EXPERIMENTAL AND CLINICAL INVESTIGATIONS OF SLOWLY DIGESTIBLE CARBOHYDRATES FOR IMPROVED PHYSIOLOGICAL OUTCOMES AND METABOLIC HEALTH

by

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A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Food Science

West Lafayette, Indiana

May 2022

THE PURDUE UNIVERSITY GRADUATE SCHOOL
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To my mother Sonia and aunt Magaly who have always been there to support me

ACKNOWLEDGMENTS

The research described in this dissertation was made possible by the support of the American People provided to the USAID Feed the Future Food Processing and Post-harvest Handling Innovation Lab. The research experiments described in Chapter 3 and 4 were additionally supported in part by research funds from Clinical and Translational Sciences Institute, global health research pilot project award. The contents are the sole responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government.

I must acknowledge the numerous individuals who have allowed me to achieve this milestone. First, I want to thank Dr. Bruce Hamaker for being my advisor and mentor. During my time at Purdue, he allowed me to pursue my academic and professional interests and offer support and advice along the way. I am especially grateful for his support during my dietetic internship since that meant I had to take time off from the lab. As a mentor, Dr. Hamaker has helped me become a better scientist by continuously challenging my ideas and always pushed me to be detail-oriented and creative in my grant proposals.

I am also appreciative for the support of my committee members, Dr. Mario Ferruzzi, Dr. Regan Bailey and Dr. Richard Mattes; not only for their time and extreme patience, but for their intellectual contributions to my development as a scientist. I am honored to be guided by such a distinguished group.

As for research collaborators, I want to thank the team at Lumen® and Metaflow; Merav Mor and Shlomi Yeshurun for their support with the planning and implementation of the experiments regarding respiratory exchange ratio in Kenya and the USA. Their help proved vital for the success of the project. To Dr. Travis Dams for facilitating the acquisition and use of continuous glucose monitors in our experiment in the USA; to Dr. Buford Nichols for his guidance with the assessment of respiratory exchange ratio metabolic flexibility, and to Mrs. Anne Wilcox and Mrs. Alexandra Cowan for their support with diet record collection and healthy eating index assessments. Particular thanks to Dr. Violet Mugalavai and Dr. Emmanuel Ayua for their logistical support to conduct experiments at University of Eldoret, Kenya. Finally, thanks to David Arthur as well as the other team members at statistical consulting for their help with experimental design, statistical modeling and data quality control.

I am thankful for the friendship and scientific debate to all the members of my laboratory and the Food Science Department, especially Dr. Anna Hayes, Dr. Jongbing Lim and Dr. Benjamin Redan who provided clarity to my ideas as well as timeline revision of the experimental design, and procedures as well as manuscripts. To Dr. Mario Martinez, who is currently faculty at Aarhus University; I am most grateful for your help and support throughout my research.

To my lab-mates, past and present members of the Hamaker lab who have also given me countless hours support and guidance. To past members – Dr. Mohammad Chegeni, Dr. Tingting Chen, Dr. Sarah Corwin, Dr. Fang Fang, Rachel Jackson, Dr. Moustapha Moussa, Dr. Elizabeth Pletsch, Dr. Leigh Schmidt, Dr. Marwa El Hindawy, Enosh Kazem, Dr. Xiaowei Zhang, Dr. Emmanuel Ayua. Dr. Yony Roman Ochoa, Ola Sangokunle, and Dr. Tianming Yao. To present members, Nuseybe Bulut, Dr. Thaisa Cantu-Jungles, Dr. Oguz Ozturk, Monica Richmond, Rosa Lopez – you have been a wonderful group to finish my Ph.D. with and I am grateful for all the memories we have together (especially the ups and downs of dealing with a pandemic!).

I am very thankful for the day-to-day support and assistance of the Purdue Food Science Department. The staff has been wonderful in helping us complete this race. I am especially grateful to Angela “Angie” Gutterman, Vicki Arnett, and Mitzi Barnett for their constant support as I have navigated my Ph.D.

Finally, to my family and friends, without your support this would have not been possible. I am eternally grateful for your unconditional love, Sonia, Magaly, Olga, Roberto and Danilo. Their selfish and noble support always kept me going. To Joan and Joseph Briller for being my adoptive grandparents while I have been at Purdue. To all the Zamorano family and in particular to Uriel Urbizo for always being ready for a camping trip. To a friend so close I can call my brother, Tuan Pham, thank you for always being kind and supportive. Finally, I am very thankful to my plus one, Dr. Victoria Greenstein, I look forward starting this new chapter with you by my side.

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LIST OF ABREVIATIONS

ALC	Amylose-lipid complexes
AgRP	Agouti-related peptide
AMG	Amyloglucosidase
AUC	Area under the curve
CART	Cocaine-and amphetamine-regulated transcript
CGM	Continuous glucose monitor
CPDR	Cumulative percent dose 13C recovery
CRH	Corticotropin-releasing hormone
DASH	Dietary Approaches to Stop Hypertension
DNS	Dinitrosalicylic acid
DOB	Delta over baseline
DP	Degree of polymerization
DPP-4	Dipeptidyl peptidase-4
GI	Glycemic Index
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide 1
GLUT2	Glucose transporter 2
HEI	Healthy eating index
MCH	Melanin-concentrating hormone
MDX DE	Maltodextrin – Dextrose equivalent
MGAM	Human maltase glucoamylase
NPY	Neuropeptide Y
PDR	Percent dose 13C recovery
POMC	Proopiomelanocortin
PRCF	Percent relative cumulative frequency
PYY	Peptide YY
RCS	Raw corn starch
RDS	Rapidly digestible starch
RER	Respiratory exchange ratio

RQ	Respiratory quotient
RS	Resistant starch
SCFA	Short chain fatty acids
SD	Standard deviation
SDC	Slowly digestible carbohydrate
SDS	Slowly digestible starch
SEM	Standard error of the mean
SGLT1	Sodium-glucose cotransporter 1
SI	Human sucrase-isomaltase
SME	Small and middle enterprises
USDA	United States Department of Agriculture
VAS	Visual analog scale
VO_2	Carbon dioxide production
VCO_2	Oxygen consumption

ABSTRACT

The world has experienced an unprecedented change in the systems responsible for food production, distribution, and commercialization with concurrent changes in diets. In developed and developing countries, the shift in consumption patterns has moved towards a Western diet pattern which has been linked to negative health outcomes including obesity, diabetes and associated non-communicable diseases. Traditional African diets have previously been associated with protective effects against the development of the above-mentioned conditions. Yet, the underlying reasons for this is not clear. One dietary factor that may contribute to its protective effect is the principal available carbohydrate, starch, which in traditional African staples is considered to contain slowly digestible carbohydrates (SDCs) and some amount of resistant starch (RS). We reported that traditional African staple starchy foods (sorghum and millet) had markedly slower gastric emptying than introduced modern starchy foods (rice, pasta and potatoes). This response was attributed to activation of enteroendocrine cells of the small intestine (L-cells) with potential to trigger physiological, hormonal, and neurological processes that affect digestion time and perception of hunger; effect known as the ileal brake. Moreover, at least in mice models, consumption of SDCs has shown to have beneficial effects on the rate and type of fuel (e.g. carbohydrate vs fat) used for metabolic processes.

The first thesis study compared the effect of diets (cohorts in the USA and Kenya) on gastric half-emptying time and metabolic fuel utilization in healthy adults. Our findings showed that gastric emptying time was not different between cohorts and that diet did not influence gastric emptying time; however, calculated respiratory exchange ratio (RER) (which is a measure of metabolic fuel utilization at the cellular level, e.g. carbohydrate vs fat) and metabolic flexibility (which is the ability to switch between metabolic fuel sources upon demand or need) was higher for the Kenyan cohort. Multivariant models were developed and corrected for multicollinearity of some diet variables. Carbohydrate and protein in multivariate model 1; total fiber, added sugars and starch in multivariate model 2; and diet quality (measured as the Healthy Eating Index based on 2015-2020 dietary guidelines, or HEI-2015) in multivariate model 3, were significantly and independently correlated with RER and metabolic flexibility.

The second study assessed if slow gastric emptying and improve metabolic fuel utilization could be induced through SDC supplementation. The objective of this study was to determine if

continual consumption of SDC for 21 days delayed the rate of gastric emptying, moderated postprandial glycemic response, decreased hunger, and/or improved metabolic fuel utilization in subjects with low diet quality (HEI-2015<65). Our results indicated that supplementation with SDC did not slow gastric emptying time or acute measures of metabolic fuel utilization; however, continuous consumption of SDC had a modest but significant effect on improving metabolic flexibility and decreasing hunger scores.

The last two chapters of this thesis focused on the use of a low-cost, high-pressure, high temperature extruder suitable for processing in Africa of whole grain pearl millet (*Pennisetum glaucum*). In Africa, emerging, entrepreneurial companies are increasingly gaining share of local markets by manufacturing and distributing high-quality locally sourced processed foods made with indigenous grains. Whole pearl millet is particularly susceptible to development of rancidity. The objective of our third study was to assess the use of the extruder on the stability and sensory attributes of whole grain pearl millet extruded flours to be used for instant thin and thick porridges. Findings showed that extrusion fully gelatinized the starch in pearl millet and prevented hydrolytic rancidity in the instant flour products. However, extrusion cooking did not stop oxidative rancidity. We concluded that while extrusion cooking is a versatile technology for whole grain processing, refinement of extrusion conditions used in the experiment and the evaluation of other unit operations (e.g. steeping, germination) in combination with extrusion cooking may improve the sensory properties of final products.

Finally, extrusion cooking has been showed to promote the formation of beneficial amylose-lipid complexes (ALCs). The objective of the last study was to evaluate the formation of ALCs in whole grain pearl millet extruded flours, characterize their composition, and assess their ability to slowly digest *in vitro*. Extrusion promoted the formation ALCs and these flours exhibited a slow enzymatic digestion *in vitro*. The findings from this thesis provide insights into the role of diets and metabolic fuel utilization, and improvement of processed pearl millet foods in Africa.

CHAPTER 1. INTRODUCTION

Carbohydrates are the main dietary energy source for humans (Shan et al., 2019) and are broadly classified as digestible (e.g. sugar, starch) and indigestible (e.g. soluble and insoluble fiber). There is a potential to improve health and prevent disease through the use of quality carbohydrates, even though carbohydrates in general are often viewed unfavorably by the public due to purported negative effects (Johnson et al., 2009; Parks & Hellerstein, 2000). Differences in carbohydrate physicochemical nature and food form affect their rate of enzymatic digestion in the body. Recent evidence suggests that consumption of carbohydrates with slow digestion rate (slowly digestible carbohydrates, SDCs) have positive effects on metabolic and physiological outcomes (Chegeni et al., 2022; Hayes AMR, 2021). Increasing the consumption of SDCs could be a strategy to prevent or even treat obesity and associated nutrition-related non-communicable diseases by activation of physiological and metabolic pathways that reduce food intake and improve metabolic fuel utilization.

Traditional African diets more commonly have SDCs in them, particularly in the thick porridges consumed in much of sub-Saharan Africa. Little is known regarding the effect of SDCs in African diets on physiology and metabolism. Moreover, food processing and introduction of Western diets to Africa is associated with rise of diet-related diseases, while the right kind of locally processed convenient foods may go counter to this trend.

1.1 Thesis organization and research hypothesis

This thesis is divided into six chapters. The first and second chapters are the Introduction and Literature Review. Chapters 3 to 6 are experimental.

Chapter 1 is the Introduction of the project background and studies' hypotheses.

Chapter 2 is the Literature Review focused on carbohydrate composition, digestion, regulatory feedback mechanisms, and the effect of diet differences on physiological and metabolic outcomes.

Chapter 3 assesses diet differences between cohorts in Kenya and the USA and determines if these differences affect gastric emptying time and metabolic fuel utilization.

Research hypothesis: differences between Kenyan and American diets might affect gastric half-emptying time and metabolic fuel utilization in healthy adults through the activation of digestive feedback mechanisms.

Chapter 4 assesses the effect of continuous consumption of SDC through a 3-week supplementation on gastric emptying time, blood glucose, satiety and hunger, and metabolic fuel utilization.

Research hypothesis: regular consumption of SDC might promote changes in the number or sensitivity of enteroendocrine L-cells in the small intestine that could affect gastric half-emptying time, postprandial blood glucose, satiety and hunger, and metabolic fuel utilization.

Chapter 5 assesses the use of high-pressure, high temperature extrusion on shelf-life stability and sensory acceptance of whole grain pearl millet flours.

Research hypothesis: using a low-cost single-screw extruder suitable for Africa, extrusion of whole grain pearl millet flours improves lipid stability (decrease peroxide and free fatty acids concentrations) and sensorial attributes (color, aroma, flavor, and texture) of the resultant porridges made from these flours stored over a period of 18 weeks.

Chapter 6 assesses the use of high-pressure, high temperature extrusion on the formation of starch (amylose)-lipid complexes with a slow digestion property.

Research hypothesis: low-cost single screw extrusion of whole grain pearl millet flours promotes the formation of amylose-lipid complexes capable of modulating starch digestion *in vitro*.

1.2 References

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CHAPTER 2. LITERATURE REVIEW

2.1 Abstract

Starch-based foods are the primary source of energy in most diets around the world. Their relatively low cost makes them an attractive target for large scale nutrition inventions. Research from our laboratory has demonstrated the ability of starch-based foods to trigger important anorexigenic and weight loss effects via hormonal secretion related to enteroendocrine cell activation and potential effect on energy utilization and metabolic flexibility. This review will provide a general context for starch-based foods and their potential role in improving health.

2.2 Introduction

Global incidence and prevalence of overweight, obesity and nutrition-related non-communicable diseases is a direct indicator of the current disruption in the balance between energy intake and expenditure. Although the broad scope and multifaceted nature of factors influencing energy intake (increased worldwide caloric availability, changes in traditional dietary patterns, lower cost of "highly processed" foods) and energy expenditure (increased sedentary lifestyles, chronic psychosocial stress, neuroendocrine dysregulation, genetic/epigenetic mechanisms) are beyond the scope of this review, it is important to note that obesity and related comorbidities affect both developed and developing nations; and developing nations suffer a double burden of disease due to the additional incidence of undernutrition (Min et al., 2018). This situation exacerbates already strained public health monetary expenditure, and decreasing funding availability for preventive care (Kushitor & Boatemaa, 2018).

Starch-based foods are the primary source of energy in most diets around the world; and carbohydrate's relatively low cost makes them an attractive target for large scale nutrition inventions. In recent years our laboratory has demonstrated the ability of ileal-targeted slowly digestible starch-based foods to trigger important anorexigenic and weight loss effects in animals (El-Hindawy, 2018; Hasek et al., 2018; Lim et al., 2021). Slowly digestible starch also was associated in mice with improvements in metabolic health (i.e. increased fat oxidation while promoting metabolic flexibility) (Hayes, 2021). Due to their high consumption, starch-based foods are uniquely positioned to serve as a therapeutic strategy to prevent rising levels of overweight,

obesity and associated diseases. This chapter will provide a general context for starch-based foods and their potential role in improving health. This will be accomplished by reviewing (1) the current state of diets in developing nations and their connection to non-communicable diseases; (2) starch structure, classification and digestion; (3) physiological and metabolic feedback mechanisms related to starch-based carbohydrates; (4) carbohydrate oxidation and glycemic control in metabolism; and (5) the current tools (*in vitro*, *in vivo*, and mathematical modeling) for studying carbohydrates related to areas mentioned above. Traditionally, carbohydrates have been primarily associated in *in vivo* studies with glycemic response, however their digestion products also influence physiological and neurobiological responses with potential impacts on health outcomes related to food intake and body weight.

2.3 Nutrition transition, tools for evaluation of healthy diets and their limitations

Global consumption of the major staple cereals, is expected to rise (rice (↑13%), corn (↑16%) and wheat (↑13%) in the next decade (OECD & FAO, 2018). The increased global availability of cereal supply, in conjunction with accelerated international trade, foreign direct investment in local food processing and retailing, as well as increased food advertising and promotion has reshaped current food systems and the diets of end consumers (Golzarand et al., 2012; Harris et al., 2020; Hawkes, 2006; Thow et al., 2011). The shift in dietary patterns towards a Western-like diet, characteristically high in refined carbohydrates, simple sugars, oils and red meat has been referred to as the "nutrition transition" (Drewnowski & Popkin, 1997). Improvements in global underweight have been observed with the nutrition transition; nevertheless, it is expected that its negative effects on public health (overweigh/obesity) and the environment (increased pollution related to production of a western like diet) outweigh this benefit (Bodirsky et al., 2020). The detrimental health effects (e.g. overweight, obesity, type 2 diabetes, cardiovascular disease) of the nutrition transition have been well documented (Popkin, 1998, 2015; Popkin et al., 2012a; Popkin & Gordon-Larsen, 2004; Reardon et al., 2021), as well as their increasing incidence among rural populations (Dalal et al., 2011; Gong et al., 2012).

International agencies in collaboration with governments, research centers and academic institutions have encouraged nutrition approaches to combat the worldwide obesity epidemic and the negative effects of the nutrition transition (Imamura et al., 2015; Schwingshackl & Hoffmann, 2015; Vecchia & Majem, 2015). Overall, nutrition research indicates that favorable dietary

patterns for health include high consumption of fruits, vegetables, beans and legumes, nuts and seeds, whole grains, low-fat dairy, fish, food items high in polyunsaturated fatty acids, plant omega-3s and dietary fiber; while unhealthy dietary patterns include high consumption of red meats, processed meats, sugars and refined carbohydrates, saturated fat, trans fat, dietary cholesterol, and sodium (Cena & Calder, 2020; Vecchia & Majem, 2015). Research on specific diets including Mediterranean (Kromhout et al., 1989) and Nordic diets (Mithril et al., 2012) have demonstrated lower mortality rates and decreased incidence of non-communicable disease (Giacosa et al., 2013; Olsen et al., 2011; Sotos-Prieto et al., 2017; Trichopoulou et al., 1995; Turati et al., 2015). These dietary patterns were identified in temperate and Nordic regions and are linked to specific food items consumed in those regions, limiting their applicability in other geographic locations. Eating indexes, including the diet quality index (Patterson et al., 1994), overall nutritional quality index (Katz et al., 2009), Dietary Approaches to Stop Hypertension (DASH) index (Appel et al., 2009), and the Healthy Eating Index (HEI-2015) (Bowman et al., 1998) have been used to characterize diet quality by determining how diets, both inside and outside North American align with specific nutrition recommendations. While reviews and research papers have highlighted the health benefits of specific food items consumed in Africa, Asia, or Latin America (Awika & Rooney, 2004; Maundu et al., 2009; Rizzo & Baroni, 2018; Saleh et al., 2013a); research on specific traditional dietary patterns from these parts of world and their protective health effects remains largely undocumented.

Currently, there is a limited number of diet assessment tools developed specifically for diets in developing countries; with most of the current tools used internationally derived from research performed for American (USA) diets. The HEI-2015 is the most frequently used tool to determine diet adequacy for populations in America and abroad. The HEI is a measure of diet quality (scored from 0-100) (Bowman et al., 1998) used to assess how diet aligns with key recommendations of the Dietary Guidelines for Americans. Since its development in 1995, its structure has been revised continuously (starting in 2005) with every new set of Dietary Guidelines. The most current version of the HEI is based on recommendations from the 2015-2020 Dietary Guidelines for Americans and is currently being updated to include Dietary Guidelines for Americans 2020-2025. Adaptions of the HEI have been successfully used for diets outside the USA. In Canada, an adaptation of the HEI which aligned with Canada's Food Guide (CFG-2007) rendered adequate validity and reliability. Results showed a strong relationship between alignment

with dietary recommendations and likelihood of obesity (Jessri et al., 2017). Similar results were found in Brazil (Fisberg et al., 2004) for adaptation of the HEI and development of the Brazilian Diet Quality Index (BDQI). These successful examples required significant effort to avoid poor translation, excessive and unjustified number of adaptations, and significant research to account for local health recommendations in each country. There is a need for diet assessment tools for traditional, developing country dietary patterns so future research can help assess if traditional diets provide protective health. Research on these diet patterns should include aspects related to carbohydrate quality that go beyond fiber concentration and glycemic index, for reasons discussed in the following sections.

2.4 Starch structure, classification systems and digestion

Starches are composed of glucose units covalently bonded by either α-1,4 or α-1,6 glycosidic linkages that form polymeric chains. The structure of these polymers is either linear or branched depending on the type of glycosidic linkage; amylose is a carbohydrate of mostly linear structure formed by α-1,4 long polymeric chains, while amylopectin's branched structure has a high concentration of α-1,6 branched structure points and short chains (degree of polymerization [DP] 15-20). These structural patterns give rise to fine microstructural features that have significant impacts on starch functionality and digestibility (Benmoussa et al., 2007; Bertoft et al., 2016; Matalanis et al., 2009; Roman et al., 2020; Vamadevan & Bertoft, 2015; G. Zhang & Hamaker, 2009a). Particularly, amylopectin's branched structure and its ratio of short-chain to long-chain fraction contribute to slow digestion properties (G. Zhang & Hamaker, 2009b). Other structure-chemical characteristics, such as starch's propensity to form intermolecular associations after gelatinization (retrogradation) can decrease its digestibility (Martinez et al., 2018). A complete review of the structural features and physicochemical interactions contributing to starch digestibility and functionality is beyond the scope of this review, nevertheless it is important to recognize because of their impact on varying rates of starch digestion.

Glucose polymers can present two types of glycosidic linkages, either α or β-linkages. Mammals can hydrolyze an array of α-linkages present in starch while β-linked glucose units form an indigestible fraction (fiber). Mammalian's starch digesting enzyme exclusively act on α-linkages (B. H. Lee et al., 2013). The indigestible fraction of some types of starches are resistant

to digestion; these "resistant starches" move intact through the upper gastrointestinal tract and arrive to the colon to be fermented by gut microbiota.

Starches differ in digestion rate due to a variety of intrinsic and extrinsic factors including, but not limited to, the nature of starch, its physical form, interactions with other food constituents (proteins, lipids, sugars), enzymatic inhibitors, processing type, and food matrix structure (hardness or porosity) (Contardo & Bouchon, 2019). Some starches are more susceptible to digestion compared to others that digest more slowly. Rapidly digestible starches generate high variations in glycemic response that are associated with substantial demands on insulin secretion, while slowly digestible starches exhibit moderated and prolonged glucose and insulin release. The high demands on insulin secretion (linked to rapidly digestible starches) can be detrimental to health; therefore, different methods have been developed to assess and change carbohydrate digestibility and its impact (Miao et al., 2015). The glycemic index (GI) was created to quantify differences in glycemic response related to carbohydrate digestibility and serves as a method of classification of carbohydrate-containing foods based on their effects on postprandial glycemia. Glycemic index is defined as the increase in blood glucose (area under the curve) 2 hours after consumption of a standard amount of carbohydrate (test meal) compared to either glucose or white bread (Jenkins et al., 1981). Limitations of the GI associated with additional dietary factors affecting postprandial glucose response led to the development of the glycemic load, which is the product of glycemic index of a specific food multiplied by its percentage of dietary energy consumed as carbohydrate (Ludwig, 2002). The glycemic load (GL) is a weighted average that integrates glycemic response and the amount of carbohydrate consumed. Despite the widespread adoption of these classification systems, glycemic index and glycemic load provide limited information regarding starch digestion profiles. Moreover, there is conflicting evidence whether consumption of low glycemic foods positively affects biomarkers for glucose homeostasis. In 2013, a panel from the International Carbohydrate Quality Consortium found strong evidence that low GI diets moderately improve glycemic response in type 1 and 2 diabetics. The panel also found good evidence for low GI diets decreasing risk of developing diabetes in cardiovascular disease, however, evidence was moderate to low for possible protective effects against cancer (Augustin et al, 2015). Similar positive effects have been found for low GI diets and plasma hormones related to glucose regulation. A clinical study showed that starches with varying grades of digestion affect GLP-1 and GIP, with postprandial blood glucose being positively correlated with both hormones

(Watchers-Hagedoorn et al, 2006). On the other hand, a crossover randomized controlled feeding trial showed that diets with low GI did not improve cardiometabolic health markers (insulin sensitivity, lipid profiles and blood pressure) compared to high glycemic index diets (Sacks et al., 2014). A second crossover trial showed no significant differences in glycemic responses between slowly and rapidly digestible starch-based foods (Eelderink et al., 2012), however slowly digestible starch had lower secretion of postprandial insulin and glucose-dependent insulinotropic polypeptide (GIP) compared to rapidly digestible starch. The lack of difference in glycemic responses was attributed to slower glucose clearance rate for the slowly digestible starch compared to the rapidly digestible counterpart. Conclusions for this study highlighted the limitations of glycemic index on identification of starches with slow digestion properties.

In 1992, Englyst and collaborators developed a system of starch characterization according to digestion rate using an *in vitro* assay with porcine pancreatin and fungal amyloglucosidase. Englyst identified three starch fractions based on digestion time; starch hydrolyzed to glucose within 20 min is classified as rapidly digestible starch (RDS), starch hydrolyzed to glucose from 20-120 min is classified as slowly digestible starch (SDS), and remaining unhydrolyzed starch after 120 min of enzymatic digestion is classified as resistant starch (RS). The Englyst classification system has been extensively studied and validated with *in vivo* glycemic response (H. Englyst et al., 1992; K. Englyst et al., 2018; K. N. Englyst et al., 1999, 2003), and it is widely used as a method to characterize nutritionally relevant starch fractions. Starches, starch derivatives, and starch ingredients can contain all these classes (RDS, SDS, RS); and isolation of each class is not possible since this classification system is based on a time-dependent experimental outcome rather than specific chemical or structural characteristics (G. Zhang & Hamaker, 2009a). Limitations of the Englyst method also include slight but significant differences in enzyme activity between human and fungal sources, and the Englyst assay uses fungal amyloglucosidase, which differs in its enzymatic hydrolysis of α-linkages compared to the mammalian α -glucosidases (B.-H. Lee et al., 2016; Lin et al., 2016; Shin et al., 2019). It is important to note the limitations and constraints of each method for translation of research findings into daily applications.

To put in perspective the current approaches used for starch classification, a detailed review of starch digestion is necessary. Glucose units from starch polymers must undergo cleavage and absorption in the small intestine before being utilized by the body. Enzymatic starch digestion begins in the oral cavity by the action of salivary α-amylase and continues in the small intestine

by the action of pancreatic α-amylase and intestinal brush border α-glucosidases. Salivary and pancreatic α-amylases are endoglycosidases which act on internal linear segments of α-1,4 linked glucose units (Fujii & Kawamura, 1985; Robyt & French, 1967). The products of amylase digestion are glucose dimers, trimers and tetramers (maltose, maltotriose, maltotetraose), as well as branched α-1-6 small, oligosaccharide size polymers (α-limit dextrins). Subsequent breakdown of these digestion products into glucose is performed by two brush border α-glucosidases; sucrase isomaltase (SI) and maltase glucoamylase (MGAM). Both SI and MGAM have N- and C-termini (Dahlqvist & Telenius, 1969; Nichols et al., 2009; Sauer et al., 2000; Sim et al., 2008). SI is exclusively expressed in the intestine, while MGAM is expressed in a variety of tissues including kidneys, stomach, bone marrow, spleen (Fagerberg et al., 2014). SI and MGAM are anchored on the luminal side of intestinal enterocytes and are concentrated in lipid rafts. For both SI and MGAM, the N-terminus is located at the enterocyte membrane and the C-terminus extends into the intestinal lumen (Sim et al., 2008). There are two enzymatic activities for each α-glucosidase (2 maltase activities from SI and 2 maltase activities from MGAM) depending on the N or C terminus (Dahlqvist & Telenius, 1969; T. P. Frandsen et al., 2002; T. P. (Carlsberg Lab. Frandsen & Svensson, 1998; Nichols et al., 2003). All α-glucosidases hydrolyze maltose and maltosides exogenously from the non-reducing end, nevertheless each terminus has unique substrate affinities. The N-terminus of the SI, isomaltase, has a strong affinity for α-1,6 linkages comprised of branched structures (α-limit dextrin), while the C-terminus of SI, sucrase, has a strong affinity for α-1,2 linkages found in sucrose. For MGAM, the N-terminus has strong activity for α-1,4 linkages in short glucose oligomer chains as well as hydrolytic activity on α-1,2 and α-1,3 glycosidic bonds (Lin et al., 2016), while the C-terminus has a strong affinity for all α-1,4 glycosidic bonds in oligomers as well as longer linear intact starch polymers.

There is balance and well-coordinated interaction to between enzyme activities and products of starch digestion. Enzymatic activity of the α-glucosidases is amplified by the products of α-amylase digestion (Chegeni, Amiri, et al., 2018), while maltase suppresses α- amylase activity (Quezada-Calvillo, Robayo-Torres, Sterchi, et al., 2007). *In vitro* studies showed that the four specific maltase activities complement each other. Quezada and collaborators (2008) demonstrated that α-amylase products amplify the activity of MGAM 2-fold and SI 10-fold, and in a feedback mechanism, the increased concentrations of maltase suppress α-amylase activity. Suppression of MGAM glucoamylase activity occurs with increased concentrations of products of starch digestion

(maltotriose, maltotetraose, and maltosides). On the other hand, there is no evidence to support that enzymatic activity of SI is regulated by maltotriose, maltotetraose, and maltosides (Quezada-Calvillo, Robayo-Torres, Ao, et al., 2007). In synthesis, MGAM is sensitive to substrate availability, while SI is not.

After complete hydrolysis of starch polymers into glucose due to the combined action of α-amylase and α-glucosidases, glucose is absorbed from the lumen into the enterocyte via the sodium-glucose cotransporter 1 (Na+-D-glucose cotransporter 1; SGLT1) located on the apical side of enterocyte lining in the small intestine (Uldry & Thorens, 2004; Wright et al., 2011). SGLT1 actively transports glucose across the apical membrane against a greater glucose concertation, glucose is moved out of the enterocyte via and glucose transporter 2 (GLUT2), as GLUT2 mediates facilitated glucose transport across the basolateral membrane into the blood capillaries (Uldry & Thorens, 2004). Other transmembrane glucose transporters have been identified. A mouse study showed that after consumption of a high carbohydrate meal, some GLUT2 transporters migrate towards the brush-border membrane, though its impact on glucose absorption was minor (Gorboulev et al., 2012). Upon exit from the enterocyte via GLUT2, glucose is taken to the liver and muscle to be used for energy or is stored.

2.5 Gastric emptying, the ileal-brake, and the gut-brain axis

Glucose and other starch digestion products have been shown to exert an effect on enteroendocrine cells of the small intestine (L-cells) (El-Hindawy, 2018) with potential to trigger physiological, hormonal, and neurological processes that affect digestion. This section will elaborate on important mechanisms affecting digestion including gastric emptying, the ileal brake, and the gut-brain axis.

The process of food breakdown starts with mastication, which facilitates mixing of food components and salivary secretions (bolus) in the oral cavity for subsequent chemical and physical breakdown. The bolus travels through the esophagus to the stomach pushed by esophageal smooth muscle. The acidic environment of the stomach is promoted by parietal cells, which in combination with activated zymogens (pepsin and lipase) secreted by chief cells located in the lining of the stomach, promote protein denaturation and hydrolysis, as well as breakdown of other food components. The stomach prepares the bolus (predigested food) for subsequent digestion in the lower gastrointestinal tract. Food's physicochemical properties including physical form (liquid vs

solid) (Collins et al., 1996; Hellmig et al., 2006; Santangelo et al., 1998), caloric content (Calbet & MacLean, 1997; Camps et al., 2016; McHugh & Moran, 1979; Moore et al., 1981), macronutrient composition (Giezenaar et al., 2018; Moore et al., 1981), viscosity (Marciani et al., 2001; Zhu et al., 2013), particle size (Meyer, 1980) affect digestion time as well as the stomach's rate of emptying into the small intestine.

Gastric emptying is defined as the rate at which stomach contents leave the stomach and pass into the duodenum. Various methods have been developed to assess gastric emptying *in vivo*. The gold standard is scintigraphy (Ma et al., 2015; Spiegel et al., 1994); this method directly measures stomach contents, however the use of radioisotopes and specialized equipment makes it expensive and cumbersome and it is not widely available. Other tests for measuring gastric emptying time have been developed, including ^{13}C breath tests (Ghoos et al., 1993; Sanaka & Nakada, 2010), magnetic resonance imaging (Marciani et al., 2001), and the paracetamol absorption test (Medhus et al., 2001; Willems et al., 2001).

Regulation of intestinal motility as a result of nutrient sensing was first demonstrated by Spiller and collaborators in 1984. The authors named this feedback mechanism "the ileal-brake system" and its effect has been studied extensively (Barreto et al., 2018; Schirra & Göke, 2005; Spiller et al., 1984). It results in inhibition of upper gastrointestinal motility (delayed gastric emptying rate) coinciding with increased satiation (short-term fullness) and satiety (longer-term fullness). Traditionally, protein and fat have been considered the strongest inhibitors of gastric emptying, however several studies have shown the effect of carbohydrates on ileal brake activation (Burn-Murdoch et al., 1978; Giezenaar et al., 2017, 2018). Studies from our laboratory have demonstrated that consumption of starch-based carbohydrates with varying digestibility decrease gastric emptying by locationally digesting starch into the ileum (Hasek et al., 2018). It was hypothesized that this response was related to postprandial feedback mechanisms related to the activation of ileal brake. A clinical study showed activation of the ileal brake by slowly digestible carbohydrates (Chegeni et al., 2022).

Specialized enterocytes in the intestinal epithelium (enteroendocrine cells) sense digestion products and trigger a hormonal response that regulate glucose homeostasis, gut motility, epithelial proliferation, and appetite (Reimann et al., 2012; Spreckley & Murphy, 2015). Despite enteroendocrine cells constituting only 1% of the cell population in the intestinal epithelium, there are at least 11 different types of currently identified enteroendocrine cells, each with a different

secretory profile and found in different concentrations along the gastrointestinal tract (Engelstoft et al., 2013; Rindi et al., 2004). A study in 2012 expanded the notion that hormonal secretion was not enteroendocrine cell specific, by demonstrating that hormonal co-secretion of GIP and GLP-1 happened in the same enteroendocrine cells (Grigoryan et al., 2012). It is generally recognized that L-cells in the duodenum and part of the jejunum secrete glucagon-like peptide-1 (GLP-1), and the more abundant L-cells in the ileum and colon secrete a GLP-1 and peptide tyrosine-tyrosine (PYY) (Engelstoft et al., 2013; Habib et al., 2012). GLP-1 is a secretagogue that promotes insulin secretion while decreasing glucagon output. GLP-1 also slows the rate of gastric emptying while decreasing hepatic glucose output (Diakogiannaki et al., 2011; Holst, 2007; Sandoval & D'Alessio, 2015). GLP-1 is also implicated in the control of food intake, and it has been experimentally shown that secretory/sensing defects in GLP-1 contribute to overeating in obesity (Steinert et al., 2017). Previous pharmacological approaches have targeted GLP-1 as a therapy due to its incretin effect (Daoudi et al., 2011; Yu et al., 2010). Several recent reviews have assessed the potential of GLP-1 to treat obesity and control food intake (Grill, 2020; Krieger, 2020). On the other hand, PYY aids in glycemic control by improving insulin sensitivity (van den Hoek et al., 2004), and stimulates pancreatic-cell proliferation while inhibiting β-cell apoptosis (Persaud & Bewick, 2014; Sam et al., 2012).

There are two main underlying mechanisms related to the ileal-brake as speculated to be triggered by GLP-1 action. First is transmission of signals locally to the enteric nervous systems and distally to the brain via vagus nerve to slow gastric emptying (Abbott et al., 2005; M. R. Hayes et al., 2011; Kanoski et al., 2011; Labouesse et al., 2012) and the second is exerted directly on the brain after GLP-1 has crossed the blood brain barrier (Kanoski et al., 2011; Labouesse et al., 2012). Their effect on gastric emptying time might not be homogenous due to GLP-1's short half-life (cleaved by dipeptidyl peptidase-4 [DPP-4]). The action of GLP-1 on gastric emptying is dose dependent (Nauck et al., 1997; Schirra et al., 1996; Schirra & Göke, 2005) and its relationship to the ileal brake has been well documented (Barreto et al., 2018; Schirra & Göke, 2005). Specifically related to carbohydrates, a recent rat study showed that slowly digestible isomaltodextrin and isomaltulose selectively increased GLP-1 secretion (Komuro et al., 2020) and humans (Martinussen et al., 2019), and additionally our laboratory showed increased GLP-1 with ileally-directed starch digestion using α-amylase inhibitors (Lim et al., 2021). These studies highlight the importance of carbohydrates digestibility to potentially trigger the ileal brake.

The other mechanism responsible for appetite, food intake, and body weight control is the gut-brain axis, which is a bilateral communication system between the gastrointestinal tract and the brain through neurological, metabolic, and humoral signals (Hussain & Bloom, 2013; Weltens et al., 2018). More recently, the gut-brain axis has been associated with the microbiome (Osadchiy et al., 2019). Despite the collective research advancements in this area, the exact mechanism(s) responsible for this bidirectional communication system is still not completely understood. Some of the more concrete evidence is derived from brain studies. Well-defined roles in appetite and food intake have been linked to specific brain regions. The hypothalamus plays a vital role in the regulation of food intake (Anand & Brobeck, 1951). The action of the area postrema and nucleus of the solitary tract also have been documented for their roles in reception and transmission food intake-related signals (Hyde & Miselis, 1983). More recent evidence supports the role of the hippocampus in food intake control. The hippocampus has been traditionally linked to memory, and reward, including aspects that can influence food intake (Davidson et al., 2007; Hsu et al., 2015; Suarez et al., 2020). In the hypothalamus, the agouti-related peptide (AgRP), neuropeptide Y (NPY), and melanin-concentrating hormone (MCH) have been identified as appetite-stimulating (orexigenic) neuropeptides, while proopiomelanocortin (POMC), cocaine-and amphetamine-regulated transcript (CART), and corticotropin-releasing hormone (CRH) have been identified as appetite-reducing (anorexigenic) neuropeptides (Hussain & Bloom, 2013). Changes in the expression of these neuropeptides can serve as an indication of gut-brain axis signaling/activity. In a previous rat study conducted by our laboratory, we found that consumption of slowly digestible starch with a high-fat diet for 12 weeks significantly decreased the expression of the appetite stimulating neuropeptides NPY and AgRP while decreasing food intake compared to high-fat diet containing rapidly digestible starch (Hasek et al., 2018). More recently, Lim et al. (2021) showed that ileally-digested starch decreased gene expression of AgRP and increased expression of POMC and CART. These findings support the ability of slowly digestible, and specifically ileal-digesting, starches to trigger the gut-brain axis and its potential effect on food intake regulation for weight loss and prevention of obesity.

2.6 L-cell activation induced by carbohydrate digestion products and mechanisms of L-cell proliferation

Intestinal enterocytes have the ability to sense and react to the presence of products of carbohydrate digestion (Chegeni, Amiri, et al., 2018). The current knowledge of the carbohydrate digesta chemosensing by enterocytes indicates its complex process involving cell membrane-embedded receptors and glucose transporters. The presence of taste receptors (found in the oral cavity) was first reported in the intestinal epithelial and enteroendocrine cells in 2002 and it was suspected that they played a role in carbohydrate sensing (Wu et al., 2002), however, later evidence showed that classic taste receptors (as the taste receptors found in the oral cavity) were not the primary detection mechanism in the intestine (Rozengurt & Sternini, 2007; Young, 2011). These receptors are G protein-coupled receptors (GPCR) and are divided into two classes: taste receptor type 1 (T1R) (Young, 2011) and taste receptor type 2 (T2R) (Rozengurt & Sternini, 2007). TR1 family of receptors is formed by two GPCRs, the sweet taste receptor (T1R2-T1R3 heterodimer), and the savory/umami receptor (T1R1-T1R3 heterodimer). On the other hand, the TR2 family of bitter taste receptors is formed by ≈30 known GPCRs. Both TR1 and TR2 receptors are expressed in enteroendocrine cells (A, K and L cells). In the distal small intestine, a receptor with a structure similar to a sweet taste receptor (T1R3 structure), is expressed by L cells. Its activation by glucose is responsible for release GLP-1 (Brown et al., 2009; Gerspach et al., 2011). T1R3 receptors are vital for glucose-stimulated GLP-1 secretion in enteroendocrine cells, in T1r3-knockout animals GLP-1 secretion was severely impaired (Geraedts et al., 2012), however TR1r2-knockout mice showed normal glycemic control and GLP-1 secretions, thus indicating a compensatory mechanism.

A strong interaction between sweet taste receptor and sodium glucose cotransporter 1 (SGLT1) has been previously documented. A mice study showed 80% reduction in glucose induced GLP-1 secretion in SGLT1-knockout mice (Gorboulev et al., 2012). In contrast, SGLT1 expression is enhanced in L-cells via release of GLP-2, which indirectly induces expression and activation SGLT1 in enterocytes, and markedly increased enzymatic activity for maltase, sucrase and lactase (Brubaker et al., 1997; Hsieh et al., 2009; Shirazi-Beechey et al., 2011). This evidence suggests the close interaction between T1R3 and SGLT1 in L-cells for GLP-1 secretion. Other transmembrane receptors for sensing fatty acids, peptides and phytochemicals have biological relevance. Finally, despite the growing evidence regarding carbohydrate sensing by

enteroendocrine cells, to date, no receptor has been reported for chemosensing starch α-amylase digestion products, such as maltose and maltotriose, as was found by our group (El-Hindawy, 2018).

