Can pea and white hemp proteins be used as a more sustainable protein source to improve nutritional and sensory results in gluten-free baked products?

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Abstract

This study hypothesized that combining HPI and PPI could provide added nutrition while achieving positive sensory results. The 'backcasting' method incorporated sustainability for the product development process, outlining objectives, priorities and critical factors, including practical application and testing. Investigation included nutritional analysis of protein, fat and fiber, glycemic testing, hunger satiety and sensory analysis. Therefore, three GF products were developed using a unique HPI/PPI protein blend up to 30% in formulations. Rheological parameters (protein, starch and hydrocolloid behavior) were investigated using Mixolab®. Blend ratios showed an 80:20 (HPI/PPI) blend exhibited approximately similar protein behavior and stability to wheat flour during mixing/heating and gelatinization stages. Torque differences were significant between starch (1.19 Nm) and no-starch (0.88 Nm) samples, with higher gelatinization intensity for the starch blend. Dough showed significantly higher gelatinization intensity with XG than without (2.21 Nm and 1.75 Nm, respectively) and starch retrogradation during cooling (1.39 Nm and 1.21 Nm, respectively), indicating prolonged shelf life. Nutritional results showed a significant increase in protein for developed products (bread/cookie, $p = 0.013$; muffin, $p = 0.022$), no significant increase in fat or fiber using the protein blend, however fiber increased by 42.9% incorporating a MG mix. Glycemic testing showed no significant spike in blood glucose after ingestion. P-value of all participants pre- and post- was *0.27* (men, $p = 0.47$; women, $p = 0.31$) while satiety results showed hunger levels of 0.73 (0-1 *Not Hungry At All*) after 30 minutes, increasing only slightly after 2 hours (1.63), indicating glycemic response and hunger levels can be controlled by incorporating certain ingredients. Sensory results showed no significant difference between products for appearance, taste and texture. 97.5% of participants rated 4 or above for taste and 95% above 4 for texture on a 5-point scale. Only 12.5% of participants believed the products were healthier options and 45% believed them to be GF. Therefore, this study confirms that nutritional improvement of GF bakery products using more sustainable plant-based proteins is possible while achieving positive sensory acceptance.

Table of Contents

LIST OF ABBREVIATIONS

Advanced Formula Protease (AFP)
Angiotensin-Converting Enzyme (ACE)
Branch Chain Amino Acid (BCAA)
Blood Glucose (BG)
Blood Pressure (BP)
Carboxymethyl Cellulose (CMC)
Chickpea Protein Isolate (CPI)
Coeliac Disease (CD)
Demineralized Whey (DM)
Egg White Protein (EWP)
Food and Drug Administration (FDA)
Free-From (FF)
Genetically Modified Organisms (GMO)
Global Warming Potential (GWP)
Gluten-Free (GF)
Glycemic Index (GI)
Glycemic Response (GR)
Greenhouse Gas (GHG)
Guar Gum (GG)
Hemp Protein Concentrate (HPC)
Hemp Protein Hydrolysate (HPH)
Hemp Protein Isolate (HPI)
Histidine (HIS)
HT Proteolytic Concentrate (HT)
Hydroxy Propyl Methyl Cellulose (HPMC)
Insoluble Dietary Fiber (IDF)
Isoleucine (ILE)
Leucine (LEU)
Lysine (LYS)
Methionine (MET)
Microbial Transglutaminase (MTG)
Milk Protein Isolate (MPI)
Modified Atmosphere Packaging (MAP)
Multigrain (MG)
Newton Meters (Nm)
Non-Coeliac Gluten Sensitivity (NCGS)
Parts Per Million (PPM)
Pea Protein Isolate (PPI)
Phenylalanine (PHE)
Protease G (Pro-G)
Rapidly Digestible Starch (RDS)
Resistant Starch (RS)

Skim Milk Powder (SMP)
Skim Milk Replacer (SMR)
Soluble Dietary Fiber (SDF)
Soy Protein Isolate (SPI)
Soybean Soluble Polysaccharide (SSPS)
Threonine (THR)
Total Dietary Fiber (TDF)
Tryptophan (TRP)
Valine (VAL)
Viral Wheat Gluten (VWG)
Whey Protein Isolate (WPI)
Xanthan Gum (XG)

List of Tables

List of Figures

1. INTRODUCTION

1.1 OBSERVATIONS OF THE GLUTEN-FREE MARKET

Coeliac Disease (CD) is a genetic autoimmune disorder associated with the body's inability to process gluten, which leads to inflammation in the small intestine causing abdominal pain, diarrhea and bone disease (Jnawali *et al.,* 2016). Individuals not affected by CD have also described gastro-intestinal symptoms related to gluten ingestion, such as bloating, abdominal pain or constipation (Lionetti *et al*., 2017). While only 1% of people in Europe and the United States are diagnosed as having CD, a growing number of people are identifying as gluten sensitive or gluten intolerant, also known as non-coeliac gluten sensitivity (NCGS). Symptoms of NCGS include fatigue, headache and fogginess (Lionetti *et al*., 2017), however these symptoms are neither related to an autoimmune reaction nor an allergy, and often self-diagnosed only when avoiding gluten-containing products results in improved conditions. NCGS is, therefore, harder to determine although indirect evidence suggests a number similar to or greater than those with CD (Uhde *et al*., 2016; Lionetti *et al*., 2017). Analysis by the National Health and Nutrition Examination Survey in the United States found that those without CD who adhere to a gluten-free (GF) diet tripled from 2010 – 2014 (Lebwohl *et al*., 2017) and that 10% of all adults in the United Kingdom have started a GF diet for various reasons (Lionetti *et al*., 2017), suggesting that consumers believe following a GF diet can improve one's health, even though heart disease and diabetes have been linked to a GF diet (Bullo *et al.,* 2013; Liu *et al.,* 2016).

The trend of consumers increasingly avoiding gluten has given rise to the popularity of GF products in the baking industry despite the higher costs associated with GF bakery products. GF bakery products have limited availability in some markets and could cost 76 - 81% more than their wheat counterparts (Tanwar and Dhillon, 2017). It was estimated that in 2018 the United States held about 59% of the total GF market value, of which bakery and confectionary products account for 46%, while in the United Kingdom alone the GF and wheat-free market grows an average of 23% per year

(Witczak *et al.,* 2016). Therefore, food manufacturers continue to look for functional ingredients, combinations and formulations that reproduce the viscoelastic properties of their wheat counterparts in structure and texture while also attempting to maintaining a competitive advantage in a global GF market.

While GF products are characterized as containing no gluten, they are also characterized by the ingredients used in them. Hydrocolloids are used to improve viscoelasticity, structure and sensory properties, starches are responsible for texture and structure while GF flours and whole grains are added to improve nutritional value (Naqash *et al*., 2017). Proteins are also highly functional and can affect nutrition, texture, water absorption and quality, however protein type can affect formulations differently (Shevkani *et al*., 2015; Naqash *et al*., 2017).

As GF products have gained popularity, concerns around product nutrition and consumer perception have become investigated topics. GF bakery products contain more sugar, sodium and starch than their gluten-containing counterparts and it has been suggested that eliminating gluten containing products from one's diet may impact an individual's dietary intake due to nutritional deficiencies often found in GF products (Nascimento *et al.,* 2013). Since natural and modified starches, such as hydrocolloids, can impact the product recipe up to 20% (Witczak *et al.,* 2016), it has been suggested that GF producers overlook focusing on nutrition when using certain ingredients because of their effects on structural improvement in the absence of gluten (Naqash *et al.,* 2017). Relying on techniques aimed at reducing the need for additives/preservatives and extending shelf-life are also used, though they are expensive options for most GF producers (Capriles *et al.,* 2015; Scazzina *et al.,* 2015; Naqash *et al.,* 2017). While it is important to look at GF packaging and shelf-life, more direct concerns related to which ingredients are being used, highlights around health-related issues, the acceptability of GF products and product sustainability should also be considered.

Most GF products result in a higher GI than their wheat counterparts (Capriles and Areas, 2016; Naqash *et al.,* 2017) though it has been suggested that adding protein might affect the GI of GF products (Scazzina *et al.,* 2015). For example, the use of high-protein ingredients, typically derived from dairy or soy products, are commonly used in GF baking and can positively impact overall nutrition. However, both have resulted in lower sensory acceptability in certain GF baked products (Ziobro *et al.,* 2016; Naqash *et al.,* 2017). Since the use of dairy proteins can be an issue for the lactose intolerant, there is room for improvement of GF products by replacing dairy proteins with plant-based proteins that result in both high consumer acceptance and lower GI. Both pea protein isolate (PPI) and hemp protein isolate (HPI) have good nutritional profiles and have shown positive results in both structure and sensory acceptance of GF products while adding nutritional value (Ziobro *et al.,* 2013; Korus *et al*., 2017). Further research could be engaged, therefore, to identify if using plant-based proteins in GF baked products can yield better nutritional results over dairy and soy-based compounds while also examining whether they might be more sustainable longer term.

Switching to more sustainable, plant-based compounds could also have a positive impact on the environment as well as consumer acceptance. For example, while chemically modified starches are classified as permitted additives, physically modified starches are considered clean label (Witczak *et al.,* 2016). Additionally, functional cellulose is derived from plant sources and xanthan gum (XG) is produced from renewable carbohydrate sources (Hublik, 2012) and as a natural origin polysaccharide may be more acceptable to consumers. As sustainability concerns gain popularity, ingredients are being looked at for their non-genetically modified origins (GMO) and potential use in GF baked products as a means to impact food waste, help reduce greenhouse gas (GHG) emissions and for the added nutrition they provide.

Research has investigated the notion that the more informed consumers are about sustainable products the less they consume, suggesting that consumers who are more educated and better informed are not only concerned with health but also more likely to have plant-based diets. Animal by-products are associated with higher GHG emissions (Lacour *et al.*, 2018) and using non-gluten flours can result in a 30-40% lower impact on the environment (Shaabani *et al*., 2018). Research exploring the functional and nutritional aspects of plant-based proteins, as more widely available and less allergenic, has also emerged and may suggest GF products that incorporate less allergenic compounds while adding nutritional value could be the advantage producers need (Mattila *et al*., 2018). Therefore, simple solutions to answering the sustainability question might involve reliance on more widely available ingredients such as locally produced plant-based ingredients, ingredients that are considered clean label and educating consumers on sustainable options with informational labeling that could influence consumption and impact production.

1.2 Research Rationale

This research was developed based on a deeply personal need. In 2012, this researcher's mom became a diabetic. She worried about making the right food choices and was told that following a GF diet may help, so this researcher began to investigate GF bakery products and recipes. Discovering products which did not spike blood sugar levels was one challenge while finding products which tasted good was another. This researcher refused to let his mom give up the pleasure she found in their shared joy for bakery items, so he began looking into making the products they both loved more nutritious. Being lactose intolerant himself, this researcher was used to using various plant-based proteins as supplementation in food applications and wondered if combining certain proteins could result in more nutritious and better tasking products. Armed with a passion for sustainability and experience in product development, achieving the goals of sustainably sourced, delicious products was less of a challenge, while creating products with improved nutritional components (and addressing diabetic concerns) was more of one.

The human body needs 21 total amino acids to survive, producing 12 non-essential amino acids on its own but requiring the remaining 9 essential amino acids from food sources. When a protein contains all 9 essential amino acids, it is considered a 'complete' protein (Piedmont, no date). The minimum percentage of total protein needed from essential amino acids is around 28.7% and whey, a dairy protein often used in bakery products, is considered a complete protein meeting 52.3% total protein needed from essential amino acids (Cudmore, 2021). The total protein from essential amino acids in PPI is around 40.4% (Cudmore, 2021). While PPI contains the necessary amino acid composition for daily protein consumption, it is not considered a complete protein because it is low in methionine. HPI contains all 9 essential amino acids the body needs and contains twice as much methionine than PPI and is also a high source of fiber, suggesting that combining plant-based proteins could result in a more complete amino acid profile and improved nutrition (Pojic *et al*., 2015; Gorissen *et al*., 2018).

Elements studied through this research were the increased nutrition, impacts of GI, consumer acceptability and sustainability. The specific contribution to science resulted in the combination of sustainable plant-based proteins which could provide nutrition often missing in GF products and potentially help to lower the glycemic response in those products. To investigate the nutritional impact and acceptance of using this protein combination in various applications, three types of GF bakery products (bread, muffin and cookie), made with widely available and cost-effective ingredients that improve product nutrition, were developed and tested. What makes this research unique is that it is, to date, the only study looking at nutritional improvement and acceptably of three distinct GF product types using a scientifically developed protein combination.

1.3 Hypotheses

This research focused on areas being investigated in the GF sector, including issues facing the baking industry with respect to GF production, health-related concerns resulting from an increase in both demand and popularity of GF products, lack of nutrition, sensory acceptance and sustainability. The aim was to demonstrate a need for improved GF bakery products and show that nutritional characteristics and consumer acceptance in the GF sector can be improved by using a more sustainable, plant-based protein combination. Therefore, a series of tests was performed to investigate the following hypothesis: Using PPI and HPI together in GF bakery products can improve the nutritional value of the finished product and have a positive impact on acceptability and product perception. Concurrently, a null hypothesis was also investigated: Using PPI and HPI together in GF baked products neither improves nutritional value nor acceptability and perception of the finished product.

1.4 Research Aim and Objectives

This study intended to show that the nutritional characteristics and consumer acceptance of GF bakery products could be improved by using a plant-based protein combination. The aim of this project was to investigate and evaluate whether a PPI/HPI combination can improve nutritional characteristics of GF baked products, appeal to consumers on a sensory level and be perceived as more sustainable sources.

The following objectives were carried out to support the project's approach:

- Assess the nutritional composition of developed GF products for improvements in protein, fat and fiber
- Assess the dough rheology of GF developed products
- Evaluate if the GI of a GF product can be affected by the recipe formulation
- Assess the sensory acceptance of developed GF products made with PPI/HPI over controls made with soy and dairy protein compounds
- Investigate whether or not a PPI/HPI protein combination is a more sustainable option for GF bakery items
- Literature Review
    - Identify the scope of research and background issues associated with GF bakery products
    - Cite studies and their results as related to the issues and offer insights into areas of research proposed by this thesis
    - Discuss techniques and compounds used in GF bakery production with focus on nutrition, rheology, glycemic response, consumer acceptability and sustainability
- Materials and Methods

    - Identify materials used and the methods engaged to obtain data for this study
    - Focus on the Nutritional Analysis (protein, fat and fiber) for 3 product types (bread, muffin and cookie)
- Results
    - Present findings to observations of engaged tests for objectives
- Discussion
    - Analyze and interpret the findings and make recommendations

2. LITERATURE REVIEW

This review concentrates primarily on the ingredients used in GF baked products and their impact on nutrition quality, rheology, GI and sustainability as related to the GF bakery sector while identifying issues and gaps in the market that this research will address.

2.1 IMPORTANT CHARACTERISTICS OF GLUTEN-FREE BAKED PRODUCTS

Products labeled as GF cannot contain more than 20 parts per million (ppm) of gluten, which includes contact with gluten through cross-contamination (Thompson, 2015). The Food and Drug Administration (FDA) does not presently require that products labeled as GF be tested for gluten content, however regulation § 101.91 (21 CFR 101.91) provides recommendations to manufacturers for ensuring GF compliance when using ingredients that might contain gluten or produced from wheat sources (Code of Federal Regulations, 2013). However, the absence/presence of gluten is not the only defining characteristic. Research has found that the characteristics of GF bakery products often include poor crumb structure, reduced volume and crumb softness, poor mouth feel and flavor, and are lacking in other nutrients, such as proteins, vitamins and minerals (Naqash *et al.*, 2017). Since producers often rely on developing rheological properties of

GF doughs similar to wheat-containing products (Gallagher *et al.,* 2003), it is therefore important to understand dough rheology within GF formulations.

2.2 Dough Rheology

Dough rheology refers to a product's viscoelasticity, or physical properties, such as dough strength, extensibility and elasticity, and usually relates to the uniqueness of gluten forming proteins and polysaccharides which are characteristic of wheat-based products and responsible for dough's viscoelastic nature (Shewry *et al*., 2002). Rheological properties determine dough, or batter, behavior during mechanical handling/mixing and influence gas development during the proofing and baking stages (Dobraszczyk and Morgenstern, 2003). Understanding the importance of dough rheology allows further investigation into GF formulation and solutions. For example, more elastic, or solid, doughs are generally needed for bread products while more extensible, or viscous, doughs are for cakes, cookies and biscuits. The rheology of a GF product is often compared to its wheat-based counterpart and requiring alternative proteins and/or other ingredients to develop a GF product's physical characteristics. Since properties like elasticity, extensibility, moisture levels and product texture are often indicators used to determine a GF product's quality, the next section looks at how gluten plays a role in defining GF dough rheology.

2.2.1 *Gluten Specific Properties*

Gluten is a storage protein found in wheat (*genus Triticum*) and is a composite of two types of protein, gliadin and glutenin. Protein molecules are made of amino acids, and those present in both gliadin and glutenin help the two proteins bind to each other to form a network that provides structure and elasticity to dough [Fig. 1]. Glutenin, stabilized by disulfide bonds, are responsible for dough strength and gluten proteins "form a continuous proteinaceous matrix in the cells of the mature dry grain and are brought together to form a continuous viscoelastic network when flour is mixed with water to form dough" (Shewry *et al.,* 2002). Gluten, therefore, is responsible for water absorption, viscosity, cohesivity and affects the crumb structure and chewiness of the finished product.

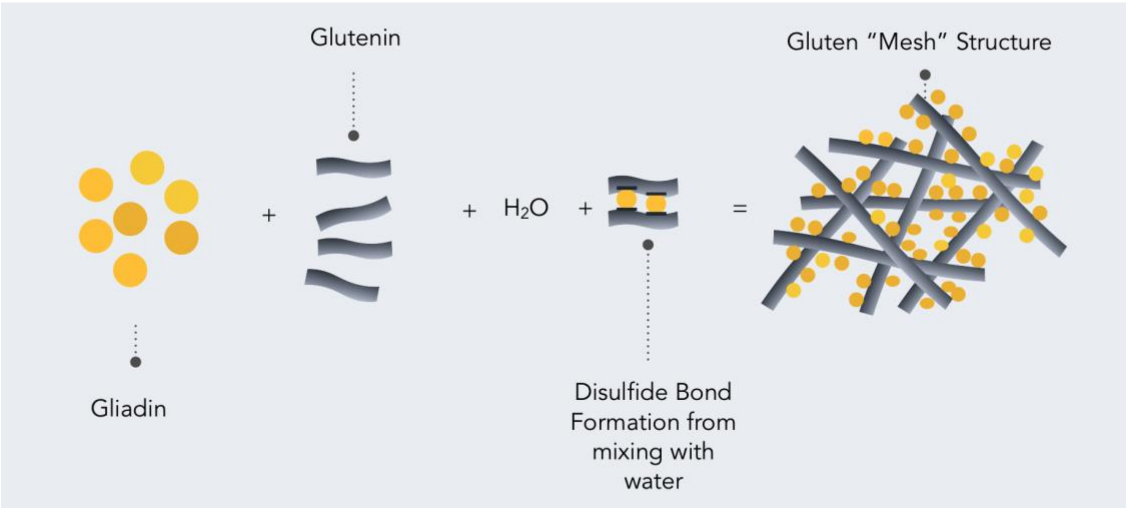


FIGURE 1: DEPICTION OF THE FORMATION OF GLUTEN STRUCTURE

Adapted from "What is Gluten," PaleoFoundation.com. Copyright 2021 by K. Pendergrass

To ensure acceptability, GF products traditionally have quality characteristics similar to wheat-based products, requiring ingredients that "mimic the viscoelastic properties of gluten" (Gallagher *et al.,* 2003) and often involve the use of dairy proteins and varying levels of hydration. The protein percentage in bread flour is typically around 10-12% and is necessary for the structure of bread products (Gallagher *et al.,* 2003; Jnawali *et al.,* 2016). Due to gluten's unique characteristics, removing it for the production of GF baked goods presents both a technical and nutritional challenge. One approach to better understanding these challenges is to use scientific equipment to help understand the rheological properties of GF products and how they relate to gluten-containing products.

2.2.1.1 The Mixolab®

The Mixolab® (*Chopin Technologies*) is a device that measures the rheological characteristics of ingredients such as protein, starch and hydrocolloids. In terms of rheological measurement, the Mixolab® replaces traditional instruments such as the Farinograph, Extensograph and Alveograph and automates the mixing, heating and cooling stages of dough. It provides information on dough development including mixing time, protein breakdown and gluten strength, starch gelatinization, additive and enzyme activity and shelf-life, which are important for the product development process. Mixolab® curves are characterized by torque measurement in five defined points that correspond to the minimums and maximums of the curve (C1 – C5), along with corresponding temperature and processing times, which were not investigated as part of this study. Definition of the torques during mixing and heating are shown in Table 1 and represented for plain bread flour in Fig. 2.

TABLE 1: MIXOLAB® TORQUE POINT DEFINITIONS

Point	Definition	Indication
C1	torque during mixing	first maximum of the curve; used to determine water absorption
CS	indicates dough stability	8 minutes after beginning of test
C2	weakening of protein	first minimum of the curve; based on mechanical work and increasing temp
C3	rate of starch gelatinization	second maximum of the curve
C4	stability of the hot-formed gel	second minimum of the curve
C5	starch retrogradation during cooling period	last point of curve and last maximum
C1 – CS	indicates the protein network stability to mixing	higher the difference = weaker the protein network is to mixing
CS – C2	Indicates the protein network stability to mixing and heating	higher the difference = weaker the protein network is to mixing and heating
C3 – C2	corresponds to starch gelatinization rate	higher the difference = higher the starch gelatinization intensity
C3 – C4	stability of the hot-formed gel to mixing at high temperature	impacted by amylase activity, damaged starch, protein and starch interactions; higher the difference, weaker the starch gel
C5 – C4	starch retrogradation at cooling phase	related to the shelf-life of end product; higher the difference = higher the starch retrogradation intensity

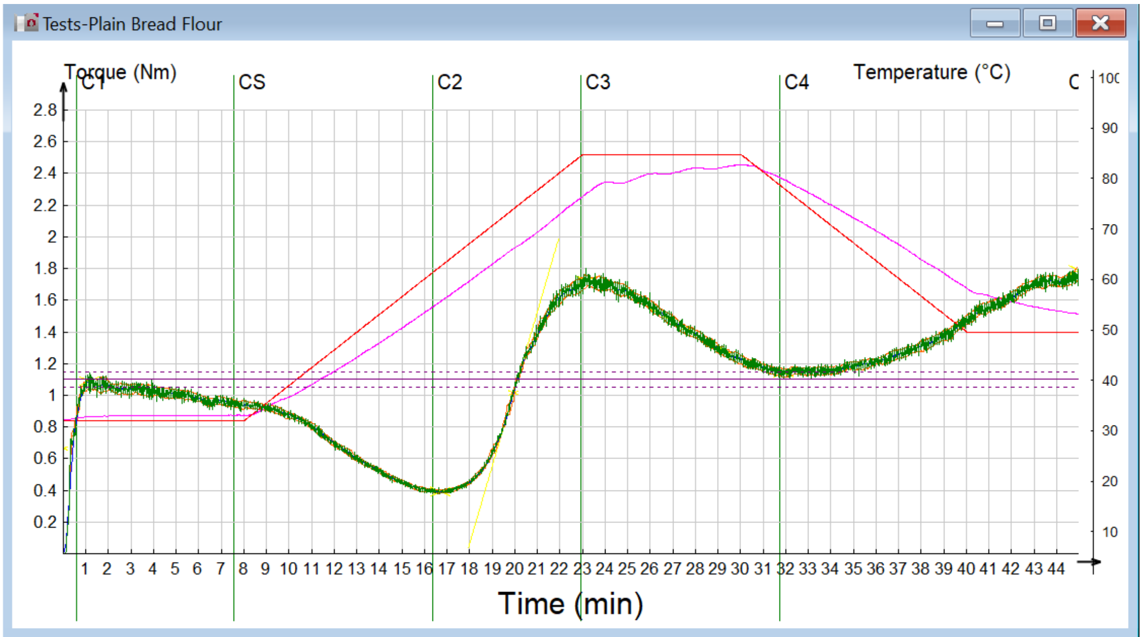


FIGURE 2: MIXOLAB® GRAPH: PLAIN BREAD FLOUR

The Mixolab® has been used to understand the rheological function of various GF ingredients, either compared to their gluten containing counterparts or for their quality features (Rosell *et al.,* 2010; Pojic *et al.,* 2015; Svec and Hrušková, 2015; Morreale *et al*., 2017; Liu *et al.,* 2018). However, overall nutrition is largely overlooked. Focusing on the development of GF products that possess both a nutritional profile and structural acceptability comparable to wheat products could be beneficial for producers. Therefore, relying solely on GF products' viscoelastic properties and their rheological comparisons to gluten-containing products should not be the only approach.

While rheology plays an important role in the development of GF products, producers also have an opportunity to address defects found in GF products as a means to develop GF formulations. It is also important to understand other characteristics of GF products, which could help impact product texture, lower GI and change consumer attitudes toward GF products by addressing and adjusting consumer perception. The next section examines issues often associated with GF products.

2.3 Issues Facing GF Production within the Baking Industry

The rise in popularity of GF products has been accompanied by certain issues amongst producers, suggesting that innovation is more of an academic approach rather than a practical one (Nascimento *et al.,* 2013). For example, labeling of GF products is intended to make consumers more aware of their options yet labelling rules can confuse both consumers and manufacturers. Thompson (2015) points out that products labeled as GF may include wheat, explaining that glucose syrup and maltodextrin are made from wheat starch and oats, which are naturally GF and often used in GF applications, may contain traces of gluten unless specially produced to be GF. European regulation No. 828/2014 places further emphasis on labelling and language of GF products to cover the reduced presence of gluten ('very low gluten') for consumers who may tolerate small amounts of gluten (European Commission, 2014). And while production issues include creating GF products with similar acceptable characteristics to their wheat counterparts (Naqash et al., 2017), focus is usually on adding ingredients which affect physical characteristics, such as volume, texture and appearance (Masure *et al*., 2016; Mir *et al*., 2016; Witczak *et al*., 2016). Therefore, most producers fail to address the nutritional component and overlook the potential health and sensory impacts of GF products, which are addressed in the following sections.

2.3.1 Lack of Nutrition

Nutritionally, wheat products are comprised of carbohydrates, minimal fiber and a protein typically derived from gluten (Jnawali *et al.,* 2016). Naqash *et al.* (2017) cited the typical nutritional characteristics of GF breads as having a protein content from 0.90 to 15.5 g/100 g, fat from 2.00 to 26.1 g/100 g and carbohydrate 68.4 to 92.2 g/100 g. Authors also suggest that GF products provide twice as much fat (mainly saturated), with the exception of bakery products, and a lower protein content than their gluten-containing counterparts [Table 2] and most GF products are often not fortified, such as with iron, folic acid and essential minerals (Nascimento *et al.,* 2013; Jnawali *et al.,* 2016). The recommended dietary allowance (RDA), the average daily dietary nutrient intake sufficient to meet the nutrient requirements of 97% - 98% of healthy individuals, varies by age and gender (National Institutes of Health, no date). However, research has shown a lower intake of essential nutrients, such as fiber, vitamins B and D, and iron, zinc and magnesium for those following a GF diet (Devlin, 2013; Jnawali *et al*. 2016), suggesting the RDA for these individuals is not being met. This suggests that the nutritional characteristics of GF products appear to be associated with the elimination of gluten while producers tend to focus on developing physical characteristics of GF products that resemble their gluten-containing counterparts rather than addressing nutritional concerns. Therefore, a variety of GF bakery products that are both nutritionally beneficial and as acceptable as gluten-containing products could address this issue.

TABLE 2: NUTRITIONAL COMPARISON OF SELECTED GF PRODUCTS

Content	Type	Flour	Cereal Bars	Breads	Bakery
Energy (kJ)	w/Gluten	1428	1739	1222	1787
	GF	1493	1550	1385	1658
Protein (g)	w/Gluten	9.77	6.86	10.0	6.38
	GF	1.43	5.67	3.47	3.79
Carbs (g)	w/Gluten	71.3	64.0	55.8	53.0
	GF	82.7	55.3	61.2	49.3
Total Fats (g)	w/Gluten	1.61	13.1	3.86	21.6
	GF	1.43	9.67	7.42	19.9
Saturated Fats (g)	w/Gluten	0.08	5.43	0.85	8.67
	GF	0.28	6.00	3.03	6.57
Fiber (g)	w/Gluten	5.23	6.57	-	3.06
	GF	2.86	15.3	-	3.18

Adapted from Naqash et al., 2017

Nascimento *et al.* (2013) analyzed 162 food labels of GF products, such as bread, cake and biscuits along with its gluten-containing counterpart and found a lower diversity of ingredients in GF product formulation. Interestingly, authors found that most GF products typically contain milk proteins which have shown to improve moisture, crumb and nutritional properties and delay staling of GF breads (Gallagher *et al*., 2003; Moroni *et al*., 2009). However, Nascimento *et al.* (2013) further mentions that many celiac sufferers are also lactose intolerant, suggesting its presence could limit choices. The lack of available, and affordable, nutrient rich ingredients for GF formulations are therefore considered contributing factors to lower nutritional quality (Tanwar and Dhillon 2017). Because the nutritional quality of GF products also has an impact on growing health-related issues, how these products address health concerns is discussed in the next section.

2.3.2 Glycemic Response

The risk of diabetes can occur when blood sugar levels are too high, with common tests including fasting and random blood sugar tests. Normal fasting blood sugar levels are 5.6 millimoles per liter (mmol/L) or below, while random blood sugar results of 11.1 (mmol/L) are considered prediabetic. Since CD is associated with a higher incidence of type 1 Diabetes (Liu *et al.,* 2016), it is recommended that people following a GF diet maintain good glycemic index (GI) control and therefore the glycemic response (GR) of GF products should be considered.

The GI for bakery products refers to the number of available carbohydrates, on a scale from zero to 100, and indicates how quickly a food causes a spike to blood sugar levels. The GI of selected GF bakery products is represented in Table 3. Most GF breads on the market today have a GI range from 83 to 96 (Naqash *et al.*, 2017), resulting in higher starch digestibility and likely due to GF products containing less protein and more carbohydrates and fats than wheat products. When combined with lower protein levels, a higher amount of soluble dietary fiber from hydrocolloids used in GF products can also contribute to a higher GI (Scazzina *et al.*, 2015), suggesting the removal of gluten directly impacts GR.

TABLE 3: GLYCEMIC INDEX OF GF BAKERY PRODUCTS

	Energy (kcal)	Fat (g)	Protein (g)	Carbohydrates (g) of which sugar (g)	Fiber (g)	Serving (g)	GI
White sourdough	282.9	5.0	5.0	50 / 3.3	8.9	132.0	52.1
White bread	284.4	7.1	3.0	50 / 5.1	4.0	124.0	61.2
White roll bread	248.9	2.8	3.4	50 / 3.7	5.0	112.0	63.3
Puffed rice cake	242.9	1.8	5.5	50 / 0.3	2.0	62.0	66.7
Cake, with yogurt	416.2	21.5	4.4	50 / 24.4	2.5	102.0	42.2
Breakfast biscuit	295.9	9.8	3.6	50 / 14	0.2	67.0	37.5

(low GI = 50, medium GI = 65); *Adapted from Scazzina et al., 2015*

It has been suggested that the impact of GR should be considered in the development of GF products as studies have shown an increased risk of heart disease in Coeliac patients, citing a higher GI diet as the possible contributing factor (Bullo *et al.,* 2013; Emilsson *et al.,* 2013). Lower GI of GF products can be achieved by replacing carbohydrates with proteins or fats and therefore the nutritional quality of GF products remains a concern. In a balanced diet, carbohydrates are necessary as an energy source, so care needs to be taken in how the GI of GF products is obtained. Pseudo-cereals have been researched as an easier way to add much needed nutrition to GF baked products, though some starches can contribute to spikes in blood sugar because of their digestibility (Naqash *et al.*, 2017). However, proteins can potentially have a greater nutritional impact while also affecting glucose response (Scazzina *et al.,* 2015). Lowering GI in GF products can be achieved by replacing added starches, such as cornstarch and potato starch, with nutrient-rich grains/flours, as they are typically higher in dietary fiber, protein and resistant starch, which can alter the rate of starch digestion and dilute the amount of available carbohydrates, which in turn reduces GI (Capriles and Areas, 2016). Although added proteins have been suggested to help lower the GI of GF bakery products, the potential health benefits of GF products with lower GI will only become a priority as long as these products taste good and are accepted by consumers, which is discussed in the next section.

2.4 Consumer Acceptance

Consumer acceptance of food is typically associated with a preference, or willingness to pay, for a product and usually measured on a 5-point scale relative to varying levels of taste, or other sensory parameters, and often obtained when a product is compared to its conventional counterpart (Verbeke, 2005). The standard approach to consumer acceptance of GF products is to compare them to their gluten-containing counterparts, usually by comparing taste, texture, aroma and visual appeal, also known as compositional approaches, or the "modification and/or incorporation of additional ingredients to counter the gluten deficiency" (Naqash *et al.,* 2017). The absence of gluten in GF baked products affects both structure and texture, therefore most GF baked products rely primarily on the use of starches and hydrocolloids and typically result in a grainy mouthfeel and poor overall eating quality (Gallagher, 2008). As a result, GF baked products are also defined by lower consumer acceptance when compared to wheat products. It is also important to note that sensory data can be acquired analytically (Masure *et al.,* 2016). For example, authors refer to analytical techniques that address aroma compounds and simulate human mastication for identifying odorants in GF bread which can be compared to wheat bread to develop the desired aroma profile in GF formulations, however exploring consumer acceptance is more than simply identifying which sensory characteristics to address.

It has been a challenge to replicate the texture, taste and aroma of gluten-containing bread products and research aimed at alternative formulations and process methods have been explored (Rosell and Matos 2010; Ziobro *et al.,* 2013; Sarabai *et al.,* 2015). However, one opportunity that still requires exploration is consumer attitude. Since GF products are typically associated with lower sensory scores, consumers often expect less from GF products. The opinion that something is 'good for being gluten-free' needs to change, which could involve changing consumer perception that GF and gluten-containing products should not be compared to as 'like for like.' Therefore, opportunities exist to improve the nutritional characteristics of GF products through the addition of nutrient rich compounds that also impact a GF product's overall taste and aroma. When viewed on their own, and for the ingredients used, GF products have the potential to also impact long-term sustainability concerns positively, which are addressed in the next section.

2.5 Sustainability

In a world where over consumption contributes to food waste and GHG and rising agriculture costs associated with GMO crops and animal-based foods begin to affect consumer choices, the increased popularity of GF products plays an important role in helping shape where these products can, and will, fit into the sustainability mix. Livestock represents a major contributing factor to increased GHG as well as a loss of biodiversity of land due to feed crops and grass (Lacour *et al.,* 2018). In Fig. 3, published by World Resources Institute, Ranganathan *et al.* (2016) compares the resource use and GHG emission of various plant and animal sources in an attempt to convey the usage impact of animal-based foods. Except for fish, GHG and land use is higher for animal-based sources. Therefore, the impact of using plant-based proteins in GF baking over animal by-products such as dairy, and whether or not this could have a positive impact on reducing GHG, should be considered. However, challenges such as one's culture, promotion of sustainable options and consumer perception play a role.

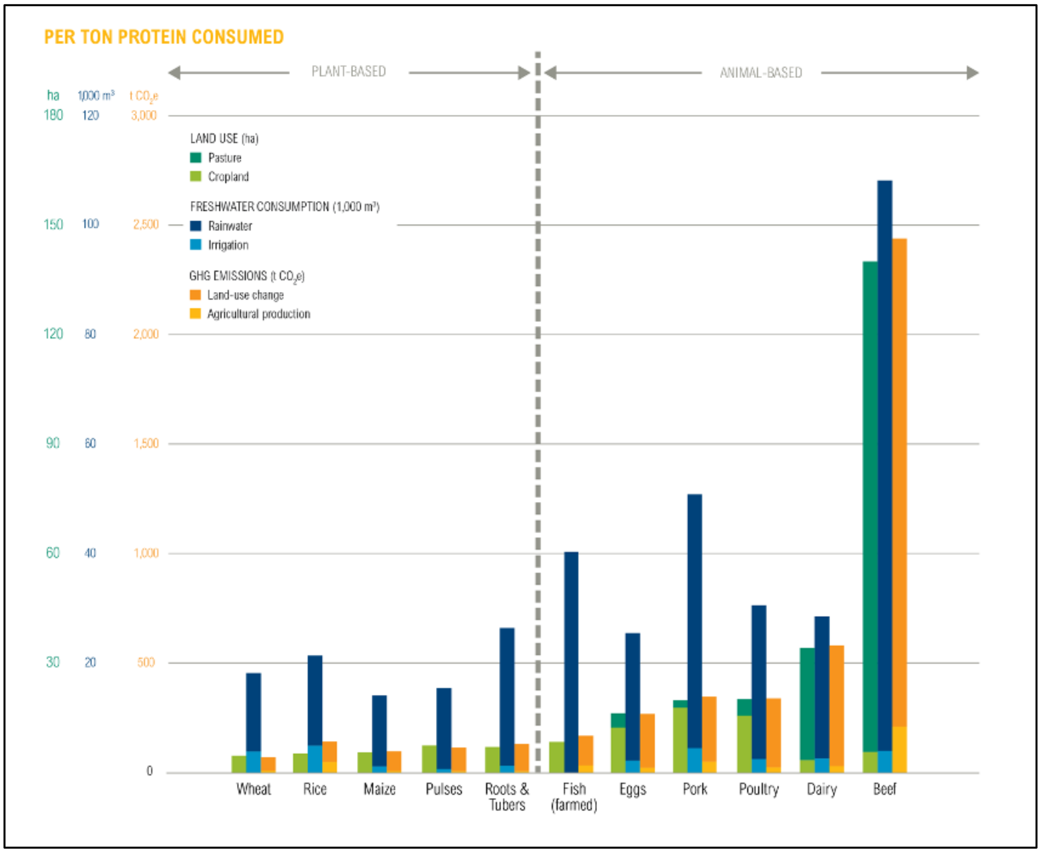


FIGURE 3: DEPICTION OF RESOURCE USE OF ANIMAL-BASED VS PLANT-BASED FOOD SOURCES

Adapted from Ranganathan et al., 2016

How one's culture influences customer perceptions of what is sustainable, and whether or not the responsibility falls upon producers or consumers to make a change, presents a challenge to approaching sustainability in the food sector. In France, Lacour *et al.* (2018) found that GHG, energy demand and land occupation were all factors that impact the acceptance of a more plant-based diet. Although it was found that a higher intake of macronutrients was reported on plant-based diets, the French were unwilling to give up cheese. A study by Milne *et al.* (2019) found that food choices and the consumption of red meat are factors preventing the United Kingdom from going climate neutral before 2050, citing that the British public do not look ready to take the necessary steps to change their lifestyle. While the focus for sustainability has primarily been thrust on producers, it seems that how consumers view sustainability is a primary factor and is affecting how policy makers react. In Switzerland, seasonal foods lead to lower energy consumption because they are neither stored or produced in greenhouses nor use energy intensive methods (Lazzarini *et al.,* 2017). However, consumers need additional information to enable their selection of food products via labeling and packaging. Though authors suggest alternative methods in which providing additional information could be improved, a key consideration might involve policymakers and retailer roles to help shape a country's local sustainability goals so that consumers are able to make better choices without the assistance of labeling and packaging. Excessive food consumption is also a concern for policymakers, therefore promoting sustainability might reduce food waste.

Farmer *et al.* (2017) found that changing consumer opinion and affecting perception by promoting sustainable options resulted in a change to how products were consumed, suggesting that sustainable information on a product influences consumer behavior. Authors also cite the knowledge of a sustainable option lessens consumption and suggest that if products are promoted as more sustainable excessive consumption can be reduced. While an approach to research on sustainable products may include more nutritious, sustainable ingredients, there is often no distinction in how the use of sustainable ingredients affect overall product view and consumption. This opens the option for further research into just how sustainable ingredients themselves might affect consumer perception of a product, rather than how a wholly sustainable product is viewed by itself. Growing sustainability concerns, as it relates to the baking sector, can be defined as a lower reliance on GHG emissions by shifting away from animal-based proteins, GMO crops and a reduction in consumer food waste, however it would be wise to further investigate the compounds and techniques used in GF bakery production.

3. TECHNIQUES AND INGREDIENTS

3.1 TECHNIQUES

Many GF producers rely on production techniques aimed at extending the shelf-life of GF products, including: Modified Atmosphere Packaging which has shown to reduce spoilage and the need for additional preservatives (Rasmussen and Hansen, 2001; Secchi *et al.,* 2017); Par-Baking and Freezing, which can reduce GI response (Capriles *et al.,* 2015; Naqash *et al.,* 2017); and Fermentation (such as sourdough), which affects the rate of available carbohydrate digestion when combined with certain pseudo-cereals (Capriles *et al.,* 2015) and increases crumb moisture without affecting shelf-life, reducing the need for additives (Rinaldi *et al.,* 2017). While these techniques have been established to extend the shelf-life of products, they remain expensive options for GF producers. Other approaches to addressing shelf-life include adjusting recipe formulations, such as adding more saturated fat because of its effect on delaying the staling process and by including preservatives, such as sugar (Nascimento *et al.,* 2013). These approaches, however, can contribute to the perception of GF products being nutritionally compromised due to consumer perceptions around added sugar and fat (Tanwar and Dhillon, 2017). Therefore, addressing the ingredients used in GF recipe formulations may be a better approach.

