-CLS</th>
<th>Purchase Intent A-CLS</th>
<th>Overall Liking B-CLS</th>
<th>Purchase Intent B-CLS</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean label</td>
<td>7.25</td>
<td>58 Yes (66.7%)</td>
<td>7.49</td>
<td>64 Yes (73.6%)</td>
<td>10.3%</td>
</tr>
<tr>
<td>Modified</td>
<td>5.38</td>
<td>20 Yes (23%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. B-CLS, before clean label statement; A-CLS, after clean label statement.
b. N=87

4.8.2 Gravy consumer study

The consumer study was held in the LSU AgCenter School of Nutrition and Food Sciences sensory laboratory and 75 people participated the study. The gender distribution indicated (Figure 13) that 51% of the population were female and 49% were male. There was no significant difference between men and women preferences between samples (data not shown). Neither had any influence in the evaluation of the gravy.

![Gravy Gender Distribution](image)

Figure 13. Gender distribution of gravy’s consumer study.

The consumer study for gravy utilized a 9-point hedonic scale to evaluate the attributes of appearance, color, overall texture, and overall flavor. The appearance and color of both samples
were significantly different. Gravy with modified starch had a higher appearance score of 6.21 compared to clean label gravy with a score of 5.6. Appearance is related to the exteroceptive cues and the recognition of particles in the product (Santagiuliana et al., 2019). The gravy can be described as clear brown semiliquid. The viscosity of the gravy is directly related to the amount of starch (Zeng et al., 1996). The gravy's viscosity was different, as is shown in table 6, but the consumers did not perceive it. The product's overall liking was 5.99 for clean label sample and 5.85 for modified starch sample; which could be because the gravy was served by itself without any side. The consumption of gravy is commonly on top of vegetables, potatoes, or meat. Liking was higher with a score of 6.5 and 6.3 in a study, where the gravy was served with mashed potatoes (Tsikritzi et al., 2015). More than 70% of the consumers gave a score above 6 to the clean label gravy, a score of 6 represents that the consumer slightly liked the product. We can conclude that the flavor was acceptable for the consumers (table 16). For the clean label starch, the flavor had the highest percentage of above 6 (72%), it is inferred that this attribute affected the overall liking score. In contrast for the modified starch gravy the score above 6 was slightly lower for flavor and overall liking.

Table 16. Gravy hedonic scale summary for consumer study.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Clean label gravy</th>
<th>Modified starch gravy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>Appearance</td>
<td>5.60 ±1.48^B</td>
<td>6</td>
</tr>
<tr>
<td>Color</td>
<td>5.55 ±1.71^B</td>
<td>6</td>
</tr>
<tr>
<td>Overall texture</td>
<td>5.55 ±1.61^A</td>
<td>6</td>
</tr>
<tr>
<td>Flavor</td>
<td>6.07 ±1.91^A</td>
<td>6</td>
</tr>
<tr>
<td>Overall liking</td>
<td>5.99 ±1.61^A</td>
<td>6</td>
</tr>
</tbody>
</table>

a. Values followed by the same letter in the row are not significantly different (P>0.05)

b. N=75
c. Data expressed as means ± standard deviation.
The purchase intent of gravy is represented in table 17. The purchase intent was significantly different after the clean label claim and increased 36%.

Table 17. Purchase intent of gravy.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Overall Liking B-CLS</th>
<th>Purchase Intent B-CLS</th>
<th>Overall Liking A-CLS</th>
<th>Purchase Intent B-CLS</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean label starch</td>
<td>5.99</td>
<td>33 Yes (44%)</td>
<td>6.43</td>
<td>45 Yes (60%)</td>
<td>36.4%</td>
</tr>
<tr>
<td>Modified starch</td>
<td>5.85</td>
<td>32 Yes (42.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. B-CLS, before clean label statement; A-CLS, after clean label statement.
b. N=75

The pudding and the gravy with clean label starch were acceptable for the consumer. These studies showed how consumers are influenced by the claim of having clean label product. This can be concluded by the increase in their purchase intent after the clean label statement.
5. CONCLUSIONS

The main objectives of this research were to develop a pudding and a gravy with clean label rice starch and test its stability under frozen and refrigeration temperatures; compare the clean label rice starch to a commercial modified starch; characterize and analyze the clean label starch; and determine consumer acceptability and purchase intent of the products.

