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Development of a Food Product Prototype Containing Added Plant Polar Lipids and Investigation of Its Postprandial Glycemic Properties

Master thesis Food technology and Nutrition
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Abstract

This degree project aimed to produce a standardized food product enriched with 14g polar lipids (PL) and compare its effects on glycemic response and appetite sensations both acutely and following a second meal in healthy individuals. In order to elucidate the viability of enriching a prebaked product with polar lipids researchers spent multiple weeks assessing variations in recipe, cook time, cook temp, mold size, and baking surface. Each sample was assessed for acceptability among the researchers and checked for repeatability. The test products made from the finalized recipe and methodology were then frozen for use in starch analysis and in later human trials. The samples for starch analysis were then thawed and dried at room temperature over night before being processed through a multi-step enzymatic degradation for assessment via photo spectrometer. The PL sample was found to have an available starch percentage of ~76% dry weight, and the control sample was ~59% available starch by dry weight.

Following product development, a pilot study was carried out with 9 healthy subjects (BMI 19-28 kg/m^2, age 20-30) 6 of which were female and 3 males. Subjects were asked to consume a standardized dinner and snack the night prior to the study and were fasted entering the lab. They were then fed a standardized breakfast at 0 minutes and standardized lunch at 210-minutes, finger prick blood glucose measurements and a subjective appetite questionnaire were completed 15 times throughout the trial day. The results from this pilot study showed significant reduction in post lunch glycemic peak ($p<0.05$), and general trends towards improved glycemic control, as well as improved appetite markers. This indicates that it was possible to produce a pre made standardized breakfast product enriched with polar lipids that can be included in a healthy diet and that reproduces results consistent with current findings.

Keywords: Plant polar lipid, glycemic response, type two diabetes mellitus, appetite regulation.

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Abbreviations

GI – Glycemic Index

PL(s) – Polar Lipid(s)

T2DM – Type Two Diabetes Mellitus

BMI – Body Mass Index

FFA – Free Fatty Acids

CHD – Coronary Heart Disease

TNF – Tumor Necrosis Factor

MCT – Medium Chain Triglycerides

TAG – Triacyl Glycerides

AUC – Area Under the Curve

iAUC – Incremental Area Under the Curve

CI – Confidence Interval

SEM – Standard Error of Mean

Preface

Before you is the master thesis titled "Development of a Food Product Prototype Containing Added Plant Polar Lipids and Investigation of Its Postprandial Glycemic Properties." This scholarly work is authored by Samuel Kirk, a Master's student in Food Technology and Nutrition at Lund University, and Emaan Binte Muhammad, an EIT student pursuing her Master's in Food Systems. As a culmination of our final semester and a mandatory component of our academic programs, this thesis marks the completion of our Master's degrees. The research and development for this project were conducted from January 2024 to June 2024.

Sam: In selecting this degree project, I, a student with 7 years of experience in the field of nutrition, pulled on previous experience working with human trials. My love for this variety of study pushed me into electing to get involved with this thesis project as a capstone to his academic career thus far. I would be remiss not to thank the many mentors I've had along the way, my family for their love and support. And of course, my wonderful thesis partner, I couldn't have done this without your support.

Emaan: Embarking on my journey in Food Chemistry and Nutrition, I enrolled in this course to fulfill my mandatory credits, bridging the gaps in my biological background. This course opened my eyes to the vast world of food science, offering not just the basics I lacked, but also a wealth of new knowledge. When the time came to choose a master's thesis, I eagerly approached Anne and Juscelino and was thrilled to join this project. Conducting this thesis was an absolute joy, with every moment filled with excitement and learning beyond my expectations. For this, I have my thesis partner, Sam, to thank. From the depths of my heart, I thank my parents for their unwavering support in helping me achieve our shared dream. I strive to be a continual source of pride for you both. To my beloved fiancée, Einar, your constant love and support have been my anchor, and I hope to always be the same for you.

We extend our heartfelt gratitude to our supervisors, Anne Nilsson and Juscelino Tovar, for granting us this incredible opportunity and guiding us with unwavering support. A special thank you to the participants who generously gave their time to be a part of our study.

Introduction

The world is facing an incredible increase in the rates of cardio-metabolic disorders and diseases as a result of lifestyle changes (1). These changes include the increase in hyper palatable calorie dense foods, and an increasingly sedentary lifestyle (2). In Europe diabetes affected approximately 61 million people with a projected increase to approximately 69 million people by 2045 the vast majority of which will develop type two diabetes (3). This large increase is set to add more strain to an already heavily burdened system. Type two diabetes is a disease characterized by impaired glucose intolerance and increased insulin resistance (3). This disease is often managed by medication however, it can lead to disability, an increased risk of other cardiometabolic diseases and even death. This variety of diabetes however can be partially treated and even largely prevented by lifestyle modification (4). The WHO suggests that steps in preventing diabetes are "reach and keep a healthy bodyweight, stay physically active with at least 30 minutes of moderate exercise per day, eat a healthy diet and avoid sugar and saturated fat, not smoke tobacco" it is thus it is imperative that researchers in the health fields continue to develop and research foods that can help achieve at least part of these recommendations (5). As such this degree project was designed to formulate a food product enriched with plant polar lipids (PL) and subsequently evaluate its impact on glycemic response.

The motivation for this investigation stemmed from findings of a previous cross-over study conducted at Lund University, which suggested that oat PLs influence acute and second-meal postprandial metabolic responses (6). Additionally, a recent investigation at Lund University examined the effects of varying concentrations of another plant PLX on blood glucose levels. This study concluded that significant reductions in postprandial blood glucose concentrations were observed when lipid-prepared test meals were administered at breakfast (6).

Further research indicates that postprandial glucose responses are elevated following a lunch that succeeds a high-GI breakfast, as compared to the same lunch preceded by a low-GI breakfast (7). Building on this foundation, the current degree project aimed to identify the optimal dosage of plant polar lipids (PL) in the food product to elicit the desired glycemic response. It is hypothesized that the PL treatment will outperform the control neutral oil with respect to glycemic response and perceived appetite sensations.

It's important to recognize that the previous research carried out at Lund University did not involve any processing of the polar lipids; they were administered either as an oil or blended into a spread for consumption by participants. Consequently, our degree project is designed to investigate whether polar lipids elicit the same effects when incorporated into baked goods that are subsequently frozen prior to consumption. Should this method prove successful, it could pave the way for a new range of baked products enriched with polar lipids, transforming everyday baked items into significantly healthier options.

1. Background

1.1 Glycemic Control and Diabetes

1.1.1 The Glycemic Index

In the 1950s, the concept of Carbohydrate counting was introduced to predict post prandial blood glucose responses by attaining a consistent carbohydrate intake. This concept was created under the assumption that simple carbohydrates illicit a greater rise in blood glucose levels than their complex counterparts. However, it also assumed that all simple carbohydrates, when taken in equal amounts, produced the same blood glucose responses. The same assumption was also made for complex carbohydrates. The glycemic index (GI) was introduced in 1981 which challenged the concept of carbohydrate counting and revealed that even if carbohydrates are consumed in equal amounts, be it complex or simple, the postprandial blood glucose levels may be different for each food (8). This is because dietary carbohydrates are digested and absorbed in the small intestine at different rates due to the different chemical and physical makeup of foods (9) Chemical factors such as the type of monosaccharide, presence of viscous soluble fibers, and the type of amylose can affect digestion and absorption. In addition, physical characteristics such as the extent of ripeness, processing, and cooking can make the carbohydrate easily digestible. Moreover, maintaining the original plant structure and the degree of refining can also influence digestibility. Furthermore, additional food components such as fats and proteins can affect gastric emptying thereby slowing starch digestion (10).

The GI curve, introduced in 1981 by Jenkins et al, as a tool for diabetic patients, takes this into consideration and describes how 50 g available carbohydrates of particular food affects the area

under the blood glucose curve when measured at regular intervals over a two-hour period after consumption. The area under the glucose curve of the test food is compared against the area of a standard food which may be white bread or pure glucose. The GI is obtained by dividing the area of the test item with the control and expressed as a percentage (8,9)

1.1.2 GI and Obesity

The hypothesis that low GI-foods promote weight control by reducing postprandial insulin release, enhancing satiety levels, and maintaining insulin sensitivity is supported by several human and animal studies (11).