It is important to note that translation of the results from animal models assessing the effect of slowly digestible carbohydrate on GLP-1 and the ileal brake needs further work for this potential application into humans. Despite this limitation, therapeutic approaches to increase L-cell proliferation that could lead to increased concentrations of GLP-1 and PYY, with their concurrent beneficial health effects related to activation of the ileal brake and gut-brain axis are promising. Earlier assessments of L-cell proliferation indicated that fermentable fructooligosaccharide (FOS) increased enteroendocrine cell count in the colon due to FFA2 activation via luminal SCFA stimulation (Kaji et al., 2010). Similar incremental effects on L-cells have been found for activation of FFA3 via GLP-2 release, however this study evaluated FFA3 activation and subsequent prevention of L-cell loss under NSAID-induced enteropathy (Said et al., 2017); and another study demonstrated L-cell proliferation after exposure to lactoglobulin *in vitro* (Gillespie et al., 2015). A promising *in vitro* study conducted by our laboratory showed that maltose induces higher gene expression level of SI genes and formation of higher molecular weight SI species in Caco-2 monolayers (Cheng et al., 2014). Further *in vitro* and human studies are necessary to clarify the mechanisms responsible for L-cell proliferation and distribution. Moreover, studies are necessary to elucidate the effect of products of carbohydrate digestion on L-cell proliferation.

2.7 Carbohydrate metabolism-oxidation and metabolic flexibility

After glucose has exited through the basolateral side of the enterocyte and is absorbed into the bloodstream, it is eventually taken into cells where it can be used for energy through oxidation in the mitochondria or stored using different metabolic pathways. For each molecule of glucose metabolized, a net total of 36 ATP molecules are produced. Several metabolic processes including glycolysis, the Krebs cycle, and oxidative phosphorylation (the electron transport chain) contribute to this net energy output.

Glucose oxidation is not the only carbohydrate related metabolic process occurring in the human body. Other processes occurring in various tissues include gluconeogenesis (conversion of non-carbohydrate source into glucose), glycogenolysis (glycogen break down to produce glucose),

glycogenesis (glycogen synthesis to store excess carbohydrate as glycogen in liver and muscle), and fructose metabolism as well as galactose metabolism.

The body can use different substrates (e.g. carbohydrate vs fat) to meet its energy needs depending on demand and availability (metabolic fuel utilization). Metabolic fuel utilization is measured by the respiratory exchange ratio (RER) that is obtained by measuring the ratio VCO_2/VO_2 in the breath as a proxy to respiratory quotient (RQ), which measures carbohydrate/lipid oxidation at the cellular level (RQ is an invasive procedure in which arterial and venous catheters are required). RER is sometimes used synonymously with respiratory quotient (RQ). The gold standard for measuring metabolic fuel utilization is indirect calorimetry. This technique allows for the measurement of the type of substrate and its rate of utilization for metabolic processes based on the gas exchange measurements of oxygen (O_2) consumption and carbon dioxide (CO_2) production (Ferrannini, 1988). This is possible because each metabolic fuel substrate requires a specific amount of O_2 in order to produce a specific amount of CO_2. This is based on the premise that per molar basis, carbohydrates contain more oxygen than fats; therefore, carbohydrates produce more CO_2 per mole O_2 consumed than fat. RER can be calculated using the following equation:

$$RER = VCO_2/VO_2 \quad [1]$$

Where VCO_2 is the volume of carbon dioxide gas produced, and VO_2 is the volume of oxygen gas consumed in the indirect calorimetry chamber system. Respiratory exchange ratio (RER) has specific values depending on to the macronutrient used by the body as the metabolic source; RER for pure carbohydrate is approximately 1.00, pure fat ≈ 0.70-0.71, and pure protein ≈ 0.80. Values for RER are a continuum (usually between range 0.7 to 1), because of the different macronutrient compositions found in foods. Experimentally, RER is generally used to only differentiate between carbohydrate and fat oxidation (and not protein oxidation). It is important to note that different fatty acids have been shown to produce different RER metabolic responses in humans, indicating that RER may vary within different types of fats (Polley et al., 2018). In regards of slowly digestible carbohydrates, a mice study in 2019 showed that females had higher carbohydrate oxidation when fed a low digestible carbohydrate diet compared to a high digestible carbohydrate diet; this effect was not observed in male mice (Fernández-Calleja et al., 2019). Further, Salto et al. (2020) and Hayes (2021) specifically showed that slowly digestible

carbohydrates reduced RQ and RER, respectively, indicating increased fat oxidation. This evidence suggests that starches with different digestibility can exert variations on RER.

Respiratory exchange ratio is an acute measure of metabolic fuel utilization. Despites its importance, on its own, it lacks the ability to quantify long-term changes in the body's ability to switch between metabolic fuel sources upon demand. Metabolic flexibility has been defined as the ability of an organism to adapt (its metabolic fuel use) according to changes in energy demand as well as the prevailing conditions or activity (Goodpaster & Sparks, 2017). In essence, metabolic flexibility can be considered as the ability to switch between carbohydrate and fat as substrates for oxidation upon need (e.g. high-carbohydrate meal, fasting, intense activity, oxygen restriction). To address these scenarios, the body must efficiently switch between metabolic fuel sources. Lack of metabolic flexibility has been associated with noncommunicable diet-related diseases including obesity, insulin resistance, and type 2 diabetes (Kelley et al., 1999; Meex et al., 2010; Sparks et al., 2008; Stull et al., 2010). RER alone does not measure the body's ability to switch between carbohydrate and fat as a metabolic fuel source. To address this limitation, Riachi and collaborators developed an approach called Percent Relative Cumulative Frequency (PRCF) (Riachi et al., 2004). This approach can be used for analyzing RER as well as energy expenditure values collected over time and is capable of detecting small differences in energy metabolism.

Dietary interventions based on caloric restriction have proved successful in improving metabolic flexibility (Mattison et al., 2017), by inducing a negative energy balance that forces lipid oxidation as an alternative metabolic substrate. However, these potential interventions are not sustainable over long periods of time (Harvie et al., 2017; Wing & Phelan, 2005). Few studies have directly assessed diet in the context of metabolic flexibility (Begaye et al., 2020; Duivenvoorde et al., 2015; Gribok et al., 2016). Evidence regarding the effect of starch digestibility on metabolic flexibility is still evolving. Fernandez-Calleja and collaborators showed improved metabolic flexibility and physiological differences (enlarged intestinal tract) in mice after three weeks of consuming a diet rich in carbohydrates with low digestibility. A study conducted by our laboratory showed that slow rate of digestion of carbohydrates exerts important effects on metabolic flexibility compared to rapidly digestible carbohydrates (Hayes AMR, 2021). These findings highlight the potential therapeutic use of slowly digestible carbohydrates to improve metabolic flexibility.

2.8 Modeling approaches related to gastric emptying and metabolism

As mentioned in the section above, several methods have been used to assess gastric emptying time *in vivo*, including scintigraphy (Spiegel et al., 1994), ^{13}C breath tests (Ghoos et al., 1993; Sanaka & Nakada, 2010), magnetic resonance imaging (Marciani et al., 2001), and the paracetamol absorption test (Medhus et al., 2001; Willems et al., 2001). Our laboratory has used the ^{13}C octanoic acid breath test in human studies to examine the potential differences in gastric emptying for different solid carbohydrate-based foods (Chegeni et al., 2022; Cisse et al., 2017, 2018; A. M. R. Hayes et al., 2021; Pletsch et al., 2022; Pletsch & Hamaker, 2018). This method is based on the rate-limiting step, in which ^{13}C tracer will appear on breath samples only after it has been emptied from the stomach.

The ^{13}C octanoic breath test uses a standard amount of ^{13}C octanoic acid (100 μL) dissolved in a carbohydrate-based test meal. Due to the hydrophobic nature of octanoic acid, ^{13}C sodium acetate has also been used. The calculation was equivalent in ^{13}C molar bases. Breath samples are collected before meal consumption (baseline) and postprandially, hours 1 to 2 at 15-min intervals, and for hours 3 to 4 at 30-min intervals. Postprandial breath samples are then compared to the baseline breath samples using a specialized infrared spectrophotometer (e.g. POCone breath analyzer, Otsuka Electronics Co., Ltd., Osaka, Japan). Differences in $^{13}CO_2/^{12}CO_2$ ratios of a breath sample compared to the ratio for baseline breath is recorded as $^{13}CO_2$ delta over baseline (DOB, ‰). These values are used to calculate the percent dose ^{13}C recovery (PDR) per hour and cumulative percent dose ^{13}C recovery (CPDR) (Sanaka & Nakada, 2010) and then are normalized for individual's body surface area (Haycock et al., 1978) to model gastric half-emptying time and lag phase. The following two equations are used to calculate gastric half-emptying time and lag phase [Equations 2 and 3]:

$$y = at^b c^{-ct} \quad [2]$$

Where y = PDR per hour (%), t = time (h), and a, b, and c = constants.

$$y = m(1 - e^{-kt})^{\beta} \quad [3]$$

Where y = CPDR over time (%), t = time (h), and m, k, and β = constants (where m = total cumulative dose recovery when time is infinite).

From these equations, lag phase (*Tlag*) and gastric half-emptying time ($T_{1/2}$) are calculated using Equations 4 and 5 as follows:

$$T_{lag} = (ln\beta)/k \quad [4]$$

$$T_{1/2} = \left(-\frac{1}{k}\right) x \ln(1 - 2^{1/\beta}) \quad [5]$$

Where β and k are constants calculated from Equation 4. In this approach, lag phase (T_{lag}) indicates the time required for the $^{13}CO_2$ excretion rate to attain its maximal level. In other words, T_{lag} is an indicator of the time it takes for a food to break down within the stomach. Gastric half-emptying time ($T_{1/2}$) is the time necessary for half of the ^{13}C ingested to be metabolized. In other words, $T_{1/2}$ represents the time necessary for half of the ingested test meal to leave the stomach (Sanaka & Nakada, 2010). An assumption is made with this experimental approach; this is the even distribution of the ^{13}C tracer within the test meal and subsequent emptying from the stomach at the same rate food would. Despite this potential limitation, the ^{13}C method is minimally invasive compared to other experimental approaches used to assess gastric emptying.

The assessment of metabolic flexibility in this thesis is based on a new modeling approach of Hayes (2021): the percent relative cumulative frequency (PRCF) analysis of RER combined with the Weibull Cumulative Distribution function (Rinne, 2009). The Weibull Distribution has been extensively used in the biological field. When fit to empirical data, it benefits from defined parameters that can be interpreted according to the conditions of the experiment. The equation for the Weibull Cumulative Distribution function is:

$$y = 1 - \exp\left(-\left[\frac{x}{x_{50}}\right]^b \ln(2)\right) \quad [6]$$

Where:

y = percent relative cumulative frequency (PRCF; 0 to 100%);

x_{50} = median respiratory exchange ratio (median RER);

b = distribution breadth constant (dimensionless), indicative of slope

The use of mathematical approaches for modeling biological processes related to gastric emptying and metabolic flexibility utilized in our experiments provides a general insight into quantifying the effect of slowly digestible starches.

2.9 Conclusions

This review has addressed the basic biological processes related to starch composition, starch digestion and metabolism, and current evidence regarding the effect of slowly digestible carbohydrates on several physiological and metabolic processes. The later part of the review touched on the current experimental tools to evaluate these outcomes.

Traditionally, carbohydrates with different digestion rates are thought to only affect glycemic response, however their consumption can be associated with other physiological and metabolic responses. Considering the prevalence and incidence of obesity and diet-related non-communicable diseases worldwide, research on diet approaches to prevent or treat these conditions will remain relevant.

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CHAPTER 3. DIFFERENCES IN DIETS BETWEEN KENYA AND USA CORRELATE WITH HIGHER CALCULATED RESPIRATORY EXCHANGE RATIO AND METABOLIC FLEXIBILITY

3.1 Abstract

Traditional African diets considered to be rich in slowly digestible carbohydrates (SDCs) have been previously associated with protective effects against the development of obesity and related co-morbidities. In this study we measured diet's, 1) macronutrient composition, 2) carbohydrate quality, and 3) overall diet quality (Healthy Eating Index HEI) for Kenyan and USA study groups and examined the relationship diet quality indices and gastric emptying time, metabolic fuel utilization, and metabolic flexibility; indicators of satiety and sourcing and efficiency of energy utilization in the body. Diets between Kenya (n=34) and USA (n=32) for randomly selected cohorts differed in several macro and micronutrients. Overall diet quality, which was measured using the HEI, was lower for the USA cohort (61.5±12.1) compared to the Kenyan cohort (66.1±10.4). No significant effect of diet on gastric emptying or associated parameters were found. On the other hand, a subset of participants showed differences by country in respiratory exchange ratio (RER) and metabolic flexibility, suggesting that diet has an effect on energy utilization. In this part of the study, a subset of participants was selected from the USA (n=14) group based on poor diet quality while in Kenya (n=23) participants were included based on ability to complete the study using the CO_2-measuring Lumen® device study; diets between these cohorts were different (USA HEI 48.9 vs Kenya HEI 68.1) suggesting that diet quality influences RER and metabolic flexibility. Overall, diet quality between the Kenya and USA study cohorts were different and were associated with change in RER and improved metabolic flexibility.

3.2 Introduction

Increased availability and consumption of foods associated with a Western diet pattern across Africa is leading to a rise in nutrition-related, non-communicable diseases, and is termed the "nutrition transition" (Popkin et al., 2012). Traditional African diets have previously been associated with protective effects against the development of obesity and related co-morbidities (BeLue et al., 2009; Delisle, 2010). Yet, the underlying reasons for this is not clear. One dietary

factor that may contribute to the nutrition transition is the principal available carbohydrate, starch, which in processed foods usually is rapidly digestible and is contrary to traditional African staples considered to be slowly digestible and to contain some amount of resistant starch. We recently reported that urban dwellers make thinner porridges than their rural counterparts and that they are less satiating (Diarra et al., 2022), and that traditional staple starchy foods had markedly slower gastric emptying than introduced modern starchy foods (Cisse et al., 2018).

Consumption of slowly digestible carbohydrates (SDCs) have recently been shown in mice to have beneficial effects on the rate and type of fuel (i.e. carbohydrate vs fat) used for metabolic processes (Saltos et al., 2020; Hayes, 2021). The gold standard for acute measurement of metabolic fuel utilization is the respiratory exchange ratio (RER) which is obtained by measuring the ratio VCO_2/VO_2 in the breath as a proxy to carbohydrate/lipid oxidation at the cellular level (respiratory quotient, RQ). Despite its advantages, RER alone lacks the capacity to assess long term changes in metabolic fuel utilization and models (e.g. Weibull) have been developed to quantify these changes. This is of importance since the ability to switch metabolic fuels at a cellular level determines the efficiency of fuel utilization. The intrinsic ability of cells to sense, store and utilize different nutrients upon availability and requirement is known as metabolic flexibility (Kelley et al., 1999; Smith et al., 2018). A growing body of evidence has demonstrated that inability to switch between carbohydrate or lipid as metabolic fuels, metabolic inflexibility, contributes to the development of noncommunicable, chronic diseases (Boyle et al., 2017; Galgani et al., 2008; Goodpaster & Sparks, 2017; Tahergorabi et al., 2016). Experimental approaches to improve metabolic flexibility have mostly focused on caloric restriction and exercise (Fernández-Verdejo et al., 2018; Goodpaster & Sparks, 2017; Mattison et al., 2017), by inducing a negative energy balance that forces lipid oxidation as an alternative metabolic substrate. However, these potential interventions are not sustainable over long periods of time (Harvie et al., 2017; Wing & Phelan, 2005). Several studies have directly assessed diet in the context of metabolic flexibility (Begaye et al., 2020; Duivenvoorde et al., 2015; Gribok et al., 2016) and, as mentioned, recent mouse studies have assessed carbohydrate digestibility (Fernández-Calleja et al., 2019; Hayes, 2021; Salto et al., 2020).

Consumption of SDCs has been shown to trigger the ileal break to slow gastric emptying rate compared to rapidly digestible carbohydrates (Chegeni et al., 2022; Hasek et al., 2020), however our previous clinical investigations demonstrate that not all individuals respond in the

same manner. The physiological mechanism that accounts for the potential activation of the ileal brake and subsequent slowing of gastric emptying lacks consistency among participants and is not fully understood. We hypothesized that the differences in the gastric emptying time between individuals relate to differences in SDC consumption between African and US participants, and that diets rich in SDC promote physiological changes that facilitate the activation of the ileal break. Diets in Kenya and USA were evaluated using three 24 h diet recalls and diet quality was assessed using the Healthy Eating Index (HEI); which is a tool (scored from 0-100) used to assess how diet aligns with key recommendations of the 2015-2020 Dietary Guidelines for Americans (Bowman et al., 1998). Additional measures including macronutrient distribution and carbohydrate quality were also quantified. In light of the recent reports of an association between SDCs and RER and metabolic flexibility, we hypothesized that Africans consuming diets containing SDCs may have improvements in these energy utilization indices.

In this study, we assessed differences in gastric emptying times and calculated RER and metabolic flexibility between a Kenyan cohort that consumes a traditional Kenyan diet and a USA cohort that consumes a Western diet.

3.3 Materials and methods

3.3.1 Participant selection and study design

A multicenter, crossover human trial was conducted at Purdue University (West Lafayette, IN, USA) and University of Eldoret (Eldoret, Rift Valley, Kenya). Eligibility criteria were as follows: males or females aged 18–50 years of age, normal body mass index (BMI, 18.5 kg/m^2 ≤ BMI ≤ 25 kg/m^2), and self-reported stable weight for the past 3 months. Participants were excluded from the study if they presented any of the following: history of current gastrointestinal diseases/disorders, diabetes (type 1 or 2), food allergies or sensitivities of any kind, actively taking any medication, active smoker, or if pregnant or nursing. For the portion of the study in Kenya, participants were required to consume a typical Kenyan diet. For the portion of the study assessing of metabolic flexibility in the US, participants were required to have a HEI ≤ 65. Participant recruitment and study participation for Kenyan and American cohorts are shown in Figure 3-1. and 3-2 respectively.

Participants that met the criteria at each location were included in a crossover trial consisting of two treatment arms. Treatment order was randomized (random computer generator) with a washout period of at least seven days between treatments. Each treatment arm consisted of two testing days. On the first testing day, the assigned test meal was consumed after an overnight fast (>10 hours) as the first morning meal. Gastric emptying was measured postprandially for a 4 h period. On the second testing day, the same test meal was consumed (same fashion) and breath samples for assessment of calculated RER/metabolic flexibility were collected postprandially for a 2 h period. The test meals were semi-thick pastes; consisting of either a slowly digestible starch powder (raw corn starch) or a rapidly digestible starch-based powder (maltodextrin DE-1) mixed in unsweetened applesauce with xanthan gum to equalize viscosity. Participants were asked to avoid intense physical activity and alcohol consumption the day before and during the test days. Additionally, a standardized dinner was provided to participants the night before gastric emptying testing days.

Approval for the study was obtained from the Institutional Review Board of Purdue University (Protocol IRB-2019-206) and the Institutional Research and Ethics Committee at Moi University in Kenya. Written consent form was obtained from each participant before his or her participation in the study. The study is registered in clinicaltrials.gov (ID NCT03630263).

3.3.2 Test Meals

The slowly digestible starch test meal was prepared using 30 g of raw corn starch (RCS) (Argo, Memphis, TN, USA) and the rapidly digestible starch meal was prepared using 30 g of maltodextrin DE-1 (MDX) (Cargill, Minneapolis, MN, USA). Each carbohydrate was mixed in 200 g of unsweetened applesauce (Musselman's, Peach Glen, PA, USA) with 0.2 g of xanthan gum (Bob's Red Mill, Milwaukie, OR, USA). Ingredients were mixed and homogenized using a hand blender immediately before consumption. Test meals were served at ambient temperature. Each participant was required to drink 100 mL of water with the test meal. The approximate nutrient and caloric contents of the test meal ingredients are shown in Table 3-1. For the assessment of gastric emptying time (test day 1), 100 mg of ^{13}C-octanoic acid (Sigma-Aldrich, Saint Louis, MO, USA) was mixed into each test meal immediately before serving. For the assessment of calculated RER/metabolic flexibility (test day 2), no tracer was added to the test meal.

3.3.3 Procedures

Statistical power analysis was performed using pilot data obtained from prior gastric half-emptying studies (two tailed, P = 0.05, 0.5 h minimum detectable difference in means, 1-β = 0.8, s_{pooled} = 0.5 h, n = 32). Ad hoc power calculation for CO_2/calculated RER for unequal variance, unbalanced groups (USA n=14, Kenya n=23)., one tail, RERμ_1= 0.92 RER μ_2= 0.87, resulted in α=0.04 β = 0.62.

Prior to any testing, participants were instructed on the protocols and procedures for the study, particularly for the assessment of calculated RER/metabolic flexibility (described below). The timeline and activities for this clinical trial are shown in Figure 3-3.

The night before testing day (Day 0) participants consumed a standardized dinner (≈700 Kcal) between 7-8 pm and were asked to fast until the next morning. The following day (Day 1), participants arrived at the testing facility between 7:45-8:15 am and upon corroboration of overnight fast (>10 h) each participant was assigned to a pre-set testing station. Subsequently, baseline breath samples for assessment of gastric emptying (two - 1.5 L bags, Cambridge Isotope Laboratories, Tewksbury, MA, USA) were collected for each participant. After completion of baseline assessment, participants were provided one test meal and water according to the assigned arm of the study. Participants were instructed to finish the test meal within 15 min. No other food or drink was allowed for the remainder of the test session. Following completion of the test meal, breath samples for assessment of gastric emptying time (0.30 L bags, Cambridge Isotope Laboratories, Tewksbury, MA, USA) were collected every 15 min for hours 1 to 2, and every 30 min for hours 3 to 4. After completion of all breath collections assessments, participants were allowed to leave the facility.

The night before second testing day participants were instructed to have a light dinner between 7 and 8 pm and subsequently fast until the next morning. No standardized dinner was provided prior the second testing day. The following day (Day 2), participants arrived at the testing facility between 7:45-8:15 am. Subsequently, baseline breath samples for assessment of calculated RER and metabolic flexibility were performed by each participant using Lumen® devices (Metaflow, Tel Aviv, Israel) (Lorenz et al., 2021). Upon successful completion of baseline sampling, participants were provided the assigned test meal (same as Day 1 without the ^{13}C tracer) and water. Similar to Day 1, participants were instructed to finish their meal within 15 min and no food or drink was allowed for the remainder of the session. Following completion of the test meal,

breath samples for assessment of RER and metabolic flexibility were collected every 10 min for hour 1, and every 15 min for hour 2. After completion of all breath collections, participants were allowed to leave the facility.

The same procedures for gastric emptying and calculated RER/metabolic flexibility were performed after a washout period of at least 7 days with the remaining treatment. For diet assessment, participants were called on non-testing days (days before and after testing were also excluded). Collection of diet records was performed three times by a registered dietitian, twice during weekdays and once during the weekend to capture variability in mealtime patterns, as explained below (Sec. 4.2.3.3). Participants were called at random to avoid unconscious bias.

3.3.4 Gastric emptying

Assessment of gastric half-emptying time using the ^{13}C octanoic acid breath test has been previously performed in our laboratory for populations in the US and Africa (Chegeni et al., 2022; Cisse et al., 2018; A. M. R. Hayes et al., 2021; Pletsch & Hamaker, 2018); the methodology has been refined from a previous method (Sanaka & Nakada, 2010) with modification accounting for differences in body surface area (Haycock et al., 1978).

For the cohort in the USA, analysis of breath samples collected for assessment of gastric emptying time was performed within 48 h after collection using a $^{13}CO_2$ breath analyzer (POCone, Otsuka Electronics Co., Ltd., Osaka, Japan). For the Kenyan cohort, the collected breath samples were stored in a dry, cool place and subsequently shipped to the US for analysis. Prior to study launch, additional testing was performed with volunteers (n=4) to determine $^{13}CO_2$ stability in stored breath samples. Breath $^{13}CO_2$ content was assessed for 4 consecutive weeks to assess changes in $^{13}CO_2$ concentration, and no changes were observed from baseline $^{13}CO2$ over the 28-day testing period.

Parameters for determining percent dose recovery and cumulative percent dose recovery of ^{13}C are shown in Appendix A and B. Only individuals with adequate data model fit ($R^2 > 0.80$) for percent dose recovery of the ^{13}C octanoic acid tracer were included in subsequent analyses. The primary outcomes calculated from these parameters were gastric half-emptying time, defined as the time required for half of the ^{13}C dose to be metabolized (Perri et al., 2005), lag phase defined as the time required for the $^{13}CO_2$ excretion rate to attain its maximal level (indicator of time for

food to break down within the stomach), and gastric emptying coefficient (overall indicator of gastric emptying).

3.3.5 CO_2, calculated respiratory exchange ratio, and metabolic flexibility

Respiratory exchange ratio is obtained by measuring the ratio VCO_2/VO_2 in the breath as a proxy to carbohydrate/lipid oxidation at the cellular level (respiratory quotient, RQ). For this study, participants used a non-invasive, individual breath device (Lumen®) to measure only CO_2 in breath; with higher amounts of CO_2 exhaled indicating carbohydrate as the main metabolic fuel source (RER =1) and lower amounts of CO_2 indicating fat as the preferred metabolic fuel source (RER = 0.7). Lumen® has been validated against indirect calorimetry and is a reliable measurement tool to assess RER (Lorenz et al., 2021).

Metabolic flexibility was assessed, using either pooled CO_2 or pooled calculated RER data for all participants followed by modeling using the Weibull Cumulative Distribution function. To accomplish this, percent relative cumulative frequency (PRCF) was first calculated per treatment and location according to the method of (Riachi et al., 2004). The procedure includes the following steps: (1) data is sorted in ascending order, (2) an interval of increment is selected (0.01 for CO_2 data, 0.001 for RER data), (3) the frequency of observations per interval is calculated, (4) the cumulative frequency is calculated, and (5) the cumulative frequency is expressed as a percentile curve. Following calculation of PRCF, plots of RER (ascending order) vs. PRCF were fit to the Weibull Cumulative Distribution function (Eq. 1).

$$y = 1 - \exp\left(-\left[\frac{x}{x_{50}}\right]^{b} ln(2)\right) \quad [1]$$

Where:

y = percent relative cumulative frequency (PRCF; 0 to 100%);

x_{50} = median CO_2 or respiratory exchange ratio (median CO_2 or RER);

b = distribution breadth constant (dimensionless), indicative of slope.

Modeling was done using the "fitnlm" function with the nonlinear least squares method option in MATLAB (R2020a, Update 5, 9.8.0.1451342, The MathWorks, Inc., Natick, MA). To ensure that x_{50} fell within the range of RER values for each dataset, bounds were placed on this parameter accordingly. Furthermore, an iterative modeling approach was used to obtain the best fit for each parameter, such that 5 initial "best guess" fits were incorporated into the modeling

approach. Modeling was performed on pooled data because only 10 data points were collected for each participant per test day, and more than 20 data points are required in order to employ the Weibull Cumulative Distribution function (Razali & Al-Wakeel, 2013; Rinne, 2009).

Prior to any testing sessions, participants were assigned an individual research account and instructed on Lumen® device usage. The device was paired and synchronized to the participant's smartphone together with the Lumen® app. In Kenya, tablets were provided to participants without smartphones. Participants practiced the Lumen® breathing technique while supervised and were only allowed to participate in the study after they showed proficiency with the device and app. Participants were instructed to practice the breathing maneuver at least 10 times, and proficiency was determined by ≥2 successful data points (valid measures provided by the app).

During the testing day, participants were continuously monitored for successful completion of the breathing technique. Participants were removed from the study if they were not able to complete two consecutive breath maneuvers. Data from breath readings was collected by Metaflow, manufacturers of Lumen®, and provided to researchers without identifiers. Incomplete or inconsistent readings were not included.

3.3.6 Diet assessment

Prior to collection of diet records, participants were provided visual aids for assessment of portion sizes. Three 24 h diet recalls were collected in person or over the phone (one weekend day, two weekdays). Participants were called randomly to avoid subconscious bias. A pre-structured interview with quality control points (multiple pass) was used to collect 24 h diet recalls (Nutrition Data System for Research software (NDSR, Minneapolis, MN, USA) (Harnack et al., 2008). The software is tailored for foods consumed in the USA; modifications and additions of specific food items and traditional Kenyan recipes were created and validated for the Kenyan diet. Overall diet quality was assessed by calculating the HEI, which measures how well a person's diet aligns with key recommendations of the Dietary Guidelines for Americans (2015-2020). Dietary data was normalized for each participant body weight (e.g. Kcal/Kg BW; nutrient g/Kg BW). Caloric intake, macronutrient assessment (carbohydrate, protein, fat, and fiber), assessment of carbohydrate quality (fiber, starch, added sugars, glycemic index), and overall diet quality (HEI-2015) were calculated for both cohorts.

3.3.7 Statistical Analyses

Statistical analysis was conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA). Two-way ANOVA (PROC MIXED) with random effects; including test meal, country and diet variables as a fixed effects and participant as a random effect was used to determine statistical significance of differences in gastric half-emptying time, gastric lag phase, and gastric emptying coefficient. Multivariate, mixed effects models assessing the impact of diet variables on gastric half-emptying time, lag phase, CO_2 and calculated RER were evaluated as follows:

Macronutrient composition | Model 1: caloric intake (Kcal/Kg B.W.), carbohydrate (g/Kg B.W.), protein (g/Kg B.W.), fat (g/Kg B.W.), including interactions between diet variables and interactions between diet variables and country.
Carbohydrate quality | Model 2: soluble fiber (g/Kg B.W.), insoluble fiber (g/Kg B.W.), starch (g/Kg B.W.), added sugars (g/Kg B.W.). Glycemic index including interactions between diet variables and interactions between diet variables and country.
Diet quality | Model 3: HEI and interactions between HEI and country

Diet variables, interactions between diet variables, and interactions between diet variables and location, were assessed for multicolinnearity and inflation.

SAS version 9.4 (SAS Institute, Cary, NC, USA) was used to perform repeated measures, two-way ANOVA (PROC MIXED) with test meal, country, diet variables and time as fixed effects and subject as random effect was conducted to determine difference in metabolic parameters (CO_2 and Calculated RER). For all repeated measures analyses, baseline values (for each outcome per its respective test day) were added as a covariate in the model (Blundell et al., 2010). Homoscedasticity and normality of residuals was assessed using histograms and quantile-quantile plots. All data was normally distributed and did not require transformation. Significance was considered at $p<0.05$, and Tukey's post hoc test for multiple comparisons were conducted when the overall model was significant ($p<0.05$ for F value).

For the Weibull modeling of pooled PRCF analyses representing metabolic flexibility, data from all participants were pooled together per group (treatment and location), and therefore there were no replicates. To statistically compare this data, 95% confidence intervals were calculated for the Weibull parameters (i.e. $x_{50,}$ b), in accordance with previous research (Gardner & Altman,

1986; Kreutz et al., 2012). Statistically significant differences ($p<0.05$) were indicated if the confidence intervals for these parameter estimates did not overlap.

3.4 Results

3.4.1 Participant characteristics

For the gastric emptying study, 34 healthy subjects (13 men, 21 women) began the study in Kenya and 34 subjects began in the US (14 men, 20 women). Clinical characteristics for participants whose results were included in the study, both Kenya and US are shown in Table 3-2. There was some missing data and participant withdrawals. For the assessment of gastric emptying in Kenya, one female participant no longer wished to participate in the study after the first session and thus was withdrawn; participant's data from the first completed treatment arm was included in the analyses. Researchers were unable to obtain dietary data for this participant. Additionally, three breath bags for the maltodextrin treatment and two for the raw corn starch treatment were damaged during transport (breath sample bags lost seal during transfer). For the assessment of gastric emptying in the USA trial, one female and one male participant withdrew before starting the study, no data was collected from these participants. Additionally, researchers were unable to reach one male participant who completed both gastric emptying sessions.

For the assessment of breath CO_2/calculated RER in Kenya, a subgroup took part in the study. Five participants were excluded from the original group due to inability to produce ≥2 successful data points during the training session, five participants were unable to comply with study procedures and one participant voluntarily withdrew from the study. Data from excluded participants and invalid sessions were not included in the analysis (Figure 3-1.).

The subgroup that participated in the assessment of breath CO_2/calculated RER in USA (n=14; 10 female/4 male) was different from the cohort that participated in the assessment of gastric emptying. No test measures were flagged during the experiment (Figure 3-2.). Clinical characteristics for the Kenya and USA breath CO_2/calculated RER groups are shown in Table 3-3.

3.4.2 Gastric emptying

A total of 126 ^{13}C octanoic acid breath evaluations (from baseline to 240 min) were collected for assessment of gastric half-emptying time. For only one participant for one treatment the R^2 model fit for percent dose recovery was below the target cut-off (sample R^2=0.78), and therefore the data was not included. For the Kenyan cohort, the average R^2 for model fit to percent dose recovery was 0.94±0.04, and the average R^2 for the model fit to cumulative percent dose recovery was 0.9993±0.0005. For the USA cohort, the average R^2 for model fit to percent dose recovery was 0.95±0.04, and the average R^2 for the model fit to cumulative percent dose recovery was 0.9993±0.0007.

Previous studies conducted by our group indicate that some subjects display a slow gastric emptying time after consumption of SDCs (Chegeni et al., 2022; Cisse et al., 2017, 2018). In the study of Chegeni et al. (2022), the slow gastric emptying designation was defined as >30 min compared to the mean for the respective treatment; and if subjects met this criterion, they were considered responders. However, in the current study, only two subjects in the USA cohort (subject 512 and 550) were responders to the rapidly digestible maltodextrin DE-1 and two were responders to the slowly digestible RCS (subjects 51 and 550). For the cohort in Kenya, only one subject (subject 15) was a responder to RCS.

Overall, gastric half-emptying time and lag phase were significantly influenced by treatment meal (p=0.01 and p=0.0001, respectively; Figure 3-4 and Table 3-4), but not by country (p=0.51), or their interaction (p=0.58). For gastric emptying coefficient, only country was statistically significant (p=0.04).

Pooled values for raw corn starch and maltodextrin DE-1 showed that the latter had a quite modest but significantly higher gastric half-emptying time and lag phase (p<0.01), and this was contrary to the original hypothesis that the SDC (RCS) would increase gastric emptying time. Gastric half-emptying time pooled values for maltodextrin DE-1 was 1.83 h ± 0.56 and for RCS was 1.68 h ± 0.27. Means for gastric half-emptying times, lag phase, and gastric emptying coefficient by country and treatment are shown in Table 3-4. Assessment of diet variables did not yield any significant results for either of the previously mentioned multivariate models.

3.4.3 CO_2 and calculated respiratory exchange ratio (RER)

Respiratory exchange ratio values were calculated from CO_2 using a conversion equation (Lorenz et al., 2021). CO_2 and calculated RER values were significantly influenced by country ($p<0.0001$; Figures 3-5 and 3-6), but not for time, test meal ($p=0.51$), or their interaction ($p=0.58$). Results for the multivariate, mixed model assessing associations between CO_2 and diet were significant and are shown in Tables 3-5 to 3-7. Multivariate models assessing associations between calculated RER and diet were also significant and are shown in Tables 3-8- to 3-10.

Several diet variables showed significant multicolinnearity (diet variables were correlated between each other, e.g. if fat increased, protein increased). After evaluation, the original proposed models were modified to avoid these confounding factors. Only variables with variance inflation factor <5 were included. All interactions between diet*diet and diet*location variables displayed multicollinearity. Individual diet variables including caloric content (Kcal/Kg BW), fat, and soluble and insoluble fiber (g/Kg BW) also displayed multicollinearity. Mixed model analyses including all participants showed that several predictors were independently associated with the production of CO_2. Of the diet variables assessed in modified model 1 (macronutrient composition), carbohydrate and protein had an effect on CO_2. Additionally, the larger the value was for the coefficient, the greater the impact was on CO_2.

For model 2 (carbohydrate quality), total fiber, starch, and added sugars had an effect on CO_2 production. For model 3, (diet quality), the diet variable HEI had an effect on CO_2 production. Since calculated RER is calculated from CO_2, the variables and models previously discussed follow the same pattern for calculated RER. Interpretation of the effect of diet is easier in the calculated RER models since RER $\approx$0.7 indicates fat oxidation, while RER$\approx$1 indicates carbohydrate oxidation.

3.4.4 Metabolic flexibility

Weibull analysis has been previously used in engineering to determine material failure and value distribution which is determined by the slope, and has been recently used by Hayes (2021) to assess RER distributions related to metabolic flexibility. With the Weibull approach, steeper slopes (larger b value) indicate more values are concentrated in a small range, and the less step the slope (smaller b value) indicates the values are more widely distributed. PRCF curves of CO_2

values illustrate a broader spread and less steep slope for participants from Kenya indicating greater distribution of CO_2 values overall (Figure 3-7). Considering that these curves represent CO_2 (carbohydrate oxidation), these characteristics are proposed to be indicative of increased metabolic flexibility.

PRCF curves for the RER values calculated from CO_2 are shown in Figure 3-8. Because the general shape and apparent trend observed between the PRCF curves of CO_2 and RER are very similar, and also because all but the baseline measurements were obtained during the postprandial period (as opposed to equally obtained during both the preprandial and postprandial periods), we performed the Weibull Cumulative Distribution modeling solely on the CO_2 PRCF curves. The results from this modeling indicate that SDC (raw corn starch) consumption by the Kenyan participants resulted in higher x_{50} (i.e., median value for carbohydrate oxidation) than for maltodextrin consumption, which suggests that SDC prompted a more complete 'switch' to carbohydrate oxidation (Figure 3-8 and 3-9). However, no differences were found between raw corn starch and maltodextrin consumption in the USA group. Intriguingly, the participants in Kenya had an overall higher x_{50} than those in USA, regardless of carbohydrate treatment, suggesting an overall diet effect.

As for the b parameter estimate (i.e., slope), there were also no differences per carbohydrate treatment by location. However, as for x_{50}, there was also a difference according to location (Kenya vs. USA) such that individuals in Kenya had significantly lower b parameter estimates than those in USA (Figure 3-9). Considering that a lower b indicates a broader spread in values (i.e., lower slope) and that the current analysis only involves CO_2 values, we propose that this lower b for the Kenyan group is suggestive of enhanced metabolic flexibility. Theoretically, a broader range in CO_2 values means more fat oxidation could be taking place, specifically at the lower CO_2 values. Overall, the participants from Kenya had higher x_{50} (increased median CO_2, representing carbohydrate oxidation) – especially for raw corn starch (SDC) – yet lower b (broader range in CO_2 values), and we propose that these results indicate the participants in Kenya had increased metabolic flexibility, notably with a slightly more pronounced switch to carbohydrate oxidation for raw corn starch than for maltodextrin.

3.4.5 Diet assessment

For the group that completed the assessment of gastric emptying time, diet variables are shown in Table 3-11. For the subgroup that completed the assessment of CO_2/calculated RER, diet variables are shown in Table 3-12. HEI-2015 values were calculated using the dietary guidelines for American 2015--2020 version.

3.5 Discussion

The objective of this study was to test the hypothesis that African vs. American diets affect gastric half-emptying time, calculated RER, and metabolic flexibility in healthy adults through the promotion of physiological changes that facilitate the activation of the ileal break and modulation of metabolic fuel utilization. Gastric half-emptying time was used as a proxy measurement for ileal brake activation (Chegeni et al., 2022). We assumed that any differences observed in gastric half-emptying time between cohorts (Kenya vs USA) would be related to the physiological ability of each cohort to sense carbohydrate digestion products and subsequently activate the ileal brake.

Considering the low number of responders to the SDC (raw corn starch) in our study and the lack of significant correlations in the multivariate model, we conclude that diet differences do not play a role in influencing gastric emptying time. Our clinical trial compares to a previous study evaluating gastric emptying response to acute consumption purified SDCs (Chegeni et al., 2022), showing that a number of subjects were responders to SDC (>30 min above treatment mean). In our study, only one participant in Kenya (n=34) and two participants in the USA (n=32) were similar responders to ingestion of the raw corn starch SDC. The test meals (30 g carbohydrate + 200 g apple sauce) were matched for energy content and viscosity; therefore, subjects' response was considered to be only related to the rate and distal location of digestion of the SDC versus the rapidly digestible maltodextrin. One notable difference between the study by Chegeni et al. (2022) and the present one is that we used an applesauce versus yogurt vehicle as a carrier for the carbohydrates.

A study performed by Hayes and collaborators showed similar gastric emptying results to our study (A. M. R. Hayes et al., 2021), with no differences in gastric half-emptying time after consumption of foods containing millet foods, which were considered to have a SDC component. Nevertheless, there was a significant effect on consumption of some millet foods on decreasing

postprandial blood glucose. Direct comparison to this study is difficult since the treatments were whole foods containing SDC (e.g. pearl millet couscous, millet porridge) and food matrix effects (matrix density, presence of phenolic compounds) cannot be ruled out as potential cofounding factors affecting gastric half-emptying time response.