3.2 Ingredients

XG, starches and proteins are used in GF bakery products, and each plays a role in the rheology, nutritional impact and acceptance of the end product. Therefore, understanding how these compounds are important to GF recipe formulations can also help improve product nutrition, consumer perception and sustainability, which are important elements for this project. Hydrocolloids are widely used in the bakery industry as structuring agents in GF formulations (Morreale *et al.,* 2017) and can improve texture and acceptability (Naqash *et al.,* 2017). Starches are required for volume, hardness and elasticity and can delay staling due to their higher water combining capacity (Martinez and Gomez, 2016). However, starches could have a negative impact on a product's nutritional properties and GI response due to their lack of fiber, micronutrients and protein (Gallagher, 2008; Capriles *et al*., 2015; Witczak *et al.,* 2016). Proteins are often responsible for improving nutritional characteristics of a product; however, they can also affect volume, decrease crumb hardness and reduce staling (Sarabai *et al*., 2015; Shevkani *et al.,* 2015; Ziobro *et al.,* 2016; Naqash *et al.,* 2017). Since gluten is responsible for structure, elasticity and provides nutrition, other proteins are necessary to replace these characteristics. For example, soy protein isolate (SPI) shows a slightly improved protein content over pea protein (Matos *et al*., 2014) and has higher protein solubility, emulsifying activity and water holding capacity than hemp (Tang *et al*., 2006). However, SPI scores lower in sensory testing (Ziobro *et al*., 2016; Korus *et al*., 2017). Pea is a good substitution for soy in terms of physical, sensory and nutritional characteristics of food products (Shevkani *et al*., 2015; Naqash *et al*., 2017). Pea protein's solubility, emulsifying, foaming and gelation properties also make it a good addition to bakery products to improve volume and texture (Ziobro et al 2013; Matos et al. 2014; Lam *et al.* 2018). Hemp has been used in food applications as a nutritive additive, to lower baking loss and time of bakery products and shows promise with structure and sensory results (Tang *et al.* 2006; Pojic *et al*., 2015; Korus *et al*., 2017; Wang and Xiong, 2019). Therefore, choosing the right protein could significantly impact a product's quality and nutrition.

Since there is a general belief among producers that most problems can be solved by technical means, the reliance on hydrocolloids and expensive techniques as a means to impact texture and structure alone in GF products has unintended consequences (Vergragt, 2006). It is necessary to balance the functional aims of GF products (providing alternatives for those avoiding gluten in their diets) with their unintended social and environmental consequences (reduced nutrition, increased food waste and reliance on GHG generating systems). However, when considering the cost of a product, especially in the GF market, producers should not only look at ingredients, particularly proteins and hydrocolloids as they are typically relied on to improve texture, but also the sustainability of such ingredients rather than more expensive processes or techniques.

The sustainability of certain foods can also have an impact on a product's perception (Siegrist *et al.,* 2015). Therefore, avoiding the use of hydrocolloids as a clean label option for GF products to reduce cost and help increase shelf life could be considered. However, since each GF product is unique based on its use of compounds and process techniques, standardizing GF bakery production to avoid or include a hydrocolloid might not be the way forward. Additionally, plant-based proteins, such as PPI and HPI, are non-GMO options which have a lower carbon production footprint and should be considered together in GF bakery production.

4. CURRENT SCOPE OF RESEARCH

In reaction to a growing GF market, research has begun to emerge around techniques, ingredients, formulations and quality parameters of GF baked products and how these applications might aid producers (Gallagher *et al*., 2003; Rosell and Matos 2012; Ziobro *et al*., 2013; Sarabai *et al*., 2015). While some research addresses the nutritional benefit of certain ingredients, techniques used to extend shelf-life and how products rate in sensory trials, the primary focus of GF research relates to how ingredients affect product structure and texture though the effect of each ingredient, such as hydrocolloids, starches, GF flours and proteins and can vary between formulations.

- Hydrocolloids improve product volume, appearance and shelf life (Sabanis and Tzia, 2010), are important for water absorption and gelatinization (Liu *et al.,* 2016; Morreale *et al.,* 2017) and can be manipulated to achieve desired textural and sensory results (Sabanis and Tzia, 2010; Morreale *et al.,* 2017; Shaabani *et al.,* 2018). Used in such small amounts they have little to no impact on price (Saha and Bhattacharya, 2010) and when combined with other functional ingredients can impact product nutrition and the sustainable perception of GF baked goods (Korus *et al.,* 2009; Hublik, 2012; Naqash *et al.,* 2017), but may not be necessary based on raw materials and hydration levels used (Rozylo *et al.,* 2015). While cellulose derivatives are more commonly used (Morreale *et al.,* 2017), XG is versatile across GF applications (i.e., not overly affected by changes in pH, temperature or the presence of salt), is cost effective (Shaabani *et al*., 2018) and results in improved product quality (Liu *et al*., 2016) making it a good option for producers.

- Starches are important for product texture (Martinez and Gomez, 2016) and can also be used to increase consumer acceptance (Korus *et al.,* 2015; Tanwar and Dhillon, 2017). Resistant starches have long been shown to improve nutritional quality reducing the energy of food, increasing satiety and improving digestive functions similar to fiber (Jenkins *et al.,* 2002; Lunn and Buttriss*,* 2007). Maize, potato and rice starches are commonly used in GF baking because they are inexpensive and widely available (Capriles *et al*., 2015) but replacing them with GF flours and pseudo-cereals can result in improved crumb structure, shelf-life, increased protein, fiber and minerals and form a similar protein network to gluten, (Alvarez-Jubete *et al.,* 2009; Korus *et al.,* 2015; Pojic *et al.,* 2015; Tanwar and Dhillon, 2017; Shaabani *et al*., 2018). While GF flours/pseudo-cereals can be expensive additions to GF formulations (Rinaldi *et al.,* 2017) they might be more sustainable options due to their increased abundance (Velazquez *et al*., 2012).
- Proteins can replace starches and flours to increase gas retention and promote viscoelasticity in GF formulations (Ziobro *et al.,* 2013; Shevkani *et al.,* 2015). When replacing hydrocolloids, proteins can improve gelatinization but can also result in lower consumer acceptance (Marco and Rosell, 2008; Sahagun *et al.,* 2018) and contribute to staling in GF breads (Ziobro *et al.,* 2016). Nutritionally, PPI contains many branch chain amino acids (BCAA), good levels of fat-soluble vitamins A, D, E and K, reduces blood pressure, cholesterol, blood sugar levels and delays gastric emptying (Li *et al.,* 2011; Mollard *et al*., 2014). HPI is a complete protein, is a good source of fiber and its pairing with hydrocolloids might not be necessary in bakery products (Pojic *et al*., 2015; Svec and Hrušková, 2015; Korus *et al*., 2017).

Since GF research is as varied as the formulations themselves, some industry critical associations are overlooked, such as how GI is affected by these various ingredients and the role of sustainability in the GF market. Higher GI levels can increase cardiovascular risk (Bullo *et al.,* 2013) and contribute to diabetes, which has long been associated with CD (Leonard *et al*., 2014). Uhde *et al.* (2016) found that subjects without CD experienced a sensitivity to wheat products based solely on immune response and intestinal activity. Lebwohl *et al.* (2017) found that while an intake of gluten was not associated with a risk of heart disease, the avoidance of gluten may result in reduced consumption of whole grains, which may impact cardiovascular risk, suggesting that a GF diet in those who are not diagnosed as Coeliac should not be encouraged. Furthermore, diabetes is also a concern when relying on a GF diet. Studies have shown that GF products typically have a higher GI than wheat products due to the higher rate of starch digestion of GF products which in turn can spike blood glucose, or blood sugar, levels (Matos and Rosell, 2011; de la Hera *et al.*, 2014; Scazzina *et al.,* 2015). While individuals claim to experience a range of symptoms to wheat ingestion, continued research around gluten-related disorders, such as identifying gluten sensitivity or the long-term effects of gluten on one's health can shape the way both consumers and producers view the GF market (Uhde *et al*., 2016; Lebwohl *et al*., 2017). A study on the glycemic response of popular Italian GF bakery products shown in Table 3, and which included breads, cakes and pasta, obtained from two groups of 10 healthy volunteers using a finger prick test indicates that increased fiber, protein and fat in GF products resulted in much lower overall numbers (Scazzina *et al.*, 2015). Authors cite a further study by Moghaddam *et al.* (2006) where the addition of up to 30% soy protein to a glucose solution (50 g glucose/250 mL water) also helped reduce glycemic response, suggesting that adding a protein source to GF products might lower GI. Other factors, such as different formulations of GF products and sourdough fermentation (up to 15-22.5% sourdough added which, when combined with par-baking and freezing, could improve product quality and shelf life) might also affect carbohydrate availability and cause a different glycemic response (Novotni *et al.,* 2012; Scazzina *et al.,* 2015). And while Scazzina *et al.* (2015) conclude that the results of their study were not enough to draw conclusions about GI values of all GF products given the variety of products on the market, the study showed that some commercial GF breads, pastas and biscuits, depending on the formulation, could be suitable to reduce GI in Coeliac patients and prevent illnesses associated with a high GI diet.

While most GF research addresses rheology, nutrition and consumer acceptance, some research attempts to link these together. However, current research has also begun to illuminate challenges facing the baking industry as GF options increase in popularity. Therefore, the next section examines some of these challenges.

4.1 Techniques and Compositional Approaches

Since gluten is responsible for viscoelasticity, gas retention and structure its removal for the development of GF bakery products presents technological challenges (Sciarini *et al*., 2010a; Sciarini *et al*., 2010b) and so producers often rely on techniques and incorporate ingredients to create a GF product that is acceptable to consumers (Moroni *et al*., 2009). While ingredients are typically used to address rheological characteristics and sensory attributes, techniques, such as active packaging, steaming and sourdough fermentation are also employed to improve shelf-life and reduce food waste.

4.1.1 Techniques

GF bakery products can stale faster due to various factors such as interactions with components like proteins and fats, moisture migration and product storage (Rinaldi *et al.,* 2017). Their lower ability to retain moisture is due to a process called starch retrogradation which refers to the recrystallization of the polysaccharides in gelatinized starch and occurs during moisture migration or when exposed to freeze/thaw cycles and is affected by storage temperature, water content, sugar, salt and fats. The inability to retain as much water can contribute to staling, an increase in food waste and have an effect on cost (Demirkesen *et al.,* 2014; Missbach *et al.,* 2015), therefore techniques aimed at extending a product's shelf-life, as well as lowering the GI response and reducing the gluten concentration in wheat, are often used in GF production. Common techniques include Modified Atmosphere Packaging (MAP), Par-Baking and Freezing and Sourdough Fermentation.

4.1.1.1 Modified Atmosphere Packaging

A promising technique to reduce product staling and promote less food waste by reducing food spoilage, MAP controls the O_2 level in packaging using very low O_2 concentrations, high CO_2 concentrations (20% or higher) and N_2 as an inert filler and anti-packaging collapse gas. This technique also reduces the need for additional preservatives, which is promising as the use of preservatives is decreasing in the baking industry due to consumer demand. In a study on cheesecakes using added whey protein MAP helped extend shelf life from 21-45 days, compared to only 11 days for the control (Secchi *et al.,* 2017). After 20 days of storage, while some hardening occurred in the product containing whey, it remained above the consumer acceptability threshold showing that the test product's sensory acceptability was not impaired. Another study of MAP on wheat bread showed no effect on starch retrogradation compared to bread stored at atmosphere (Rasmussen and Hansen, 2001). However, authors cited the firmness of the bread packaged in 100% CO_2 reached its maximum value after approximately 35 days, suggesting MAP may help extend shelf life compared to bread packaged at atmosphere. This technique has been shown to also reduce food spoilage in GF fresh filled pasta. A study by Sanguinetti *et al.* (2016) showed the shelf life of GF pasta was 42 days compared to 14 days for the control which, combined with lower storage temperatures, resulted in a control of mold growth within the pasta filling. While this indicates potential reduction in food spoilage for GF products, more research is needed to determine if MAP reduces staling in GF bread and whether or not it could extend to GF bakery products as well.

4.1.1.2 Par-baking and Freezing

Par-Baking, or partially baking, is an emerging method used as a means to combat the staling process in GF bread products. Conventional baking requires a one step process, whereas par-baking involves baking in two steps: an initial baking stage at generally two-thirds total baking time (Rosell and Gomez, 2007), followed by cooling/storage/freezing and then a second step which includes thawing and then final baking. Since most GF products contain a higher amount of starch, retrogradation occurs after a product is baked. Although retrogradation affects the products shelf-life, the process is reversed in the second phase of baking for par-baked products because the second baking step has been shown to melt amylopectin (Sciarini *et al*., 2012). However, while the results are positive, they are dependent on the addition of a hydrocolloid to maintain bread quality.

4.1.1.3 Fermentation

The process of fermentation has been known to improve the quality of GF breads when combined with certain pseudo-cereals such as amaranth, sorghum, chickpea, buckwheat and quinoa (Coda *et al.,* 2010; Arendt *et al*., 2011). Sourdough fermentation improves volume, texture and delays staling of GF breads and adding 15 and 22.5% sourdough to GF bread formulations has shown to significantly reduce GR because it affects the rate of available carbohydrate digestion (Novotni *et al.,* 2012). Rinaldi *et al.* (2017) also found that sourdough fermentation has been shown to increase crumb moisture content with no negative effect on shelf life. Authors therefore concluded that fermentation reduces the need for expensive additives while also resulting in higher acceptance from consumers. Additionally, in terms of consumer perception, the fermentation technique is also considered clean label and natural.

4.1.2 Compositional Approaches to Gluten-Free Baked Products

While GF baked products are identified as containing no gluten, they are also characterized by the ingredients used in them. Studies have suggested that different starch, flour and hydrocolloid combinations are best for certain baked products – ranging from cookies, bread, cake, pasta and muffins. Price, though, will always play a determining factor in how GF products are both sourced and produced. GF products tend to cost more when compared to their wheat-containing counterparts (Stevens and Rashid, 2008) and producers could feel that using more nutritious proteins, cereals and pseudo-cereals in GF products negatively impacts cost, although studies show potential in this area. For example, potato is the fourth most important crop in the world (next to rice, wheat and maize) and is suggested to have a lower overall cost, a balanced amino acid composition and vitamins/minerals making potato flour, or potato starch, a good substitute in GF products (Liu *et al.,* 2016). Chickpea protein is more expensive based on the costs associated with chickpea production, however adding 2-15% to a GF product may increase product value but the overall price is still lower than that of a gluten containing product (Shaabani *et al*., 2018). A study of GF cookies suggests the addition of nutrient rich ingredients such as buckwheat and/or millet flours could be a cost-effective way to improve the nutrition of some GF products (Tanwar and Dhillon, 2017). While whole meal flours can provide more vitamins and minerals, such as B vitamins and iron, they are not always suitable substitutions in baked goods. Similarly, egg and dairy are protein sources used in GF baking however they are not suitable for lactose-free or vegan diets. Removing gluten can present producers with an opportunity to replace it with ingredients which can add more nutritional value, however it also changes the structure of GF products and requires the addition of compounds to improve a product's textural characteristics. When considering product cost and quality, especially in the GF market, producers should look at hydrocolloids, starches, cereals and proteins to address rheological and sensory attributes before moving to more expensive processes or techniques. Therefore, the next sections look at how these compounds are used in GF recipes.

4.1.2.1 Influence of Hydrocolloids on GF Products

Primarily used as thickening and gelling agents in food, hydrocolloids function as stabilizers and emulsifiers in GF products. Since GF flours lack the necessary proteins to provide the same elasticity as gluten, hydrocolloids are added to improve texture and elasticity and include starches, fibers, gums, pectin and gelatin. Research of various hydrocolloids, particularly HPMC, within GF bread making is extensive (Morreale *et al.,* 2017) and includes understanding batter/dough rheology during heating and cooling stages (Rosell *et al.,* 2011), the importance of hydration on crumb hardness during these stages (Matos and Rosell, 2013) and, with respect to the raw materials and additives used, the ability to control loaf volume by hydration level (Lazaridou *et al.,* 2007; de la Hera *et al.*, 2013; Hager and Arendt, 2013). Therefore, Table 4 summarizes selected studies pertaining to GF bread making and the effect of HPMC and XG, because of their impact on product quality (Sciarini *et al*., 2010b; Hager and Arendt, 2013; Mancebo *et al.,* 2015), as more versatile hydrocolloids for GF applications. However, the nature of testing relies on dough rheology and how GF products rate rather than their nutritional value. A concern, therefore, is that relying on hydrocolloids from a technical perspective might lower the need to improve the nutritional value of GF baked products (Ziobro *et al*., 2013). Although the use of fibers has led to the improvement of volume, crust and crumb of GF cakes (Gularte *et al.,* 2012), how hydrocolloids impact a GF product's nutrition, consumer acceptance and shelf life should also be investigated.

TABLE 4: THE INFLUENCE OF SELECTED HYDROCOLLOIDS ON GF BREAD DEVELOPMENT

Source	Functional Ingredients	Non-Functional Ingredients
Lazaridou et al. (2007)	Pectin, CMC, Agarose, XG, Oat ß-glucan	Corn Starch, Rice Flour, Sodium Caseinate
Among hydrocolloid levels of 1% and 2%, XG yields the stronger dough and a farinograph curve typical to wheat-flour doughs. Except for XG, increased hydrocolloid levels improved loaf volume, but decreased with pectic added. Higher crumb porosity was observed with 1% CMC and ß-glucan and 2% pectin, and higher crumb elasticity at 2% CMC, pectin and XG. ß-glucan increased crumb lightness and XG improved whiteness of crumb. Overall highest acceptability seen in 2% CMC formulation.		
Sabanis and Tzia (2010)	HPMC, XG, Carrageenan, GG	Corn Starch, Rice Flour
Hydrocolloid levels of 1% and 1.5% (except XG) resulted in GF bread with higher volume, better color and increased shelf life due to their moisture absorption ability. Apart from XG, the addition of hydrocolloids resulted in softer crumb structure over control and with similar moisture contents, with HPMC and GG being the lowest. Crumb firmness decreased with addition of 1.5% hydrocolloid. Sensory results showed preference for 1.5% HPMC due to volume, appearance and firmness.		
Velazquez et al. (2012)	HPMC, Sorghum Flour	Corn Starch

HPMC (1%, 2% and 3%) and sorghum flour (0.11, 0.55 and 1.00) ratios were tested with hydration levels (90%, 100% and 110%) on GF bread for softness, volume and crumb. Each factor had a linear effect on softness, with no relationship found with volume/crumb. Bread volume increased with HPMC and increased starch/flour ratio likely due to improved gas retention during fermentation/baking. Crumb depends on each starch/flour, water and HPMC addition, increasing with starch/flour but decreasing with water.

Hager and Arendt (2013)	HPMC, XG, Pectin	Buckwheat Flour, Maize Flour, Teff Flour, Rice Flour

Effect of hydrocolloids on GF breads varied based on raw materials used. HPMC had a positive effect on volume, except for rice breads, whereas XG had a negative effect on volume of all breads. HPMC reduced crumb hardness, whereas XG increased it for teff and buckwheat but reduced for maize. Results concur with previous studies that formulation and ingredient quality play a role in using XG and HPMC as improvers to GF bread. However, were contradictory to past observations that XG decreases bread quality, suggesting size of baking tin used (relative to heat transfer and mechanical support from bottom and sides of tins) may be a contributing factor.

Mancebo et al. (2015)	HPMC, Psyllium	Rice Flour

The addition of both HPMC and psyllium increased elasticity and viscosity of dough. While psyllium reduced the pasting temperature, the addition of HPMC had no effect. Psyllium reduced bread volume and increased hardness, whereas HPMC had little effect on either parameter. Interestingly, both hydrocolloids resulted in a decrease on dough rheology, volume and hardness as hydration level was increased.

Martinez and Gomez (2016)	HPMC	Maize Flour, Rice Flour, Maize Starch, Potato Starch, Wheat Starch

The use of HPMC (2 g/100 g) with common GF flours in dough preparations showed the starch-hydrocolloid matrix dependent on the type of starch used. Changes during the fermentation and baking processes were dependent on the structure of starch granules, as well as water absorption and pasting temperature. Larger granules helped with dough viscoelasticity but produced loaves with lower volume and texture properties. Wheat starch produced more CO_2 during fermentation, forming a continuous starch-hydrocolloid matrix likely due its bimodal size distribution, and producing highest volume/best texture.

Liu et al. (2016)	HPMC, CMC, XG, Pectin	Potato Flour

Hydrocolloids increased gelatinization temperatures and water absorption of GF potato flour bread dough. Steamed breads with hydrocolloids resulted in significantly higher volume, lower hardness, decreased rapidly digestible starch and lower GI. HPMC resulted in largest specific volume, likely due to dough stability formed by potato protein/starch and hydrocolloid interaction. Addition of all hydrocolloids increased crumb porosity. *In vitro* starch digestibility tests showed RDS was significantly decreased with hydrocolloid addition and RS significantly increased, especially with HPMC and XG, which correlates to lower GI of steamed breads. HPMC and XG performed the best as improvers.

Morreale et al. (2017)	HPMC	Rice Flour

HPMC levels (1%, 2%, 3%) and hydration levels (90%, 100%, 110%) were used to understand the role of viscosity in GF breads. Results show hydration level is important to GF batter viscosity and dough rheology. HPMC amounts can be manipulated to improve desired product texture, such as crumb hardness, cohesiveness and resilience. Optimal quality was obtained from 2.2% HPMC and 110% hydration.

4.1.2.1.1 Nutritional Component

Producers overlook focusing on nutrition when hydrocolloids are used because of their effects on structural improvement in the absence of gluten (Sabanis and Tzia 2010; Velazquez *et al.,* 2012; Martinez and Gomez, 2016). However, hydrocolloids can increase the nutritional value of GF products because some, including cellulose and its modified forms such as HPMC, also serve as a source of dietary fiber (Morreale *et al.*, 2017). Fiber is thought to possess nutritive and physiological effects, such as appetite regulation, bowel function and prevention of coronary heart diseases and diabetes by influencing digestion and absorption of available carbohydrates (Lairon *et al.,* 2005; Gularte *et al.,* 2012). Korus *et al.* (2015) found that the fiber present in acorn flour significantly modifies water binding and influences the rheological properties of dough, suggesting that higher levels of fiber in GF formulations can have a positive effect on dough rheology as well as nutrition. Conversely, Mancebo *et al.* (2015) found that while HPMC (in 2 and 4 g/100 g) resulted in minimal differences between bread volume and hardness, the addition of psyllium had a greater (and negative) effect on these parameters. However, authors neglected to investigate the impact of added fibers on nutritional quality or consumer acceptance.

4.1.2.1.2 Rheology

Gluten is responsible for the viscoelasticity of dough; therefore, its absence presents a challenge that hydrocolloids attempt to address. Proteins in GF cereals do not have the same structural and viscoelastic properties of the protein in gluten therefore GF dough is more viscous and resembles a batter (Morreale *et al.*, 2017). Hydrocolloids are used to improve texture, increase the moisture content and extend the overall quality of GF baked products because they consist of a number of water-soluble polysaccharides with varied chemical structures that improve bread dough development and gas retention through an increase in viscosity. As a polysaccharide, XG is comprised of glucose, mannose and glucuronic acid (Kulkarni and Shaw, 2016) and important properties include being highly viscous in low concentrations and acting as a suspending agent in GF batters (Sharma *et al.,* 2014). However, the impact of hydrocolloids on the characteristics of GF dough is dependent on the chemical structure, quantity and interaction with raw materials used (Hager and Arendt, 2013).

Sabanis and Tzia (2010) tested GF bread formulations with varied levels (1%, 1.5%, 2%) of HPMC, XG, GG and carrageenan to determine the optimal amount. Authors found 1% and 1.5% formulations (except XG) resulted in bread with higher volume, better color and increased shelf life due to moisture absorption ability, however increased hydration levels were needed. Morreale *et al.* (2017) looked at the viscosity and hydration of rice-based, GF breads using HPMC (1%, 2%, 3%) with varied hydration levels (90%, 100%, 110%) and found that while the hydration level is crucial, HPMC can be manipulated to improve desired textural features, such as crumb hardness, cohesiveness and resilience with desirable levels being 2.2% HPMC and 110% hydration. Velazquez *et al.* (2012) looked at the relationship between batter softness, volume and crumb with GF breads based on cornstarch/sorghum flours with varied hydration and HPMC levels. Authors found that HPMC improves gas retention during fermentation and baking, and that constant levels of HPMC increase batter softness as hydration is increased. However, there is no relationship between volume and crumb. Liu *et al.* (2016) studied the effects of XG, HPMC, CMC and apple pectin on steamed vs. baked GF potato flour bread formulations and found that all hydrocolloids increase the gelatinization temperatures (from 52.0 to 64.2 C) and water absorption (from 56.22 to 65.50%) of steamed breads, resulting in higher volume and lower hardness. HPMC and XG performed the best as improvers, with XG particularly shown to have a softening effect on steamed breads. Shaabani *et al*. (2018) concluded that XG is one of the best choices to be used in GF products because not only does it improve texture and moisture retention it is an extracellular heteropolysaccharide with a high molecular weight which reduces flour sedimentation and improves gas retention, forming more viscous batters that are not affected much by temperature, pH changes or the presence of salts. Despite attempts to use various production methods and hydrocolloids to affect or improve GF dough rheology, there is no clear link between bread quality and dough rheology.

Lazaridou *et al.* (2007) found that bread quality was dependent on type of hydrocolloid used and supplementation level. For example, while XG improved dough strength it had no effect on volume whereas increased levels of pectin decreased it. And while XG improved crumb lightness and elasticity, 1% CMC and 2% pectin resulted in significantly higher loaf volumes and crumb porosity and elasticity values. Similarly, Hager and Arendt (2013) found that results for HPMC and XG are often contradictory, acknowledging HPMC is more suitable for loaf volume and quality while XG impacts dough stability and viscosity. However, authors cite hydrocolloid quality also plays a role, as well as the formulation and surrounding matrix which supports findings that suggest there are no optimal means of producing GF breads because of the various ingredient combinations and process techniques used (Martinez and Gomez, 2016). Furthermore, it has been suggested that the combination of hydrocolloids has a synergistic effect on GF formulations (Haque and Morris, 1994; Arendt and Dal Bello, 2008) however neither Hager and Arendt (2013) nor Mancebo *et al*. (2015) found this. And while most studies support the theory that hydrocolloids are needed to produce a quality GF product, other factors such as GI levels must also be considered. Therefore, the next section explores how hydrocolloids impact the GI of GF products.

4.1.2.1.3 Glycemic Response

According to Liu *et al.* (2016) the use of XG during steaming, as a technique to improve the quality of GF breads, not only has a softening effect on the end product but also reduces GI by decreasing starch solubility and digestibility, resulting in decreased rapidly digestible starch (from 45.51 to 20.64) and a lower estimated GI (from 58.89 to 73.35). And while hydrocolloids control water binding, authors found that they also decreased the density of the potato protein bands in GF steamed breads, which suggests a change in protein solubility. And although hydrocolloids may change the starch gelatinization qualities of GF steamed products by influencing starch digestibility, this would not apply to all GF production methods and could be expensive for producers. Still, soluble fibers, such as guar gum (GG), ß-glucans and psyllium, as additives to wheat bread have been widely researched and can delay gastric emptying and therefore effect GI response by reducing digestibility (Scazzina *et al.,* 2013; Bae *et al*., 2016) which may be a more cost-effective approach to GF formulations but would need to be investigated further.

4.1.2.1.4 Consumer Acceptance

Sabanis and Tzia (2010) found that HPMC and GG in GF breads resulted in higher loaves, crumb softness, appealing dark crust as well as uniform and finely grained crumb texture when comparable to wheat products. And while sensory results showed 1.5% HPMC was preferential due to volume, appearance and firmness, authors state that all GF breads with hydrocolloids were acceptable, rating higher than 5 (5.5 to 7.5) on a 9-point hedonic scale. Similarly, Lazaridou *et al.* (2007) found all GF formulations tested resulted in acceptable sensory scores (6.1 to 7.5). Research has also suggested that process techniques and various ingredient combinations could produce different results. For example, Shaabani *et al*. (2018) found that using the enzyme microbial transglutaminase (MTG) with chickpea protein isolate (CPI) in GF millet muffin formulations could not form the desired texture that XG could, however when using MTG and XG together produced acceptable results. And while Sabanis and Tzia (2010) found using XG produced loaves which were denser and with higher crumb firmness values than control loaves, authors suggest a smaller loaf size could have impacted crumb firmness. In fact, Hager and Arendt (2013) also suggest pan size could explain contradictions in hydrocolloid performance studied in GF breads. Therefore, sensory acceptance could extend beyond investigation of product quality parameters. Liu *et al*. (2018) also suggests that the higher crumb porosity found with the addition of hydrocolloids influences sensory scores and that using hydrocolloids, regardless of type, would result in higher customer acceptance of GF breads. And while hydrocolloids can improve sensory acceptance, they offer little in the way of nutrition and are dependent on the specific formulation (Morreale *et al.*, 2017), which suggests that it might be possible to produce acceptable results without the use of a hydrocolloid, though this would need to be investigated.

4.1.2.1.5 Sustainability

Research has shown the benefit of using hydrocolloids and how they affect both product and producer alike. Martinez and Gomez (2016) looked at the evolution of the most common GF flours and starches (rice/maize flours and wheat/potato starches) using HPMC to gain insight into the starch-hydrocolloid matrix formed during fermentation and baking in an attempt to better predict the quality of GF bread, which could have industry implications towards sustainable practices. Authors found that granule size directly correlates to volume and texture, citing GF wheat starch as having both large and small granules contributing to its ability to retain CO_2, assisted by HPMC, and improved dough integrity over maize flour and potato starches. Hydrocolloids not only improve a product's sensory and technical characteristics, but they can also extend shelf-life (Demirkesen *et al*., 2014) which could lead to less waste. Shaabani *et al*. (2018) found that hydrocolloids are often used by producers of GF baked products as a more cost-effective option for both product quality and shelf-life. Hager and Arendt (2013) urge hydrocolloids be used at lower levels due to price, while Shaabani *et al*. (2018) concluded that when used in small amounts XG does not affect price. And as sustainability concerns grow, so does the need to consider consumer perceptions and clean label options.

Lazzarini *et al.* (2017) found that consumers consider organic and fair-trade labels make little difference and suggest that increased education around labeling and sustainable transparency on food packaging might further influence consumer behavior. Authors further suggested that if hydrocolloids are seen as unnecessary, sustainability goals could be reshaped so that consumers are able to make better choices without the assistance of labeling and packaging and offer better guidance to producers. While using different types of flours and hydrocolloid combinations show positive effects on GF products, Mir *et al.* (2016) has suggested that, unlike traditional baking methods, mass production of GF baked products is generally more difficult to streamline as the variables for each recipe are vastly different. Morreale *et al.* (2017) reinforces this notion that each recipe, including its use of hydrocolloids, is unique and depend on the technique used. Therefore, one has to wonder if adding ingredients such as a hydrocolloid, which may or may not be considered clean label, is really addressing the issue and needs to be investigated further.

4.1.2.2 Influence of Starch on GF Products

Starches can directly impact a GF product's properties, ranging from physical appearance and water binding capacity to nutrition (Martinez and Gomez, 2016). Components of starches, such as storage proteins, non-starch carbohydrates, fats, minerals and vitamins depend on its origin and processing method, and only starch containing flours can be used as replacements for wheat flour (De Leyn, 2014). Therefore, it is important to consider how starches in various forms can affect the properties of GF bakery products. Table 5 summarizes the effect of more sustainable starch/flour combinations on product parameters for GF product development. Table 6 summarizes the composition and physical properties of commonly used GF flours and starches.

TABLE 5: THE INFLUENCE OF STARCH/FLOUR ON THE PROPERTIES OF GF PRODUCTS

Source	Functional Ingredients	Non-Functional Ingredients
Korus et al. (2015)	Acorn Flour	Corn Starch, Potato Starch, GG, Pectin
Partial replacement of starch with acorn flour in GF breads increased dough firmness, volume, improved crumb characteristics and slowed staling. Acorn supplementation at 20% and 40% also improved sensory acceptance as well as enriching bread with protein (37% - 105%), fiber (76% - 220%) and decreased carb content (between 5% - 13%). Bread supplemented with 20% also resulted in decrease in staling.		
Pojic et al. (2015)	Hemp Flour	Wheat Flour

Hemp and wheat flour were mixed in various ratios for GF bread (0/100, 5/95, 10/90, 20/80). Hemp flour lowered water absorption, dough development time, volume, color and structure regardless of level of substitution. 20% resulted in a significant decrease in both dough stability and strength, higher in macro- and micro-elements, protein and decrease in starch content. Supplemented breads also showed a reduction in metabolized energy from carbohydrates.

Svec & Hrušková (2015)	Hemp Flour	Wheat Flour

Hemp flour in defatted forms was added to wheat bread formulations in 5, 10, 15 and 20% and quality parameters tested using Mixolab©. Supplementation level resulted in greater torque differences than hemp flour type. Mixing and starch retrogradation phases showing the most observed differences, showing a possible prolongation of shelf-life with hemp. Protein content gradually increased at by adding hemp, but decreased bread volume. However, hemp origin affects protein quality, which gradually worsened without respect to total protein content increase.

Martinez & Gomez, (2016)	Rice Flour, Maize Flour, Maize Starch, Wheat Starch, Potato Starch	HPMC

GF bread made with flours resulted in lower volume than those with starch with volume correlating to crumb hardness. Loaves with starch show higher specific volume and lower hardness, especially when made with wheat starch, in part due to water absorption capacity. Potato starch did not form a continuous starch-hydrocolloid matrix, possibly due to larger granule size, resulting in breads with lower volume, elasticity and increased hardness.

Rinaldi et al. (2017)	Chestnut Flour	Corn Starch, Tapioca Starch

Sourdough and chestnut flour showed reduced volume loaves. Chestnut darkened crumb and crust while sourdough showed no effects on color. Sourdough and/or chestnut addition showed significant increase in crumb hardness at day 0 with significant reduction of staling only at day 5. Sourdough allowed for increased moisture content with no significant variation in shelf life.

TABLE 6: COMPOSITION AND PHYSICAL PROPERTIES OF GF FLOURS AND STARCHES

Ingredient	Moisture (g water/100g)	Protein (g/100g)	Water Binding Capacity (g water/g solid)
Maize flour	9.37	6.10	1.421
Rice flour	8.70	7.80	1.291
Maize starch	10.54	ND	1.337
Wheat starch	11.10	ND	0.626
Potato starch	14.66	ND	0.171

Adapted from Martinez and Gomez, 2016 (ND = not determined)

4.1.2.2.1 Nutritional Component

Potato, rice, maize and tapioca flours are the sources of starch more commonly used in GF baked products and result in less protein than similar products made with starch containing grains such as buckwheat, millet or chestnut (Gallagher, 2008; Martinez and Gomez, 2016; Rinaldi *et al.,* 2017). For example, buckwheat contains 58.9% starch compared to 63% starch found in wheat and studies have shown that replacing potato starch with buckwheat in GF bread applications can result in a significantly lower total starch content (Alvarez-Jubete *et al*., 2009). Potato flour has a balanced amino acid composition superior to cereal proteins and contains higher levels of vitamins and minerals than wheat. It also contains phytochemicals, such as phenolics, flavonoids, polyamines and carotenoids (Liu *et al.,* 2018). However, potato starch, as well as maize and wheat starch, offer no added protein (Martinez and Gomez, 2016) whereas resistant starches can improve the fiber content of GF bread up to 89% (Korus *et al*., 2009). Chestnut flour has also gained popularity for its nutritional and health benefits. It contains essential amino acids, dietary fiber and essential vitamins, such as E, B group, potassium and magnesium and increases fiber content and antioxidant capacity of GF bread (Rinaldi *et al.,* 2017). Nutritionally, chestnut flour is made up of carbohydrates (76.1 g/100 g) [24 g/100 g sugar], protein (6.3 g/100 g), fiber (9.4 g/100 g) and fat (3.6 g/100 g), although authors state it remains a pricey option for adding nutrition. Compared to wheat flour, acorn flour contains less starch (458 vs. 618-739 g kg^{-1}), protein (54 vs. 80-134 g kg^{-1}) and total carbohydrates (400-439 vs. 532-735 g kg^{-1}), but more total fiber (181 vs. 38 g kg^{-1}) (Korus *et al.*, 2015). However, in GF bread formulations authors found acorn flour substituted for starch at 20%, 40% and 60% resulted in enriched bread with protein levels between 37% - 105%, fiber levels between 76% - 220% and a decrease in total carbohydrate content from 5% - 13%. And ratios of hemp/wheat flours (0/100, 5/95, 10/90, 20/80) resulted in GF bread higher in macro- and micro-elements, especially iron, and higher protein (2-5 g/100 g) as levels of hemp flour were increased (Pojic *et al.,* 2015). Additionally, hemp flour at 20% resulted in a 17% decrease in starch content. This was confirmed by Svec and Hrušková (2015) who tested dough with 5%, 10%, 15%, 20% defatted hemp flour from various regions. Authors found protein content gradually increased at the addition of hemp, but protein quality gradually worsened without respect to total protein content increase, suggesting that ingredient quality does play a role to nutrition.

4.1.2.2.2 Rheology

Starches seem to be required for volume, hardness of crumb and elasticity. Tapioca starch and maltodextrins (chemically modified, resistant starches) are best when used in GF products because modified starches are used to improve the texture and staling of a product (Witczak *et al.,* 2010; Pongjaruvat *et al.,* 2014). However, Martinez and Gomez (2016) found that rice and maize flours are used in GF baking because they are highly produced and affordable and have a higher water binding capacity which can delay staling. Authors also found that GF wheat starch results in higher volume and lower hardness of GF products and that starches can be modified with hydrocolloids, often of polysaccharide origin, which work together to provide stabilizing, densifying, gelling and emulsifying properties. Pojic *et al.* (2015) found hemp flour lowered water absorption and dough development time as well volume, color and structure regardless of substitution level, suggesting dough supplemented with hemp flour is less elastic. However, dough stability and strength were not affected up to 10% whereas 20% resulted in a decrease ($p < 0.05$) in both parameters. Additionally, both chestnut and acorn flours had the ability to reduce staling in GF breadmaking (Rinaldi *et al.,* 2017; Korus *et al.*, 2015) but with mixed results which requires more investigation.

4.1.2.2.3 Glycemic Response

Matos and Rosell (2012) found that the high digestibility GF rice bread was due to the starches used while Scazzina *et al.* (2015) discovered that it is the absence of gluten that affects starch digestibility, resulting in a higher GI. Alcantara *et al.* 2020 recently found that wheat bread supplemented with corn and banana flours (40% and 20% respectively) resulted in a peak of BG levels compared to wheat control while Johnston *et al.* 2017 found GR for GF pasta made with corn and rice was higher than pasta made with wheat. Birt *et al.* (2013) suggests that resistant starches have a beneficial effect on glucose tolerance, reducing its level in the blood after ingestion. Liu *et al.* (2018) found that steamed bread with potato flour produced lower GI than steamed wheat flour bread. Although both breads had the same starch/protein content, authors cite slower starch digestibility for GF bread because potato starch has more resistant starch than wheat and likely because the specific volume and the granule surface area of GF steamed bread were lower than those of wheat steamed bread. Rinaldi *et al.* (2017) looked at the shelf life of GF bread made from chestnut flour/sourdough variations tested in a 5-day study. Authors found that sourdough reduced the percentage of hydrolyzed starch during in vitro digestion due to lactic and acetic acids, implying a lower glycemic index is probable in GF products using sourdough fermentation. Additionally, Korus *et al.* (2017) found a decreased carbohydrate content between 5% - 13% for GF breads supplemented with acorn flour and Pojic *et al*. (2015) found the metabolizable energy from carbohydrates reduced 13% in bread supplemented with hemp flour, indicating they could be used in lower carbohydrate baked goods. Since digestible carbohydrates effect GI, using acorn and hemp flours as a low-carb substitutes could help control glycemic response in GF products.

4.1.2.2.4 Consumer Acceptance

While one strategy to address the low nutritional value of GF products is to replace ingredients with nutritionally valuable ones, some interactions of these ingredients with certain starches could result in improved sensory parameters and extended shelf-life. For example, replacing acorn flour for starch to GF breads increases dough firmness, volume and improved crumb characteristics for overall improved sensory acceptance (Korus *et al.,* 2015) whereas adding chestnut flour results in a more uniformed structure, decreased hardness and reduced staling (Demirkesen *et al*., 2013; Demirkesen *et al*., 2014), which can impact consumer acceptance. And Rinaldi *et al.* (2017) found that while both sourdough and chestnut flour reduced loaf volume, chestnut flour darkened crumb and crust while sourdough allowed for increased crumb moisture. Reduced staling was only observable after 5 days, however authors concluded that sourdough fermentation could be a commercially viable way to impact consumer acceptability of industrial GF bread production.

4.1.2.2.5 Sustainability

Although essential in GF baking, starches are rarely considered for their sustainable characteristics. Svec and Hrušková (2015) and Pojic *et al.* (2015) acknowledge the commercialization of hemp seed, bred to maximize fiber and oil, as a non-traditional, industrial ingredient compared to soybean for its nutritional benefit and versatility as a sustainable food ingredient. Liu *et al.* (2016; 2018) refers to potato as a widely available food source as well as convenient to store and circulate. And while acorn is more expensive than potato or corn starch, Korus *et al*. (2015) found using it in GF formulations below the suggested 40% replacement would result in a minimal influence on price. Therefore, research is growing on more sustainable sources of naturally occurring starch, both nutritionally and economically, and should be considered.