A systematic review and meta-analysis on the long-term effects of low glycemic vs. high glycemic diets in the management of obesity and prevention of obesity associated risks such as Type 2 Diabetes Mellitus (T2DM) provided evidence for the beneficial effects of a low-GI diet in the primary prevention of diseases related to obesity (12).

Another systematic review and meta-analysis reviewing the metabolic effects of high GI diets in rodents. Although the mechanisms for how a low GI-diet benefits metabolic health are unclear, this review revealed that when fed a high GI diet, an increase in body weight, adiposity, fasting insulin levels, and area under the curve (AUC) for glucose and insulin levels during a glucose tolerance test (GTT) in male rats and mice as compared to a low GI diet (13).

An acute crossover study evaluating the effects of low, medium, and high GI diets on voluntary food intake and energy metabolism in obese teenage boys revealed that a high GI diet sequentially generates hormonal and metabolic changes resulting in voluntary excess food intake (14). Another similar study performed a randomized control trial with obese adolescents to evaluate the effects of a low GI diet on the body mass index (BMI) and other obesity indices. The results conclude that the low GI group recorded a significantly higher decrease in BMI, body weight, and waist circumference as compared to the control group (15).

Long-term studies performed in animal models showed rats that were fed high GI starch over a five-week period had higher epididymal fat (abdominal fat storage in rodents (16)) and bigger adipocyte volume than the group given the low GI-starch (11).

In another experiment, for 32 weeks, one group of young adult rats were fed a high GI starch diet, and the other group was given a low GI starch diet. To ensure the diet was like modern western diets, the test meals comprised of 45% carbohydrate, 20% protein, and 35% fat as a proportion of energy. In addition, the diet consisted of two large meals per day that were isoenergetic. This was done to simulate meal feeding in humans and provide a sufficient time difference between each meal's postprandial hyperglycemic response. After this 32-week period, it was observed that the weight of the low-GI group remained stable whereas the high-GI group gained 16% more body weight (11).

1.1.3 High GI Diet and Insulin Resistance

Although the loss in pancreatic function is a key characteristic of T2DM, it is not clear whether this defect is caused by β-cell exhaustion or cell toxicity due to hyperglycemia. Nonetheless, both mechanisms point to a high GI diet resulting in a high blood glucose concentration and a greater insulin response. Therefore, it is plausible that a high GI diet increases the risk of developing T2DM by increasing insulin resistance (17).

A 3-week study consisting of 28 women with and without a history of coronary heart disease (CHD) were randomly assigned to consume a low and high GI diet. After the study period was over, the subjects' insulin resistance in vivo was measured along with in vitro adipocyte insulin sensitivity and production of TNF-α. The study concluded that a low GI diet was shown to improve insulin sensitivity in adipocytes in subjects with a history of CHD and in vivo insulin sensitivity in women both with and without a history of CHD (17,18).

The study found that although insulin resistance both in vivo and in vitro was greater in women consuming a high GI diet, it was the greatest in women that also had a history of CHD as this group already had higher baseline insulin resistance. The reason for the adverse effects elicited by the high GI diet seemed to be due to a higher generation of free fatty acids (FFA) produced in response to the hypoglycemia typical of a high GI diet (17).

Counterregulatory hormones (catecholamines, cortisol, glucagon, and growth hormone (GH)), are also secreted in response to hypoglycemia to restore cellular homeostasis which further contribute to insulin resistance. They do this by reducing the secretion of insulin by making the pancreatic alpha cells more active (16).

Since the purpose of cortisol is to increase energy, it acutely impairs the secretion of insulin and increases release of hepatic glucose while simultaneously reducing the uptake of glucose in peripheral tissues (19).

In addition, catecholamines reduce insulin binding and the activation of insulin by suppressing the activity of tyrosine kinase (insulin receptor) as well as reducing glucose uptake in the peripheral tissues by GLUT-4 (19).

GLUT4 (Glucose transporter type 4) is a glucose transporter that is regulated by insulin and is responsible for the uptake of glucose into fat and muscle cells (20).

How an individual respond to a particular glycemic load depends on their insulin resistance at baseline which is mainly determined by diet, physical activity, adiposity measure, and genetics. Therefore, we can expect that the metabolic consequences of a high GI-diet would be aggravated in overweight, genetically predisposed individuals leading a sedentary lifestyle (17).

1.1.4 Type Two Diabetes Mellites (T2DM)

Type 2 Diabetes Mellitus (T2DM) is a common metabolic disorder caused by a progressive decline in insulin sensitivity and defective β-cell function. Since the body can no longer main glucose homeostasis, T2DM is characterized by chronic hyperglycemia which can eventually damage the heart, eyes, nerves, kidneys, and the vascular system. A common characteristic of people with diabetes is obesity or a high percentage of body fat mainly in the abdominal area (21).

The adipose tissue is an endocrine organ and one of the main sites for the deposition of glucose. It stores fat and provides the body with energy whenever required. In addition, it secretes hormones and cytokines which are collectively called adipokines as well as free fatty acids. The adipose tissue is known to promote insulin resistance due to an increased release of FFA and adipokine deregulation in T2DM (21,22).

To restore homeostasis, insulin is released in the blood and transported to peripheral tissues where it binds to insulin receptors resulting in their autophosphorylation. Consequently, two main pathways are activated namely the phosphatidylinositol 3-kinase (PI3K) signaling pathway and mitogen-activated protein (MAP) kinase pathway. The PI3K pathway is involved in the synthesis

of glycogen, lipogenesis, and the uptake of glucose via GLUT4 in the peripheral tissues. It also plays an active role in gluconeogenesis and lipolysis (22).

Looking at the above mechanism closely, the binding of insulin to the alpha and beta subunits of the insulin receptors results in their autophosphorylation which leads to the phosphorylation of insulin receptor substrates which in turn activate the PI3K pathway (23,24). FFA released by the adipose tissue promote insulin resistance in T2DM by interfering in this mechanism through the inactivation of the insulin receptor substrate-1 thereby getting in the way of the PI3K pathway and consequently, hindering glucose uptake by peripheral tissues (22).

In addition to the increased production of FFA, adipokine deregulation also promotes insulin resistance.

An adipokine called adiponectin maintains homeostasis by regulating blood glucose concentration, insulin sensitivity, and lipid metabolism and has anti-inflammatory, antioxidant, and anti-atherogenic activity (25). Another adipokine secreted by the adipose tissue is leptin, a hormone that regulates appetite and plays a role in energy homeostasis. Obese patients with metabolic syndrome are shown to have low adiponectin levels and a leptin-resistant state (26).

Tumor necrosis factor (TNF)-α, also an adipokine, was shown to be elevated in adipose tissue and associated with insulin resistance in obese mice. Moreover, inflammation mediators such as C-reactive protein, interleukin (IL)-6, and plasminogen activator inhibitor were elevated in the plasma of these mice ((27).

1.2 Lipids and Lipid Regulation

1.2.1 Dietary Lipids

1.2.1.1 Lipid Classifications

Lipids are a classification of biologically important molecules characterized by the presence of a number of fatty acid “tails” comprised of long carbon chains connected to a central “backbone” such as glycerol (28). Lipids in the diet are primarily divided into three separate categories, saturated, trans, and unsaturated fatty acids. These classifications depend entirely on the presence of double bonds which cause kinks in the otherwise straight fatty acid chain and how these double

bonds are formed. According to recommendations in the Nordic Nutrition recommendations (NNR) total dietary fat intake should make up somewhere between 25-40% of total energy intake (29). Intakes levels which are too low may lead to deleterious health effects such as impaired hormonal production and impaired intake/storage of fat-soluble vitamins (28). Saturated fats are fats containing only single carbon-carbon bonds resulting in a straight chain and more efficient packing, giving them in general higher melting points than unsaturated fats. Trans-fats are fats largely derived from unsaturated fats but made to resemble saturated fats through a process known as hydrogenation (30). These fats in particular are the most damaging for cardiovascular health and it is suggested they be limited to as low as reasonably possible in the human diet (29). Unsaturated fats are, in general, the most health promoting of all the fat categories and can be subdivided into two main categories mono (those containing only one carbon-carbon double bond) and poly-unsaturated (those containing two or more carbon-carbon double bonds) (28). The double bonds in these fatty acids cause them to kink resulting in less efficient packing, a lower melting point, and more rapid oxidation (31).