On the other hand, CO_2 and calculated RER were significantly influenced by country and diet, but not for treatment (SDC vs. RDC). The Kenyan cohort had a significantly higher CO_2/calculated RER compared to the USA cohort. It is suggested that African populations consume healthier diets (comparably high in SDCs) while USA populations have a Western diet pattern (Harmon et al., 2015). This may be related to the respective HEI scores for Kenya and the USA (68.1 vs 48.9) and the values for each subgroup regarding carbohydrate variables (Table 3-12). Despite that carbohydrate quality (slow vs fast digesting) cannot be directly assessed from these values due to limitations in NDSR database, it is speculated that some diet variables (e.g. higher fiber, low simple sugars) might be associated in the Kenyan cohort with slowly digestible carbohydrate consumption (USDA, 2022).

Regarding diet variables and the multivariate models, several variables showed multicollinearity. Energy intake was speculated to show multicollinearity since it is the sum of individual macronutrients multiplied by their caloric content (carbohydrate 4 Kcal/g, protein 4 Kcal/g, fat 9 Kcal/g); therefore, as overall nutrient content increases so does caloric intake. Also, as one macronutrient increases in the diet, often another decreases. However, protein and fat displayed multicollinearity with both increasing in the diet. Only protein was selected to be included in the model 1. A similar relationship was observed for soluble and insoluble dietary fiber, with both increasing in higher fiber-containing diets.

Model 1 results suggested that carbohydrate and protein negatively affect CO_2 production, the latter having the greater impact (coeffcient -0.05). For the variable “carbohydrates”, NDSR aggregates all carbohydrates in the diet including simple and refined carbohydrates, added sugars, as well as fast and slow digesting starches. Thus, there is no way to discern the rate of digestion of any of the elements composing this diet variable. It was previously shown that high fat and high sucrose diets impair muscle mitochondrial function decreasing metabolic flexibility in rats exposed for 12 weeks to those diet (Jørgensen et al, 2020). Similar findings have been found in overweight men after consumption of high fat diet for 3 weeks; with the control group (low fat diet) showed a

slight but significant increase in RQ (+0.02) while the high fat diet group decreased (-0.05) (van Herper et al. 2011).

For model 2, several variables related to carbohydrate quality, including total fiber, starch, and added sugars were independently correlated to CO_2 production. The Jørgensen paper showed that diets high in sucrose exert a negative effect on metabolic flexibility partially supporting our positive finding for added sugars. It is speculated that starch follows a similar rational and contributes to total added sugar in diet (NDSR does not distinguish between slow and fast digesting starches). Fiber showed the highest coefficient (+0.12), indicating the highest impact on CO_2 production.

It is notable that for the assessment of CO_2/calculated RER, participants in the USA had a significantly lower mean HEI-2015 score (48.9) compared to the average American HEI-2015 score of 59 (USDA, 2022). This is because they were screened and selected based on their poor diet quality. In a recent study assessing overall healthy diet patterns (high in fruits and vegetables, pulses, fibers, nuts, fatty fish, and low in high-glycemic carbohydrates) vs a typical Western diet in obese and overweight adults for 6 weeks; the researchers found no significant effect of healthy diet on metabolic flexibility after the intervention (Fechner et al, 2020). This contradicts our findings in model 3, however the researchers noted that diet alone does not affect metabolic flexibility, however, diet in combination with adequate exercise might have a significant effect. This study was performed in obese and overweight individual in which metabolic flexibility might already be compromised.

Regarding carbohydrate quality, our findings are partly supported by previous animal studies (Fernández-Calleja et al., 2018; Hayes, 2021; Santos et al., 2020). Fernandez Calleja and collaborators demonstrated that continuous consumption of carbohydrates with low digestibility in early life exerted acute effects on intestinal morphology (weight and size of intestine and colon), however this effect was lost after such supplementation was discontinued. The researchers also found lasting effects on metabolic fuel utilization that carried throughout life. This effect was only found in females; with female mice displaying higher metabolic flexibility after consumption of a high fat diet for 8 weeks. On the other hand, both studies by Saltos et al. (2020) and Hayes (2021) demonstrated that consumption of SDC improves metabolic flexibility in mice. These outcomes demonstrate the potentially protective metabolic effect of SDC consumption and supports our

findings. Tailored nutrition interventions in humans could be done to assess the effect of diet on RER modulation and its potential improvement of metabolic flexibility.

The lack of statistical significance of treatments (SDC vs. RDC) supports that the ability to utilize specific substrates for metabolic fuel utilization is rather linked to the cohort and the diet that potentially shapes their metabolic response. No differences in CO_2/calculated RER were found between treatments (MDX vs RCS), which were carbohydrate rich test meals (30 g MDX vs RCS+ ≈18 g fructose from unsweetened applesauce). We expected to observe a higher CO_2/calculated RER after consumption of the fast-digesting carbohydrate (MDX DE-1). This could, in retrospect, be attributed to the fructose (from applesauce) masking differences in metabolic fuel utilization between treatments. The assessment of RER and metabolic flexibility was experimentally added *post hoc* to the study design. In an animal study conducted by Hayes and collaborators, the researchers showed that SDCs shifted RER downwards towards fat oxidation. This effect was attributed to the slower carbohydrate digestion related to a decreased availability of postprandial blood glucose that subsequently would promote fat oxidation (Hayes, 2021). It is important to note that cohorts' age and body composition (i.e. total lean body mass, adipose/muscle mass ratio) may influence RER and metabolic flexibility. As age increases, lean body mass decreases with a concomitant decrease in muscle O_2 consumption that affect basal energy expenditure and (basal metabolic rate) (Tzankoff & Norris, 1977). This decrease in O_2 consumption could have potential implications in metabolic flexibility. Similary, though not measured here, total lean body mass and adipose/muscle mass ratio differences in the populations between Kenya and USA might impact calculated RER and metabolic flexibility as it has been shown that reduced metabolic flexibility is associated with increased body fat (Sparks et al., 2009).

3.6 Conclusion

Although no significant effect of diet on gastric emptying was found, our findings substantiate that diet exerts a direct effect on metabolic fuel utilization, and specifically that overall the traditional Kenyan diet, based significantly on the thick starchy porridge *ugali*, leads to better metabolic flexibility that is associated with good health. While not a causal proof of SDCs leading to better utilization of metabolic fuels in the body, these results show the interesting possibility that dietary differences in carbohydrate quality affect this fundamental metabolic process.

Thus, a further experimental nutrition intervention is necessary to assess diet, specifically related to dietary carbohydrate quality, and its potential to modulate metabolic fuel utilization and metabolic flexibility. Finally, whether SDC effect on metabolic fuel utilization is dependent on continuous consumption of SDC or is it a protective carryover effect of tissue plasticity from early stages of life remains to be answered.

3.7 Acknowledgment

Thanks to Anne Wilcox for her support with dietary data collection; and to Alexandra Cowan and David Arthur for their support with calculation of Healthy Eating Index, data analysis and modeling. This work was made possible by the support of the American People provided to the Feed the Future Innovation Lab for Food Processing and Post-harvest Handling Innovation through the United States Agency for International Development (USAID) under Cooperative Agreement No. AID-OAA-A-13-00047 and the support provided through the Indiana Clinical and Translational Sciences Institute (CTSI), global grant awards. The contents are the sole responsibility of the authors and do not necessarily reflect the views of CTSI, USAID or the United States Government. We are also grateful to Prof. Dennis Savaiano (Purdue University, and Prof Regan Bailey (Texas A&M), for facilitating the tools for dietary assessment; and to Merav Mor and Shlomi Yeshurun (Metaflow) for facilitating the use of Lumen®.

3.8 References

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Table 3-1. Approximate nutrient and caloric content of the test meal and ingredients.

Ingredient	Quantity (g)	Caloric content (Kcal)	Moisture (%)
Unsweetened Apple Sauce	200	68	91
Carbohydrate treatment	30	106.8	11
Xanthan gum	0.2	-	-
Total	230.2	174.8	-

Table 3-2. Clinical characteristics of the gastric emptying study cohort.

Characteristic	Kenya n=(34)	SD	USA n=(32)	SD
Age (years)	32.7	9.5	25.3	5.1
Height (cm)	166.5	9.1	169.2	6.9
Weight (kg)	63.7	9.7	65.0	9.5
BMI (kg/m^2)	22.9	2.2	22.7	2.2
Sex	14 M, 20 F		12 M, 20 F	

M, male; F, female
SD = standard deviation

Table 3-3. Clinical characteristics of the CO_2/calculated RER study subgroup.

Characteristic	Kenya n=(23)	SD	USA n=(14)	SD
Age (years)	32.9	9.2	23.9	7.2
Height (cm)	167.7	9.1	166.2	9.9
Weight (kg)	66.6	8.0	65.2	11.1
BMI (kg/m^2)	23.7	1.8	23.5	2.8
Sex	12 M, 11 F		4 M, 10 F	

M, male; F, female
SD = standard deviation

Table 3-4. Mean half-gastric emptying, lag phase, and gastric emptying coefficient values for Kenya and USA participants after consumption of either standard testslow digesting meal (30 g RCS + 200 g applesauce) or rapidly digestible starch meal (30 g MDX

	Kenya n=(34)		**USA n=(32)**	
	Raw Corn Starch	**Maltodextrin**	**Raw Corn Starch**	**Maltodextrin**
Half-gastric emptying (h)	1.65±0.25	1.79±0.65	1.70±0.28	1.87±0.43
Lag phase (h)	0.83±0.22	0.93±0.33	0.71±0.18	0.91±0.20
Gastric Coeff	3.88±0.38	3.81±0.36	3.74±0.38	3.63±0.35

± values represent standard deviations

Table 3-5. Multivariable associations between CO_2 response and macronutrient diet variables (Model 1).

Effect	Coefficient	CL		P value
(Intercept)	5.38	5.21	5.55	<.0001
Location (USA vs Kenya)	-0.20	-0.32	-0.09	0.00
Carbohydrate (g/Kg BW)	-0.06	-0.11	-0.01	0.02
Protein (g/Kg BW)	-0.32	-0.48	-0.15	0.00

Bolded P values are statistically significant after correction for multiple comparisons using Tukey mean separation.
BW = body weight
CL = 95% confidence intervals

Table 3-6. Multivariable associations between CO_2 response and carbohydrate quality diet variables (Model 2).

Effect	Coefficient	CL		P value
(Intercept)	5.56	5.33	5.79	<.0001
Location (USA vs Kenya)	-0.49	-0.60	-0.38	<.0001
Total fiber (g/Kg BW)	0.73	0.09	1.37	0.026
Starch (g/Kg BW)	-0.22	-0.33	-0.11	0.0001
Added sugars (g/Kg BW)	-0.36	-0.50	-0.23	<.0001

Bolded P values are statistically significant after correction for multiple comparisons using Tukey mean separation.
CL = 95% confidence intervals
BW = body weight

Table 3-7. Multivariable associations between CO_2 response and healthy eating index (HEI) (Model 3).

Effect	Coefficient	CL		P value
(Intercept)	4.304	4.026	4.583	<.0001
Treatment (maltodextrin vs raw corn starch)	-0.036	-0.078	0.006	0.094
Location (USA vs Kenya)	-0.149	-0.263	-0.035	**0.010***
HEI	0.009	0.005	0.013	**<.0001***

Bolded P values are statistically significant after correction for multiple comparisons Tukey
CL = 95% confidence intervals

Table 3-8. Multivariable associations between calculated RER response and macronutrient diet variables (Model 1).

Effect	Coefficient	CL		P value
(Intercept)	1.00	0.97	1.03	<.0001
Location (USA vs Kenya)	-0.03	-0.05	-0.01	0.00
Carbohydrate (g/Kg BW)	-0.01	-0.02	0.00	0.02
Protein (g/Kg BW)	-0.05	-0.08	-0.03	0.00

Bolded P values are statistically significant after correction for multiple comparisons Tukey
CL = 95% confidence intervals
BW = body weight

Table 3-9. Multivariable associations between calculated RER response and carbohydrate quality diet variables (Model 2).

Effect	Coefficient	CL		P value
(Intercept)	1.03	0.99	1.07	<.0001
Location (USA vs Kenya)	-0.08	-0.10	-0.06	<.0001
Total fiber (g/Kg BW)	0.12	0.01	0.22	0.026
Starch (g/Kg BW)	-0.04	-0.05	-0.02	0.0001
Added sugars (g/Kg BW)	-0.06	-0.08	-0.04	<.0001

Bolded P values are statistically significant after correction for multiple comparisons using Tukey mean separation.
CL = 95% confidence intervals
BW = body weight

Table 3-10. Multivariable associations between calculated RER response and healthy eating index (HEI) (Model 3).

Effect	Coefficient	CL		P value
Intercept	0.826	0.781	0.872	<.0001
Treatment (maltodextrin vs raw corn starch)	-0.006	-0.013	0.001	0.094
Location (USA vs Kenya)	-0.024	-0.043	-0.006	**0.010**
HEI	0.001	0.001	0.002	**<.0001**

Bolded P values are statistically significant after correction for multiple comparisons using Tukey mean separation.
CL = 95% confidence intervals

Table 3-11. Diet characteristics for assessment of gastric emptying.

		USA n =34		Kenya n=32	
Variable	**Units**	**Mean**	**SD**	**Mean**	**SD**
Caloric content	Kcal/kg BW	27.95	7.49	25.37	7.95
Carbohydrates	g/Kg BW	3.29	0.93	3.75	1.10
Protein	g/Kg BW	1.16	0.33	0.89	0.34
Fat	g/Kg BW	1.14	0.43	0.80	0.32
Total fiber	g/Kg BW	0.32	0.10	0.36	0.13
Soluble fiber	g/Kg BW	0.10	0.04	0.08	0.03
Insoluble fiber	g/Kg BW	0.22	0.08	0.28	0.11
Starch	g/Kg BW	1.41	0.49	2.06	0.62
Fructose	g/Kg BW	0.28	0.16	0.16	0.11
Galactose	g/Kg BW	0.01	0.02	0.00	0.01
Glucose	g/Kg BW	0.26	0.13	0.14	0.10
Lactose	g/Kg BW	0.18	0.11	0.34	0.24
Maltose	g/Kg BW	0.04	0.02	0.02	0.02
Sucrose	g/Kg BW	0.52	0.27	0.46	0.26
Added Sugars	g/Kg BW	0.68	0.38	1.42	0.25
Glycemic Index		56.86	4.04	124.89	50.68
HEI		61.46	12.08	66.06	10.42

SD = standard deviation
BW = body weight
HEI = healthy eating index

Table 3-12. Diet characteristics for assessment of CO_2/calculated RER.

		USA n=14		Kenya n=23	
Variable	**Units**	**Mean**	**SD**	**Mean**	**SD**
Caloric content	Kcal/kg BW	29.04	5.16	23.98	6.96
Carbohydrates	g/Kg BW	3.14	0.71	3.50	0.93
Protein	g/Kg BW	1.25	0.26	0.84	0.28
Fat	g/Kg BW	1.28	0.27	0.77	0.29
Total fiber	g/Kg BW	0.26	0.06	0.33	0.10
Soluble fiber	g/Kg BW	0.08	0.02	0.07	0.02
Insoluble fiber	g/Kg BW	0.17	0.05	0.26	0.08
Starch	g/Kg BW	1.69	0.51	1.93	0.53
Fructose	g/Kg BW	0.19	0.10	0.15	0.11
Galactose	g/Kg BW	0.00	0.01	0.00	0.00
Glucose	g/Kg BW	0.22	0.08	0.13	0.09
Lactose	g/Kg BW	0.09	0.05	0.33	0.24
Maltose	g/Kg BW	0.04	0.01	0.01	0.01
Sucrose	g/Kg BW	0.40	0.28	0.41	0.19
Added Sugars	g/Kg BW	0.83	0.45	1.34	0.17
Glycemic Index		59.46	4.87	130.04	48.43
HEI		48.89	9.10	68.08	9.62

SD = standard deviation
BW = body weight
HEI = healthy eating index

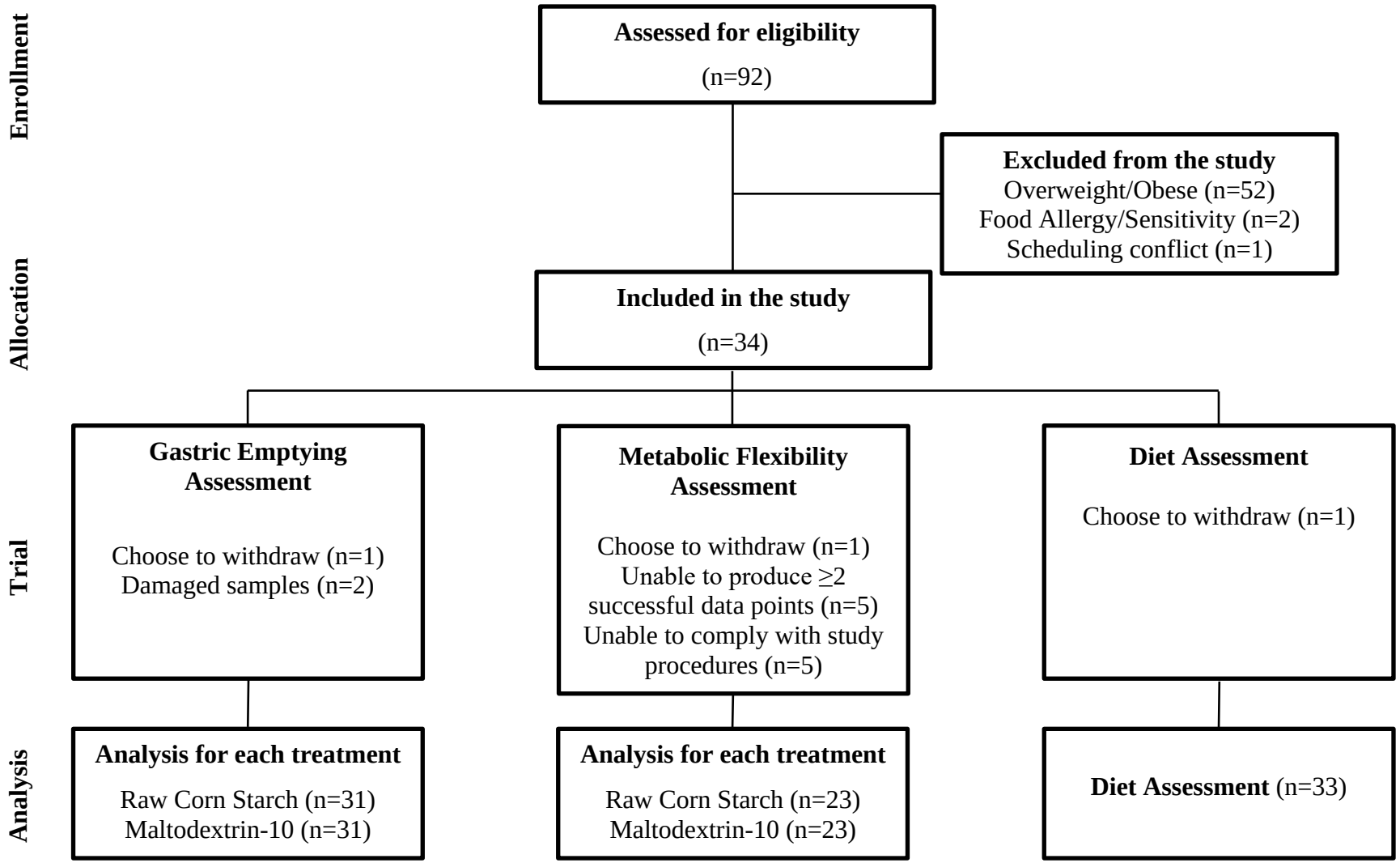

Figure 3-1. Participant recruitment and participation flow diagram - Kenya

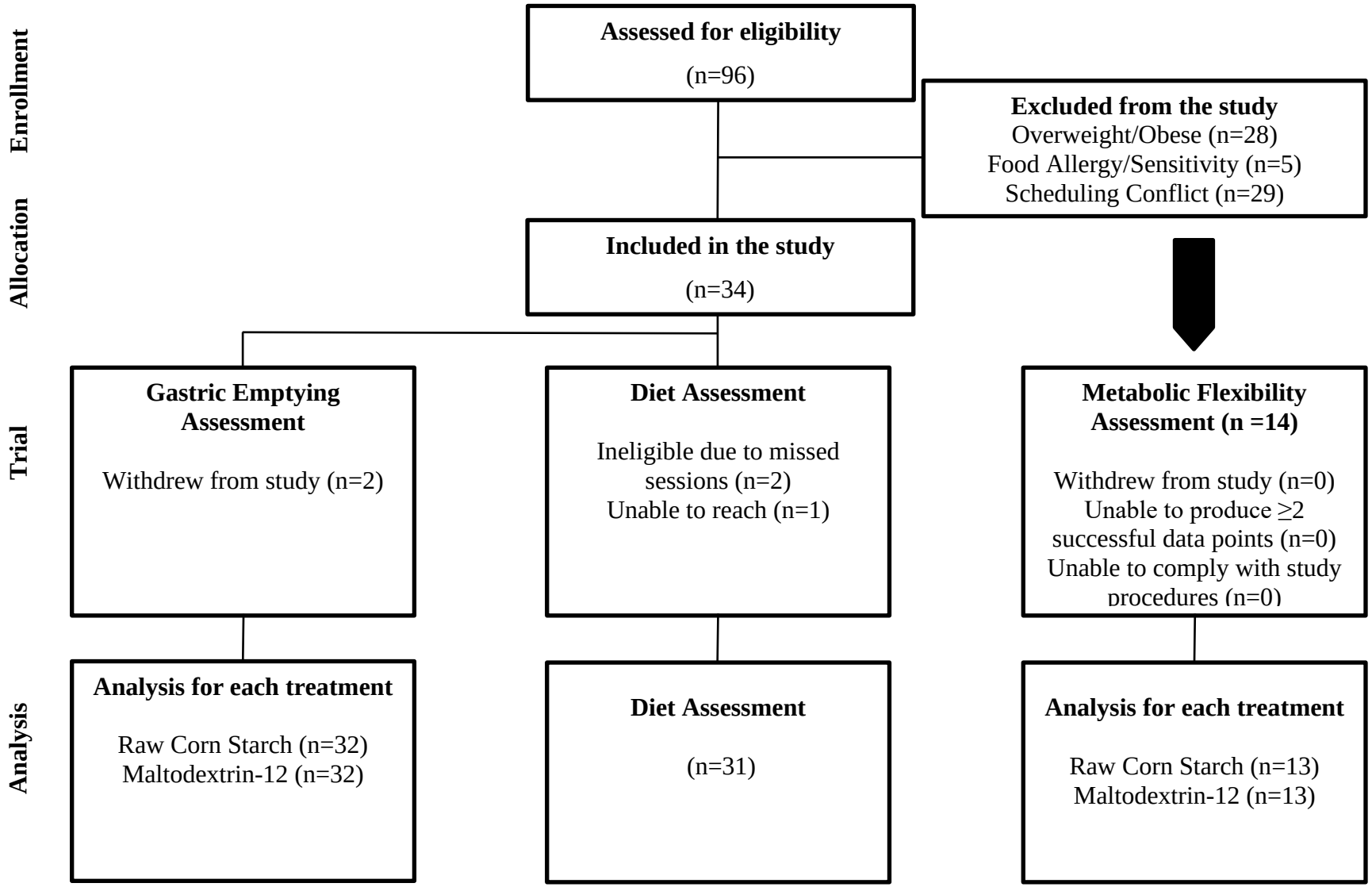

Figure 3-2. Participant recruitment and participation flow diagram - USA.

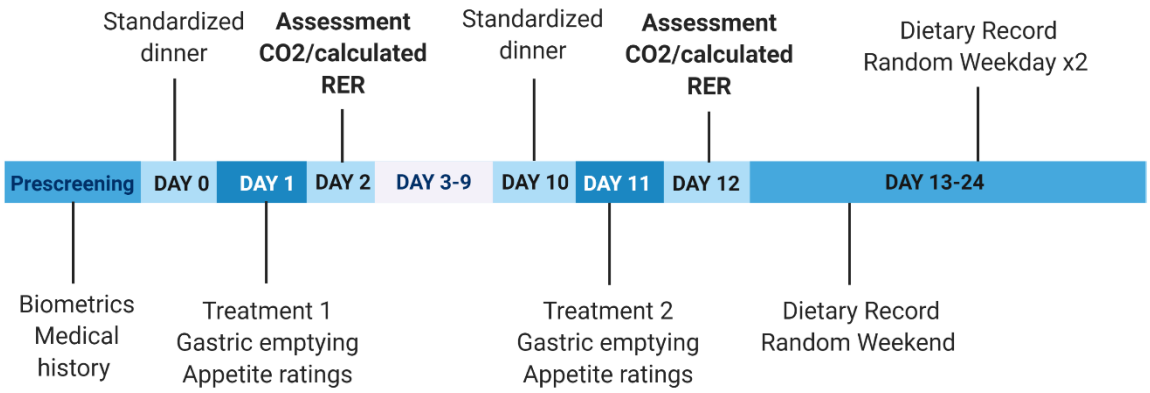

Figure 3-3 Clinical trial events timeline.

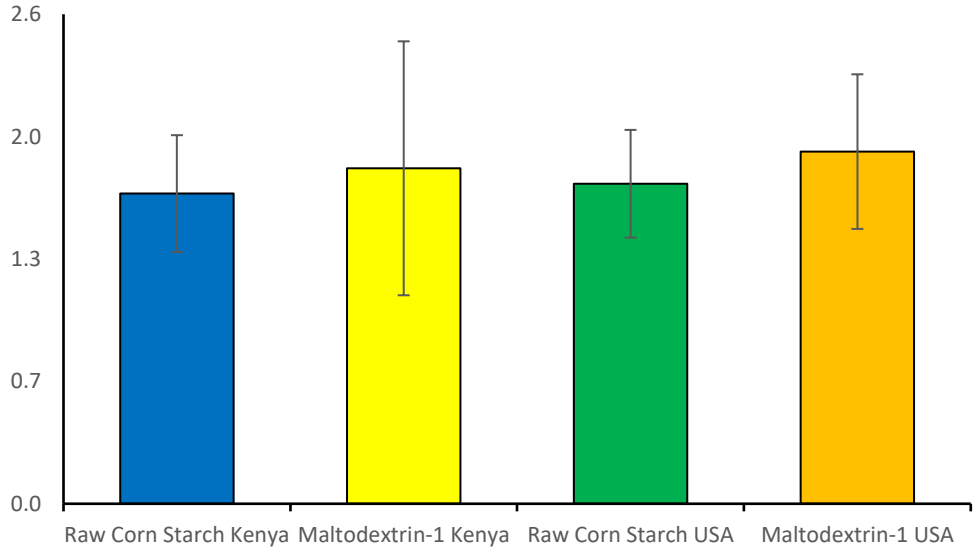

Figure 3-4. Mean half-gastric emptying values for Kenya and USA after consumption of either slowly digestible carbohydrate meal (30 g RCS + 200 g applesauce) or rapidly digestible starch meal (30 g MDX + 200 g applesauce). Raw corn starch – Kenya (Blue), maltodextrin DE-1 – Kenya (Yellow), raw corn starch – USA (Green), maltodextrin DE-1 – USA (Orange).

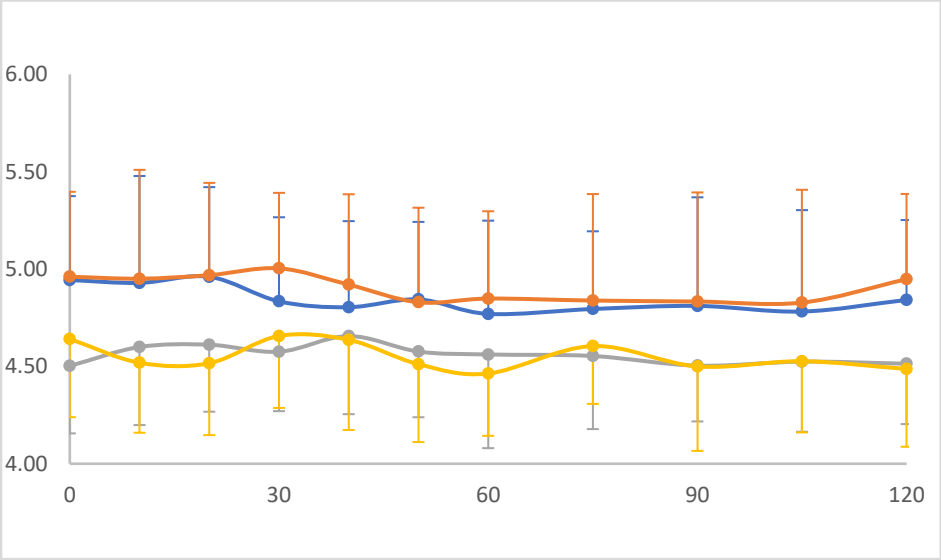

Figure 3-5. Assessment of CO_2 values over a 2-hour period after consumption of either slowly digestible starch meal (30 g RCS + 200 g applesauce) or rapidly digestible starch meal (30 g MDX + 200 g applesauce). Raw corn starch – Kenya (Orange), maltodextrin DE-1 – Kenya (Blue), raw corn starch – USA (Yellow), maltodextrin DE-1 – USA (Grey).

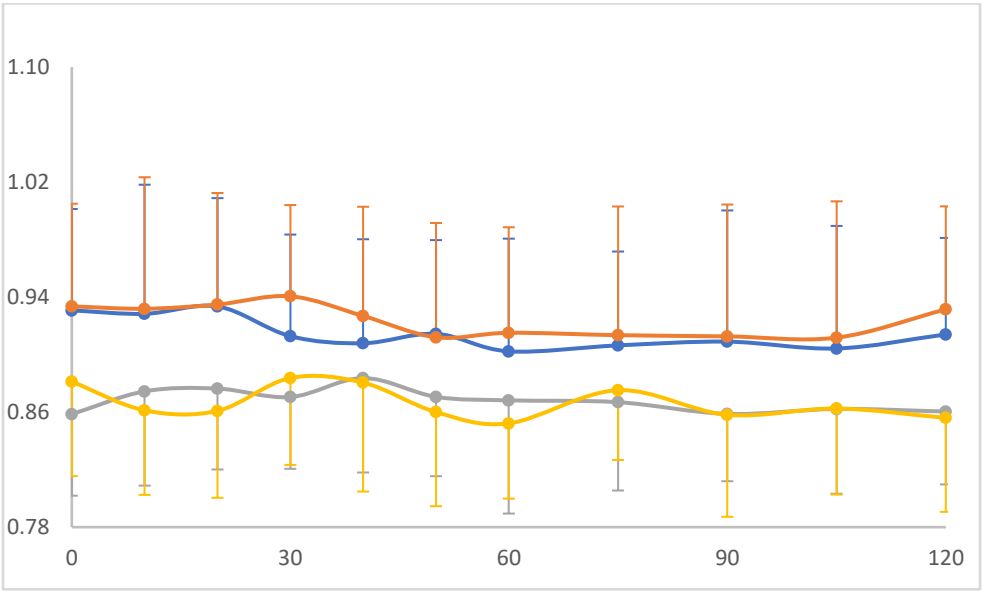

Figure 3-6. Assessment of calculated RER values over a 2-hour period after consumption of either slowly digestible startch meal (30 g RCS + 200 g applesauce) or rapidly digestible starch meal (30 g MDX + 200 g applesauce). Raw corn starch – Kenya (Orange), maltodextrin DE-1 – Kenya (Blue), raw corn starch – USA (Yellow), maltodextrin DE-1 – USA (Grey).

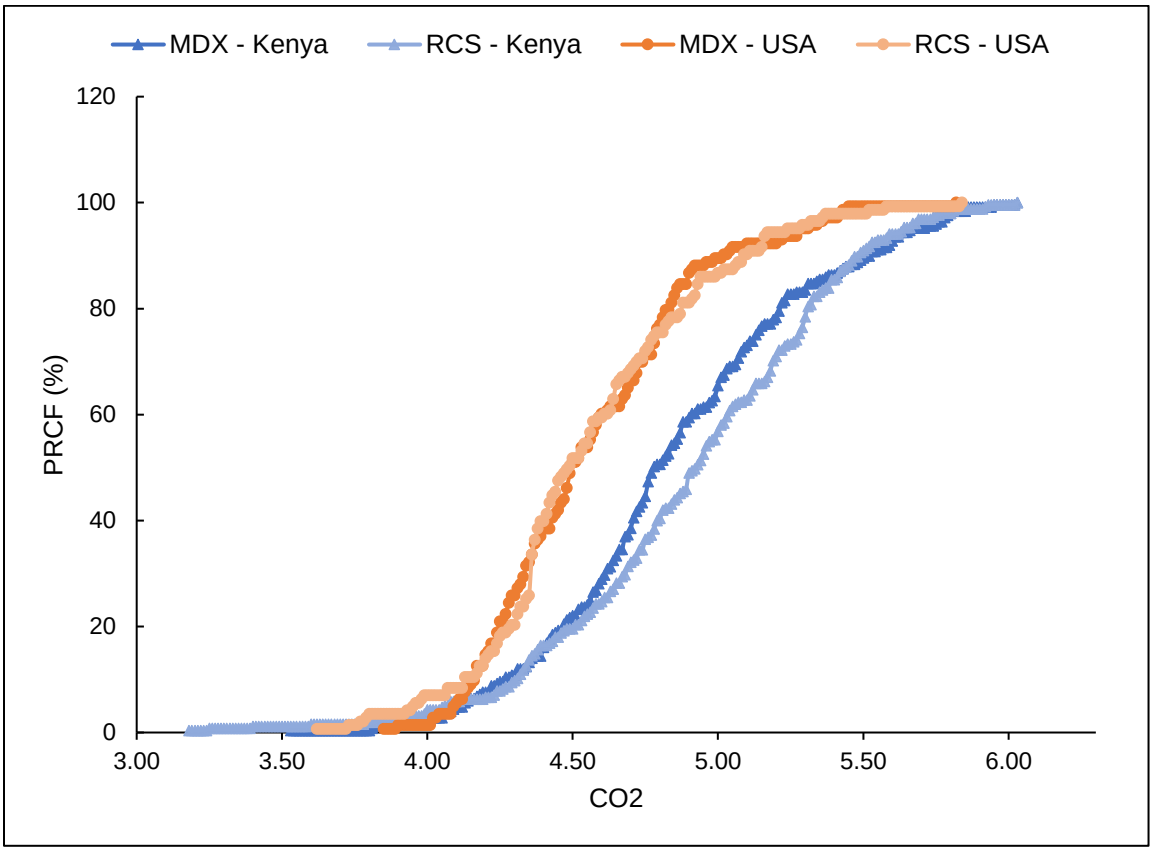

Figure 3-7. Percent relative cumulative frequency (PRCF) of pooled respiratory exchange ratio (RER) values calculated from CO_2 among all participants (USA n=14, Kenya n=23) following consumption of raw corn starch (RCS) or maltodextrin (MDX-1). Because this is pooled data, there are no error bars. MDX-1, maltodextrin; RCS, raw corn starch. USA MDX-1 (orange); USA RCS (pink); Kenya MDX-1 (dark blue); Kenya RCS (light blue).

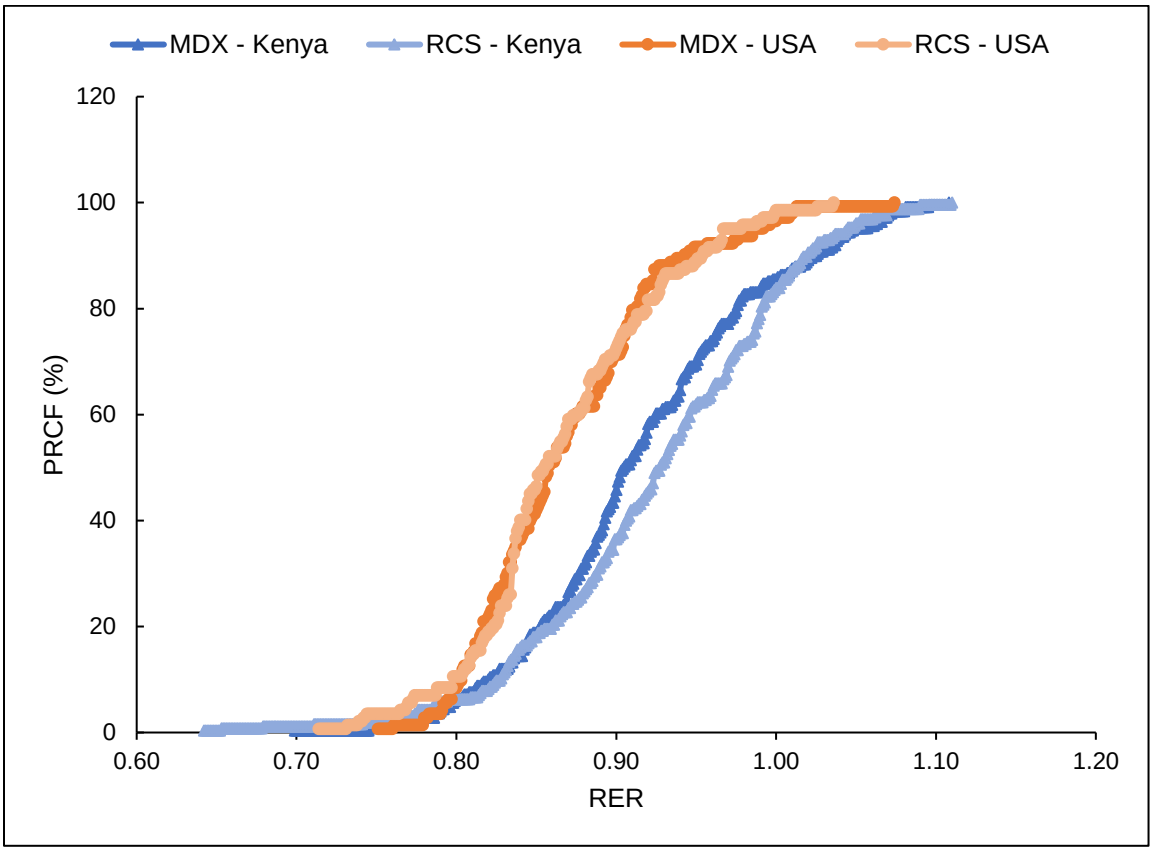

Figure 3-8. Percent relative cumulative frequency (PRCF) of pooled respiratory exchange ratio (RER) values calculated from CO2 among all participants (USA n=14, Kenya n=23) following consumption of raw corn starch (RCS) or maltodextrin (MDX-1). Because this is pooled, there are no error bars. MDX-1, maltodextrin; RCS, raw corn starch. USA MDX-1 (orange); USA RCS (pink); Kenya MDX-1 (dark blue); Kenya RCS (light blue).

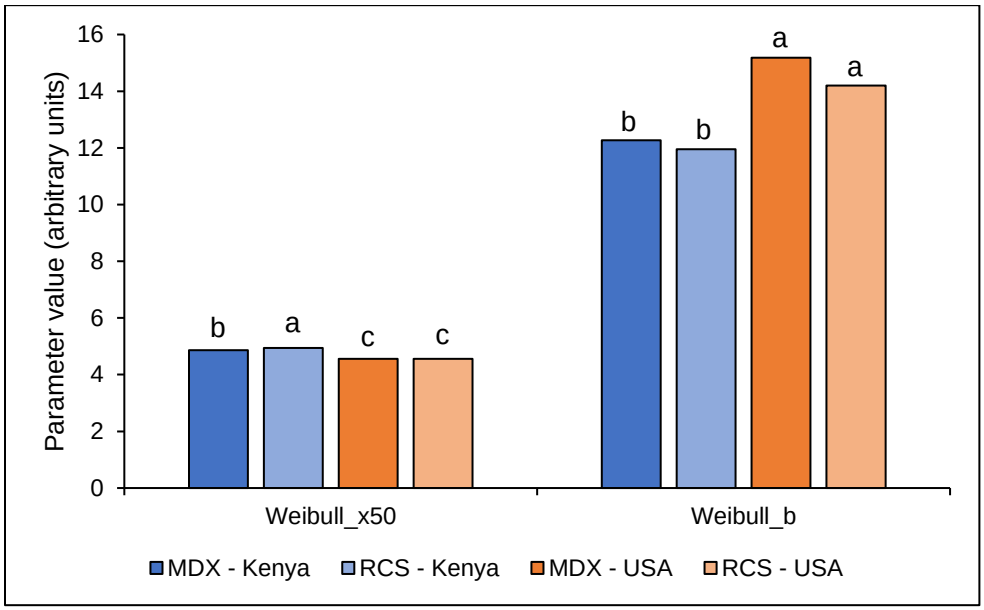

Figure 3-9. Weibull Cumulative Distribution parameter estimates from modeling percent relative cumulative frequency (PRCF) of pooled CO2 values among all participants (USA n=14, Kenya n=23) following consumption of raw corn starch (RCS) or maltodextrin (MDX-1). x_{50} represents the median CO_2 during the postprandial testing period, whereas *b* signifies the distribution breadth constant or slope (dimensionless). Because this data is specifically depicting carbohydrate oxidation, a higher x_{50} may signify a more complete switch to carbohydrate oxidation (vs. fat oxidation), which is a hallmark of superior metabolic flexibility. Furthermore, a lower *b* indicates a broader spread in values, which suggests enhanced metabolic flexibility when specifically examining CO_2 values. Different letters indicate statistically significant differences in parameter estimates per group (no overlap in 95% confidence intervals). Because this is pooled data, there are no error bars. MDX-1, maltodextrin; RCS, raw corn starch. MDX-1, maltodextrin; RCS, raw corn starch. USA MDX-1 (orange); USA RCS (pink); Kenya MDX-1 (dark blue); Kenya RCS (light blue).