4.1.2.3 Influence of Cereals and Pseudo-Cereals on GF Products

Cereals and pseudo-cereals, such as sorghum, teff and buckwheat, have become popular additions to GF bakery products because of their perceived health characteristics, however research is primarily focused on the physical properties of GF bread (Velazquez *et al.*, 2012; Hager and Arendt, 2013; Korus *et al*., 2015; Rozylo *et al.,* 2015). Therefore, Table 7 summarizes the composition and physical properties of popular GF cereals for a selection of studies that represent a variety of GF applications. These studies will show whole grains can result in better textural characteristics, overall volume and color, are able to withstand higher baking temperatures and improve nutritional composition. Table 8 summarizes the average nutritional value of various dry cereal and pseudo-cereal grains. And while they can be a great substitute for added nutrition, producers feel they negatively impact overall production costs (Tanwar and

Dhillon, 2017). Therefore, higher costs associated with GF ingredients might contribute to inadequate nutrient intake as long as producers perceive them as a barrier.

TABLE 7: THE INFLUENCE OF CEREALS ON THE PROPERTIES OF GF PRODUCTS

Source	Functional Ingredients	Non-Functional Ingredients
Alvarez-Jubete et al. (2009)	Amaranth Flour, Buckwheat Flour, Quinoa Flour	Rice Flour, Wheat Flour, Potato Starch
GF breads supplemented with pseudo-cereals at 50 and 100% were analyzed for nutritional quality. All supplemented breads resulted in significantly higher levels of protein, fat, fiber and mineral composition over controls. Protein content for amaranth and quinoa were significantly higher than that of wheat and with amino acid profiles higher in lysine than common grains and are a suitable replacement for GF flours and starches.		
Velazquez et al. (2012)	Sorghum Flour	Corn starch, HPMC
No good relationship found with specific volume and crumb grain. Volume increased with HPMC and increased starch/flour ratio with crumb depending on each starch/flour, water and HPMC addition, increasing with starch/flour but decreasing with water.		
Duta and Culetu (2015)	Oat Bran	Oat Flour
Rheological and nutritional characteristics of oat bran were measured in GF oat-flour cookies. OB increased protein weakening, water absorption and decreased stability of gelatinized starch vs control and significantly enhanced protein, fat and fiber levels.		
Tanwar and Dhillon (2017)	Bajra Flour, Buckwheat Flour, Millet Flour	N/A
Moisture, ash, fiber, carbohydrate, fat and protein contents were measured. Wheat flour achieved best result, though not significantly, to moisture, ash and protein content, with higher fat and fiber in test flours, resulting in nutritional composition of test flours possible over wheat flour formulations.		
Shaabani et al. (2018)	Chickpea Protein Isolate (CPI)	MTG, XG, Millet Flour
XG increased volume and porosity and decreased hardness. Browning of crust decreased with XG and CPI but increased with MTG. Results show that a combination of CPI, XG and MTG in varied levels mark improvements to millet-based muffins and that using CPI in small amounts could lower production costs.		

TABLE 8: NUTRITIONAL VALUE OF GRAINS USED AS ALTERNATE FLOUR SOURCE IN GF PRODUCTS

Cereals		Protein (g/100g)	Fat (g/100g)	Dietary Fiber (g/100g)	Minerals (g/100g)
Cereals	Rice	7.70	2.20	2.20	1.20
	Corn	8.80	3.80	2.20	1.30
	Sorghum	10.40	1.90	9.80	1.60
	Teff	9.60	2.00	ND	2.90
	Millet	14.80	4.86	ND	1.64
Pseudo-Cereals	Buckwheat	12.50	2.10	29.50	1.42
	Quinoa	16.50	5.20	14.20	2.70
	Amaranth	16.50	5.70	20.60	3.25
Legumes	Chickpea	23.64	6.48	18.00-22.00	ND
	Lentil	22.70	0.70	14.60	ND
	Soybean	36.00	19.00	17.00	5.00
	Pea	21.30	0.60	18.40	5.00
	Wheat	10.50	0.90	2.80-12.10	ND

Adapted from Jnawali et al., 2016 (ND = not determined)

4.1.2.3.1 NUTRITIONAL COMPONENT

An increase in nutritional characteristics of GF products is possible by the addition of cereals/pseudo-cereals and certain proteins (Duta and Culetu, 2015; Pojic *et al.,* 2015; Tanwar and Dhillon, 2017; Korus *et al.,* 2017), however there is room for improvement. Studies have shown that replacing starch with pseudo-cereals in GF baked products increases protein, fiber, calcium, iron and vitamin E. For example, oat-based GF cookies supplemented with oat bran (in 30%, 50%, 70% and 100% addition) produced cookies with significantly increased protein, fat and fiber levels at each percentage added (Duta and Culetu, 2015). Cookies made from buckwheat flour, millet flour and bajra flour (in levels of 40 g, 60 g and 100 g to total 200 g) all provide more fiber (0.3% - 2.3%) than wheat flour alone, however wheat improved moisture, ash and protein contents though not significantly (Tanwar and Dhillon, 2017). Additionally, buckwheat contains higher amount of fiber (29.5% compared to 17.4% of wheat) and Alvarez-Jubete *et al*. (2009) found the fiber content of buckwheat bread three times that of GF control bread made with potato starch. Sorghum flour is 6.3% protein, 80% starch and 2.5% fiber and can be substituted as naturally occurring starch in GF bread applications (Velazquez *et al.*, 2012). Chickpea is rich in protein, dietary fibers, carbohydrates, folate and trace minerals and could be used to improve the nutrition of GF muffins (Shaabani *et al.*, 2018). And when combined with pea protein, chickpea has the potential to produce GF breads with good product and sensory characteristics (Minarro *et al*., 2012).

4.1.2.3.2 Rheology

Shaabani *et al*. (2018) found that adding CPI to GF millet-based muffins made it possible to form a similar protein network to gluten but relied on the use of a hydrocolloid. Authors found that when MTG and XG were added at added at lower levels the protein/enzyme combination decreased specific gravity but increased when added at higher levels, suggesting that the efficiency of the enzyme was dependent on the protein content and level of enzyme concentration. Browning decreased with increased concentration of XG/CPI, whereas it increased with higher levels of MTG. And while MTG/CPI concentrations produced satisfactory results, desired texture was only achieved when XG was used. Velazquez *et al.* (2012) tested bread made from seven varied combinations of cornstarch/sorghum, water and HPMC with mixed results. Volume increased with higher HPMC and starch/flour ratios due to improved gas retention, however crumb depended on starch/flour, water and HPMC combinations, increasing with added starch/flour but decreasing with added water. Therefore, no good relationship was found between specific volume and crumb however optimal results appeared to be 0.55 starch/flour ratio; 90% water; 3% HPMC.

4.1.2.3.3 Glycemic Response

There is little research to show specifically how cereals and pseudo-cereals impact GI. Studies have also shown that GF products typically have a higher GI than wheat products due to the higher rate of starch digestion, which in turn can spike blood glucose levels (Berti *et al.* 2004; Scazzina *et al.,* 2015). Berti *et al.* (2004) looked at the *in vivo* glucose response between GF products supplemented with quinoa and their gluten-containing counterparts on healthy participants. While GI levels for quinoa products were slightly lower, GR for GF bread was significantly higher than bread with gluten. Those without CD and relying on a GF diet often lack an intake of beneficial whole grains, placing them at an increased cardiovascular risk (Lebwohl *et al.,* 2017) and that higher GI levels can increase this risk (Bullo *et al.,* 2013). And while Duta and Culetu (2015) found an increase in fiber possible with the addition of oat bran in GF cookies, Gularte *et al.* (2012) found that oat fiber also resulted in lower estimated GI when used in GF layer cake. However, the estimated GI levels increased when GG or inulin was added to the formulation. Therefore, further research into how GF cereals and pseudo-cereals, and possibly with the use of hydrocolloids as a fiber, affect the GI of GF products should be encouraged.

4.1.2.3.4 Consumer Acceptance

Tanwar and Dhillon (2017) suggest that replacing non-wheat flours up to 50% will neither adversely affect the physical characteristics nor sensory properties of GF cookies. Similarly, Alvarez-Jubete *et al*. (2009) and Kim and Yokoyama (2011) found that replacing pseudo-cereals for starch in GF bread products also had no difference in acceptability over control bread. While Duta and Culetu (2015) found that the addition of oat bran to GF cookies resulted in lower overall acceptability, they were deemed acceptable because the values were greater than 5 on a 9-point hedonic scale. Marcilio *et al.* (2005) found, when combined with fat, amaranth in GF cookies had a positive influence on flavor. Similarly, Schoenlechner *et al.* (2008) found that using pseudo-cereals, such as amaranth, buckwheat and quinoa, in GF cookies formulated with white bean flour resulted in overall acceptability. Interestingly, Bouasla *et al.* (2017) found that legume flours (chickpea, lentil and yellow pea) when added to GF pasta also resulted in acceptable scores (values > 5). And while authors note the overall acceptability decreased with each increasing amount of legume flour added, it was not significant.

4.1.2.3.5 Sustainability

A variety of flours, such as millet, chestnut, oat and sorghum, have been researched as more sustainable substitutions in GF baking but use is limited. Tanwar and Dhillon (2017) introduce bajra, buckwheat and ragi flours as lower cost and more widely available options in GF formulations. Shaabani *et al*. (2018) cites millet flour as 30-40% lower than wheat flour, and while CPI is more expensive would only add a value of up to 30% total product price which would still be considered below expected price for GF products. White sorghum flour was found to be a more sustainable option because of its wide use in semiarid zones in Africa with 40% of the world production of sorghum being used for food consumption (Velazquez *et al*., 2012). Also, it is closer to maize than oats, barley or rye making it suitable for coeliac sufferers. However, authors also concluded that several factors, such as product type, quality of substituted flour, manufacturing process and appearance must be considered when using alternative flours since GF production requires different technologies due to batter and dough consistencies.

4.1.2.4 Influence of Proteins on GF Products

Studies have shown that replacing gluten with various protein sources can produce structural and textural characteristics similar to wheat containing products; increased volume and gas cell retention, increased dough elasticity, decreased crumb hardness, reduced staling and increased protein and fiber levels have been achieved (Alvarez-Jubete *et al*., 2009; Ziobro *et al*., 2013; Matos *et al*., 2014; Gani *et al*., 2015). Authors also note milk and soy proteins in particular can improve dough handling, texture and nutrition and while dairy is a good source of protein (content ranging from 7-90% per source), these protein sources might not be suitable for those with CD or who are lactose intolerant. Proteins are essential to the structure of GF products and can help improve nutritional quality, as outlined in Table 9.

TABLE 9: THE INFLUENCE OF PROTEIN ON THE PROPERTIES OF GF PRODUCTS

Source	Functional Ingredient	Non-Functional Ingredient
Lang et al. (1998)	Egg White, Albumin, Casein, Gelatin, Soy, Pea, Wheat Gluten	N/A

Protein type has on satiety, energy and macronutrient intakes or glucose/insulin concentrations. All results differ from previous studies suggesting proteins may be differentiated in terms of their satiety capacities. Varying protein source in a mixed meal does not affect food behavior, perhaps due to co-ingestion of fat/carbs with protein acting as a buffer to satiety of protein load.

Gallagher et al. (2003)	Whey, SMR, SMP, Casein, MPI	Wheat Starch, GF flour

Dairy proteins reduced loaf volume by up to 8% and increased crumb hardness with fixed hydration, except for demineralized whey. 10% and 20% more hydration resulted in higher volume, softer crumb and crust with preference for skim milk replacer, sodium caseinate and milk protein isolate.

Marco and Rosell (2008)	Pea, Soy, Albumin, Whey	MTG, Rice Flour

Proteins significantly modified gelatinization and gelling of dough. Pea, soy and whey significantly decreased final viscosity with whey promoting a 27.3% decrease in peak viscosity. Pea and soy increased elasticity, egg and whey decreased it. Use of MTG and protein together resulted in increased protein content.

Meulen et al. (2010)	Pea, Fava Bean, Fava Bean Hulls	Soy

Piglets were given enterotoxigenic Escherichia coli and intestinal samples were taken after 21 days. From 10 days afterward, no ETEC was found. Microbial profile of showed no distinct difference between pea/fava suggesting legumes can be used to manipulate gut health.

Ziobro et al. (2013)	Albumin, Collagen, Pea, Lupine, Soy	Corn Starch, Potato Starch

Proteins significantly affect viscoelastic properties of dough. Soy and collagen reduced volume, lupine and albumin increased it while most proteins decrease crumb hardness and chewiness. The addition of proteins retard staling of starch-based bread. Pea was most acceptable with soy being the least.

Landero et al. (2014)	Pea	Soy, Wheat

Pea substituted for soy in wheat-based feed showed a reduced ($p < 0.01$) average daily gain and feed efficiency in first week. Growth performance, feed intake, ADG or feed efficiency were not affected. Possible substitution of up to 400 g pea for soy, reducing tract digestibility 7% and gross energy 2%.

Matos et al. (2014)	Soy, Pea, EWP, Casein, VWG	Rice Flour

Soy, pea and casein significantly increased hardening in GF muffins. Casein and EWP increased volume. Soy did not affect parameters, whereas pea made softer and springier muffins. Results show rheological and technological characteristics of muffins depend on type of protein used.

Shevkani et al. (2015)	White and Red Cowpea	Rice Flour

White cowpea showed higher solubility, foaming and emulsification but lower water absorption than red cowpea. Both increased batter viscosity. Firmness, cohesiveness, chewiness and springiness increased for both (above 8 g/100 g incorporation). White increased volume while red decreased it. Results suggest that both level of proteins and their properties influence muffins.

White et al. (2015)	Pea	Soy, Fava Bean

Upon carcass examination diets showed that feeding treatments did not affect performance data. Pea based diets showed a greater dressing percentage over fava bean, but pea and soy showed greater lean meat percentages to fava bean.		
Sanguinetti et al. (2016)	Whey	Rice Flour, Corn starch
Air packaged samples of GF fresh-filled pasta spoiled at 14 days while MAP samples (70/30 of N_2/CO_2 stored at 4°C) lasted 42 days. Sensory quality was maintained with overall acceptability using MAP, resulting in a threefold extension of shelf life.		
Ziobro et al. (2016)	Albumin, Collagen, Pea, Lupine, Soy	GG, Pectin
Soy and pea resulted in lower volume. Albumin was significantly greater than control. All showed higher porosity, decrease in cell density, a darker color and increased bread hardness. Substituting proteins for gums contributes to staling. Pea and lupine showed improved sensory acceptance, more acceptable color and smell to control. Soy was least acceptable.		
Secchi et al. (2017)	Whey	N/A
Use of whey in cheesecakes stored for 60 days at 20°C extended shelf life to 21 and 45 days (with added 4% and 8% whey respectively) compared to control which spoiled at 11 days. Combining whey and MAP further increased shelf life to 60 days.		
Sahagun et al. (2018)	Pea, Rice, EWP, Whey	Rice Flour
Added protein increased dough viscosity with pea having the highest overall effect. EWP and whey led to higher volumes and increased cake hardness. Pea and rice hardly modified cake volume, hardness and color. Sensory was lower in all cakes with protein but more pronounced in rice and EWP due to taste, odor and texture. Whey had the highest acceptability.		

Gallagher *et al*. (2003) concluded that using dairy proteins in GF bread products resembles those with gluten in structural and sensory tests, resulting in higher volume, softer crumb and crust texture. Alvarez-Jubete *et al*., 2009 found overall bread quality improved with dairy proteins, whereas Ziobro *et al.* (2016) found they resulted in lower bread volume and a harder crust. While diary proteins increase water absorption, when used at minimal levels they have no impact on nutritional value (Gani *et al*., 2015). Though, whey has the potential to reduce staling and increase shelf-life. Secchi *et al.* (2017) found that adding bovine whey powder to cheesecakes (4% and 8% over control) stored at 20°C extended shelf life to 21 and 45 days respectively, whereas the control spoiled at 11 days. Combining whey and MAP further increased shelf life to 60 days. Sanguinetti *et al.* (2016) found that GF fresh filled pasta with MAP stored at 4°C lasted 42 days whereas non-MAP samples spoiled after 14 days. However, the environmental impact of using dairy proteins is a concern. Livestock is believed to be responsible for 18% of GHG emissions globally, with milk being responsible for 20% of emissions (Lacour *et al.,* 2018). Substantial energy is required for feed, breeding, electric production and operations which also affect GHG. Because of their non-allergenic characteristics and low GHG profiles, plant-based proteins might be the way forward.

Proteins such as lupine and albumen impact higher GF bread volumes and show the potential to reduce product staling although SPI caused a decrease in these parameters (Ziobro *et al*., 2013). Marco and Rosell (2008) found that both SPI and PPI can increase elasticity and protein content in rice flour dough formulations of 5% protein and 1% MTG. Used in GF muffins PPI resulted in softer, springier texture (Matos *et al.,* 2014) and improved sensory acceptance in bread products (Ziobro *et al.,* 2013; Ziobro *et al.,* 2016). However, studies using SPI have concluded that while soy improves nutritional value, it rates low in sensory testing (Ziobro *et al*., 2013; Sarabai *et al.,* 2015). Most GF products also have lower contents of iron, folic acid, B-complex vitamins and dietary fiber (Thompson, 2000). Therefore, replacing proteins in GF baking to enhance or improve the structure, texture and taste to that of a wheat product presents one universal challenge; adding more nutritional value presents another.

Proteins are made of amino acids and needed by the body for tissue repair and growth, immune function and certain enzymatic reactions. Essential amino acids include histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val). While attempts are made to add protein, protein quality and more complete nutritional improvement are often overlooked as a standard for comparison. Table 10 represents essential amino acids compared by protein source. Quality of various samples of the same protein source can vary by supplier, with wheat protein ranging from 74 to 88%; soy protein ranging from 61 to 91%; pea protein ranging from 77 to 81%; and whey protein ranging from 72 to 84% (Gorissen *et al.*, 2018). Therefore, choosing a single protein source, depending on the supplier, may not fully address nutritional concerns. Furthermore, there are other factors to consider when determining which protein to use.

TABLE 10: AMINO ACIDS BY PROTEIN SOURCE

Amino Acid	HPI	PPI	SPI	Whey	Wheat
His	3.2	2.5	2.8	1.4	1.4
Ile	3.6	2.3	4.4	3.8	2.0
Leu	6.5	5.7	6.8	8.6	5.0
Lys	2.7	4.7	5.2	7.1	1.1
Met	1.9	0.3	0.9	1.8	0.7
Phe	4.7	3.7	5.1	2.5	3.7
Thr	3.4	2.5	3.9	5.4	1.8
Trp	1.0	ND	ND	1.4	ND
Val	4.7	2.7	4.3	3.5	2.3
Sum	31.7	24.4	33.4	35.5	18.0

Values presented in g of amino acid per 100 g protein (ND = not determined)

Adapted from Wang et al., 2007; Gorissen et al., 2018;; Wang and Xiong, 2019

Marco and Rosell (2008) found increased protein content possible in GF dough processing but only when MTG was used. However, Ziobro *et al.* (2016) found that substituting proteins for gums in GF breads contributes to staling, which presents an issue for producers attempting to add nutrition while considering clean label options. Research also suggests that added protein might affect GI (Li *et al.,* 2011; Mollard *et al*., 2014; Scazzina *et al.,* 2015). High animal protein intake has been linked to increased risk of diabetes and cardiovascular disease while plant proteins have shown protective effects (Matilla *et al.,* 2018). For example, Meulen *et al.* (2010) conducted a study on piglets where E-coli was introduced after weaning and fed diets of pea or soy. The carcasses were tested 10 days after being sacrificed, those fed pea protein showed no E-coli in the gut suggesting that legume protein sources can improve intestinal disorders and supporting the claim that pea produces less allergenic reactions in the body. White *et al.* (2015) conducted a study on mature pigs and found pea could aid in weight loss, based in part on the lean meat percentage of the carcasses that were fed a diet of pea. Landero *et al.* (2014) studied the diets of weaned piglets by replacing wheat and soy feed with pea and found a reduction in tract digestibility of 7% suggesting an effect on satiety. Although Lang *et al.* (1998) found protein type does not affect satiety, pea proteins can be used to reduce overall production costs (Landero *et al.,* 2014; White *et al.,* 2015).

While research indicates rheological and technological characteristics depend on the type of protein used (Ziobro *et al.,* 2013; Matos *et al.,* 2014; Ziobro *et al.,* 2016; Sahagun *et al.,* 2018), it also suggests that a mix of protein could provide acceptable texture to GF products. No formal research exists on the combination of both PPI and HPI as enhancements in GF and FF products. Therefore, the following sections address both proteins, their nutritional and dietary benefits and how they may complement each other.

4.1.2.4.1 Pea Protein

Pea protein (*Pisum sativum*) comes from yellow and green peas, which are also classified as legumes. Pea is composed of several classes of proteins, the two major types being globulin and albumin which account for 10-20% and 70-80% of the protein in the pea seed, respectively (Lam *et al*., 2018). Albumins are water soluble and considered the metabolic and enzymatic proteins, while globulins are salt soluble and act as the storage proteins. Their composition, molecular structure and charge distribution determine PPI's physical and chemical properties. Processing conditions, such as temperature, pH, ionic strength and the presence of other ingredients can also affect the functional properties of PPI and their applications in the food industry (Lu *et al*., 2019).

Studies have been conducted looking at PPI as a potential supplement to GF baked products, ranging from bread to layer cakes and muffins, with very positive results. Pea has been shown to reduce blood pressure, cholesterol, blood sugar levels and because of its effect on the hormone ghrelin in the stomach is also known to aid weight loss by delaying gastric emptying (Li *et al.,* 2011; Mollard *et al*., 2014). Pea proteins have shown increased viscoelasticity, firmness, springiness and chewiness of GF baked products and is more acceptable to consumers, however it often requires the inclusion of a hydrocolloid (Matos *et al.,* 2014; Ziobro *et al.,* 2016).

4.1.2.4.1.1 Nutritional Component

Due to PPI's nutritional profile and promising results in baking, it makes for an exceptional supplement to GF baked goods. Pea protein naturally contains many BCAA's, and high levels of lysine, though is not considered a complete protein source. Pea is not rich in methionine, which converts to the amino acid cysteine, an amino acid that is produced by the body and necessary in times of illness and stress. Legume proteins are primarily comprised of globulin protein, which are of 2 main types: legumin and vicilin, and both exhibit functional attributes due to their amino acid profile, size and structure. It also contains albumins, which are water soluble. Pea protein is about 23-31% protein and 1.5-2% fat, with carbohydrates being primarily starch (35-40%) and fiber ranging from 60-65% (10-15% insoluble/2-9% soluble) (Tiwari *et al.,* 2011; Lam *et al.,* 2018) though it may form weaker and less elastic gels compared to soy. In terms of bioavailability, whey is considered 99% digestible (Hoffman and Falvo, 2004) and has a better amino acid profile than PPI [Table 10]. However, during an eight-week weight training study where participants ingested both whey and pea proteins, PPI produced similar measurement outcomes to body composition and muscle thickness (Banaszec *et al.,* 2019), suggesting bioavailability is not an issue.

4.1.2.4.1.2 Rheology

Shevkani *et al.* (2015) found that pea increases viscoelasticity, firmness, springiness and chewiness of GF rice muffins and that pea protein's emulsification and foaming characteristics were necessary for functional improvement. Matos *et al.* (2014) found that 13% PPI (based on percentage of milk and egg in muffin formulations) used in rice-based muffin batter resulted in a higher viscoelasticity and a more compact crumb, whereas egg white protein (EWP) had a higher volume but much less density. Marco and Rosell (2008) also showed that PPI proved to be more elastic than whey and egg proteins when used in rice flour applications. In layer cakes, Sahagun *et al.* (2018) showed that PPI, when substituted at 30% total flour mixture, had no effect on batter density but provided a greater number of bubbles and greater viscosity, though required increased moisture levels.

4.1.2.4.1.3 Glycemic Response

Lang *et al.* (1998) studied the satiety of protein sources by feeding participants lunches supplemented with an additional 22% protein. Satiety was assessed 8 hours after ingestion and energy/macronutrients after 24 hours, which included blood tests for glucose concentrations. Results showed protein type had no effect on blood glucose concentrations, satiety, energy or macronutrient intakes. Conversely, Smith *et al.* (2012) studied the effects of short-term (30 minutes and 120 minutes) food intake and glycemic response on young men and found that pea protein in 10/20 g supplementation levels resulted in lower BG up to 30 minutes against a low carb soup while 20 g suppressed food intake 30 minutes after meal. And fiber alone had no effect on outcomes. Mollard *et al.* (2014) studied pea components (pea hull fiber, pea protein and yellow peas) on appetite and blood glucose responses of men before and after meal ingestion. While no differences were observed in appetite levels, a combination of protein/fiber/yellow peas led to lower blood glucose levels post meal than fiber alone. While results were not significant, it suggests pea components can be used in food products to improve glycemic control. However, more research is required to understand pea's role in affecting glycemic response.

4.1.2.4.1.4 Consumer Acceptance

Ziobro *et al.* (2016) found pea protein more acceptable in color and smell than soy in bread products supplemented with 10% added protein replacing gum/starch total. However, Sahagun *et al.* (2018) found pea protein resulted in a reduced overall sensory score to whey protein in GF layer cakes, represented in Table 11. Authors suggest this was due to pea's product appearance, likely due to an irregular surface. However, authors also point out that pea scored higher than rice and EWP, showing promise as a plant-based option when dairy or egg cannot be used.

TABLE 11: PROTEIN SOURCES SUBSTITUTED AT 30% IN GLUTEN-FREE LAYER CAKES

	Appearance	Odor	Texture	Taste	Overall Acceptability
Control	6.83 ± 1.19b	6.8 ± 1.31d	6.74 ± 1.68d	6.65 ± 1.50e	7.02 ± 1.42d
Rice	5.72 ± 1.67a	4.55 ± 2.03a	4.86 ± 2.19b	3.49 ± 1.93a	4.09 ± 1.85a
Pea	5.78 ± 1.64a	5.34 ± 1.83b	6.02 ± 1.84c	4.73 ± 1.95c	5.33 ± 1.71b
Egg White	6.94 ± 1.17b	4.78 ± 1.61a	3.71 ± 1.75a	4.02 ± 1.89b	4.45 ± 1.64a
Whey	6.79 ± 1.47b	6.01 ± 1.51c	5.62 ± 1.78c	6.07 ± 1.82d	6.07 ± 1.59c

Consumer data expressed as means ± SD
Values with the same letter in the same column do not present significant differences

Adapted from Sahagun et al., 2018

4.1.2.4.1.5 Sustainability

Ziobro *et al.* (2016) attempted to test if hydrocolloids could be avoided with protein supplementation in order to make a GF product clean label. Results showed that GG or pectin were needed for staling, but protein combined with a polysaccharide hydrocolloid could retard staling, suggesting further research might be needed to explore the protein/hydrocolloid relationship. From a cost perspective, Landero *et al.* (2014) explored the economic impacts to feed by substituting pea for soy in wheat-based feed and found it was possible to substitute up to 400 g pea for soy without affecting growth performance, which could be promising for producers considering plant-based proteins as cost effective ingredients. Still, because pea scores high in crumb structure, odor and flavor in sensory testing, is non-GMO, low allergy, highly available and low in cost, it is a good option as a more sustainable protein source.

4.1.2.4.2 Hemp Protein

Hempseed (*cannabis sativa*) consists mainly of two classes of proteins, globulin (edestin) and albumin. Edestin accounts for approximately 60% to 80% of the total protein content while albumin constitutes the rest (Tang *et al.,* 2006), which makes HPI 91-98% digestible (Amino Science, 2020). Known for its universal benefits, hemp flour has been studied in recent years and mostly in bread applications. However, hemp protein in various forms has been studied for its nutritional and health benefits and represented in Table 12. Both hemp flour and hemp protein come from the hemp seed, which is about 30% oil. The seeds are pressed to extract the oil, resulting in hemp seed cake (Pojic *et al.,* 2015). The hemp seed cake is then milled to produce a flour, creating a high fiber product which is generally lower in protein. This flour is then sifted to remove most of the fiber leaving a higher concentration of protein, which contains more protein and less fat than hemp flour. Studies involving HPI in GF baking have resulted in a higher intake of proteins, fiber and macro and micro-elements, such as iron (Pojic *et al*., 2015; Korus *et al*., 2017), and reducing blood pressure (Girgih *et al.,* 2014). Other benefits of HPI include boosting the immune system, lowering cholesterol and supporting weight loss.

TABLE 12: SELECTED STUDIES ON GF PRODUCT DEVELOPMENT AND SYSTEMS

Source	Functional Ingredients	Non-Functional Ingredients
Girgih et al. (2010)	Hemp Protein Hydrolysate	Pepsin, Pancreatin
HPH showed metal chelation activity greater than the activities of fractioned peptides with significant improvement in ferric reducing power, showing HPH's potential use for the treatment of oxidative stress-related diseases.		
Girgih et al. (2014)	Hemp Protein Hydrolysate, HPI	Casein, Pepsin, Pancreatin
HPH showed significant reduction in systolic blood pressure over casein control in adult, hypertensive rats (from 145 mmHg to 119 mmHg). Plasma levels of renin and angiotensin (ACE) were also significantly suppressed over control (ACE from 0.123 U/ml to 0.047-0.059 U/ml and renin 0.151 µg/ml to 0.040-0.054 µg/ml), suggesting HPH could be used to prevent and treat hypertension.		
Teh et al. (2016)	HPI	Proteases (AFP, HT, Pro-G, Actinidin, Zingibain)
Alkali- and acid-soluble HPI were used with various proteases. Degree of bioactivity varied by hydrolysis time, type of protease used and HPI. Alkali-soluble was best, and when combined with HT resulted in highest bioactivities in shortest amount of time, suggesting HPI can be used to prevent and treat hypertension.		
Korus et al. (2017)	HPI, hemp flour	Corn Starch, Potato Starch, GG, Pectin
Replacing starch with hemp flour resulted in weakened dough structure, while 20% hemp protein reinforced dough structure. Both protein and flour improved nutritional value of bread, with higher total fiber (15.2 to 61.0 g/kg) and dietary fiber (29.3 to 90.0 g/kg). HPI influenced crumb color by reducing lightness from 62.3 to 40.8 and increased volume 633 to 878 mL and limited hardening during storage.		

Mollard et al. (2018)	HPC	Soy Protein

Hemp and soy proteins were tested for their blood glucose and insulin responses before and after a meal. BG response was dose dependent, resulting in significantly lower mean BG pre-meal levels at 40 g whereas each 20 g treatment resulted in significantly lower BG levels compared to control. No difference was observed in post-meal mean response, though BG response varied by treatment over time (0-200 minutes). Insulin was significantly reduced for 40 g treatments pre-meal but no mean differences in post-meal insulin response.

4.1.2.4.2.1 Nutritional Component

Hemp protein is considered a complete protein source because it contains 20 amino acids, including all of the 9 essential amino acids the body needs, in a favorable ratio, and is considered superior to soy protein (Pojic *et al*., 2015). It is also a great source of B vitamins, vitamin E, calcium, magnesium and thiamin with an ash content higher with hemp protein than flour alone (Pojic *et al*., 2015; Korus *et al*., 2017). And while the fat content of hemp is high, hemp oil is rich in polyunsaturated fatty acids and linoleic and α-linolenic acids which are seen as nutritionally beneficial. Hemp is also a good source of dietary fiber (Mattila *et al.,* 2018). Shown in Table 13, products supplemented with hemp result in a higher intake of protein and fiber and studies indicate that higher levels of hemp replacement could contribute to lower carb baked products (Pojic *et al*., 2015; Korus *et al*., 2017).

TABLE 13: NUTRITIONAL VALUES OF HEMP PROTEIN IN GF BREAD

	Protein (g/kg)	**Fat (g/kg)**	**Ash (g/kg)**	**Total Dietary Fiber (g/kg)**	**Insoluble Dietary Fiber (g/kg)**	**Soluble Dietary Fiber (g/kg)**
Control	15.2	21.2	1.1	29.3	16.1	13.2
10%	36.8	24.6	17.2	40.1	26.3	13.9
20%	61.0	29.1	23.1	52.9	37.8	15.1

Adapted from Korus et al., 2017

4.1.2.4.2.2 Rheology

Results for GF bread made with HPI and hemp flour substituted at 10% and 20% for other starches found the foaming qualities of hemp protein created and stabilized gas bubbles while also limiting crumb hardening during storage, which slowed the aging of bread (Korus *et al.*, 2017). Ziobro *et al.* (2016) found that because of the high viscosity one could obtain by adding the right protein to GF baked products, the use of a hydrocolloid might not be necessary if the right proteins and particle sizes were employed. However, Teh *et al*. (2016) found that there have been issues with hemp protein solubility on its own, requiring the need for proteases to improve solubility. Since gelation is considered one of the most important functional properties to modify GF food texture and solubility is based on particle size (De Leyn, 2014), this might suggest that milling could be a factor and therefore more research could be explored.

4.1.2.4.2.3 Glycemic Response

Mollard *et al.,* 2018 investigated the effects of HPC on blood glucose and insulin responses with a before and after fixed meal design. Participants randomly ingested 20/40 g of hemp and soy proteins against a carbohydrate control in the form of a fruit shake prior to the consumption of a meal. Glucose levels were taken pre- and post-meal and all protein supplementations resulted in significantly lower pre-meal blood glucose levels compared to control. However, there was no difference in post-meal levels, although each protein variation had a different post meal response to treatment over time, suggesting that hemp protein leads to lower blood glucose levels but is dose dependent. Girgih *et al.* (2010) found that the antioxidant properties of hemp seed protein isolate in vitro tests exhibited a weaker scavenging of free radicals and greater metal chelation activity, or bonding to metal ions, which disrupts enzyme function. Due to these characteristics, the authors concluded that the use of hemp protein could be used to potentially treat oxidative stress-related diseases, such as high blood pressure (BP), heart disease, boost the immune system, lower cholesterol and support weight loss. In fact, Girgih *et al.* (2014) found HPH when tested against casein in rats significantly lowered systolic blood pressure as well as plasma ACE and renin levels and suggests that hemp in the form of protein hydrolysates provides more rapid BP response. Studies have shown an increase in heart disease in Coeliac suffers, possibly due to a high GI diet, and lower GI in GF foods could be achieved by replacing carbohydrates with proteins and fats (Bullo *et al.,* 2013; Lebwohl *et al.,* 2017; Matilla *et al.,* 2018). Hemp is both high in protein and fat and shown to impact other areas of health. However, more research involving HPI in GF products in relation to GI is needed.

4.1.2.4.2.4 Consumer Acceptance

Hemp has shown improved acceptance in volume and crumb color of GF breads (Korus *et al.,* 2017). And while Svec and Hrušková (2015) concluded that hemp flour can successfully replace wheat flour in bread formulations up to 20% with little change in quality (providing 30-33 g/100 g protein, 7-13 g/100 g fat, more than 40 g/100 g carbs, and result in a possible prolongation of shelf life), results were obtained using Mixolab®. Because hemp protein consists primarily of edestin, a highly digestive storage protein, HPI is easily digestible and considered one of the least allergenic protein sources when taken as a powder compared to SPI (Wang *et al.,* 2007). And while HPI has also shown to potentially benefit consumers by reducing hypertension and adding nutrition without sacrificing quality (Wang *et al.,* 2007; Teh *et al.,* 2016; Korus *et al.,* 2017) studies showing real consumer data are limited.

4.1.2.4.2.5 Sustainability

Hemp seed is cultivated as a food source, oil, animal feed, fiber and medicine and is even sufficient for daily requirements of amino acids in children and infants (Wang *et al.,* 2007; Teh *et al.,* 2016) making it a highly sustainable option. Hemp cultivation has grown in recent years and includes countries such as Great Britain, United States, Canada, France, Spain, China and Australia (Rodriguez-Leyva and Pierce, 2010). And since there is a global interest in using more sustainable plant sources as food ingredients, more research using hemp protein isolate as a sustainable and cost-effective solution to GF product development within bakery is necessary.

4.2 Sustainable Considerations to Gluten-Free Baked Products

Understanding the impact of plant vs animal sources and how ingredients can help lower food production cost, reduce waste and increase product nutrition are important. But so are considerations like consumer perception and choices that influence consumption, such as cultural identity. A longitudinal study conducted by Siegrist *et al.* (2015) on the eating behaviors of the Swiss examined how consumer perception of environmentally related consumption patterns changed from 2010 – 2014. Authors found that convictions around sustainable perceptions, such as reduced meat consumption being more beneficial for the environment and seasonal fruits and vegetables as better tasting and cheaper options, influenced consumer beliefs. Farmer *et al.* (2017) also found that promoting sustainability reduces consumption, however a drawback to the study is that it was conducted on college students who might arguably have greater knowledge and understanding of the impacts to sustainable options. Therefore, factors pertaining to sustainability need to be addressed with a wider consumer scope when considering the future of GF bakery products.

Lazzarini *et al.* (2017) investigated the factors that influence how the Swiss view plant-based foods and found that enforcing ideas around local markets and production was a relevant factor. While they eventually found that locally produced products and foods have the highest impact on customer choices, some consumers only consider large transport distances as affecting sustainability and suggest that increased education around labeling and sustainable transparency on food packaging could further influence consumer behavior. A study conducted in Canada and Germany on consumer preferences for potato and ground beef found that irrespective of product or country, high subjective knowledge (i.e., what consumers think they know) and objective knowledge (i.e., what is actually memorized) drive sustainable food choices (Peschel *et al.,* 2016). Authors found that 20% of consumers were ready to adopt footprint labels on food, and another 10-20% could be targeted by enhancing product knowledge. Siegrist *et al.* (2015) also suggest consumption patterns can be affected the more educated consumers are about choices that affect the environment, noting consumers lacked product-specific environmental footprints. Interestingly, the United Kingdom had footprint labels but discontinued their use when people would not pay premiums for sustainably labeled products (Peschel *et al.*, 2016). And while Lazzarini *et al.* (2017) found that premium labeled products, such as organic and fair-trade, make little difference in consumer choice, whereas a study conducted by O'Connor *et al.* (2017) on a focus group of 11 participants responsible for making household grocery purchasing decisions found that purchasing intentions and consumption behavior with regard to fair-trade labels relate to 'thoughtful' shopping. Authors found that consumers are influenced by doing the right thing and motivated by a sense of moral obligation with regard to purchasing behavior.

Verain *et al.* (2015) also investigated behavior and whether sustainable consumption was defined by product choice (i.e., the way it is produced) or curtailment (i.e., reduced quantity). Looking at product choices, dietary patterns and consumer segmentation, 942 people filled out a questionnaire geared toward food choice motivations, personal views to sustainable behavior, personal regard and social norms to sustainability and health, knowledge of sustainable food and their ability to judge sustainable options. Results showed that an approach to behavioral strategies relied both on both the level (i.e., motivations, knowledge, food involvement and personal norms toward sustainability) of sustainable consumption and type, or segment, of consumer behavior. Citing "theoretical routes toward more sustainable, plant-based diets" authors suggest consumer acceptance is based on either food innovations that are not very noticeable (i.e., hybrid meat products), smaller patterns to food consumption behavior (i.e., smaller portions or meatless days) or cultural change (i.e., taking production methods/environment into account).

Lacour *et al.* (2018) found that substituting meat at 35 g/day with plants like potato, vegetables and nuts could reduce GHG by 12% and a 60% decrease in daily meat consumption replaced by plant-based products resulted in up to a 38% decrease in energy demand. Because substantial energy is required for feed, breeding, production and operations it could be suggested that understanding of food production systems might also help influence consumer behavior rather than simply stating how a plant-based diet can affect the environment or one's health. Interestingly, Visschers and Siegrist (2015) conducted a study of taste rating compared to global warming potential (GWP). Part 1 compared taste results between climate-friendly and unfriendly meals and part 2 introduced climate friendly informational labels. Results showed GWP was unrelated to taste and offering more climate friendly meals did not impact satisfaction. And although climate friendly meal information increased food purchase, it did not affect satisfaction.

Visschers and Siegrist (2015) also cite that diets with lower environmental impact are often healthier, but a climate friendly diet can conflict with health. For example, a diet high in complex carbohydrates and low intake of meat sources might be good for the environment but does not always translate to a healthier option. This same comparison can be drawn to the consumer perception of GF products as being healthier options: GF options may be perceived as healthier even though they may not be. Therefore, products that are sustainable for both the environment and consumer health are preferable. However, taste and consumer acceptability will remain factors, as will product knowledge, availability of sustainable choices and the consumers' need to save time (Farmer *et al.,* 2017). Therefore, consideration of sustainable transparency, consumer education, of both sustainable sources and production methods, and product quality are important to changing consumer perception and acceptability.

5. MATERIALS AND METHODS

5.1 MATERIALS AND EQUIPMENT USED

The materials used in each test consisted of high-quality ingredients.