1.2.1.2 Polar Lipids

Polar lipids (PL) are a variety of lipid molecule consisting of a hydrophilic head group and at least one hydrophobic tail group. These lipids are most commonly found in the membranes of cells and are vital in supporting cellular function by creating a barrier between distinct intra and extracellular environments (32). These lipids are a small proportion of the dietary fats consumed from plant sources (33). The role that these lipids play in glucose control is not yet fully elucidated however previous studies performed at Lund University have pointed to their ability to strongly influence both glycemia and appetite regulation post-prandially and after a second meal. These studies also established an appropriate dose to elicit these responses, this dose being around 13-15g (7).

1.2.1.3 Lipid Digestion and Absorption

Lipid digestion start as all digestion starts, in the mouth. Though not much happens enzymatically in the mouth it is still an important part of the process as the mastication that occurs at this stage aids in increasing the available surface area of food (34). The bolus produced at this stage passes through the esophagus and stomach where further churning occurs, and proteins are largely digested. After this the main portion of lipid digestion occurs beginning with the introduction of bile salts and pancreatic enzymes, these salts and enzymes and salts digest and emulsify larger

fatty molecules, called triacyl-glycerides (TAG), into absorbable single fatty acids (34). These fatty acids are packed into transport molecules called micelles for absorption into the body at the brush border of the small intestine. Post absorption fat molecules are often used for energy in the body through beta oxidation at the cellular level, or they may be used in important fatty molecules throughout the body (28). They may also be stored in adipocytes in their TAG form for later use, this is in a "normal" diet a potentially beneficial thing as it can be used in cases of prolonged energy deficit. However, prolonged excessive caloric intake and by consequence excessive storage of lipids in adipose tissue can lead to obesity which is a net negative condition for the overall health of a person or population (35,36).

1.3 Bread/Cake Making

1.3.1 Overview

Bread dough, although made with the same basic ingredients of flour, water, yeast, and salt, has remained a staple food product worldwide. Over the years, bread has evolved from a basic to a nutritious food used as a vehicle for functional ingredients which reflects the changing dietary habits and health consciousness of consumers. Bread today can be enriched with various nutrients and functional ingredients to address specific dietary needs or deficiencies in the population making this staple food not only a source of energy, but also a way to improve health (37).

The conversion of the basic bread ingredients into bread has changed with time, however, the variations are small, and the central standard process has remained the same. The process starts with mixing the correct ratios of flour, water, yeast, and salt together. Next, the gluten structure is formed by mixing or kneading the dough. During this time, air bubbles are incorporated into the dough. Breadmaking processes differ from one another due to how they are mixed and kneaded, the incorporation of air, and the development of the gluten structure (38).

Mixing is a crucial stage that is responsible for the mechanical characteristics of the dough, directly impacting the final product's quality. The mixing process ensures a uniform distribution of all ingredients, hydrates the wheat flour, provides the required mechanical energy to form the protein structure, and introduces air bubbles into the dough (37).

Further work may be done on the dough to improve its rheological properties followed by improving the flavor profile of the bread. The dough may then be divided into serving sizes and then shaped appropriately. Resting the dough at this stage allows further modification of the rheological properties (38).

1.3.2 Gluten

During the process of making bread, a number of physical transformations take place where gluten proteins primarily contribute to the formation of the structure of the bread dough, while starch plays a crucial role in determining the final texture and maintaining the stability of the bread (37).

Gluten is a storage protein present in wheat, barley, and rye and is a mixture of distinct proteins, mainly gliadin and glutenin. The proteins can be divided into sub-categories based on their sulfur content and molecular weight. Further classification is done depending on the primary structure into alpha, beta, gamma, and omega (α, β, γ, and ω) gliadins. The gluten proteins are bound to one another by strong covalent and non-covalent bonds. The structure of these proteins and how they interact with one another give gluten its unique properties (39).

The rheological characteristics of the dough are primarily determined by the interaction of starch, proteins, and water. The proteins in the flour can create a continuous, large-scale viscoelastic network of gluten, but only if there is enough water for hydration and adequate mechanical energy is applied during mixing. This viscoelastic network is crucial for the dough's machinability and influences the texture of the final bread product (37).

More specifically, gluten's rheological and functional properties depend on the ratio of glutenins to gliadins, and their interactions with one another since both proteins individually play an important role in controlling the viscoelastic properties and quality of the final product. For example, gliadins are responsible for the dough's viscosity and extensibility whereas glutenins determine the elasticity and strength due to their cohesive characteristics (39).

During the proofing or fermentation stage, the metabolic activity of yeast leads to the release of carbon dioxide and the expansion of pre-existing air bubbles from the mixing process. This causes the dough to inflate, increasing in volume and thinning the cell walls before they eventually collapse. The growth of these gas bubbles during proofing and baking shapes the structure of the

bread, influencing the final volume and texture of the product. The yeast metabolizes carbohydrates (starch and sugars) into carbon dioxide and alcohol during alcoholic fermentation. Enzymes found in both yeast and flour aid in accelerating this reaction. The carbon dioxide generated in these reactions causes the dough to rise, while the alcohol mostly evaporates during baking. As fermentation progresses, each yeast gives off carbon dioxide bubbles. Thousands of these tiny bubbles, each encased in a thin gluten film, grow as the fermentation progresses. The kneading or remixing of the dough encourages the release of larger gas bubbles, leading to a more uniform distribution of bubbles within the dough (37).

The characteristics of the gas bubbles that form during the proofing and baking stages, such as their size, distribution, growth, and collapse, significantly influence the final quality of the bread, affecting both its texture and volume. As the intense heat from the oven permeates the dough, the gases within the dough expand, leading to an increase in the dough's size. As the temperature continues to rise, the rate of fermentation and the production of gas cells also increase. This process continues until the yeast is deactivated at around 45°C. When the proteins denature, the gluten strands that encase each gas cell transform into a semi-rigid structure that forms the bread crumb. The enzymes naturally present in the dough are deactivated at various temperatures during baking. The sugars and protein breakdown products released from the enzymatic activity then sweeten the bread crumb and participate in Maillard or non-enzymatic browning reactions, which give the crust its brown color (37).

1.3.3 The role of fats

The role fats play in baking can in general be described as both contributing to the flavour overall and most importantly to the structure and perceived moistness or softness. They do this by preventing some of the water from absorbing into the flour by coating starch granules in a fatty layer, this results in a more tender final product (40).

2. Methodology

2.1 Food Product Development

Our master's thesis began by designing a breakfast product incorporated with polar lipids (PLs). Given that a preceding master's thesis had utilized a high dosage of 17.6 g of PLs per serving (7), we resolved to incorporate 14 g of PLs into our breakfast product. Uncertainties regarding the impact of 14 g of PLs per serving on the structural properties of the food product led us to also experiment with a lower dosage of 7 g of PLs, especially since the lower dose in the prior study was 8 g (7).

Since we planned on creating a breakfast item, we decided to try to create a type of muffin. To minimize potential food waste and facilitate recipe adjustments, we began with small-scale preparations which focused on creating a muffin incorporated with a single serving of PLs (either 7 or 14 g).

We adapted a mug cake recipe specifically designed to suit single servings and modified the flour content from 31 g to 60 g to maintain appropriate dry ingredient ratios and to ensure 50 g of available carbohydrates per serving. The absence of oil and milk in our formulation necessitated the substitution of the original recipe's total liquid content (104 ml) with water, which was later refined to 89 ml for optimal consistency (41).