CHAPTER 4. SUPPLEMENTATION OF SLOWLY DIGESTIBLE STARCH SLIGHTLY DECREASES HUNGER WHILE IMPROVING METABOLIC FLEXIBILITY IN INDIVIDUALS WITH LOW DIET QUALITY

4.1 Abstract

To gain a better understanding about the effect of continuous consumption of slowly digestible carbohydrates (SDCs) on metabolic and physiological conditioning; this study monitored changes in gastric emptying, hunger/fullness, glycemic profile, and metabolic flexibility after consumption of a purified, single source of SDC for 21 days. To test the hypothesis that SDCs affect such parameters, a crossover design human trial was conducted (n=14). Healthy participants (BMI 23.63±2.63) with poor diet quality (healthy eating index of 50.8±9.5) consumed either a SDC (raw corn starch) or rapidly digestible starch hydrolyzate (maltodextrin DE-10) for 21 days with a washout period of at least two weeks between treatments. Measures were collected at baseline and after intervention. Contrary to our expectations, no differences in gastric half-emptying time, fullness ratings, or glycemic profile were observed before and after supplement treatments. Nevertheless, small but significant differences in hunger ratings were found with the SDC showing less hunger post-treatment. This was accompanied by improvement in metabolic flexibility after 21 days of consumption of the SDC. In summary, the findings from this study provide a valuable starting point for understanding the effect of continuous feeding of SDCs on feelings of hunger and potential improvement of metabolic fuel utilization.

4.2 Introduction

Consumption of slowly digestible carbohydrates (SDCs) can elicit higher satiety feeling and lower glycemic response (A. M. R. Hayes et al., 2020), compared to rapidly digestible carbohydrates, and was shown to slow gastric emptying time through the ileal brake (Chegeni et al., 2022), however in the latter the satiety response was not observed. Results from a previous human study conducted in Mali by our laboratory showed that traditional starchy West African foods made from pearl millet and sorghum delayed rates of gastric emptying compared to Western foods (Cisse et al., 2018). Malian diets are characterized by a high consumption of foods rich in slowly digestible carbohydrates (sorghum and millet) (Diarra et al., 2022) compared to traditional

Western diets high in simple sugars and refined carbohydrates. We speculated that differences in gastric emptying times between Malian and American populations might be diet mediated, where continuous consumption of a minimal dose of SDC might induce physiological changes in the distal small intestine (proliferation of L-cells) that activate the ileal brake and affect energy utilization by the body.

Energy utilization in the body is measured using respiratory exchange ratio (RER) which is obtained by measuring the ratio VCO_2/VO_2 in the breath as a proxy to carbohydrate/lipid oxidation at the cellular level (respiratory quotient, RQ). RER is the current gold standard for the assessment of metabolic fuel utilization. Despite its advantages, RER alone lacks the capacity to assess long term changes in metabolic fuel utilization and models (e.g. Weibull) have been developed to quantify these changes. This is of importance since the ability to switch metabolic fuels at a cellular level contributes to the development of noncommunicable, chronic diseases (Boyle et al., 2017; Galgani et al., 2008; Goodpaster & Sparks, 2017; Tahergorabi et al., 2016). The intrinsic ability of cells to sense, store and utilize different nutrients upon availability and requirement is known as metabolic flexibility (Kelley et al., 1999; Smith et al., 2018) while the inability to switch has been termed metabolic inflexibility. Several studies have directly assessed diet in the context of metabolic flexibility (Begaye et al., 2020; Duivenvoorde et al., 2015; Gribok et al., 2016), and mouse studies have assessed carbohydrate digestibility (Fernández-Calleja et al., 2019; Hayes AMR, 2021; Salto et al., 2020). Consumption of slowly digestible carbohydrates (SDCs) have recently been shown in mice to have beneficial effects on the rate and type of fuel (i.e. carbohydrate vs fat) used for metabolic processes (Hayes AMR, 2021; Salto et al., 2020).

The ileal brake is a feedback mechanism that inhibits gastrointestinal motility, modulates meal transit time in the intestinal tract and decreases subsequent food intake (Maljaars et al., 2008; Spiller et al., 1984). The ileal-brake is mediated by hormones, glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY), released from jejunal and ileal L-cells. This hormonal response to food facilitates bilateral communication between the gastrointestinal tract and the brain (van Citters & Lin, 2006). Human studies have showed that ileal infusion of glucose increased secretion of GLP-1 and PYY while simultaneously decreasing post-meal energy intake, suggesting the activation of the ileal brake (Alleleyn et al., 2016) A recent L-cell culture study conducted by our laboratory compared the secretory ability (GLP-1 secretion) of maltotriose and maltotetrose compared to glucose and propionate, indicating that products of carbohydrate digestion are potent

activators of the ileal brake (El-Hindawy, 2018) This was followed by a study in mice showing that starch digestion directed to the ileum markedly increased plasma GLP-1 and activated the gut-brain axis with reduced food intake and weight gain (Lim et al., 2021) The slower rate of digestion of SDC can provide targeted intestinal delivery of products of carbohydrate digestion that modify the luminal intestinal environment and facilitate the activation of the ileal brake.

Our hypothesis for the present study was that metabolic and physiological changes can be induced by consuming SDC over time. Thus, the first aim of this research was to determine if continual consumption of SDC induces non-responder subjects (i.e. with rapid gastric emptying when administered SDCs) to activate the ileal brake, delaying rate of gastric emptying and moderating postprandial glycemic response while decreasing hunger and increasing fullness. The second aim of this research was to determine whether continual consumption of SDC influences on metabolic fuel utilization using a portable breath CO_2 device to measure respiratory exchange ratio (RER). The findings from this study may provide insights into designing healthy carbohydrate-based foods for weight maintenance by activating the ileal brake and the gut-brain axis, and through altering RER and metabolic flexibility. This study was approved by the institutional review board at Purdue University (IRB protocol # 1706019377) and is registered at clinicaltrials.gov.

4.3 Materials and methods

4.3.1 Participant selection and study design

A nutrition supplementation, crossover trial was conducted at Purdue University (West Lafayette, IN, USA). Eligibility criteria were as follows: males or females aged 18–50 years of age, normal body mass index (BMI, 18.5 $kg/m^2 \leq$ BMI $\leq$ 25 kg/m^2), self-reported stable weight for the past 3 months, and consumption of a typical American diet. Diet assessment was evaluated through collection of dietary data (3 diet recalls) and calculation of healthy eating index (HEI) for potential participants. Only individuals with a HEI $\leq$ 65 were included in the study. Participants were excluded from the study if they presented history of current gastrointestinal diseases/disorders; diabetes (Type I and II); food allergies or sensitivities of any kind; actively taking any medication, active smoker; or if pregnant or nursing. Participant recruitment and study participation are shown in Figure 4-1.

Participants that met the criteria were included in a crossover, supplementation trial consisting of two treatment arms. The treatment was composed of 21 days supplementation of slowly digestible carbohydrate (SDC) and the control arm consisted of 21 days supplementation rapidly digesting carbohydrate (RDC). Treatment order was randomized (random computer generator) with a washout period of at least two weeks between treatments. Figure 4-2 presents an example of the sequence of events for the clinical trial if the participant was randomly assigned to start with treatment SDC (raw corn starch). The order of events would be inverted if participant started with control RDC (maltodextrin DE-10). For this study, participants were assessed for blood glucose, gastric half-emptying time, hunger/fullness, CO_2/calculated RER, and metabolic flexibility.

For the assessment of postprandial blood glucose, gastric half-emptying time, and hunger/fullness; participants were assessed at baseline and after 21 days of continuous consumption of the assigned carbohydrate. The assessments were performed using a slowly digestible starch meal which is described in the section below.

For the assessment of CO_2/calculated RER; participants were only assessed after completion of 21 days of continuous consumption of the assigned carbohydrate. Participants were evaluated a total of three times. After 21 days of consumption of the SDC, participants were evaluated once using the slowly digestible starch meal (description below). On the other hand, participants were evaluated twice after 21 days of consumption the RDC; the first time using the slowly digestible starch meal and the second time using the RDC meal (description for both meals below). Data obtained from CO_2/calculated RER assessments was used to calculate metabolic flexibility.

4.3.2 Materials for supplementation study and test meals

The slowly digestible carbohydrate (SDC) used was raw corn starch (Argo, Memphis, TN, USA) and the rapidly digestible carbohydrate (RDC) was maltodextrin DE-10 (Cargill, Minneapolis, MN, USA).

For the 21-day supplementation, each participant consumed a daily carbohydrate dose of 30 g, either SDC or RDC. The assigned carbohydrate was mixed in ~115 g of unsweetened applesauce (Musselman's, Peach Glen, PA, USA). A 21-day supply of unsweetened applesauce (individual 4 oz containers) and individually prepacked (30 g) SDC or RSC were provided.

Participants were asked to consume the supplement at ~10 am every day. Compliance was assessed by randomly requesting participants to submit an image of the empty containers after consumption.

All the slowly digestible starch meals were composed of 30 g of raw corn starch, 200 g unsweetened applesauce, and 0.2 g of xanthan gum (Bob's Red Mill, Milwaukie, OR, USA) to equalize viscosity. Ingredients were mixed and homogenized using a hand blender immediately before consumption each time. Test meals were served at ambient temperature. Each participant was required to drink 100 mL with the test meal. The RDC meal was composed of 30 g of maltodextrin DE-1, 200 g of applesauce, and 0.2 g of xanthan.

4.3.3 Procedures

Measurements for gastric emptying time, hunger/fullness, and postprandial blood glucose were done on the first testing day. Measurements for CO_2/calculated RER were performed the second day.

Performed before experimental data collection began

Participants were instructed on the protocols and procedures used for the study before data collection began. For the assessment of RER, participants were assigned an individual research account and instructed on Lumen® device usage (Metaflow, Tel Aviv, Israel). Lumen® is a miniaturized, portable, device that indirectly measures metabolic fuel utilization via a CO_2 and flow sensor from breath samples. The CO_2 data collected from Lumen® was used to calculate RER (Lorenz et al., 2021). The device was paired and synchronized to the participant's smartphone together with the Lumen® app. Participants were taught and practiced the Lumen® breathing technique while supervised and were only allowed to participate in the study after they showed proficiency with the device and app. Participants were instructed to practice the breathing maneuver at least 10 times, and proficiency was determined by ≥ 2 successful data points (valid measures provided by the app).

Day before testing day (gastric half-emptying time, hunger/fullness, and postprandial blood glucose)

The morning before the testing day, participants arrived at the Department of Food Science between 8:00-8:30 a.m. A commercially available continuous glucose monitor (CGM) with a wireless receiver (FreeStyle Libre, Abbott Nutrition, OH, USA) was placed to monitor participant's blood glucose levels during the testing sessions. The CGM patch was be placed on the tricep of each subject while he/she was sitting. Abbott's FreeStyle Libre CGM sensors are self-calibrating and required no further adjustments. Continuous glucose readings were collected every 15 min by an accompanying wireless receiver that was carried by participants at all times. To prevent carryover effect from previous meals, the night before testing day participants consumed a standardized dinner (~700 Kcal) between 7-8 pm and were asked to fast until the next morning. The use of the CGM was overseen by Dr. Travis Dam (IU Health, Lafayette, IN, USA).

Testing Day (assessment of gastric emptying time, hunger/fullness, and postprandial blood glucose)

Participants arrived at the testing facility between 7:45-8:15 am and upon corroboration of overnight fast (>10 h) each participant was assigned to a pre-set testing station. Subsequently, baseline breath samples for assessment of gastric emptying (two - 1.5 L bags, Cambridge Isotope Laboratories, Tewksbury, MA, USA) and baseline appetitive/satiety sensation scores (Visual Analog Scale, VAS) were collected for each participant. After completion of baseline assessments, participants were provided one test meal (30 g of raw corn starch, 200 g unsweetened applesauce, and 0.2 g of xanthan gum) containing 57 mg of ^{13}C sodium acetate as a tracer and 100 mL of water. Participants were instructed to finish the test meal within 15 min. No other food or drink was allowed for the remainder of the test session. Following completion of the test meal, breath samples for assessment of gastric emptying time (0.30 L bags, Cambridge Isotope Laboratories, Tewksbury, MA, USA) were collected every 15 min for hours 1 to 2, and every 30 min for hours 3 to 4. During the same time (4 h post meal), participants completed appetitive sensation surveys (VAS) every 30 min for the assessment of hunger/fullness sensations. After completion of all breath collections and hunger/fullness sensation assessments and removal of the CGM (> 4 h postprandial glucose), participants were allowed to leave the facility.

Testing Day (assessment of CO_2/RER)

The night before the CO_2/RER test day; participants were instructed to have a light dinner between 7 and 8 pm and subsequently fast until the next morning. No standardized dinner was provided. On the CO_2/RER test day, participants arrived at the testing facility between 7:45-8:15 am. Subsequently, baseline breath samples for assessment of CO_2/RER were performed by each participant using the Lumen® device. Upon successful completion of baseline sampling, participants were provided the test meal (without the ^{13}C tracer) and water, participants were instructed to finish their meal within 15 min and no food or drink was allowed for the remainder of the session. Following completion of the meal, breath samples for assessment of CO_2/RER were collected every 10 min for hour 1, and every 15 min for hour 2. After completion of all breath collections participants were allowed to leave the facility.

According to our protocol, after competition of control (21 days of consumption of RDC), participants were also evaluated using an RDC meal, which was composed of 30 g of maltodextrin DE-1, 200 g of applesauce, and 0.2 g of xanthan.

Analysis of gastric half-emptying time

Assessment of gastric half-emptying time using ^{13}C breath test has been previously performed by our laboratory for populations in the USA and Africa (Chegeni et al., 2022; Cisse et al., 2017; A. M. R. Hayes et al., 2021; Pletsch & Hamaker, 2018); the methodology has been refined from a previous method (Sanaka & Nakada, 2010) with modifications accounting for differences in body surface area (Haycock et al., 1978) as well as considerations included due to the water soluble nature of the test meal (Gonzalez et al., 1998).

Analysis of breath samples collected for assessment of gastric half-emptying time was performed within 24 h after collection using a $^{13}CO2$ breath analyzer (POCone, Otsuka Electronics Co., Ltd., Osaka, Japan). Parameters for determining percent dose recovery and cumulative percent dose recovery of ^{13}C are shown in Appendix C. Only individuals with adequate data model fit (R2 > 0.80) for percent dose recovery of the ^{13}C sodium acetate tracer were included. The primary outcome calculated from these parameters was gastric half-emptying time, defined as the time required for half of the 13C dose to be metabolized (Perri et al., 2005). Lag phase, defined as the time required for the $^{13}CO2$ excretion rate to attain its maximal level (indicator of food to break

down within the stomach) and gastric emptying coefficient values (overall indicator of gastric emptying) were calculated as well.

Analysis of glycemic response

Postprandial glycemia was the primary outcome measured by the CGM (FreeStyle Libre, Abbott Nutrition, OH, USA) to represent glycemic response (Hall et al., 2019a; Li et al., 2019). Glucose values during the postprandial period (4 h) were corrected by subtracting each participant's baseline glucose from the subsequently taken values (after meal ingestion) to obtain change in glucose. Corrected glucose values are presented as area under the curve ($AUC_{corrected}$, mg×min/dL, including values above and below baseline). Glycemic response characteristics of peak glucose values (mg/dL) and time of peak glucose value (min) were also calculated.

CO_2, calculated respiratory quotient and metabolic flexibility

Respiratory exchange ratio (RER) is obtained by measuring the ratio VCO_2/VO_2 in the breath as a proxy to carbohydrate/lipid oxidation at the cellular level (Respiratory Quotient, RQ). For this study, participants used a non-invasive, individual breath device; Lumen®, which measures only CO_2 in breath; with higher amounts of CO_2 exhaled indicating carbohydrate as the main metabolic fuel source (RER =1) and lower amounts of CO_2 indicating fat as the preferred metabolic fuel source (RER = 0.7). Lumen® has been validated against indirect calorimetry and is a reliable measurement tool to assess RER (Lorenz et al., 2021).

Metabolic flexibility was assessed using either pooled CO_2 or pooled calculated RER data for all participants followed by modeling using the Weibull Cumulative Distribution function. To accomplish this, percent relative cumulative frequency (PRCF) was first calculated per treatment and location according to the method of Riachi et al. (2004). The procedure included the following steps: (1) data was sorted in ascending order, (2) an interval of increment was selected (0.01 for CO_2 data, 0.001 for RER data), (3) the frequency of observations per interval was calculated, (4) the cumulative frequency was calculated, and (5) the cumulative frequency was expressed as a percentile curve. Following calculation of PRCF, plots of CO_2 and calculated RER (ascending order) vs. PRCF were fit to the Weibull Cumulative Distribution function (Eq. 1).

$$y = 1 - \exp\left(-\left[\frac{x}{x_{50}}\right]^{b} ln(2)\right)$$

Where:

y = percent relative cumulative frequency (PRCF; 0 to 100%);

x_{50} = median CO_2 or respiratory exchange ratio (median CO_2 or RER);

b = distribution breadth constant (dimensionless), indicative of slope.

Modeling was done using the "fitnlm" function with the nonlinear least squares method option in MATLAB (R2020a, Update 5, 9.8.0.1451342, The MathWorks, Inc., Natick, MA). To ensure that x_{50} fell within the range of RER values for each dataset, bounds were placed on this parameter accordingly. Furthermore, an iterative modeling approach was used to obtain the best fit for each parameter, such that 5 initial "best guess" fits were incorporated into the modeling approach. Modeling was performed on pooled data because only 10 data points were collected for each participant per test day, and more than 20 data points are required in order to employ the Weibull Cumulative Distribution function (Razali & Al-Wakeel, 2013; Rinne, 2009).

Appetitive/satiety sensations

Visual analog scales (VAS) were used to assess hunger and fullness (linear scale in mm) before test meal consumption (baseline) and every 30 min in the 4 h postprandial period using an online questionnaire (Qualtrics, Provo, Utah, USA) (Appendix D). Participants were provided with an email link to answer the questionnaire (i.e. using a personal laptop or cell phone) for all arms of the study.

4.3.4 Statistical Analysis

Statistical power analysis was performed using pilot data obtained from prior gastric half-emptying studies (P = 0.05, 0.75 h minimum detectable difference in means, 1-β = 0.8, s_{pooled} = 0.5 h, n = 16). Statistical analysis was conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA). Two-way ANOVA (PROC MIXED) with treatment (raw corn starch vs maltodextrin), time of assessment (baseline vs 21 days after consumption) as fixed effect, and participant as a random effect was used to determine statistical significance of differences in glycemic response

characteristics and gastric emptying parameters (∫0-120 min and ∫120-240 min, time of peak, peak glucose value, gastric half-emptying time, gastric lag phase, gastric emptying coefficient).

Two-way ANOVA with repeated measures (PROC MIXED) with treatment (raw corn starch vs maltodextrin), time of assessment (baseline vs 21 days after consumption) as fixed effects was conducted for appetitive sensations (hunger and fullness) and CO_2/calculated RER. Baseline values (for each outcome per its respective test day) were added as a covariate in the model (Blundell et al., 2010). Homoscedasticity and normality of residuals was assessed using histograms and quantile-quantile plots. All data was normally distributed and did not require transformation. Significance was considered at $p<0.05$, and Tukey's post hoc test for multiple comparisons was conducted when the overall model was significant ($p<0.05$ for F value).

For the Weibull modeling of pooled PRCF analyses representing metabolic flexibility, data from all participants was pooled together per group (treatment and location), and therefore there were no replicates. To statistically compare this data, 95% confidence intervals were calculated for the Weibull parameters (i.e. x_{50}, b), in accordance with previous research. Statistically significant differences ($p<0.05$) were indicated if the confidence intervals for these parameter estimates did not overlap.

4.4 Results

4.4.1 Participant characteristics

Sixteen individuals (11 women, 5 men) participated initially in the study and two dropped out. Participant characteristics are shown in Table 4-1. One female participant no longer wished to participate in the study after the first, baseline session and thus was withdrawn and her data was not included in the analyses. One male participant repeatedly missed the scheduled baseline session and thus was withdrawn; no data was collected for this participant.

4.4.2 Gastric half-emptying time

A total of the 56 ^{13}C sodium acetate breath evaluations (from baseline to 240 min) were collected for assessment of gastric half-emptying time. All tests fitted the established modeling approach adequately ($R^2>0.90$). There were three gastric half-emptying times that were >30 min compared to the mean for the respective treatment (Appendix C). Previous studies conducted by

our laboratory indicate that a subset of subjects displayed a slower gastric emptying response to consumption of slowly digestible carbohydrates (Chegeni et al., 2022; Cisse et al., 2017, 2018). We termed these subjects as responders. Two subjects (subject 2 and 13) were responders to the RDC (maltodextrin DE-10) and only one of them (subject 13) was responder to the SDC (raw corn starch). All these observations were found during baseline assessment; no responders were found after 21 days of continuous supplementation for either carbohydrate. Overall, there were no significant differences before and after treatment for gastric half-emptying time, lag phase, or gastric emptying coefficient (Table 4-2). Similarly, there were no significant differences between treatments for the above-mentioned parameters.

4.4.3 Glycemic response

Initial exploratory analysis of glycemic response was performed using total area under the curve (AUC_{total}). Values were integrated from 0 to 120 min to demonstrate acute glycemic changes in response to the test meal. Additionally, integration of all glycemic response values was performed from 0 to 240 min to show overall response to the test meal. Figure 4-3 indicates AUC_{total} (∫0-120 min) at baseline and 21 days after consumption of either maltodextrin DE-10 or raw corn starch. Figure 4-4 indicates AUC_{total} (∫0-240 min) at baseline and 21 days after consumption of either raw corn starch or maltodextrin DE-10.

The large values in AUC_{total} potentially masked small differences in postprandial blood glucose. A correction was performed by subtracting each participant's fasting glucose from the subsequent postprandial values to obtain change in glucose (ΔGlucose). Values were expressed as glucose incremental area under the curve ($AUC_{corrected}$), including values below baseline. Figure 4-5 indicates $AUC_{corrected}$ (∫0-120 min) at baseline and 21 days after consumption of either raw corn starch or maltodextrin DE-10. Figure 4-6 indicates $AUC_{corrected}$ (∫0-240 min) at baseline and 21 days after consumption of either raw corn starch or maltodextrin DE-10. There were no differences in glycemic responses (neither $AUC_{corrected}$ ∫0-120 min or $AUC_{corrected}$ ∫0-240 min) before and after 21 days of supplementation for either of the carbohydrate sources (p 0.05). Similarly, no differences were found between treatment (21 days consumption SDC) and control (21 days consumption RDC).

A subsequent detailed analysis of corrected values was performed for integrated values (∫0-240 min) above baseline ($AUC_{positive}$) and below baseline ($AUC_{negative}$) (Table 4-3). There were

no differences in glycemic profile for neither integrated glycemic values above ($AUC_{positive}$) or below baseline ($AUC_{negative}$). Finally, glycemic response characteristics of peak glucose (mg/dL) and time of peak glucose value (min) are shown in Table 4-4.

4.4.4 Appetite/satiety ratings

Results for the repeated measures ANOVA demonstrated that hunger was significantly influenced by treatment (raw corn starch vs maltodextrin), time of assessment (baseline vs 21 days after consumption), and timepoint ($p<0.0001$; Figure 4-9), but not for their interaction ($p=0.76$). Additionally, there were significant differences between mean values of hunger in the postprandial period for different test meals (before and after test meal consumption). After 21 days of continuous consumption of raw corn starch participants showed the lowest hunger ratings ($p<0.0001$). Fullness was significantly influenced by both time of assessment (baseline vs 21 days after consumption) ($p<0.03$), and timepoint ($p<0.0001$; Figure 4-10), but not their interaction ($p=0.88$). Treatment (raw corn starch vs maltodextrin) was not significant ($p=0.22$) indicating that participants did not feel fuller for either treatment.

4.4.5 CO_2/calculated RER

Respiratory exchange ratios were calculated from CO_2 using a conversion equation (Lorenz et al., 2021). CO_2 and calculated RER values did not significantly differ before and after 21-days of supplementation for either carbohydrate. There were no differences between the standard SDC meal and control RDC meal (different meals were assessed only after 21 days of supplementation of FDC). Results for the repeated measures ANOVA with treatment and time of assessment as fixed effects, did not show any differences between time of assessment, treatment or their interaction. CO_2 response is shown is Figure 4-7. Calculated RER response is shown in Figure 4-8.

4.4.6 Metabolic flexibility

PRCF curves for CO_2 and calculated RER are shown in Figures 4-11 and 4-12, respectively. The slope and spread of the curves indicate the effect of supplementation of SDC vs RDC in the diet. Given that this data is specifically representing carbohydrate oxidation, a broader spread and

less steep curve is reasoned to be indicative of enhanced metabolic flexibility. It can be observed that after supplementation of raw corn starch for 21 days (assessed with the slowly digestible starch meal) the dispersion of values is wider compared to the other two curves, indicating a wider range of values for CO_2 and calculated RER, thus greater fluctuations (which is desirable) in the previously mentioned values. Regarding the slope, a steep slope is indicative of a greater concentration of values in a narrow range; after supplementation of raw corn starch for 21 days (assessed with slowly digestible starch meal) presents the smallest slope indicating that values are not concentrated in a narrow range.

Values for the subsequent analysis of Weibull modeling (performed only on CO_2 curves) is shown in Figure 4-13. The Weibull provides a mathematical quantification of the values for the above-mentioned curves; x_{50} represents the median CO_2 during the postprandial testing period, whereas b signifies the distribution breadth constant or slope (dimensionless). In an acute assessment; x_{50} will depend on the substrate consumed. For instance, if a subject consumes a fat rich meal, we would expect x_{50} values ≈ 0.7 (indicative of fat oxidation); while if subject the subject consumes a simple carbohydrate rich meal (sucrose), we would expect x_{50} values ≈ 1 (indicative of carbohydrate oxidation). With a SDC, a lower x_{50} is desirable since it indicates that SDC is slowly releasing glucose postprandially, favoring fat oxidation in the body. In this study, after supplementation of raw corn starch for 21 days (assessed with slowly digestible starch meal) participants displayed marginally higher however statistically significant x_{50}. For b (slope), after supplementation of raw corn starch for 21 days (assessed with slowly digestible starch meal) participants displayed the lowest values (less steep slope) as indicated in the paragraph above.

4.5 Discussion and conclusion

The first aim was to assess the effect of continuous SDC supplementation on gastric half-emptying time and hunger/fullness ratings via proposed ileal brake activation due to L-cell proliferation. Contrary to our expectations, no differences in gastric half-emptying time or fullness ratings were observed. Nevertheless, small but significant differences in hunger ratings were found. Our results contrast from those by (Chegeni et al., (2022), who showed moderate differences in gastric half emptying time after consumption of SDC (either raw corn starch or isomaltooligosaccharides) but no effects on hunger/fullness rating scores. A sub-division of participants into two groups (responder vs non-responder) showed a larger effect on gastric half-

emptying time. In our study, only one participant (subject 13) was responder to raw corn starch at baseline assessment. None of the participants were responders after 21 days of supplementation for either raw corn starch or maltodextrin DE-10. Participants were preselected based on diet quality (HEI 50.8±9.5) and it was hypothesized that none would be a responder to the SDC (raw corn starch) treatment at baseline (i.e. due to lack of SDCs in the diet), nevertheless we expected to see a slow gastric emptying response after the intervention. It is possible that the lack of L-cell activation might be related to SDC dosage; while we provided an SDC amount previously documented to activate the ileal-break (Chegeni et al., 2022), continuous consumption might have unintended consequences related to enhanced digestion. In this regard, it is well documented that specific diet patterns across species cause genetic and physiological adaptations that maximize efficiency of digestion (Axelsson et al., 2013; Guilloteau et al., 2009; Janiak, 2016). Also, in our study, the dose of SDC might have not been enough to promote physiological changes or induce activation of the ileal brake after 21 days of continuous consumption.

The lack of difference between baseline and assessment of gastric half-emptying time after 21 days of continuous supplementation of SDC indicates that the ileal brake activation did not occur. This response suggests either efficient digestion of the SDC by the study group or the lack of L-cell proliferation in the distal small intestine. *In vitro* studies conducted by our laboratory indicate that products of starch digestion promote secretion of GLP-1 by L-cells and promote regulation of gene expression related to GLP-1 secretion (El-Hindawy et al., 2019). To date few studies have assessed L-cell proliferation related to dietary factors. Gillespie and collaborators demonstrated increased numbers of L-cells after exposure to lactoglobulin *in vitro* (Gillespie et al., 2015). An earlier assessment of L-cell proliferation indicated that continuous consumption of a fermentable carbohydrate (FOS) increased enteroendocrine cells in the colon via luminal SCFA stimulus of FFA2 receptor activation (Kaji et al., 2010). Similar incremental effects on L-cells have been found for activation of FFA3 via GLP-2 release, however in this study evaluated a chemoprotective effect (prevented loss of L-cells) of FFA3 activation under NSAID-induced enteropathy (Said et al., 2017). Further *in vitro* and human studies would be necessary to understand better the products of carbohydrate digestion on L-cell activation in the distal small intestine, as well as the ability of different food matrixes to provide locational digestion and expose L-cells to products of carbohydrate digestion.

The second aim of our clinical trial was to determine the effect of continual consumption of SDC on metabolic fuel utilization through monitoring the RER and metabolic flexibility. Significant differences in metabolic flexibility were found (Weibull b and x_{50}), though no differences were found in RER (CO_2 and calculated RER curves). PRCF curves of CO_2 values indicate a fairly broad spread and less steep slope was observed following 21 days of the SDC (raw corn starch) supplementation period, which are thought to be characteristic of increased metabolic flexibility, compared to the maltodextrin RDC control (Figure 4-11). PRCF curves for the RER values calculated from CO_2 are shown in Figure 4-12. Because all data points except the baseline measurements were obtained during the postprandial period (as opposed to equally obtained during both the preprandial and postprandial periods), the present data more accurately represent carbohydrate oxidation (and not fat oxidation). Accordingly, we performed Weibull Cumulative Distribution modeling solely on the CO_2 PRCF curves, and not on the calculated RER PRCF curves.

After 21 days of supplementation with maltrodextrin DE-10, modeling showed no differences for the x_{50} or b parameter when assessed using either the slowly digestible starch meal (SDC) or the rapidly digesting (RDC) test meal (Figure 4-13). However, following the supplementation period with the SDC (raw corn starch), x_{50} increased (assessed with slowly digestible starch meal) compared to the RDC (maltodextrin DE-10) [for both the slowly digestible starch meal (SDC) and the rapidly digesting (RDC) test meal], which indicates a more complete 'switch' to carbohydrate oxidation. Furthermore, b decreased with raw corn starch supplementation compared to maltodextrin DE-10 supplementation (assessed with the RDC test meal). This indicates a broader spread in CO_2 values was observed for the treatment involving the highest exposure to raw corn starch. A broader range in CO_2 values suggests that more fat oxidation was taking place, notably at the lower CO_2 values, and supports the view that raw corn starch supplementation improves metabolic flexibility. Though not differing from the other treatments, the intermediate b value observed after 21 days of maltodextrin DE-10 supplementation [assessed with rapidly digesting (RDC) test meal] may indicate that greater exposure to RCS is needed in order to elicit an effect on metabolic flexibility. In summary, this data suggests that increased exposure to SDCs, specifically related to continuous supplementation for 3 weeks, improves metabolic flexibility.

In summary, the findings from this study may provide valuable insights into designing nutrition therapies based on SDC carbohydrate-based foods with the ability to improve metabolic flexibility.

4.6 Acknowledgment

Thanks to Hannah Von Weder for assisting with sample analysis, and to Alexandra Cowan and David Arthur for their support with calculation of Healthy Eating Index, data analysis and modeling. This work was made possible by the support of the American People provided to the Feed the Future Innovation Lab for Food Processing and Post-harvest Handling Innovation through the United States Agency for International Development (USAID) under Cooperative Agreement No. AID-OAA-A-13-00047 and the support provided through the Indiana Clinical and Translational Sciences Institute (CTSI), global grant awards. The contents are the sole responsibility of the authors and do not necessarily reflect the views of CTSI, USAID or the United States Government. We are also grateful to Dr. Travis Dam (IU Health) for his oversight using CGMs, to Merav Mor and Shlomi Yeshurun (Metaflow) for facilitating the use of Lumen®; and to Prof Dennis Savaiano (Purdue University) and Regan Bailey (Texas A&M), for facilitating the tools for dietary assessment.

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Table 4-1. Participant baseline characteristics (n=16).

Characteristic	**n=(16)**
Age (years)	25.4±6.8
Height (cm)	166.6±9.4
Weight (kg)	65.8±10.4
BMI (kg/m^2)	23.6±2.6
Sex	5 M, 11 F

± values represent standard deviations
M, male; F, female

Table 4-2. Mean half-gastric emptying, lag phase, and gastric emptying coefficient values (n=14) after consumption of slowly digestible starch test meal (30 g SDC + 200 g applesauce).

	Raw Corn Starch		Maltodextrin-1	
Parameter	**Baseline**	**Post**	**Baseline**	**Post**
Half-gastric emptying (hr)	3.1±0.5	3.1±0.3	3.0±0.6	3.1±0.3
Lag phase (hr)	1.5±0.4	1.6±0.2	1.6±0.3	1.7±0.2
Gastric Coefficient	2.7±0.2	2.6±0.2	2.7±0.3	2.7±0.2

± values represent standard deviations

No statistically significant differences were found for half gastric emptying time, lag phase, or gastric coefficient.

Table 4-3. Mean positive (above baseline) and negative (below baseline) postprandial glucose response (n=14) after consumption of slowly digestible starch meal (30 g SDC + 200 g applesauce).

		AUC positive §	**AUC negative §**
Maltodextrin DE-10	Baseline	2709.8±1718.7	-502.8±607.6
	Post	2253.3±1297.0	-547.0±646.6
Raw Corn Starch	Baseline	2948.7±1798.3	-369.9±613.7
	Post	1994.3±945.1	-376.3±495.4

§ Values for $AUC_{positive}$ (∫0-240 min) and $AUC_{negative}$ (∫0-240 min)
± values represent standard deviations
No statistically significant differences were found between baseline and 21 days after supplementation (post).
No statistically significant differences were found between treatments.

Table 4-4. Glycemic response characteristics (n=14) after consumption of slowly digestible starch meal (30 g SDC + 200 g applesauce).

		Peak glucose (mg/dL)	Time of peak glucose (min)
Maltodextrin DE-10	Baseline	124.9±32.3	46.3±8.6
	Post	115.1±19.7	45.3±11.2
Raw Corn Starch	Baseline	117.8±16.6	45.9±10.0
	Post	112.9±13.5	42.1±9.3

± values represent standard deviations

No statistically significant differences were found between baseline and 21 days after supplementation (post).

No statistically significant differences were found between treatments.

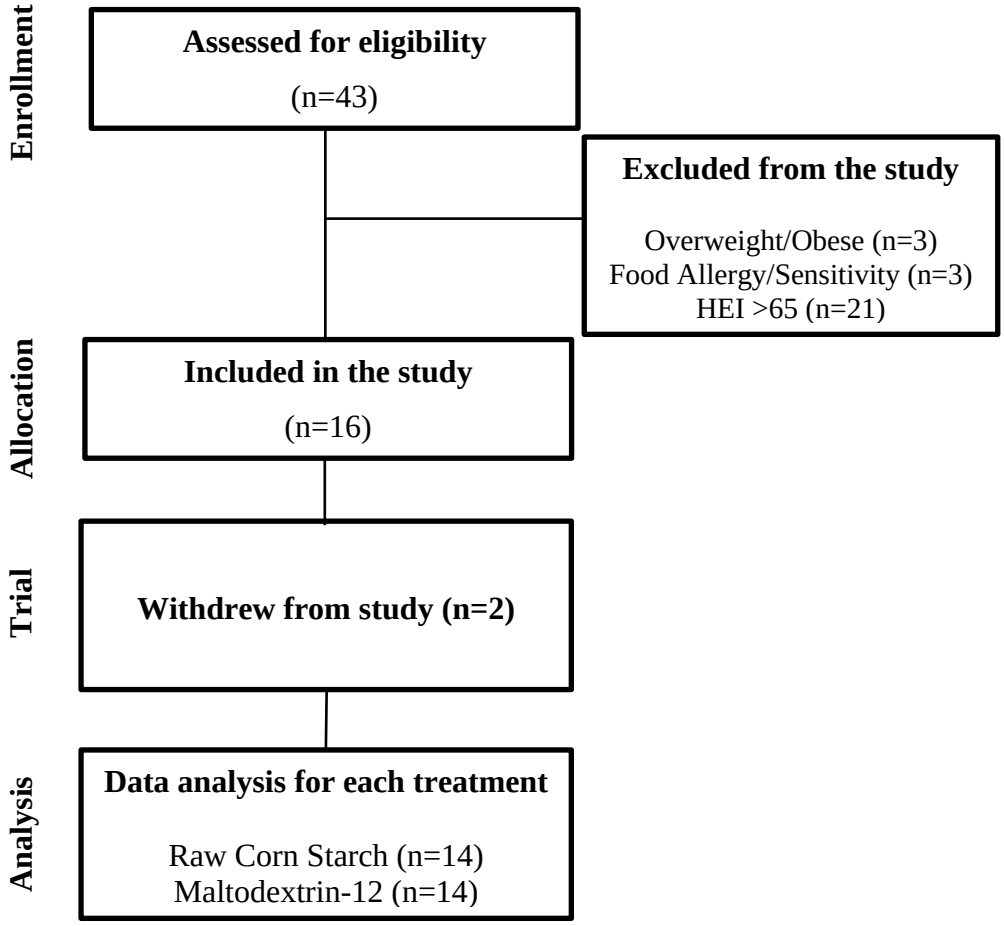

Figure 4-1. Participant recruitment and participation flow diagram.

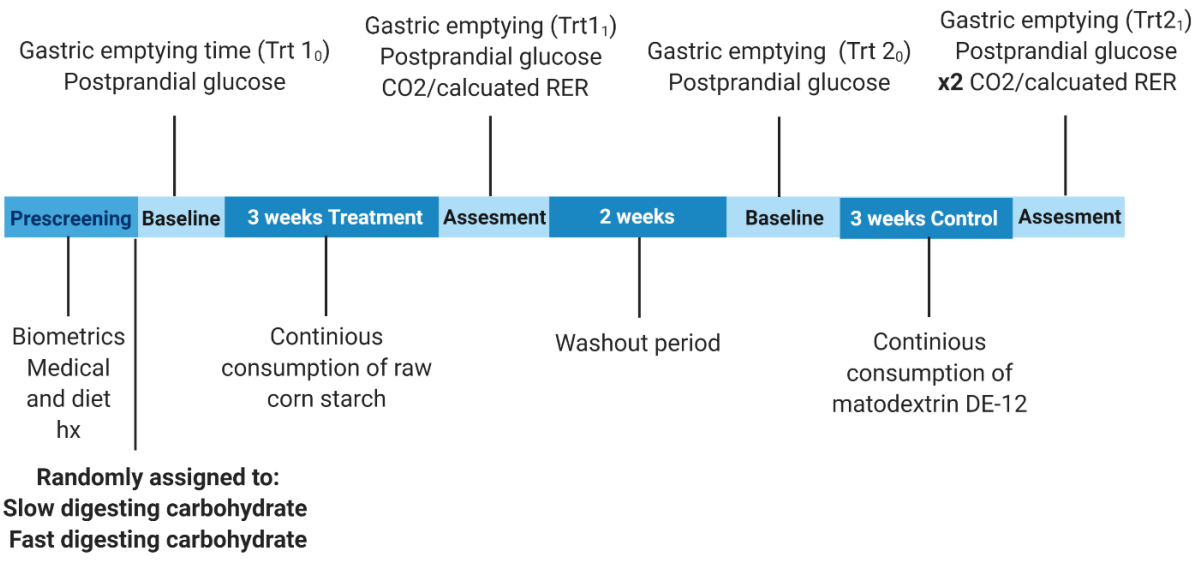

Figure 4-2. Clinical trial timeline (e.g. started with treatment; SDC/raw corn starch).