5.1.1 PROTEINS

The following were purchased from *MyProtein.com, Manchester, United Kingdom:*

- PPI (per 100 g: protein 75 g/kg, fat 5 g/kg, fiber 0 g/kg, carbohydrates 3 g/kg)

- SPI (*MyProtein.com, Manchester, United Kingdom*) (per 100 g: protein 90 g/kg, fat 0.5 g/kg, fiber 0 g/kg, carbohydrates 5 g/kg)
- Whey Protein Isolate (WPI) (*MyProtein.com, Manchester, United Kingdom*) (per 100 g: protein 90 g/kg, fat 0.3 g/kg, fiber 0 g/kg, carbohydrates 2.5 g/kg)

The following was purchased from *Real Food Source, Musselburgh, East Lothian:*

- HPI (per 100 g: protein 75.8 g/kg, fat 3.8 g/kg, fiber 5.5 g/kg, carbohydrates 1.1 g/kg)

5.1.2 Flours, Starches and Cereals

The following were purchased from *Shipton Mill Ltd., United Kingdom:*

- All Purpose Flour (GF); Organic Brown Rice Flour (GF); Organic White Rice Flour (GF); Organic Buckwheat Flour (GF); Oat Flour (GF); Sorghum Flour (GF); Organic Millet Flour (GF); Organic Potato Starch (GF); Organic Tapioca Starch (GF)

5.1.3 Other Ingredients

The following were purchased from *Kudos Blends Ltd., Worcestershire, United Kingdom:*

- KODA™ Potassium Bicarbonate
- PELL™ Gluten-Free Baking Powder

The following were provided by *The National Bakery School, London:*

- Blanched, Ground Almond Flour; Cornstarch; Margarine; Pectin; Sugar; Salt; Vanilla; Vegetable Oil; Water; XG; Yeast

5.1.4 Laboratory Apparatus

All laboratory equipment used for testing and analysis was located at London South Bank University and consisted of the following: Sartorius Entris Analytical

Balance (ENTRIS224-1S); Kjeldahl Protein Analyzer (KT2100); Soxtec™ Extraction Unit (ST243); Mixolab® (provided by *Chopin Technologies, Paris*). All glassware used was standard laboratory issue.

5.1.5 Equipment for Test Baking and Preparation

All equipment used for the preparation and baking of both test and developed products included standard and commercial equipment located at the National Bakery School.

5.2 Methodology

5.2.1 *Rheological Properties of Dough*

The rheological properties of each compound tested were analyzed using the Mixolab® (*the Mixolab® was introduced in 2004 by Chopin Technologies, Paris, and is the accepted ICC standard method*).

Samples were analyzed using the universally accepted Chopin+ (standard method: NF V 03 - 764 / ICC N 173 / AACC 54 - 60.01) and Chopin+ 90 g protocols. The protocols provide a complete analysis of a sample in 45 minutes and during five phases: mixing behavior, protein quality, gelatinization, amylase activity and starch retrogradation. The difference between the Chopin + and Chopin+ 90 g protocols is the weight of the dough being analyzed. As the standard protocol used, the Chopin+ relies on total dough weight of 75 g while the Chopin+ 90 g protocol relies on a dough weight of 90 g and is used for more accuracy during testing. With the Chopin+ protocol, the flour weight is around 45-55 g, which varies according to the hydration applied. Flour weight for the Chopin+ 90 g protocol is generally around 55-65 g and varies according to the hydration applied. The Blending Law, a predictive feature of the Mixolab® software, is a tool that can assist in predicting the potential behavior of a blend. It compares the theoretical result of a blend to the actual result as a validation measure. For testing purposes, the tool determined the blend that would yield similar rheology to a sample of a commercially used white flour (per 100 g: protein 13.7 g/kg, fat 1.9 g/kg, fiber 12.2 g/kg, carbohydrates 72.6 g/kg), represented in Fig. 2 [page 23].

5.2.1.1 The Mixolab®

Tests were carried out to analyze the rheological properties of compounds for each sample as follows: for the Chopin+ protocol a standard of 55% water per 47.59 g samples at 12% moisture and 14% hydration base was selected; for the Chopin+ 90 g protocol a standard of 55% water per 56.74 g samples at 12% moisture and 14% hydration base was also selected to test for consistency of results in some cases. Mixing speed was 80 rpm and the temperature regime was 8 minutes at 30°C, heating rate of 4°C/min until 90°C, 7 minutes held at 90°C, cooling rate 4°C/min until 55°C, then 5 minutes held at 55°C (total time of analysis was 45 minutes). To prepare the test, dry samples were measured manually using a digital scale and the device's water tank was filled with distilled water. To begin the test, the Mixolab® software was opened and the option to run a new test was selected. The protocol, water, moisture and hydration levels were set and the test initiated. Once the test began, dry samples were placed directly into the machine using the Mixolab® funnel. A small brush was used to remove any residual dry sample left in the funnel. Funnel was removed and replaced with the hydration hose (attached to the device's water tank). The test ran automatically for 45 minutes before generating working files consisting of all relevant rheological data.

5.2.2 *Formulation of Gluten-Free Recipes*

Protein composites were tested in percentages of HPI/PPI at 100:0, 0:100, 50:50 and 80:20 (w/w) respectively. To determine the importance and impact of starch, a GF multi-grain (MG) mix was developed [Table 14]. The mix resulted in a ratio of 70% GF flours and 30% added starches. Tests included GF formulations for bread, muffin and cookie products and detailed in the following sections.

TABLE 14: MG GF MIX RECIPE

Ingredient	Amount (g)
Almond Flour	100
Brown Rice Flour	100
Buckwheat Flour	100
Millet Flour	100
Sorghum Flour	100
Oat Flour	200
Cornstarch	100
Potato Starch	50
Tapioca Starch	50
White Rice Flour	100

5.2.2.1 Bread Dough

An experimental plan was established for the GF bread recipe which included testing the levels of total overall added protein, substituted in place of dry ingredients (starches) to maintain total of 600 g per recipe (see Appendix 5). Hydration levels were not adjusted. Test recipe ingredients [Table 15] were mixed, fermented at 29°C / 85% humidity, remixed by hand and baked in 250 g loaves and cooled completely at ambient temperature. Based on observations made to the visible and physical characteristics of each test product, total starch used in the control recipe was reduced by 42% to scale back on the amount of ingredients needed.

TABLE 15: GF BREAD RECIPE COMPARISION - TEST VS CONTROL

	Test	Control
Ingredient	Amount (g)	Amount (g)
Cornstarch	480	200
Potato Starch	120	50
Guar Gum	10	0
Xanthan Gum	0	4
Pectin	0	4
Sugar	12	5
Salt	11	4
Oil	18	7.5
Water	570	258
Dried Yeast	30	0
Fresh Yeast	0	12.5

Control recipe ingredients were measured using a digital scale and mixed using a Hobart N50 mixer (speed 1 for 2 minutes, speed 2 for 6 minutes). Dough was transferred into 250 g loaf tins, placed in a prover (Foster DRP RBC MK 3 Controller, 30°C, 93% humidity) and fermented for 20 minutes. Dough was then removed and baked in a Tom Chandley Compacta Deck Oven at 200°C for 30 minutes. After removing from the oven, the bread was cooled for 10 minutes before being removed from the tins, placed on a wire rack and cooled completely at ambient temperature. Blend ratios for the control recipe at 30% and 40% are represented in Table 16.

TABLE 16: PROTEIN BLEND RATIOS FOR GF BREAD RECIPE

Variation	Starch (g)	HPI (g)	PPI (g)
Control	250	0	0
30% Protein, Blend 1 (100:0)	175	75	0
30% Protein, Blend 2 (0:100)	175	0	75
30% Protein, Blend 3 (50:50)	175	37.5	37.5
30% Protein, Blend 4 (80:20)	175	60	15
40% Protein, Blend 5 (100:0)	150	100	0
40% Protein, Blend 6 (0:100)	150	0	100
40% Protein, Blend 7 (50:50)	150	50	50
40% Protein, Blend 8 (80:20)	150	80	20

The GF MG mix was used in the developed bread recipe and the blend ratios are represented in Table 17. Once baked, the samples were cooled completely at ambient temperature before being used for further lab analysis.

TABLE 17: PROTEIN BLEND RATIOS FOR GF BREAD RECIPE USING MG MIX

Variation	GF Mix (g)	HPI (g)	PPI (g)
Control	250	0	0
30% Protein, 80:20 blend	175	60	15

5.2.2.2 MUFFIN BATTER

An experimental plan was established for the muffin recipe which included testing protein types at 17.3 g per recipe (see Appendix 6). Test recipe ingredients [Table 18] were scaled using a digital scale and mixed by hand in large bowl, weighed into 60 g portions and baked in a lined muffin tin at 180°C for 25 minutes in a Tom Chandley

Compacta Deck Oven. The muffins were removed from the oven, cooled for 10 minutes then removed from the tin, placed on a wire rack and cooled completely at ambient temperature.

TABLE 18: GF MUFFIN RECIPE COMPARISION - TEST VS CONTROL

	Test	Control
Ingredient	**Amount (g)**	**Amount (g)**
GF Flour Blend	100	100
Protein Isolate	17.3	17.3
Sugar	75	75
Bicarbonate of Soda	4	4
Citric Acid	3	0
Salt	1.5	1.5
Xanthan Gum	0.5	0.5
Oil	46	46
Water	100	100

Based on observations made to the visible and physical characteristics of each test product, an adjustment was made for the control recipe, removing an additional the leavening agent. Hydration levels were not adjusted. The control variations were baked under the same processing conditions as the test muffins. Blend ratios for the proteins tested are represented in Table 19.

TABLE 19: PROTEIN BLEND RATIOS FOR GF MUFFIN RECIPE

Variation	WPI (g)	HPI (g)	PPI (g)
Control	17.3	0	0
Blend 1 (100:0)	0	17.3	0
Blend 2 (0:100)	0	0	17.3
Blend 3 (50:50)	0	8.65	8.65
Blend 4 (80:20)	0	13.84	3.46

The GF MG mix was used in the developed muffin recipe and SPI was substituted for WPI. The blend ratios are represented in Table 20. Once baked, the samples were cooled completely at ambient temperature before being used for further lab analysis.

TABLE 20: PROTEIN BLEND RATIOS FOR GF MUFFIN RECIPE USING MG MIX

Variation	SPI (g)	HPI (g)	PPI (g)
Control	17.3	0	0
MG Control	17.3	0	0
MG 80:20	0	13.84	3.46

5.2.2.3 COOKIE DOUGH

An experimental plan was established for the cookie recipe which included testing the combined protein percentages, substituted in place of dry ingredients (GF flour blend) to maintain total of 200 g per recipe (see Appendix 7). The test recipe ingredients [Table 21] were scaled using a digital scale and mixed using a Hobart N50 mixer (speed 1 for 2 minutes, speed 2 for 2 minutes). Dough was weighed into 40 g portions, rolled into balls by hand and baked on a lined cookie sheet at 180°C for 12 minutes in a Tom Chandley Compacta Deck Oven. The cookies were removed from the oven, cooled for 10 minutes, placed on a wire rack and cooled completely at ambient temperature.

TABLE 21: GF COOKIE RECIPE COMPARISION - TEST VS CONTROL

	Test	Control
Ingredient	**Amount (g)**	**Amount (g)**
GF Flour Blend	200	200
Baking Powder	14	4
Xanthan Gum	3	3
Sugar	125	125
Margarine	125	125
Water	15	15
Vanilla	4	2

Based on observations made to the visible and physical characteristics of each test product, an adjustment was made for the control recipe, reducing the leavening agent and vanilla. Hydration levels were not adjusted. The control variations were baked under the same processing conditions as the test cookies. Blend ratios for the proteins tested are represented in Table 22.

TABLE 22: PROTEIN BLEND RATIOS FOR GF COOKIE RECIPE

Variation	Starch (g)	HPI (g)	PPI (g)
Control	200	0	0
50:50	140	30	30
80:20	140	48	12

The GF MG mix was used in the developed cookie recipe. The blend ratios are represented in Table 23. Once baked, the samples were cooled completely at ambient temperature before being used for further lab analysis.

TABLE 23: PROTEIN BLEND RATIOS FOR GF COOKIE RECIPE USING MG MIX

Variation	MG Mix (g)	HPI (g)	PPI (g)
Control	200	0	0
80:20	140	48	12

5.2.3 *Nutritional Analysis*

Laboratory tests were conducted to collect nutritional data for each sample formulation to determine the protein and fat percentages. Fiber content was tested in the developed products only. Testing also included assessing the protein's impact on blood glucose levels and hunger satiety levels of participants during the product development phase.

5.2.3.1 Protein

The Kjeldahl Protein Analyzer was used to determine the protein content of each sample. The Kjeldahl method consisted of a three-step process: 1) digesting samples in a sulfuric acid solution along with a catalyst, which converted nitrogen to ammonium ions; 2) ammonium ions were converted to ammonium gas, heated and distilled, then dissolved in a trapping solution becoming ammonium ion once again; 3) the trapped ammonia was titrated with a solution and calculations were made. This process took 2.5 hours to complete.

To begin step one (sample digestion), 1-2 g samples were weighed using a digital scale and placed into a digestion tube along with a catalyst tablet. Digestion tubes were placed into a stand. Next, 15 ml sulfuric acid (95%, low in nitrogen specific gravity 1.83) were added to each tube and placed into the digestor unit. The start/stop button was pressed to begin the process automatically, taking 2 hours to complete. Step two (distillation) consisted of removing the digested tubes from the digestor unit and adding 50 ml distilled water to each tube. Next, a flask was filled with 20 ml boric acid (4% w/v with indicator). The digestion tube was then attached to one nozzle of the distillation unit and the flask was placed under the other nozzle of the unit. The start button was pressed to begin the process and when the light was orange, pressed again to run the process, automatically taking 4 minutes. Upon completion, the digestion tube was removed and contents discarded. The flask was also removed and set aside. Step three (titration) consisted of filling a burette with hydrochloric acid. Next, the flask was placed under the burette and acid was slowly added to the flask until the solution changed color to an orangish-yellow, at which point the volume of acid used was recorded.

The following equation was used to determine the amount of protein per sample:

$$\% \text{ protein} = \frac{(\text{ml standard acid} - \text{ml blank}) \times \text{N of acid} \times 1.4007}{\text{weight of sample in grams}} \times \text{protein factor}$$

In the equation, 'N' represents normality, 'ml standard acid – ml blank' refers to the milliliters of acid needed to back titrate and 'protein factor' refers to a numeric factor of which the nitrogen content of a protein is multiplied to approximate the protein amount. Because the protein used was a combination of two proteins with different protein factors, the standard of 6.25 was used for all calculations.

5.2.3.2 Fat

The Soxtec™ Extraction Unit was used to determine the crude fat content of each sample. This method consists of isolating soluble material by using solvents in a two-stage process followed by a recovery cycle. The process takes 90 minutes to complete.

Step one consisted of weighing out clean and dry extraction cups using a digital scale, then 40 ml of mixed ether 50-50 (diethyl ether/petroleum spirit 40-60) was measured into each cup and placed into the cup holder. Step two consisted of attaching thimbles to thimble adapters, with 2-3 g samples weighed into each thimble and placed in a thimble stand. Next, defatted cotton dipped into mixed ether was placed on top of samples. On the extraction unit, each knob was moved to the boiling position, thimbles were fastened onto the magnet and the knobs were lowered to the rinsing position. Step three consisted of placing the cup holder (with the extraction cups) onto the unit and lowering the handle to lock them into place. Next, the knob was moved back to the boiling position and the condenser valve was opened to a 90° angle.

To start the program, the start/stop button on the control unit was pressed. The unit started automatically when the appropriate temperature was reached. After 30 minutes (boiling cycle), the control unit beeped. The knobs were then moved to the rinsing position, the condenser valve adjusted to a 45° angle and the timer button pressed to start the rinsing cycle. After 30 minutes the control unit beeped again. The timer button was pressed to start the cooling cycle. Once all the solvent in the thimbles was evaporated, the cups were removed from the hot plate and cooled completely before the extraction cups were weighed.

The following equation was used to determine the amount of fat per sample:

$$\% \text{ fat} = \frac{\text{weight of metal cup after process} - \text{weight of metal cup before process}}{\text{sample weight in kg}} \times 100$$

5.2.3.3 FIBER

Total dietary fiber (TDF) was determined by the AOAC method (American Association of Analytical Chemists) on all three developed products and provided by Campden BRI Limited (*Chipping, Campden*) whereby 1 g dried food samples were subjected to sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglucosidase. Insoluble dietary fiber (IDF) was filtered, and the residue was washed with warm distilled water. A combined solution of filtrate and water washings were precipitated with 95% ethanol for soluble dietary fiber (SDF) determination. Precipitate was then filtered and dried. Both SDF and IDF residues were corrected for protein, ash and blank for the final calculation of SDF and IDF values. TDF was determined by precipitating the SDF with ethanol and the residue was filtered, dried and weighed, and TDF calculated as weight of residue minus protein and ash plus the SDF.

5.2.3.4 GLYCEMIC INDEX

In vivo research was conducted on a group of healthy volunteers to determine if consuming GF products developed in this study would result in increased GI levels. Participants were separate from those involved in the sensory testing, and this approach was similar to that followed by Scazzina *et al.* (2015). The blood glucose levels of participants were collected via thumb prick testing and used the Accutrend Plus Blood Test Meter (by *Roche Labs*), Accutrend Glucose Control Solution (High/Low Level), Accutrend Glucose Test Strips and Accu-Check Safe-T-Pro single use lancet. An application for ethical approval was submitted to LSBU for permission involving all testing on participants (see Appendix 1). Pursuant to Ethics Approval - SAS1735 (see Appendix 2), consent forms were obtained from each participant (see Appendix 3 & 4) prior to testing. Glucose tests were administered before/after ingestion and no more than 2.5 hours apart, the length of time for blood glucose levels to be impacted by food.

To begin, the test meter was calibrated for quality control. The meter was powered on and a sample test strip was inserted with the measurement chamber closed. After the beep, the 'M' button was pressed to indicate this as a test. The chamber flap was opened, and a drop of the Low-Level Control Solution applied to the strip. The chamber flap was closed, and the test ran automatically. Steps were repeated for the High-Level Control Solution. Test strips were discarded, and the high and low levels recorded. This was performed only once and before glucose testing began. To perform a glucose test for each participant, a test strip was inserted with the application area facing up with the measurement chamber flap closed. After the beep, the chamber flap was opened, and the test was strip removed. Participant's fingertip was cleaned with an alcohol wipe. The lancet was placed on the tip of the finger and the trigger button pressed. The finger was gently massaged until a drop of blood appeared and was applied immediately to the test strip. The strip was inserted into the meter and the chamber flap was closed. The device began the automatically, taking 12 seconds. The displayed result was recorded onto a summary sheet. The test strip and lancet were removed and discarded according to safety guidelines.

Based on the evaluation of GI response to GF products conducted in prior research (Lang *et al.,* 1998; Atkinson *et al.,* 2008; Gularte *et al.,* 2012; Scazzina *et al.,* 2015) average participant numbers for this trial were determined to be 12 (10 being the average participant size plus 20% attrition and using a 5% statistical significance standard), with testing on healthy participants and using no other criteria in order to gain the most varied set of data. The evaluation group for this study consisted of 11 volunteers (7 men and 4 women) with no known health issues. A total of three GF products (bread, muffin and cookie) were randomly assigned to participants. Randomization was used to determine if the ingredients used in the GF products were responsible for the GI levels rather than attributing it to the individual products themselves. Therefore, this theoretical approach involved assigning no control product. Subjects arrived the day of the test in a fasted state and asked to consume a random sampling of 50 g of product and allowed to drink only water. The blood glucose levels of each participant were measured by using a thumb prick test before ingestion and 2 hours after ingestion.

5.2.3.4.1 Hunger Satiety

A hunger satiety questionnaire developed to record satiety levels was administered to participants of the GI testing (see Appendix 27). Each participant began the study on an empty stomach, ingested a randomly selected 50 g serving size of product containing HPI/PPI (bread, muffin or cookie) and recorded, over a period of time, how hungry/full they felt. Participants recorded their responses on a 7-point scale (0 being 'Not Hungry At All' and 7 being 'Extremely Hungry). Data was collected and aggregated to determine participant hunger levels in relation to the developed products. Participants were allowed to drink only water during the course of the test. Satiety levels were assessed by each participant in a fasted state prior to ingestion, and then every 30 minutes for up to 2 hours after ingesting the samples.

5.2.4 *Sensory Analysis*

Sensory analysis was conducted on a total of 66 participants who engaged in discrimination testing. Consent forms were obtained from each participant (see Appendix 3 & 4) prior to testing and pursuant to Ethics Approval - SAS1735 (see Appendix 2). A sensory evaluation questionnaire (see Appendix 26) was administered to obtain the acceptability of developed products. Preference Testing was conducted according to *British Standard Institution* (BS 5929). Participants were asked a series of questions, using a 5-point Hedonic Scale, for the quantitative portion of the tests focused on attribute scoring; 1 (very much dislike) to 5 (very much like). Sensory attributes consisted of appearance, taste and texture. Sensory testing of the final products also included *Affective* testing to determine consumer perception of the product to obtain greater data understanding of the product acceptability. In the form of yes/no questions, the *Affective* testing portion included whether or not the products were healthier based on appearance, taste and texture and whether or not consumers would purchase the products. Testing was divided into two sessions on different community groups to gain the most varied set of data on consumer response. The two sessions were conducted on random participants consisting of men and women. The first session took place at The National Bakery School and consisted of students and staff, most of whom were familiar with the expectations of quality for baked products. The second session was conducted at Pure Gym, Borough on random participants who had little knowledge of bakery products but a wider opinion on healthier options.

5.2.5 *SUSTAINABILITY*

The strategic approach to sustainable product development for this research involved a method known as backcasting, which defines a desired vision by working backwards to identify steps that will connect future predictions to present circumstances (Robinson, 1990). Backcasting essentially asks one fundamental question: In order to achieve a specific goal tomorrow, what actions or steps must be taken today to be successful? This method identifies six main steps for outlining objectives, goals, variables, current system constraints and scenario/impact analysis. To integrate sustainability into the product development process, sustainable considerations needed to be imbedded into the process before product planning began so that sustainable outcomes could be achieved efficiently and cost-effectively. Therefore, a process for this research was adapted using a method for sustainable product development proposed by Byggeth *et al.* (2006) and involved incorporating a series of questions used to guide developmental decisions.

Step one of the backcasting method, investigation, involved an initial brainstorming session of the proposed product by asking 'what is needed?' Based on that answer, step two involved identifying critical objectives relevant to the product, taking into considering clean label concerns, nutrition, use of sustainable ingredients and product type. Step three began with prioritization, and included identifying which sustainable, economic and technical changes were a priority by asking 'is the change headed in the right direction based on the principles of sustainability, flexible enough to adapt to future sustainable changes and cost-effective for businesses/producers?' Once complete, step four involved defining the product development matrix based on the identified objectives and priorities.

The product development matrix, comprised of four development phases (Product, Research, Production and Launch), consisted of an additional brainstorming session for each development phase which involved assessing the existing product and identifying potential solutions to problems/issues during assessment. Assessment was achieved by asking, 'how does the product contribute to sustainable views' and 'how does it violate them?' Solutions were obtained by asking, 'how does the product need to be re-designed to fulfill the sustainable vision' and 'does it create opportunities to fulfill customer needs when sustainability is more accessible?' The answers were then applied to key areas critical to each phase. Within each phase there were five areas that needed to be considered (Purpose, Design, Ingredients, Production and Purchase). Each phase would have a different set of critical areas to consider. Simultaneous to brainstorming at each phase, assessing the sustainability of the product based on set objectives was critical to identifying potential systems, resources or activities critical to the product and generating questions for possible improvements. The final step involved addressing the same questions as in the prioritization stage to identify any possible changes that might impact sustainability concerns. Both the backcasting method and product development matrix are represented in Fig. 4 and Fig. 5.

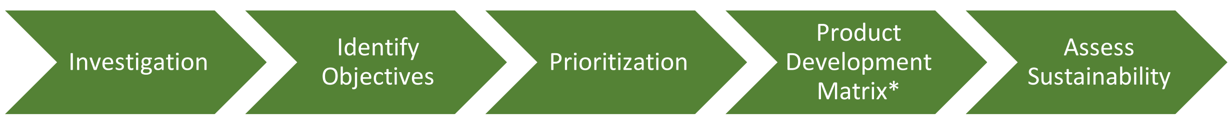


FIGURE 4: VISUAL DEPICTION OF THE BACKCASTING METHOD

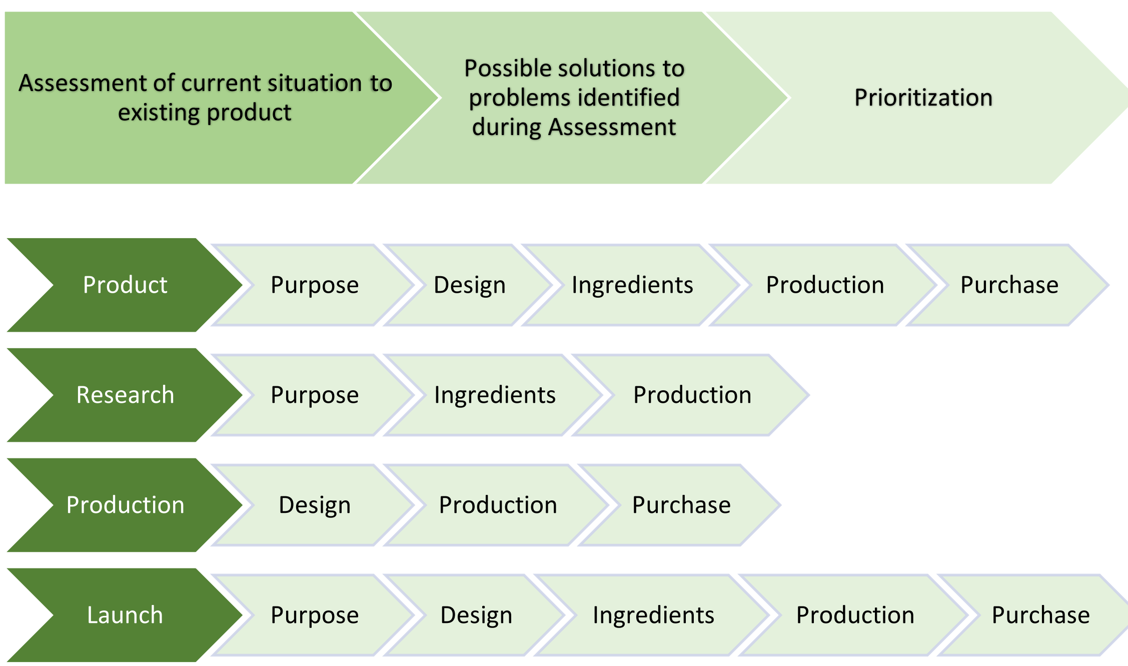


FIGURE 5: VISUAL DEPICTION DEFINING THE PRODUCT DEVELOPMENT MATRIX

5.2.6 *Statistical Analysis*

Analyses was performed, in triplicate for nutritional results of developed products and in duplicate for test products, and the mean values with standard deviation were reported. Microsoft Excel 2016 (*version 16.35, 20030802*) and SPSS Statistics software (*IBM, version 26*) were used for analysis of variance (ANOVA) with a level of significance set to 95%. Where necessary the Bonferroni Correction was used to assess statistical differences between samples and was chosen because it lowers the area of correction where one might reject the null hypothesis. Statistical significance is normally set at $P < 0.05$ however the differences using the Bonferroni Correction were considered as significantly different at a value of $P < 0.0167$ for test products and $P < 0.025$ for developed products. As a confidence measure, a Descriptive Statistics approach was also implemented as a follow up to validate the statistical analysis, using coefficient of variance and relying on maximum difference between samples to determine significance. Differences were considered statistically different at a value of $P < 0.05$.

6. Results

6.1 Rheological Properties of Dough

The rheological properties of PPI and HPI were analyzed using the Mixolab® by mixing each protein isolate with the GF flour to make up 100 g (17.3 g protein added to 82.7 g GF flour). To measure the protein's behavior under development conditions, protocol parameters for standard wheat flour were applied to determine if adjustments to the protocol needed to be made after initial testing. The parameters for hydration selected were 55% water, 12% moisture and 14% hydration base, and correlated to the hydration level used for commercial wheat flours. Tests included 100% PPI added to GF flour and 100% HPI added to GF flour to understand the basic rheological differences between the proteins and yield a baseline for comparison. Due to the precision of the Mixolab® testing, tests were performed twice to ensure the repeatability of the method. Tests are represented in Fig. 6 and Table 24.

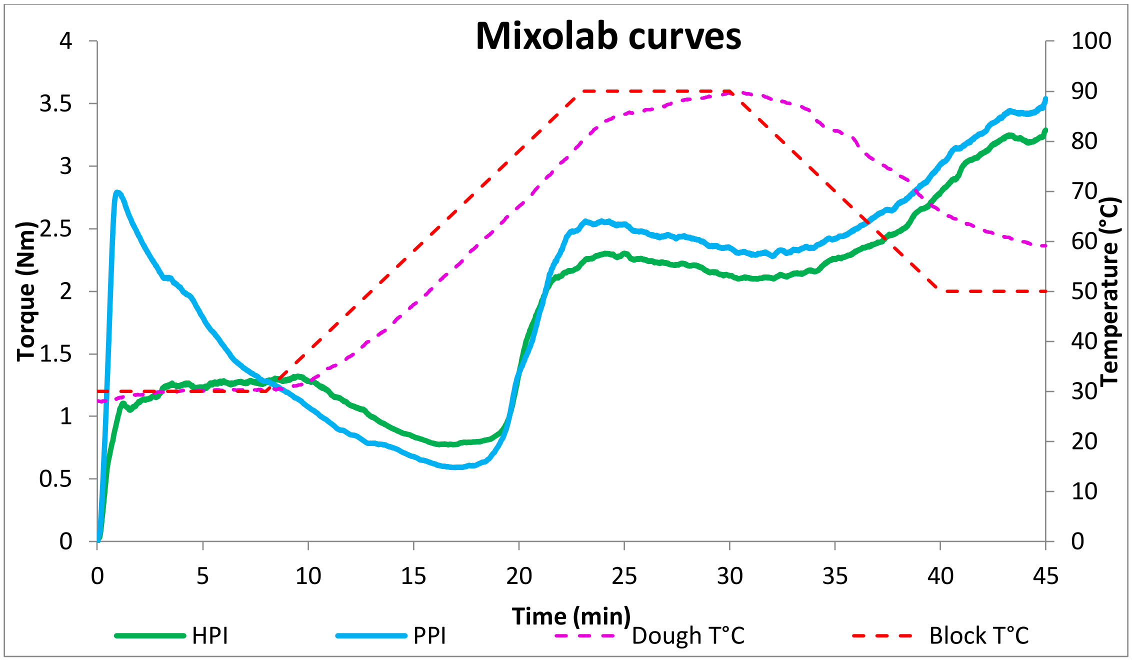


FIGURE 6: MIXOLAB® GRAPH: COMPARING 100% PPI & 100% HPI

TABLE 24: RHEOLOGICAL PARAMETERS OF PROTEINS AT 100%

Sample[1]	PPI	HPI
C1 (N m)	2.73 ± 0.08*	1.33 ± 0.01*
CS (N m)	1.27 ± 0.01 ns	1.28 ± 0.01 ns
C2 (N m)	0.57 ± 0.03*	0.77 ± 0.002*
C3 (N m)	2.55 ± 0.02*	2.30 ± 0.01*
C4 (N m)	2.28 ± 0.01*	2.09 ± 0.02*
C5 (N m)	3.54 ± 0.01*	3.25 ± 0.05ns

[1] mean value of 2 replication ± standard deviation

*Values with * are significant ($P \leq 0.05$); those with ns are not significant ($P > 0.05$)*

ANOVA results showed significance between proteins at start of mixing (C1). Post Hoc tests were performed and showed there is significance for the rate of gelatinization (C3), gel stability (C4) and starch retrogradation (C5). When compared against the curve for standard wheat flour [Fig. 7], differences between the curves, particularly in the initial mixing/heating stages, become apparent. Therefore, combining the two proteins might produce an even closer behavior to wheat flour and the next section will discuss the steps to determine the correct proportion of each.

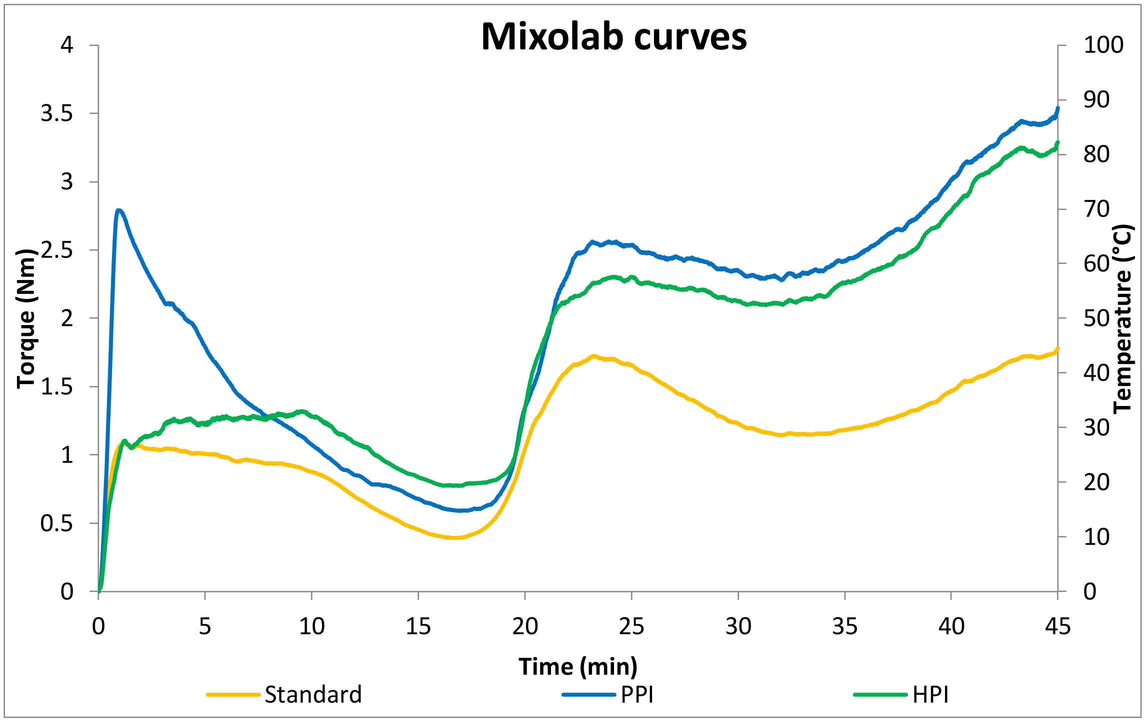


FIGURE 7: MIXOLAB® GRAPH: COMPARING 100% PPI, 100% HPI & WHEAT FLOUR

6.1.1 Determining the Correct Protein Mix

In order to optimize the protein combination, tests were run using the Blending Law tool of the Mixolab® software to determine the correct mix of PPI and HPI proteins which would exhibit similar rheological properties to wheat flour. To test the accuracy of the Blending Law predictions, comparison of a 50% blend ratio for HPI/PPI from a Mixolab® test against the Blending Law software was required [Fig. 8 and Table 25].

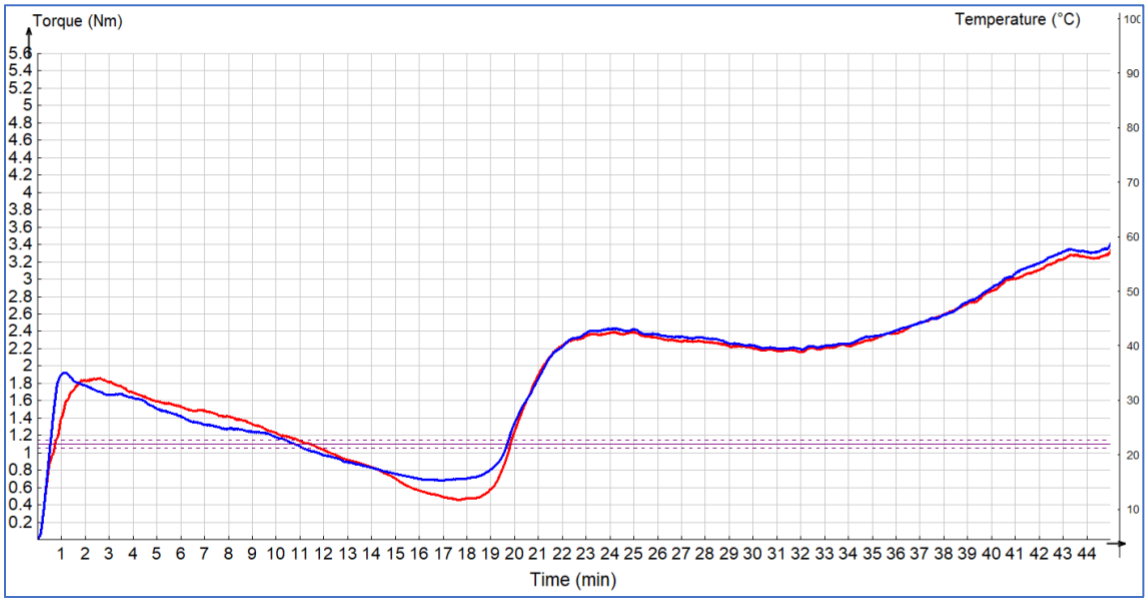


FIGURE 8: MIXOLAB® GRAPH: COMPARING 50:50 BLENDS - ACTUAL VS BLENDING LAW

TABLE 25: RHEOLOGICAL PARAMETERS OF PROTEIN RATIOS AT 50% - BLENDING LAW VS ACTUAL

Sample[1]	BL	50:50
C1 (N m)	1.92 ± 0.00 ns	1.91 ± 0.01 ns
CS (N m)	1.28 ± 0.00 *	1.42 ± 0.001*
C2 (N m)	0.68 ± 0.00*	0.45 ± 0.01*
C3 (N m)	2.43 ± 0.00 ns	2.41 ± 0.11 ns
C4 (N m)	2.19 ± 0.00 ns	2.17 ± 0.01 ns
C5 (N m)	3.42 ± 0.00 ns	3.32 ± 0.03ns

[1] mean value of 2 replication ± standard deviation

*Values with * are significant (P ≤ 0.05); those with ns are not significant (P > 0.05)*

Based on ANOVA results there was no statistical difference between tests at the start of mixing (C1), rate of gelatinization (C3), gel stability (C4) or starch retrogradation (C5). Ultimately results showed the behavior of the curves were similar enough to confirm that the Blending Law could be used to predict the best protein mix. Therefore, combinations of PPI and HPI using the Blending Law tool [Fig. 9], each at 10 percent additions +/- a 50:50 blend baseline [Table 26], show the prediction of each formulation's behavior compared to wheat flour.

TABLE 26: BLEND RATIOS OBSERVED USING BLENDING LAW SOFTWARE

	Percentage PPI	Percentage HPI
Test 1	10%	90%
Test 2	20%	80%
Test 3	30%	70%
Test 4	40%	60%
Test 5	50%	50%
Test 6	60%	40%
Test 7	70%	30%
Test 8	80%	20%
Test 9	90%	10%

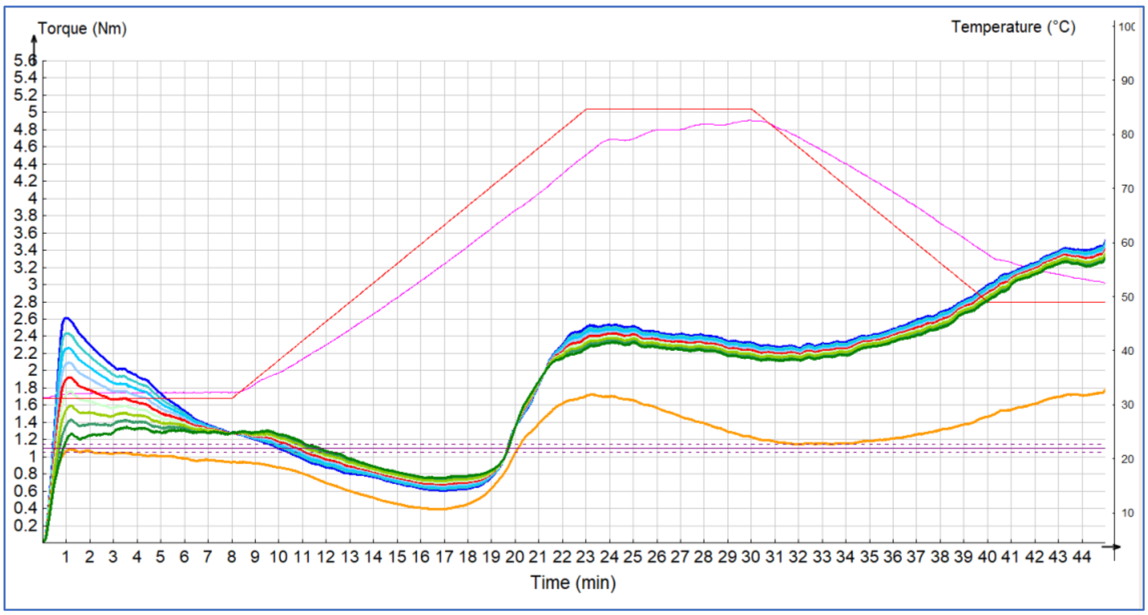


FIGURE 9: MIXOLAB® GRAPH: COMPARING BLENDING LAW PROTEIN BLEND RATIOS

The curve for the 50:50 blend (noted in red) indicates an intermediate behavior between the 100% PPI (noted in dark blue) and HPI (noted in dark green) curves. The main differences between each curve occurred in the initial mixing stage only (i.e., the protein behavior during mixing). Comparing each curve to wheat flour (noted in orange) and understanding from previous tests that more HPI would likely need to be included in the protein concentration, it was determined that 80:20 HPI/PPI blend would likely yield similar results to wheat flour. This comparison is examined closer in Fig. 10.