The baking process involved setting the oven at 200°C and initially, using a large 14 cm aluminum muffin mold. The initial baking time of 27 minutes resulted in an undercooked product, prompting further trials of 37 and 47 minutes. Observations revealed that the batter with 14 g of PLs was noticeably thicker and darker compared to the 7 g variant. Sensory evaluations indicated a mild PL flavor, with the overall taste being satisfactory. A snippet of lab notes made during the entire production process can be seen in appendix A.

2.1.1 Baking Powder vs. Yeast

Initially, we prepared the first batch of our product using 1.2 grams of baking powder, in accordance with the original recipe. This method proved to be successful. In subsequent experiments conducted over the following days, we opted to test yeast as an alternative leavening

agent, employing 1.6 g of yeast. The liquid ratio was maintained, and the yeast was pre-dissolved in warm water before being mixed with the dry ingredients.

The yeast muffins produced with both 14 and 7 g of PLs exhibited significant browning and hardness on their upper surfaces while remaining undercooked internally. To address the issue of excessive browning, we adjusted the position of the oven rack to a lower level and covered the cakes with aluminum foil after a 20-minute rise period in the oven. Despite these adjustments, the cakes continued to exhibit undercooking in the center and overcooking at the top. Further trials using 1 g of yeast failed to rectify these issues and produced similar results.

Based on these observations, it was determined that yeast caused the cake to brown more rapidly and rise more significantly than baking powder, necessitating longer baking times. Consequently, we resolved to use baking powder in the final product formulation to achieve a more desirable baking outcome.

2.1.2 Testing with Various Molds

Throughout the ten-week baking period, we experimented with three different types of molds to prepare our muffins. Initially, we utilized individual aluminum muffin molds discovered in the study kitchen, each measuring approximately 14 cm in diameter, suitable for a single serving of batter. This initial trial yielded muffins that were soft on the exterior and perfectly cooked internally. Unfortunately, however, these molds were subsequently unavailable, and we therefore replaced them with alternatives.

We tested both rectangular and circular aluminum molds of similar size. However, the batter, particularly the 14 g variant, proved too thick to evenly distribute within these molds since these were not specifically designed for muffins. In contrast, although the 7 g PL batter was sufficiently fluid, the resulting cakes came out excessively hard and crisp.

After numerous trials, we opted for muffin paper cups with a diameter of 6 cm, dividing the batter between two cups as a single cup proved too small for one serving. A primary challenge during the mold transition was achieving the correct crust texture. Attempts to soften the overly hard crust by reducing baking time invariably resulted in an undercooked center.

To address this, we first attempted using only bottom heat, which unfortunately led to burning. Incorporating both top and bottom heat while introducing a pot of water to generate steam within the oven did not successfully mitigate the issue; the baked products remained undercooked. Although they appeared set, the cooling process revealed incomplete gelatinization of the starch.

Ultimately, we experimented with eliminating molds altogether, opting for a glass baking tray to prepare a larger batch. Despite various adjustments to the baking process, this method consistently produced a cake that was undercooked in the middle with an overbaked outer crust.

2.1.3 The Final Recipe

Our finalized recipe comprised 60 g of flour, 1.2 g of baking soda, and 0.7 g of salt as the dry components. For the liquid ingredients, 14 g of PLX were combined with 89 ml of water. In the control recipe, the liquid portion consisted of 14 g of sunflower oil mixed with 75 ml of water, totaling 89 ml, while the dry ingredients remained unchanged. This made two small muffins as one serving.

The batter preparation involved gently mixing the wet and dry ingredients until just combined, taking care to avoid overmixing. Equal quantities of the batter were then dispensed into two paper cups, constituting a single serving. Instead of making one large batch, the batter for each dose was prepared individually and dispensed into the paper cups to ensure correct dosage.

The oven was preheated to 200°C, and the cups were placed within a glass baking dish to mitigate excessive browning of the muffin bases and to maintain their softness. At the 20-minute mark, following their rise, the cups were removed from the oven and covered with aluminum foil to prevent over-browning and hardening of the tops. Both the top and bottom heat settings of the oven were utilized.

The total baking duration was set at 1 hour and 25 minutes, followed by a cooling period of 20 minutes. Subsequently, the muffins were placed into freezer bags and stored in the freezer in preparation for use in human trials. See appendix B for the pictures of the final product, both control and PL muffins.

2.2 Starch Analysis

A refined enzymatic method, developed by Holm et al. in 1986 (42), was employed for the starch analysis of our developed baked products (muffins). This method is notable for its ability to achieve high accuracy and precision in starch recovery, even with minimal incubation times.

The analytical procedure commences with the mixing of the milled sample in Termamyl—a thermostable α-amylase—and distilled water, followed by the gelatinization of the sample through boiling. Upon cooling, amyloglucosidase, along with sodium acetate buffer, is introduced to facilitate further breakdown of the starch. After this enzymatic reaction, glucose oxidase/peroxidase (GLOX) is added to detect and quantify glucose via spectrophotometric analysis.

Finally, the starch content is quantified using the measured glucose concentrations, adjusted by specific dilution factors and a conversion factor to account for molecular differences between glucose and starch. These calculations are standardized on a dry matter basis.

2.3 The Human Trials

2.3.1 The Study Subjects

The study involved nine healthy participants, consisting of six females and three males, aged between 20 and 30 years. These individuals were all non-smokers with a normal BMI ranging from 19 to 28 kg/m², and did not suffer from any metabolic disorders, food allergies, or consume medications that could influence the study's results. Additionally, none of the participants had ingested probiotics for a minimum of four weeks before the commencement of the study. All subjects adhered to an omnivorous diet. The research was carried out in the spring of 2024 at the Division of Food and Pharma, Lund University. Ethical approval for the study was granted by the Regional Ethics Review Board in Lund, Sweden. The participants were thoroughly informed about the study's protocol and objectives, and their participation was voluntary, with informed consent obtained for all involved.

2.3.2 Study Procedure

We conducted a randomized cross-over study that involved two separate test days for each participant, with each day featuring a distinct test breakfast. The breakfast options included a test product enriched with polar lipids and a control product based on sunflower oil. A standardized lunch, consisting of instant mashed potatoes and vegetarian meatballs, was served 3.5 hours post-breakfast. An interval of approximately seven days, serving as a washout period, separated the two test days for each participant.

The parameters evaluated were postprandial glucose levels and subjective appetite responses during both the acute and the second meal postprandial periods following the test breakfast and the standardized lunch.

Participants were instructed to follow specific guidelines on the day preceding each test day to ensure uniform conditions among all subjects. These guidelines included abstaining from alcohol, high-fiber foods, and strenuous physical activities. Moreover, at 21:00 on the eve of the test days, participants were required to consume a standardized evening snack of white bread provided by the research center. Following this snack, participants were asked to fast, refraining from consuming any food or beverages until the initial meal at the test site the following day. They were also instructed to maintain a similar routine on the days preceding each test to standardize meal patterns.

Participants were expected to arrive at the test site by 7:30 AM and were allotted approximately 20 minutes to acclimatize. During this period, their weight and height were measured, and an initial finger prick was taken to record fasting glucose levels. A second measurement was subsequently taken, and an average of the two was calculated to establish the fasting glucose level for that day. Breakfast was served at approximately 8:00 AM, and participants were instructed to consume their meal within 10 minutes.

Postprandial glucose concentrations were measured at multiple intervals after breakfast—15, 30, 45, 60, 90, 120, 150, 180, and 210 minutes. Participants were also required to complete an information sheet recording their subjective appetite scores (satiety, hunger, and desire to eat) before each glucose measurement.

The standardized lunch was provided at the 210-minute mark, and similar to breakfast, participants were expected to consume it within 10 minutes. The same monitoring routine was repeated post-lunch with glucose concentrations and appetite scores recorded at 225, 240, 255, 270, 300, and 330 minutes.

Throughout each test day, which lasted for a total of six hours, participants were provided with 250 ml of water at both breakfast and lunch, and an additional 150 ml at the 150-minute mark. They were not permitted to consume any food or beverages other than those provided during the trial.

