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Development of Gluten-Free Sugar Cookies Using Kudzu Starch as a Partial Fat Replacement Ingredient

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ABSTRACT

The prevalence of kudzu has risen in the United States since its introduction in 1876. Though its invasiveness may play a negative role in our ecosystems, its roots have been used nutritionally in China for over 2,000 years because of their high starch content. In the United States, the use of kudzu starch as food products is limited. This study compared the moisture content and spread of reduced-fat gluten-free cookies prepared with kudzu starch-thickened aqueous mixture to similar gluten-free cookies prepared with rice flour and gluten-containing all-purpose wheat flour sugar cookies with kudzu starch, to gluten-containing sugar cookies (control). Moisture content was determined gravimetrically, while cookie spread was measured in centimeters. The results indicated that, compared to the control wheat flour (6.2%) and rice flour cookie (3.4%), the kudzu rice flour and kudzu wheat flour cookie had mean moisture contents (14%) which were significantly higher than control cookies (p=0.001). Substitution of kudzu starch for margarine in rice flour cookies (8.11 +/- 0.32 cm) and wheat flour cookies (7.18 +/- 0.33 cm) resulted in a significant difference in spread between the two. However, control rice flour cookies prepared with margarine (9.94 +/- 0.40cm) were significantly different compared to control wheat flour cookies (7.41 +/- 0.30cm) and kudzu rice flour cookies due to lack of gluten and moisture-thickened starch. This study shows that kudzu starch can be successfully incorporated into cookies to reduce fat content and combined with gluten-free flours to achieve moisture and maintain structure.

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List of Abbreviations

ANOVA	Analysis of Variance
COVID-19	Coronavirus disease 2019
CVD	Cardiovascular disease
g	Gram
K-cal	Kilocalories
LDL	Low-density Lipoprotein
ml	Milliliter
qt	Quart

Chapter One

Introduction and Literature Review

1.1 Diet-Related Health Conditions

Foods can provide an abundant amount of nutrients and energy but can also lead to certain food-related health conditions depending on the type of food and the individual. Examples of these conditions include heart disease (Bhupathiraju and Tucker, 2011) and Celiac disease (Littlejohns et al., 2021)

1.1.1 Heart Disease

Heart disease, specifically coronary artery disease, is prevalent worldwide among children and adults and is one of the main causes of morbidity and mortality (Formentini et al., 2019). In 2015, cardiovascular disease (CVD) reached over 400 million cases worldwide with 17.7 million deaths. (Formentini et al., 2019). These deaths represented 31% of all global deaths (Zhu et al., 2019). Formation of arterial coronary plaque is the ultimate mechanism that leads to CVD progression (Wang et al., 2020). A diet high in both saturated and trans saturated fat has been shown to contribute to the development of heart disease (Fattore and Massa, 2018). The Dietary Guidelines for Americans, recommends limiting saturated fats to less than 10% of total energy intake (Lichtenstein, 2019). If consumed in high amounts, saturated fats can cause a buildup of lipids within arterial walls which can lead to limited blood supply for the body (Vinay et al., 2020). Low-density lipoprotein (LDL) is also a major causal factor in the development of CVD (Domanski et al., 2020) because it is the primary carrier of cholesterol that contributes to the development of fatty deposits in the blood vessels (Albarrati et al., 2018). Epidemiological research has shown that the replacement of dietary saturated fats with unsaturated fats or fat substitutes is associated with the reduction in cardiovascular disease events and deaths (Kris-Etherton and Krauss, 2020).

1.1.2 Celiac Disease

Celiac Disease was once viewed as a rare disorder, but now affects millions of lives worldwide (Garner, 2016). This disease causes an autoimmune reaction to gluten which damages the small intestine when consumed (Bascunan et al., 2017). Gluten is part of a family of storage proteins that are found in grains, such as wheat, rye, spelt, and barley (Bascunan et al., 2017)). Individuals are either genetically predisposed or exposed to certain non-dietary environmental factors that can contribute to its pathogenesis rather than being caused by certain diets or foods (Ciao et al., 2019). When people with Celiac Disease eat gluten, their body automatically switches into defense mode against the gluten protein by primarily attacking and affecting the small intestine (Fueyo-Diaz et al., 2019). This autoimmune response results in damaged villi, and various GI symptoms including diarrhea, abdominal pain, and sometimes constipation (Caio et al., 2019). The only known treatment for Celiac Disease is the complete avoidance of gluten-containing foods (Otman et al.,2020)

1.2 Functions of Fat and Gluten in Foods

1.2.1 Fat and modifications

Most baked goods contain some form of fat in their recipes because of the crucial structural and textural properties fat plays (Biguzzi et al., 2014).