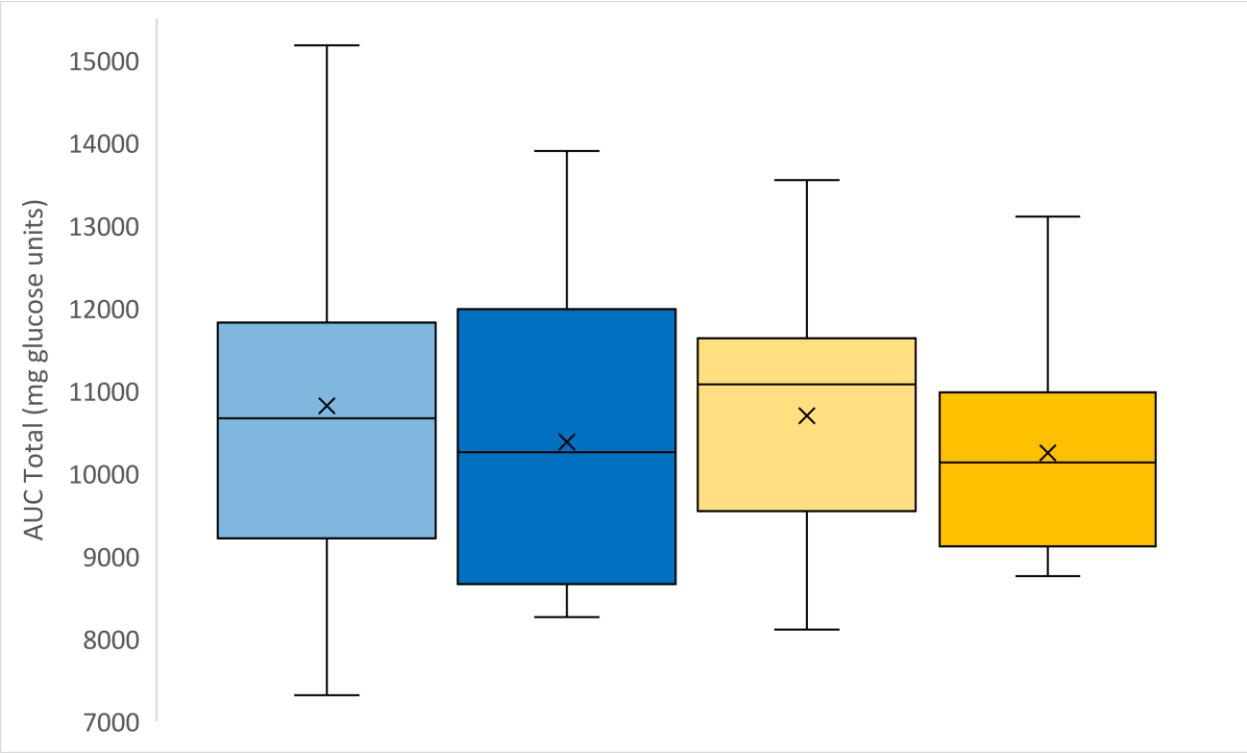

Figure 4-3. Area under the curve (AUC total ∫0-120) min at baseline and 21 days after consumption of either maltodextrin DE-10 or raw corn starch. Glucose AUC $_{total}$ ∫0-120. Baseline maltodextrin DE-10 (light blue), 21-days post consumption maltodextrin DE-10 (dark blue), baseline raw corn starch (light orange), 21-days post consumption raw corn starch (dark orange).

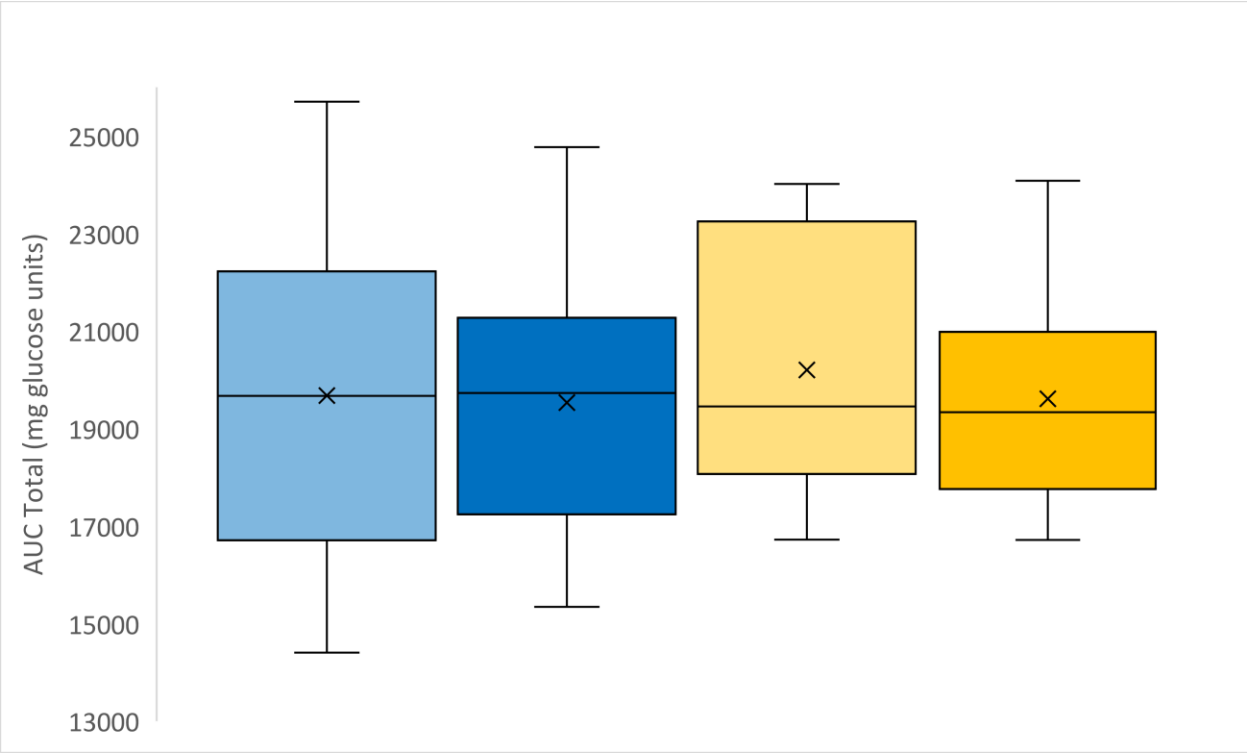

Figure 4-4. Area under the curve (AUC total ∫0-240) min at baseline and 21 days after consumption of either maltodextrin DE-10 or raw corn starch. Glucose AUC $_{total}$ ∫0-240. Baseline maltodextrin DE-10 (light blue), 21-days post consumption maltodextrin DE-10 (dark blue), baseline raw corn starch (light orange), 21-days post consumption raw corn starch (dark orange).

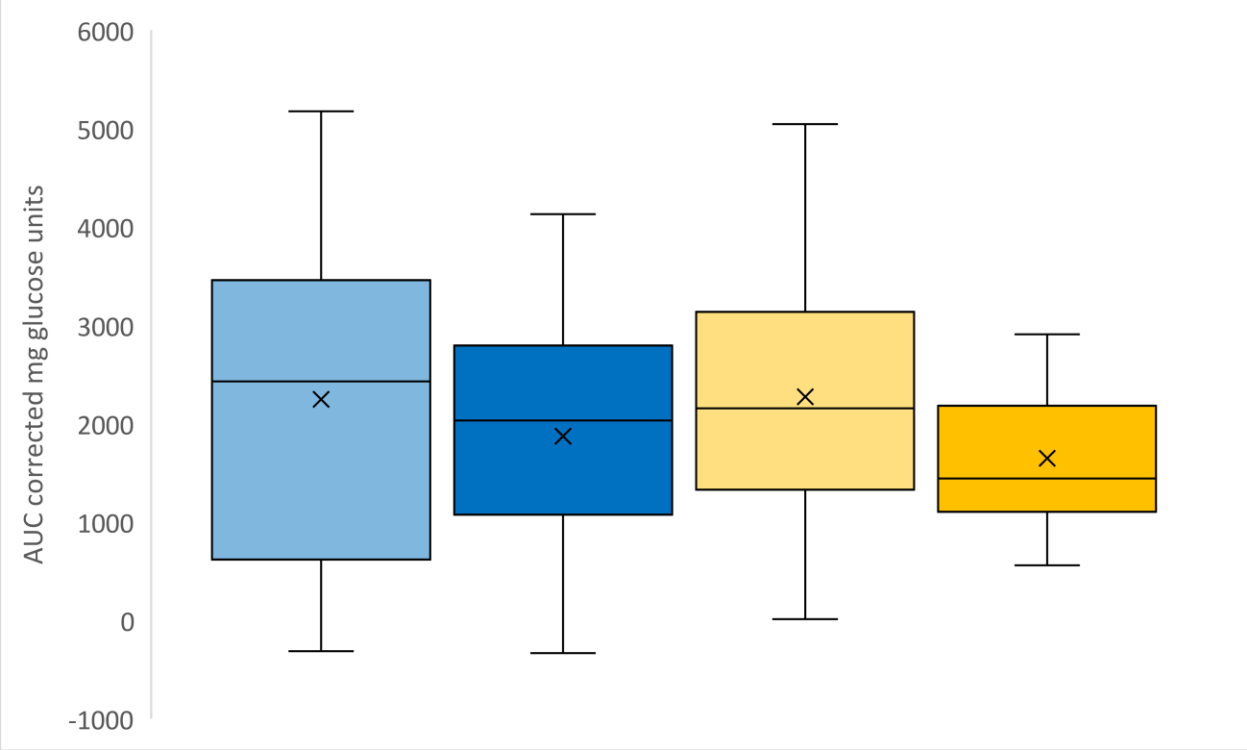

Figure 4-5. Area under the curve (AUC corrected ∫0-120) min at baseline and 21 days after consumption of either maltodextrin DE-10 or raw corn starch. Glucose AUC $_{\text{corrected}}$ ∫0-120. Baseline maltodextrin DE-10 (light blue), 21-days post consumption maltodextrin DE-10 (dark blue), baseline raw corn starch (light orange), 21-days post consumption raw corn starch (dark orange).

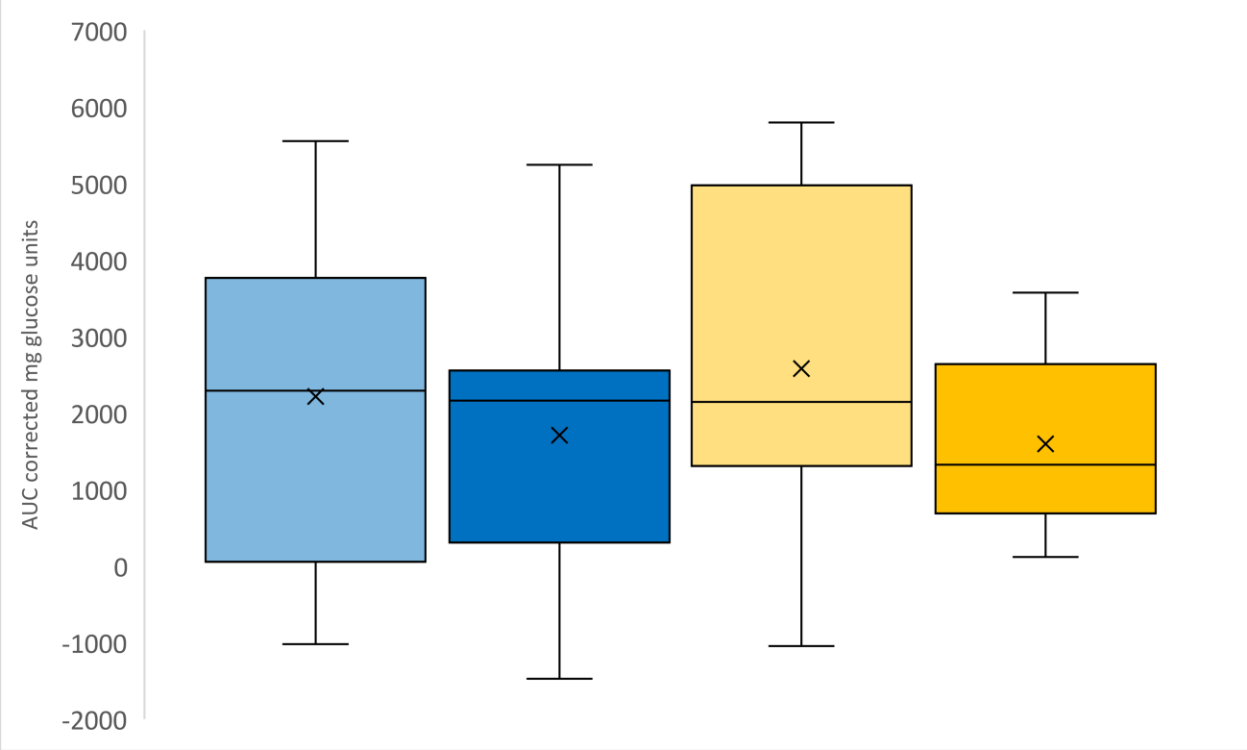

Figure 4-6. Area under the curve (AUC corrected ∫0-240) min at baseline and 21 days after consumption of either maltodextrin DE-10 or raw corn starch. Glucose AUC $_{\text{corrected}}$ ∫0-240. Baseline maltodextrin DE-10 (light blue), 21-days post consumption maltodextrin DE-10 (dark blue), baseline raw corn starch (light orange), 21-days post consumption raw corn starch (dark orange).

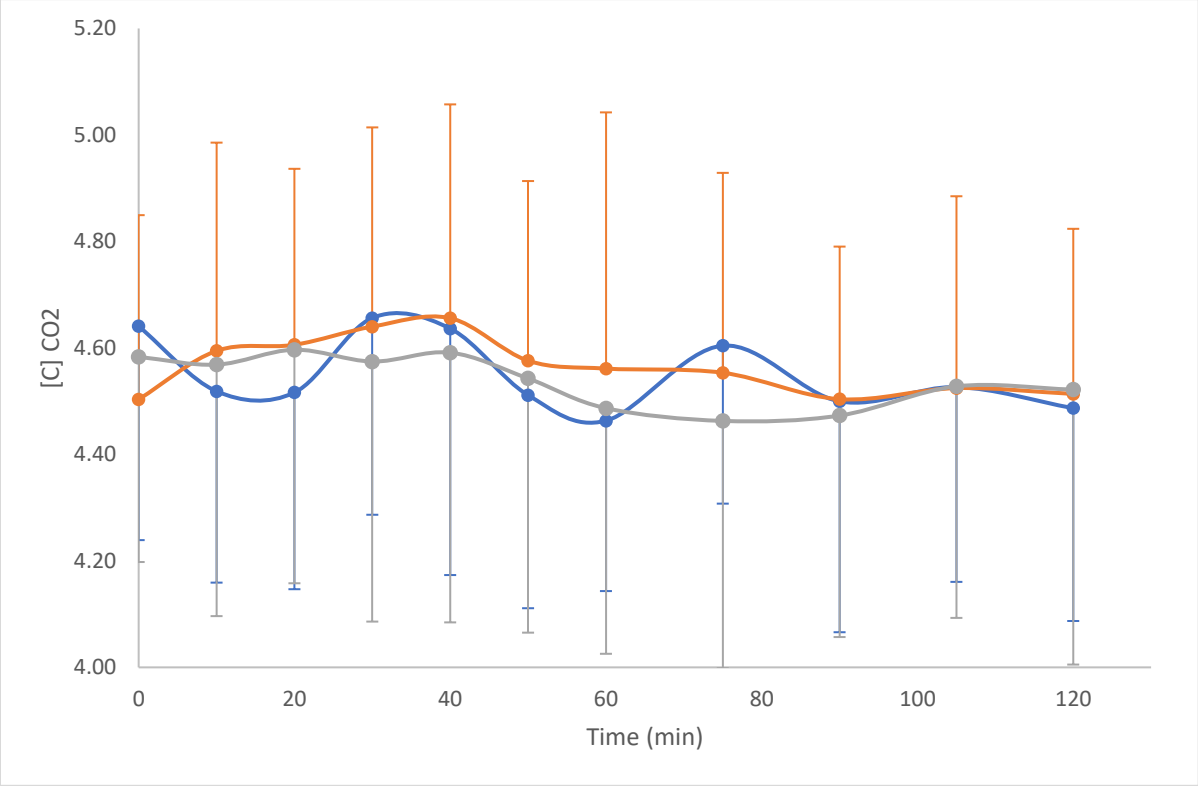

Figure 4-7. Assessment of CO2 values over 2-hour period. Twenty-one days after consumption of maltodextrin DE-10; measured with slowly digestible starch meal (SDC) (blue), 21 days after consumption of maltodextrin DE-10; measured with FDC test meals (orange), 21 days after consumption of raw corn starch; measured with slowly digestible starch meals (SDC) (light grey).

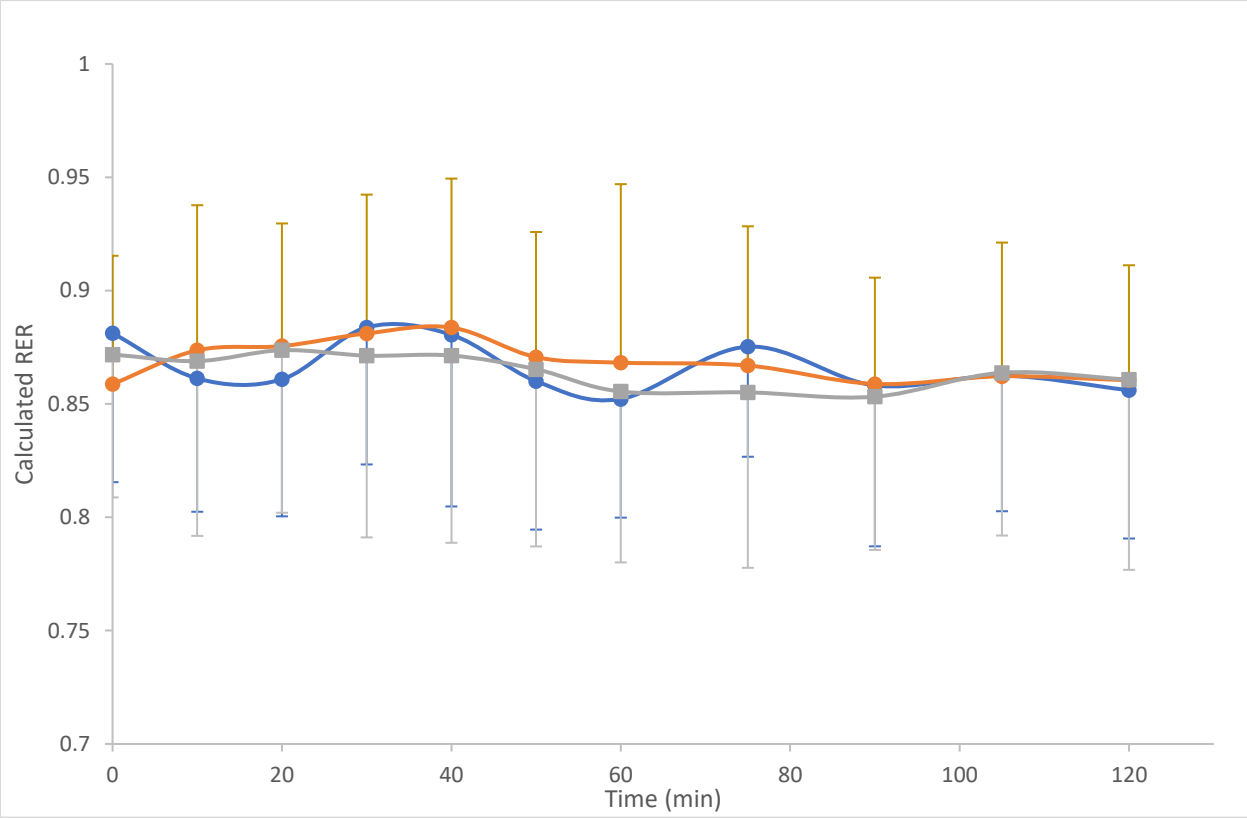

Figure 4-8. Assessment of calculated RER values over 2-hour period. Twenty-one days after consumption of maltodextrin DE-10; measured with slowly digestible starch meal (SDC) (blue), 21 days after consumption of maltodextrin DE-10; measured with FDC test meals (orange), 21 days after consumption of raw corn starch; measured with slowly digestible starch meal (SDC) (light grey).

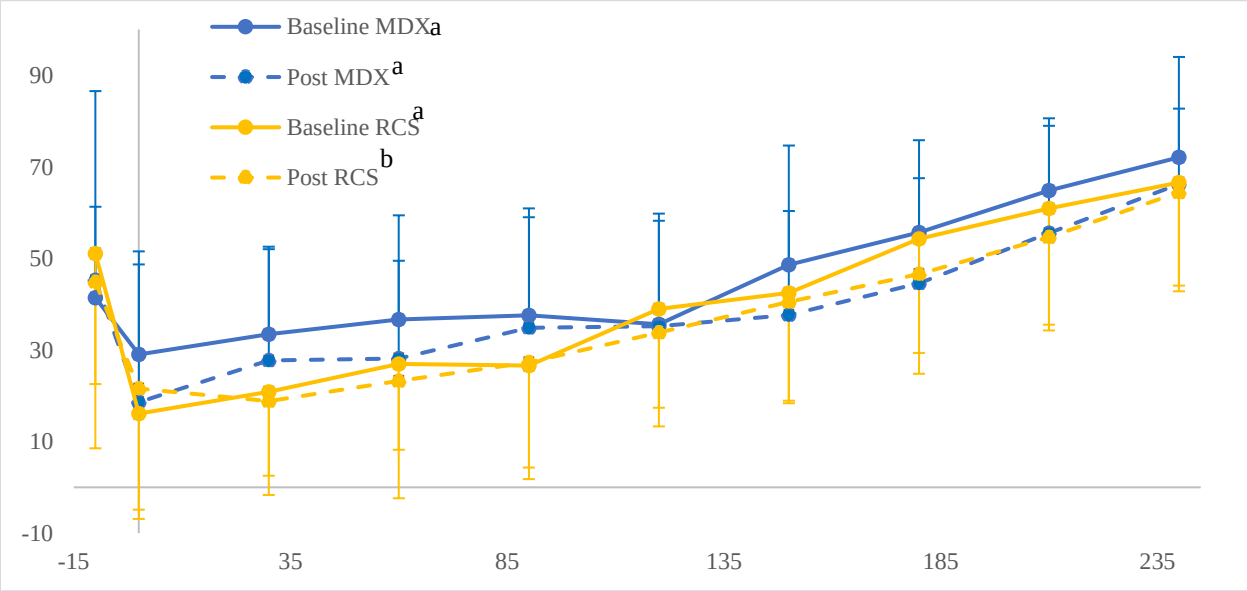

Figure 4-9. Mean hunger ratings (Visual Analog Scale) immediately prior to and 4 h following consumption of standard carbohydrate meal. Baseline maltodextrin DE-10 (blue), 21-days post consumption maltodextrin DE-10 (dotted blue), baseline raw corn starch (orange), 21-days post consumption raw corn starch (dotted orange).

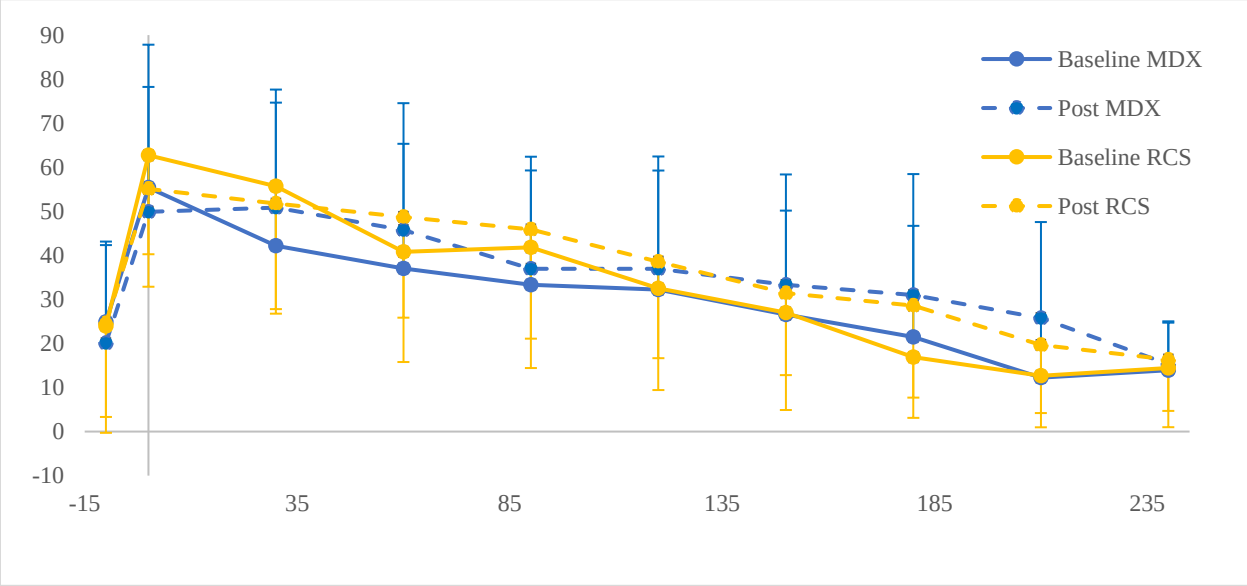

Figure 4-10. Mean fullness ratings (Visual Analog Scale) immediately prior to and 4 h following consumption of standard meal. Baseline maltodextrin DE-10 (blue), 21-days post consumption maltodextrin DE-10 (dotted blue), baseline raw corn starch (orange), 21-days post consumption raw corn starch (dotted orange).

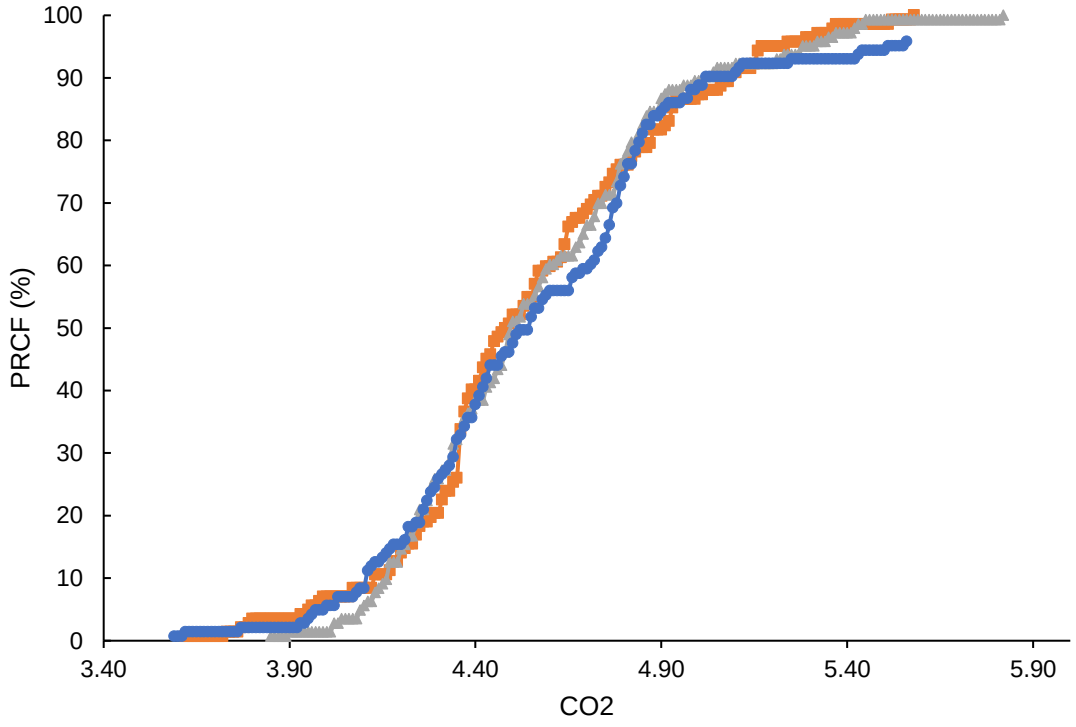

Figure 4-11. Percent relative cumulative frequency (PRCF) of pooled CO_2 values among all participants (n=14) following consumption of raw corn starch (RCS) or maltodextrin (MDX-10) after supplementation of either of these types of carbohydrates. Given that this data is specifically representing carbohydrate oxidation, a broader spread and less steep curve is reasoned to be indicative of enhanced metabolic flexibility. Because this is pooled data, there are no error bars. MDX-10, maltodextrin; RCS, raw corn starch. Post MDX-10 tested w/ RCS (orange); post RCS tested w/ RCS (blue); post MDX-10 tested with MDX-1 (grey).

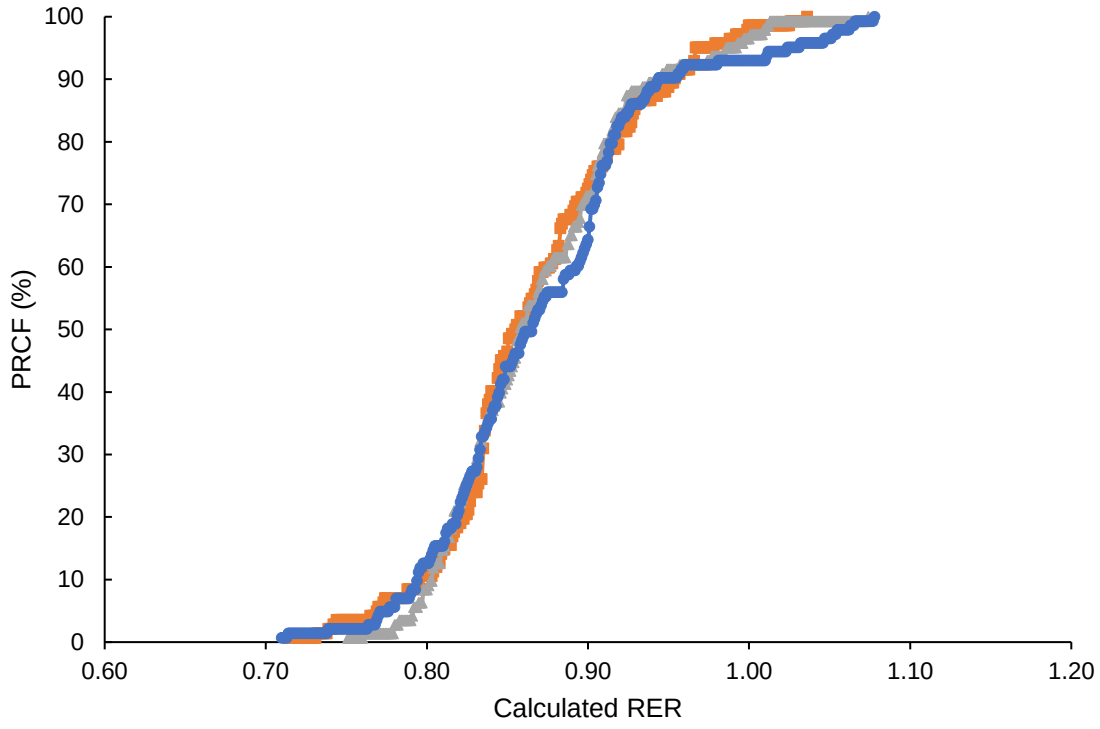

Figure 4-12. Percent relative cumulative frequency (PRCF) of pooled respiratory exchange ratio (RER) values calculated from CO_2 among all participants (n=14) following consumption of raw corn starch (RCS) or maltodextrin (MDX-10) after supplementation of either of these types of carbohydrates. Because this is pooled data, there are no error bars. MDX-10, maltodextrin; RCS, raw corn starch. Post MDX-10 tested w/ RCS (orange); post RCS tested w/ RCS (blue); post MDX-10 tested with MDX-1 (grey).

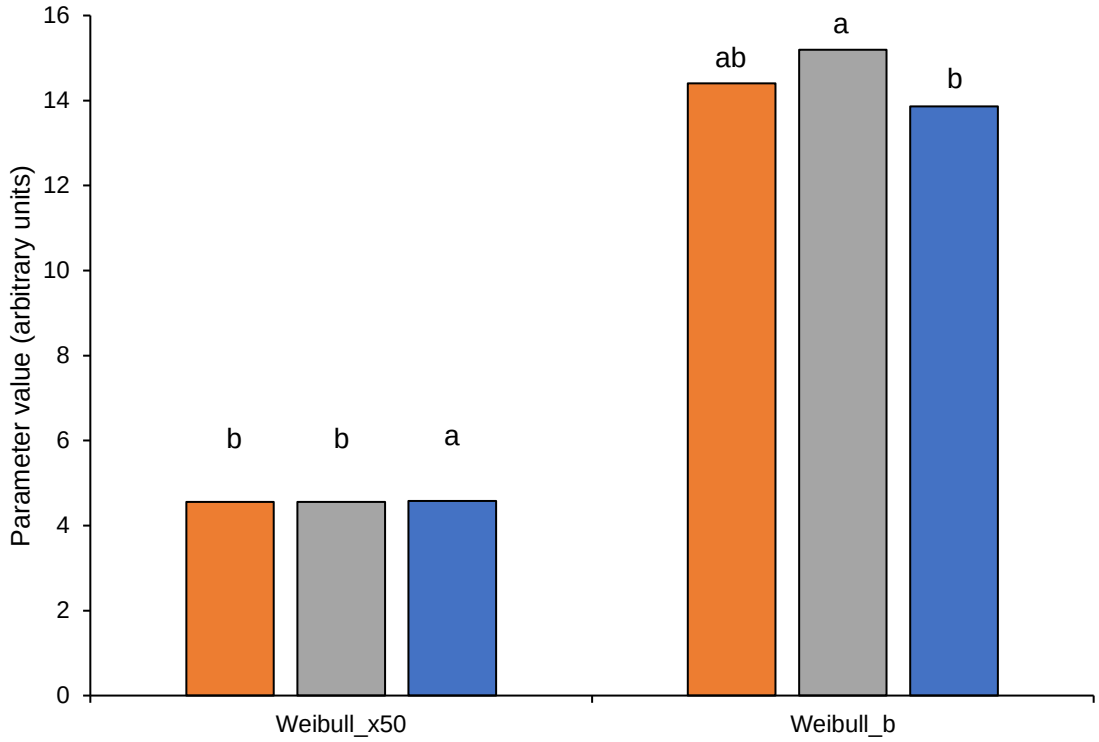

Figure 4-13. Weibull Cumulative Distribution parameter estimates from modeling percent relative cumulative frequency (PRCF) of pooled CO_2 values among all participants (n=14) following consumption of raw corn starch (RCS) or maltodextrin (MDX-10) after supplementation of either of these types of carbohydrates. x_{50} represents the median CO_2 during the postprandial testing period, whereas b signifies the distribution breadth constant or slope (dimensionless). Because this data is specifically depicting carbohydrate oxidation, a higher x_{50} may signify a more complete switch to carbohydrate oxidation (vs. fat oxidation), which is a hallmark of superior metabolic flexibility. Furthermore, a lower b indicates a broader spread in values, which suggests enhanced metabolic flexibility when specifically examining CO_2 values. Different letters indicate statistically significant differences in parameter estimates per group (no overlap in 95% confidence intervals). Because this is pooled data, there are no error bars. MDX-10, maltodextrin; RCS, raw corn starch. Post MDX-10 tested w/ RCS (orange); post RCS tested w/ RCS (blue); post MDX-10 tested with MDX-1 (grey).

CHAPTER 5. EXTRUSION COOKING IMPROVES LIPID STABILITY FOR WHOLE GRAIN PEAL MILLET (PENNISETUM GLAUCUM) INSTANT FLOURS IN AFRICA

5.1 Abstract

Emerging, local entrepreneurial companies in Africa are increasingly gaining share of local markets from foreign competitors by manufacturing and distributing high-quality, low-cost processed foods. The implementation and use of food processing technologies can be important for their success. In this study, we assessed the use of a low-cost, single screw extruder as a feasible technology to develop shelf-stable whole grain pearl millet (*Pennisetum glaucum*) flours to be used for instant thin and thick porridges. We compared oxidative and hydrolytic rancidity of whole grain and decorticated extruded and native flours over an 18-week period, and sensory attributes of the prepared porridges (color, aroma, texture and flavor, n=75 US consumers) were analyzed. Extrusion prevented the development of hydrolytic rancidity, but did not slow the production of products of oxidative rancidity over time. Contrary to expectation, consumers preferred the lighter color, and texture and flavor of porridges of native (un-extruded) flours compared to their extruded counterparts. This work shows that extrusion using a low-cost extruder suitable for processing by small to medium-scale enterprises adds value to whole grain pearl millet by generating fully gelatinized instant flours with enhanced shelf stability.

5.2 Introduction

Whole grain cereal products in sub-Saharan Africa have the potential to improve the health of nutritionally vulnerable consumers by providing enhanced levels of iron, and to a lesser degree zinc, and dietary fiber. Whole grains are beneficial to urban consumers with overnutrition and metabolic syndrome diseases of obesity, diabetes, and cardiovascular disease. Yet, cereal grains of West, East, and Southern Africa are typically debranned/degermed or decorticated before consumption and whole grain traditional foods are not common. The biggest challenge to processing whole grain cereal products is the problem of enzymatic and oxidative lipid rancidity, which shortens shelf life. Extrusion processing is one way of curtailing the rancidity process and extending shelf life by inactivating the lipid breakdown enzymes, lipase and lipoxgenase (Camire

et al., 1990). Our group has been active in recent years, through US Agency for International Development projects in West and East Africa, in transferring low-cost extrusion processing technology and providing know-how to local entrepreneur processors to make regular and nutrient-fortified instant flours based on pearl millet, sorghum, and maize. The work described here is on extrusion of whole grain and decorticated pearl millet instant flours, to reduce the rancidity processes and improve shelf life.

Pearl millet (*Pennisetum glaucum*) is a staple crop in the semi-arid regions of the world. It has the ability to thrive under severe conditions, including low rainfall, intense heat, and soils with low fertility. This strengthens pearl millet as a staple crop among low income populations (*FAOSTAT*, n.d.; Saleh et al., 2013b). In Sahelian West and Central Africa, pearl millet is a desirable grain for making a range of dishes, though is most commonly consumed as thin and thick porridges from decorticated grain milled to flour. Industrially processed whole grain pearl millet products are not common in the region, though their availability would help to address some micronutrient deficiencies as well as the growing problem of diet-related diseases of overnutrition in cities (Obilana, 2003). Similar to other whole grains, pearl millet has a relatively high concentration of unsaturated triglycerides which renders it susceptible to lipolysis, oxidative rancidity, and the development of undesirable flavors during storage (Lai & Varriano-Marston, 1980). Extensive decortication, which is commonly practiced, is laborious and leads to a significant loss of important minerals including Fe, Zn, Mn, Mg, and Cu (Pucher et al., 2014). Even after decortication, small amounts of lipids remain in the endosperm that are susceptible to oxidation (Lai & Varriano-Marston, 1980). Treatments such as dry heat and blanching, have limited ability to prevent degradation of lipids in pearl millet flours (Kadlag et al., 1995). Alternatively, toasting and toasting/boiling millet before milling has been shown to extend the shelf stability of whole grain pearl millet flours, but these techniques can impart undesirable sensory characteristics like burnt aroma and bitter taste (Nantanga et al., 2008).

High temperature, high-shear extrusion cooking is a physical treatment in which cereal meals or coarse flours are gelatinized, and to some degree starch is fragmented. Extrusion cooking can be further used to customize the rheological and hydration properties of starch, allowing it to exhibit specific functionalities (Martínez, Calviño, et al., 2014; Martínez, Rosell, et al., 2014). Desirable changes from extrusion include protein denaturation, microbial reduction, and enzyme inactivation (Camire et al., 1990; Hagenimana et al., 2006).

In sub-Saharan West and Central Africa pearl millet is usually consumed as thin and thick porridge, which are prepared by boiling decorticated flour with water until complete starch gelatinization. Extrusion cooking offers the possibility of making pre-cooked instant porridge flours, and further permits natural fortificants to be added in the extrusion process (Debelo et al., 2020). Instant flours gives the consumer the advantage of preparing porridge without the need of energy-intensive cooking, and also offers a convenience food for mid-level consumers. Extrusion-type processes have been used to produce instant flours from sorghum (using a low pressure, high temperature, high-shear continuous processor) (Moussa et al., 2011), rice (Martínez, Calviño, et al., 2014), and wheat (Martínez, Rosell, et al., 2014). Although operational cost and initial capital are barriers for widespread dissemination of extrusion technology in Africa (Filli et al., 2014), we have introduced a low-cost, small-scale extruder suitable for African SMEs through projects funded by the US Agency for International Development (USAID) (Ponrajan, 2016).

In the present work, we hypothesized that the use of extrusion cooking to process millet could: 1) extend the shelf life of whole grain pearl millet flour; 2) improve the sensory characteristics of pearl millet flours. We investigated the effect of low-cost single screw extrusion on the lipid stability (peroxides and free fatty acids) and color of whole and decorticated pearl millet flours, as well as acceptance of the sensory attributes (color, aroma, flavor, and texture) of the resultant porridges made from these flours stored over a period of 18 weeks.

5.3 Materials and Methods

5.3.1 Materials

Whole pearl millet kernels were obtained from Cisco Co. (Indianapolis, IN, USA). PET/PE impermeable bags were purchased from ProAmpac (Cincinatti, OH, USA). Peroxides and free fatty acids were assessed using commercial kits, PeroxiSafe™ and AciSafe™, respectively (MP Biomedicals, Tempe, AZ, USA).