It can be concluded that based on the results of the RVA, is evident that the freeze-drying process affected the starch in a negative way. The freeze-drying increased the breakdown value for the clean label starch; this could lower the starch's stability during food processing since freeze-drying makes significant changes in the granule structure. The results of the DSC supported this. The starch subjected to freeze-dried treatment had a lower $\Delta H$ and a lower $T_o$. It is highly recommended to try different processing methods to produce the clean label starch, avoiding processes that will cause significant damages to the granules.

The pudding was kept refrigerated for three weeks; during which the pH was maintained constant, indicating no fermentation. This was confirmed by the microbiology tests, in which no bacteria were detected over the three weeks. The quality of the rice starch pudding and the clean label starch pudding were affected as syneresis increased. It is recommended to try different packages and conditions for the pudding. Due to the clean label starch's low pasting temperature, it is recommended to make trials for an instant pudding.

It can be concluded that reheating the gravy several times can affect its viscosity and quality since it tends to dehydrate during microwave eating. The viscosity of the gravy tends to increase over the weeks. This an essential factor to be considered for future processing.
All the formulations were kept constant to prepare both products, but for both products, the amount of modified starch was not enough. To obtain a similar consistency as the other it was necessary to increase the amount of modified starch. By increasing the starch amount, factors such as flavor and mouthfeel (overall consistency of the product) were negatively affected. The consumer gave a higher score in attributes to the clean label starch. The purchase intent of the product was positively influenced by the statement of the clean label product. For the gravy, the increase was 36.4 % and for the pudding was 10%. It can be concluded that the trend of clean label products affects the purchase intent of consumers.
APPENDIX A. IRB - Approval

Research Consent Form (EXAMPLE)

I., agree to participate in the research entitled “Consumer Acceptance and Perception of New and Healthier Food Products” which is being conducted by Dr. Wittern Pyne, a Professor of the School of Nutrition and Food Sciences at Louisiana State University, Agricultural Center, phone number (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experiential records, or destroyed. Up to 300 consumers will participate in this research. For this particular research, about 15-20 minutes participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.

2. The reason for the research is to gather information on sensory acceptability, emotion and purchase intent of new and healthier food products. The benefit that I may expect from it is a satisfaction that I have contributed to quality improvement of these products.

3. The procedures are as follows: A 3-5 coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk. The only risk which can be envisioned is that of an allergic reaction toward common food ingredients (red beans, bell pepper, onion, garlic, celery, thyme, cayenne pepper, bay leaf, pork products, rice and rice products, milk and dairy products, yogurt or fermented milk products, peanuts, mayonnaise products, wheat flour, tapioca flour, eggs, table sugar, vanilla, soy products, sweet potato, salt (sodium chloride) and salt substitute (potassium chloride and common amino acids such as glycine and lysine), and plain unsalted crackers). However, because it is known to me beforehand that the food to be tested contains common food ingredients, he situation can normally be avoided.

5. The results of this study will not be released to any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand that the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Michael Keenan, Chair of LSU AgCenter IRB, (225) 578-1708. I agree with the terms above and acknowledge.