Specifically, fat plays a significant role in leavening products, tenderizing, as well as contributing moisture and creaminess in food products (Huang, 2019). There are many forms of fats used in baked products.

Milk fats primarily come from the milk of cattle and consist of the fatty portion of milk. The use of butter, buttermilk, ghee, and cheese are a few of the common milk fat products utilized in baked goods (Huang et al., 2019). Milk fats represent the most important dietary lipids in Western countries because they are known to perform various roles in bakery products (Huang et al., 2019). Some of their functions include dough strengthening, textural softeners, filling fats, coating lipids, and flavor improvers (Huang et al., 2019). They play more of a strengthening role in yeast-leavened bread dough, whereas in cakes, fats mainly contribute to aeration (Huang et al., 2019). As for cookies, milk fats are mainly responsible for textural effects like crisping and puffing of cookies (Huang et al., 2019).

The semi-solid soft white fat located in the adipose tissue of a pig is lard (Munir et al., 2019). This dairy and protein free fat was commonly used back in the 19th century but is now looked down upon due to its association with nutrition-related health conditions (Yanty et al., 2018). Aside from its popularity, lard is primarily used in the creation of pie crust because of its larger crystalline nature which helps to create more layers (Engelmann et al., 2018). For that reason, lard can also be incorporated into biscuits for ideal texture. Commercially, lard is not used in cookie making but still creates softer cookie dough texture with uniformed cookie spread (Manaf et al., 2019).

Unlike lard which consists of 80% animal fat and 20% water, vegetable shortening contains 100% vegetable fat (Marcus, 2013). It is commonly made from the extraction of plant oils such as soybean, cottonseed, and palm oil (Parsons, 1922). It has the ability to lubricate, weaken, and shorten the structure of bakery products (Huang et al., 2019). This solid fat is often used as a replacement to butter and lard because of its stability at high temperatures during heat transfer (Smith and Johansson, 2004). This ingredient has been through a process of hydrogenation to accomplish that same that partially hydrogenated oils once performed.

Reduction of fat in baked goods is considered important to maintain or improve the health of American citizens (Kriss-Etherton and Krauss, 2020). Popular fats including milk fats, lard, and shortening contain high proportions of saturated or hydrogenated fat that could be replaced with polyunsaturated fats or fat substitutes to help reduce the risk of coronary heart disease (Hamley, 2017).

1.2.2 Modification of Fat Content in Baked Goods

It may be more so the type of fat being consumed rather than the amount that can increase the risk of certain health conditions. Researchers say that replacement of saturated fats with monounsaturated or polyunsaturated fats will lower cardiovascular heart disease events (Kriss-Etherton and Krauss, 2020). Specifically, saturated and trans-saturated fats which come from animal fats or partially hydrogenated oils are often incorporated into baked goods (Slining and Popkin, 2013). These fats are associated with an increased risk of heart disease and other nutrition related conditions due to their abilities to elevate blood cholesterol levels and prompt the blockage in arteries in the heart and other vessels (Sudheendran et al., 2020). Many modifications to baked goods have been made to decrease total fat content, including the use of healthier fat alternatives.

From apple peels to applesauce, apples have started to play significant roles in the food and baking industry (Zbikowska and Kowalska, 2017). Apples contain health promoting ingredients such as polyphenols that are antioxidants and fiber which can help lower blood cholesterol and glucose (Zbikowska and Kowalska, 2017). One of the easiest ways to reduce fat in baked goods is substituting applesauce in place of fat because its pectin content allows gel formation with flour (Min, 2010). Researchers have also used apple fiber in cakes, which resulted in an increase in density and crumb hardness in short crust biscuits (Zbikowska and Kowalska, 2015).