5.3.2 Decortication of whole pearl millet grain and milling into flour

Pearl millet kernels were debranned in an electric abrasive decorticator (Nutana Machine Ltd, Saskatoon, Canada) for 15 min to obtain a 12% decortication yield (88% weight difference). Bran and endosperm were separated by sieving, discarding the fraction that passed through a 300

µm mesh (bran powder). For the whole grain native millet sample, whole millet grains were ground to grits (≈1-1.5 mm) using a pin mill (Alpine American Corp., Natick, MA, USA). Decorticated flours were processed with the decorticated grain similarly. Grits were subsequently used for extrusion.

Whole and decorticated native flours were ground to flour (300 µm mesh) using the pin mill.

5.3.3 Extrusion of flours

A 35 kg/h small-scale single screw extruder with restrictions on the screw (Technochem Inc., Boone, IA, USA) was used. The design of the extruder is based on the scale-down of a 300 kg/h extruder by Insta-Pro International (Des Moines, Iowa, USA). A preliminary study was carried out to optimize the extrusion conditions. Whole and decorticated pearl millet grits were equilibrated to 30 and 32% moisture content, respectively, prior to extrusion. Temperature was measured using a thermocouple (HH309A 4-channel data logger thermometer, Omega Engineering Inc., Stamford, CT, USA) placed immediately before the exit on the die opening. The temperature of the extrudate, measured when equilibrium was reached, was above 120°C. The screw speed (700 rpm) was measured using a tachometer (HHT13, Omega Engineering Inc., Stamford, CT, USA) by fixing a laser reflective tape on a pulley attached to the extruder shaft behind the feeding section of the extruder. The length to diameter (L/D) ratio and die diameter for the extruder were 40:1 and 6 mm, respectively. Extruded material was dried by convection air at 50°C (Blue M Oven, Blue Island, IL, USA) and then ground with a roller mill (Alice Chalmers, West Allis, WI, USA). Flours were stored in PET/PE bags (ProAmpac, Cincinatti, OH, USA) and held at 4, 20 and 35°C for up to 18 weeks.

5.3.4 Determination of peroxides and free fatty acids in pearl millet flours

Evaluation of intermediate and final products of lipid oxidation were determined in flour samples stored at 4, 20 and 35°C for up to 18 weeks to understand their evolution in retarded, standard, and accelerated conditions. Peroxide values and free fatty acids were assessed using commercial kits (PeroxiSafe™ and AciSafe™) based on established methods (Jiang, Woollard, & Wolff, 1991); (Lowry & Tinsley, 1976); (Hamilton, R. J.; Rossell, 1986) (MP Biomedicals, Tempe,

AZ, USA). All analyses were performed according to the procedures provided by the manufacturer. Absorbance was measured at 570 nm for peroxide and fatty acids using a Spectromax 190 spectrometer (Molecular Devices, Sunnyvale, California, USA). All samples were measured in duplicate.

5.3.5 Porridge preparation for sensory analysis

Native flour samples stored at 20°C were cooked at a flour-water ratio of 1:3 in boiling DI water for 15 min under constant mixing. Due to the high absorption capacity of extruded flours, the water amount had to be adjusted to keep comparable viscosity. Accordingly, extruded flour samples stored at 20°C were reconstituted at a flour-water ratio of 1:2 in boiling DI water and mixed for 10 min at room temperature. After preparation, samples were transferred to Crockpots and kept at constant temperature (50°C).

5.3.6 Color stability of pearl millet porridges

Color was measured using a LabScan XE colorimeter (HunterLab, Reston, VA, USA) with the D65 standard illuminant and 10 observer angle. CIE parameters L* (lightness), a* (redness), and b* (yellowness) were recorded. Color determinations were made on flours and prepared porridges at 50^{o}C in triplicate.

5.3.7 Sensory evaluation

Consumer evaluation of porridge prepared from the four treatments was conducted at five different storage time intervals (0, 4, 8, 12, and 18 weeks) in the sensory laboratory at the Food Science Department at Purdue University. Only samples stored at 20°C were evaluated, and they were prepared and served at 50±2^{o}C. Only samples from a specific time interval were evaluated per testing session. Five different testing sessions were performed in total. For each testing session, 75 consumers (18-65 years) were recruited through flyers and email communications to participate in the evaluation of pearl millet porridge samples. Participants were screened and accepted into the study based on their frequency of consumption of hot cereals (e.g. oatmeal, cream of wheat, porridges). Prior to initiation of the testing session, participants read an electronic form indicating the details of the study and informed consent was obtained. Four prepared porridge samples (20 g

each) in styrofoam cups coded with three-digit random numbers were presented simultaneously to participants under white light. Participants were asked to rank the four samples based on preference (from 1st = most preferred to 4th = least preferred) and to indicate the rationale for the choice (the results are not discussed in this paper). Subsequently, while still having all samples, participants were asked to rate the color, aroma, flavor, and texture of the samples using a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely). The order in which each individual sample was presented for evaluation was randomized. Data was input directly on Compusense *five* (Guelph, ON, Canada). Prior to conducting the study, approval was obtained from the Purdue University Institutional Review Board, protocol number 1601016938.

5.3.8 Statistical analysis

For assessment of lipid oxidation products (free fatty acids and peroxides), ANOVA (PROC MIXED) with treatment, storage temparature and time as fixed effects was used to determine the effects of decortication and extrusion on the of pearl millet flours. Post-hoc (Tukey) test for significant differences ($p < 0.05$) was performed to compare means.

Porridge color (L*a*,b*) was assessed using ANOVA (PROC MIXED) ($p < 0.05$) with treatment and storage temperature as fixed effects, comparisons were performed between baseline values (week 0) and after 18 weeks of storage.

For assessment of sensory attributes, multiple comparisons sensory results were analyzed using ANOVA (PROC MIXED) with treatment and time as fixed effects and subject as random effect. Tukey's least significant difference post-hoc test was performed to compare the means at the same significance level ($p < 0.05$). Models for determining the effect of free fatty acids and peroxide concetration on flavor and aroma response were assessed. Free fatty acids, peroxide values and their interactions were treated as fixed effects, subject was treated as a random effect. All statistical analyses were performed with SAS 9.4 for Windows (SAS Institute, Inc., Cary, NC, USA). Additionally, Principal Components Analysis (PCA) was performed at the University of Pretoria to characterize study samples. Principal Component Analysis (PCA) is a multivariate data analysis method for visualization (projection method) of correlations between multiple quantitative observations and variables in a 2-dimensional space (information is measured here through the total variance of the scatter plots) from the initial dimensions (0, 0 points in axes). In this study, the information presented in the 2 axes represented a sufficient percentage of the total variability

of the scatter plot. PCA was used to produce trajectories (biplot) allowing identification of uniform groups of observations. The vectors indicate the loadings (assessment of variance) for sensory attributes while the position of the sample codes indicate the score values (XLSTAT, 2022). Internal preference maps (IPM) were generated from the acceptability test, based on the overall impression results for each parameter (color, aroma, flavor, and texture) (McEwan, 1996). These statistical analyses were carried out using XLSTAT software, version 2018.3 (Addinsoft, New York, NY, USA).

5.4 Results and discussion

5.4.1 Evaluation of products of hydrolytic and oxidative lipid degradation

The development of hydrolytic and oxidative rancidity of native and extruded whole grain and decorticated pearl millet flours stored at 20^{o}C for a period of up to 18 weeks, and for extruded flours only at 4, 20, and 35°C, is shown in Figures 5-1 to 5-4. Hydrolytic rancidity develops when fatty acids are hydrolyzed from the glycerol backbone by the action of lipases, generating free fatty acids that are in turn more susceptible to oxidative rancidity. Only native flours showed increase in free fatty acid accumulation, indicating the development of hydrolytic rancidity (Figure 5-1); extruded flour samples were stable in this regard. At day 0, free fatty acid concentration of extruded pearl millet flours did not significantly differ from native flours ($p < 0.05$); however, differences were apparent at week 1 and increased continually until week 18 ($p < 0.01$). Native whole flour showed a 4-fold increase in free fatty acid concentration over the 18 weeks of storage, while native decorticated flours showed a 2-fold increase during the same time period. Nantanga and collaborators (Nantanga et al., 2008) reported that fat acidity (indicative of free fatty acids) increased over a 12-week period in untreated millet samples due to the effect of enzymatic lipolysis following milling. For thermally-treated samples to inactivate lipase, lower fat acidity values were reported and remained consistently low over the 12-week period. Similar results were reported in thermally-treated pearl millet and sorghum samples in which fat acidity and free fatty acid production were negatively correlated to the intensity of thermal treatment (Meera et al., 2011; Tiwari et al., 2014). Corroborating previous findings, our study indicates that extrusion prevents the development of hydrolytic rancidity, presumably by promoting lipase inactivation. Storage

temperature (4 or 35ºC) did not exert a significant effect in free fatty acid concentration. There were no significant interactions ($p < 0.05$) between storage temperature and extrusion treatment.

Oxidative rancidity occurs as double bonds of unsaturated fatty acids undergo cleavage, forming unstable peroxyl-fatty acid radical species which are further degraded into volatile aldehyde compounds. In this study, oxidative rancidity changes in the concentration of peroxides, which are intermediate products of oxidative rancidity, were monitored (Figure 5-2). Starting from day 0, extruded pearl millet flours displayed higher ($p < 0.05$) concentrations of peroxides which increased over storage time to week 6 compared to native flours. The higher concentration of peroxides indicates that extrusion promoted the initiation stage of oxidative rancidity, as these increments were not observed in native flours. Peroxide levels increased over time, reached their maximal concentration after 6 weeks of storage, and decreased to their initial levels by week 8, indicating the propagation and termination stages of oxidative rancidity. Threshold levels of detection of end products of hydrolytic rancidity (aldehydes) are dependent on the type of aldehyde, their detection range is as low as 0.24 ppm for pentanal (described as woody, bitter, oily) or 0.32 ppm for hexanal (described as fatty, powerful, oily, grassy) and octanal (sharp, citrus) (Morales & Przybylski, 2013). Storage temperature played a role in the development of peroxides, as whole extruded samples stored at 35°C contained the highest peroxide concentrations. There was a significant interaction ($p < 0.01$) between storage temperature and extrusion treatment. Overall whole extruded and decorticated extruded samples stored at 4°C were not significantly different from samples stored at 20°C, though samples stored at 35°C had statistically higher peroxide concentrations. Lower peroxide concentrations were found in decorticated flours compared to whole flours, resulting from reduction in lipid levels.

We expected that extrusion would reduce oxidative rancidity due to lipoxygenase inactivation. Zhu, Riaz, and Lusas showed that extrusion conditions similar to the ones used in our experiment inactivated lipoxygenase in soybeans, however we did not observe a positive effect of extrusion on peroxide reduction. On the contrary, extruded flours exhibited increased concentrations of reactive oxidative species. This could be because extrusion promotes cell disruption which liberates lipid and metal catalyzers (Fe and Cu), facilitating the oxidative process (Camire et al., 1990). In our study, we did not test for lipoxygenase activity, and further work may lead to a better understanding of extrusion conditions that would not cause oxidative rancidity to occur.

5.4.2 Color stability of pearl millet porridges

The color results of porridges prepared with fresh (time 0) and stored flours at 20°C for 18 weeks are shown in Figure 5-5 to 5-7. Significant differences ($p < 0.05$) in the color parameters L*, a*, and b* were observed for porridges prepared from fresh and stored flours. Porridges prepared from fresh decorticated flours (day 0) presented higher ($p < 0.05$) lightness values (L*) compared to whole grain counterparts. Similarly, Taylor and Dewar (Taylor & Dewar, 2001) showed that decortication improved lightness of sorghum products by removing pigments concentrated in the outer layers of the grain.

Extrusion decreased L* values, as porridges prepared with fresh extruded flours had lower lightness values (L*) compared to their native counterparts (Figure 5-5). This effect could be attributed to the Maillard reaction during extrusion, the result of the chemical reaction between an amino group and a reducing sugar at elevated temperatures (140-165°C), forming brown-colored, heterocyclic compounds (Fennema, 2017). Moreover, relatively high concentration of lysine and proline in pearl millet could increase the formation of melanoidins and other compounds, resulting in darker porridges (Indira & Naik, 1971). The largest discrepancy in lightness values occurred between porridge prepared with whole grain and decorticated native flours ($p < 0.01$). This indicates that the effect of decortication on lightness (L*) of pearl millet porridges is greater than that of extrusion.

Porridges prepared with extruded flours presented higher values (a*) compared to the native counterparts (Figure 5-6), which indicates an intensification of red color. Porridge prepared with native decorticated flour had the lowest a* value. On the other hand, porridges prepared with extruded flours presented lower b* values compared to the native counterparts (Figure 5-7), which indicates an intensification of blue color. These changes denote that extrusion has a significant effect on L*, a*, and b* parameters, especially in decorticated grain. Similar to our results, Tiwari and Jha, Pal, Sethi & Krishan (2012) demonstrated that decortication increased L* values (decorticated 69.3 vs native 53.3), followed by extrusion (63.6). The authors reported minor effects on a* and b*.

Flour storage at 20°C over 18 weeks also had a significant effect on the color of porridges. All porridges, except for the one prepared with decorticated native flour, increased in lightness values (L*) at 18 weeks of storage compared to day 0 (Figure 5-5). Porridges prepared with decorticated native flours ($L^*_{day\ 0} = 59.22$; $L^*_{week\ 18} = 45.96$) showed a greater difference compared

to the other samples (whole grain native, $L^*_{day\ 0} = 51.75$; $L^*_{week\ 18} = 58.50$). Similarly, a* values increased for porridges prepared with whole grain extruded and decorticated native flours, which means an intensification of the red color (Figure 5-6). The b* values decreased for all porridges, which indicates an intensification of blue color, except for the one prepared with whole grain extruded flour (Figure 5-7). Color changes over time might be attributed to changes in oxidation of phenolic compounds in the flours. While there are no studies to date assessing the stability of pearl millet polyphenols during storage, de Oliveira and collaborators (de Oliveira et al., 2017) indicated a modest reduction in total phenols in sorghum after 18 weeks of storage, which significantly contributed to changes in lightness value (L*). Phenolic profiles are not directly comparable between sorghum and pearl millet (Dykes & Rooney, 2006); however, the dynamics of degradation might play a similar role for pearl millet flour.

5.4.3 Sensory evaluation

Hedonic rating results for the sensory attributes of pearl millet porridges prepared with flour stored for an 18-week period are shown in Table 5-1. For the acceptability tests, panelists assessed porridge samples for each sensory attribute using a 9-point hedonic scale. Figure 5-8 (a) shows the multivariate distribution of liking ratings for the color of the porridge samples. The position of a grey dot on the plot indicates the direction of positive acceptability (see example on Figure 5-8 (a) for an individual consumer. Note that all four of the porridge samples are positioned on the right side of the plot. The first dimension (PC1) on Figure 5-8 (a) explains 66% of the variation in the acceptability ratings for color of the porridges. Considering the ratings of all the consumers for the four porridges, PC1 separates consumers on the right that were more positive about the color of the samples from those on the left of the plot who gave low ratings and therefore disliked the color of all the porridges. PC2 separates consumers at the top of the plot with a preference for the color of the decorticated native and whole native porridge samples from those at the bottom that were more positive towards the color of whole extruded and decorticated extruded porridge samples. In our study, both treatment type (extruded vs native flours) and storage time exerted a significant effect on the color perception; participants preferred the color of the porridges prepared with native flours over the ones prepared with extruded counterparts (Table 5-1). The lower scores for extruded samples might relate to darkening due to the presence of brown-colored, heterocyclic compounds characteristic of Maillard browning (Fennema, 2017)

which allows consumers to discriminate between samples and possibly associate the extruded samples with stronger flavor. Liking of the color of a product is a personal choice and is largely determined by past experiences with other products and expectations formed about the flavor from color associations. For example, darker colored cereal products are associated with more intense flavor and toasted flavor notes as well as attributes linked to bitterness (e.g. wholegrain products) (Heiniö et al., 2016; Kobue-Lekalake et al., 2007).

Previous research on sorghum porridges consumed by sub-Saharan populations has demonstrated that consumers prefer lighter colored porridges over darker ones (Aboubacar et al., 1999; Moussa et al., 2011), and this trend might extrapolate to porridges prepared with pearl millet. Similarly, Western consumers have shown similar preference for light colored versus dark colored cereal products, indicating a perceived expectation for unpleasant taste and poor texture as a driving factor limiting consumption and acceptability (Schaffer-Lequart et al., 2017).

Figure 5-8 (b) presents a summary of consumer perceptions of aroma/smell of the porridges. The PCA plot explains 86% of the variations in opinion among the consumers (PC1 71%, PC2 15%). Contrary to the color sensory attribute assessed before, participants had diminished ability to discriminate between the aroma of native and extruded porridge samples. This is indicated by the relatively small difference between hedonic aroma scores (Table 5-1). We expected participants to be able to differentiate porridge samples based on the increasing concentration of products of oxidative hydrolytic rancidity however no significant differences were found (Figure 5-2; 5-4; and table 5-1). Peroxides peaked at week 6 and subsequently decreased to their previous levels by week 8, indicating continuation of oxidative reaction and formation of volatile molecules, including aldehydes, ketones, alcohols, and heterocyclic compounds. It has been reported that 1-octen-3-ol, (E)-2-octen-1-ol, (E)-2-octenal, 3-nonen-2-one, (E)-2-nonenal, decanal, 2-undecanone, and 2-methylnaphthalene are the most prominent volatiles in finger millet degradation (R. Wang et al., 2014), and particularly 1-octen-3-ol and (E)-2-nonenal play a key role in unpleasant aroma resulting from oxidation. Even though we observed an increase in peroxide concentration and can assume subsequent formation of aldehydes in all samples, participants did not show a preference based on peroxide concentration. This might be related to potential dilution of aldehydes below a level of detection, as well as potential loss from porridge preparation. A model to assess the impact of free fatty acid and peroxide concentration on aroma response indicated that only peroxide

concetration and the interaction between peroxides and free fatty acid had signicant effects. However, when these parameters were plotted into a equation the fit was poor (R^2=0.014).

In addition to the explanation of the orthonasal aroma perception, Fig 3c provides insight on the acceptability of the in-mouth perceived flavor of the different porridge samples. The internal preference map explains 80% of the variations in opinion among the consumers (PC1 63%, PC2 17%). Teatment type (extruded vs native flours) and storage time exerted a significant effect on the flavor perception (Table 5-1); participants' preferences might relate to a combination of factors, including flavors developed during extrusion as a result of Maillard reaction, the presence of compounds of hydrolytic and oxidative rancidity, as well as the removal of grain fractions responsible for bitterness and astringency during decortication. Previous research on non-wheat, hot-extruded cereal products has shown the development of sweet and starchy flavors as a result of the Maillard reaction (Zhou et al., 1999). These flavor notes have traditionally been considered pleasant, however in our experiment extruded samples (whole and decorticated) received lower hedonic flavor scores compared to native samples (Table 5-1). Recent studies using whole grain have indicated the formation of bitter compounds in extruded and baked products prepared with whole grains (Bin et al., 2014; L. Zhang & Peterson, 2018). Tryptophan, tryptophan derivatives (Tryptophol and N-(1-deoxy-D-fructos-1-yl)-L-tryptophan), and Maillard reaction products including Acortatarins A, Acortatarins C, 5-hydroxymethyl)furfural (HMF), 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP), 2-(2-formyl-5-(hydroxymethyl-1H-pyrrole-1-yl) butanoic acid (PBA) previously been shown to give rise to bitter flavor in whole grain wheat bread (Bin et al., 2014) while L-tryptophan, chae-norpine, N1,N5-Di-[E]-p-coumaroyl-spermidine, and terrestribisamid are responsible for bitter flavor in whole extruded corn products (L. Zhang & Peterson, 2018). While the dynamics of formation of bitter products in whole grain is not well-understood, it is hypothesized that proteolic enzymes in the outer layers of the kernel generate small molecular weight peptides that take part in the Maillard reaction (Heiniö et al., 2016). It is also important to note that pearl millet has a high concentration of tryptophan compared to other grains, potentially exacerbating the development of bitter notes (Indira & Naik, 1971).

Similarly, products of hydrolytic rancidity have traditionally been associated with undesirable taste. The fatty acid profile of millet is composed of a combination of long (C16 to C22) saturated and unsaturated fatty acids (Sridhar & Lakshminarayana, 1994). Flavors developed due to hydrolytic rancidity differ based on molecular weight; while liberation of short-chain fatty

acids results in volatile rancid aromas, liberation of long chain fatty acids such as the ones present in pearl millet results in off-flavors with "soapy" and "paint-like" notes. As shown in Figure 5-1; 5-3, extrusion inhibited the development of hydrolytic rancidity; native flours produced a significantly larger amount of free fatty acids over storage in comparison to extruded flours. Despite native samples presenting higher amounts of free fatty acids, native samples received higher hedonic flavor ratings (Table 5-1). Mattes, 2009 indicated wide variability in human subjects' ability to detect low concentrations of free fatty acids (linoleic, stearic, lauric, and caproic acids). As samples were reconstituted to make porridge, free fatty acid concentration might have decreased below detection levels. This is supported by the comments section of the questionnaire which did not indicate "painty" or "soapy" flavors for extruded flours.

Decortication also played an important role in the assessment of porridge flavor (Table 5-1). Porridge prepared with decorticated native flour received the highest score and this might be attributed to the lower polyphenol content. Studies using whole grain pearl millet and sorghum have shown that tannins and phenolic acids in the pericarp, testa, and aleurone layer contribute to bitterness and astringency (Dykes & Rooney, 2006), and removal of these portions increases flavor liking scores (Rathi et al., 2004; Shobana & Malleshi, 2007). The concentration of phenolic compounds affects bitter intensity in a dose dependent manner. Prior studies using pearl millet have shown that bitter taste perception is directly proportional to the concentration of millet flour in composite products(McSweeney et al., 2016; Tiwari et al., 2014). Despite the flavor improvement observed with decortication, decortication negatively affects nutritional quality since phenolic compounds in whole grains offer protection against the development of chronic and degenerative diseases (van Dokkum et al., 2008). The assessment of flavor is difficult due to its multifactorial nature; here we have discussed the effect of potential flavor compounds responsible for specific flavor notes, however it is important to highlight that flavor compounds can act in synergy/in conjunction to affect flavor scores.

A model to assess the impact of free fatty acid and peroxide concentration on flavor response was performed. None of the factors or their interactions showed a significant effect on flavor response. Figure 5-8 (d) shows consumer acceptability based on texture perception of the samples. Texture is a complex sensory attribute that encompasses an array of factors including smoothness, thickness, and stickiness. The preference map explains 82% of the variance (PC1 60%, PC2 22%) in consumers' opinions. (Fig 3d). In our experiment we adjusted the flour to water ratios

to match porridge viscosity. This was necessary due to changes in hydration power related to extrusion cooking (Nkama & Bulus Filli, 2006). Porridges from native flours received higher scores compared to extruded flour porridge, with decorticated native porridge receiving the highest score (Table 5-1). Adjectives from the comments section of the questionnaire used to describe this sample included phrasing such as “smooth” and “not sticky”. For porridge prepared with whole flours (both native and extruded), participants used descriptors such as “sandy”, “grainy”, “gritty”, and “rough”. The insoluble fiber present in the porridges prepared with whole flour might contribute to the “gritty” texture described by participants. Finally, adjectives used to describe decorticated extruded porridge were “glue-like”, “too smooth”, “pasty”, and “gummy”.

Extrusion of cereal products containing insoluble fiber has been shown to increase hardness and bulk density of the extrudates while increasing peak viscosity (Robin et al., 2015). In our experiment, extrusion might have rendered flours that created porridges with smoother textures, which were disliked by consumers. However, smoother textures are preferred in some parts of Africa, as Moussa et al., 2011, demonstrated that Nigerien consumers prefer sorghum porridges with smooth over more coarse texture; however, their evaluation was only performed on native flours.

Overall, there were differences in participants’ liking of extruded and native samples, with extruded and native samples clustering on opposite sides of the second component axis (Fig 3a-d). This was observed for all the parameters assessed in the study. The same behavior was observed when samples were separated by time points (results not shown). At day 0, extruded and native samples also clustered on opposite sides of the second component axis for liking of color, aroma, flavor, and texture. Similarly, at week 18, extruded and native samples clustered separately except for flavor, for which the porridge prepared with whole extruded flour clustered with the ones prepared with native flours. Further assessment of specific flavor and aroma notes by a descriptive panel might yield valuable information to improve the acceptability of extruded whole millet products.

5.5 Conclusions

Demand for more convenient and nutritious food items is rising among urban consumers in Africa. In this study, we evaluated the use of extrusion cooking as a potential technology to add value and prevent detrimental changes to whole grain pearl millet flours for use as porridges. Our

results demonstrate that small-scale extrusion can be used to extend shelf stability of instant flours made with whole grain pearl millet by preventing the development of products related to hydrolytic rancidity. Nevertheless, extrusion cooking created challenges related to undesirable sensory properties. While extrusion cooking is a versatile technology for whole grain processing, refinement of extrusion conditions and the evaluation of other unit operations (e.g. steeping, germination) in combination with extrusion cooking may be necessary to improve the sensory properties of final products.

5.6 Acknowledgment

Thanks to Anna Hayes for her support editing the paper. This work was made possible by the support of the American People provided to the Feed the Future Innovation Lab for Food Processing and Post-harvest Handling Innovation through the United States Agency for International Development (USAID) under Cooperative Agreement No. AID-OAA-A-13-00047. The contents are the sole responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. We are also grateful to Prof. Mario Martinez, University of Guelph, for his help in setting up the extrusion process.

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Table 5-1. Sensory scores for pearl millet porridges (n=75).

		Week																			
	Treatment	**0**				**4**				**8**				**12**				**18**			
Color	Whole Native	5.50	Aa	±	0.22	5.55	Aa	±	0.22	6.04	Aa	±	0.22	5.21	Aa	±	0.22	5.27	Aa	±	0.22
	Whole Extruded	4.77	Ba	±	0.22	4.60	Ba	±	0.22	4.95	Ba	±	0.22	4.45	Ba	±	0.22	4.12	Ba	±	0.22
	Decorticated Native	6.04	Aab	±	0.22	6.03	Aab	±	0.22	6.20	Aa	±	0.22	5.52	Aab	±	0.22	5.36	Ab	±	0.22
	Decorticated Extruded	4.77	Ba	±	0.22	4.31	Ba	±	0.22	4.52	Ba	±	0.22	4.07	Ba	±	0.22	3.97	Ba	±	0.22
Aroma	Whole Native	5.22	Aa	±	0.19	5.19	Aa	±	0.19	5.39	Aa	±	0.19	4.93	Aa	±	0.19	4.89	Aa	±	0.19
	Whole Extruded	5.55	Aa	±	0.19	5.27	Aa	±	0.19	5.55	Aa	±	0.19	4.84	Aa	±	0.19	5.19	Aa	±	0.19
	Decorticated Native	5.55	Aa	±	0.19	5.35	Aa	±	0.19	5.61	Aa	±	0.19	4.99	Aa	±	0.19	5.05	Aa	±	0.19
	Decorticated Extruded	5.45	Aa	±	0.19	5.01	Aa	±	0.19	5.29	Aa	±	0.19	4.81	Aa	±	0.19	4.96	Aa	±	0.19
Flavor	Whole Native	4.77	Ba	±	0.23	4.16	BCab	±	0.22	4.73	ABab	±	0.22	3.97	ABab	±	0.22	3.89	Ab	±	0.22
	Whole Extruded	4.62	Ba	±	0.23	4.71	ABa	±	0.22	4.63	Ba	±	0.22	3.85	Ba	±	0.22	3.99	Aa	±	0.22
	Decorticated Native	5.76	Aa	±	0.23	5.13	Aab	±	0.22	5.21	Aab	±	0.22	4.55	Ab	±	0.22	4.44	Ab	±	0.22
	Decorticated Extruded	4.32	Ba	±	0.23	4.11	Cab	±	0.22	3.87	Cab	±	0.22	3.43	Bb	±	0.22	3.28	Bb	±	0.22
Texture	Whole Native	5.24	Ba	±	0.24	5.03	ABa	±	0.24	5.32	Aa	±	0.24	4.60	Aa	±	0.24	4.63	ABa	±	0.24
	Whole Extruded	4.22	Ca	±	0.24	4.91	Ba	±	0.24	4.92	Aa	±	0.24	4.19	Aa	±	0.24	4.07	Ba	±	0.24
	Decorticated Native	5.99	Aa	±	0.24	5.56	Aab	±	0.24	5.56	Aab	±	0.24	4.71	Ab	±	0.24	4.79	Ab	±	0.24
	Decorticated Extruded	3.32	Da	±	0.24	3.51	Ca	±	0.24	2.85	Ba	±	0.24	2.75	Ba	±	0.24	2.93	Ca	±	0.24

Uppercase letters indicate differences between samples at each time point (e.g. at week 0 scores for color are significantly different between decorticated native > whole native, $p<0.0001$).
Lowercase letters denote differences between time points for the same sample (e.g. color scores for whole native flours at week 0 are not significantly different compared to whole native flour stored for 18 weeks, $p=0.0565$).
± SEM, Standard error of the mean.

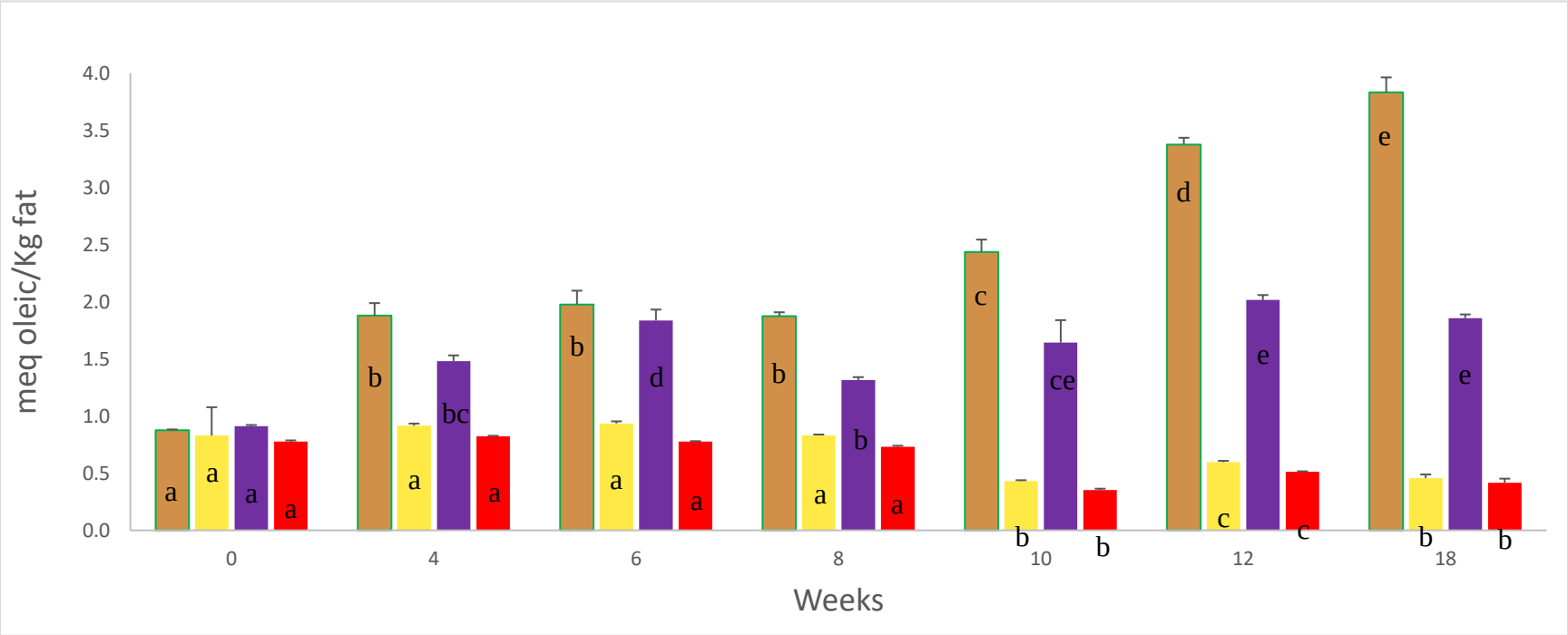

Figure 5-1. Concentration of free fatty acids, representing hydrolytic rancidity, for native and extruded whole grain and decorticated pearl millet flours over an 18-week period. Whole grain native (green), whole grain extruded (yellow), decorticated native (purple), decorticated extruded 20°C (red). All samples were stored at 20 °C. Letters represent Tukey mean differences for repeated measures within treatments over time points.

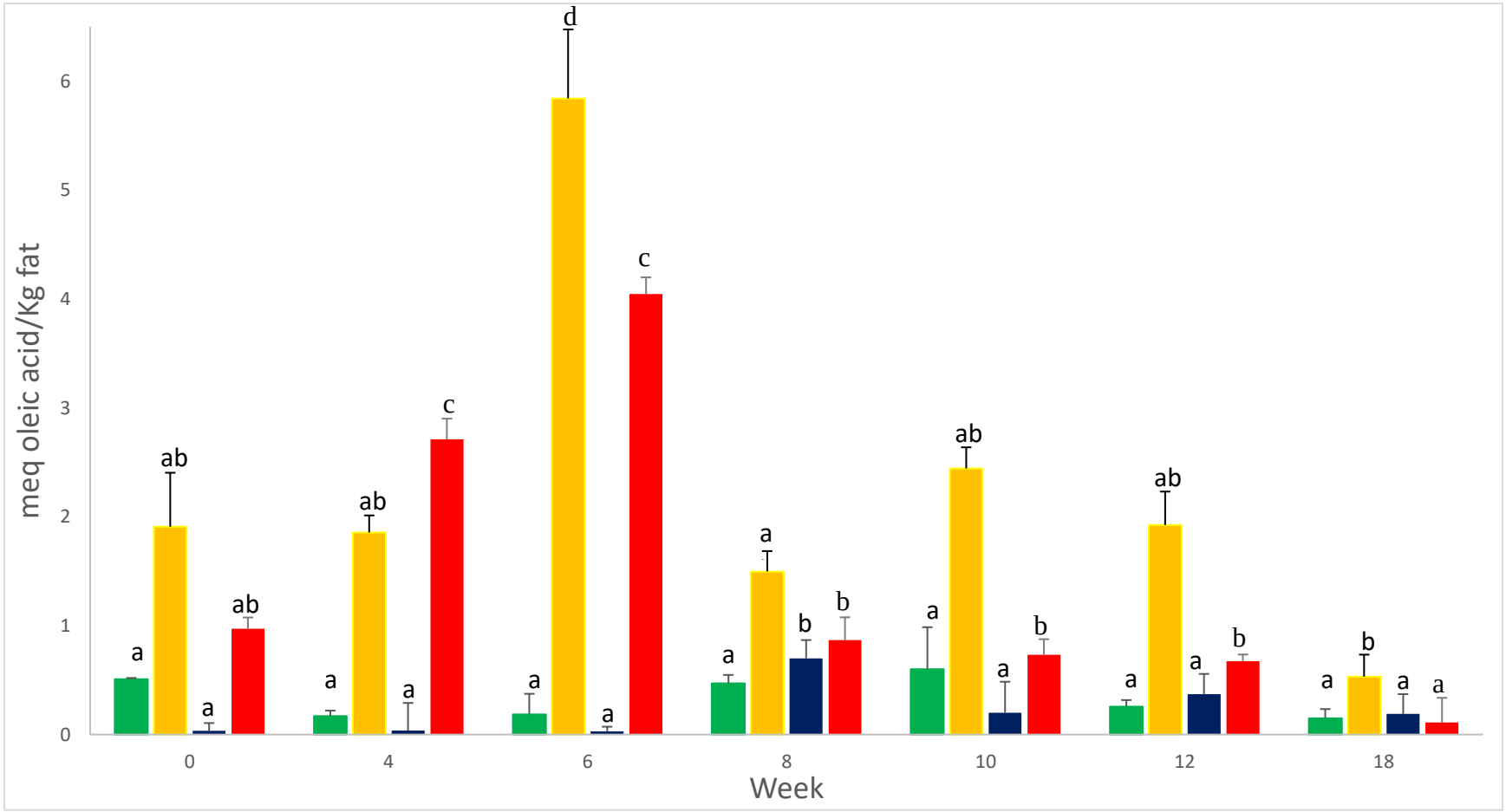

Figure 5-2. Concentration of peroxides, representing oxidative rancidity, for native and extruded whole grain and decorticated pearl millet flours over an 18-week period. Whole grain native (green), whole grain extruded (yellow), decorticated native (purple), decorticated extruded 20°C (red). All were stored at 20 °C. Letters represent Tukey mean differences for repeated measures within treatments over time points.

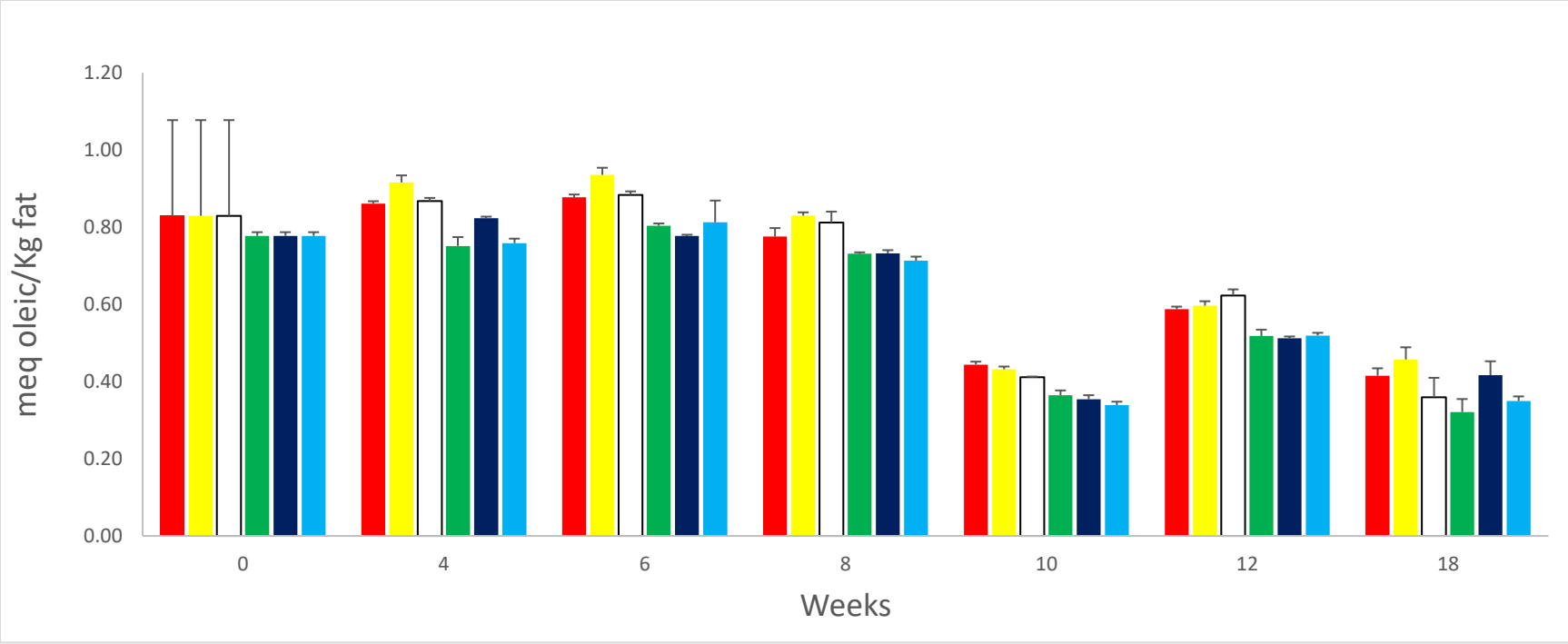

Figure 5-3. Concentration of free fatty acids, representing hydrolytic rancidity under retarded (4°C), standard (20°C), and accelerated (35°C) storage conditions, for extruded whole grain and extruded decorticated pearl millet flours stored over an 18-week period. Whole grain extruded 20 °C (yellow), whole grain extruded 4 °C (red), whole grain extruded 35 °C (white), decorticated extruded 20°C (purple), decorticated extruded 4°C (green), decorticated extruded 35°C (light blue). No mean differences were found for repeated measures within treatments over time points.

