STUDIES ON EXTRUSION PROCESSING OF INSTANT PORRIDGE FLOURS FOR AFRICAN PROCESSOR OPTIMIZATION, ACCEPTANCE, MARKETABILITY FOR CONSUMERS, AND IMPROVEMENT IN *IN VITRO* FECAL FIBER FERMENTATION

by

Emmanuel O. Ayua

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 2021

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Bruce R. Hamaker, Chair

Department of Food Science

Dr. Fernanda S. Martin

Department of Food Science

Dr. Osvaldo H. Campanella

Department of Food Science and Technology
Ohio State University

Dr. Stephen R. Lindemann

Department of Food Science

Approved by:

Dr. Arun K. Bhunia

Dedicated to my parents,

Rose Ayuma and William Ayua (Deceased)

My Wife, Martha Aluoch Odhacha

ACKNOWLEDGMENTS

First, I want to thank God for good health during my entire graduate life

I am grateful to my advisors and lab mates for helping throughout my research during my stay in the department. I am thankful to my advisor Dr. Bruce Hamaker for his support and guidance and for driving me to maximize my potential. Your wisdom, research experience and encouragement have shaped me up during the graduate school and will be very useful later in my career.

I am also grateful to My committee members: Dr. Fernanda San Martin-Gonzalez, Dr. Osvaldo Campanella and Dr. Stephen Robert Lindemann for useful insights in my studies. They improved my way of looking at ideas.

I want to thank Professor Violet Mugalavai from University of Eldoret, Hugo De Groote and his team from International Center for maize and Wheat Improvement for you're the help you accorded me.

I want to thank my lab mates especially Jongbim Lim, Tianming Yao, Marwa El-Hindawy, Yunus Tuncil, Enosh Kazem, Fang Fang, Nuseybe Bulut, Aminatta Diatta and Smith Nkhata for your help. You created a conducive environment for me to work and accomplish my research goals.

Thanks to all staff in the Food Science department for helping me throughout my PhD journey. May God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AMG	Amyloglucosidase
FPIL	Feed the Future Food Processing and Post-harvest Handling Innovation Lab
FtF	Food-to-food fortification
HPSEC	High performance size-exclusion chromatography
IL-6	Interleukin 6
IL-10	Interleukin 10
NF-kβ	Nuclear factor kappa B
OTUs	Operational taxonomic units
RDS	Rapidly digestible starch
RS	Resistant starch
RVA	Rapid Visco-Analyzer
SCFAs	Short chain fatty acids
SD	Standard deviation
SDS	Slowly digestible starch
SE	Standard error
SEM	Scanning electron microscopy
SMIL	Sorghum and Millet Innovation Lab
USAID	United States Aid for International Development
USD	United States dollar
WTP	Willingness to pay

ABSTRACT

The Food Processing and Postharvest Handling Innovation Lab (FPIL) project seeks to reduce food loss and link up consumers with food-to-food fortified instant products that are enriched with micronutrient sources that target vitamin A, zinc, and iron deficiencies. These are mostly maize-based products, but may be combined with other cereals, such as sorghum, and pseudocereals, such as amaranth. The general goal of this thesis study was to facilitate the adoption of extrusion technology to process instant flours, assess the acceptance and willingness to pay (WTP) for these products, and to assess the health impacts of the products on gut health. A low-cost, single-screw extruder was used that was developed at Purdue, and has been placed in different locations in Africa country study sites. The first study aimed to optimize process conditions of a low-cost single-screw extruder, currently done at 35% feed moisture, for African small- to medium-scale entrepreneurs to produce good quality and low-cost pregelatinized instant pearl millet porridge flours and other whole grains by relating feed moisture (27, 29, 31, 33, and 35%) to extrusion energy, drying time and physicochemical properties. We found that we could lower the feed moisture to 27% and still attain good pasting profiles of the porridges, reduce drying time, have better expansion of the extrudates, obtain increased L^* color values of the flours, and with a higher extrusion energy but lower drying time. In conclusion, the single screw extruder can be efficiently operated at 27% feed moisture compared to the currently used 35% feed moisture and obtain instant flours with desired quality. It is not known whether higher extrudate energy consumption may be offset by the lower drying time representing lower drying energy. In the second study, we investigated extrusion enhance *in vitro* fecal fermentation of maize bran, which has been characterized by a poor gut microbiota fermentation property due to its highly crosslinked and densely branched arabinoxylan chemical structure, making it poorly available to the gut microbiota. We hypothesized that this dense cell wall matrix can be opened for better fermentation by applying extrusion. Test conditions of a twin-screw extruder at Purdue were low (200 RPM) and high (400 RPM) shear rates applied to a maize meal and bran mixture (60:40) at different feed moisture conditions (20, 25, 30%). *In vitro* fermentation of test materials was conducted on stool samples from three donors. Extrusion increased total short chain fatty acids and produced individualized donor effects on the gut microbiota. Some extruder test condition effects were observed on certain bacteria. For example, extrusion at 30% feed moisture and 400 RPM tended