Avocado puree is a medium energy dense fruit that has a buttery texture and can be utilized as a fat replacer in baked goods (Nurulain et al., 2016). Because avocado is high in monounsaturated fats, unlike lard and butter, it promotes reduced blood glucose concentrations and lower plasma LDL cholesterol blood lipid profiles as well as regulates blood glucose due to its bioactive phytochemicals (Park et al., 2018). In muffin production, the use of avocado puree had no significant effects on physical properties like hardness, cohesiveness, and chewiness of muffins, making it an appropriate natural ingredient as a butter replacement in baked goods. (Nurulain et al., 2016). In cookies, avocado puree created a cake-like texture and lower cookie spread (Wekwete and Navder, 2008).

While pumpkin may alter the color of baked products, its high fiber content and low calories helps it perform well as a substitute for shortening (Dadkhah et al., 2017). Researchers have used pumpkin powder as a shortening replacer in cakes (Dadkhah et al., 2017). This substitution resulted in cakes with high moisture contents (Dadkhah et al., 2017). This may have been due to the high fiber content in pumpkin and its water binding ability to maintain that moisture and weight in the cake (Dadkhah et al., 2017).

Plant gums such as those contained in okra can also be used as fat replacers, due to their high water-soluble fiber content and binding properties. Specifically, okra gum is contained in the plant's immature pods (Hu and Lai, 2017). Okra gum has been used as an acceptable fat replacer in chocolate bar cookies (Romanchik-Cerpovicz et al., 2002). It can also replace fat in drop batter breads such as banana bread while enhancing nutritional qualities and not changing the color or flavor of the product (Hu and Lai, 2017). When using okra gum for bakery products, ingredients like the amount of egg may have to be reduced and flour content increased due to the gum's liquid-like consistency (Hu and Lai, 2017).

Scientists have also utilized mungbean starch in the production of cakes with acceptable results of texture, color,and quality (Punia et al., 2019). Mungbeans are mainly cultivated in the Eastern and Southern Asian regions, as well as the Indian subcontinent and they are typically used as bean sprouts for soups, salads, and stir-frys (Kyoung-Mo Koo et al., 2003). A few studies have shown that mungbean starch can successfully be used as fat replacer in baked goods, such as cakes (Punia et al., 2019). Sneh Punia used mungbean starch in cake, but the starch was modified with octenyl succinic anhydride which helped to prevent separation of oil and water (Punia et al., 2019).

1.2.3 Gluten

In the baking industry, wheat is primarily used in the form of flour, to create many types of commercialized products including loaf bread, cakes, cookies, and noodles (Li et al., 2016). It has played a critical role in historic civilization and agriculture. In the 3rd millennium BC, common crops such as wheat and barley were cultivated in Northwestern India (Pokharia et al., 2016). The most common reason for their utilization is its gluten content (Li et al., 2016).

Gluten is a combination of storage proteins found in certain grains like wheat, rye, and barley. These long chain proteins are quite elastic (Li et al., 2016), so when combined with moisture and leavening agents, such as yeast, the inflation in the food product results in its unique structure (Lucas et al., 2020). Because gluten is a protein, it tends to harden when heated, so the hardening of the gluten molecules is what contributes to the baked goods appearance, texture, and quality (Heredia-Sandoval et al., 2016).

1.2.4 Modifications to Gluten-Containing Products to Create Gluten-free products

There is a growing trend in the availability of gluten-free products on the market due to a worldwide rise in the prevalence of Celiac disease (Glissen Brown and Singh, 2019). Although there are still nutritional and sensory challenges in gluten-free product development, many replacements have been shown to have consumer appeal (Stantiall and Serventi, 2018).

Because the quality of wheat greatly depends on viscoelastic properties of gluten, various ingredients have been examined to mimic the properties of gluten (Yano et al., 2019). Researchers have used rice flour and mung bean starch to create gluten-free instant noodles (Sutheeves et al., 2020) and using chickpea protein isolate to enhance the protein content of noodles (Sofi et al., 2020). Gluten-free crackers have also been developed with teff flour, which is also a more nutritive alternative to all-purpose wheat flour due to higher protein and fiber content (Rico et al., 2019). In addition, teff flour has been shown to enhance antioxidant properties of crackers and shows a similar sensory profile to rice crackers (Rico et al., 2019).

Buckwheat flour is also a gluten-free flour which provides high protein and fiber content (Urubkov et al., 2020). It is nutritionally beneficial as it contains biologically active elements (Urubkov et al., 2020). Its binding properties allow buckwheat to become very gelatinous when moisture is added making it suitable for various types of food products like noodles, pancakes, and jellies (Dragomir and Bahaciu, 2019). When used in cookies, however, the products appear darker in color compared to wheat flour. In addition, it was crumbly in nature. (Torbica et al., 2012).