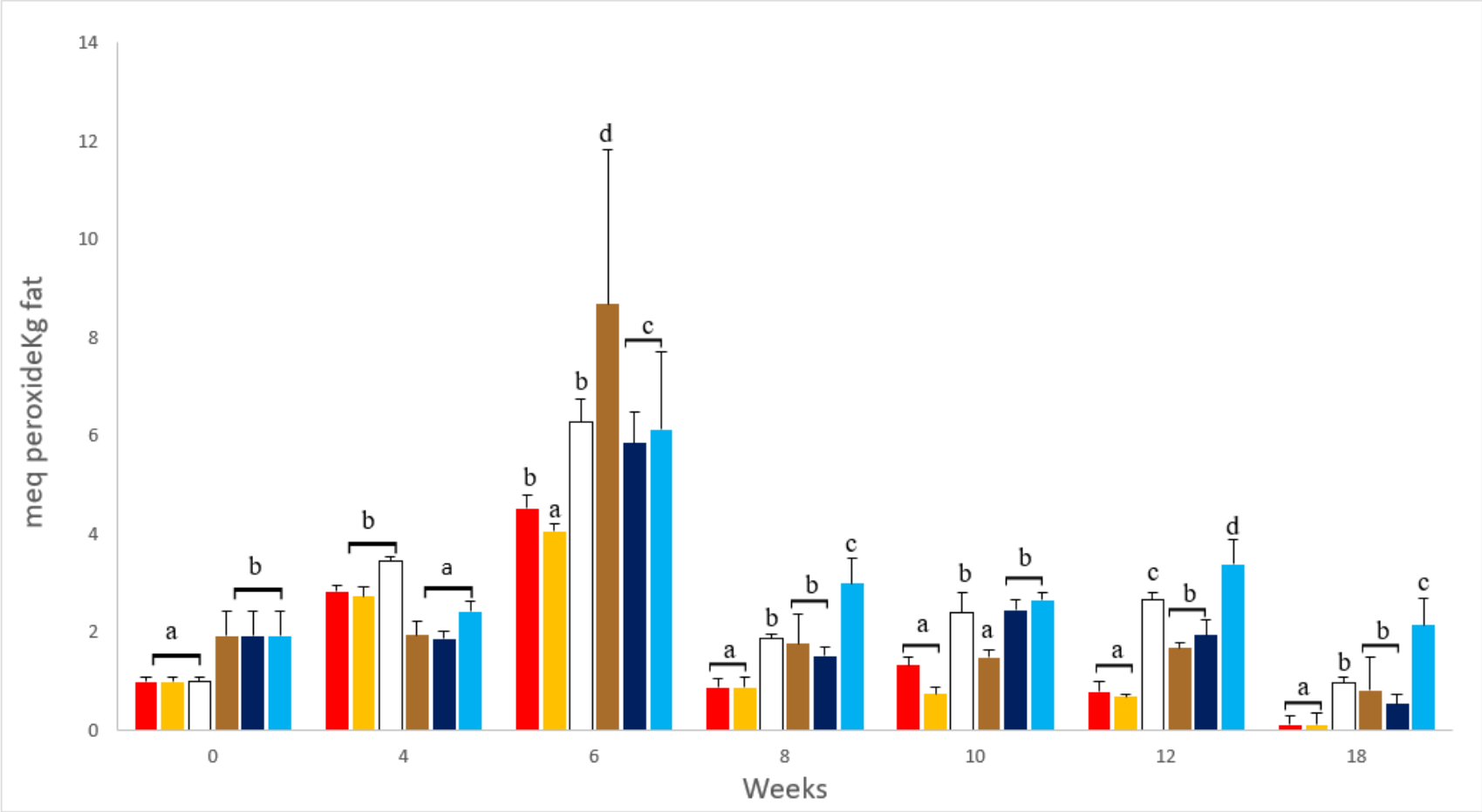

Figure 5-4. Concentration of peroxides, representing oxidative rancidity under retarded (4°C), standard (20°C), and accelerated (35°C) conditions, for extruded whole grain and extruded decorticated pearl millet flours stored over an 18-week period. Whole grain extruded 20 °C (yellow), whole grain extruded 4 °C (red), whole grain extruded 35 °C (white), decorticated extruded 20°C (purple), decorticated extruded 4°C (green), decorticated extruded 35°C (light blue). Letters represent Tukey mean differences for repeated measures within treatments over time points.

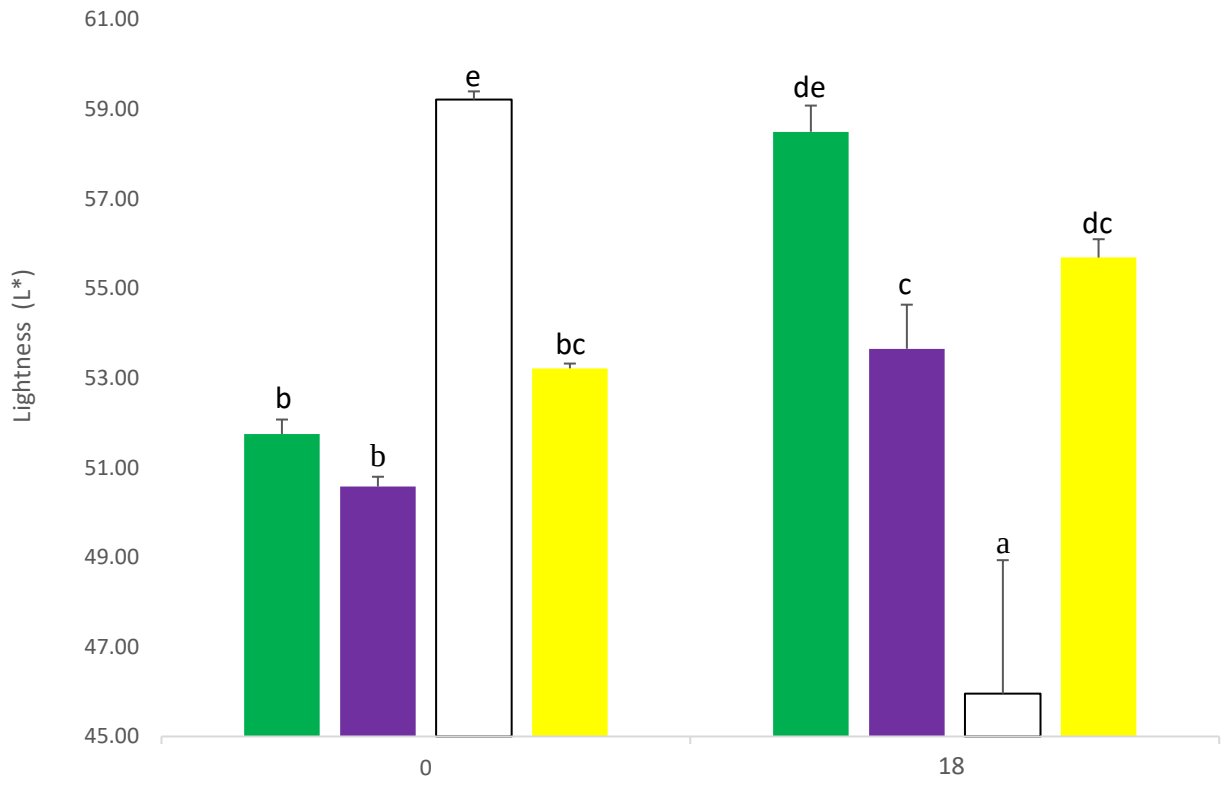

Figure 5-5. Lightness (L*) value changes of porridges prepared with flours made from native and extruded whole grain and decorticated pearl millet flours stored over an 18-week period. Whole grain native (green), whole grain extruded (purple), decorticated native (white), decorticated extruded 20°C (yellow). All were stored at 20 °C. Letters represent Tukey mean differences for repeated measures within treatments.

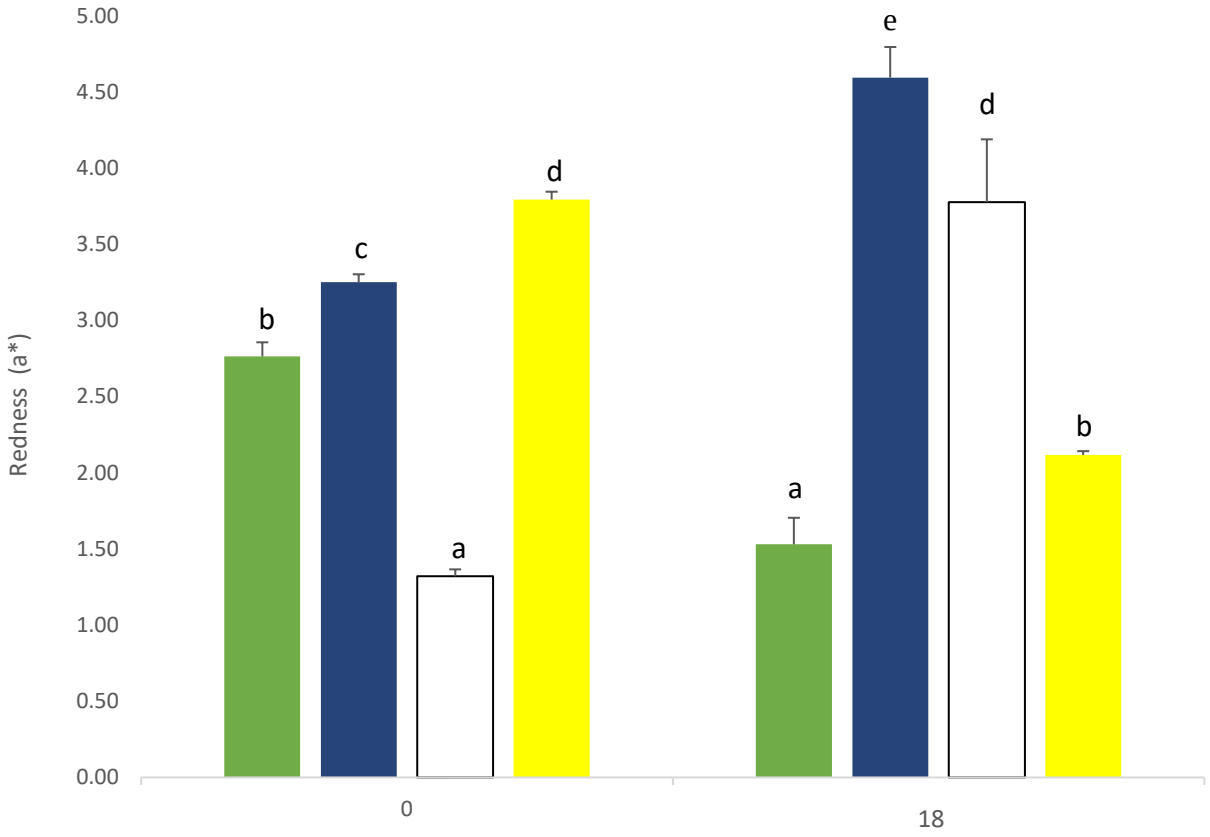

Figure 5-6. Redness (a*) value changes in porridges prepared with flours made from native and extruded whole grain and decorticated pearl millet flours stored over an 18-week period. Whole grain native (green), whole grain extruded (purple), decorticated native (white), decorticated extruded 20°C (yellow). All were stored at 20 °C. Letters represent Tukey mean differences for repeated measures within treatments.

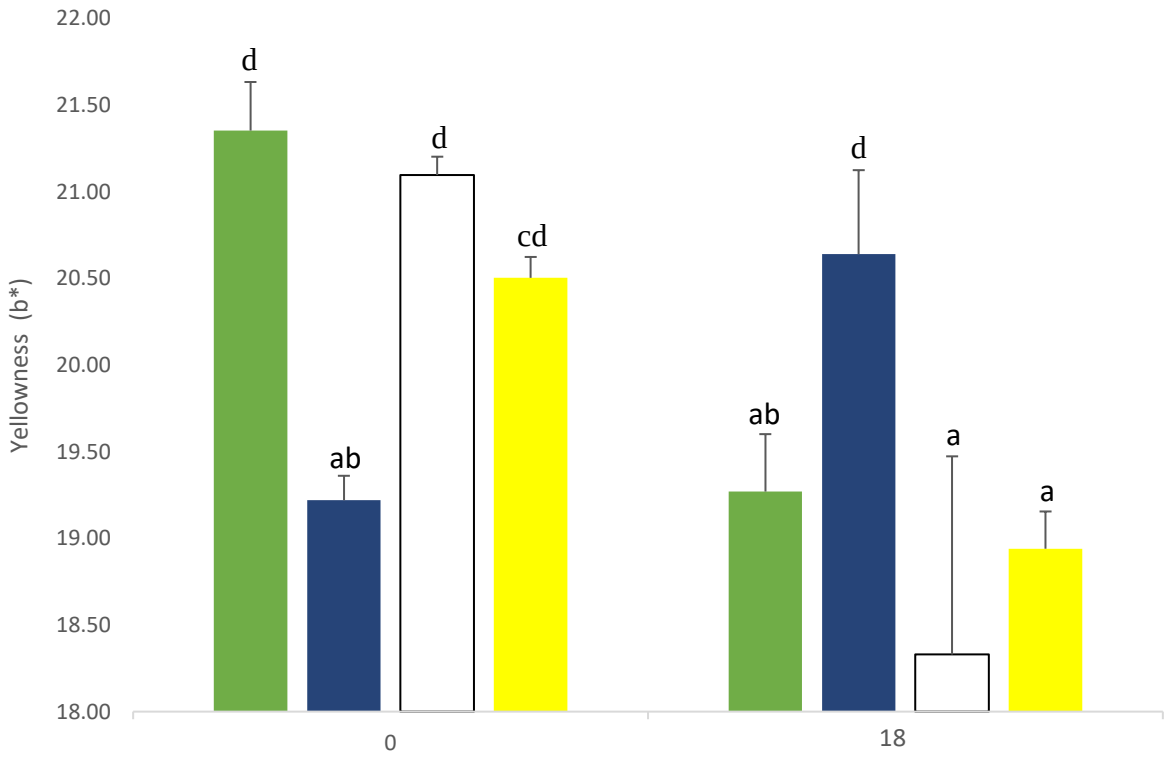

Figure 5-7. Yellowness (b*) value changes in porridges prepared with flours made from native and extruded whole grain and decorticated pearl millet flours stored over an 18-week period. Whole grain native (green), whole grain extruded (purple), decorticated native (white), decorticated extruded 20°C (yellow). All were stored at 20 °C. Letters represent Tukey mean differences for repeated measures within treatments.

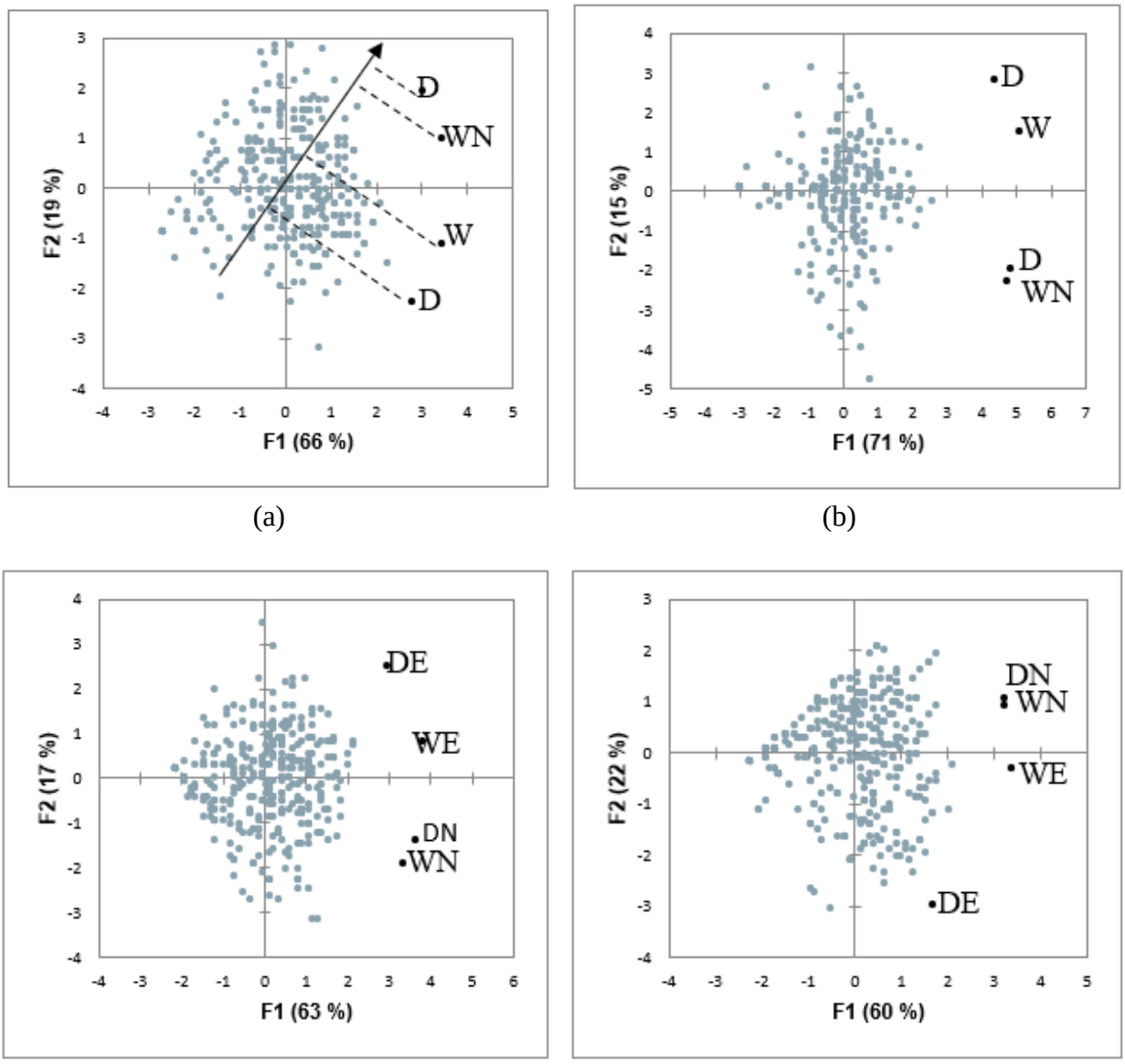

Figure 5-8. Internal preference maps of liking ratings for (a) color, (b) aroma, (c) flavor, and (d) texture of pearl millet porridges. Principal Component Analysis (PCA) of the sensory profiles of the porridges prepared from decorticated native (DN), decorticated extruded (DE), whole native (WN) and whole extruded (WE) flours stored from 0 to 18 weeks. The vectors indicate the loadings (assessment of variance) for sensory attributes while the position of the sample codes indicate the score values.

CHAPTER 6. DIFFERENCES EXTRUSION OF WHOLE GRAIN PEARL MILLET CONTRIBUTES TO THE FORMATION OF AMYLOSE-LIPID COMPLEXES THAT ARE SLOWLY DIGESTIBLE

6.1 Abstract

Pearl millet (*Pennisetum glaucum*) is one of the most promising but underutilized crops in Sub-Saharan Africa. Herein we evaluated the use of extrusion cooking for whole pearl millet processing related to the formation of amylose-lipid complexes and their potential effect on slowing starch digestion. Thermal properties (differential scanning calorimetry), fatty acid profiles (derivatization followed by gas chromatography), amylose-lipid complex dissociation (hexane extraction followed by derivatization and gas chromatography), and *in vitro* digestibility (α-amylase digestion assay) were determined for whole and decorticated pearl millet flours in both native and extruded states. Extrusion cooking melted stable type-VII complexes naturally present in native flours into Type-VI complexes characterized by ΔH (enthalpy) in the 82-112°C range. Extrusion caused the formation of amylose-lipid complexes containing mono- and polyunsaturated fatty acids, which were not found in native flours. Finally, extruded whole and decorticated instant flours exhibited a delayed release of reducing sugars compared to cooked native samples, although this effect was lost when the extruded samples were cooked.

6.2 Introduction

The prevalence of obesity and related comorbidities has steadily risen in the African continent as sociocultural and economic shifts have prompted changes in dietary patterns and physical activity (Popkin et al., 2012b). Globalization of the food supply chain, urbanization, and increased household income have allowed greater access and consumption of foods distinctive of a Western diet, including fried foods, animal source products, and refined carbohydrates, thus replacing traditional diets characterized by high consumption of indigenous grains and tubers, legumes, fruits, and vegetables. As middle and upper classes grow and young consumers gain purchasing power, the demand for shelf stable, convenient and easy to prepare foods and beverages is expected to rise (Signé et al., 2018).

The African food-processing sector is still at an early stage of development; and most locally-grown staple food products are sold in local markets as raw ingredients such as flours, still requiring time and labor for preparation. In order to overcome this limitation, non-governmental organizations, multilateral financing institutions and development agencies have invested in local processors' technical capabilities as a strategy to cover the growing demand for high-quality, affordable, and nutritious products. Emerging local companies are incorporating technologies, such as low-cost extrusion cooking, for the processing of staple grains and legumes (Technoserve, 2017). During extrusion cooking, high temperature and shear melt and gelatinize the crystalline structure of native starch granules, changing rheological and hydration properties to generate instant flours (Martínez, Calviño, et al., 2014; Martínez, Rosell, et al., 2014).

As starch is gelatinized, amylose chains gain enough mobility and a percentage of them interact with ligands including free fatty acids or mono-acyl glycerol, generating products with slow digesting property, termed amylose-lipid complexes (ALCs) (Putseys et al., 2010). In ALCs, amylose forms a single, left-handed helix encapsulating the aliphatic tail of long chain fatty acids, known as Type-I ALC. As these amorphous complexes reorganize, they form more stable, larger crystallites with higher melting temperatures, known as Type-II ALCs (Panyoo & Emmambux, 2017; Putseys et al., 2010). The rate and extent of amylolytic digestion of starch in these complexes depends on a variety of factors. For Type-I ALCs, fatty acid chain length, fatty acid degree of unsaturation, and stereochemistry (cis-trans configuration) determine degree of hydrolysis. For Type-II ALCs, the organization of helices into larger, aggregated structures drives their lower rate of starch digestion (Putseys et al., 2010). The diversity of lipid ligands in ALCs creates nano- and micromolecular structures, that additionally affect starch digestion rate (Lesmes et al., 2009). While extensive research has been performed on ALCs from isolated materials using model systems (Putseys, Lamberts & Delcour, 2010; De Pilli, Derossi, Talja, Jouppila, & Severini, 2011; Seo, Kim & Lim, 2015; Wang, Wang, Yu & Wang, 2016), there is limited applicability of this research using whole grains. We speculate that extrusion of whole grains with inherently diverse lipid profiles may generate an array of ALCs compared to refined grains

Recent studies have proposed a relationship between consumption of ultra-processed, highly refined foods and high caloric intake and weight gain, and intermediate risk factors for non-communicable diseases in adults (Hall et al., 2019b; Lane et al., 2021). Specific attributes of foods such as slow digestion rate may exert protective effects against the development of such metabolic

conditions. The benefits of slow digesting carbohydrates on glycemic management are established (Miao et al., 2015).

The most common staple food across sub-Saharan Africa is thick porridge, which involves gelatinizing the raw starch in grains or tubers. The starch source in thick porridge varies from geographic location with pearl millet (*Pennisetum glaucum*) and sorghum comprising the principal grains of the semi-arid regions of Africa, and particularly the Sahel. In 2020, Africa produced 13.8 million metric tons of pearl millet grain, and most of it was commercialized in local markets with minimal added value (FAOSTAT, 2022). Compared to other carbohydrate sources, pearl millet offers agronomic and nutritional advantages, because it is resistant to adverse environmental conditions, such as low rainfall and intense heat. Iit is also suitable for growing in soils with low fertility. In addition, its short growing season and ability to resist pests and disease allows pearl millet to be a staple crop in low-income populations (Saleh et al., 2013b).

In this study, we evaluated the formation of amylose-lipid complexes (ALCs) in whole grain pearl millet extruded flours using a low-cost small-scale extruder suited for entrepreneur processors in Africa. Our objective was to evaluate the characteristics of the complexes and assess their ability to modulate starch digestion.

6.3 Materials and Methods

6.3.1 Materials

Whole pearl millet kernels were obtained from Cisco Co. (Indianapolis, IN, USA). PET/PE bags were purchased from ProAmpac (Cincinnati, OH, USA). Porcine pancreatic α-amylase (Sigma A6255), 3-(N-morpholino)-propanesulfonic acid sodium (MOPS) salt, 3,5-dinitrosalicylic acid (DNS reagent), and dodecanoic acid as an internal standard were purchased from Sigma-Aldrich (St. Louis, MO, USA).

6.3.2 Preparation of whole and decorticated pearl millet flours

Pearl millet kernels were debranned in an electric abrasive decorticator (Natana Machine Ltd, Saskatoon, Canada) for 15 min to obtain a 12% decortication yield (88% weight difference). Bran and endosperm were separated by sieving, collecting the fraction that passed through a mesh of 300 μm. For the whole millet sample, whole millet grains were ground to grits using a pin mill

(Alpine American Corp., Natick, MA, USA). Decorticated kernels were processed in similar fashion.

6.3.3 Extrusion of flours

In the present work, a 35 kg/h small-scale single screw extruder with restrictions on the screw (Technochem Inc., Boone, Iowa, USA) was used. This extruder was developed at Purdue for the National Aeronautical and Space Administration (NASA) and its operating conditions have been published elsewhere (Ponrajan, 2016). The design of the extruder was based on the scale-down of a 300 kg/h extruder by Insta-Pro International (Des Moines, Iowa, USA). A preliminary study was carried out to optimize the extrusion conditions. Whole pearl millet kernels were pin-milled to grits (Alpine American Corp., Natick, MA, USA) before extrusion. Whole and decorticated pearl millet grits were equilibrated for 12 h at 4^{o}C to 30 and 32% moisture content prior to extrusion. Extruder temperature was measured using a thermocouple (HH309A 4-channel data logger thermometer, Omega Engineering Inc., Stamford, Connecticut, USA) placed before the exit at the die opening. The equilibrium temperature of the extrudates was above 120°C. The screw speed (700 rpm) was measured using a tachometer (HHT13, Omega Engineering Inc., Norwalk, CT, USA) by fixing a laser reflective tape on a pulley attached to the extruder shaft behind the feeding section of the extruder. The length to diameter (L/D) ratio and die hole for the extruder were 40:1 and 6 mm, respectively. Extruded material was then dried by convection air at 50°C until no weight changes were detected (Blue M Oven, Blue Island, IL, USA) and then ground with a roller mill (~150 µm particle size) (Allis Chalmers, WI, USA). Extruded flours were stored in PET/PE bags at room temperature until further analysis.

6.3.4 Thermal properties of native and extruded pearl millet flours.

Thermal properties were measured using a differential scanning calorimeter DSC-Q2000 (TA instruments, New Castle, Delaware, USA), with high volume pans (TA instruments, New Castle, Delaware, USA). The equipment was calibrated with indium and an empty pan was used as the reference. Flour (6 mg) and water (18 µL) were loaded into the pan and hermetically sealed. Samples were allowed to stand for 1 h at room temperature for equilibration before analysis. Samples were kept at 30°C for 2 min and heated from 30 to 180^{o}C at 10^{o}C/min. Onset (T_o), peak

(T_p), and conclusion temperatures (T_c) as well as the enthalpy (ΔH_g, expressed as J/g of sample) were calculated for amylopectin gelatinization and the melting of the amylose-lipid complexes. All tests were run in duplicate per sample type.

6.3.5 Determination of Fatty Acid Profile

Extracted oil from pearl millet was derivatized to fatty acid methyl esters (FAME) as described by Kiefer (1997), and then analyzed with gas chromatography according to AOAC Official Method 996.06 (AOAC, 2005). A gas chromatograph with a flame ionization detector (GC-2010, Shimadzu, Kyoto, Japan) was used to analyze fatty acid composition, with a polar ionic liquid column SLB-IL60 30 m x 0.25 mm x 0.20 μm (Sigma-Aldrich, St. Louis, MO, USA). Helium was used as the carrier gas with a flow rate of 1 ml/min. The column temperature was set from 150 to 280°C, at a heating rate of 5°C/min. A volume of 1 μl was injected, with a split mode 50:1. Supelco 37-component FAME mix was used as an external fatty acid standard to identify components (10 mg/ml; Sigma-Aldrich, St. Louis, MO, USA). Dodecanoic acid (Sigma-Aldrich, St. Louis, MO, USA) was added as an internal standard.

6.3.6 Amylose-lipid complex dissociation and characterization of FAs

Dried native and extruded samples were defatted using hexane in a Soxhlet extractor for 8 h. Defatted samples were dried overnight at 50^{o}C in a convection oven. To corroborate total free lipid extraction, hexane was added to defatted flours at 10:1 proportion and centrifuged for 15 min at 3000 rpm (Avanti J25I, Beckman Coulter, Brea, CA, USA). Supernatants were decanted, dried, and derivatized to determine fatty acid methyl ester residues (Kiefer, 1997). None of the samples contained residual free fatty acids. Fifty (50) mg of defatted flour was suspended in 10 mL of MOPS buffer (50 mM, pH 7). The mixture was incubated at 125^{o}C with constant stirring for 30 min. After the final incubation time was reached, the mixture was immediately placed in ice. The mixture was washed three times with hexane (40 mL). Supernatants were collected, centrifuged, and dried at 20^{o}C. Fatty acids liberated from the amylose-lipid complexes were derivatized to fatty acid methyl esters (FAME) and identified using the procedure described above (Kiefer K., 1997).

6.3.7 *In vitro* starch digestibility

Enzymatic digestion was performed on native and extruded samples. A second set of both native and extruded samples was thermally treated at 100°C for 20 min with gentle stirring prior to digestion of α-amylase. Hydrothermal treatment was performed to demonstrate the effect of fully gelatinized starch on glucose release of pearl millet flours. Enzymatic digestion was carried out using 3.7 units of porcine pancreatic α-amylase (A6255, Sigma-Aldrich, St. Louis, MO, USA) per 50 mg of flour. Flour (50 mg) was suspended in 9 mL of MOPS buffer (50 mM, pH 7). The mixture was incubated for 10 min at 37°C with constant stirring at 350 rpm with a 3×6 mm magnetic stirrer bar. At defined time intervals, 50 mL of aliquot was immediately placed in boiling water to stop the enzymatic reaction. The supernatant was used to determine the reducing sugar content compared to a maltose standard using the DNS method (Edwards et al., 2014; Miller, 1959). Samples were collected up to 60 min of amylolysis. Total area under the curve was used to quantify digestion products through determination of reducing sugars. To assess the content of endogenous reducing sugars, the addition of α-amylase was omitted for control assays (no hydrothermal treatment), showing that the amount of reducing sugar present in these samples was negligible. Tests were run in triplicate.

6.3.8 Statistical analysis

Analysis of variance was used to determine the effects of decortication and extrusion of whole grain flours on ALC characteristics (thermal properties, FA composition) as well as enzymatic digestibility of the samples. Tukey's multiple comparison test for significant differences was used to calculate the means and 95% confidence intervals. Statistical analysis was performed with SAS 9.3 for Windows (SAS Institute, Inc., Cary, NC, USA).

6.4 Results and discussion

6.4.1 Effect of extrusion on the thermal properties of pearl millet flours

The formation of amylose-lipid complexes (ALCs) during extrusion has been extensively studied in model systems (e.g. C16, C18, C18:1, C18:2 and isolated amylose) (Putseys et al., 2010; Seo et al., 2015), and some studies have been done using extrusion to create ALCs (Cervantes-Ramírez et al., 2020; de Pilli et al., 2011; Panyoo & Emmambux, 2017; Thachil et al., 2014). *In*

vivo and *in vitro* models of ALC dissociation have shown their capacity to modulate glucose release (Holm et al., 1983). In this study, we evaluated the physicochemical changes derived from ALC formation, preferential fatty acid uptake of ALC in a whole grain matrix and its potential nutritional effect using an *in vitro* digestion model as an indicator of postprandial glucose release.

Table 6-1 shows the effect of extrusion treatment on the thermal properties of pearl millet flours. Native pearl millet flours displayed an endothermic transition at around 67^{o}C, corresponding to starch gelatinization, and this peak was not observed in extruded flours, indicating that full gelatinization was achieved during extrusion. Decorticated pearl millet flour starch displayed lower enthalpy, T_c and T_c-T_0 than the whole flour counterpart. Morrison, Tester and Gidley (Morrison et al., 1994) reported how the mechanical energy obtained during ball-milling can disrupt the structure of wheat and maize starches, and it is possible that the abrasive forces during decortication could account for the differences in the results. Abrasive forces could decrease starch crystallinity and double-helix content and/or break the continuity of the more ordered crystalline layers, facilitating gelatinization (less energy required for disordering crystalline lamellas) (Tran et al., 2011).

The other two endothermic transitions detected in millet flours correspond to Type-I and Type-II ALCs, with melting temperatures about 98 and 120^{o}C, respectively (Table 6-1). High concentrations of ALCs in millet flour have been reported (Annor et al., 2017). Decorticated native flours presented lower enthalpy for the melting of Type-II amylose-lipid complexes compared to whole native flours, and conversely showed higher enthalpy for the melting of Type-I ALCs when compared to their native counterparts. This indicates that during decortication, the most stable Type-II complexes are partially disrupted leading to of the less stable Type-I complexes. Likewise, extrusion led to total disruption of the more stable Type-II complexes and the formation of Type-I complexes, especially for extruded whole flours.

Dissociation temperatures of ALCs in whole extruded flours ranged from 82.2 to 112.8°C (Table 6-1). The wide temperature range for the complexes present in whole extruded flours relates to the higher concentration of ALC as well as greater diversity of ALC species present in the sample. A narrower range of 82.6 to 95.2°C was measured for decorticated extruded flours, related to the formation of fewer, more homogenous ALCs.

The differences observed in dissociation temperatures are attributed to ALC lipid chain length and degree of unsaturation of the fatty acid present in the hydrophilic cavity. As fatty acid

chain length increases, so does ALC dissociation temperature. For instance, the dissociation temperature for ALC containing palmitic acid (C16:0) ranged between 81.8 to 94.5, while for stearic acid (C18:0) ranges between 83.7 to 95.7 (Seo et al., 2015). Higher dissociation temperatures are attributed to the lower hydrophilicity of longer lipid chains, resulting its stronger hydrogen bonding within the hydrophobic helix cavity (Putseys et al., 2010). Similar effects in dissociation temperature are observed due to degree of unsaturation, with higher thermal stability for saturated fatty acids over mono- and polyunsaturated fatty acids (Eliasson & Krog, 1985; Panyoo & Emmambux, 2017; Putseys et al., 2010). Molecular dynamics largely favor linear chains over bent molecules in model systems by facilitating full inclusion in the hydrophobic helix cavity and greater steric hindrance (Panyoo & Emmambux, 2017; Putseys et al., 2010; Yamada et al., 1998).

The wider enthalpy range in whole extruded flours indicates a larger structural heterogeneity of Type-I amylose-lipid complexes. De Pilli and et al. (2012) reported the formation of diverse amylose-lipid complexes in rice starch-nut flour models during extrusion. The authors emphasized that differences in fatty acid length contributed to the amount and heterogeneity of the Type-I complexes (de Pilli et al., 2012), which can explain the greater enthalpy and heterogeneity (T_c-T_0) for whole extruded compared to decorticated extruded flour observed in our study. In particular, the higher enthalpy for ALCs in whole extruded flours may be due to the higher concentration of lipids present in whole pearl millet flours, since the germ is the major site of lipid storage in the grain.

During cereal grain extrusion, high-shear and temperature gelatinize the starch in flours and denature proteins networks. Previous research has shown that changes in triglyceride structure at that temperature, specifically cleavage of ester bonds, are minimal [enthalpy of ester bond dissociation is significantly higher than the energy achieved during extrusion (~400 kJ/mol) (El-Nahas et al., 2007). This is of particular importance since the presence of free fatty acids is a limiting factor for the formation of ALCs. Amylose preferentially complexes with free fatty acids compared to monoglycerides (Tufvesson et al., 2003), therefore pre-existing free-fatty acids present in the flour matrix will be complexed during extrusion, with limited release of new fatty acids occurring during the extrusion process.

6.4.2 Fatty acid composition of pearl millet flours

Table 6-2 shows the fatty acid composition of the fat fraction of pearl millet flour, extracted from whole and decorticated native flours and the whole and decorticated extruded flours. The decortication process removed 26-29% of the total content of fat from the whole grain. The fatty acid composition of the fat extracted from native pearl millet flours was mainly comprised polyunsaturated fatty acids, comprised of 49-51% of linoleic acid (C18:2) and 3.3% of linolenic acid for whole and decorticated native flour. The high concentration of unsaturated fatty acids makes these native flours susceptible to lipid oxidation. Linolenic acid (C18:3) is oxidized 2.4 times faster than linoleic acid (C18:2), and the latter 40 times faster than oleic acid (C18:1) (Labuza & Dugan, 1971). Lipid profiles for pearl millet flours similar to the ones found in our study have been previously reported (Rooney, 1978). Similar to other grains, pearl millet flours usually present a high degree of mono- and polyunsaturated fatty acids, ranging from C16 to C20.

6.4.3 Identification of fatty acids liberated after thermal treatment

The amount of fat extracted from the extruded flours was significantly lower than the amount extracted from the native flours ($p<0.05$) (Table 6-2), with 39-44% of the fat content remained in the extruded flours. This reduction is likely due to the formation of ALCs, where the long hydrocarbon chain of fatty acids is embedded into the hydrophobic helix cavity of the amylose and is unextractable in its crystalline form (Lalush et al., 2004; Singh et al., 2007).

Differences in fatty acid profiles between whole grain native and whole extruded flours show a significant reduction in linoleic acid content (C18:2) (Table 6-2). Table 6-3 shows fatty acid profiles of ALCs found in the extruded flours. It has been reported that ALCs are preferentially formed in the presence of longer chain fatty acids and that the addition of double bonds in fatty acids (unsaturation) interrupts the Type-I amylose complex formation (Eliasson & Krog, 1985; Zabar et al., 2009, 2010). However, affinity for polyunsaturated fatty acids to complex with amylose is also dependent on processing conditions and not just the degree of unsaturation (de Pilli et al., 2011; Seo et al., 2015).

6.4.4 Starch digestibility of native and hydrothermally-treated (cooked) pearl millet flours

The susceptibility of native and extruded pearl millet flours to enzymatic amylolysis was assessed by quantification of reducing sugars (expressed in maltose equivalents). *In vitro* digestibility of samples was assessed in native and extruded flours (Figure 6-1 (A): native with α-amylase; Figure 6-1 (B): extruded with α-amylase); and after cooking at 100^{o}C for 20 min (Figure 6-1 (C): native; Figure 6-1 (D): extruded; both with α-amylase). Area under the curve (AUC) was used to assess total reducing sugar release and is expressed as total maltose equivalents (maltose Eq) released in 60 min. All decorticated samples presented higher total reducing sugar release compared to their whole grain counterparts. Decorticated (AUC 377.4±9.1 maltose Eq) and whole extruded (AUC 360.7±11.4 maltose Eq) cooked flours presenting the highest AUC. As expected, neither whole native (AUC 27.2±2.3 maltose Eq) nor decorticated native flours (AUC 22.5±1.8 maltose Eq) significantly released reducing sugars after 60 min of α-amylase digestion as shown in Figure 6-1 (A). Reduced ability of the enzyme to physically access native starch granules produces less significant reducing sugars release (Tester et al., 2006; G. Zhang et al., 2006). The total amount of reducing sugar released in decorticated cooked flours (AUC 281.5±58.0 maltose Eq) was slightly, though significantly, higher compared to its whole grain counterpart (AUC 254.3±51.5 maltose Eq). During hydrothermal treatment, the crystalline regions in native starch are melted and granule architecture is permanently lost which subsequently facilitates enzymatic hydrolysis (S. Wang & Copeland, 2013).

Extrusion cooking affected starch digestibility of both whole grain (AUC 215.7±33.1 maltose Eq) and decorticated flours (AUC 253.7±41.1 maltose Eq), however the change in digestibility was only significant for whole grain extruded flour ($p=0.0064$) (Figure 6-1 (B)). Total reducing sugar release for decorticated extruded flour (AUC 253.7±41.1 maltose Eq) was slightly lower and not statistically different ($p=0.34$) from cooked whole grain native flour (AUC 254.3±51.5 maltose Eq). The decrease in digestibility in whole grain extruded flour was attributed to the formation of ALCs. It is suggested that ALC's compact structure decreases hydrolytic α-amylase activity due to inability to access α-(1-4) glycosidic bonds (Panyoo & Emmambux, 2017). A human study suggested that postprandial glucose regulation related to ALCs relied on starch indigestibility by the formation of resistant starch, therefore completely preventing enzymatic digestion (Hasjim et al., 2010). The authors showed a significant reduction of postprandial plasma glucose and insulin responses in humans by consumption of Type-II (palmitic acid) ALCs.

Because we still observed a release of reducing sugars for extruded flours, the results from our experiment indicate that the lower AUC values of ALC-containing extruded flours relate to a slower digestion rate rather than the formation of resistant starch. Even through the extrusion conditions used in this study were sufficient to promote the formation of Type-II ALC polymorphisms, we only found Type-I complexes in extruded flours which may have a slow rather than resistant digestion property.

Digestibility of extruded samples might also relate to the type of fatty acid present in ALCs. Evidence suggests that ALCs containing unsaturated fatty acids display unique morphological characteristics at micro- and nanoscopic levels that could help modulate enzymatic digestion (Lesmes et al., 2009). After dissociation of the amylose-lipid complexes present in defatted flours, the whole extruded sample contained 39.1% of unsaturated fatty acids while decorticated flours had 43.2%. The same authors found that fatty acids with greater degree of unsaturation formed larger ALCs which were less susceptible to enzymatic degradation. This supports our findings related to reducing sugar AUCs, as polyunsaturated fatty acids accounted for most of the species released after dissociation in whole extruded and decorticated extruded flour. These samples presented the lowest AUC. Finally, cooked extruded flours displayed the highest release of reducing sugars, indicating re-melting of the amylose-lipid complexes erases the potential benefits of ALCs regarding modulation of digestion rate.