Signature of Investigator

Signature of Participant

Witness: ___________________________ Date: ___________________________
LSU AgCenter Institutional Review Board (IRB)
Dr. Michael J. Keenan, Chair
School of Nutrition & Food Sciences
209 Knapp Hall
225-578-1708
mkeenan@agcirls.edu

Application for Exemption from Institutional Oversight

All research projects using living humans as subjects, or samples or data obtained from humans must be
approved or exempted in advance by the LSU AgCenter IRB. This form helps the principal investigator

- Applicant, please fill out the application in its entirety and include the completed application as
  well as parts A-E, listed below, when submitting to the LSU AgCenter IRB. Once the application
  is completed, please submit the original and one copy to the chair, Dr. Michael J. Keenan, in 209
  Knapp Hall.

- A Complete Application includes All of the Following:
  (A) The original and a copy of this completed form and a copy of part B through E.
  (B) A brief project description (adequate to evaluate risks to subjects and to explain your
      responses to Parts 1 & 2)
  (C) Copies of all instruments and all recruitment material to be used.
      • If this proposal is part of a grant proposal, include a copy of the proposal.
  (D) The consent form you will use in the study (see part 3 for more information)
  (E) Beginning January 1, 2009: Certificate of Completion of Human Subjects Protection Training
     for all personnel involved in the project, including students who are involved with testing and
     handling data, unless already on file with the LSU AgCenter IRB.

1) Principal Investigator: Dr. Wisnon Prasayawathul
   Rank: Professor
   Student: Y/N, NO
   Dept: School of Nutrition & Food Sciences Ph: (225) 578-5155
   E-mail: wprasayawatulu@lsu.edu

2) Co-Investigator(s): Please include department, rank, phone and e-mail for each
   • If student is principal or co-investigator(s), please identify and name supervising professor in this
     space
     o Ashley Gutierrez, Research Associate, School of Nutrition & Food Sciences
     o (225) 578-5423, agutierrez@agcenter.lsu.edu

3) Project Title: Consumer acceptance and perception of New and Healthier Food Products

4) Grant Proposal? (yes or no) NO
   If Yes, Proposal Number and funding Agency
   Also, if Yes, either: this application completely matches the scope of work in the grant Y/N
   OR
   more IRB applications will be filed later Y/N

5) Subject pool (e.g. Nutrition Student) LSU Faculty, Staff, Students and off-campus consumers
   • Circle any "vulnerable population" to be used: children<18, the mentally impaired, pregnant
     women, the aged, other. Projects with incarcerated persons (may be exempted.

6) PI signature
   **Date 8/23/18 (no signatures)

**I certify that my responses are accurate and complete. If the project scope or design is later changed
I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-
LSU AgCenter institutions in which the study is conducted. I also understand that it is my responsibility to
maintain copies of all consent forms at the LSU AgCenter for three years after completion of the study. If I
leave the LSU AgCenter before that time, the consent forms should be preserved in the Departmental
Office.

Committee Action: Exempted □ Not Exempted □
IRB #: HE 18-22
Reviewer: Michael Keenan Signature: Michael Keenan Date: 9-5-2018
APPENDIX B. Consumer Study Questionaries

Pudding Consumer Study

Research Consent Form

I, _______________________, agree to participate in the research entitled “Development of Pudding and Gravy with Rice Starch, Stearic Acid and Lysine” conducted by Dr. Joan King, Professor of the School of Nutrition and Food Sciences at Louisiana State University, Agricultural Center, phone number (225) 620-2617.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participants returned to me, removed from the experimental records, or destroyed. Up to 75 consumers will participate in this research. For this particular research, about 5-10 minutes of participation will be required for each consumer. The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.

2. The reason for the research is to gather information on sensory acceptability, liking, and purchase intent of new products. The benefit that I may expect from it is a satisfaction that I have contributed to the quality improvement of these products.

3. The procedures are as follows: 2 coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk: The only risk which can be envisioned is that of an allergy to milk, or intolerance to lactose, wheat (from unsalted crackers); or adverse reaction to common food ingredients [milk, butter, salt, vanilla, or sugar]. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.

5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems
regarding these activities should be addressed to Dr. Michael Keenan, Chair of LSU AgCenter IRB, (225) 578-1708. I agree with the terms above and acknowledge.