Almond flour has become a popular flour due to its gluten free components (Granato and Ellendersen, 2009). It is made from ground and blanched almonds and is commonly used in baked goods and alternative grain-free breadcrumbs

(Almeida et al., 2019). It contains many minerals like iron, magnesium, and calcium. However, it contains a higher fat content which, unlike wheat flour, can result in a significant increase in oiliness and shininess of food products (Jia et al., 2008).

When whole-grain oats are ground together, the resulting product is oat flour. Though this flour is gluten-free, cross contamination of oats with gluten may occur in the supply chain (Koerner et al., 2013). The grain infrastructure, which includes growing, transporting, and milling, increases the risk for naturally gluten-free cereals to be contaminated with gluten (Koerner et al., 2013). Oat flour is high in soluble fiber, beta-glucan, and rich in protein and magnesium (Qian et al., 2020). When used for bread baking, the product will likely be moister, with a chewier, and more crumbly texture than when wheat flour is used. Unfortunately though, it may result in an unpleasant flavor in the finished product (Majzoobi, 2016).

Rice flour is considered to be a suitable replacement for wheat, due to its worldwide availability and tolerance (Yano et al., 2019). Rice flour is rich in easily digestible carbohydrates and contains low amounts of protein but high amounts of fiber (Sciarini et al., 2010). It can be used as a thickening agent because it prevents liquid separation, making it useful for soups, sauces, and gravies (Aryupong et al., 2019). Rice flour can also be used in cakes and cookies but may result in lower volume with a crispier texture due to its fine, softer texture (Dragomir and Bahaciu, 2019).

Additional ingredients like chickpea flour can be added to rice flour mixtures to increase fat and protein content and produce products with higher foaming capacity and stability (Kahraman et al., 2018). Another protein-enhancing ingredient added to rice flour is Bombay locust powder (Patanga succincta L.) which comes from a locust species in India and Southeast Asia (Indriani et al., 2020), Researchers used Bombay locust powder mixed with rice flour for cake development. This cake had an increased protein content consumer acceptability (Indriani et al., 2020).

For an increase of viscosity, Konjac flour, an herb also known as glucomannan, was used in cookies, and resulted in higher spread ratio with an increase in panelist acceptance, regarding texture and overall acceptability (Akesowan, 2016)

Pea protein, an easily digestive protein source made from yellow peas, was added to rice flour cookie and researchers observed an increased in hydration, but limited cookie spread measurement during baking time (Mancebo et al., 2016). The study revealed a successful trial with pea protein cookies having high consumer acceptability (Mancebo et al., 2016).

1.3 Kudzu

Kudzu starch comes from the root of the kudzu plant (Li et al., 2019). In North America, specifically the southern region, the invasive species kudzu is known for its fast growth rate (Hudson and Aborn, 2020). Its large, perennial woody vine and deep tap roots blanket many native plant species, while dominating the sun light and nutrients of where its planted (Alderman, 2015). It is native to Southeastern Asia and is commonly used in traditional Chinese medicine (Wong et al., 2011). Over 2000 years, kudzu root has been used to treat fever, acute dysentery, diarrhea, diabetes, and heart disease due to its abundant number of phytochemicals (Wong et al., 2011). Kudzu starch also contains several bioactive isoflavones including daidzein, which may possess anti-cancer properties (Li et al., 2019).

Aside from the kudzu root being nutritionally beneficial, it is also a good source of starch giving it high paste viscosity properties (Reddy et al., 2017). It possesses a neutral flavor which may promote its usage in baked goods (Li et al., 2019). The starch has not been utilized in the Western food industry due to limited information regarding its molecular structure (Reddy et al., 2017) but researchers have begun using the starch in food products like noodles, puddings, and cakes (Romanchik et al., 2020).

Goals of This Project

While some studies have examined the physical and sensory properties of kudzu starch as a cornstarch replacement, no studies to date have used the starch as a fat substitute in cookies, especially cookies which have been altered in to remove gluten. Therefore, the principle goal of this project is to determine changes in the objective quality, including spread and percent moisture, of sugar cookies with and without gluten in regular and lower fat versions. It is hypothesized that a kudzu rice flour sugar cookie will spread the same as the wheat flour sugar cookie and that the overall percent moisture will be higher than gluten-containing and gluten-free sugar cookies without the replacement of margarine with moisture-thickened kudzu starch.