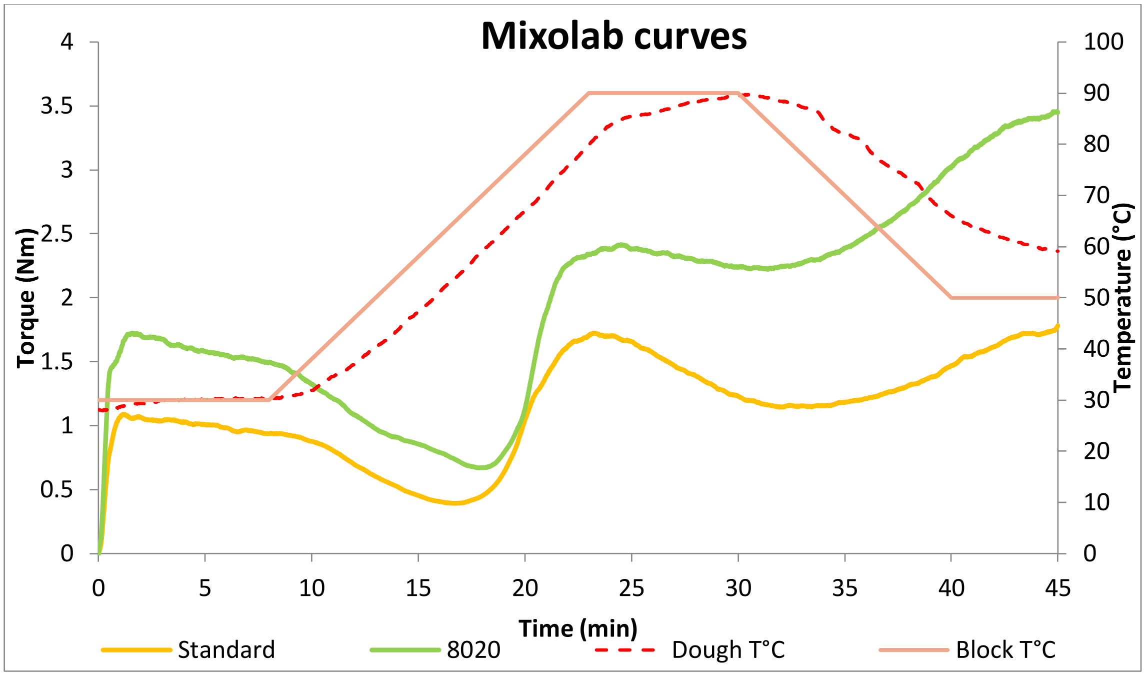


FIGURE 10: MIXOLAB® GRAPH: COMPARING 80:20 HPI/PPI BLEND & WHEAT FLOUR

The resulting curves show the stability of the 80:20 HPI/PPI blend when compared to wheat flour during the initial mixing and heating stages. To further validate this, a comparison was performed using the Mixolab® on the actual 80:20 blend curve and the Blending Law predicted 80:20 curve [Fig. 11 and Table 27].

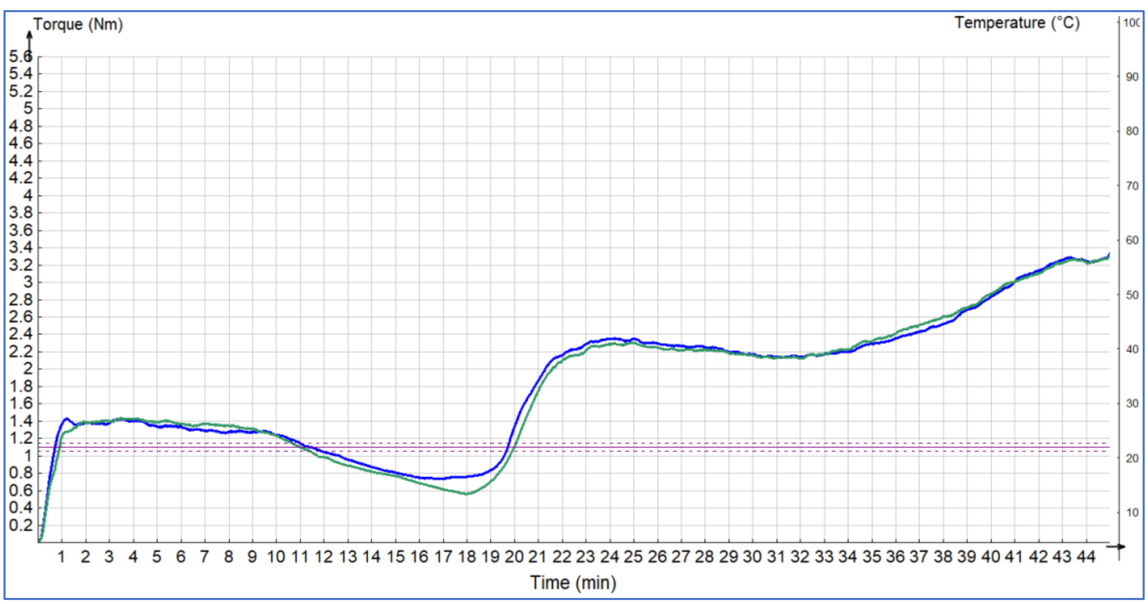


FIGURE 11: MIXOLAB® GRAPH: COMPARING 80:20 HPI/PPI BLENDS - ACTUAL VS BLENDING LAW

TABLE 27: RHEOLOGICAL PARAMETERS OF 80:20 HPI/PPI BLEND - BLENDING LAW VS ACTUAL

Sample[1]	BL	80:20
C1 (N m)	1.92 ± 0.00*	2.44 ± 0.01*
CS (N m)	1.28 ± 0.00 ns	1.29 ± 0.02 ns
C2 (N m)	0.68 ± 0.00 ns	0.69 ± 0.09 ns
C3 (N m)	2.43 ± 0.00 ns	2.50 ± 0.01 ns
C4 (N m)	2.19 ± 0.00 ns	2.22 ± 0.03 ns
C5 (N m)	3.42 ± 0.00 ns	3.47 ± 0.03 ns

[1] *mean value of 2 replication ± standard deviation*

*Values with * are significant (P ≤ 0.05); those with ns are not significant (P > 0.05)*

Based on ANOVA results there was no statistical difference between tests at the heating (C2), rate of gelatinization (C3), gel stability (C4) or starch retrogradation (C5) stages. Therefore, the resulting curves are similar enough to confirm the Blending Law. To better understand how the tested blends compared to each other, 100% PPI, 100% HPI, 80:20 blend and wheat flour curves are shown in Fig. 12.

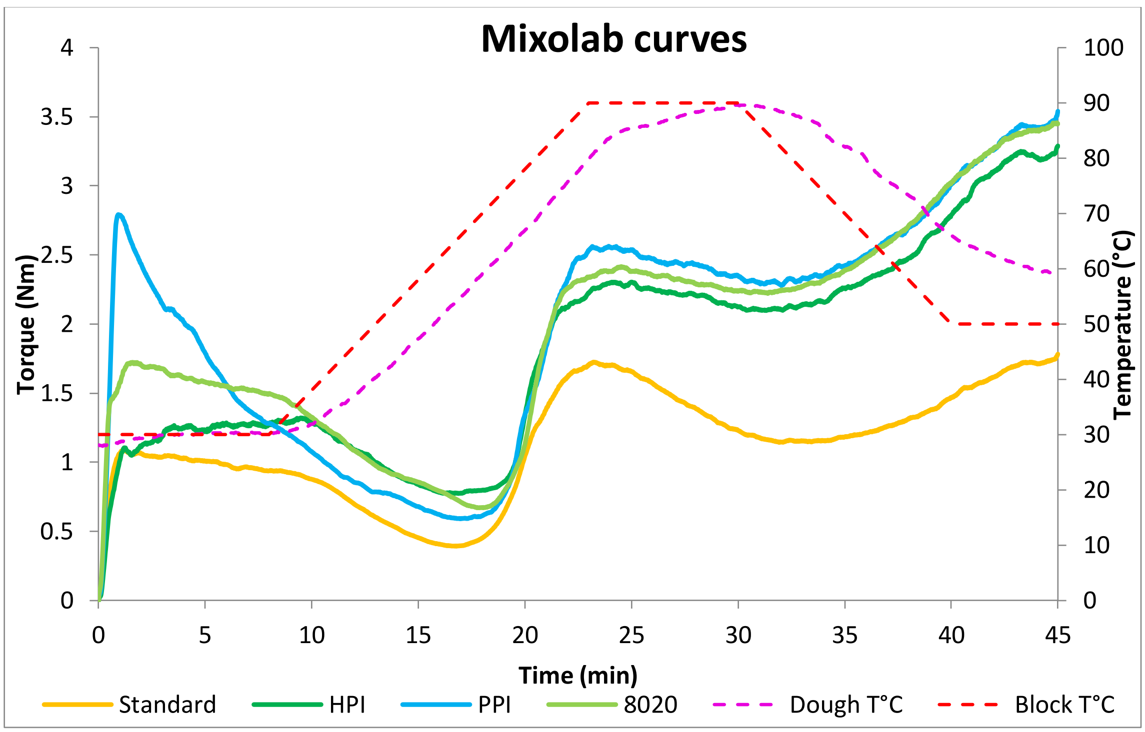


FIGURE 12: MIXOLAB® GRAPH: COMPARING 100% PPI & HPI, 80:20 BLEND & WHEAT FLOUR

Visually, the 80:20 curve appears more stable during mixing with similar resistance to heating/mixing and gelatinization, yielding an approximately similar protein behavior to that of wheat flour. It was determined that the 80:20 blend was the better option for product development. Initial recipe formulation and testing focused on the addition of protein to a GF blend that was entirely starch based, the next section will investigate the use of this blend in relation to starch.

6.1.2 Starch Activity

The developed GF MG mix was tested to understand any variations in rheological behavior between naturally occurring starches in GF flours and from added starches [Table 14, pg. 77], as well as the addition of the developed protein blend in 10% and 20% increments (see Appendix 8 - 11). Since initial tests included 17.3% protein, it was determined that the 20% protein formulation would be used to test for starch activity. The comparison of the 20% blend with and without added starch is shown in Fig. 13 and Table 28.

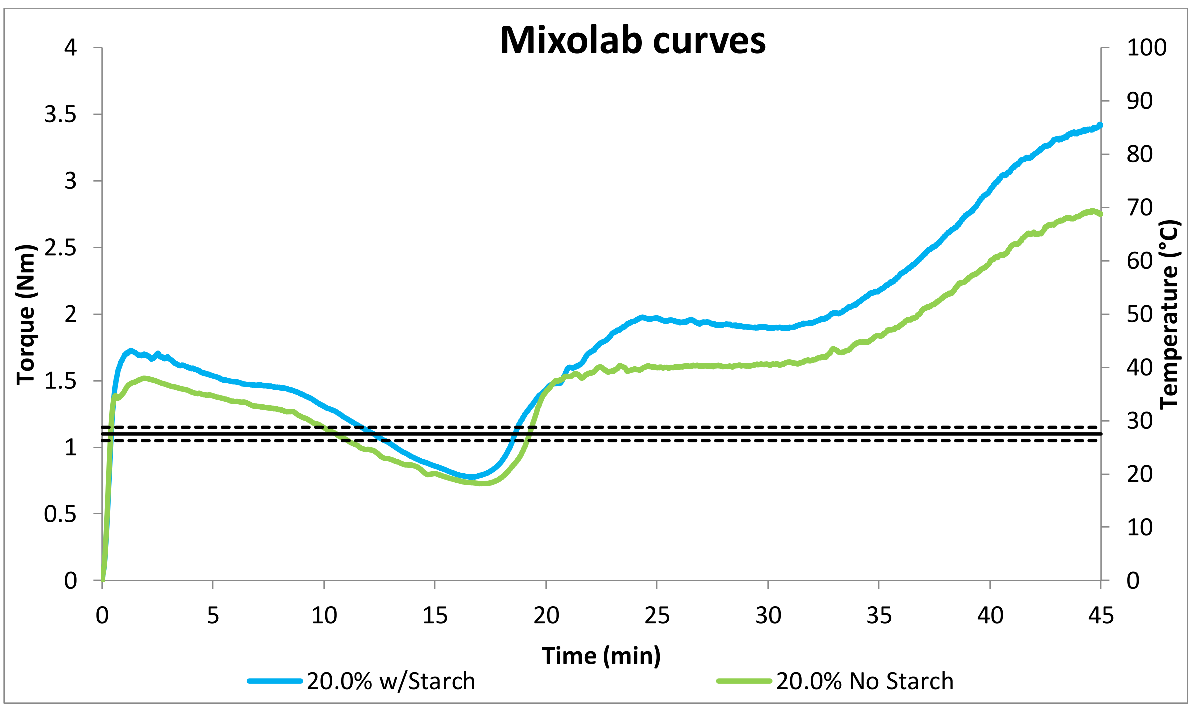


FIGURE 13: MIXOLAB® GRAPH: COMPARING MG MIX + 20% PROTEIN WITH/WITHOUT STARCH

TABLE 28: RHEOLOGICAL PARAMETERS OF 20% PROTEIN AND STARCH FOR MG MIX

Sample[1]	20% with Starch	20% w/o Starch
C1 (N m)	1.73 ± 0.01*	1.53 ± 0.01*
CS (N m)	1.48 ± 0.04ns	1.28 ± 0.0ns
C2 (N m)	0.78 ± 0.01ns	0.73 ± 0.01ns
C3 (N m)	1.97 ± 0.01*	1.61 ± 0.01*
C4 (N m)	1.89 ± 0.01*	1.57 ± 0.001*
C5 (N m)	3.41 ± 0.01*	2.75 ± 0.003*

[1] mean value of 2 replication ± standard deviation

*Values with * are significant ($P \leq 0.05$); those with ns are not significant ($P > 0.05$)*

Results showed significance between samples at C1. Post Hoc tests were performed and showed significance for rate of gelatinization (C3), gel stability (C4) and starch retrogradation (C5). The C3-C2 torque, 1.19 and 0.88 respectively, shows a higher gelatinization intensity for the starch blend. Similarly, the C5-C4 torque for the starch blend is higher (1.52 Nm). The next step to product development was understanding the importance of hydrocolloid use, which is investigated in the following section.

6.1.3 Determination of Xanthan Gum

The behavior of each protein blend was compared with and without XG (see Appendix 13 - 15). Because this research relies on the development of a blended protein, Fig. 14 and Table 29 below show how the 80:20 blend compared with and without XG.

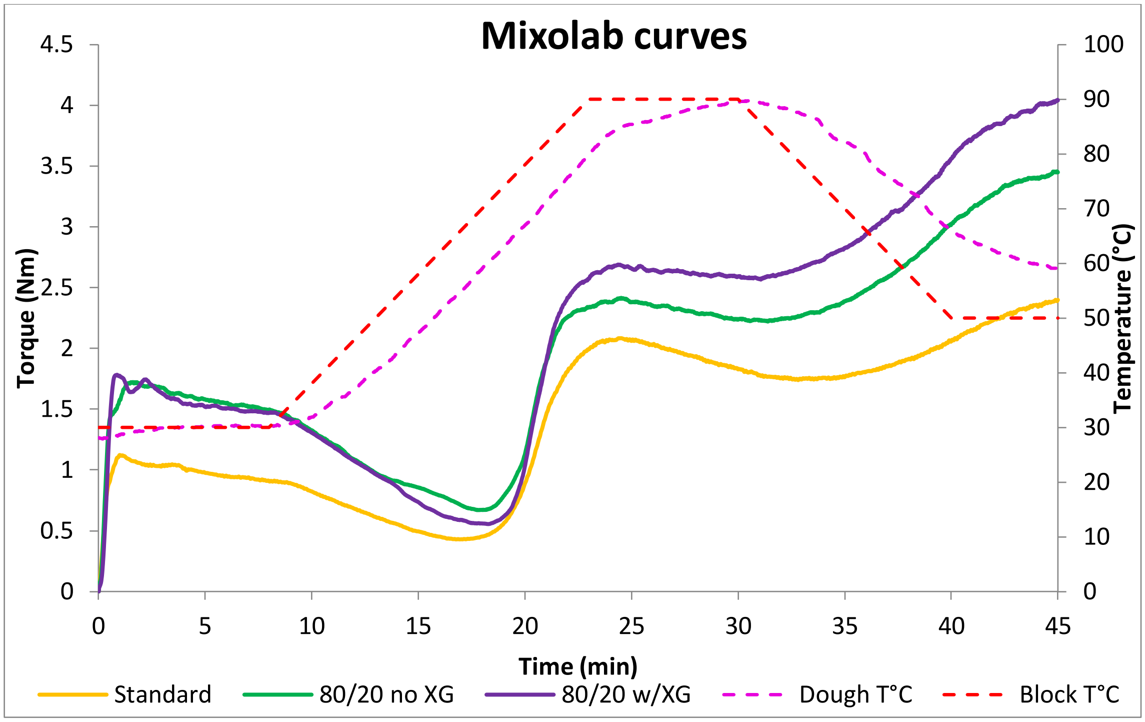


FIGURE 14: MIXOLAB® GRAPH: COMPARING 80:20 BLEND WITH/WITHOUT XG & WHEAT FLOUR

TABLE 29: RHEOLOGICAL PARAMETERS OF 80:20 BLEND WITH AND WITHOUT XANTHAN GUM

Parameter	Sample[1]	With XG	w/o XG
C1 (N m)	80:20	1.69 ± 0.14*	1.44 ± 0.00*
CS (N m)	80:20	1.40 ± 0.10ns	1.35 ± 0.00ns
C2 (N m)	80:20	0.56 ± 0.01ns	0.56 ± 0.00ns
C3 (N m)	80:20	2.68 ± 0.01*	2.31 ± 0.00*
C4 (N m)	80:20	2.55 ± 0.02*	2.12 ± 0.00*
C5 (N m)	80:20	3.94 ± 0.15*	3.33 ± 0.00*

[1] *mean value of 2 replications ± standard deviation*

*Values with * are significant ($P \leq 0.05$); those with ns are not significant ($P > 0.05$)*

ANOVA results show that there is a significant difference when XG is used. Post Hoc tests were performed and showed significance for the rate of starch gelatinization (C3), validated by C3-C2 values (2.12 and 1.75 respectively), showing higher gelatinization intensity with XG as temperature increases. The stability of the hot-formed gel (C4) and starch retrogradation during cooling (C5) are also significant, and C5-C4 is higher with XG than without (1.39 and 1.21).

6.2 Formulation of GF Recipes

After completion of the Mixolab® tests, products were developed and produced in the bakeries of the National Bakery School, London. An experimental plan was established to test formulations where both the levels of total added protein and combined protein percentages were examined.

6.2.1 Bread Dough

Basic bread formulations included the 80:20 protein blend added at 30% and 40% of the total applied starches used (75 g or 100 g respectively). When baked, initial results showed that while the addition of protein imparted a unique, nutty flavor it was not unpleasant. Visible observations concluded the 30% added protein formulation maintained an acceptable crumb structure and color, with a slightly ashier crust than the 40% added protein formulation. The 40% sample appeared darker in appearance, with a much ashier crust and an obvious odor which was unappealing. For this reason, attempts to improve the 40% formulation were abandoned. It was also determined that the MG mix would be used, and protein added at 30% of the total dry ingredients. Development bakes included removing dairy and trialing starch and no starch formulations, however it was determined that added starch was needed for the correct structure of the finished product. See Appendix 12 for final recipe.

6.2.2 Muffin Batter

Basic muffin formulations were tested with whey and 80:20 blends using the established 17.3 g added protein per recipe. Each sample baked normally and resulted in golden brown tops and good crumb structure. There was no unpleasant odor present, and the taste was acceptable for each blend. Initial visual observations showed an acceptance of color and texture to the sample with HPI/PPI blend, with the least acceptable being the whey sample which appeared darker due to browning. It was also determined that the MG mix would be used, and development bakes included removing dairy, XG, sugar and trialing starch and no starch formulations. It was determined that XG was needed for the correct structure of the finished product, however added starch was not. See Appendix 14 for final recipe.

6.2.3 Cookie Dough

Basic cookie formulations included the 80:20 protein blend protein added at 30% and 40% of the total applied starches used (60 g or 80 g respectively). Initial results showed that each sample baked normally with golden brown tops and good crumb structure. Visual observations for cookies baked at 30% showed an acceptable color and texture and had more chew than the control. The 40% formulation did not spread resulting in dense, round samples and possessed an odor that would have been unacceptable during sensory testing. Therefore, these observations led to 30% added protein being used for development. It was also determined that the MG mix would be used, and development bakes included removing dairy, XG and trialing starch and no starch formulations. It was determined that added starch was not needed for the correct structure of the finished product. See Appendix 13 for final recipe.

6.3 Nutritional Analysis

Nutritional data was collected on all test formulations and results were analyzed using ANOVA and Descriptive Statistics approaches in order to understand the impacts of adding additional protein to GF formulations and whether or not the combined protein levels have an effect on nutritional characteristics. As indicated in Table 30, the percentages of both protein and fat were measured for three variations of GF products; a control (without added protein), protein added at 50:50 and protein added at 80:20.

Tests were performed twice on each product and the standard deviation of each product, and for each measurement, was included. The variations of protein in the test products were not considered statistically different according to ANOVA results. Similarly, variations of fat in the test products were not considered statistically different, however measured difference was achieved over the control.

TABLE 30: NUTRITIONAL CHARACTERISTICS OF GF TEST PRODUCTS

Sample (g/100 g)	Control	50:50	80:20
Bread Formulations			
% protein[1]	14.75 ± 3.36	32.10 ± 2.30 ^{ns}	31.14 ± 0.50 ^{ns}
% fat[1]	0.66 ± 0.03	1.91 ± 0.13 ^{ns}	2.035 ± 0.09 ^{ns}
Muffin Formulations			
% protein[1]	10.63 ± 0.22	11.12 ± 0.37 ^{ns}	15.33 ± 1.79 ^{ns}
% fat[1]	14.47 ± 2.19	16.45 ± 1.50 ^{ns}	18.08 ± 3.00 ^{ns}
Cookie Formulations			
% protein[1]	11.44 ± 0.76	20.54 ± 1.74 ^{ns}	46.71 ± 1.23 ^{ns}
% fat[1]	20.83 ± 0.41	23.59 ± 0.04 ^{ns}	26.81 ± 5.44 ^{ns}

[1] mean value of 2 replication ± standard deviation

*Values with * represent significance ($P \leq 0.0167$); those with ^{ns} are not significant ($P > 0.0167$)*

The next step was to understand if there was a significant nutritional impact from the control to the 80:20 formulation to determine if the adjustment of protein mix affected nutritional quality, as this is the basis of all remaining testing and results. The p-values for each product variation are shown [Table 31] in relation to its incremental cohort, indicating whether the probability of a change in product variation for either protein or fat is statistically significant. The change in protein between variations and formulations do not show statistically significant increases based on the Bonferroni Correction. However, the variation of fat between the control to 80:20 cookie formulation was statistically significant.

TABLE 31: P-VALUES OF GF TEST PRODUCTS

Sample (g/100 g)	Control[a] / 50:50[b]	50:50[b] / 80:20[c]	Control[a] / 80:20[c]
Bread Formulations			
% protein[1]	*0.144[a-b ns]*	*0.59[b-c ns]*	*0.105[a-c ns]*
% fat[1]	*0.056[a-b ns]*	*0.126[b-c ns]*	*0.039[a-c ns]*
Muffin Formulations			
% protein[1]	*0.138[a-b ns]*	*0.222[b-c ns]*	*0.187[a-c ns]*
% fat[1]	-	-	*0.10[a-c ns]*
Cookie Formulations			
% protein[1]	*0.046[a-b ns]*	*0.051[b-c ns]*	*0.025[a-c ns]*
% fat[1]	-	-	*0.014[a-c]**

[1] mean value of 2 replication

*Values with * represent significance ($P \leq 0.0167$); those with [ns] are not significant ($P > 0.0167$)*

These results were further analyzed using descriptive statistics indicated in Table 32 to determine the degree of variation from one data series to another. The coefficient of variance for both protein and fat in the test products supports the ANOVA results, indicating a low variance. However, the maximum difference between variations indicates the potential for nutritional improvement is possible. Maximum difference is used to gain understanding of potential consumer preferences between paired comparisons, therefore the difference between the data points quantifies the relative importance of each compared item.

TABLE 32: COEFFICIENT OF VARIANCE OF GF TEST PRODUCTS

Sample (g/100 g)	Control [a]	50:50 [b]	80:20 [c]
Bread Formulations			
CV protein[1]	22.78	7.18	1.59
Max Difference	*21.36 [a-b]*	*2.24 [b-c]*	*19.12 [a-c]*
CV fat[1]	4.29	6.66	4.25
Max Difference	*1.36 [a-b]*	*0.15 [b-c]*	*1.46 [a-c]*
Muffin Formulations			
CV protein[1]	2.04	3.33	11.69
Max Difference	*0.60 [a-b]*	*5.74 [b-c]*	*6.12 [a-c]*
CV fat[1]	15.11	9.11	16.59
Max Difference	*2.47[a-b]*	*2.69[b-c]*	*4.19[a-c]*
Cookie Formulations			
CV protein[1]	6.60	8.46	2.64
Max Difference	*9.80[a-b]*	*28.28[b-c]*	*36.67[a-c]*
CV fat[1]	1.97	0.15	20.31
Max Difference	*3.02[a-b]*	*7.10[b-c]*	*10.12[a-c]*

[1] based on mean value and standard deviation of 2 replications

CV<10 = very good; 10-20 = good; 20-30 = acceptable; CV>30 = unacceptable

6.3.1 PROTEIN

Based on the nutritional learnings of the GF test products, the next approach was to develop products for each formulation and conduct tests to measure any potential increase in total protein percentages. After obtaining Mixolab® results indicating that more HPI would be used in all applications moving forward, products were developed with the 80:20 protein blend and tested against a control version without additional added protein. The results [Table 33] were then compared to measure potential impacts. In all three formulations, the protein increase over the control product was statistically significant, with p-values of 0.013 for bread and cookie and 0.022 for muffin.

TABLE 33: PROTEIN PERCENTAGE OF GF DEVELOPED PRODUCTS

Sample (g/100 g)	Control		80:20
Bread Formulations			
% protein²	43.46 ± 3.50		67.86 ± 1.43
P-value		*0.013**	
Muffin Formulations (MG)			
% protein²	19.01 ± 0.17		20.09 ± 0.17
P-value		*0.022**	
Cookie Formulations			
% protein²	107.66 ± 3.81		173.98 ± 10.48
P-value		*0.013**	

² mean value of 3 replications ± standard deviation

*Values with * are significant (P ≤ 0.025); those with ns are not significant (P > 0.025)*

6.3.1.1 Bread

As indicated in Table 33, the mean protein percentage and standard deviation of the control product was 43.46 ± 3.50 and the mean protein percentage and standard deviation for the 80:20 product was 67.86 ± 1.43. The addition of protein over the control formulation was found to be statistically significant with a p-value of *0.013.* These results were supported by the descriptive approach [Table 34], where the coefficient of variance for the developed bread product supports the ANOVA results, indicating a low variance. The max difference is 29.09, resulted in a 66.7% increase in protein over the control product.

TABLE 34: COEFFICIENT OF VARIANCE OF DEVELOPED BREAD PRODUCT

Sample (g/100 g)	Control ᵃ	80:20 ᵇ
CV protein²	8.06	2.12
Max Difference	*29.09 ᵃ⁻ᵇ*	

² based on mean value and standard deviation of 3 replications

CV<10 = very good; 10-20 = good; 20-30 = acceptable; CV>30 = unacceptable

6.3.1.2 Muffin

The mean protein percentage and standard deviation of the control MG control muffin was 19.01 ± 0.17 and the mean protein percentage and standard deviation for the 80:20 muffin was 20.09 ± 0.17. The addition of protein over the MG control formulation was found to be statistically significant with a p-value of *0.022* and indicated in Table 35. The results of all three formulations were compared and although there was a slight increase in protein percentage from the control to the MG control, the increase was not significant.

TABLE 35: COMPARED PROTEIN PERCENTAGES OF DEVELOPED MUFFINS

Sample (g/100 g)	Control (Soy)		MG Control (Soy)		MG (HPI/PPI)
% protein[2]	16.91 ± 1.51		19.05 ± 0.17		20.09 ± 0.17
P-value		*0.155 [ns]*		*0.022**	

[2] mean value of 3 replications ± standard deviation

*Values with * are significant (P ≤ 0.025); those with [ns] are not significant (P > 0.025)*

These results were compared against data obtained using the descriptive approach [Table 36], where the coefficient of variance for the developed MG muffins supports the ANOVA results, indicating an extremely low variance. The max difference was 3.44 and 1.36 respectively, resulting in a 12.60% increase from control (soy) to MG control (soy) and a 5.52% increase from MG soy to MG HPI/PPI.

TABLE 36: COEFFICIENT OF VARIANCE OF DEVELOPED MUFFIN PRODUCT

Sample (g/100 g)	Control (Soy) [a]	MG Control (Soy) [b]	MG (HPI/PPI) [c]
CV protein[2]	8.09	0.09	0.86
Max Difference	*3.44 [a-b]*	*1.36 [b-c]*	-

[2] based on mean value and standard deviation of 3 replications

CV<10 = very good; 10-20 = good; 20-30 = acceptable; CV>30 = unacceptable

6.3.1.3 COOKIE

The mean protein percentage and standard deviation of the control product was 107.66 ± 3.81 and the mean protein percentage and standard deviation for the 80:20 product was 173.98 ± 10.48. The addition of protein over the control formulation was found to be statistically significant with a p-value of *0.013.* Followed by the descriptive approach [Table 37], where the coefficient of variance for the developed cookie product supports the ANOVA results, indicating a low variance. The max difference was 81.62, resulting in an 61.60% increase in protein over the control product.

TABLE 37: COEFFICIENT OF VARIANCE OF DEVELOPED COOKIE PRODUCT

Sample (g/100 g)	Control [a]	80:20 [b]
CV protein[2]	3.53	6.02
Max Difference	*81.62 [a-b]*	

[2] based on mean value and standard deviation of 3 replications

CV<10 = very good; 10-20 = good; 20-30 = acceptable; CV>30 = unacceptable

6.3.2 Fat

Given HPI's higher fat content, products were tested to determine if there is a significant increase in overall fat per recipe. Table 38 represents the total tested fat percentages of the developed products.

TABLE 38: TOTAL FAT PERCENTAGE OF GF DEVELOPED PRODUCTS

Sample (g/100 g)	Control		80:20
Bread Formulations			
% fat[2]	1.95 ± 0.92		7.19 ± 1.35
P-value		*0.039 [ns]*	
Muffin Formulations (MG)			
% fat[2]	18.95 ± 0.75		20.84 ± 0.16
P-value		*0.058 [ns]*	
Cookie Formulations			
% fat[2]	21.20 ± 0.70		24.92 ± 5.06
P-value		-	

[2] mean value of 3 replications ± standard deviation

*Values with * are significant (P ≤ 0.025); those with [ns] are not significant (P > 0.025)*

6.3.2.1 Bread

As indicated in Table 39, the mean protein percentage and standard deviation of the control product was 1.95 ± 0.92 and the mean protein percentage and standard deviation for the 80:20 product was 7.19 ± 1.35. The addition of 80:20 protein blend over the control formulation was not found to be statistically significant with a p-value of *0.039.* As indicated in Table 28 the coefficient of variance for the developed bread product supports the ANOVA results, indicating a higher variance. The max difference was 7.33, resulting in a 274% increase in fat over the control product.

TABLE 39: COEFFICIENT OF VARIANCE OF DEVELOPED BREAD PRODUCT

Sample (g/100 g)	Control [a]	80:20 [b]
CV fat[2]	48.06	18.75
Max Difference	*7.33 [a-b]*	

[2] based on mean value and standard deviation of 3 replications

CV<10 = very good; 10-20 = good; 20-30 = acceptable; CV>30 = unacceptable

6.3.2.2 Muffin

The mean protein percentage and standard deviation of the control MG control muffin with soy was 18.95 ± 0.75 and the mean protein percentage and standard deviation for the 80:20 muffin with HPI/PPI was 20.84 ± 0.16. The addition of protein over the MG control formulation was not found to be statistically significant with a p-value of *0.058* and compared in Table 40. The results of all three formulations were compared and although there is a slight increase in fat percentage from the control (soy) to the MG control (soy), the increase was also not significant.

TABLE 40: COMPARED FAT PERCENTAGES OF DEVELOPED MUFFINS

Sample (g/100 g)	Control (Soy)	MG Control (Soy)	MG (HPI/PPI)
% fat[2]	16.04 ± 0.63	18.95 ± 0.75	20.84 ± 0.16
P-value	*0.062 [ns]*	*0.058 [ns]*	

[2] mean value of 3 replications ± standard deviation

*Values with * are significant ($P \leq 0.025$); those with [ns] are not significant ($P > 0.025$)*

These results were compared against data obtained using the descriptive approach [Table 41], where the coefficient of variance for the developed MG muffins supports the ANOVA results, indicating an extremely low variance. The max difference was 3.93 and 2.57, respectively, resulting in a 18.14% increase in fat from control soy muffin to MG control soy muffin and only a 9.97% increase from MG control soy to MG HPI/PPI.

TABLE 41: COEFFICIENT OF VARIANCE OF DEVELOPED MUFFIN PRODUCT

Sample (g/100 g)	Control (Soy) [a]	MG Control (Soy) [b]	MG (HPI/PPI) [c]
CV fat[2]	3.90	3.93	0.77
Max Difference	*3.93 [a-b]*	*2.57 [b-c]*	-

[2] based on mean value and standard deviation of 3 replications

CV<10 = very good; 10-20 = good; 20-30 = acceptable; CV>30 = unacceptable

6.3.2.3 COOKIE

As indicated in Table 38, the mean protein percentage and standard deviation of the control product was 21.20 ± 0.70 and the mean protein percentage and standard deviation for the 80:20 product was 24.92 ± 5.06. The addition of 80:20 protein mixture over the control formulation was not found to be statistically significant as the ANOVA test could not reject the null hypothesis. As shown in Table 42 the coefficient of variance for the developed cookie product supports the ANOVA results, indicating a higher variance. The max difference was 9.55, resulting in a 17.55% increase in fat over the control product.

TABLE 42: COEFFICIENT OF VARIANCE OF DEVELOPED COOKIE PRODUCT

Sample (g/100 g)	Control [a]	80:20 [b]
CV fat[2]	3.32	20.29
Max Difference	*9.55 [a-b]*	

[2] based on mean value and standard deviation of 3 replications

CV<10 = very good; 10-20 = good; 20-30 = acceptable; CV>30 = unacceptable

6.3.3 FIBER

Fiber testing was provided by Campden BRI, TES-AC-203 (UKAS) and indicated as total dietary fiber (TDI) per sample provided and shown in Table 43. Due to the high level of testing accuracy, validation was provided randomly on one sample and the uncertainty for the method ± 0.57% assigned to one duplicate sample which was applied to all samples. Since results were provided by external sources, a descriptive statistics approach was used, and maximum difference was determined between samples. Testing carried out on developed products only.

TABLE 43: TOTAL FIBER PERCENTAGE FOR DEVELOPED PRODUCTS

Formulation	Fiber[3] (g/ 100 g) (Mean ± Standard Deviation)
Control Bread	7.6 ± 0.57
Bread w/Protein	7.7 ± 0.57
Control Muffin	2.0 ± 0.57
Muffin MG w/Soy	3.5 ± 0.57
Muffin MG w/HPI/PPI	3.7 ± 0.57
Control Cookie	4.0 ± 0.57
Cookie w/Protein	4.1 ± 0.57

[3] mean value of 1 replication ± standard deviation

6.3.3.1 BREAD

The max difference between bread samples is 0.1, which translates to a 1.3% increase in fiber over the control formulation.

6.3.3.2 MUFFIN

The max difference between muffin samples was calculated for the difference between flours (normal GF flours and the MG mix) and between proteins (soy and the HPI/PPI blend). The control soy to control MG soy comparison resulted in a max difference of 1.5, which is a 42.9% increase in fiber. The MG soy to MG HPI/PPI comparison resulted in a max difference of 0.2, which is an increase of 5.7%.

6.3.3.3 COOKIE

The maximum difference between cookie samples is 0.1, which translates into a 2.5% overall increase.

6.3.4 GLYCEMIC ANALYSIS

Participant data was recorded and statistical analysis using ANOVA was run on the results with a standard p-value of 0.05. The results are shown in Table 44 and Table 45. The average mean blood glucose levels for all participants pre- and post-test were 4.33 and 4.68, respectively. The p-value between the pre- and post- results for all participants was 0.27. Further analysis was conducted to look at pre- and post- results by participant group (men/women). P-values for each group were 0.47 and 0.31, respectively, and not significant. Blood glucose data by participant (see Appendix 28) indicates that 22% of participant levels remained the same 2 hours after ingestion, 33% resulted in lowered blood glucose levels and 44% showed an increase.

TABLE 44: IN VIVO BLOOD GLUCOSE RESULTS FOR ALL PARTICIPANTS

Parameter	Pre	Post
Glycemic Index	4.33 ± 0.89	4.68 ± 0.53
Variance	0.79	0.23
P-value	*0.27* ^{ns}	

Pre/Post values represent mean ± standard deviation
*Values with * are significant (P ≤ 0.025); those with* ^{ns} *are not significant (P > 0.025)*

TABLE 45: ANOVA STATISTICAL RESULTS FOR ALL PARTICIPANTS

Men	***Pre***	***Post***
Mean	4.32857143	4.68571429
Variance	1.29571429	0.28809524
Observations	7	7
Pearson Correlation	0.41541794	
Hypothesized Mean Difference	0	
df	6	
t Stat	-0.9108491	
P(T<=t) one-tail	0.19874534	
t Critical one-tail	1.94318028	
P(T<=t) two-tail	0.39749069	
t Critical two-tail	2.44691185	
P-value	*0.4672258^{ns}*	
Women	***Pre***	***Post***
Mean	4.325	4.675
Variance	0.04916667	0.34916667
Observations	4	4
Pearson Correlation	0.69325575	
Hypothesized Mean Difference	0	
df	3	
t Stat	-1.5038412	
P(T<=t) one-tail	0.11483193	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.22966386	
t Critical two-tail	3.18244631	
P-value	*0.3098478^{ns}*	

t-Test: Paired Two Sample for Means: Pre/Post values represent mean of men/women
*Values with * are significant (P ≤ 0.025); those with ^{ns} are not significant (P > 0.025)*

6.3.5 HUNGER SATIETY ANALYSIS

In tandem to the GI data collection, a hunger satiety test was conducted on the intake of the developed GF products to determine if the added proteins impact hunger levels. Participants consisted of a group of 10 volunteers: 7 men and 3 women (age range

21-46 years) with no known health issues. Results are displayed in Table 46 showing the mean ± standard deviation at each 30 min interval.

TABLE 46: HUNGER SATIETY DATA BY TIME INTERVAL

Minutes	Average Hunger Level
0	5.56 ± 0.42
30	0.73 ± 1.04
60	0.88 ± 1.14
90	1.24 ± 1.18
120	1.63 ± 1.27

Values represent mean ± standard deviation

To interpret individual hunger levels, participants recorded how hungry they felt based on the following descriptors: 0-1 *Not Hungry At All*; 2-3 *Somewhat Hungry*; 4-5 *Very Hungry*; 6-7 *Extremely Hungry*. All participants began the test on an empty stomach and in a state of being *Extremely Hungry*, between 5-6 on the scale. Their results were recorded prior to ingestion and averages calculated. Thirty minutes after ingestion recorded levels of satiety were 0.73, indicating *Not Hungry At All* on the scale. Hunger levels increased slightly over the course of 2 hours though stayed at or below 2 at the 60, 90 and 120 minute marks (0.88, 1.24 and 1.63 respectively), with the exception of one participant. Participant 3 appeared less satieated than other participants from the beginning of the test, though their individual responses followed the same trend as other participants and can be seen visually in Fig. 15.

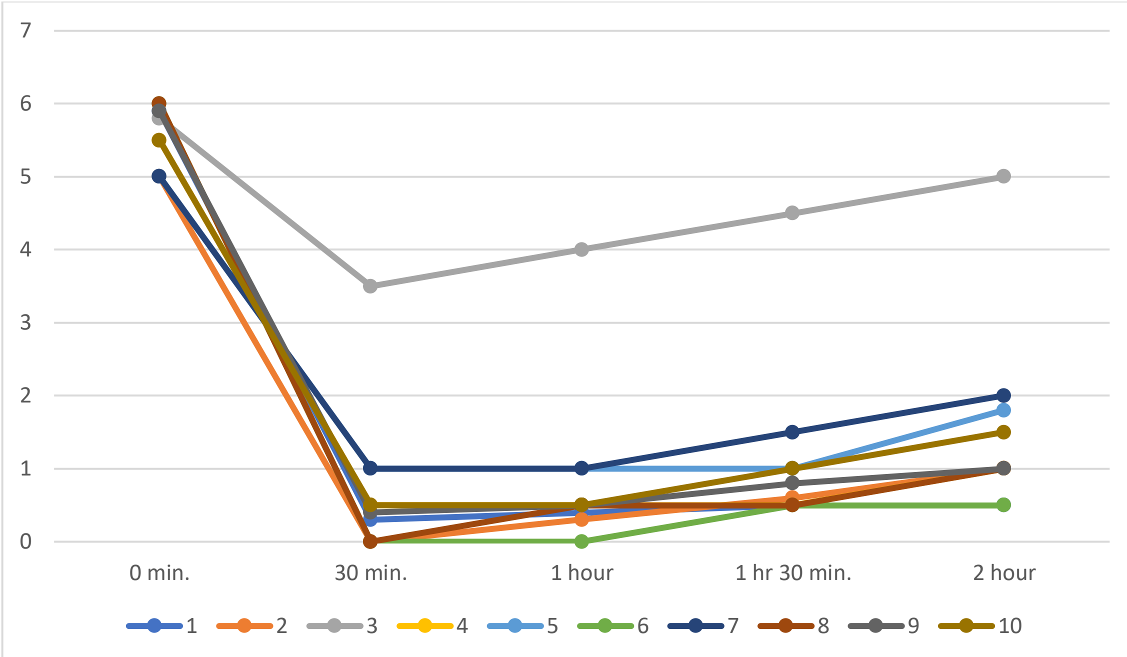


FIGURE 15: DEPICTION OF HUNGER SATIETY LEVELS BY PARTICIPANT

6.4 Sensory Analysis

Sensory testing was conducted on two groups of random participants, totaling 66 men and women. Preference Testing was used for the quantitative portion while Affective testing used to determine consumer perception of each product. The means of each parameter are represented in Table 47 and are based on a 5-point hedonic scale (5 being 'very much like'). The total mean for each product's appearance, taste and texture was 4.43, 4.37 and 4.25 respectively. ANOVA results showed p-values being 0.00, 0.001 and 0.00 respectively meaning that the null hypothesis was rejected and there is a statistical significance between at least 2 of the 3 product formulations for appearance, taste and texture. Post Hoc tests further showed the formulations that were not statistically significant were between the muffin and cookie samples.