2.3.3 Test Breakfast

Approximately one week prior to the commencement of the study, the test muffins were prepared and subsequently frozen for use on the trial days. We would arrive at the venue 30 minutes ahead of the participants to microwave the muffins until they were suitably warm before serving.

Participants were randomly assigned to receive one of the breakfast products on each of the two trial days. Each breakfast serving consisted of two muffins, with each muffin containing 7 grams of fat, totaling 14 grams of fat per serving. These muffins were crafted using either polar lipids (PLX) or sunflower oil. The PLs used in the muffins were produced by AAK Sweden AB, located in Karlshamn, Sweden.

Alongside the muffins, a 250 ml glass of water was provided. Participants were required to consume both the muffins and the water within a 10-minute timeframe.

Table 1. Ingredients in PL enriched and control muffins.

	PL Muffins per serving	**Control muffins per serving**
Flour (g)	60	60
Baking powder (g)	1,2	1,2
Salt (g)	0,7	0,7

Fat (g)	14	14
Water (ml)	89	89

2.3.4 Standardized Lunch

On both test days, a standardized lunch was served, consisting of approximately 60 g of vegan mashed potato powder (Eldorado, Stockholm, Sweden) mixed with 350 ml of water. This was accompanied by 60 grams of vegetarian meatballs (Felix, Eslöv, Sweden) and a 250-ml glass of water. According to the nutritional information provided by the manufacturers, the meal contained 54 grams of carbohydrates. Once again, participants were required to consume both the lunch and the water within a 10-minute timeframe.

Table 2. Composition of the standardized lunch meals.

	Mashed Potatoes	**Vegetarian Meatballs**	**Standardized Lunch**
Total Energy in Kcal	227	137	364
Carbohydrates (g)	48	6	54
Fat (g)	<1	10	10
Protein (g)	5	7	12
Fiber (g)	-	2	2

2.3.5 Determining Blood Glucose Concentrations

To determine the blood glucose concentrations, the HemoCue® Glucose 201+ System was employed. Microcuvettes, which had been stored in the refrigerator, were removed in advance of the experiment to allow them to reach room temperature. The system was calibrated for accuracy before the commencement of the experiment on each of the trial days.

Blood sampling involved pricking the middle and ring fingers. Prior to each puncture, the selected finger was sterilized with chlorhexidine. Blood was drawn using a Hemocue safety lancet to obtain capillary blood. This blood was then carefully collected into the microcuvette, taking care to avoid air bubble entrapment, and immediately placed into the HemoCue® Glucose 201+ System for measurement. The blood glucose readings were promptly recorded as soon as they appeared on the screen, and the used microcuvette was disposed of in a biohazard waste bin. For each participant, the total volume of blood drawn per trial did not exceed 500 µl, with the total amount for both test days approximately 1000 µl.

2.3.6 Determining Subjective Appetite Parameters

In addition to blood glucose measurements, subjective appetite parameters were also assessed. Prior to each finger prick, participants were asked to evaluate their levels of satiety, hunger, and desire to eat by marking a vertical line on a 10 cm visual analog scale. Appendix C provides an example of the marking sheets given to participants. Marks were permitted anywhere along the scale, except at the extreme ends.

2.4 Statistical Analysis

A variety of statistical analyses were performed to determine any statistically significant differences in the results and allow the researchers to draw conclusions regarding the effectiveness of the PL intervention versus the sunflower oil intervention. Firstly, blood glucose data was transferred directly from a recording sheet to Microsoft Excel (43) and categorized by trial day and sample ID. Subjective appetite measures were transposed from the 10cm linear scale to a numerical value by measuring the distance of the mark from the 0cm baseline, the transposed values were then recorded in Microsoft Excel and divided by the parameters used in the blood glucose Excel sheets. Area under the curve (AUC) calculations were completed in GraphPad Prism ver. 10.2.3 (44). To test for significant differences between data sets (PL vs. control) and establish the means and standard error of mean (SEM) results were entered in Minitab (45). In order to produce this data a variety of analyses were performed in Minitab, initially descriptive statistics were calculated to define the means and SEMs. Following this all-data sets were tested for normality; any non-normal data was transformed using a Box-Cox transformation. The normalized data sets were then run through a general linear model ANOVA to establish any significant

differences at a 95% CI ($p<0.05$). Finally, all data was graphed in GraphPad Prism for visual representation.

3. Results

3.1 Starch Analysis

Table 3 shows the results of a starch analysis carried out on both the PL and control products. Both tests were done with replicates in order to improve data quality (46). It was found that the PL product contained 37.18% available starch by fresh weight and 76.12% available starch by dry weight, and the control product contained 11.8% available starch by fresh weight and 59.36% available starch by dry weight. As can be clearly described from the table the PL caused substantially greater amounts of starch to remain available during baking and drying.

Table 3. Percentage of available starch in products by dry and fresh weight

Sample Name	**Sample Weight**	**Average % Starch Dry Sample**	**% Starch Fresh Weight**
Control	500mg	59.36	11.80
PL	500mg	76.12	37.18

3.2 Postprandial Blood Glucose Responses, Acute and Second Meal

Figure 1 shows the plotted incremental changes in blood glucose concentration from the first fasting value at 0 minutes through the end of the trial days at 330 minutes. The dotted line seen in the figure denotes the time at which the standardized lunch meal was served (210 minutes). As can be seen in the curve some differences can be noted though only one is of statistical significance. This being the post lunch peak, the difference in mean for the peak values between the two groups

was found to be 6.1%. However, differences in mean iAUC can still be observed, the polar lipid test showed a 22.6% lower iAUC than the control treatment. Additionally, the iAUC post standardized lunch (210min) saw the largest non-significant difference with the PL group having a 29.2% lower iAUC from 210-330 minutes. Table 4 outlines the mean responses ± SEM within the two treatment groups, all values in a single row with a differing superscript letter are significantly different.

Table 4. Blood glucose concentrations and iAUC data from trial days[1]

Measurements	**Polar Lipids**	**Control**	**Delta %**
Fasting Blood Glucose (mmol/L)	5.7 ± 0.1^{A}	5.8 ± 0.1^{A}	-1.7%
Blood Glucose at 210min (mmol/L)	5.4 ± 0.1^{A}	5.5 ± 0.1^{A}	-1.8%
iAUC 0-330min (mmol*min/L)	231.4 ± 54.1^{A}	290.3 ± 41.8^{A}	-22.6%
iAUC 0-120min (mmol*min/L)	93.7 ± 19.3^{A}	109.6 ± 12.6^{A}	-15.6%
iAUC 210-330min (mmol*min/L)	115.1 ± 32.0^{A}	154.4 ± 25.5^{A}	-29.2%
After Breakfast Peak (mmol/L)	7.9 ± 0.3^{A}	7.8 ± 0.2^{A}	+1.3%
After Lunch Peak (mmol/L)	8.0 ± 0.3^{A}	8.5 ± 0.3^{B}	-6.1%

[1]Data presented as mean values ± SEM, all values in a row with differing superscript letter are significantly different at a 95% CI using a general linear model ANOVA test for analysis.