Aim 1

To determine the moisture content of reduced-in-fat, gluten-free, sugar cookies following rice flour incorporation and substitution of kudzu starch for margarine.

Aim 2

To determine the spread of reduced fat, gluten-free, sugar cookies containing rice flour and kudzu starch.

Aim 3

To compare the moisture content to gluten-free, reduced fat, rice flour sugar cookies with kudzu and reduced fat all-purpose wheat flour sugar cookies with kudzu to all-purpose wheat flour sugar cookies and gluten-free rice flour sugar cookies.

Aim 4

To compare the spread of gluten-free, reduced fat, rice flour sugar cookies and reduced fat, all-purpose wheat flour sugar cookies with kudzu to all-purpose wheat flour sugar cookies and gluten-free rice flour sugar cookies.

Chapter 2

Materials and Methods

2.1 Materials

Ingredient	All-purpose Wheat Flour Cookies (g)	Gluten-free Rice Flour Cookies (g)	All-purpose Wheat flour cookies with Kudzu (g)	Gluten-free Rice Flour Cookies with Kudzu (g)
Sugar	227	227	227	227
All-purpose Wheat Flour	212.8	------	212.8	-----
Rice Flour	------	212.8	-----	212.8
Margarine	127.5	127.5	-----	-----
Kudzu Starch-Thickened Water	------	------	127.5	127.5
Shortening	113.5	113.5	113.5	113.5
Eggs	37.7	37.7	37.7	
Baking Soda	2.9	2.9	2.9	2.9
Cream of Tartar	1.5	1.5	1.5	1.5
Salt	0.8	0.8	0.8	0.8

Other Materials:

Balance

Mixing bowls

KitchenAid Classic standing mixer

Wax paper

Parchment paper

Cookie Sheet (15" x 10.3" x 0.7")

2.2 Standardization of Drop Sugar Cookie Recipe

The recipe was first modified to yield 2 dozen cookies instead of 8 dozen cookies. The method for this procedure was as follows:

1. Each ingredient was converted to grams using the USDA Food Data Central tool.
2. Once converted to grams, each ingredient was divided by four.
3. Each ingredient was placed on a scale with wax paper to weigh the specific grams needed.

2.3 Preparation of Kudzu Starch

The preparation of kudzu starch was developed based upon the method for cornstarch -thickened mixture published in the Experimental Foods Laboratory Manual 9th edition (2017). The method is as follows:

1. Water (472ml) was slowly stirred into 32 g of kudzu starch in a 1-qt heavy aluminum saucepan.

2. The mixture was cooked over direct heat while stirring constantly to prevent lumping and heated to boiling. The mixture was brought to a full boil and stirred continuously for one minute.
3. The Kudzu starch mixture was then placed in a bowl and cooled in a shallow pan of ice water to room temperature, while being stirred occasionally.

2.4 Preparation of Sugar Cookies

2.4.1 Control Sugar Cookie Recipe

Shortening, margarine, and sugar were combined in a KitchenAid Classic mixer bowl with flat beater attachment and mixed on low speed 2 for 30 seconds. After scraping the bowl, the ingredients were further mixed on medium speed 4 for 30 seconds, and then after another scraping, they were mixed on high speed 10 for 5 minutes. Egg and vanilla were added to the creamed mixture and beaten on speed 10 for 1 minute. The bowl was then scraped. Dry ingredients including all-purpose flour, cream of tartar, baking soda, and salt were combined in a separate bowl. Then, half of the dry mixture was added to creamed mixture and mixed on medium speed 4 for 30 seconds. After scraping the bowl, the rest of the dry ingredients were added and mixed again on speed 4 for another 30 seconds. Using a No. 40 dipper, individual cookie dough balls were dropped onto a baking sheet lined with parchment paper. Cookies were baked in oven at (190°C) 375°F for eight minutes.

2.4.2 Preparation of Rice Flour Sugar Cookies

The rice flour cookie recipe used with the same mixing methods as the control sugar cookies in Section 2.4.1 with the exception that in place of all-purpose wheat flour, rice flour was combined with the other dry ingredients. Using a No. 40 dipper, cookie dough balls were dropped onto a baking sheet lined with parchment paper. Cookies were placed in the oven at (190°C) 375°F for eight minutes.