6.5 Conclusions

Amylose-lipid complex formation, thermal properties, and enzymatic hydrolysis of extruded flours containing ALCs were investigated. Extrusion of the whole pearl millet grain food matrix produced flours with a significant concentration of ALCs compared to native flours. Lipid reduction in extruded flours was attributed to lipid complexation with amylose following a rapid cool-down, promoting fast nucleation and subsequent formation of Type-I ALCs. Endothermic transitions corresponded only to Type-I ALCs, with melting temperatures between 82 to 112^{o}C. Subsequent dissociation of ALCs present in extruded flours demonstrated a significantly high concentration of mono- and polyunsaturated fatty acids. Prior studies have demonstrated that ALCs are preferentially formed in the presence of long, unsaturated fatty acids, however amylose-lipid complexation depends also on processing conditions. Starch digestion tests suggest moderated reducing sugar release. This effect was attributed to the compact structure of ALCs and

inability of enzymes to access substrate. The unique chemical composition of the ALCs present in extruded flours makes them good candidates for slowly digestible functional foods.

6.6 Declaration of interest

All authors declare no financial or personal interest, relationships with other people or organizations that could inappropriately influence or bias the results of the current study.

6.7 Acknowledgement

This work was made possible by the support of the American People provided to the Feed The Future Innovation Lab for Food Processing and Post-harvest Handling Innovation through the United States Agency for International Development (USAID) under Cooperative Agreement No. AID-OAA-A-13-00047. The contents are the sole responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government.

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Table 6-1. Effect of extrusion treatment on the thermal properties of pearl millet flours.

		Whole Native	Whole Extruded	Decorticated Native	Decorticated Extruded
Starch gelatinization	To	66.7±0.2[a]	nd	67.3±0.3[a]	nd
	Tp	72.2±0.3[a]	nd	72.6±0.3[a]	nd
	Tc	85.9±0.6[a]	nd	82.3±0.1[b]	nd
	ΔTc-To	19.2±0.8[a]	nd	15.0±0.2[b]	nd
	ΔH (J/g) (d.b.)	6.0±0.1[a]	nd	4.3±0.2[b]	nd
100 (100 V-Type-I)	To	94.0±1.3[a]	82.15±0.4[b]	94.36±0.1[a]	82.6±0.1[b]
	Tp	97.6±1.3a[b]	95.54±0.8[b]	100.8±0.3[a]	85.7±0.1[c]
	Tc	101.2±2.6[b]	112.77±0.3[a]	110.0±0.1[a]	95.1±2.4[b]
	ΔTc-To	7.2±1.3[c]	30.62±0.2[a]	15.6±0.0[b]	12.8±2.6[bc]
	ΔH (J/g) (d.b.)	0.0±0.0[b]	1.30±0.2[a]	0.1±0.0[b]	0.20±0.0[b]
120 (100 V-Type-II)	To	113.2±11.0[a]	nd	130.7±0.4[a]	nd
	Tp	120.1±17.5[a]	nd	134.2±1.6[a]	nd
	Tc	134.2±26.9[a]	nd	143.0±0.3[a]	nd
	ΔTc-To	20.9±15.9[a]	nd	12.3±0.1[a]	nd
	Enthalpy (J/g) (d.b.)	1.3±1.3[a]	nd	0.2±0.0[a]	nd

Values are means ± standard error of the mean. Different letters indicate significant differences ($p<0.05$). To, onset temperature; Tp, peak temperature; Tc, completion temperature; ΔH, enthalpy; nd, not detected.

Table 6-2. Fatty acid composition of fat extracted from native and extruded pearl millet flour (%).

	Whole Native	Whole Extruded	Decorticated Native	Decorticated Extruded
Palmitic C16:0	14.9±0.0^{c}	17.7±0.6^{b}	14.4±0.1^{d}	20.6±3.3^{a}
Palmitoleic C16:1	0.2±0.0^{a}	0±0.0^{b}	0.2±0.0^{a}	0±0.0^{b}
Stearic C18:0	4.1±0.1^{b}	4.7±0.9^{ab}	3.8±0.0^{c}	5.8±2.2^{a}
Oleic C18:1	25.6±0.2^{b}	28.8±0.6^{a}	25.5±0.1^{b}	30.7±3.6^{a}
Linoleic C18:2-9c,12c	51.3±0.3^{b}	43.7±2.7^{c}	52.1±0.2^{a}	38±1.5^{d}
Linolenic C18:3-9c,12c,15c	3.3±0.1^{b}	4.3±0.4^{a}	3.3±0.1^{b}	4.1±1.2^{ab}
Arachidic C20:0	0.7±0.1^{a}	0.8±0.2^{a}	0.6±0.1^{a}	0.9±0.2^{a}
Saturated	19.7	23.2	18.8	27.2
Monounsaturated	25.8	28.8	25.7	30.7
Polyunsaturated	54.5	48.0	55.4	42.0
Fat content	7.7±0.3^{a}	4.7±0.1^{c}	5.5±0.2^{b}	3.1±0.1^{d}

Values are means ± standard error of the mean. Different letters indicate significant differences ($p<0.05$).

Table 6-3. Fatty acid composition of dissociated amylose-lipid complexes in defatted flours (%).

	Whole extruded	Decorticated Extruded
Palmitic C16:0	30.2 ± 1.4[a]	31.4 ± 6.5[a]
Palmitoleic C16:1	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Stearic C18:0	8.9 ± 2.7[a]	11.8 ± 7.0[a]
Oleic C18:1	20.7 ± 5.0[a]	20.3 ± 9.1[a]
Linoleic C18:2-9c,12c	33.7 ± 0.2[a]	33.8 ± 0.4[a]
Linolenic C18:3-9c,12c,15c	6.3 ± 1.1[a]	2.8 ± 3.9[a]
Arachidic C20:0	0.0 ± 0.0[a]	0.0 ± 0.0[a]

Values are means ± standard error of the mean. Different letters indicate significant differences ($p<0.05$).

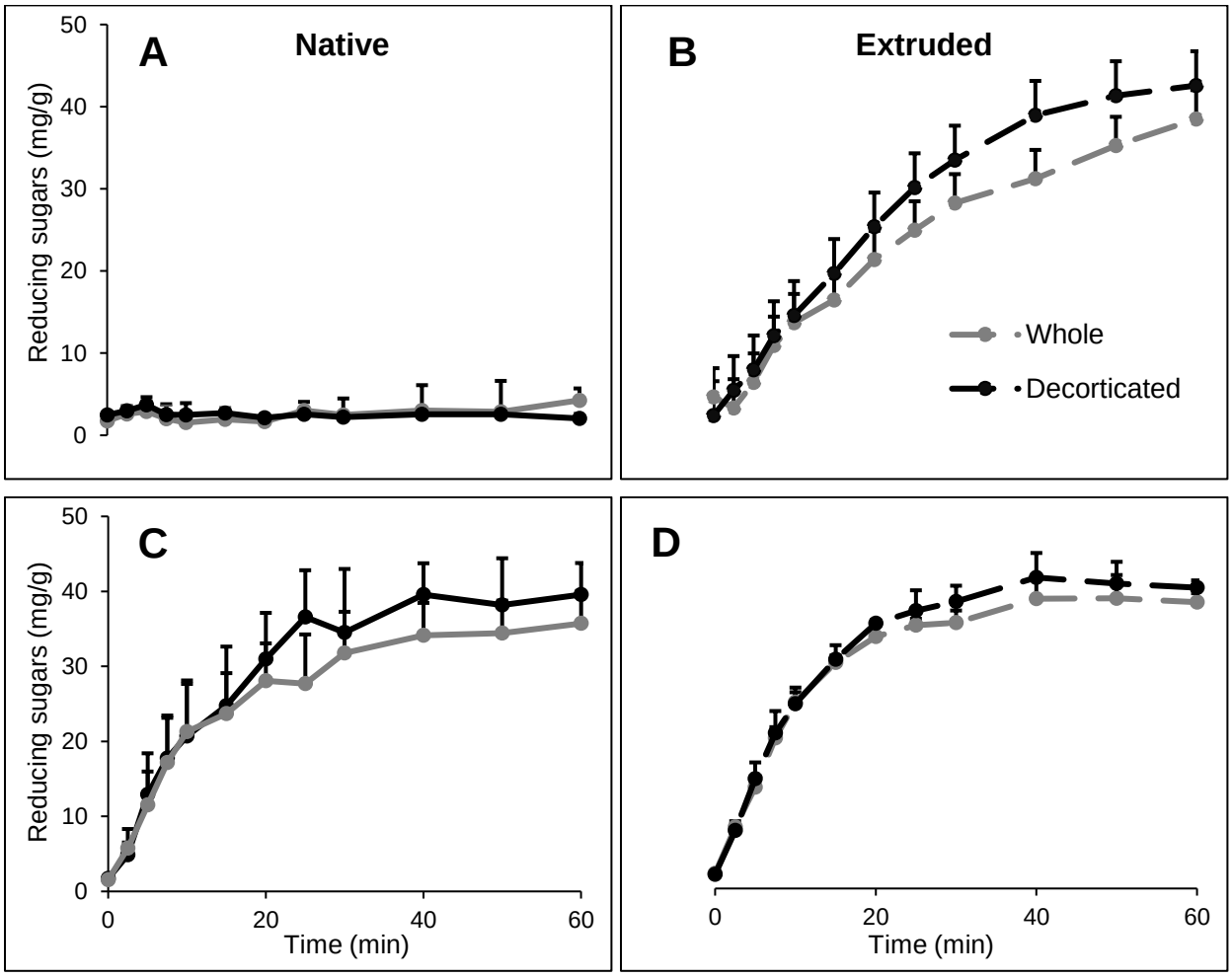


Figure 6-1. Digestion of a) native flours with α-amylase, and digestion by α-amylase of b) extruded flours, c) hydrothermally treated native flours and d) hydrothermally treated extruded flours. Black lines indicate decorticated flours, while grey lines represent whole flours.

CHAPTER 7. OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

7.1 Summary and overall conclussions

Starchy foods with a slow digesting characteristic have previously been associated with slow gastric emptying; however, this response was not consistent between African and USA cohorts and the underlying mechanism for this observation was not clear. Moreover; at least in animal models, consumption of SDCs have been associated with positive metabolic outcomes. The potential role of quality carbohyrates to promote biological adaptations that improve phsysiological and metabolic outcomes in humans was hypothesized. Chapters 3 and 4 of this work examined retrospectively (through diet assessment) and prospectively (through a carbohydrate supplementation study) the effect of a known SDC on gastric emptying time, postprandial blood glucose, appetite/hunger sensations, RER, and metabolic flexibility. Chapters 5 and 6 examined the role of low-cost extrusion on shelf-life stability, sensory acceptance, and development of amylose-lipid complexes with slow digesting property of whole pearl millet (*Pennisetum glaucum*) instant flours.

Summary of research findings, study limitations, as well as considerations for future work to advance our understanding about the role of SDCs and diets on health as well as the potential use of low-cost technologies to improve shelf-life stability of whole extruded flours containing carbohydrates with slow digesting characteristics are descibred below.

The first experimental chapter, Chapter 3, assessed differences in gastric emptying times, calculated RER, and metabolic flexibility between a Kenyan cohort that consumed a traditional Kenyan diet and a USA cohort that consumed a Western diet. Our results suggest that although no significant effect of SDC or diet on gastric emptying was found; specific diet factors such as increasing total fiber, and decreasing added sugars and starches could potentially improve metabolic fuel utilization in humans. The second experimental chapter, Chapter 4, tested the hypothesis that continual consumption of a SDC supplement induces a slow gastric emptying time via activation of the ileal brake, and if the same consumption of SDC influences metabolic fuel utilization, measured with CO_2 as a proxy to RER. Although continual consumption of the SDC resulted in no significant effect on gastric emptying time, hunger ratings or acute measures of RER; the same consumption of SDC resulted in a modest but significant effect on metabolic flexibility

and exhibited a wider range of RER values. Continual consumption of SDC marginally but significantly was associated with utilization of carbohydrate as metabolic fuel, which was contraary to a previous animal study where SDC was associated with fat utilization as a metabolic fuel.

Chapter 5 assessed the effect of extrusion on shelf-life stability and sensory characteristics of whole grain pearl millet flour, while Chapter 6 evaluated the formation and characteristics of ALCs in extruded whole grain pearl millet flour and its hypothesized modulation of starch digestion rate *in vitro*. Results of Chapter 5 demonstrated that small-scale extrusion can be used to extend shelf stability of instant whole whole grain pearl millet flour by preventing the development of products related to hydrolytic rancidity. However, extrusion cooking did not stop the development of products of oxidative rancidity and created a challenge related to some undesirable sensory properties. On the other hand, results of calorimetric analysis in Chapter 6 showed the presence of type-I ALCs in whole grain pearl millet flours, particularly in extruded ones. Free lipid reduction in extruded flours was attributed to lipid complexation with amylose following the rapid cool-down after the extrusion melt leaves the die, promoting fast nucleation and subsequent formation of type-I ALCs. A starch digestion test showed moderated reducing sugar release for whole grain extruded pearl millet flour that was attributed to the ALCs.

7.2 Future directions

In Chapter 3, potential differences in total lean body mass and adipose/muscle mass ratio could potentially explain the metabolic fuel utilization differences observed between the Kenyan and USA cohorts. A future study should control for these variables. A future direction could consider diet interventions, such as different levels of dietary fiber, added sugars, and starch as in improving metabolic flexibility. Additionally, to better answer the potential effect of SDCs on both gastric emptying, appetitive response, and metabolic fuel utilization, a careful consideration of the SDC carrier should be done. It is possible that the fructose in the the unsweetened applesauce carrier using in our studies could have acted as a signaling molecule in the oral cavity affecting subsequent digestion and physiological response of the SDC. A study design might incorporate a different SDC carrier (e.g. yogurt or a macronutrient-free food material). Evaluation of RER using a challenge test (feeding-refeeding challenge using fat then glucose) might be beneficial to enhance

the differences in metabolic fuel utilization. These recommendations apply to both Chapter 3 and 4 studies.

For the Chapter 4 study, a recommendation may be to include the use of a biomarker (tracer in urine) to measure subject compliance of taking the supplement rather than reliance on the random request for photographic proof of SDC consumption that was used in our study. Additionally, it is important to assess palatability since repeated consumption of SDC (repetitive sensations) might influence consumers' perception of hunger/fullness. Future directions for this work include assessment of continuous consumption of SDC on metabolic flexibility in humans using a higher SDC dose (e.g. 60 g) for longer time (e.g. 8 weeks) and divided throughout the day, since it could potentially enhance the improvement observed in metabolic flexibility.

For Chapter 5, limitations of the study included the lack of direct quantification of the final products of oxidative rancidity (volatile aldehydes and alcohols) through the assessment of headspace analysis. Quantification and characterization of these compounds would help to better determine the type of undesirable flavors developed during storage and to link them to descriptive sensory analysis. Also, a study design that includes a negative control with an antioxidant, e.g. BHA or BHT, might be useful. Future directions for this experiment might include work to improve extrusion conditions to inactivate lipooxigenase, thereby reducing xidative rancidity, as well as sensory assessment performed with African consumers who typically eat pearl millet porridges.

Finally, for Chapter 6, extrusion promoted the formation of ALCs in whole pearl millet flour and starch digestion tests suggested moderated modulation of sugar release *in vitro*. A future study assessing, in human subjects, slow digestion properties using a CGM for postprandial blood glucose would be beneficial.

APPENDIX A

Parameters from modeling percent dose recovery (PDR) and cumulative percent dose recovery (CPDR) of ^{13}C (corrected for endogenous ^{13}C) for each participant in Kenya. Parameters were used for calculating gastric half-emptying time, lag phase, and gastric emptying coefficient.

Test meal	Participant number	PDR model				CPDR model				
		a	b	c	R2	m	k	B	R2	t Half (h)
Raw Corn Starch	1	82.35	0.83	1.25	0.97	53.30	0.87	1.80	1.00	1.30
	2	58.35	0.53	0.98	0.89	55.55	0.71	1.49	1.00	1.39
	5	43.98	0.36	0.74	0.98	58.05	0.66	1.50	1.00	1.49
	6	55.95	0.65	1.05	0.95	48.17	0.76	1.63	1.00	1.39
	9	56.33	1.03	1.15	0.94	45.02	0.77	1.98	1.00	1.59
	10	103.41	1.42	1.55	0.88	48.25	0.90	2.30	1.00	1.49
	11	49.88	0.61	1.01	0.90	45.22	0.73	1.58	1.00	1.41
	12	58.73	0.94	1.25	0.92	38.25	0.88	1.95	1.00	1.38
	13	91.57	1.17	1.30	0.95	58.21	0.88	2.18	1.00	1.48
	14	52.77	1.47	1.26	0.95	40.51	0.78	2.48	1.00	1.80
	15	28.94	0.68	0.64	0.90	56.25	0.49	1.70	1.00	2.21
	16	109.20	1.23	1.62	0.93	45.29	0.95	2.03	1.00	1.31
	17	39.02	0.56	0.75	0.92	55.05	0.60	1.61	1.00	1.74
	18	44.09	0.63	0.80	0.98	56.66	0.65	1.70	1.00	1.69
	19	37.46	0.60	0.60	0.94	75.75	0.48	1.63	1.00	2.20

	20	44.88	0.70	0.75	0.88	70.03	0.54	1.68	1.00	2.00
	21	54.51	0.59	0.98	0.93	51.13	0.74	1.60	1.00	1.41
	22	54.97	0.92	1.11	0.91	45.72	0.75	1.86	1.00	1.56
	23	47.82	0.78	0.83	0.86	63.12	0.60	1.76	1.00	1.86
	24	80.53	1.22	1.26	0.85	57.71	0.77	2.09	1.00	1.65
	25	30.03	0.00	0.44	0.96	61.68	0.54	1.27	1.00	1.60
	26	41.52	1.06	1.24	0.96	28.41	0.84	2.05	1.00	1.48
	27	62.85	0.58	0.88	0.93	69.32	0.68	1.59	1.00	1.53
	29	56.77	0.70	0.90	0.98	59.85	0.76	1.85	1.00	1.53
	30	47.67	1.11	1.03	0.95	48.38	0.70	2.11	1.00	1.82
	31	28.18	0.13	0.71	0.96	37.53	0.68	1.31	1.00	1.30
	32	31.75	0.39	0.53	0.98	65.47	0.52	1.58	1.00	2.00
	33	46.95	0.67	0.94	0.94	48.10	0.70	1.66	1.00	1.54
	34	53.03	1.12	1.21	0.87	39.72	0.76	2.01	1.00	1.62
Maltodextrin DE-1	1	55.80	0.82	0.95	0.98	58.43	0.72	1.89	1.00	1.64
	2	35.46	0.23	0.83	0.98	38.97	0.78	1.41	1.00	1.22
	5	47.58	0.58	0.79	0.99	60.42	0.67	1.70	1.00	1.63
	6	51.50	0.94	1.02	0.97	49.47	0.73	1.95	1.00	1.66
	7	40.37	0.92	0.88	0.95	51.31	0.63	1.93	1.00	1.90
	9	67.23	1.58	1.47	0.95	36.42	0.91	2.60	1.00	1.60
	10	40.15	0.89	0.87	0.98	50.10	0.65	1.95	1.00	1.84
	11	69.53	1.14	1.39	0.97	37.99	0.93	2.13	1.00	1.38
	12	55.42	0.85	1.15	0.98	41.02	0.84	1.88	1.00	1.39

13	83.22	1.08	1.21	0.97	59.94	0.82	2.08	1.00	1.53
14	52.10	1.31	1.09	1.00	51.50	0.74	2.40	1.00	1.88
15	14.65	0.66	0.27	0.98	120.73	0.21	1.69	1.00	5.15
16	69.09	1.15	1.32	0.89	43.90	0.83	2.06	1.00	1.51
17	33.62	0.73	0.77	0.99	47.91	0.62	1.81	1.00	1.86
18	33.06	0.71	0.64	0.99	65.18	0.51	1.80	1.00	2.22
19	38.91	0.63	0.65	0.99	70.87	0.54	1.74	1.00	2.07
20	49.68	1.04	0.89	0.97	65.05	0.64	2.10	1.00	1.98
21	33.25	0.45	0.62	0.99	56.92	0.57	1.60	1.00	1.84
22	43.09	0.57	0.79	0.92	56.02	0.62	1.60	1.00	1.68
23	32.20	0.17	0.55	0.96	58.56	0.54	1.33	1.00	1.67
24	41.47	0.51	0.81	0.95	50.69	0.66	1.57	1.00	1.56
25	39.89	0.45	0.80	0.82	43.45	0.85	1.74	1.00	1.31
27	59.81	0.83	0.96	0.96	61.31	0.71	1.85	1.00	1.64
28	46.17	0.74	0.81	0.99	62.46	0.61	1.76	1.00	1.84
29	59.51	0.88	0.98	0.99	58.84	0.77	2.01	1.00	1.61
30	34.41	0.84	0.80	0.99	48.52	0.63	1.94	1.00	1.92
31	116.19	1.27	1.87	0.90	34.73	1.06	2.01	1.00	1.17
32	32.83	0.48	0.58	0.96	62.90	0.55	1.68	1.00	1.98
33	46.51	0.81	0.95	0.99	47.54	0.74	1.90	1.00	1.60
34	48.36	0.91	1.01	0.96	46.81	0.72	1.92	1.00	1.65

CPDR, cumulative percent dose recovery; PDR, percent dose recovery; tHalf, gastric half-emptying time.

APPENDIX B

Parameters from modeling percent dose recovery (PDR) and cumulative percent dose recovery (CPDR) of ^{13}C (corrected for endogenous ^{13}C) for each participant in USA. Parameters were used for calculating gastric half-emptying time, lag phase, and gastric emptying coefficient.

Test meal	Participant number	PDR model				CPDR model				
		a	b	c	R2	m	k	B	R2	t Half (h)
Raw Corn Starch	50	100.95	1.42	1.48	0.91	53.02	0.86	2.29	1.00	1.56
	51	22.92	0.03	0.33	0.93	67.02	0.37	1.20	1.00	2.24
	60	32.35	0.37	0.61	0.90	57.00	0.52	1.42	1.00	1.84
	70	30.16	0.28	0.63	0.98	46.51	0.63	1.47	1.00	1.55
	102	45.08	0.77	0.83	0.93	59.16	0.62	1.78	1.00	1.83
	135	45.79	0.31	0.75	0.95	59.71	0.65	1.41	1.00	1.46
	145	35.34	0.15	0.63	0.99	52.23	0.68	1.40	1.00	1.38
	190	43.73	0.47	0.75	0.98	58.50	0.64	1.57	1.00	1.60
	220	24.00	0.12	0.38	0.95	63.43	0.40	1.27	1.00	2.15
	223	43.55	0.50	0.77	0.91	57.32	0.62	1.54	1.00	1.63
	246	46.60	0.35	0.75	0.96	59.93	0.66	1.46	1.00	1.47
	261	39.13	0.36	0.65	0.97	60.95	0.60	1.49	1.00	1.66
	320	31.99	0.40	0.57	0.95	60.22	0.53	1.53	1.00	1.92
	332	31.97	0.49	0.57	0.98	65.46	0.49	1.58	1.00	2.12
	381	27.87	0.22	0.46	0.88	63.58	0.44	1.31	1.00	2.05

	400	40.60	0.33	0.67	0.98	60.02	0.63	1.48	1.00	1.57
	414	60.11	0.64	0.90	0.89	66.85	0.65	1.61	1.00	1.61
	490	98.60	1.31	1.58	0.83	44.78	0.85	2.00	1.00	1.45
	512	29.17	0.43	0.66	0.95	45.10	0.60	1.55	1.00	1.71
	550	27.48	0.45	0.49	0.92	68.20	0.42	1.51	1.00	2.39
	555	36.74	0.54	0.75	0.89	52.24	0.58	1.55	1.00	1.76
	567	51.58	0.83	1.05	0.81	47.06	0.70	1.73	1.00	1.59
	600	68.37	1.35	1.27	0.94	48.76	0.81	2.35	1.00	1.68
	610	60.78	0.43	0.95	0.93	59.17	0.74	1.45	1.00	1.32
	614	31.91	0.30	0.54	0.94	61.70	0.50	1.40	1.00	1.87
	642	43.87	0.82	0.79	0.86	66.35	0.55	1.78	1.00	2.04
	651	40.14	0.04	0.64	0.98	58.20	0.68	1.28	1.00	1.28
	679	38.01	0.43	0.99	0.96	33.50	0.84	1.54	1.00	1.21
	719	89.05	1.23	1.28	0.89	62.03	0.78	2.11	1.00	1.64
	849	29.80	0.36	0.61	0.91	50.74	0.54	1.43	1.00	1.77
	949	45.38	0.40	0.84	0.92	52.54	0.66	1.43	1.00	1.44
	970	57.65	0.82	1.04	0.87	52.98	0.70	1.74	1.00	1.58
Maltodextrin DE-1	50	51.39	1.02	0.96	0.99	56.81	0.71	2.13	1.00	1.81
	51	28.72	0.46	0.52	0.97	65.34	0.45	1.55	1.00	2.24
	60	26.57	0.28	0.51	0.99	53.33	0.53	1.49	1.00	1.87
	70	31.89	0.57	0.80	0.94	39.42	0.70	1.72	1.00	1.59
	102	44.62	0.73	0.85	0.98	53.18	0.68	1.81	1.00	1.68
	135	37.79	0.56	0.65	1.00	65.13	0.57	1.70	1.00	1.93

145	41.13	0.56	0.89	0.97	41.49	0.83	1.80	1.00	1.37
190	40.52	0.52	0.76	0.99	53.20	0.68	1.68	1.00	1.60
220	27.27	0.18	0.46	0.99	59.39	0.49	1.38	1.00	1.89
223	36.13	0.55	0.71	0.98	54.84	0.60	1.65	1.00	1.80
246	47.60	0.78	0.93	0.98	50.08	0.72	1.85	1.00	1.61
261	31.82	0.49	0.65	0.99	52.01	0.60	1.66	1.00	1.80
320	26.27	0.44	0.50	0.94	64.83	0.42	1.52	1.00	2.37
332	35.22	0.61	0.61	0.99	69.64	0.51	1.70	1.00	2.16
381	35.74	0.51	0.64	0.97	62.33	0.54	1.61	1.00	1.93
400	48.10	0.52	0.77	0.97	63.02	0.65	1.61	1.00	1.62
414	53.33	0.72	0.85	0.94	66.05	0.64	1.74	1.00	1.74
490	77.25	1.05	1.22	0.90	56.61	0.76	1.92	1.00	1.58
512	10.06	0.55	0.31	0.97	52.17	0.27	1.60	1.00	3.85
550	28.62	0.62	0.55	0.98	66.82	0.47	1.74	1.00	2.38
555	24.55	0.29	0.45	0.96	59.02	0.44	1.43	1.00	2.16
567	47.64	1.01	1.08	0.96	42.31	0.76	2.03	1.00	1.64
600	38.62	0.83	0.77	0.97	59.95	0.58	1.87	1.00	2.04
610	54.43	0.85	0.93	0.99	58.90	0.72	1.96	1.00	1.67
614	44.43	0.74	0.83	0.98	55.62	0.66	1.82	1.00	1.73
642	61.62	1.06	1.00	0.96	64.31	0.70	2.09	1.00	1.80
651	37.85	0.54	0.71	1.00	55.36	0.63	1.68	1.00	1.73
679	32.89	0.87	0.90	0.98	38.38	0.70	1.99	1.00	1.75
719	61.39	1.07	0.99	0.93	66.94	0.68	2.08	1.00	1.86

849	35.02	0.63	0.73	0.96	52.18	0.60	1.71	1.00	1.84
949	38.55	0.62	0.78	0.96	51.75	0.62	1.67	1.00	1.74
970	62.18	0.66	1.27	0.89	41.57	0.76	1.46	1.00	1.28

CPDR, cumulative percent dose recovery; PDR, percent dose recovery; tHalf, gastric half-emptying time.

APPENDIX C

Parameters from modelling percent dose recovery (PDR) and cumulative percent dose recovery (CPDR) of ^{13}C for each participant. These parameters were used for calculating gastric half-emptying time, lag phase, and gastric emptying coefficient.

Test meal		Participant number	PDR model				CPDR model					
			a	b	c	R2	m	k	B	R2	t (h)	Half
Raw Corn Starch	Baseline	1	13.04	0.60	0.49	0.95	37.84	0.38	1.63	1.00		2.77
		2	12.42	0.69	0.44	0.97	47.00	0.34	1.82	1.00		3.27
		3	17.31	0.58	0.53	0.97	41.67	0.45	1.65	1.00		2.40
		4	9.02	0.31	0.30	0.99	36.92	0.30	1.44	1.00		3.17
		5	20.21	0.67	0.54	0.99	50.59	0.44	1.74	1.00		2.52
		6	16.96	1.19	0.65	0.98	49.71	0.44	2.24	1.00		3.00
		7	13.39	0.39	0.34	0.99	51.33	0.32	1.50	1.00		3.08
		8	13.75	0.59	0.42	0.95	50.81	0.33	1.62	1.00		3.20
		9	14.70	0.76	0.50	0.96	47.47	0.39	1.84	1.00		2.98
		10	15.18	0.81	0.46	0.99	59.47	0.35	1.85	1.00		3.34
		11	15.71	0.72	0.43	0.99	61.63	0.34	1.79	1.00		3.30
		12	15.29	1.15	0.66	0.93	40.52	0.46	2.21	1.00		2.85
		13	9.21	0.97	0.41	0.98	56.40	0.28	1.98	1.00		4.29
		14	16.05	0.73	0.48	0.99	52.54	0.38	1.79	1.00		2.99
	After 21 days	1	16.81	1.28	0.79	0.99	37.48	0.54	2.36	1.00		2.55
		2	12.70	0.59	0.41	0.98	45.95	0.35	1.67	1.00		3.09

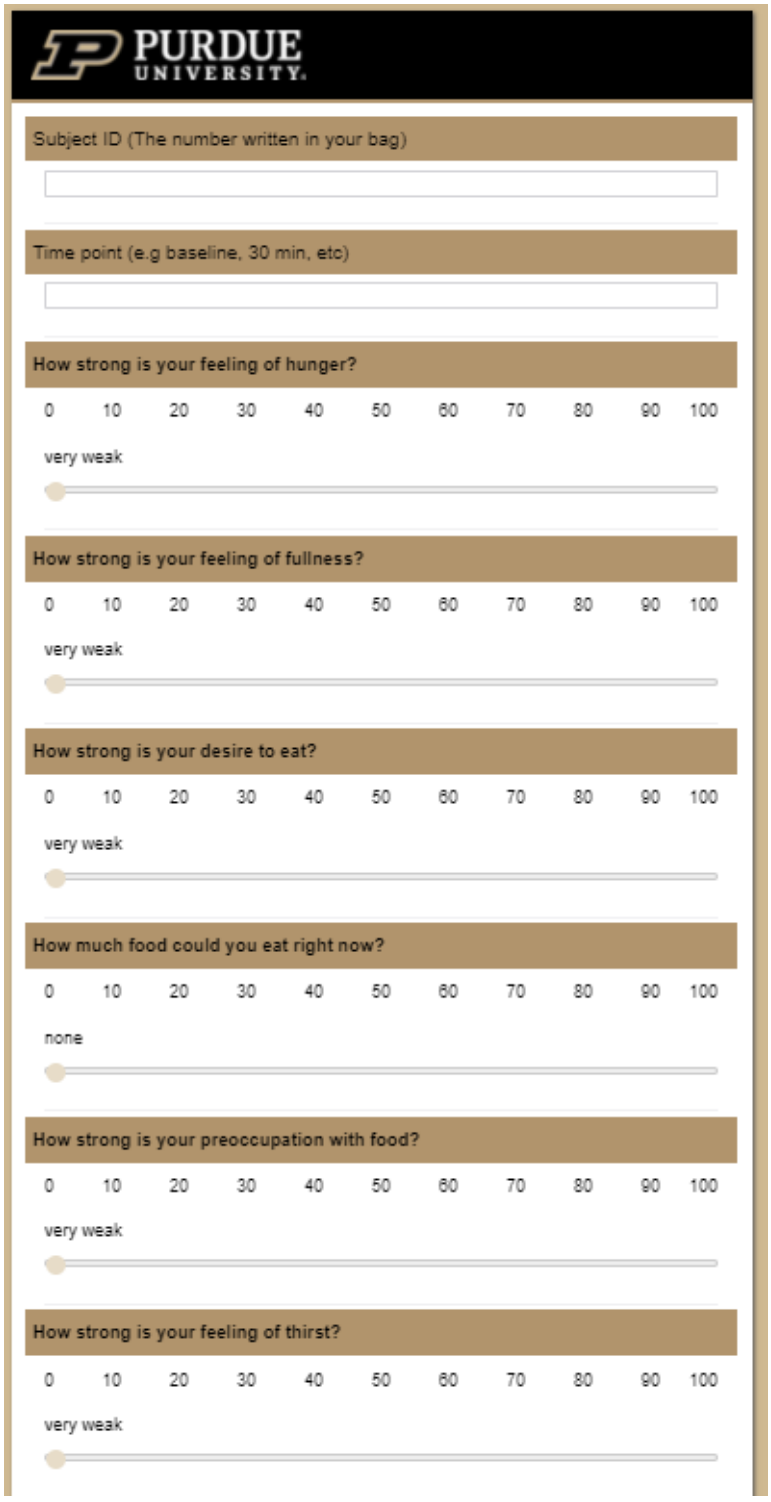
		3	13.78	0.60	0.39	0.99	53.11	0.34	1.68	1.00	3.20
		4	11.61	0.48	0.40	0.96	40.50	0.34	1.56	1.00	2.99
		5	16.10	0.90	0.54	1.00	50.37	0.41	1.97	1.00	2.97
		6	13.75	1.06	0.57	0.98	47.30	0.39	2.07	1.00	3.23
		7	11.82	0.79	0.43	0.99	49.53	0.34	1.86	1.00	3.44
		8	14.09	0.89	0.59	0.95	36.85	0.43	1.90	1.00	2.73
		9	15.20	0.63	0.43	0.99	53.73	0.36	1.72	2.00	3.06
		10	12.99	0.50	0.39	0.98	46.44	0.34	1.58	1.00	3.03
		11	18.32	0.71	0.47	0.99	60.96	0.37	1.77	1.00	3.00
		12	14.69	0.89	0.52	0.95	51.62	0.37	1.90	1.00	3.23
		13	12.51	0.97	0.49	0.98	51.69	0.36	2.03	1.00	3.45
		14	17.59	0.76	0.55	0.99	46.21	0.43	1.81	1.00	2.66
Maltodextrin DE-1	Baseline	1	16.02	0.91	0.67	0.98	34.00	0.50	1.98	1.00	2.44
		2	7.84	0.44	0.23	0.99	53.69	0.22	1.53	1.00	4.56
		3	29.84	1.11	0.72	0.99	42.90	0.51	2.18	1.00	2.55
		4	13.51	0.86	0.51	0.96	45.08	0.39	1.91	1.00	3.07
		5	19.79	0.85	0.59	0.99	49.68	0.45	1.91	1.00	2.63
		6	15.22	1.10	0.59	0.99	49.65	0.41	2.14	1.00	3.12
		7	14.72	1.14	0.60	0.99	48.96	0.41	2.19	1.00	3.15
		8	10.39	0.58	0.44	0.99	32.36	0.39	1.70	1.00	2.77
		9	14.11	0.59	0.44	0.96	45.76	0.39	1.72	1.00	2.86
		10	17.13	1.04	0.64	0.95	44.58	0.45	2.08	1.00	2.80
		11	16.81	0.92	0.55	0.98	51.71	0.41	1.96	1.00	2.98
		12	14.29	0.46	0.41	0.94	42.18	0.41	1.60	1.00	2.57

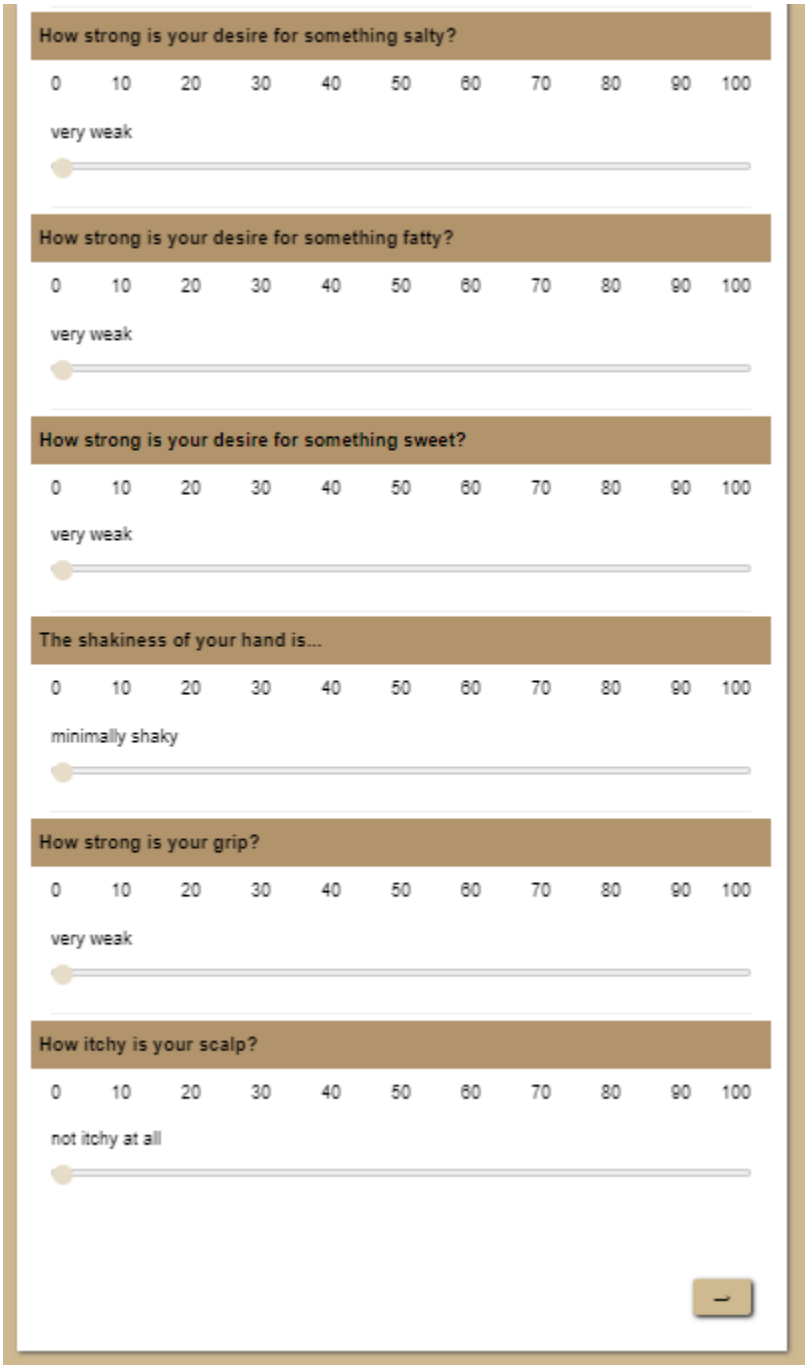
	13	11.34	0.88	0.45	0.97	52.32	0.32	1.88	1.00	3.74
	14	15.71	1.20	0.65	0.95	46.05	0.45	2.27	1.00	2.98
After 21 days	1	11.52	0.83	0.44	0.93	48.10	0.34	1.87	1.00	3.47
	2	14.48	0.98	0.56	0.97	45.17	0.41	2.01	1.00	3.02
	3	16.20	0.86	0.58	0.98	43.50	0.43	1.91	1.00	2.75
	4	10.59	0.38	0.29	0.98	49.85	0.28	1.48	1.00	3.55
	5	17.13	0.74	0.49	1.00	53.62	0.40	1.82	1.00	2.88
	6	16.26	0.93	0.52	0.96	55.48	0.39	1.97	1.00	3.14
	7	14.36	0.78	0.46	0.96	53.86	0.35	1.81	1.00	3.27
	8	16.93	0.75	0.52	0.97	49.84	0.40	1.79	1.00	2.84
	9	14.71	0.79	0.47	0.99	54.66	0.35	1.81	1.00	3.28
	10	18.42	0.79	0.55	0.92	51.23	0.41	1.82	1.00	2.78
	11	15.31	0.93	0.52	1.00	54.33	0.38	1.97	1.00	3.21
	12	16.45	0.72	0.51	0.96	48.69	0.40	1.76	1.00	2.83
	13	10.40	1.27	0.57	0.98	42.74	0.39	2.35	1.00	3.48
	14	14.71	0.79	0.52	0.96	45.64	0.39	1.83	1.00	2.95

CPDR, cumulative percent dose recovery; PDR, percent dose recovery; tHalf, gastric half-emptying time.

APPENDIX D

Example of visual analog scales for assessment appetite and hunger in Qualtrics.




How strong is your desire for something salty?
0 10 20 30 40 50 60 70 80 90 100
very weak
How strong is your desire for something fatty?
0 10 20 30 40 50 60 70 80 90 100
very weak
How strong is your desire for something sweet?
0 10 20 30 40 50 60 70 80 90 100
very weak
The shakiness of your hand is...
0 10 20 30 40 50 60 70 80 90 100
minimally shaky
How strong is your grip?
0 10 20 30 40 50 60 70 80 90 100
very weak
How itchy is your scalp?
0 10 20 30 40 50 60 70 80 90 100
not itchy at all