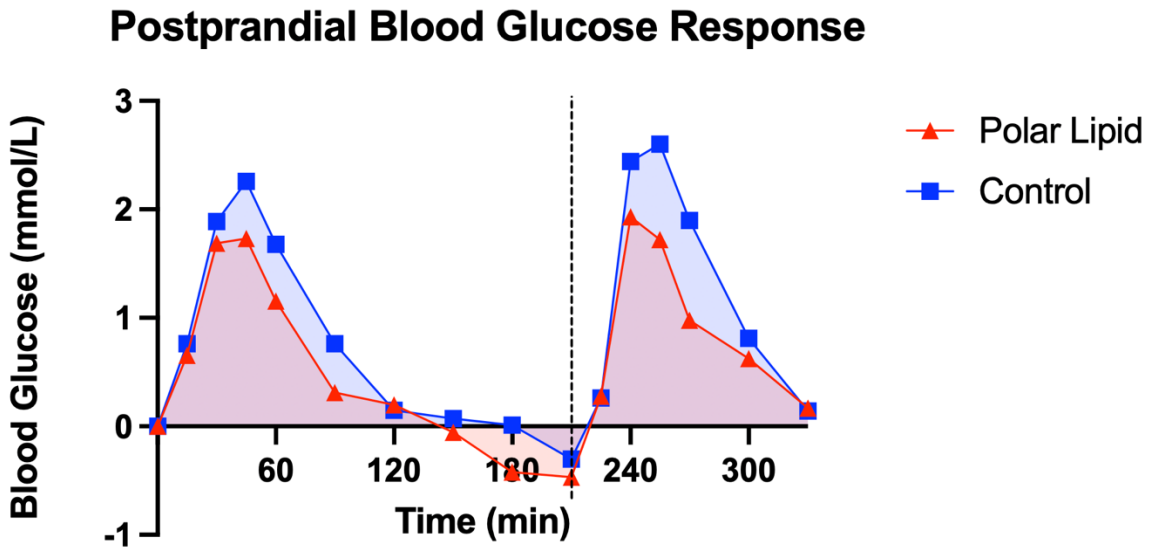

Figure 1. Graphical representation of incremental blood glucose response after test breakfast and standardized lunch meals. Values are presented as mean values, n=9 healthy subjects.

3.3 Subjective Postprandial Appetite Responses, Acute and Second Meal

Figures 2, 3 and 4 show the subjective postprandial appetite responses both acute and second meal (Fullness, Hunger, and Desire to Eat respectively). The Y-axis shows the subjective scores ranked on a linear scale from 0 to 10 centimeters, and the dotted line denotes the time at which the standardized lunch was served, 210 minutes. No statistically significant differences were observed between either treatment for any of the tested measurements. However general trends can be seen as all subjective appetite markers improved by various delta percentages as outlined in tables 5, 6, and 7.

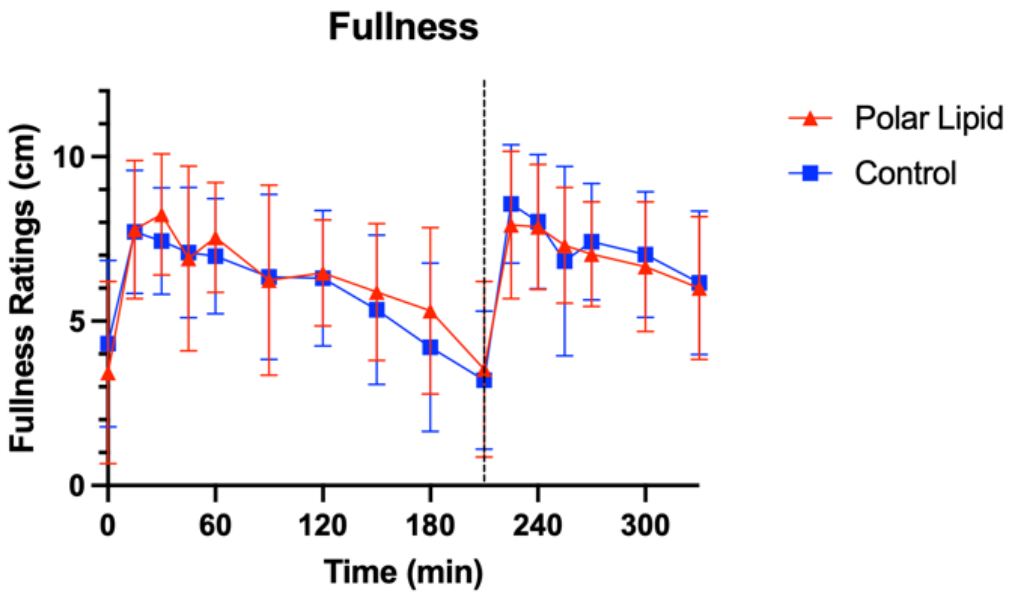

Figure 2. Graphical representation of subjective feelings of fullness after test breakfast and standardized lunch meals. Values are presented as mean values ± standard deviations, n=9 healthy subjects.

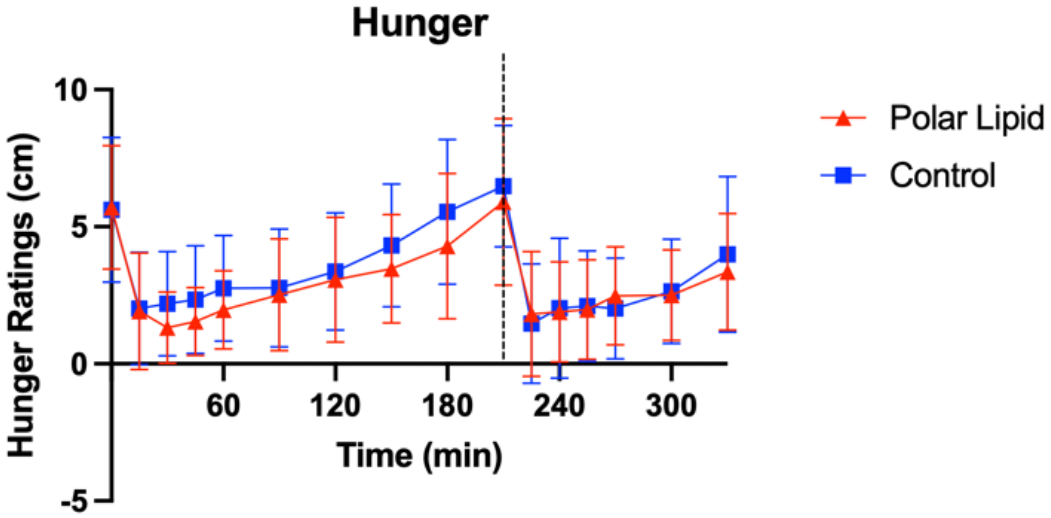

Figure 3. Graphical representation of subjective feelings of hunger after test breakfast and standardized lunch meals. Values are presented as mean values ± standard deviations, n=9 healthy subjects.

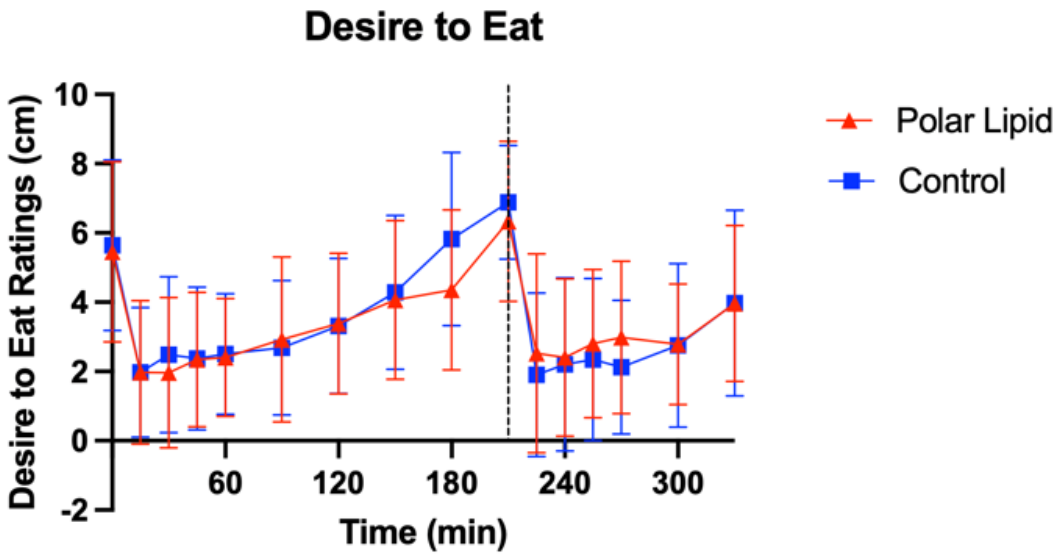

Figure 4. Graphical representation of subjective feelings of desire to eat after test breakfast and standardized lunch meals. Values are presented as mean values ± standard deviations, n=9 healthy subjects.