2.4.3 Preparation of Control Sugar Cookies with Kudzu Starch

Shortening, and sugar were combined in a KitchenAid Classic mixer bowl with flat beater attachment and mixed on low speed 2 for 30 seconds. After scraping the bowl, the ingredients were further mixed on medium speed 4 for 30 seconds, and then after another scraping the bowl, they were mixed on high speed 10 for 5 minutes. Egg and vanilla were added to the creamed mixture and beaten on speed 10 for 1 minute. Moisture-thickened kudzu starch mixture was added, in place of margarine, to creamed mixture and mixed on speed 2 for 1 minute. The bowl was then scraped. Dry ingredients including all-purpose flour, cream of tartar, baking soda, and salt were combined in a separate mixing bowl. Then, half of dry mixture was added to the creamed mixture and mixed on speed 4 for 30 seconds. After scraping the bowl, the rest of the dry ingredients were added and mixed again on speed 4 for another 30 seconds. Using a No. 40 dipper, three cookie dough balls were dropped onto a baking sheet lined with parchment paper. Cookies were baked in the oven at (190°C) 375°F for eight minutes

2.4.4 Preparation of Rice Flour Cookies with Kudzu Starch

The rice flour cookies with kudzu starch were prepared using the same mixing methods as the control cookies in Section 2.4.3 with the exception that in place of all-purpose wheat flour, an equal amount of rice flour was combined with the other dry ingredients. Using a No. 40 dipper, cookie dough balls were dropped onto a baking sheet lined with parchment paper. Cookies were placed in oven at (190°C) 375°F for eight minutes.

2.5 Objective Evaluation Methods

2.5.1 Measurement of Moisture Content

Moisture content (%) was determined as {(initial mass-dried mass)/initial mass} x 100. Samples (n=3) of each type of freshly prepared cookies were analyzed for moisture content. The cookies were cooled to (25°C) 77°F. The Thermogravimetric Moisture Analysis Method utilized drying the samples to a constant weight (105 degrees Celsius; 3 hours) (AOAC, 2000).

2.5.2 Measurement of Cookie Spread

Cookie samples freshly baked and cooled to (25°C) 77°F were analyzed for spread (diameter) using a 30 cm ruler. Each type of cookie was assessed 12 times.

2.6 Statistical Analysis

Cookie spread and moisture content (mean +/- standard deviation) were analyzed statistically using one-way analysis of variance (ANOVA) with post-hoc testing by Tukey-Kramer multiple comparisons using InStat Instant

BioStatistics (version 3.0 for Windows, 1998-1999, Graph Pad Software, Inc, San Diego, CA). Significance was determined at $p < 0.05$.

Chapter 3

Results

3.1 Moisture Content of Cookies

The moisture content of the wheat flour cookies prepared with margarine averaged 6.2% while that of the rice cookies prepared with margarine averaged 3.3% (**Table 1)**. There was a trend which showed wheat flour cookies prepared with margarine may have more moisture in comparison to the rice flour cookies. However, the difference in mean values was not significant.

The lower fat version of rice flour cookies prepared with kudzu starch had a mean moisture content of 14% which was significantly higher than control rice flour cookies (p=0.001). The wheat flour cookies with kudzu similarly averaged 14% moisture. This moisture was significantly higher than the moisture in wheat flour cookies prepared with margarine (p=0.01). Similarly, the substitution of Kudzu for margarine in rice flour sugar cookies resulted in a product with significantly more moisture (p=0.001).

3.2 Spread of Cookies

The average spread for the wheat flour cookies prepared with margarine (7.4 cm) was significantly less than that of the rice flour cookies prepared with margarine (9.9 cm) (p=0.001) (**Table 1)**.

The lower fat version of rice flour cookies prepared with kudzu starch also had an average spread (8.1 cm) which was significantly less than the control rice flour control cookies (9.9cm) (p=0.001) while the spread of the wheat flour cookies with kudzu (7.2 cm) was not quite significantly different from the control wheat flour cookies prepared with margarine (7.4 cm) ($p=0.05$) (Table 1).