TABLE 47: SENSORY RESULTS FOR GF DEVELOPED PRODUCTS

Parameter	Mean ± St. Dev.	P-values
Appearance		
Bread[a]	3.65 ± 0.81*	0.00[a-b], 0.00[a-c]
Muffin[b]	4.59 ± 0.50[ns]	0.00[b-a], 0.98[b-c]
Cookie[c]	4.61 ± 0.49[ns]	0.00[c-a], 0.98[c-b]
Total	**4.43 ± 0.67***	***0.00***
Taste		
Bread[a]	3.85 ± 0.67*	0.001[a-b], 0.001[a-c]
Muffin[b]	4.48 ± 0.62[ns]	0.001[b-a], 1.00[b-c]
Cookie[c]	4.48 ± 0.62[ns]	0.001[c-a], 1.00[c-b]
Total	**4.37 ± 0.67***	***0.001***
Texture		
Bread[a]	3.55 ± 0.83*	0.001[a-b], 0.00[a-c]
Muffin[b]	4.33 ± 0.94[ns]	0.001[b-a], 0.63[b-c]
Cookie[c]	4.48 ± 0.62[ns]	0.00[c-a], 0.63[c-b]
Total	**4.25 ± 0.87***	***0.00***

For hedonic test n = 46 (muffin/cookie) and n = 20 (bread)
Values represent mean ± standard deviation
*Values with * are significant (P ≤ 0.05); those with [ns] are not (P > 0.05)*

Homogeneity of variances and subsets was also tested. Using the Levene Statistic data showed that the overall taste and texture of the muffin and cookie samples were not statistically significant from each other, showing that consumers find both taste and texture between the samples equivalent. Homogeneity of subsets was also examined, and results showed that for overall appearance, taste and texture bread differs significantly with consumers showing a preference for muffin and cookie formulations. Results are presented visually in Fig. 16.

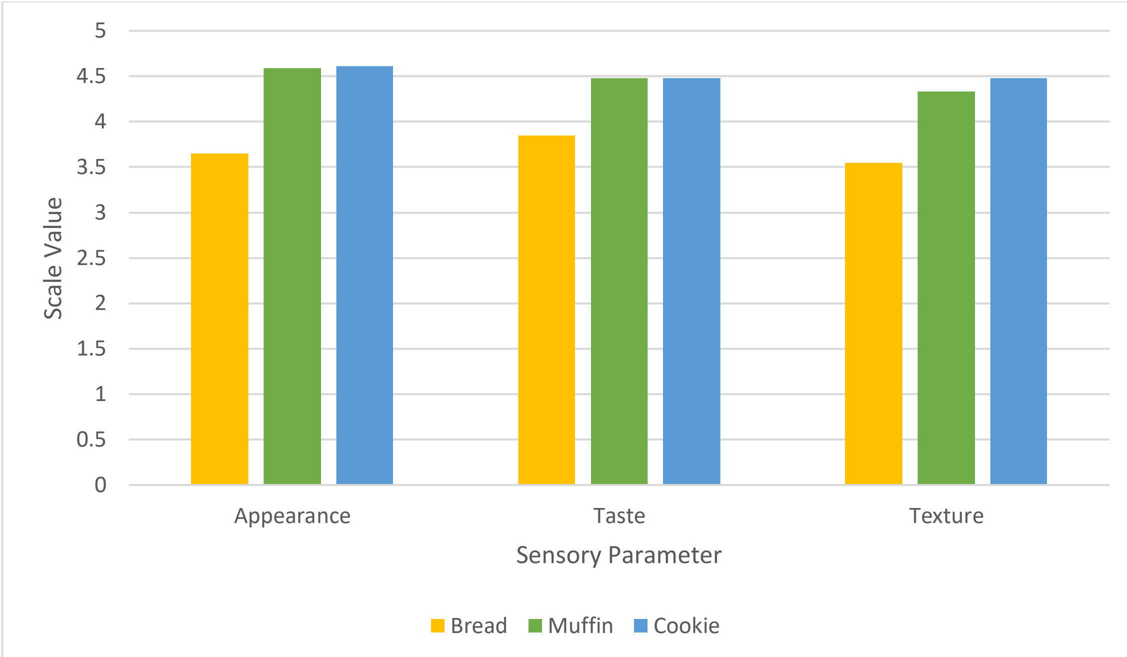


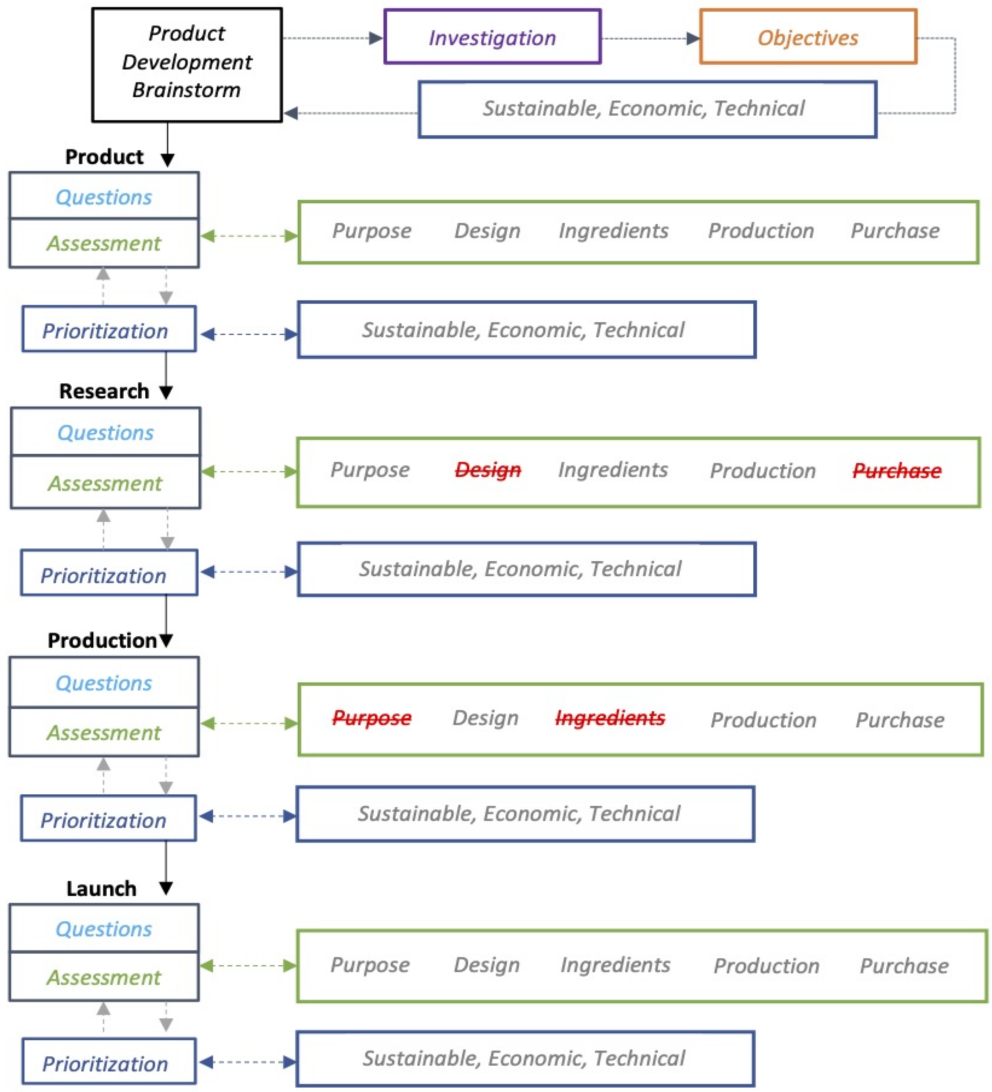
FIGURE 16: CHART OF CONSUMER PREFERENCE BY PRODUCT TYPE AND PARAMETER

(n = 46 (muffin/cookie) ± SD; n = 20 (bread) ± SD)

Results for the Affective testing included yes/no questions regarding whether or not consumers felt the products were healthier versions to products similar in appearance, taste and texture and would consumers purchase the product if given the option. Regarding taste, 97.5% of participants rated 4 or above, with 45% scoring 5 of 5. Texture scored 95% above 4 with 75% of participants rating a 5 of 5. When asked if they would purchase the products, 95% of all participants answered 'yes.' Additionally, consumers believed the bread formulation to be a healthier option over the muffin and cookie formulations, with 12.5% of participants believing the products were healthier and 45% of participants believed the products were GF.

6.5 Sustainability

Sustainable measures were achieved by following the backcasting method, which outlined objectives, set priorities and identified critical factors for the product development process. Products were then assessed for their sustainability, both from a theoretical and practical approach. A visual representation of the steps outlined for the product development process using the backcasting method is represented in Fig. 17.



Step 1: *Investigation* - Identify 'What is Needed?'
Step 2: Identify the *Objectives*
Step 3: Identify Factors for *Prioritization*
Step 4: Develop **Product Development Matrix** (Product, Research, Production, Launch)

- Phase 1: Identify **Product**
  - Step 1: Ask *Questions* to identify solutions to potential problems/issues
  - Step 2: *Assessment* of critical areas at each development phase
  - Step 3: Confirm factors for *Prioritization*
  - If successful, move to next phase
- Phase 2-4: Identify **Research**, **Production** and **Launch**
  - Repeat steps 1-3 above for each phase of **Product Development Matrix**

FIGURE 17: DEPICTION OF THE PRODUCT DEVELOPMENT BRAINSTORMING PROCESS

The initial brainstorming session began by identifying what exactly was needed (Step 1: Investigation), which involved theoretical research of GF bakery products, ingredients used and how consumers perceive them (i.e., research of available literature, articles and review/investigation of studies pertaining to the development of GF bakery products). This was followed by practical research (i.e., test baking and experimentation) which suggested that measurable nutritional improvements are possible without sacrificing product quality or flavor.

After the initial investigation, it was determined that improving product nutrition by using nutrient rich ingredients, choosing ingredient sources with more sustainable origins and creating products that were acceptable to consumers would be the focus of the product development process (Step 2: Objectives). Variables considered included ingredients (such as hydrocolloids, plant-based proteins, fibers, grains and cereals), the impact of improved nutrition (on both product quality and consumer perception) and sustainability factors (such as sustainable source and application techniques). Possible constraints included existing consumer perception of GF products, lack of available nutrient rich ingredients within GF production and production costs. Therefore, based on the defined objectives, critical success measures were identified (Step 3: Prioritization), which included sustainable, economic and technical factors. Product types were then identified to test whether or not the objectives could be achieved across three distinct GF applications: bread, muffin and cookie.

The product development process (Step 4: Product Development Matrix) was then identified and consisted of four phases: Product, Research, Production and Launch. Each phase considered all three products simultaneously and began with identifying potential issues (Step 1: Questions) by asking the following questions:

1. Is the product considered sustainable (i.e., through use of sustainable ingredients or processed in a way that minimizes loss/waste)?
2. Is the product (formulation, process technique, etc.) flexible enough to adapt to sustainable changes, if necessary?

3. Is the product cost-effective (both affordable to produce and widely available to consumers)?

Based on the scientific principles of sustainability, nutrient cycling was identified as the highest priority being both flexible enough for future sustainable changes and cost-effective for producers. Pea and hemp proteins were chosen for their lower environmental impacts and for their ability to address factors such as allergies, nutritional characteristics and cost. Sustainable perception, such as clean label concerns and use of sustainable ingredients, and consumer acceptance were identified as secondary priorities. Most hydrocolloids used in GF baking are not clean label options and investigation into whether or not they were necessary was considered. The sustainability of certain ingredients, such as starches and cereals, was also researched which investigated bioavailability, cost-effectivity and environmental impact.

Once solutions were identified for each potential issue, the products were assessed against five critical areas (Step 2: Assessment*), which included:

1. Purpose (*What is the product's purpose?*)
2. Design (*What will the product look like?*)
3. Ingredients (*Which ingredients will be used/What is the formulation?*)
4. Production (*How will the product be made?*)
5. Purchase (*How will the product be bought/sold?*)

(*It is important to note that products were assessed for areas unique to each particular phase in the event that the product needed to change due to addressing the potential issues/solutions at the start of each new phase.) The success factors (Step 3: Prioritization) were once again examined to ensure compliance with the established objectives. Once the Product Development Matrix was completed, the development of each test product began. Practical application and testing included laboratory and bakery evaluation, measurement of nutritional factors (i.e., the analysis of protein, fat and fiber contents and blood glucose testing to determine the GI impact of developed products) and sensory evaluation, with results previously outlined in this study.

7. DISCUSSION

7.1 CHALLENGES TO INTERPRETING THE MIXOLAB® DATA

There were a few challenges to interpreting and understanding the Mixolab® data. Mixolab® protocols are often adjusted to mixing and heating times, hydration levels, and amount of dough in the mixer because GF flours contain varying levels of protein, starch and fiber and can require more hydration than wheat flours. In fact, Masure *et al.* (2016) reviewed over 132 articles to establish that a conventional process does not exist for producing GF bread since a range of hydration levels, mixing and fermentation steps are used based on the formulation, resulting in widely varied techniques for analyzing the batter or dough. Therefore, one challenge encountered during this study pertained to testing at constant hydration.

Constant hydration was chosen for testing to allow for direct comparison of the protein's rheological behavior relative to wheat flour (Ziobro *et al*., 2013). The intention was not to obtain the same curve, rather obtain a comparative curve from the protein that best resembled the rheological behavior of wheat flour. The results [Fig. 6 and Table 24, page 90] showed significant differences between dough behaviors of PPI and HPI at the start of each phase (C1 - C5). Because torque differences at C1-CS (PPI 1.46 Nm; HPI 0.05 Nm) and CS-C2 (PPI 0.70 Nm; HPI 0.51 Nm) determine water absorption, the differences at the start of mixing (C1) indicated that PPI absorbs more water. As temperature increased at the start of gelatinization (C2), torque for PPI was lower than HPI indicating HPI is more resistant to mixing. The rate of gelatinization (C3), validated by C3-C2 (PPI 1.98 Nm; HPI 1.53 Nm), indicates a higher gelatinization intensity for PPI. However, the torque differences for C3-C4 (PPI 0.27 Nm; HPI 0.21 Nm) and C5-C4 (PPI 1.26 Nm; HPI 1.16 Nm) are similar, indicating that protein choice would have little impact on shelf-life. HPI exhibits the closest rheological behavior at constant hydration to that of wheat flour [Fig. 7, page 91], supporting research that HPI can be substituted for wheat flour (Pojic *et al.*, 2015; Svec and Hrušková, 2015). HPI shows greater resistance to mixing with a more stable protein network compared to PPI at constant hydration, however PPI allows for a higher gelatinization intensity. This implies that absorption potential for each protein was different and hydration levels should be adjusted if attempting to achieve similar results to wheat flour applications, which supports previous findings that protein isolates exhibit different rheological behavior at constant hydration (Marco and Rosell, 2008) and that varied hydration levels are required to achieve desired rheological properties of GF dough (Mancebo *et al.,* 2015; Rozylo *et al.,* 2015). However, if hydration levels of GF formulations are adjusted during the testing phase, and compared against wheat flour results, this might address which technical challenges need to be examined but may not address if they are necessary to achieve acceptable products.

Although the Mixolab® was designed to analyze the rheological properties of compounds used in the development of wheat-based products, it has been used to successfully obtain rheological information on GF compounds and applications, such as; sorghum flour in GF breadmaking (Velazquez *et al.,* 2012): hemp flour as an additive to wheat flour composites (Pojic *et al.*, 2015; Svec and Hrušková, 2015): effect of protein isolates (egg albumen, pea, soy and whey) and MTG on rice flour applications (Marco and Rosell, 2008): effect of chickpea protein isolate with MTG and XG in GF millet flour muffins (Shaabani *et al.,* 2018): and the role of hydrocolloids on GF bread/dough (Sabanis and Tzia, 2010; Morreale *et al.,* 2017) and fiber-rich formulations (Rosell *et al.,* 2010; Duta and Culetu, 2015). However, authors have acknowledged that rheological characteristics of GF products are based on observations of the gluten network characteristic of wheat-based products (Velazquez *et al.,* 2012; Marco and Rosell, 2008; Shaabani *et al.,* 2018). Gluten, as a specific protein, has rheological properties that are consistent across applications (Sapone *et al.,* 2012) and GF recipe formulations do not exhibit the same rheological properties as wheat-based products. Starches, hydrocolloids, enzymes, proteins, flours and fibers are included in the compositional approaches to developing GF products, although ingredient combination, hydration level, production and storage all effect the end product differently. Therefore, a direct comparison of the data obtained via testing of a GF product to its wheat counterpart can be a concern if acceptability is tied directly to uniquely wheat-based rheological and textural properties or overall baking quality (Mazzeo *et al.,* 2013) when acceptable results are still achievable (Lazaridou *et al.* (2007) and could be influenced by other factors (Siegrist *et al.,* 2015; Peschel *at al.,* 2016).

Studies using the Mixolab® have successfully shown the comparisons of product and dough quality in order to improve the textural and nutritional characteristics of bakery products. However, the quality features of the GF products were interpreted analytically, which suggests more of an academic concern to improving the nutritional quality of GF products (Sciarini *et al.,* 2010b; Sumnu *et al.,* 2010). For example, Duta and Culetu (2015) found that the addition of oat bran to GF cookies resulted in lower overall acceptability but were deemed acceptable because the scores were higher than 5 (from 5.6 – 7) on a 9-point hedonic scale. However, authors findings were based on ANOVA results. And while data can tell us if there is a significant difference between samples based on calculated means, observed differences within practical applications may be too slight to be considered significant and may not address concerns that available GF products result in lower consumer satisfaction (Araujo and Araujo, 2011). Therefore, data alone should not be responsible for the narrative. It might be suggested that interpreting the Mixolab® data could extend beyond simply comparing dough rheology and how the behavior of GF compounds relates to our understanding of wheat-based products, however, using and interpreting the data and applying the results to developing products that also rate high among consumers is also important for producers.

7.2 Developing the Correct Protein Mix

The primary differences between PPI and HPI are at the initial mixing and heating stages. When observing the behavior of each protein, initial results showed that PPI is less resistant to mixing and has a higher gelatinization intensity than HPI at constant hydration, drawing the conclusion that the additional free water in the PPI mix contributes to its gelatinization intensity. This corroborates the previous findings on cowpea protein isolates in GF rice muffins (Shevkani *et al.,* 2015) and that protein type influences the availability and amount of water absorbed for starch granules, which influences paste viscosity and temperature, an indicator of starch gelatinization. Preliminary tests also showed both HPI and wheat flour appear to have similar mixing behaviors, stability and rate of starch gelatinization and supported by previous research on incremental additions of hemp to wheat flour composites (Pojic *et al.*, 2015; Svec and Hrušková, 2015), suggesting that less PPI and more HPI added to GF formulations may result in products that exhibit similar rheological properties to that of wheat-based products. Existing research shows that protein source varies depending on formulation and type of starches/flours used, and that a mix of proteins could provide acceptable texture for GF products (Shaabani *et al*., 2018). PPI can retard staling of starch-based breads while HPI can also extend shelf life (Ziobro *et al.,* 2016). Therefore, the Blending Law tool of the Mixolab® software was run to verify if it could be used to predict the protein combination that would yield a similar rheology to wheat flour.

ANOVA results showed differences between the actual and simulated tests at the start of heating and gelatinization (C2) [Fig. 8 and Table 25, page 92] but no significant difference between proteins. However, Post Hoc tests revealed significance at C2 with p-values of 0.004 and 0.02 and differences between CS-C2 for PPI and HPI being 0.6 and 0.97, respectively. A similar challenge occurred comparing the actual and simulated 80:20 blend. ANOVA results showed differences between the two tests at the start of mixing (C1) [Fig. 11 and Table 27, pages 94 - 95] and Post Hoc tests revealed significance with a p-value of 0.01. And while further testing would be needed to fully understand what caused this difference, ultimately the curves of both tests between blends were similar enough to confirm the use of the Blending Law. Therefore, examination of the predicted blends showed the 80:20 HPI/PPI blend most similar to that of wheat flour, being less resistant to mixing and heating and with a higher absorption potential due to the PPI. Further comparison of the Blending Law predicted results to the actual 80:20 tested results confirmed this decision.

7.3 Importance of Starch Activity

The development process presented an opportunity to replace the original GF blend include with a developed GF multi-grain (MG) mix to determine the importance and impact of starch from naturally occurring GF flours and more commonly used starch sources. Initial recipe formulation focused on the addition of protein only, however concerns came up during the development process that adding proteins while not reducing sugar, starch or increasing available nutrients as further means to positively impact GF formulations may be counterproductive to this research. As recipe formulations are unique to each product, this presented an opportunity to trial formulations that use ingredients that would naturally add fiber and protein as well as positively impact nutritional characteristics of developed products. If successful, such a formulation that was versatile enough for various GF applications could have wider industry impact. Therefore, a GF MG mix which could be applied to all recipe formulations was developed (see Appendix 29 – 32 for nutritional breakdown and impact on RDA). Understanding the potential rheological impacts of the MG mix enables producers to make more informed decisions about product development. Therefore, the mix was tested with 10% and 20% added protein blend, with and without added starch (see Appendix 8 - 11).

Besides water absorption, curves for each added protein percentage for the MG blend with added starch were basically parallel (see Appendix 8), which supports the importance of hydration (Rozylo *et al.,* 2015). Torque differences were also similar indicating that the protein blend does not impact the mixing behavior nor the starch behavior in this formulation (see Appendix 9). Torque differences for C3-C2, C3-C4 and C5-C4 were also similar and would suggest the addition of protein levels makes no difference in terms of shelf-life impact (Ziobro *et al*., 2013; Ziobro *et al.*, 2016). Therefore, the next test performed on the MG mix was to determine the importance of starch used.

Results [Fig. 13 and Table 28, pages 96 - 97] showed significance at C1 only for the 20% addition of protein and indicate that the added protein can absorb more water and is less resistant to mixing (Svec and Hrušková, 2015; Sahagun *et al.,* 2018). The torque for the 20% blend is also significantly higher at start of gelatinization (C2) and suggests adding protein promotes emulsification. However, in order to truly understand the impact of starch, results for the 20% added protein formulation were compared with and without added starch. The torque for two blends at start of gelatinization (C2) is relatively similar and not significant, suggesting that a quality of the protein mix used promotes emulsification (Ziobro *et al*., 2013; Ziobro *et al*., 2016) which was indicated in initial protein tests. However, the C5-C4 torque for the starch blend is higher indicating a shorter shelf-life. Essentially, the significance of starch seems to be higher water absorption potential and shorter shelf-life (Hager and Arendt, 2013; Korus *et al.*, 2015; Martinez and Gomez, 2016). And while statistical results during Mixolab® testing might not show significant difference between tested versions, practical applications could result in differences that may not affect sensory acceptance and should be considered.

7.4 IMPORTANCE OF XANTHAN GUM

XG is among the most commonly used hydrocolloids in GF baking and was chosen for this research because of its availability, cost and sustainable impact, as well as its water binding capabilities and overall effect on GF formulations, including its nutritive potential as a source of available fiber and its effect on lowering the GI of the final product (Lazaridou *et al*., 2007; Sciarini *et al*., 2010b; Hublik, 2012; Matos and Rosell, 2015; Liu *et al*., 2018; Shaabani *et al*., 2018). Therefore, all protein blends were tested with and without XG (see Appendix 13 - 15 for results). Though the resulting curves visually indicate that the torque differences are not much higher with XG than without, data analysis confirmed that there is a significant difference when XG is used [Fig. 14 and Table 29, page 98].

It would appear XG impacts the weakening of the dough during mixing (C1), with the exception of the 50:50 blend where significance was not obtained, meaning that formulations including XG would be less resistant to mixing (Lazaridou *et al.,* 2007; Hager and Arendt, 2013). For each blend, C5 and C5-C4 are higher with XG than without indicating the addition of XG may shorten product shelf-life (HPI 1.55/1.16; PPI

1.51/1.26; 50:50 1.47/1.15; 80:20 1.39/1.21) contradicting previous research that combining hydrocolloids and proteins could retard staling (Ziobro *et al.,* 2016). However, for the 80:20 blend Post Hoc tests showed there was no significance at CS which implies XG does not affect dough stability for that formulation. The 80:20 blend was compared to wheat flour with and without XG [Fig. 14, page 98] and it was noticed that while both curves appeared similar the blend without XG resulted in a more parallel curve (i.e., behavior) to wheat flour especially at C3-C2, suggesting that in the context of this formulation XG is unnecessary. The main difference between samples is that higher gelatinization intensity is achieved with XG than without, however results would ultimately depend on the final recipe formulation and sensory testing.

Previous research investigating GF formulations have applied various hydrocolloids to mimic the viscoelastic properties of gluten, some suggesting that the use of a hydrocolloid is dependent on the formulation and might not be necessary. Ziobro *et al*. (2013) found using non-gluten proteins as structure forming agents in GF bread produced acceptable results but required optimization of blends due to differences in water binding capacity and emulsification properties. Sciarini *et al.* (2012b) showed that additives, such as emulsifiers, hydrocolloids and enzymes are not essential for GF breadmaking. Therefore, it can be concluded that while XG does affect the gelatinization intensity of GF dough, it is not a necessity and could be removed in certain recipes if clean label concerns are an issue as long as the product itself passes sensory testing among consumers.

Witczak *et al.* (2012) and Ziobro *et al.* (2012) suggest that some hydrocolloids can have an impact on the product recipe of up to 15% while Krupa *et al.* (2010) suggests that clean label hydrocolloids might be more acceptable to consumers. Mixolab® testing showed that XG was not necessary and would likely depend on the acceptance of the end product, which could help address clean label concerns while sensory testing without the use of XG showed positive consumer acceptance and the potential to create GF formulations without a hydrocolloid, supporting claims that quality GF bread products can be produced without the need for additives (Sciarini and Ribotta *et al*., 2012). Mixolab® results also indicated that a quality of HPI promotes emulsification, suggesting it may also be possible to reduce the need for hydrocolloids in GF baking by using use fiber-rich ingredients, but this would need to be investigated further.

7.5 Recipe formulations

Previous research indicates that 20-30% additional protein seems to be the standard threshold for acceptability in sensory testing of bread products (Korus *et al*., 2017; Sahagun *et al*., 2018) and positive results up to 10% added for testing of protein-rich flour mixes in cookies (Tanwar and Dhillon, 2017). Since objectives of this study include understanding both the rheological and nutritional impacts of higher protein levels and associating them with high consumer acceptability, initial test bakes were performed with 30% and 40% total protein added (see Appendix 5 and 7). The purpose was to understand if there would be any rheological impacts on combining these proteins together and if higher amounts of added protein could be achieved.

7.5.1 Bread Dough Development

For purposes of data collection, bread recipes were tested at both 50:50 and 80:20 HPI/PPI concentrations to show the impact of additional HPI. Initial formulations showed both 50:50 and 80:20 blends resulted in dough that held its shape quite similar to wheat dough and appeared resistant to mixing, which could be an indication of the quality of HPI used and dough rheological properties during mixing (Svec and Hrušková, 2015). When baked, both blends appeared similar in terms of visible observations to crumb structure, ash and color. Initial sensory observations showed that while the addition of protein imparted a unique flavor to the dough, it was not unpleasant and had a nutty taste. These observations support previous research which suggests that adding protein at 20%-30% is an acceptance threshold and supports previous research (Korus *et al*., 2017; Sahagun *et al*., 2018), but also suggest a measured success is possible. And while the 40% added protein samples appeared darker in appearance with an ashier crust most likely due to the additional hemp protein, they were more dense and likely due to the hydration not being adjusted to compensate for the extra protein and may have resulted in better bread quality (Rozylo *et al.,* 2015). Developed bread recipes also included XG, as research has shown its effect on overall bread quality (Lazaridou *et al.,* 2007; Sciarini *et al*., 2010b; Hager and Arendt, 2013; Liu *et al.,* 2018).

7.5.2 Muffin Batter Development

Because muffins require a protein source (typically from egg and milk), the control sample contained a protein. The amount of added protein was assessed to be 13% which was determined as a 75% contribution of protein from milk/egg [(13 x 100)/75 = 17.3 g] (Matos *et al*., 2014). Therefore, protein was substituted in ratios to maintain total of 17.3 g per recipe. To understand the protein's effect on the rheological properties of the muffin batter and to understand the impact of the HPI, samples were tested with 50:50 and 80:20 blends. The control formulation included WPI. However, soy protein was substituted in the development stage to better compare the nutritional impacts of plant-based proteins within the formulation. Each sample baked normally and resulted in acceptable color and crumb structure, the least acceptable being the whey sample which appeared darker due to browning, supporting previous findings (Gallagher *et al.,* 2003; Matos *et al.,* 2014). All samples had acceptable taste and likely due to the amount of sugar (Sahagun *et al.,* 2018).

7.5.3 Cookie Dough Development

For the cookie formulations, recipes included protein added at 30% and 40% with both 50:50 and 80:20 blends to understand how the protein would affect each recipe. Initial results showed that each sample baked normally however more spreading occurred in the control sample (i.e., no protein) indicating that the protein added affects hydration slightly and might need adjustment (Rozylo *et al.,* 2015). Visual observations for cookies baked at 30% with both blends showed an acceptable color and texture, with the least acceptable being the control sample which appeared darker due to browning and may have been due to the GF flours not replaced by protein (Tanwar and Dhillon, 2017). The 80:20 blend sample had more chew than expected which could be due to HPI's ability to retain more water during the baking process. In fact, the 80:20 blend showed very little staling when left at room temperature for 3 days uncovered, supporting findings that hemp supplementation prolongs shelf-life (Svec and Hrušková, 2015). The 40% formulation was unacceptable in both appearance and odor.

7.6 Nutritional Improvements

The next approach was to compare test and developed formulations to see if there is a significant increase in protein, fat and fiber and to what degree formulation plays a role in nutritional characteristics, including glycemic response and hunger satiety.

7.6.1 Protein

For the bread and cookie products, 30% total added protein was determined in test production. Therefore, during the development phase a total amount of 30% protein was factored in to maintain consistency and resulted in two samples; a control without added protein but around 5% existing protein from GF flours used, and one with 25% added HPI/PPI protein. Although no statistical significance was achieved in the testing phase, significance was observed in protein levels of the developed products.

The max differences for the test/developed bread products were 19.12 and 29.09 respectively, resulting in a 66.7% protein increase over the control product. For the text/developed cookie products the max differences were 36.67 and 81.62, respectively, resulting in a 61.6% protein increase over the control product. Results indicate that nutritional improvement is possible, and findings concur with previous research that partial replacement of starch with protein in GF formulations can increase overall nutrition (Alvarez-Jubete *et al*., 2009; Ziobro *et al*., 2013; Matos *et al*., 2014; Korus *et al*. 2015 and Tanwar and Dhillon, 2017). Interestingly, the amount of protein added between both recipes was similar while the cookie resulted in a greater overall increase. The calculated protein levels of the cookies differ significantly in the developed products with calculations supporting those similar to the Kjeldahl analysis method for higher protein foods (Mæhre *et al.,* 2018). These results are likely due to the reliance on higher protein flours in addition to the existing proteins in the formulation itself, and possibly the ratio of total protein to recipe weight, suggesting that serving size relative to formulation might also be a factor.

WPI was used in the test muffin formulations and did not differ significantly from the soy test products. Therefore, the control formulations for the developed products used soy for a more accurate comparison of the impact of plant-based proteins. The development phase also included a secondary test involving the MG mix developed during rheological testing. For the test muffin formulations, protein percentage for the control (whey), 50:50 and 80:20 formulations were 10.63, 11.12 and 15.33 respectively [Table 30, page 101]. Because the muffin formulation required a protein source, the amount was the same for all three variations. Surprisingly, the whey and 50:50 HPI/PPI variations were similar and showed no significant difference, which differs with prior research on dairy powders as protein enhancements to GF products (Gallagher *et al*., 2003; Matos *et al*., 2014) but confirms that combinations of plant-based proteins could provide protein content comparable to animal-based proteins (Gorissen *et al.*, 2018). And while the difference between protein contents for the control and 80:20 test formulations was not statistically significant (10.63% and 15.33%) results indicate measured improvement is possible. Additionally, there was significance between the developed MG Soy and MG HPI/PPI formulations (19.05 and 20.09 respectively [Table 35, page 105]), suggesting that the type of protein used could have an impact on protein quality. This reinforces previous findings that hemp can significantly increases nutrition (Pojic *et al.,* 2015; Korus *et al.,* 2017). And although there is an increase in protein percentage from the developed control (soy) to the MG control (soy) (16.91 and 19.05 respectively), the increase was not significant. However, the mean protein values for the control test and developed products were 10.63 and 16.91, respectively. Interestingly, the control sample with WPI contains less overall protein which supports research that there is potential to replace animal-based proteins in GF formulations with plant-based proteins without compromising nutritional quality (Mattila *et al.,* 2018). And while whey protein is more acceptable than soy in protein-enriched GF cakes (Sahagun *et al.,* 2018) there is potential to substitute plant-based proteins in GF products for those who suffer from lactose intolerance, as long as sensory acceptance can be achieved.

Based on the initial nutritional findings, there was no significant impact on the protein percentage within the test products however there was significant increase with the developed products. This indicates that while adding protein can have an impact, formulations play a critical role to significantly adding protein and that nutritional improvement is possible incrementally by protein source, either by protein isolate or high protein multigrain. Since PPI and HPI are rich in amino acids and, as studies have shown, produce less allergic reactions in the body they could be used to significantly improve the nutritional value of GF baked products, suggesting plant-based proteins might be the way forward.

7.6.2 FAT

Fat was evaluated as part of the research because it is a necessary component to most GF baked products, often coming from saturated fats (Thompson*.,* 2000). However, hemp protein is high in omega fatty acids so this research investigated whether potential fat increases can be attributed to healthier fats.

The mean fat values for the test control, 50:50 and 80:20 bread formulations were 14.75, 32.10 and 31.14 respectively and values for the developed control and 80:20 bread formulations were 1.95 and 7.19 respectively. And while the results were varied, fat values between bread versions did not differ significantly. However, the max difference of the developed bread product [Table 39, page 107] resulted in a 274% increase over the control product, indicating that fat found in hemp does have an impact on the nutritional characteristics of bread products.

For the muffin formulations, the mean fat percentages for the tested control (whey), 50:50 and 80:20 variations were 14.47, 16.45 and 18.08 respectively, and not significant. Testing of the developed muffin formulations also consisted of the fat impact between plant-based proteins (i.e., soy vs. HPI/PPI) and a developed MG mix. The mean fat percentages of the developed control and 80:20 formulations were 18.95 and 20.84 respectively, and not significant, indicating the fat from HPI had little effect on the nutritional characteristics. Interestingly, the biggest increase in fat was from control (soy) to MG control (soy), where the max difference was 3.93 based solely on the MG mix [Table 41, page 106]. The max difference between the soy and HPI/PPI blends was 2.57, indicating a greater variability of fat content from the alternative grains used in the MG mix (Alvarez-Jubete *et al.,* 2009; Moreno *et al.,* 2014).

Results for the mean fat values of cookie formulations also did not differ significantly between versions, nor did they differ significantly between test/developed products. Furthermore, the max difference for the control/80:20 test cookie comparison was 10.12 (versus 3.02 for the control/50:50 comparison) and the max difference for the developed control/80:20 comparison was 9.55, a 17.55% increase over the control product [Table 42, page 108], again suggesting that the added hemp protein could have an effect on nutritional quality (Pojic *et al.,* 2015; Korus *et al.,* 2017).

Overall, there was no significant increase in fat in the test products except with the control to 80:20 cookie formulation where the p-value was 0.014, which was significant [Table 31, page 102], indicating the increase in fat might come from the added hemp protein (Mattila *et al.,* 2018). Interestingly, the fat measurements between the control and 50:50 bread and cookie test formulations within this study showed a p-value of 0.06 (rounded from 0.05587) and 0.06104 respectively. And while the results were not significant, the data indicates that it might be possible to achieve statistical significance with these formulations if more replications were included in the mean values. Additionally, the higher fat percentages and lack of significance between variations could be a result from the overall fat required of the formulation and not from the addition of the proteins (Missbach *et al.,* 2015). Interestingly, Marcilio *et al.* (2005) found that fat combined with amaranth in GF biscuits had a positive influence on flavor, suggesting that fat could have mechanical and sensory value rather than nutritional one (Matos and Rosell, 2015). And if so, it would be worth investigating if quality of fat source plays a role in consumer perception.

7.6.3 Fiber

Fiber can be added to GF bakery products either by adding fiber-rich flours or hydrocolloids, and hemp is considered a good source of dietary fiber (Mattila *et al.,* 2018). Therefore, the purpose of this research was to investigate if fiber could be added to GF baked products simply by including HPI to the formulation. The developed bread formulation resulted in a 1.3% increase of fiber over the control, while developed cookie formulation resulted with an increase of 2.5% over the control. This supports research that hemp can replace starch/flour to incrementally improve nutrition (Pojic *et al.,* 2015). And although a good source of fiber, HPI provides less overall fiber than hemp flour (Korus *et al.,* 2017). Surprisingly, the protein added muffin formulations resulted in an increase of 42.9% from soy control to MG soy. However, the MG soy to MG HPI/PPI comparison resulted in an increase of just 5.7%. Soy protein isolate contains 6 g / 100 g fiber while the nutritional label for hemp protein isolate used contains 5.5 g / 100 g fiber, which indicates a loss of fiber during the refinement process from hemp seeds, which contain up to 16.4 g / 100 g dietary fiber (Svec and Hrušková, 2015). The increase in fiber from control/MG versions supports findings that fiber-rich ingredients might replace up to 20% rice flour in GF formulations (Gularte *et al.,* 2012). Also, the slight increase from MG soy and MG HPI/PPI versions indicates that the use of proteins does not have a negative impact on overall fiber levels, and it is possible to increase fiber levels of GF products incrementally by the addition of HPI (Mattila *et al.,* 2018), though perhaps not significantly. The results found in this study could also support findings that HPI can be used as a nutritive supplement (Girgih *et al.,* 2010) although TDF may have a greater impact when added via fiber-rich flour sources rather than by added protein.

7.6.4 Glycemic Response

This study followed the approaches outlined by Scazzina *et al.* (2015) and Mollard *et al.* (2018), whereby the GI for a variety of GF products was compared against industry established low, medium and high levels and treatments were randomly consumed. Results for the average mean BG levels of all participants, shown in Table 44 and Table

45 [pages 110 - 111], were 4.33 (pre) and 4.68 (post). Blood glucose levels for healthy individuals range from 3.6 (low) to 11.4 (high) (Mayo Clinic, 2020). The p-value between pre- and post- results was 0.27, indicating there is no significant increase in blood glucose after ingesting the products supplemented with the HPI/PPI mix. In fact, 33% of participants had lower BG levels two hours after ingestion, which supports previous findings that GF bakery products with certain ingredients might lower postprandial glycemic response (Scazzina *et al*., 2015). Results also indicate support of previous research that pea and fiber together control glycemic response (Smith *et al.*, 2012; Mollard *et al*., 2014) and that hemp protein can improve glycemic control (Mollard *et al*., 2018). Findings also support research that up to 30% protein added to GF products can help lower GI (Moghaddam *et al*., 2006). Due to the COVID pandemic, participants were only able to participate in one testing session. And although findings to the current study are limited, there were limitations to previous studies as well.

Smith *et al.* (2012) asked participants to fast overnight, however subjects ate a standardized breakfast containing a sugared cereal, milk and orange juice before the test. Also, the time between breakfast and the start of the test varied by subject. Mollard *et al*. (2018) also obtained GI levels after the ingestion of a fixed meal. And while results of these studies show ingredients can impact BG levels, pairing them with meals could have impacted the results. In fact, Smith *et al.* (2012) found that when an ad lib meal was consumed 120 minutes later, pea protein had no effect on BG or appetite before or after the meal and Mollard *et al*. (2018) found that 40 g treatment of hemp protein resulted in higher BG response following a meal consumed 60 minutes later. Additionally, while Smith *et al.* (2012) and Mollard *et al*. (2014) show the presence of certain ingredients, such as fiber and protein, can reduce glycemic response in bakery items, the studies did not involve GF products which typically have a higher GI than wheat products and can spike BG levels (Matos and Rosell, 2011; de la Hera *et al.*, 2014). And while Scazzina *et al.* (2015) obtained results from developed GF products, the study did not have a standardized approach to the actual ingredients used.