Table 5. Averaged values for subjective feelings of fullness.[1]

Measurements	Polar Lipids	Control	Delta %
Fasting value (cm)	3.4 ± 0.9[A]	4.3 ± 0.8[A]	-23.4%
Value at lunch, 210min (cm)	3.5 ± 0.9[A]	3.2 ± 0.7[A]	+9.0%
iAUC (cm/min)	1123.7 ± 330.8[A]	819.9 ± 227.7[A]	+31.3%
iAUC (0-120min) (cm/min)	435.7 ± 124.6[A]	313.7 ± 91.4[A]	+32.6%
iAUC (210-330min) (cm/min)	418.3 ± 91.0[A]	464.1 ± 84.1[A]	-10.4%
Peak Value (0-210min) (cm)	8.5 ± 0.5[A]	8.2 ± 0.6[A]	+3.6%

Peak Value (210-330min) (cm)	8.5 ± 0.6^{A}	8.7 ± 0.5^{A}	-2.3%

[1]Data presented as mean values ± SEM, all values in a row with differing superscript letter are significantly different at a 95% CI using a general linear model ANOVA test for analysis.

Table 6. Averaged values for subjective feelings of hunger.[1]

Measurements	**Polar Lipids**	**Control**	**Delta%**
Fasting value (cm)	6.0 ± 0.6^{A}	5.6 ± 0.9^{A}	+8.5%
Value at lunch, 210min (cm)	5.9 ± 1.0^{A}	6.5 ± 0.7^{A}	-9.7%
AUC (cm/min)	958.7 ± 172.6^{A}	1097.2 ± 193.6^{A}	-13.5%
AUC (0-120min) (cm/min)	280.6 ± 60.1^{A}	336.0 ± 71.8^{A}	-18.0%
AUC (210-330min) (cm/min)	310.8 ± 67.3^{A}	317.5 ± 74.7^{A}	-2.1%
Peak Value (0-210min) (cm)	6.0 ± 0.7^{A}	5.9 ± 0.8^{A}	+1.7%
Peak Value (210-330min) (cm)	6.3 ± 0.8^{A}	6.6 ± 0.8^{A}	-4.7

[1]Data presented as mean values ± SEM, all values in a row with differing superscript letter are significantly different at a 95% CI using a general linear model ANOVA test for analysis.

Table 7. Averaged values for subjective feelings of desire to eat.[1]

Measurements	**Polar Lipids**	**Control**	**Delta %**

Fasting value (cm)	5.5 ± 0.9^{A}	5.6 ± 0.8^{A}	-1.8%
Value at lunch, 210min (cm)	6.3 ± 0.8^{A}	6.9 ± 0.5^{A}	-9.1%
AUC (cm/min)	1100.0 ± 200.1^{A}	1126.4 ± 195.7^{A}	-2.4%
AUC (0-120min) (cm/min)	327.8 ± 76.5^{A}	331.6 ± 69.8^{A}	-1.2%
AUC (210-330min) (cm/min)	373.8 ± 78.1^{A}	338.5 ± 83.2^{A}	+9.9%
Peak Value (0-210min) (cm)	5.7 ± 0.8^{A}	5.9 ± 0.7^{A}	-3.4%
Peak Value (210-330min) (cm)	6.6 ± 0.6^{A}	6.9 ± 0.6^{A}	-4.4%

[1]Data presented as mean values ± SEM, all values in a row with differing superscript letter are significantly different at a 95% CI using a general linear model ANOVA test for analysis.

4. Discussion

This degree project aimed to develop a food product enriched with plant polar lipids (PL) and evaluate its impact on glycemic response. The motivation originated from previous studies at Lund University, which showed that oat PLs influence postprandial metabolic responses (6) and that varying concentrations of plant PLs significantly reduce postprandial blood glucose levels when administered after breakfast (7). Additional research indicated that postprandial glucose responses are higher after a high-GI breakfast compared to a low-GI breakfast. The current project sought to determine the optimal dosage of plant polar lipids in the food product to achieve the desired glycemic response (17).

The PL dose established was 14g per person incorporated in 2 muffins, 6 cm in diameter each (see Appendix B). Results were obtained once all the participants had completed trials under the

standardized conditions described previously. Figure 1 shows the blood glucose differences obtained between control and PL groups. Blood glucose concentrations remain steady at about 5 mmol/l during a fasting period and in healthy individuals, seldom falls below this point. Peptide hormones such as the gastric inhibitory peptide (GIP) secreted from the enteroendocrine cells within the mucosa of the small intestine prepare the pancreas and other exocrine tissues to expect an increase in blood glucose levels. After a meal, the glucose concentration begins to rise above 5 mmol/l and as it does, these peptide hormones facilitate the secretion of insulin (47,48). This pattern can be observed in figure 1 for both the control and PL breakfast. Both groups start at baseline (0 mmol/L) and experience an initial increase in blood glucose levels, peaking at around 60 minutes. The Control group reaches a higher peak (around 2.5 mmol/L) compared to the Polar Lipid group (around 2 mmol/L). Following this peak, both groups show a decline in blood glucose levels, returning close to baseline by 120 minutes. Between 120 and 180 minutes, the levels remain near baseline, with the Polar Lipid group showing a slight dip below zero.

The different metabolic effects elicited by low and high GI foods are believed to be caused by the rate at which glucose is absorbed in the small intestine (8). High GI foods are absorbed rapidly which results in a blood glucose spike causing insulin to be released from the pancreatic beta cells while simultaneously inhibiting the release of glucagon from the pancreatic alpha cells. Insulin lowers the blood glucose levels by facilitating its uptake into the liver, skeletal muscles, and adipocytes and promoting its conversion to glycogen as storage (8,48,49).

However, to counter this high blood glucose spike, the high insulin and low glucagon levels cause the blood glucose to fall below the baseline resulting in hypoglycemia. In response to this state, glycogenolysis and gluconeogenesis occur to restore the blood glucose in the normal glycemic range. A low GI-meal, in contrast, will not cause a blood glucose spike due to more gradual absorption of glucose in the small intestine which in turn will prevent hypoglycemia in the postprandial period (8). In addition, the hypoglycemia induced by the high GI meal may result in increased levels of hunger and may therefore lead to the consumption of more high GI foods. In contrast, as seen in several short-term intervention studies, low GI meals increase satiety and reduce hunger levels in the later meal (50). This second meal effect can be attributed to the slower absorption rates resulting in a longer fasting period between meals (8). Although hypoglycemia can be observed in for both control and PL at the 210-minute mark after breakfast, the dip below

baseline for the control group is slightly lower than for the PL group which stays closer to the baseline.

Around 240 minutes, there is a second increase in blood glucose levels for both groups. The Control group again reaches a higher peak (around 2.5 mmol/L) compared to the Polar Lipid group (around 2 mmol/L). Blood glucose levels then decline after the second peak, returning to near baseline levels by 330 minutes, with the decline appearing slightly steeper for the Control group. The Control group consistently shows higher peaks compared to the PL group during both increases in blood glucose levels. The areas under the curves suggest that overall, the Control group has higher blood glucose levels over time compared to PL. The graph indicates that PL intake might result in lower blood glucose peaks compared to the Control group, suggesting a possible moderating effect on blood glucose levels.

The differences observed between the two types of test breakfasts align with findings from other studies on the effects of various fats on glycemic responses and gastric emptying. For instance, a study by Celgg et al. examined how adding different types of fats to a carbohydrate-rich food (a pancake with 50 g of available carbohydrate) affects gastric emptying, glycemic response, and satiety in vivo. The results indicated that adding fats to the pancakes impacted the glycemic response, with olive oil and MCT (medium chain triglycerides) oil having the greatest effect in reducing glycemic response. Regarding gastric emptying, the study found that the type of fat added to a meal can significantly influence the rate of gastric emptying, which subsequently affects glycemic response and satiety (51).