Table 1. Moisture Content (%) and Spread of Cookies (cm)

Moisture Content (n=3)	**(%)**
Wheat Flour Cookies (Control)	6.23 +/- 0.23
Rice Flour Cookies (Control)	3.43 +/- 0.23^{A}
Kudzu Rice Flour Cookies	14.07 +/- 3.35^{A}
Kudzu Wheat Flour Cookies	14.77 +/- 0.43
Spread (n=12)	**(cm)**
Wheat Flour Cookies (Control)*	7.41 +/- 0.30^{B}
Rice Flour Cookies (Control)*	9.94 +/- 0.40^{B,C}
Kudzu Rice Flour Cookies	8.11 +/- 0.32^{C,D}
Kudzu Wheat Flour Cookies	7.18 +/- 0.33^{D}

*ANOVA; significant difference (P<0.001)

^{A,B,C,D} Indicated significant (P<0.001) difference between each cookie type.

3.3 Compositional Analysis

As shown in **Table 2**, both types of control sugar cookies prepared with margarine were comparable in Calorie and macronutrient composition. Per 100g, the rice flour cookies (control) had the highest number of calories (568.8). Both types of cookies prepared with kudzu were almost 20% lower in Calories compared to their respective control cookies because the replacement of margarine with kudzu starch substantially reduced their fat content by 5 kcals/gram for those ingredients. Specifically, the wheat and rice flour cookies (control) had a fat content of 54.3 % while the cookies prepared with kudzu had 38.7% fat. Both cookies prepared with rice flour had higher carbohydrate content due to the amount of starch in both the rice flour and in the kudzu starch.

Table 2. Compositional Analysis of Cookies*

Nutrient	Wheat Flour Cookies	Rice Flour Cookies	Kudzu Wheat Flour Cookies	Kudzu Rice Flour Cookies
Energy (Kcal)	532.9	568.8	451.8	454.8
Carbohydrate (g; % k-cal)	56.1 g; 42.1%	62.8 g; 44.2%	63.9 g; 56.6%	67.5 g; 59.4%
Total Fat (g; % k-cal)	32.1 g; 54.2%	34.4 g; 54.4%	19.4 g; 38.6%	19.6 g; 38.8%
Protein (g; % k-cal)	4.9 g; 3.7%	2.0 g; 1.4%	5.4 g; 4.8%	2.1 g; 1.8%

*Macronutrient content per 100 g prepared product

Source: USDA Food Data Central; http://fdc.nal.usda.gov;

Accessed March 10th, 2021

Chapter 4

Summary and Discussion

This study examined, for the first time, the properties of both gluten-containing and gluten-free sugar cookies in which kudzu starch was substituted for margarine. Specifically, this study compared the moisture content and cookie spread of gluten-free, reduced fat rice flour and wheat flour cookies with kudzu to full-fat all-purpose wheat flour sugar cookies and gluten-free rice flour sugar cookies. The goal of this project was to determine if kudzu starch substitution for margarine could result in cookies with similar moisture content and spread to gluten-containing and gluten-free cookies containing margarine.

Consistent with the aims of this project, moisture contents of all four cookie types were evaluated. The results indicated that the rice flour and wheat flour cookies prepared with kudzu had significantly higher moisture content than control cookies prepared with margarine. It is possible that differences in fat and water content between margarine and kudzu starch may have influenced the moisture content of the cookies. Margarine contributes to the tenderization of baked goods by providing an illusion of wetness that is actually contributed by its high fat content (80%) (Silva et al., 2021). In contrast, kudzu starch thickened water is primarily water based on McWilliams, 2017. These differences may explain why the average moisture content of the control wheat flour cookies and rice flour cookies were significantly lower than to the cookies containing kudzu starch. Starch, in general, plays a role in maintaining moisture because it contains two polysaccharides: amylose and amylopectin (White and

Chiaramonte, 2021). These structural components have the ability to absorb water unlike the vegetable oil in margarine which is hydrophobic in nature (Debabhuti et al., 2022). In addition, the process of gelatinizing the kudzu starch includes the incorporation of water to create the kudzu-thickened mixture. The lower-in-fat version of rice flour cookies had a similar mean moisture content to the lower-in-fat versions of wheat flour cookies both substituted with kudzu.