Furthermore, Westman *et al.* (2008) found that dietary modification of low-carbohydrate products resulted in improved glycemic control over a low glycemic diet in those with type 2 diabetes. Moghaddam *et al.* (2006) found that protein and fiber together in food products control glycemic response more so than added fat. And while products tested in this study were neither low in carbohydrate nor low fat, it would be interesting to test low carbohydrate/fat products supplemented with HPI/PPI mix to see if results remain consistent.

Although the current study did not compare blood glucose levels against a GF or wheat control, results were obtained from developed GF bakery products as independent samples and for the ingredients used. Therefore, taking into consideration that GR for GF products is higher than those containing wheat (Berti *et al.,* 2004; Scazzina *et al.,* 2015; Johnston *et al.* 2017), the goal was to understand if products containing a combination of proteins and ingredients could help keep blood glucose levels low. For now, the results conclude that the HPI/PPI mix has the potential to control GI levels when added to GF bakery products. However, studies should be engaged to ensure repeatability and perhaps testing against non-protein versions and by product type for more accurate comparison.

7.6.5 Hunger Satiety

Prior research suggests that PPI affects hunger levels (Meulen *et al.,* 2010; Landero *et al.,* 2014; Mollard *et al.,* 2014) and soluble fibers can delay gastric emptying (Scazzina *et al.,* 2015; Bae *et al*., 2016) and thereby affecting satiety. The purpose of the satiety test was to determine if using a unique HPI/PPI combination within bakery items supports previous findings. Although participants recorded their responses on a 7-point scale, the only two levels clearly defined were 0, being *Not Hungry At All*, and 7, being *Extremely Hungry*. It was important not to influence response and necessary for participants to be as honest as possible with how hungry they felt, so they were not given descriptive hunger levels from points 1 through 6. Even without these markers, participants recorded their individual hunger levels at similar points on the scale, with the exception of one participant who appeared approximately 50% less satieated than other participants from the beginning of the test. Results have shown individual responses to hunger levels from fasted state through the end of the test followed the same linear trend, suggesting that PPI and HPI together can affect appetite suppression [Fig. 15, page 113]. These findings support previous research that pea protein has an effect on satiety (Landero *et al.*, 2014; Smith *et al.*, 2012) and seem to contradict findings that protein and fiber levels do no effect appetite levels (Mollard *et al.*, 2014). Since the amount of HPI (80%) was greater than PPI (20%) it could be suggested that the fiber level found in the HPI has an effect on satiety, however HPI has previously not been studied for its satiating effects. Interestingly, this could contradict previous findings that manipulating protein source has no effect on satiety levels (Lang *et al.,* 1998) although more testing should be done.

7.7 Consumer Acceptance

During this study, the approach to consumer acceptance was twofold; to identify if acceptance of healthier GF baked products was possible based solely on taste and texture without comparing them to gluten-containing products, and to gage acceptance of the products without the use of a hydrocolloid. Sensory testing was therefore conducted on finished products made with the developed protein blend, making this research unique. The intent was to understand whether consumer preference exists for more nutritious GF products, and if this factor is important to them. The results support previously published results on the increased desire, need and market for GF products, specifically research around GF products lacking nutrition.

Regarding overall taste, 97.5% of participants rated the products 4 or above on a 5-point hedonic scale (5 being 'Very Much Like'), with 45% of participants rating them 5 of 5. Regarding overall texture, 95% of participants rated products 4 or above for acceptance, with 75% of participants rating 5 of 5. When asked if they believed the products to be GF, 55% of participants answered 'no.' These results indicate that not only is it possible for GF products made without a hydrocolloid to achieve high consumer acceptance, doing so without comparing them to rheological or sensory qualities of gluten-containing products resulted in acceptance of the qualities of the product itself. This supports previous research that food innovation and taste play critical roles to consumer acceptance (Farmer *et al.,* 2017; Verain *et al.*, 2015). And while most research attempts to identify which combination of ingredients in GF applications can replace the qualities of gluten resulting in a more gluten-like product and often with lower sensory acceptance scores when compared to a gluten containing product, one approach to increased consumer acceptance could involve changing perception to avoid a like for like comparison.

Regarding the perception of nutrition, participants believed the bread formulation to be a healthier option over the muffin and cookie formulations, however this did not affect acceptance. In fact, only 12.5% of participants believed the products were healthier options suggesting consumer preference was based on taste and texture rather than any perception of health. And 95% of all participants surveyed during the sensory evaluation said they would purchase the products if offered, suggesting that purchase motivations are based on taste rather than on labeling or education (Siegrist *et al.*, 2015). This supports findings that positive sensory results can be achieved by adding HPI up to 30% to GF bread products (Korus *et al.,* 2017) and questions the common belief that taste acceptance in GF breads is a challenge while acknowledging the variability of formulation (Gallagher *et al.,* 2003; Matos and Rosell, 2011; Matos and Rosell, 2013). Interestingly, 30% is a higher percentage than has previously been tested as 'acceptable' in GF products (Svec and Hrušková, 2015; Korus *et al.,* 2017; Sahagun *et al.*, 2018). However, it is not clear if consumer acceptance would be affected by higher protein levels. These results suggest that making GF products more nutritious would not have an adverse effect on acceptability and positive consumer acceptance is possible for GF products based solely on their taste, texture and appearance, rather than a rheological comparison to gluten-containing products.

Measuring the acceptability of a GF product against the characteristics obtained from a product containing gluten, especially if consumer acceptance is based on this correlation, could have an effect on otherwise acceptable products. And while Sabanis and Tzia (2010) actually conducted sensory testing with a trained panel, panelists still compared the GF bread samples to 'real bread.' And although acceptance scores were overwhelmingly high for this study, investigation into consumer acceptance of developed GF products was not compared with properties of gluten-containing products. Perhaps, then, the approach is not about finding a combination that replaces gluten rather mimics its behavior enough to results in a quality product and involves changing consumer perception away from a like for like comparison.

The nutritional information for the developed GF formulations used during sensory testing were also compared against nutrition labels of similar competitor products per 100 g samples (see Appendix 19 - 25). For example, the developed bread formulation contains nearly 3 times the amount of protein (and less carbohydrates and sugars) than the compared commercial GF bread product. The developed muffin formulation contains nearly twice as much protein (with zero sugars and less fat) compared to the commercial GF muffin product, even with added frosting. The developed cookie formulation resulted in more protein and less fat than its commercial GF counterpart. And while these results might not be absolute comparisons, results show that it is possible for GF formulations to provide producers with cost-effective, nutritionally improved, acceptable products (Wang *et al.,* 2007; Minarro *et al*., 2012; Duta and Culetu, 2015; Korus *et al.,* 2015; Shevkani *et al*., 2015; Tanwar and Dhillon 2017; Teh *et al.,* 2016; Witczak *et al.,* 2016; Mattila *et al*., 2018; Shaabani *et al*., 2018).

7.8 Sustainability

Challenges to approaching sustainability include how culture influences perceptions of what is sustainable. Lazzarini *et al.* (2017) explored factors that influence how consumers view plant-based foods finding support for local markets and production was a relevant factor. For example, seasonal foods lead to lower energy consumption because they are neither stored or produced in greenhouses nor use energy intensive methods. However, authors also suggest that consumers need additional information to enable their selection of food products. While authors suggest alternative methods in which labeling could be improved, a key consideration might involve shaping a country's local sustainability goals so that consumers are able to make better choices without the assistance of labeling and packaging. Lacour *et al.* (2018) found that GHG, energy demand and land use were all factors that influence the acceptance of more plant-based diets. However, culture seems to play a huge role in acceptance of these factors as the French love their cheese too much to make a lasting change. To address concerns, a method for sustainable product development was defined for this study while simultaneously clarifying those factors which were quantifiable and deemed essential to success.

Backcasting was used to discover factors of sustainable development of GF products as it relates to consumer perception and product development. Research showed that there is room for nutritional improvement of GF products that taste good and choosing the right ingredients can have an impact on a product's sustainable perception (Wang *et al.,* 2007; Pojic *et al.,* 2015; Korus *et al*., 2015; Tanwar and Dhillon, 2017; Teh *et al.,* 2016). Supported by theoretical research and statistical data obtained through laboratory work, factors such as nutrition, cost and resource availability were easily quantified. Recent research showed that nutritional improvements are achievable using plant-based proteins without compromising quality (Gorissen *et al.,* 2018; Mattila *et al.,* 2018). Research showed that while commercial GF products tend to cost more (Stevens and Rashid, 2008) lower costs can be achieved by using more widely availability ingredients (Velazquez *et al*., 2012; Landero *et al.*, 2014; Korus *et al*., 2015; Pojic *et al.*, 2015; Svec and Hrušková, 2015; Liu *et al.,* 2016; Liu *et al.,* 2018; Shaabani *et al*., 2018) and that lower GHG is achievable by substituting plant-based sources (Lacour *et al.,* 2018). However, consumer acceptance was more difficult to quantify.

Working backwards to identify acceptability as the most important factor of the product development stage supported the idea that taste is subjective and can influence the sustainable perceptions of a product. For example, the halo effect, or the influence of one trait on another, as it pertained to consumer perception was supported in one study with regard to sustainable food behaviors and their health effects (Siegrist *et al*., 2015) however it was not observed in a follow up study from the same authors on sustainable foods being less satisfying to consumers (Visschers and Siegrist, 2015). This suggests that an association of attributes towards sustainable products is unique to consumer experience and less reliant on education or labeling. And while research suggests that greater knowledge influences sustainable choices more so than individual experience (Peschel *et al.,* 2016), results from this study have shown that consumer acceptance can be achieved without such knowledge being shared initially. Therefore, this might support the notion that customer segmentation is a factor to sustainable food consumption (Verain *et al.,* 2015), however may be less important of a factor if better tasting products are available.

Sustainability concerns play an important role in the increasing popularity of GF bakery products. And while these concerns include the impact of using plant-based proteins over animal by-products and whether or not this could reduce GHG, food cost and waste, consumer acceptance is also a factor. The potential impact involves improved sustainability for producers; lower reliance on GHG due to the shift from animal-based proteins and GMO crops, a lesser impact on agriculture (as pea and hemp crops require less energy and cost to produce), a reduction in consumer food waste and products that rely on taste to change consumer perception rather than more expensive labeling options. Farmer *et al.,* (2017) found that excessive food consumption is a concern for policy makers, having an impact on both the planet and consumer health, but that promoting sustainability was also a factor to reducing consumption. Therefore, knowledge of a sustainable option lessened consumption suggesting that if products are promoted as more sustainable, excessive consumption can be reduced. And while research shows that sustainable perception of products is possible through education and labeling (i.e., GF, FF, fair trade, organic), one variable to this study is that while it was conducted on sustainable products, this study did not record consumer perception to sustainable ingredients used and whether those ingredients affected consumer views on product sustainability which opens further discussion into how sustainable ingredients might affect the perception of a product rather than a wholly sustainable product.

It is understood that GF products, being mostly starch-based, tend to have a shorter shelf-life which has an impact on food waste, and ultimately consumption. And while Mixolab® results for this study indicate that protein choice would have little impact on shelf-life, it does not pertain to consumer perception. Research also does not address if product staling influences consumption, or more specifically consumer choice. During initial testing, products with added protein hardened by the next day. However, it was observed that the added HPI/PPI protein mix had an effect on the softness of developed cookies after several days when left unpackaged at ambient temperature. Research indicates that HPI can extend shelf-life (Korus *et al.,* 2017) and similar shelf-lives would be achievable between wheat-based and GF formulations if hemp is used as supplementation (Svec and Hrušková, 2015). Interestingly, HPI does not require labeling to indicate it as a priority allergen (Malomo *et al.,* 2014), which could be used in product labeling to influence consumption. And while this would have to be investigated properly, it suggests that the HPI/PPI mix could extend shelf-life and result in improved perception of product quality (therefore changing consumption habits) and suggest a cost savings over more expensive packaging solutions that attempt to address staling and food waste.

7.9 Limitations

It is important to acknowledge some limitations to this research. While investigation for this project was extensive, it attempted to address more recent findings within the last 10-15 years where possible in relation to GF bakery products. However, due to a rapidly changing GF landscape it is possible that assumptions, challenges and support by section might have evolved as research on this project began. Additionally, testing for this project commenced just weeks before the Covid-19 outbreak and subsequent cancelling of all in-person and laboratory work. Therefore, follow up testing and repeatability was affected. However, results and findings show promise for what was obtained and are still considered relevant to this project.

7.10 Research Impact

The purpose of the initial testing was purely to understand the nutritional benefits of the proteins added. Therefore, hydration levels were not adjusted, and results were compared to wheat flour at the same hydration level. However, the addition of HPI did require a slight modification to hydration levels during the product development stage to accurately account for potential production concerns, supporting findings that GF product quality can be affected by hydration adjustment (Rozylo, *et al.,* 2015). And while test results indicated HPI holds on to water during the gelatinization stage, which confirms HPI's potential effect on extending shelf-life (Ziobro *et al.,* 2016), more research into this could potentially impact production of GF products if constant hydration levels were achieved.

We also know that the addition of protein isolates can have a positive effect on potential rice flour applications in the baking industry (Marco and Rosell, 2008). HPI contains higher levels of amino acids and dietary fiber compared to soy (Tang *et al.,* 2006) and replacing soy with pea protein can help to reduce costs (Landero *et al.,* 2014). However, more research is needed to fully understand the production and cost benefits to using the HPI/PPI protein blend.

Additionally, consideration should be given to HPI as a replacement for hydrocolloid functionality. Yin *et al.* (2015) found the emulsification properties of PPI was improved when combined with soybean soluble polysaccharide (SSPS) in laboratory tests suggesting binding properties similar to hydrocolloids. SSPS is a water-soluble polysaccharide extracted and refined from soybean and consists mainly of the dietary fiber of the bean, is considered a functional food and clean label. The oil-water interface was helped by the PPI/SSPS complex, resulting in stability against changes to pH and salt. Shaabani *et al*. (2018) found that XG is also shown to produce similar stability in GF batters in the presence of pH changes and the presence of salts. Mancebo *et al.* (2015)

found the gel structure of psyllium is similar to XG, resulting in similar rheological behaviors suggesting fiber as a replacement for XG. Within this research's objectives it was hypothesized that HPI's higher level of available fiber might impact GF applications by reducing the need of a hydrocolloid. When initial tests were run using the Mixolab® the results obtained with HPI blends suggested this could be true. HPI alone is not as soluble as PPI, so if the extracted fiber in SSPS helps PPI's emulsification properties it is not unrealistic to hypothesize that the fiber content in HPI would result in a similar binding property when combined with PPI, though this would need to be investigated further. And whether it is to impact nutrition or production, HPI has the potential to be used as a more universal protein substitute in the UK.

This study has produced results which could greatly impact industrial production. Consistent hydration, if achieved for GF products, could potentially reduce GF production costs. Combining HPI/PPI has shown improved nutrition and a positive impact on GR, and as widely available and cost-effective ingredients could help address the perception of higher ingredient costs associated with GF products. Combining plant-based proteins has the potential to reduce the carbon footprint associated with dairy proteins and shows potential at reducing or eliminating hydrocolloid use in GF bakery applications. Finally, this study relies on results generated from commercially available ingredients under technological conditions like those used in GF production, allowing this research to be easily adapted to industrial GF bakery production.

8. Conclusion and Recommendations

8.1 Conclusion

The goal of this study was to examine the characteristics of PPI and HPI in relation to their nutritional and rheological properties within bakery applications and create GF bakery products that are nutritious, sustainable and acceptable by consumers. Aims included: determining if combining the two proteins could improve nutritional composition of developed products; understanding how the proteins impact dough rheology, GI and consumer acceptance; and whether or not they can be used as sustainable options in GF production. This study's originality lies in the development of a plant-based protein blend which complements each protein's nutritional and structural characteristics in bakery applications and shown to resemble the rheology of gluten in mechanical testing. This blend was also used to create three distinct products which obtained high levels of sensory acceptance, improved nutritional characteristics, showed potential at reducing starch from sources other than those naturally occurring in GF flours and at reducing the need for hydrocolloid use in GF bakery products. Finally, the protein blend appeared to have a positive impact on keeping blood sugar levels from spiking after consuming GF bakery products, which addresses the personal concerns of this researcher's attempt to help a diabetic relative. Therefore, as more sustainable ingredients these proteins used in GF production can both define and improve GF bakery products.

One can control dough/batter behavior by understanding the hydrocolloid used, extend product shelf life, improve nutrition, digestibility and sensory qualities, but results showed that their use might not be necessary. As most GF products contain hydrocolloids, this needed to be investigated as well if sustainable perceptions were to be considered. However, experimental techniques that avoid hydrocolloids are often costly for producers and further research is needed to explore whether such techniques are more favorable than avoiding them. Producers may also overlook a focus on nutrition when hydrocolloids are used, creating an opportunity to address improved product nutrition while reducing GI of GF products. Starches and pseudo-cereals might be an easier way to add more nutrition to GF baked products, but protein source varies depending on the recipe type and on the starch/flour used. Results showed consumer acceptance for developed formulations using a GF MG starch-free mix, supporting previous research that there is no significant relationship between starch nutrition and rheology (Velazquez *et al.*, 2012). Proteins, however, can have a greater potential nutritional impact while also affecting glucose response. It has been suggested that the removal of gluten directly impacts glycemic response, and that the addition of protein, fiber and fat can lower the GI of GF products (Scazzina *et al.*, 2015). Proteins also shape the overall cost and perception of GF products and whether plant-based options can satisfy consumer demand. PPI and HPI are less allergenic than dairy options, could address nutritional and GI concerns for those relying on a GF diet and are considered more sustainable options due to their origin and abundance. Research has shown both pea and hemp proteins have scored high in structural, textural, sensory and nutritional analysis of GF baked products. It has also been suggested that the gas forming/emulsification characteristics of both proteins could partially replace and reduce the reliance on hydrocolloids in GF baking while also providing added nutrition.

For the purposes of product development, product characteristics were identified and prioritized based on how each GF product is compared against the quality perceptions of each product type. Characteristics identified for bread assessment were softness, volume and color. Research showed hydrocolloids are often responsible for softness and volume of most GF bread products, therefore understanding how to replace the hydrocolloid was necessary. Although some studies involving breads have tested GF against wheat formulations to ascertain consumer acceptability in terms of volume, crumb structure and color with positive results (Sabanis and Tzia 2010; Velazquez *et al.,* 2012; Martinez and Gomez, 2016), most overlook taste and nutrition as key acceptance factors. For example, chestnut, millet and acorn flours have shown improved consumer acceptance for taste (Korus *et al*., 2015; Tanwar and Dhillon, 2017; Shaabani *et al.,* 2018) while also improving texture and nutrition. Color, defined by ingredients used and processing method, can also be accompanied by negative consumer perceptions and was considered a priority. Muffin characteristics were identified as density, moistness and sweetness. Research showed hydration plays a role in density and moisture of GF muffins, as does fat and protein source. Sweetness is associated with consumer acceptance but also has a negative nutritional perception which could be challenged. Cookie characteristics were identified as texture, flavor and nutrition. Research showed cookie texture is often driven by consumer preference however it can also be associated with product quality. And while flavor plays a role in consumer acceptance, nutrition is overlooked. Therefore, the main priorities in product development were to create products that compare to the identified characteristics of each product type while incorporating nutritious ingredients and appealing to consumer taste.

Assessment also identified that most GF products consist of animal-based proteins, which are not wholly sustainable. Therefore, replacing the protein source was necessary. Dairy and egg proteins have rated high in both sensory and structural results (Gallagher *et al.,* 2003; Matos *et al*., 2014) while Sahagun *et al.* (2018) found that adding protein to GF cakes decreases acceptability overall. However, Rinaldi *et al*. (2017) found that using a sourdough technique improved sensory acceptance, suggesting that acceptance could be related to the formulation and/or application used. Furthermore, most GF bakery products are associated with a perception of being healthier, which has been contradicted in research. Therefore, factors such as GI, fiber and protein levels were also assessed. If plant-based options can satisfy consumer demand, producers could realize a more cost-effective way to increase nutritional value of GF products by incorporating more environmentally sustainable plant-based proteins. Finally, assessment consisted of solutions to problems associated with consumer perceptions of GF products. Research showed that most GF products are assessed against quality characteristics associated with gluten-containing products rather than for the quality characteristics achieved from the GF sources themselves. Acceptance is therefore based on a like for like comparison rather than for the quality characteristics of the product itself, which can include long-term sustainable factors.

From a sustainability point of view, it has been shown that excessive consumption can be reduced if a product is seen as more sustainable. And while techniques such as packaging and sourdough fermentation could reduce the need for additives thus increasing product shelf life and help reduce food waste, they are expensive. Simple solutions to answering the sustainability question might involve using locally produced, plant-based and/or clean label ingredients, techniques to extend shelf life and reduce the need for preservatives and educating consumers on sustainable options. PPI and HPI have scored high in structural, textural, sensory and nutritional analysis of GF baked products. Both proteins are considered more sustainable options due to their origin and abundance and could potentially serve as a solution to addressing rising GHG and lower carbon footprint concerns while addressing nutritional and GI issues for those relying on a GF diet. And while more research is needed to determine if the combination of PPI and HPI protein can address these potential concerns, gaps in the research still exist.

There is currently a gap in research around portion size. If education and processing can affect product stability and health, study and research could be performed to determine if, using labeling that promotes sustainability, consumers would react favorably to reduced portion sizes. Research shows the more informed consumers are about sustainable products the less they consume, and that consumers who are more educated and better informed are not only concerned with health but also more likely to have plant-based diets. Particle size affects gelation in GF recipes and hydrocolloids are not a necessity, however there is a gap in research that could address whether or not milling plays a more cost-effective role in GF production and ingredient choices (de la Hera *et al.,* 2013). Educating consumers and informational labeling could influence a change in portion size to help reduce consumption and impact production and food waste. Therefore, the use of pea and hemp together, as finer milled proteins, could have an impact on production, nutrition and sustainability.

8.2 Recommendations

As a result of this research the following recommendations for future work can be made:

The Mixolab® provides information on the rheological properties of compounds, additive and enzyme activity.

- Mixolab® tests showed XG would not be necessary depending on the formulation. This research suggested that XG might not be necessary due to the fiber content of HPI. Previous research using the Mixolab® showed the functionality of fiber rich ingredients. Therefore, research into the comparison of fiber found in HPI (or the HPI/PPI blend) and if it is responsible for replacing a hydrocolloid (i.e., XG in this case) could be warranted.

Starch is important in GF formulations and this research tested starch in relation to a developed MG Blend.

- Results indicate having more starch positively affects gas and dough development, with 10% added protein having the most potential. Therefore, additional tests using Chopin's Rheo F4® to validate or further investigate the Mixolab® findings would be recommended to see how this protein blend affects gas and dough development within the MG blend.

Techniques (such as packaging or milling) paired with these compounds might have a positive impact on shelf-life, preserving product texture and cost.

- Particle size affects gelation in GF recipes and hydrocolloids are not a necessity (Pojic *et al*., 2015; Svec and Hrušková, 2015; Korus *et al*., 2017; Liu *et al.*, 2018). De la Hera *et al.* (2013) suggesting processing methods, such as milling, which take the size of starch granules into account (smaller starch granules mean quicker digestion and higher glycemic response), could be considered by producers for GF formulations. And Teh *et al*. (2016) suggests the bioavailability of hemp can be adjusted through processing means. However, there is a gap in research that could address whether or not milling can play a more cost-effective role in GF production and ingredient choices and should be investigated.

Results indicate that the HPI/PPI blend has a positive effect on GI and satiety levels.

- Repeatability for both BG and satiety tests to show validation is necessary. It is also recommended to compare results to a GF control and perhaps by product type to see just how much formulation plays a role to GI response.

There is currently a gap in the research around portion size related to reduced consumption/food waste.

- If education and processing can affect health and product stability, study and research could be performed to determine if, using labeling that promotes sustainability, consumers would react favorably to reduced portion sizes to further impact consumption levels.

Research has shown that labeling, packaging and knowledge of sustainable ingredients affects consumer perception.

- Further sensory tests could be conducted which explore how the ingredients used affect taste or perception of product quality if information on the ingredients is provided beforehand.

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APPENDICES

Appendix 1: Ethics Application Form

London South Bank University

EST 1892

Ethics Application Form

Project Title: A study of pea and white hemp proteins on the structural, sensory and nutritional characteristics of gluten-free products. **School:** School of Applied Sciences, in cooperation with the National Bakery School

Lead Applicant	Supervisor (Doctoral students only)
Name: Clay Niccum **Address:** 63 Borough Rd, Flat 3, SE1 1DZ **Email:** niccumc@lsbu.ac.uk **Phone number:** 07380331123	**Name:** Dr. Amar Aouzelleg **Address:** 103 Borough Road, SE1 0AA **Email:** aouzella@lsbu.ac.uk **Phone number:** 02078157945

Theoretical rationale (~500 words)
Gluten, a composite of the proteins gliadin and glutenin, is necessary for the structure of bread products (Cauvain, 2015). Removing gluten in gluten-free baking not only changes the product's structure and texture it also removes protein. While most gluten-free producers are concerned with using ingredients that mimic the characteristics of gluten, they often overlook the nutritional component of the finished product. Gluten-free products typically lack essential nutrition, suggesting that nutritional quality of gluten-free products might be compromised (Nascimento *et al*. 2013). While replacing gluten with dairy proteins mimics more closely the characteristics of wheat products, these gluten-free versions rate low in sensory tests (Gallagher *et al.* 2003) and presents a challenge for free-from products. Because it is a plant-based protein, soy has been used with success in gluten-free and free-from products because it not only acts like gluten but also enriches the product with minerals. Still, soy products rate low in sensory testing (Sarabai *et al*. 2015; Ziobro *et al*. 2013). Both pea protein isolate and white hemp proteins have excellent nutritional profiles, adding amino acids, good fats and other nutrients, however they have not been as widely tested in gluten-free products although preliminary tests have shown positive sensory results (Ziobro *et al*. 2013; Korus *et al*. 2017). Previous gluten-free studies have shown that combining multiple ingredients (proteins, additives, starches, etc.) have produced better quality products yet no study combines both pea and white hemp together. My research will attempt to leverage, and further research, these compounds' higher sensory and structural ratings in gluten-free and free-from products as well as attempt to significantly improve the overall nutritional value of the finished product. I will also attempt to draw conclusions that using more nutritious, plant-based compounds in gluten-free and free-from products can be more environmentally sustainable.

Ethical guidelines
National Health Service National Patient Safety Agency: National Research Ethics Service

Participants

University staff and students will be recruited through flyers posted on campus and contacted via email once they have registered their interest (see *Appendix 1*, attached). For acceptance tests, assessors will be bread users. For sensory profiling, tasters will be screened for sensitivity and trained for the assessment of various flavor/texture/color attribute assessments. Participants will engage in blind taste testing, using sensory rooms located in LSBU labs. Participants will be recruited by me, as the gatekeeper of the study. No sensitivity to participation is expected. Disclosure that gluten will be present will be communicated in advance. Prior to product testing, a complete list of ingredients and potential allergy information will be provided to each participant. Any participant suffering from a food allergy or gluten intolerance will be excluded from the study.

Recruitment

Recruitment will take place on campus, through posted flyers (*Appendix 1*). They will be asked to contact me via email, when I shall reply with further information about the study as well as the Participant Information Sheet and Consent Form for them to review (*Appendix 2*). They will be asked to then reply with their written consent to participate in the study and show up at the date/time specified to fill out the consent form.

Data types:

Non-intrusive physiological data (including blood pressure, kinematics, reaction time data, eye tracking, etc.)

For the purpose of collecting information on the satiety properties of both pea and hemp proteins as a possible means of weight loss, participant data will be collected using a visual analogue scale. This Hunger Satiety Form (Appendix ??) allows the participant to record how hungry/full they feel, in 30-minute intervals, for up to two hours after ingesting a product with pea and hemp protein. The data collected will represent the progression of hunger. This data will then be aggregated to represent a pattern to the participants hunger (ultimately, the rise and fall of their hunger level). Participant data will be compared and, if proven correct, should follow the same trend and show whether the protein combination affects satiety.

Physiological materials / human tissue collection (incl. bloods and saliva)

Screened participants' (myself included) blood glucose levels will be measured, as both pea and hemp proteins are known to help reduce blood sugar. This would be obtained by means of a thumb prick test and automated sampler at the beginning and end of the test, which should last no more than 2.5 hours - the length of time for blood glucose levels to be impacted by food. Sampling in this way is minimally invasive and produces only a momentary stinging sensation. Subjects will be pre-warned at the moment of sampling. Post sampling the site of skin puncture will be alcohol swabbed and subjects given a cotton swab to hold against the pinprick site for 4 minutes to ensure no residual blood drops. Swabs and used sharps will be disposed of safely in sharps and medical waste disposal. The samples are not retained following automated analysis; therefore, no tissue license implications exist.

Other forms of data / special procedures

Blind taste testing/sensory testing will be conducted, with participants verbally recording their reactions to, and impressions of, the product's texture, structure and flavor. This data will then be recorded manually and later put into a chart to analyze the results of all participants. Sensory testing will include Acceptance/Preferential and Profiling/Descriptive Testing.

Timespan

Data collection will commence when ethics is granted and should be concluded by the end of June, 2018.

Disclosure and Barring Service (formerly Criminal Records Bureau check)

This does not apply to this study as it does not rely on any personal participant information.

Informed consent
Study Manager/Researcher will obtain consent in writing from each participant, both in the form of email communication as well as through the Consent Form participants will sign prior to the study (see *Appendix 1* and *Appendix 2*). There will be multiple testing times available so that both student and staff can attend without impacting their study or work. Participants can withdraw at anytime until data analysis through email contact.

Anonymity & data management
The only personal data collected by participants will be their names and contact info for the purposes of coordinating the testing time/participation. This information will exist via university email, housed on LSBU servers, not downloaded or shared publicly and will be kept for no more than 10 years. All data used after the study and in the thesis will be anonymous, as outlined in the Participation Form. Electronic files will be stored in a password protected location and paper copies in a locked cabinet. All data will be kept for a minimum of 10 years pursuant to LSBU University's Data Protection Policy.

Incentives
No incentives will be provided, other than convenient testing locations and timeframes.

Procedure
Once a participant responds to the posted flyer, they will receive both the Participation and Consent forms via email. If they agree to participate by confirming via email, locations and timeframes will be shared. Potential participants will be screened and selected for sensitivity for the profiling test and those with any food allergies or intolerance to gluten will be excluded, only those with no food allergies or an intolerance to gluten will be chosen to participate. Upon arrival, participants will sign and present their Consent forms, ensuring their participation - participants may leave at any stage during the testing, however all data collected to that point will still be used unless the participant withdraws from the study. Testing will take place in a small group or 1:1 setting in a university approved location, and consist of two types of testing, Acceptance/Preferential and Profile/Descriptive. All participants will take part in the Acceptance/Preferential testing however a small group of volunteers (no more than 10) will be asked to take part in the Profile/Descriptive testing. Acceptance/Preferential Testing will consist of sensory comparison (blind testing 2-3 products and recording the products structure, texture, appearance, flavor, etc.). Profile/Descriptive testing will require some training but will take no more than an hour and can be conducted the day of the testing and both types of testing will take place on the same day. Products tested will be those developed for this study against popular in-store items. Once testing has completed, data will be collected and compiled. Participants will have the opportunity to then express an interest to stay informed of the results and how or if this data will be shared. The compiled data will then be analyzed and presented to the supervisor prior to publication and/or use.

Risk	
Please tick 'X' where applicable	
	Use of environmentally toxic chemicals.
x	**Use of radioactive substances, ingestion of foods, fluids or drugs.**
	Refraining from eating, drinking or usual medication.
	Contravention of legislation on any of: gender, race, human rights, data protection, obscenity.
	Potential psychological intrusion from questionnaires, interview schedules, observation techniques.
	Bodily contact.

x	**Sampling of human tissue or body fluids (including by venipuncture).**
	Sensory deprivation.
	Defamation.
	Misunderstanding of social / cultural boundaries nudity; loss of dignity.
	Compromising professional boundaries with participants, students, or colleagues.
	Involves the study of terrorism or radicalization or use of any information associated with such study.
	Other risk (please indicate what these consist of):

How will these risks be mitigated?
A risk assessment form has been completed/submitted which details potential risks and how they will be mitigated. All testing will be conducted on campus and supervised by staff in areas approved for all health and safety requirements. Allergy information will be provided prior to testing and participants with allergies will be excluded to avoid risk or complication.

Debriefing
A debriefing form has been submitted (see Appendix 3)

Analysis
T-test and ANOVA statistical analysis will be used.

Collaborations
This research does not include other organizations or individuals.

Training
There is no special training needed to complete this research.

Beneficiaries
There are no beneficiaries of this research.

References

1) Cauvain, S. (2015) *Technology of Breadmaking*. London: Springer.

2) Gallagher, E., Gormley, T.R. and Arendt, E.K. (2003) *Crust and crumb characteristics of gluten free breads.* London: Journal of Food Engineering, pp. 153-161.

3) Korus, J., Witczak, M., Ziobro, R. and Juszczak, L. (2017) *Hemp (Cannabis sativa subsp. sativa) flour and protein preparation as natural nutrients and structure forming agents in starch based gluten-free bread.* London: LWT - Food Science and Technology, pp. 143-150.

4) Nascimento, A., Fiates, G., Anjos, A. and Teixeira, E. (2013) *Analysis of ingredient lists of commercially available gluten-free and gluten-containing food products using text mining technique.* Florianopolis: International Journal of Food Science and Nutrition, 217-222.

5) Sarabai, S., Indrani, D., Vijaykrishnaraj, M., Kumar, V. and Prabhasankar, P. (2015) *Effect of protein concentrates, emulsifiers on textural and sensory characteristics of gluten-free cookies and its immunochemical validation.* New Delhi: Journal of Food Science & Technology, pp. 3763-3772.

6) Ziobro, R., Witczak, T., Juszczak, L. and Korus, J. (2013) *Supplementation of gluten-free bread with non-gluten proteins. Effect on dough rheological properties and bread characteristic.* London: Food Hydrocolloids, pp. 213-220.

Appendix checklist		
Document	**Yes**	**Not applicable**
Letters requesting / confirming permission to conduct the study		*x*
Recruitment poster or other recruitment material	*x*	
Indicative letter / email of invitation to the participant	*x*	
Participant information sheet	*x*	
Consent form	*x*	
Debriefing form	*x*	
Questionnaire(s)		*x*
Interview guide (questions)		*x*
Observation guide		*x*
Light Touch Review Eligibility Assessment	*x*	
Scan of application signature page	*x*	
Other(s) (Please list):		

Signatures

Send your application scanned copy to the School Ethics Panel OR University Ethics panel as appropriate (see Appendix 1 in the Code of Practice).

<u>Lead Applicant</u> *I confirm that this ethics proposal accurately details the research I intend to undertake. I also confirm that I understand ethical approval must be granted before research commences.* **Name:** **Signature:** **Date:**
<u>Supervisor (Doctoral students only)</u> *I confirm that I have given feedback on an initial draft of this proposal, and that this feedback has been taken into account in the submitted document.* **Name:** **Signature:** **Date:**

Appendix 2: Ethics Approval Letter

London South Bank University
EST 1892

School of Applied Sciences

Direct line: 0207 815 5422
E-mail: dawkinl3@lsbu.ac.uk
Ref: SAS1735

Monday 21st May 2018

Dear Clay,

RE: A study of pea and white hemp proteins on the structural, sensory and nutritional characteristics of gluten-free products

Thank you for submitting your application.

I am pleased to inform you that full Chair's Approval has been given by Dr. Lynne Dawkins, on behalf of the School of Applied Sciences.

I wish you every success with your research.

Yours sincerely,

L. Dawkins

Dr. Lynne Dawkins
Chair, Research Ethics Coordinator
School of Applied Sciences

Appendix 3: Participant Information Sheet

London South Bank University
EST 1892

Participant Information Sheet

17 April, 2018

A study of pea and white hemp proteins on the structural, sensory and nutritional characteristics of gluten-free products.

Study Manager: Clay Niccum, PhD Scholar
Contact Details: niccumc@lsbu.ac.uk
Study Location: J503

You are being invited to take part in a research study on gluten-free products. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

Purpose of the study:

- Gluten-free products are typically low in nutritional value. This study aims to determine if using certain plant-based proteins in gluten-free baking yield similar or better structural and textural results while adding nutritional value.
- This study will run for 3 months and is designed to test participant reactions to taste and texture of test products against current market products in a sensory testing panel.
- You have been asked to participate because of your knowledge and/or consumption of baked products. You will be one of 10 total participants in this study.
- Prior to product testing a complete list of ingredients and potential allergy information will be provided. Should you possess an allergy to any used ingredients, you will be excluded from the study.

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. **If you decide to take part you are still free to withdraw at any time and without giving a reason.** Simply email your withdraw to the contact above. Your final chance to withdraw from the study will be up to 7 days after you have received your debriefing form. NOTE: If you are a student, choosing to either take part or not take part in the study will have no impact on your marks, assessment or future studies.

What to expect for participation

- You will be asked to participate in a taste test of baked products.
- You will observe, taste and comment on product structure, texture and flavour.
- By agreeing to participate, you also agree to have you hunger satiety and blood sugar levels tested.
- Participants will be able to withdraw from the study at any stage up to the conclusion and collection of the data, which coincides with receiving a debriefing form. This form will be sent to all participants via email informing them of their final opportunity to withdraw from the study prior to data analysis, which will take place 1 week after the debriefing form is sent.

- Data collected from the testing may be quoted in publications, reports, posters, web pages, and other research outputs.
- To sign up, simply send an email to the details above for a list of times and any further information.

Possible benefits and risks to participation

- Your voluntary participation will contribute to further knowledge in the development of gluten-free products.
- Not all products tested will be gluten-free, nor will they all be made in a gluten-free facility, therefore should you suffer from celiac disease be aware gluten, and traces of gluten, will be present.

All the information collected about you and other participants will be kept strictly confidential (subject to legal limitations). Data generated by the study must be retained in accordance with the University's Code of Practice. All data generated in the course of the research must be kept securely in paper or electronic form for a period of 10 years after the completion of a research project. Participant privacy and anonymity will be ensured in the collection, storage and publication of research material.

What will happen to the results of the research study on completion?

1. All results of this research will be used for the study manager's PhD thesis in Food Science.
2. Results will be published by study manager to LSBU academic staff but may also be presented in upcoming conferences or journals.
3. Should you wish to obtain a copy of the published research, simply email the study manager.

Who is organising and funding the research?

- The study manager is conducting the research as a student at London South Bank University, on behalf of the School of Applied Sciences in cooperation with the National Bakery School.
- Research has been approved by London South Bank University.

Who to contact for further information

- For further information about this study and/or possible uses of the data collected, please contact:
  - Clay Niccum, PhD Scholar - niccumc@lsbu.ac.uk (Study Manager)
  - Dr. Amar Aouzelleg - aouzella@lsbu.ac.uk (Study Supervisor)
- If you have any concerns about the way in which the study is conducted, please contact the Head of Division, Mandy Maidment - maidmem@lsbu.ac.uk

Thank you for taking time to read the information sheet!

_______________________   _____________________

Clay Niccum   Date

PhD Scholar, LSBU

niccumc@lsbu.ac.uk

Appendix 4: Participant Consent Form

London South Bank University
EST 1892

Research Project Consent Form

Full Title of Project: **A study of pea and white hemp proteins on the structural, sensory and nutritional characteristics of gluten-free products.**

Ethics Approval Registration Number: SAS1735

Name: Clay Niccum

Researcher Position: PhD Student

Contact details of Researcher: niccumc@lsbu.ac.uk

Taking part (please tick the box that applies)	Yes	No
I confirm that I have read and understood the information sheet/project brief and/or the student has explained the above study. I have had the opportunity to ask questions.	☐	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without providing a reason. I further understand that I will be given one final opportunity to withdraw upon receipt of a debriefing form, after which data will be analyzed and I'll be unable to withdraw from the study.	☐	☐
I agree to take part in the above study.	☐	☐

Use of my information (please tick the box that applies)	Yes	No
I understand my personal details will not be revealed to people outside the project.	☐	☐
I understand that my data may be quoted in publications, reports, posters, web pages, and other research outputs.	☐	☐
I agree for the data I provide to be stored (after it has been anonymized) in a specialist data center and I understand it may be used for future research.	☐	☐

______________________ ____

Participant Name (print) Date Signature

______________________ ____

Clay Niccum Date Signature
PhD Scholar, LSBU
niccumc@lsbu.ac.uk

Project contact details for further information;
Supervisor: Dr. Amar Aouzelleg
Phone: +44 (0)20 7815 7945
Email: aouzella@lsbu.ac.uk

APPENDIX 5: EXPERIMENTAL PLAN, GF BREAD

Parameter	*Description*
Ingredients:	Corn starch, potato starch, GG, dried yeast, sugar, salt, oil, water, PPI, HPI
Samples:	Control (no protein), 10% protein, 20% protein, 30% protein and 40% protein

- The plan for the GF bread recipe included testing the levels of added protein, substituted in place of starches in 10% increments, to understand protein impact.