One mechanism explaining the different rates of gastric emptying for fats involves the release of gut peptides such as cholecystokinin (CCK) and peptide YY (PYY) in the small intestine. A study by Maljaars et al. concluded that triacylglycerols containing unsaturated fatty acids increase satiety more effectively than those with saturated fatty acids, primarily due to higher CCK secretion (52). Since the inhibition of gastric emptying is one of the physiological actions of CCK, varying concentrations of CCK secretion will result in different rates of gastric emptying (51,53).

Another mechanism affecting both gastric emptying and glycemic response is the formation of amylose-lipid complexes, which reduce carbohydrate digestibility by making it resistant to enzymatic digestion. Factors influencing the digestibility of these complexes include

gelatinization, lipid chain length, lipid solubility in water, and degree of saturation. A study by Lau et al. examined how different types of fats in bread affect the formation of amylose-lipid complexes (ALCs) and the subsequent glycemic response. The control product had the highest glycemic response, while the test products with olive oil (containing oleic acid) and coconut oil (containing MCTs like lauric and myristic acid) showed lower glycemic responses compared to butter and grapeseed oil (54). Thus, the differences observed in the results of this study may be attributed to the different types of fats used in the two test products.

This mechanism is particularly useful not only for producing food products that elicit a lower glycemic response but also for managing T2DM. A study investigating the impact of different fatty acids on postprandial insulin, glucose, and triglyceride responses in subjects with T2DM found that meals rich in oleic acid or EPA and DHA resulted in a lower postprandial insulin response compared to meals rich in palmitic acid or linoleic acid. Although no significant differences were observed in postprandial glucose responses before lunch (210-minute mark), the study noted limitations related to its small sample size, consisting of only 11 subjects (55). This limitation can also be attributed to the design of this study as well, where the lack of statistical significance may be attributed to the small sample size of only nine subjects. This issue is further compounded by two of the nine subjects having higher fasting glucose levels, suggesting an underlying degree of insulin resistance. Therefore, we recommend conducting the test with a larger sample size in the future as it may yield statistically significant results.

However, it is important to note that the differences observed in the after-lunch peak are in fact statistically significant with a delta percentage of 6.1%. This is consistent with other studies that have shown a similar second-meal effect where the glucose response was lower after a standardized lunch when given a low-GI breakfast as compared to a high-GI one (56–58).

Moving on to the subjective appetite parameters, Figure 2 shows the fullness obtained between control and PL groups. Both groups start with an increase in fullness ratings, peaking around 30 minutes. The Control group reaches a slightly lower peak compared to the Polar Lipid group, which peaks at 8.6cm. Though not statistically significant this indicates that on average the PL treatment achieved 2% greater subjective feelings of fullness. After the initial peak, fullness ratings gradually declined for both groups, with intermittent fluctuations. At 210 minutes, the fullness levels for both groups achieve levels close to baseline this corresponds directly with the peaks of

both the hunger and desire to eat graphs (figures 3 and 4). Following the serving of the standardized lunch at 210 minutes the average fullness ratings then jump back up to peak levels at 225 minutes with the PL treatment registering at an average of 8.5cm and the control group at an average of 8.7cm. This peak corresponds directly with the lowest average ratings of both hunger and desire to eat after the 210-minute mark. The area under the curve for both treatments is shown in table 5, the PL treatment had an average total iAUC of 1123.7 (cm/min) and the control treatment had an average total AUC of 819.9 (cm/min) a non-statistically significant delta of ~31.3%. This delta suggests a general trend towards improvement in feelings of fullness in the PL group. This fullness data indicates that PL X may present a benefit over sunflower oil in terms of increasing overall satiety. However, this conclusion requires more research as no statistically significant benefit was detected. This outcome is in line with current research comparing PL to neutral oils such as soy, sunflower, and rapeseed (6,7). This finding though not statistically significant lends strength to preexisting data and warrants further exploration to elucidate greater clarity in the findings.

Figure 3 shows the hunger parameter obtained between control and PL groups. Initially, both groups start with similar fasting hunger ratings around 6 cm, which quickly drop to approximately 2 cm within the first 30 minutes. After this initial decrease, hunger ratings for both groups gradually increased over time. Hunger levels peaked at 210 minutes right before the standardized lunch meal was serves with the Control group showing slightly higher hunger levels than the PL group, a delta of approximately 10%. Following the lunch meal both groups experience a rapid decline in perceived hunger rating until ~225 minutes, after which both groups see a steady rise towards baseline levels. Figure 4 shows desire to eat rating which follow a similar trend, with the PL treatment outperforming the control treatment at almost all the analyzed time stamps. This observed trend is in line with previous studies performed at the department (6,7). In summation, this data suggests that though not statistically significant the introduction of polar lipids seemed to improve perceived fullness, hunger, and desire to eat as compared to the neutral reference oil. This could potentially decrease the caloric intake of potential consumers over time resulting in favourable outcomes with respect to obesity and T2DM (35). These observed effects on appetite regulation can be attributable to a multitude of mechanisms. The main potential mechanism is due to modulation of gastric emptying rate. As the presence of fats in a diet or meal led to delayed gastric emptying and thus higher perceived satiety (36). This is supported by the other potent mechanism of upregulation of satiety inducing hormones GLP-1, PYY, and CCK. As shown in

previous studies the addition of PLs appear to substantially increase the circulating levels of these hormones (6,59). These results were consistent with those seen by Hossain et al. and van den Elzen et al. (6,7). However further research is needed to overcome limitations in the present study and reveal further details with respect to mechanisms of action and long-term effects of PL consumption.

These results could potentially be improved by improving the statistical significance between products, the lack of statistical significance is likely attributable to the overall lack of participants (60). This is one of the main limitations that we observed in the study as there was an overall lack of interest in participation amongst eligible participants, and we experienced multiple dropouts. Thus, we would propose the following changes for further investigating the effects of PL in a pre-made breakfast product. Acquire a larger sample of eligible participants, provide greater time for the acquisition of participants, and run multiple recruitment rounds. Additionally due to a lack of participants researchers were required to participate in the study, this removed all blinding during testing and therefor introduced another potential source or error/bias (61).

5. Conclusion

In concluding this project, the objectives of creating a standardized food product enriched with PL and measuring its effects on glycemic control and subjective appetite sensations both acutely and following a second meal were successfully completed. The results obtained during this project suggest that PL is viably incorporated into a standardized food product and has effects that in general trend towards positively improving all test parameters. Due to the limitations however, it is difficult to draw strong conclusions as much of the data remains non statistically significant. In the future it is suggested that researchers measure more biomarkers in order to better quantify the mechanisms through which the PLs affect both the subjective and biochemical responses of test subjects. Additionally, it would be prudent for future research to focus on longer term consumption of PLs to investigate any long-lasting effects, positive or negative, that might be caused by the addition of PLs in the diet.

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Appendices

Appendix A: Snippet of Lab Notes taken during Food Product Development

Trial Number + Type	Notable Elements/Changes	Cook Temp (°C)	Cook Time (min)	Notes	Pictures
Batch 1	Covered the tops with Aluminium foil, used both top and bottom heat, and placed the rack in the middle of the oven.	200	47	Came out light brown with soft tops. However, not fully cooked. Some areas do not have gelatinized starch and it is notably visible while others do.	  

Appendix B: Final Food Product, Control (above) and PL-enriched (below)

Appendix C: Subjective Appetite Parameters

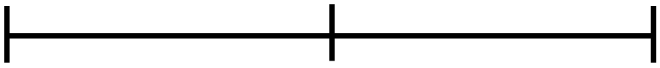
Participant Code: Date:

Time: 0 min
(Just Before Breakfast)

Mark with a line the position on the scale that best corresponds to your appetite sensation.

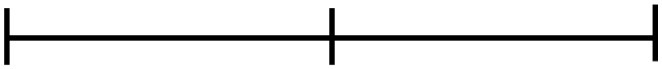
How FULL do you feel right now?

Not FULL at all **Very FULL**



How HUNGRY do you feel right now?

Not HUNGRY at all **Very HUNGRY**



How much do you want to EAT right now?

Do not want to EAT **Strong desire to EAT**