Please, type your first and last name:

Gender

☐ Male ☐ Female

Age

☐ 18-25 ☐ 26-35 ☐ 36-45 ☐ 46-59 ☐ 60 +

Please select your highest level of education achieved.

☐ Highschool degree ☐ Undergraduate degree ☐ Master degree ☐ PhD degree ☐ Other professional degrees

Please drink water and eat unsalted crackers to cleanse your palate between samples

PLEASE CLOSELY OBSERVE THE PUDDING SAMPLE 769.

Please answer the following questions **BY VISUAL EVALUATION ONLY (DO NOT TASTE THE SAMPLE YET):**

Please rate your liking of the APPEARANCE of sample 769.

Dislike extremely ☐ Dislike very much ☐ Dislike moderately ☐ Dislike slightly ☐ Neither like or dislike ☐ Like slightly ☐ Like moderately ☐ Like very much ☐ Like extremely

Please rate your liking of the COLOR of sample 769.

Dislike extremely ☐ Dislike very much ☐ Dislike moderately ☐ Dislike slightly ☐ Neither like or dislike ☐ Like slightly ☐ Like moderately ☐ Like very much ☐ Like extremely

PLEASE TASTE SAMPLE SAMPLE 769.

AFTER THIS, ANSWER THE FOLLOWING QUESTIONS:

Please rate your liking of the OVERALL TEXTURE of sample 769.

Dislike extremely ☐ Dislike very much ☐ Dislike moderately ☐ Dislike slightly ☐ Neither like or dislike ☐ Like slightly ☐ Like moderately ☐ Like very much ☐ Like extremely
Please rate your liking of the **Flavor** of sample 769.

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>○</td>
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<td>○</td>
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<td>○</td>
</tr>
</tbody>
</table>

Please rate your **OVERALL LIKING** of sample 769.

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
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<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

Would you purchase this product?

Yes ☐

No ☐

*This is a clean label pudding. According to The Institute of Food Technologists (IFT) "Clean Label" is making products with natural, simple, non-synthetic chemicals and easy to recognize ingredients.*

**AFTER KNOWING THIS ADDITIONAL INFORMATION, PLEASE ANSWER THE FOLLOWING QUESTIONS.**

Please rate your **OVERALL LIKING** of sample 769.

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>○</td>
<td>○</td>
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<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

How likely would you purchase this product?

Yes ☐

No ☐

Please drink water and eat unsalted crackers to cleanse your palate between samples.

**PLEASE CLOSELY OBSERVE THE PUDDING SAMPLE 418.**

Please answer the following questions **BY VISUAL EVALUATION ONLY (DO NOT TASTE THE SAMPLE YET):**

Please rate your liking of the **APPEARANCE** of sample 418.
Please rate your liking of the COLOR of sample 418.

Please rate your liking of the OVERALL TEXTURE of sample 418.

Please rate your liking of the Flavor of sample 418.

Please rate your OVERALL LIKING of sample 418.

Would you purchase this product?

Yes  No

Thank you for your participation, please slide your tray through the sliding door in front of you.

Have a nice day!
Gravy Consumer Study

Research Consent Form

I, ______________________, agree to participate in the research entitled “Development of Pudding and Gravy with Rice Starch, Stearic Acid and Lysine” conducted by Dr. Joan King, Professor of the School of Nutrition and Food Sciences at Louisiana State University, Agricultural Center, phone number (225) 620-2617.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participants returned to me, removed from the experimental records, or destroyed. Up to 75 consumers will participate in this research. For this particular research, about 5-10 minutes of participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.

2. The reason for the research is to gather information on sensory acceptability, liking, and purchase intent of new products. The benefit that I may expect from it is a satisfaction that I have contributed to the quality improvement of these products.

3. The procedures are as follows: 2 coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk: The only risk which can be envisioned is that of an allergy to milk and milk products, wheat (from unsalted crackers); or adverse reaction to common food ingredients [Beef broth, butter, salt, pepper, or garlic]. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.