BLEND RATIOS OF TESTED PROTEIN – BREAD EXPERIMENTAL

	Starch (g)	**HPI (g)**	**PPI (g)**
Base Recipe	600	0	0
Recipe 1 (10% HPI)	540	60	0
Recipe 2 (10% PPI)	540	0	60
Recipe 3 (20% HPI)	480	120	0
Recipe 4 (20% PPI)	480	0	120
Recipe 5 (30% HPI)	420	180	0
Recipe 6 (30% PPI)	420	0	180
Recipe 7 (40% HPI)	360	240	0
Recipe 8 (40% PPI)	360	0	240

APPENDIX 6: EXPERIMENTAL PLAN, GF MUFFIN

Parameter	*Description*
Ingredients:	GF flour blend, water, protein (soy, whey, HPI/PPI), sugar, oil, baking soda, citric acid, salt, XG
Samples:	Control (WPI), HPI/PPI (80/20 and 50/50)

- The plan for the GF muffin recipe included testing protein type and combination, substituted for 17.3 g total protein per recipe, to understand protein impact.

APPENDIX 7: EXPERIMENTAL PLAN, GF COOKIE

Parameter	*Description*
Ingredients	GF flour blend, water, protein, sugar, margarine/butter, baking powder, vanilla, XG
Samples	Control (no protein), HPI/PPI (80/20 and 50/50) at 30% & 40%

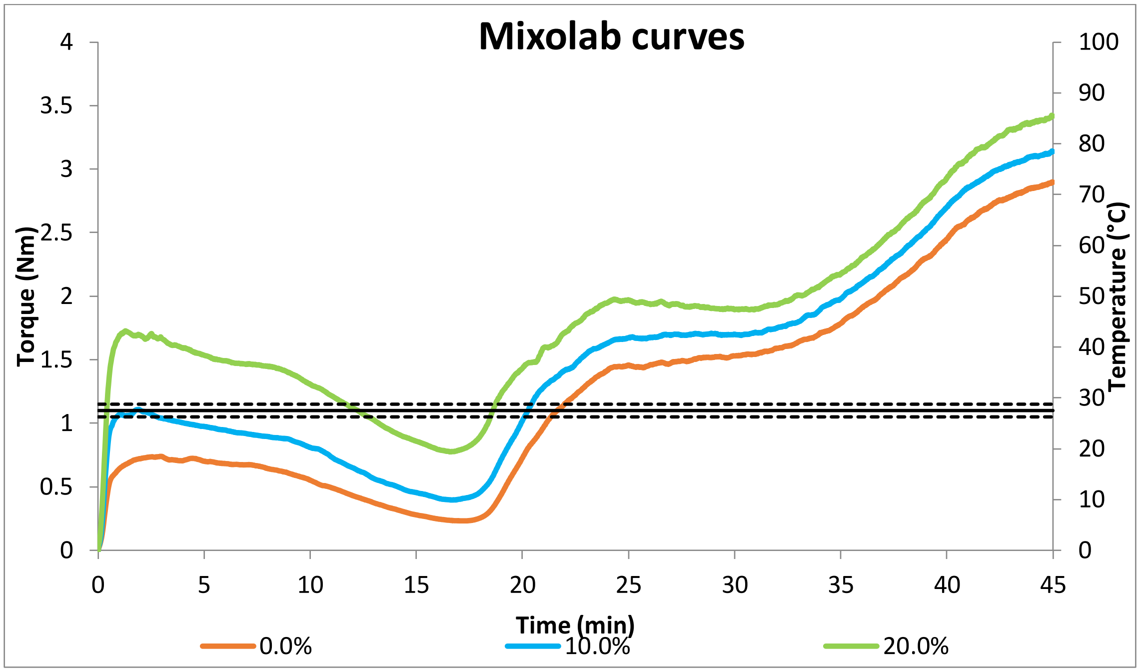
- The plan for the GF cookie recipe included testing protein combination, substituted for starch, to understand protein impact.

BLEND RATIOS OF TESTED PROTEIN – COOKIE EXPERIMENTAL

	Starch (g)	**HPI (g)**	**PPI (g)**
Base Recipe	200	0	0
Recipe 1 (30% HPI)	140	60	0
Recipe 2 (30% PPI)	140	0	60
Recipe 3 (40% HPI)	120	80	0
Recipe 4 (40% PPI)	120	0	80

Starch Activity: Determine the amount of protein with and without starch using MG Mix.

Appendix 8: MG Mix with Starch and 0%, 10% and 20% added Protein



APPENDIX 9: COMPARISON OF PROTEIN LEVELS FOR MG MIX USING MIXOLAB®

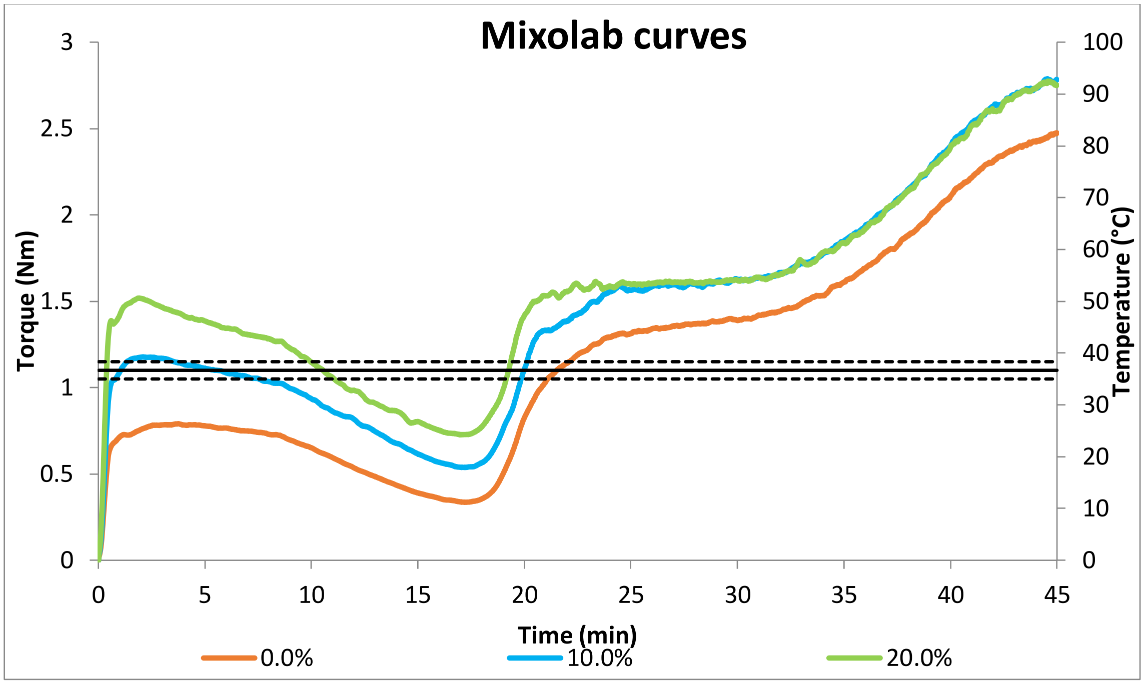
Sample[1]	0%	10%	20%
C1 (N m)	1.54 ± 0.02*	1.08 ± 0.001*	1.73 ± 0.01*
CS (N m)	1.30 ± 0.03*	0.90 ± 0.001*	1.48 ± 0.04*
C2 (N m)	0.71 ± 0.01*	0.43 ± 0.01*	0.78 ± 0.01*
C3 (N m)	1.64 ± 0.001*	1.06 ± 0.0*	1.97 ± 0.01*
C4 (N m)	1.58 ± 0.02*	0.36 ± 0.001*	1.89 ± 0.01*
C5 (N m)	2.23 ± 0.61 ns	2.39 ± 0.01 ns	3.41 ± 0.01 ns

[1] mean value of 2 replication ± standard deviation

*Values with * are significant (P ≤ 0.05); those with ns are not significant (P > 0.05)*

- Significance between samples at C1 indicate the 20% blend can absorb more water and is less resistant to mixing. Post Hoc tests showed significance for rate of gelatinization (C3) and gel stability (C4), although visually each curve appears similar in terms of behavior. However, there is no significance noted for starch retrogradation (C5). This indicates that while torque differences are similar (C3-C2, C3-C4 and C5-C4), the protein blend does not impact the mixing behavior or the starch behavior in this formulation – only hydration. Therefore, the addition of protein levels makes no difference in terms of shelf-life impact.

APPENDIX 10: MG Mix without Starch and 0%, 10% and 20% added Protein



APPENDIX 11: COMPARISON OF PROTEIN LEVELS FOR MG MIX WITHOUT STARCH

Sample[1]	0%	10%	20%
C1 (N m)	0.96 ± 0.23^{ns}	1.18 ± 0.01^{ns}	1.53 ± 0.01*
CS (N m)	0.86 ± 0.19^{ns}	1.04 ± 0.01^{ns}	1.28 ± 0.0^{ns}
C2 (N m)	0.41 ± 0.11^{ns}	0.55 ± 0.02^{ns}	0.73 ± 0.01*
C5 (N m)	2.72 ± 0.34^{ns}	2.79 ± 0.01^{ns}	2.75 ± 0.003^{ns}

C3 and C4 values were not generated because they relate to starch activity

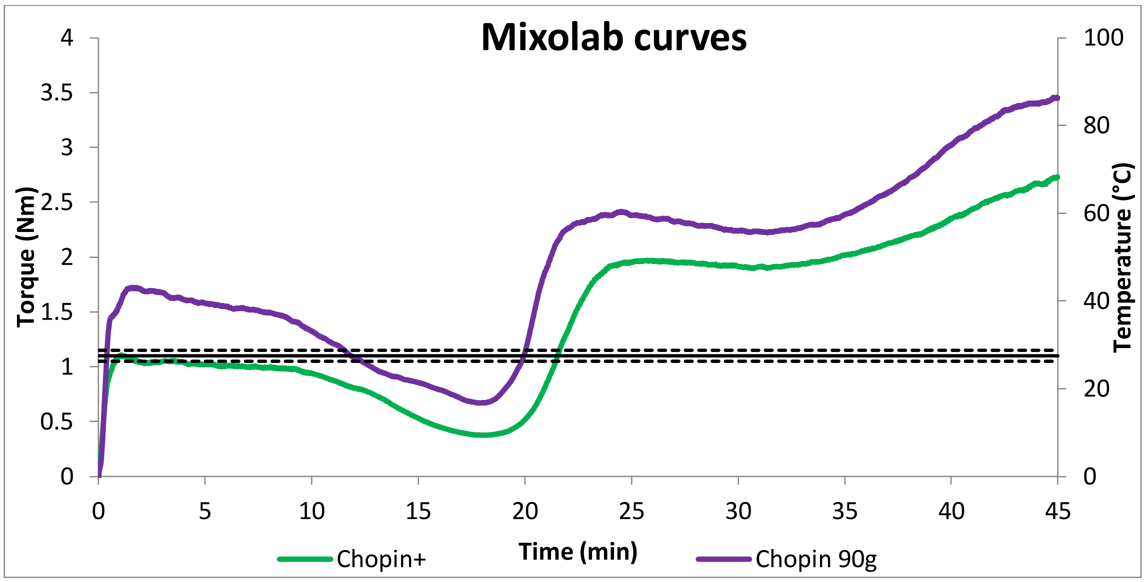
[1] mean value of 2 replication ± standard deviation

*Values with * are significant (P ≤ 0.05); those with ^{ns} are not significant (P > 0.05)*

- There was no significant difference between torque levels with the added protein, with the exception of the mixing (C1) and gelatinization (C2) stages for the 20% version. This indicates that the added protein can absorb more water and is less resistant to mixing, and the added protein promotes emulsification (allowing the absorption of water). There is no decrease between C3 and C4 for the 10% and 20% curves so mathematically the Mixolab® did not automatically generate torque differences. No significance at C5 (starch retrogradation) indicates that the added protein would have no significant impact on shelf-life.

Protocol Test: To validate for accuracy, tests for the 80:20 blend were run using both the Chopin+ and Chopin+90 g protocols.

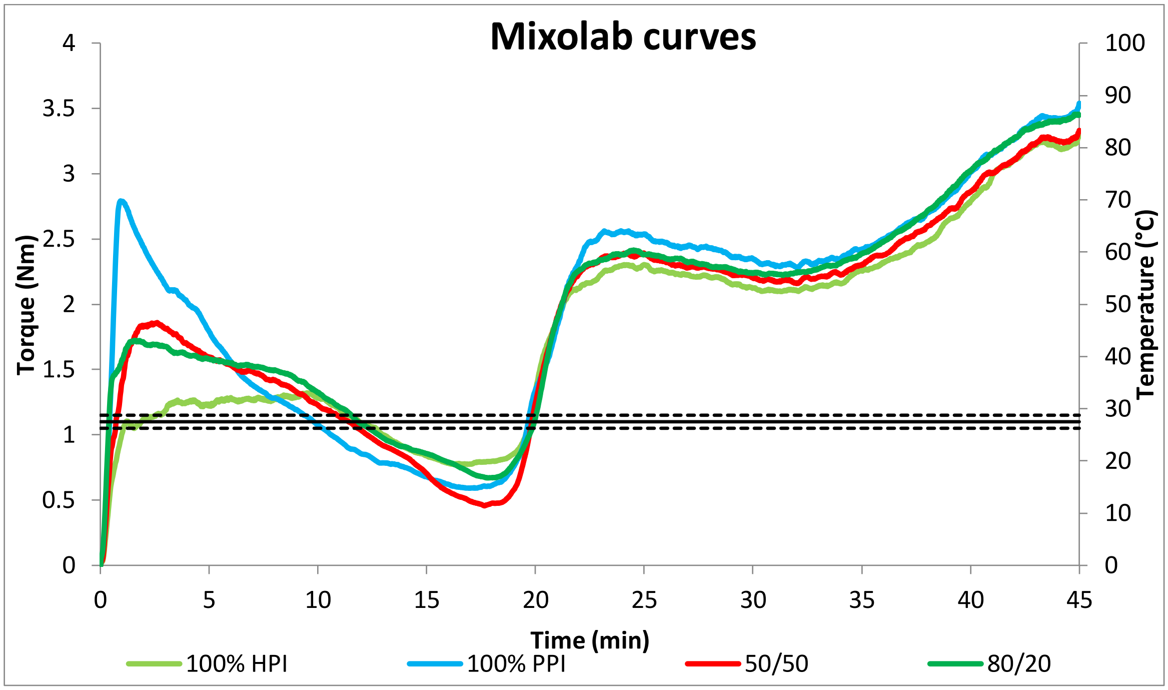
APPENDIX 12: PROTOCOL COMPARISON: 80:20 BLEND WITH CHOPIN+ AND CHOPIN 90G



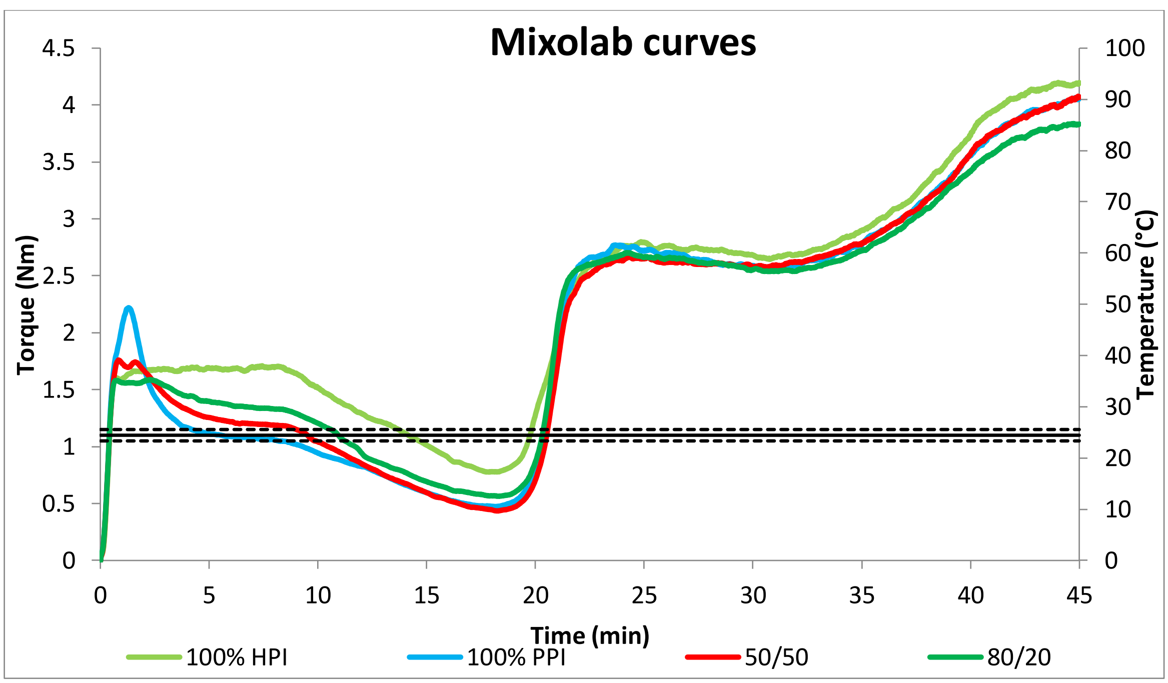
- Results showed similar behaviors with parallel curves indicating both protocols could be used.

XG Tests: To validate for accuracy, all protein blends were tested with and without XG.

APPENDIX 13: PROTEIN BLEND COMPARISONS WITHOUT XANTHAN GUM



APPENDIX 14: PROTEIN BLEND COMPARISONS WITH XANTHAN GUM



APPENDIX 15: COMPARISON OF PROTEIN BLENDS AND XANTHAN GUM

Parameter	Sample[1]	With XG	w/o XG
C1 (N m)	100% HPI	1.72 ± 0.01*	1.33 ± 0.01*
	100% PPI	2.22 ± 0.01*	2.73 ± 0.08*
	50:50	1.77 ± 0.01ns	1.91 ± 0.07ns
	80:20	1.69 ± 0.14*	1.44 ± 0.00*
CS (N m)	100% HPI	1.68 ± 0.01*	1.28 ± 0.01*
	100% PPI	1.06 ± 0.01*	1.27 ± 0.01*
	50:50	1.20 ± 0.02*	1.42 ± 0.001*
	80:20	1.40 ± 0.10ns	1.35 ± 0.00ns
C2 (N m)	100% HPI	0.76 ± 0.002ns	0.77 ± 0.002ns
	100% PPI	0.47 ± 0.01*	0.57 ± 0.03*
	50:50	0.44 ± 0.00ns	0.45 ± 0.01ns
	80:20	0.56 ± 0.01ns	0.56 ± 0.00ns
C3 (N m)	100% HPI	2.80 ± 0.01*	2.30 ± 0.01*
	100% PPI	2.78 ± 0.01*	2.55 ± 0.02*
	50:50	2.68 ± 0.01*	2.41 ± 0.02*
	80:20	2.68 ± 0.01*	2.31 ± 0.00*
C4 (N m)	100% HPI	2.63 ± 0.02*	2.09 ± 0.02*
	100% PPI	2.55 ± 0.01*	2.28 ± 0.01*
	50:50	2.59 ± 0.02*	2.17 ± 0.01*
	80:20	2.55 ± 0.02*	2.12 ± 0.00*
C5 (N m)	100% HPI	4.18 ± 0.02*	3.25 ± 0.05*
	100% PPI	4.06 ± 0.02*	3.54 ± 0.01*
	50:50	4.06 ± 0.01*	3.32 ± 0.03*
	80:20	3.94 ± 0.15*	3.33 ± 0.00*

[1] *mean value of 2 replication ± standard deviation*

*Values with * are significant (P ≤ 0.05); those with ns are not significant (P > 0.05)*

- Although ANOVA results showed significance between blends, torque differences were greatest at C1 indicating XG impacts the weakening of the dough during mixing. Therefore, formulations with XG would likely be less resistant to mixing.

Appendix 16: Developed Bread Recipe Formulation

The GF MG mix for this formulation included starch and recipe used XG.

- 241 g GF MG mix
- 104 g 80:20 protein blend
- 8 g XG
- 7 g salt
- 8 g dry, active yeast
- 280 g warm water
- 30 g vegetable oil
- 22 g honey
- 2 g lemon juice
- 112 g egg

Dry ingredients were combined in a large bowl and set aside. In smaller bowl, yeast was activated by adding to warm water until foamy, about 10-15 minutes. Proofed yeast liquid was added to dry ingredients with oil, honey, lemon juice and egg. Mixture was beat to form a soft batter and transferred to loaf tin coated in release spray. Tin was covered with plastic wrap and left to rise 45-60 minutes in a warm spot. Loaf was baked in preheated oven at 180C for 45-50 minutes. Loaf was then removed from tin and placed back in oven for additional 8-10 minutes.

Appendix 17: Developed Cookie Recipe Formulation

The GF MG mix for this formulation did not include starch and XG was not used.

- 174 g GF MG mix (no starch)
- 58 g 80:20 protein blend
- 124 g margarine
- 160 g dark brown sugar
- 23 g agave syrup
- 56 g egg
- 4 g soda
- 2 g salt
- 2 g vanilla extract
- 100 g dark chocolate

Oven was preheated to 180C. Cookie sheet was lined with parchment paper and set aside. Dry ingredients were combined in small bowl and set aside. In large bowl, dark brown sugar and margarine were creamed together. Egg, agave and vanilla were added and mixed well. Dry ingredients were added to wet ingredients and mixed completely. Dark chocolate was stirred in. Dough was formed into balls, placed on prepared cookie sheet and baked 8-10 minutes.

Appendix 18: Developed Muffin Recipe Formulation

The GF MG mix for this formulation did not include starch and XG was not used

- 100 g GF MG mix (no starch)
- 17.3 g 80:20 protein blend
- 75 g Erythritol Gold (brown sugar substitute)
- 100 g water
- 46 g coconut oil, melted
- 4 g soda
- 1 g salt

Oven was preheated to 180C. Muffin tray was lined with paper cups and set aside. Dry ingredients and sugar were combined in large bowl and mixed well. Water and oil were added and mixed well. Muffin cups were filled halfway and baked 15-18 minutes and cooled completely before being frosted.

Buttercream Frosting

- 226 g margarine
- 2 g Synergy creamy mouthfeel vanilla flavoring
- 2 g Synergy butterscotch flavoring
- 250 g icing sugar
- Dairy-free, sugar free butterscotch syrup

In a large bowl margarine, flavorings and sugar were beaten until smooth and spreadable. Each muffin was frosted with a tablespoon of icing and drizzled with a teaspoon of butterscotch syrup before serving.

Nutritional Comparison Charts: Nutritional reports for developed recipes were created using NutriCalc Nutritional Software (Copyright© 2022, NutriCalc Limited)

Appendix 19: Combined Nutritional Report for Developed GF Bread

Developed Bread

Report date: 3/26/2022

Nutrition

	per 100g
Energy	957kJ/228kcal
Fat	8.3g
of which Saturates	1.0g
Carbohydrate	22g
of which Sugars	2.5g
Protein	15g
Salt	0.91g

Ingredient Declaration

Water, Egg, Pean Protein Isolate, Oats, Rice Flour, Oil, Vegetable, Average, Almonds [Nuts], Flour, Buckwheat, Millet Flour, Sorghum Flour, Whole-Grain, Cornflour, Honey, Pea Protein Isolate, Potato Flour, Cassava Flour, Thickener: Xanthan Gum E415, Yeast, Salt, Lemon Juice

Allergens

Contains Eggs !

Contains Nuts !

Front of Pack

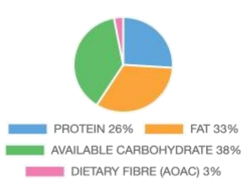
A 100g serving contains

Energy	Fat	Saturates	Sugars	Salt
957kJ 228kcal	8.3g	1.0g	2.5g	0.91g
11%	12%	5%	3%	15%

of an adult's reference intake

Typical values per 100g: 957kJ/228kcal

Energy Contributions



EU Reference Intakes

Per 100g		RI	Quantity	% RI
Energy	kJ	8400	957	11%
Total Fat	g	70.0	8.3	12%
Saturates	g	20.0	1.0	5%
Carbohydrate	g	260.0	21.6	8%
Sugars	g	90.0	2.5	3%
Protein	g	50.0	15.0	30%
Salt	g	6.0	0.91	15%

POWERED BY NutriCalc® NUTRITION CALCULATION

Appendix 20: Schär GF Wholesome White Loaf Labelling

Ingredients:

maize starch, water, sour dough (rice flour, water) 14%, rice starch, rice syrup, vegetable fibre (psyllium), sunflower oil, millet flour 2,6%, **soya protein**, quinoa flour 1,7%, thickener: hydroxypropyl methyl cellulose; yeast, iodised salt (salt, potassium iodide), honey 0,5%. **May also contain lupin and mustard. LACTOSE FREE**.

Analysis per 100 g

Energy Value	1009 / 239 kj/kcal
Fat	3.4 g
Of Which Saturates	0.5 g
Carbohydrates	45 g
Of Which Sugars	3.3 g
Fibre	7.3 g
Protein	3.5 g
Salt	1 g

Weight: 300 g
Weight per product: 27.0 g

Adapted from Schaer.com. Copyright 2021

Appendix 21: Combined Nutritional Report for Developed GF Muffin

Control Muffin (MG)

Report date: 3/26/2022

Nutrition

	per 100g
Energy	1428kJ/341kcal
Fat	16g
of which Saturates	1.2g
Carbohydrate	41g
of which Sugars	22g
Protein	7.4g
Salt	1.4g

Ingredient Declaration

Water, Sugar, Oil, Vegetable, Average, Oats, Rice Flour, Soya Protein Isolate (Typical), Almonds [Nuts], Flour, Buckwheat, Millet Flour, Sorghum Flour, Whole-Grain, Cornflour, Potato Flour, Cassava Flour, Raising Agent: Sodium Hydrogen Carbonate E500, Salt, Thickener: Xanthan Gum E415

Allergens

Contains Nuts !

Front of Pack

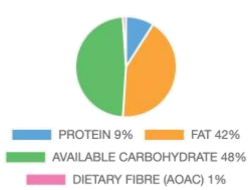
A 100g serving contains

Energy	Fat	Saturates	Sugars	Salt
1428kJ 341kcal	16g	1.2g	22g	1.4g
17%	23%	6%	25%	23%

of an adult's reference intake

Typical values per 100g: 1428kJ/341kcal

Energy Contributions



EU Reference Intakes

Per 100g		RI	Quantity	% RI
Energy	kJ	8400	1428	17%
Total Fat	g	70.0	16.0	23%
Saturates	g	20.0	1.2	6%
Carbohydrate	g	260.0	40.8	16%
Sugars	g	90.0	22.2	25%
Protein	g	50.0	7.4	15%
Salt	g	6.0	1.4	23%

POWERED BY NutriCalc® NUTRITION CALCULATION

Appendix 22: Schär GF Mini Muffins Choco Chips Labelling

Ingredients

egg *, sugar, high oleic sunflower oil, lactose free dark chocolate drops 9% (sugar, cocoa mass, cocoa butter, emulsifier: sunflower lecithin; natural vanilla flavour), maize starch, rice flour, potato starch, stabilizer: sorbitol; **lactose free yoghurt** 3,9% (lactose free milk), maize flour, glucose syrup, rice starch, vegetable fibre (citrus), **egg white powder**, emulsifier: mono- and diglycerides of fatty acids; raising agents: disodium diphosphate, sodium bicarbonate; thickeners: guar gum, xanthan gum; salt, natural flavours. **May contain traces of soya**. *Barn eggs. **LACTOSE FREE**.

Analysis per 100 g

Energy Value	1793 / 428 kj/kcal
Fat	21 g
Of Which Saturates	4.2 g
Carbohydrates	54 g
Of Which Sugars	30 g
Fiber	2.2 g
Protein	4.8 g
Salt	0.81 g

Weight: 240 g
Weight per Product: 40 g

Adapted from Schaer.com. Copyright 2021

Appendix 23: Combined Nutritional Report for Developed GF Muffin (with frosting)

Developed Muffin with Frosting

Report date: 3/26/2022

Nutrition

	per 100g
Energy	1753kJ/421kcal
Fat	28g
of which Saturates	12g
Carbohydrate	47g
of which Sugars	30g
Protein	3.2g
Salt	0.88g

Ingredient Declaration

Sugar, Margarine, Water, Sweetener: Erythritol E968, Coconut Oil, Oats, Almonds [Nuts], Rice Flour, Flour, Buckwheat, Millet Flour, Sorghum Flour, Whole-Grain, Pean Protein Isolate, Raising Agent: Sodium Hydrogen Carbonate E500, Pea Protein Isolate, Salt

Allergens

Contains Nuts !

Front of Pack

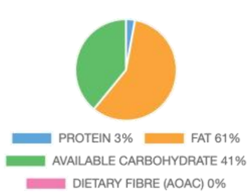
A 100g serving contains

Energy	Fat	Saturates	Sugars	Salt
1753kJ 421kcal	28g	12g	30g	0.88g
21%	40%	62%	34%	15%

of an adult's reference intake

Typical values per 100g: 1753kJ/421kcal

Energy Contributions



EU Reference Intakes

Per 100g		RI	Quantity	% RI
Energy	kJ	8400	1753	21%
Total Fat	g	70.0	28.3	40%
Saturates	g	20.0	12.3	62%
Carbohydrate	g	260.0	46.9	18%
Sugars	g	90.0	30.4	34%
Protein	g	50.0	3.2	6%
Salt	g	6.0	0.88	15%

POWERED BY NutriCalc® NUTRITION CALCULATION

Appendix 24: Combined Nutritional Report for Developed GF Cookie

Developed Cookie

Report date: 3/26/2022

Nutrition

	per 100g
Energy	1894kJ/453kcal
Fat	24g
of which Saturates	8.8g
Carbohydrate	46g
of which Sugars	28g
Protein	12g
Salt	1.00g

Ingredient Declaration

Brown Sugar, Margarine, Dark Chocolate, Egg, Oats, Pean Protein Isolate, Almonds [Nuts], Rice Flour, Flour, Buckwheat, Millet Flour, Sorghum Flour, Whole-Grain, Agave Syrup (Typical), Pea Protein Isolate, Raising Agent: Sodium Hydrogen Carbonate E500, Salt

Allergens

Contains Eggs !

Contains Nuts !

Front of Pack

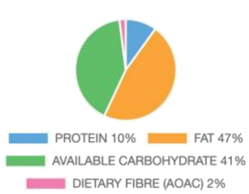
A 100g serving contains

Energy	Fat	Saturates	Sugars	Salt
1894kJ 453kcal	24g	8.8g	28g	1.00g
23%	34%	44%	31%	17%

of an adult's reference intake

Typical values per 100g: 1894kJ/453kcal

Energy Contributions



EU Reference Intakes

Per 100g		RI	Quantity	% RI
Energy	kJ	8400	1894	23%
Total Fat	g	70.0	23.8	34%
Saturates	g	20.0	8.8	44%
Carbohydrate	g	260.0	46.1	18%
Sugars	g	90.0	28.2	31%
Protein	g	50.0	11.7	23%
Salt	g	6.0	1.00	17%

POWERED BY NutriCalc® NUTRITION CALCULATION

Appendix 25: Tesco FF Chocolate Chip Cookie Labelling

Ingredients

INGREDIENTS: Gluten Free **Oat** Flour, Sugar, Palm Oil, Dark Chocolate (13%) [Sugar, Cocoa Mass, Cocoa Butter, Emulsifier (**Soya** Lecithins), Flavouring], Tapioca Starch, Rice Flour, Rapeseed Oil, Partially Inverted Sugar Syrup, Raising Agents (Sodium Bicarbonate, Ammonium Bicarbonate), Salt, Flavouring, Stabiliser (Xanthan Gum), Emulsifier (Mono- and Di-Glycerides of Fatty Acids).
Dark Chocolate contains: Cocoa solids 35% minimum.

Allergy Information

May contain peanuts and nuts. Contains soya. For allergens, see ingredients in bold.

Nutrition

Typical Values	One cookie (12g)	Per 100g
Energy	245kJ / 59kcal	2045kJ / 488kcal
Fat	2.6g	22.0g
Saturates	1.2g	10.2g
Carbohydrate	7.9g	65.6g
Sugars	3.5g	29.4g
Fibre	0.4g	3.4g
Protein	0.6g	5.2g
Salt	0.1g	0.4g

Adapted from Tesco.com. Copyright 2021

Appendix 26: Sensory Evaluation Questionnaire

London South Bank University
EST 1892

Sensory Evaluation Questionnaire

A study of pea and white hemp proteins on the structural, sensory and nutritional characteristics of gluten-free products.

The purpose of the test is to determine if using certain plant-based proteins in gluten free baked products affects both taste and texture. You will be asked to sample each product and then answer the questions below.

Muffin

Based on how the product(s) tastes, please select the product(s) rating from 1 – 5
(1 being the least tasty, 5 being the tastiest)

1	2	3	4	5

Based on the texture of the product(s), please select the product(s) rating from 1 – 5
(1 having the worst texture, 5 being the best texture)

1	2	3	4	5

Would you consider this a healthy product?	Y	N
Would you buy this product?	Y	N

Cookie

Based on how the product(s) tastes, please select the product(s) rating from 1 – 5
(1 being the least tasty, 5 being the tastiest)

1	2	3	4	5

Based on the texture of the product(s), please select the product(s) rating from 1 – 5
(1 having the worst texture, 5 being the best texture)

1	2	3	4	5

Would you consider this a healthy product?	Y	N
Would you buy this product?	Y	N

Bread

Based on how the product(s) tastes, please select the product(s) rating from 1 – 5
(1 being the least tasty, 5 being the tastiest)

1	2	3	4	5

Based on the texture of the product(s), please select the product(s) rating from 1 – 5
(1 having the worst texture, 5 being the best texture)

1	2	3	4	5

Would you consider this a healthy product?	Y	N
Would you buy this product?	Y	N

Thank you for agreeing to take part in this survey.

Contact for Further Information

For further information, please contact:

Doctoral Research Student – Clay Niccum (niccumc@lsbu.ac.uk)

If you have any concerns about the way in which the study has been conducted, please contact the School of Applied Sciences ethics coordinator at sasethics@lsbu.ac.uk

Appendix 27: Hunger Satiety Form for Participants

London South Bank University

EST 1892

Hunger Satiety

Introduction

This test will record your reaction to hunger based on how you feel, incrementally in time intervals, after ingesting gluten-free products. It will also test your blood glucose level before and after your 2 hour session. Total time for this test will take approximately 2.5 hours.

The purpose of the test is to determine if using certain plant-based proteins in gluten free baked products satiates hunger and lowers blood sugar.

Directions:

You will start hungry and on an empty stomach. Your blood glucose level will be tested, recorded on the sheet provided, then you will ingest one full serving of the product. You then simply record at each interval how hungry you feel.

Using the table on the following sheet, mark an X along the line based on how you feel, somewhere between very hungry indeed and not hungry at all. At the end of the 2 hour period, your blood glucose level will be tested again and recorded.

You will not be allowed to eat or drink anything else during this test, as this could skew results.

Blood Glucose Measurements and Visual Analogue Scale

Step 1: Record your blood glucose level and start time
Step 2: Mark an X along the first line, 0 min, to indicate how hungry you are
Step 3: After 30 min, mark an X along the line to indicate how hungry you are
Step 4: After 1 hour, mark an X along the line to indicate how hungry you are
Step 5: After 1 hr 30 min, mark an X along the line to indicate how hungry you are
Step 6: After 2 hours, mark an X along the line to indicate how hungry you are
Step 7: Record your blood glucose level and end time

Blood Glucose Level	
start time:	
end time:	

Very Hungry Indeed — Not Hungry At All

0 min |____|

30 min

1 hour

1 hour 30 min

2 hours

Thank you for your participation in this survey!

Contact for Further Information

For further information, please contact:

Doctoral Research Student – Clay Niccum (niccumc@lsbu.ac.uk)

If you have any concerns about the way in which the study has been conducted, please contact the School of Applied Sciences ethics coordinator at sasethics@lsbu.ac.uk

Appendix 28: Blood Glucose Levels by Participant

Parameter	Pre	Post
M	3.9	3.9
M	4.6	5.5
M	2.1	4.4
M	5.5	4.7
M	5.4	4.9
F	4.2	4.1
F	4.6	4.9
F	4.4	5.4
M	4.3	4.3
M	4.5	5.1
F	4.1	4.3

Pre/Post values represent single trial GI results; M = male, F = female

Appendix 29: Combined Nutritional Report for MG Mix Recipe

MG Mix

Report date: 3/28/2022

Nutrition

	per 100g
Energy	1658kJ/393kcal
Fat	8.7g
of which Saturates	1.0g
Carbohydrate	65g
of which Sugars	1.4g
Fibre	6.5g
Protein	10g
Salt	0.02g

Ingredient Declaration

Oats, Rice Flour, Almonds [Nuts], Flour, Buckwheat, Millet Flour, Sorghum Flour, Whole-Grain, Cornflour, Potato Flour, Cassava Flour

Allergens

Contains Nuts !

Front of Pack

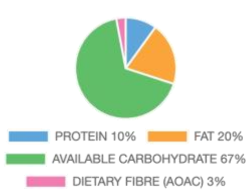
A 100g serving contains

Energy	Fat	Saturates	Sugars	Salt
1658kJ 393kcal	8.7g	1.0g	1.4g	0.02g
20%	12%	5%	2%	0%

of an adult's reference intake

Typical values per 100g: 1658kJ/393kcal

Energy Contributions



EU Reference Intakes

Per 100g		RI	Quantity	% RI
Energy	kJ	8400	1658	20%
Total Fat	g	70.0	8.7	12%
Saturates	g	20.0	1.0	5%
Carbohydrate	g	260.0	65.4	25%
Sugars	g	90.0	1.4	2%
Protein	g	50.0	10.2	20%
Salt	g	6.0	0	0%

POWERED BY NutriCalc® NUTRITION CALCULATION

Appendix 30: MG Mix Recipe Compared to Recommended Daily Allowance

MG Mix

Per 100g

		RDA	Quantity	% RDA
Vitamin A	µg	800	0	0%
Vitamin D	µg	5.0	0.0	0%
Vitamin E	mg	12.00	2.47	21%
Vitamin C	mg	80	1	1%
Thiamin (Vitamin B1)	mg	1.10	0.28	25%
Riboflavin (Vitamin B2)	mg	1.40	0.13	9%
Niacin (Vitamin B3)	mg	16.0	1.1	7%
Vitamin B6	mg	1.40	0.09	7%
Folate	µg	200	25	12%
Vitamin B12	µg	2.5	0.0	0%
Biotin	µg	50.0	6.5	13%
Pantothenate	mg	6.00	0.52	9%
Potassium	mg	2000	431	22%
Chloride	mg	800	23	3%
Calcium	mg	800	46	6%
Phosphorus	mg	700	255	36%
Magnesium	mg	375	97	26%
Iron	mg	14.0	3.0	21%
Zinc	mg	10.0	1.7	17%
Copper	mg	1.00	0.37	37%
Manganese	mg	2.0	1.4	72%
Selenium	µg	55	5	9%
Iodine	µg	150	1	1%

Appendix 31: Combined Nutritional Report for MG Mix Recipe (No Starch)

MG Mix (No Starch)

Report date: 3/28/2022

Nutrition

	per 100g
Energy	1751kJ/416kcal
Fat	12g
of which Saturates	1.4g
Carbohydrate	60g
of which Sugars	1.9g
Fibre	8.2g
Protein	13g
Salt	0.02g

Ingredient Declaration

Oats, Almonds [Nuts], Rice Flour, Flour, Buckwheat, Millet Flour, Sorghum Flour, Whole-Grain

Allergens

Contains Nuts !

Front of Pack

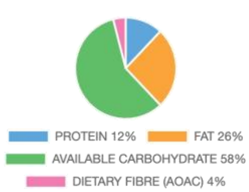
A 100g serving contains

Energy	Fat	Saturates	Sugars	Salt
1751kJ 416kcal	12g	1.4g	1.9g	0.02g
21%	17%	7%	2%	0%

of an adult's reference intake

Typical values per 100g: 1751kJ/416kcal

Energy Contributions



EU Reference Intakes

Per 100g		RI	Quantity	% RI
Energy	kJ	8400	1751	21%
Total Fat	g	70.0	12.1	17%
Saturates	g	20.0	1.4	7%
Carbohydrate	g	260.0	60.0	23%
Sugars	g	90.0	1.9	2%
Protein	g	50.0	12.8	26%
Salt	g	6.0	0	0%

POWERED BY NutriCalc® NUTRITION CALCULATION

Appendix 32: MG Mix (No Starch) Compared to Recommended Daily Allowance

MG Mix (No Starch)

Per 100g

		RDA	Quantity	% RDA
Vitamin A	µg	800	0	0%
Vitamin D	µg	5.0	0.0	0%
Vitamin E	mg	12.00	3.51	29%
Vitamin C	mg	80	0	0%
Thiamin (Vitamin B1)	mg	1.10	0.37	33%
Riboflavin (Vitamin B2)	mg	1.40	0.17	12%
Niacin (Vitamin B3)	mg	16.0	1.1	7%
Vitamin B6	mg	1.40	0.10	7%
Folate	µg	200	32	16%
Vitamin B12	µg	2.5	0.0	0%
Biotin	µg	50.0	9.1	18%
Pantothenate	mg	6.00	0.71	12%
Potassium	mg	2000	436	22%
Chloride	mg	800	3	0%
Calcium	mg	800	57	7%
Phosphorus	mg	700	327	47%
Magnesium	mg	375	127	34%
Iron	mg	14.0	3.7	27%
Zinc	mg	10.0	2.2	22%
Copper	mg	1.00	0.46	46%
Manganese	mg	2.0	2.0	100%
Selenium	µg	55	7	12%
Iodine	µg	150	0	0%