Gluten may play a role in maintaining the spread of the wheat flour control cookie. During the mixing process, when flour was combined with the fat and egg mixture, this created a gluten matrix which helped hold in the melted fat and sugar preventing the cookie from spreading too far while influencing the density of the dough. The average spread for the wheat flour cookie was significantly smaller than the control rice flour cookie ($p<0.001$). Cookie spread of kudzu rice flour cookie was significantly bigger than the spread of the kudzu wheat flour cookie ($p<0.001$). The incorporation of kudzu starch into the wheat flour and rice flour cookies may have enhanced the thickening properties from the starch molecules, resulting in a decrease in spread compared to control rice flour cookie (Rojhani and Naranjo, 2017). Swelling power is significantly related to both amylose and amylopectin content (Farooq et al., 2021). During the baking process, these molecules swell and retain even more liquid which may have helped the cookies to spread less. According to Adeleye (2016), researchers used a starch-based instant food thickener as a fat replacer in chocolate chip cookies which resulted in a reduce of cookie spread. This analysis supports the theory that kudzu starch can help reduce spread and increase the height of baked goods or cookies. When cookie spread was evaluated, the majority of cookie types ranged in 7.2 to 8.1 cm in spread. However, the control rice flour cookie was significantly larger (9.9 cm). This data would be expected because the lack of gluten in rice flour may compromise the structure of baked goods.

In addition, the types of flour used in each cookie suggest that their composition in relationship to water retention may contribute to observed differences. Gluten-containing flour is known to absorb water due to its glutenin and gliadin content. This fact may explain why the control wheat flour cookie had a higher moisture content than the control rice flour cookie which didn't contain gluten (Adeyeye et al., 2019). Another important thing to note is the starch in rice flour. With rice flour lacking gliadin and glutenin, it is mostly composed of starch, specifically, 20% amylose, which has a lower solubility value than glutinous rice flour and gluten in wheat flour (Farooq et al., 2021) (Yunos et al., 2021). The rice flour used in the cookies is known to not absorb liquid well because of amylose having a more compact structure and not being non-easily ruptured (Farooq et al., 2021). In fact, when rice flour is combined with water, it only creates a suspension (Netrprachit et al., 2019). As such, it is more effective to combine rice flour with other flours, gums, or starches like kudzu when developing gluten-free baked goods, that may help mimic the gluten matrix and increase water absorption.

The absence of margarine in the both kudzu-containing rice and kudzu-containing wheat flour cookies reduced the Calories substantially. Whereas fat, such as margarine, supplies nine Calories per gram, kudzu, as a starch-based carbohydrate provides just 4 Calories per gram. As such, the control rice flour cookies had approximately 6.3% more Calories than the control wheat flour cookies (532.9 Kcals). However, the removal of fat, margarine, reduced both the fat content and Calorie content of the rice cookies compared to control rice flour cookies with margarine.

This project shows that the use of kudzu starch as a substitute for margarine in rice flour cookies significantly helps maintain structure and increase moisture content compared to wheat flour cookies. This knowledge may help researchers develop other acceptable gluten-free products for people who have Celiac Disease or people who are following a low-fat diet to control other aspects of their health.

One of the limitations of this study was the inability to obtain sensory data about consumer acceptability of the cookies. The time that the project was started, COVID-19 emerged and spread throughout the United States. As such, the study was conducted strictly as a product development-study with objective analysis of the resulting cookies.

In the future, consumer acceptability of the products may be determined. In addition, more objective tests such as standing height may be used to determine how kudzu starch additionally affects the structural composition of the cookie types. Cookie hardness could also be used to see how soft or hard the final product is, after substitutions are made (Riaz et al., 2018).

The study showed that it is feasible to use kudzu starch such as a fat replacer in gluten-free sugar cookies. The reduction in Calories in the cookies and similarity in objective assessment, may facilitate its future use as food ingredient commercially for individuals diagnosed with Celiac Disease or Cardiovascular Disease.

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APPENDICES

Appendix A: Time Schedule of Study

Appendix A: Time Schedule of Study

Thesis Proposal Submitted	December 2020
Data Collection	September-October 2020
Data Analysis	January 2021
Final Thesis Submitted to Honors Program Office	November 2021
Presentation of Thesis	November 2021

Appendix B: Biographical Summary

Appendix B: Biographical Summary

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Bachelor of Science: Nutrition and Food Science

Emphasis: Food Science/Food Systems Administration

Thesis Title: Development of Gluten-Free Sugar Cookies Using Kudzu Starch as a Partial Fat Replacement Ingredient

Mentor: Dr. Joelle Romanchik-Cerpovicz