5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Michael Keenan, Chair of LSU AgCenter IRB, (225) 578-1708. I agree with the terms above and acknowledge.

Please, type your first and last name:
Gender
☐ Male ☐ Female

Age
☐ 18-25  ☐ 26-35  ☐ 36-45  ☐ 46-59  ☐ 60 +

Please select your highest level of education achieved.
☐ Highschool degree  ☐ Undergraduate degree  ☐ Master degree
☐ PhD degree  ☐ Other professional degrees

Please drink water and eat unsalted crackers to cleanse your palate between samples

PLEASE CLOSELY OBSERVE THE GRAVY SAMPLE 502.

Please answer the following questions **BY VISUAL EVALUATION ONLY (DO NOT TASTE THE SAMPLE YET):**

Please rate your liking of the **APPEARANCE** of sample 502.

Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like or dislike

Please rate your liking of the **COLOR** of sample 502.

Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like or dislike

PLEASE TASTE SAMPLE SAMPLE 502.

**AFTER THIS, ANSWER THE FOLLOWING QUESTIONS:**

Please rate your liking of the **OVERALL CONSISTENCY** of sample 502.

Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like or dislike

Please rate your liking of the **Flavor** of sample 502.

Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like or dislike
Please rate your **OVERALL LIKING** of sample 502.

Dislike extremely 
Dislike very much 
Dislike moderately 
Dislike slightly 
Neither like or dislike 
Like slightly 
Like moderately 
Like very much 
Like extremely

Would you purchase this product?

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This is a clean label gravy. According to The Institute of Food Technologists (IFT) "Clean Label" is making products with natural, simple, non-synthetic chemicals and easy to recognize ingredients.

AFTER KNOWING THIS ADDITIONAL INFORMATION, PLEASE ANSWER THE FOLLOWING QUESTIONS.

Please rate your **OVERALL LIKING** of sample 502.

Dislike extremely 
Dislike very much 
Dislike moderately 
Dislike slightly 
Neither like or dislike 
Like slightly 
Like moderately 
Like very much 
Like extremely

How likely would you purchase this product?

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Please drink water and eat unsalted crackers to cleanse your palate between samples.

PLEASE CLOSELY OBSERVE THE GRAVY SAMPLE 270.

Please answer the following questions **BY VISUAL EVALUATION ONLY (DO NOT TASTE THE SAMPLE YET):**

Please rate your liking of the **APPEARANCE** of sample 270.

Dislike extremely 
Dislike very much 
Dislike moderately 
Dislike slightly 
Neither like or dislike 
Like slightly 
Like moderately 
Like very much 
Like extremely

Please rate your liking of the **COLOR** of sample 270.

Dislike extremely 
Dislike very much 
Dislike moderately 
Dislike slightly 
Neither like or dislike 
Like slightly 
Like moderately 
Like very much 
Like extremely

65
PLEASE TASTE SAMPLE SAMPLE 270.

AFTER THIS, ANSWER THE FOLLOWING QUESTIONS:

Please rate your liking of the OVERALL CONSISTENCY of sample 270.

![Rating scale for overall consistency]

Please rate your liking of the Flavor of sample 270.

![Rating scale for flavor]

Please rate your OVERALL LIKING of sample 270.

![Rating scale for overall liking]

Would you purchase this product?

Yes

No

Thank you for your participation, please slide your tray through the sliding door in front of you.

Have a nice day!
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1. Permission for figure 1

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VITA

Andrea Suazo Rosales was born in 1996 in Tegucigalpa, Honduras. She left her hometown Siguatepeque in 2015 to begin her studies in Escuela Agricola Panamericana (Zamorano), where she obtained her bachelor's degree in Food Science and Technology in 2018. In 2019 she started her master's studies at Louisiana state university, currently pursuing her master's in Food Science and technology. She will receive her degree in May 2021.