to increase genera of *Subdoligranulum* and *Eubacterium hallii* and *Ruminococcus torques* groups in Donor 1 compared to non-extruded bran. There was also a trend of increase in *Subdoligranulum* and *Blautia* in extruded compared to non-extruded bran in Donor 2. In Donor 3, *Lachnospiraceae NK4A136* group was increased at 20 and 25% feed moistures at 200 RPM and 30% feed moisture at 400 RPM compared to non-extruded bran. In the final study, we investigated the acceptance and WTP for instant fortified flours using the Becker-DeGroot-Marschak mechanism when consumers are incrementally given nutrition information and demonstration how to reconstitute instant flours. This study was conducted in Eldoret, Kenya. Participants preferred the fortified thick porridge higher in maize content than fortified thin porridge prepared from the same blend. Contrarily, thin porridge made from fortified flour with higher sorghum content was ranked more highly than for the corresponding thick porridge. Participants were willing to pay more for instant fortified products higher in sorghum when given product name and nutrient composition, even without a practical demonstration of how to reconstitute the flours. For the instant product higher in maize, consumers needed demonstration of how to reconstitute the instant flour for them pay a higher premium. These findings suggest that food-to-food fortified instant porridge flours have the potential to be adopted and can be used as a vehicle to deliver micronutrients to these populations and that extrusion somewhat enhances fermentation of whole grain fibers by the gut microbiome.

CHAPTER 1. LITERATURE REVIEW

Note: parts of this chapter was published in the following paper, that is included in Appendix section. Ayua, E.O., Kazem, A.E., Hamaker, B.R. 2020. Whole grain cereal fibers and their support of the gut commensal Clostridia for health. *Bioactive Carbohydrates and Dietary Fibre* 24:100245.

1.1. Extrusion technology for processing instant foods

Extrusion process involves a combination of shear, mixing, kneading, shaping, and forming at high temperature and pressure with the forcing of food materials through a die leading to chemical, molecular, and structural alterations of the food materials (Bouvier and Campanella, 2014; Singh et al., 2007; Kamau et al., 2020; Brennan et al., 2011). This process is used to produce a variety of food materials that are convenient to use such as breakfast cereals, instant flours, and other foods with varied texture (Ndiaye et al., 2020; Brennan et al., 2013; Meng et al., 2010). There are two types of high temperature, pressure extruders, the single-screw type that has one rotating screw and the twin-screw type with two co-rotating screws (Figure 1.1).

Extruders have a motor that provides the mechanical energy to create a material "melt" inside the barrel that gelatinizes starch and, when released to atmospheric pressure, expands the product. Condition variables that can be manipulated of the extruder to produce instantized products include moisture, temperature, and screw speed producing different shear rates (Sacchetti et al., 2004; Salgado et al., 2017).

Purdue projects of the US Agency for International Development (USAID) Feed the Future Food Processing and Post-harvest Handling Innovation Lab (FPL), the Sorghum and Millet Innovation Lab (SMIL), Rockefeller Foundation Strengthening African Food Processors have used a low-cost small-scale extruder (Figure 1.2) developed at Purdue University by Profs. Okos and Campanella (Ponrajan et al., 2020). The extruder was commercialized by Technochem Inc. (Boone, Iowa, USA). The extruder was placed in different Incubation Center laboratories formed by the Labs to make instant flours for thin and thick porridges that are typically consumed in East and West Africa. Local entrepreneurs continue to be trained on extrusion processing, and in some cases are obtaining the extruder.

The extruder is relatively inexpensive, easy to operate, and can help drive industrialization of food processing in African countries and to meet sustainable development goals. One of the goals of this project was to investigate the use of the single-screw extruder to make whole grain instant products and their potential effect on whole grain fiber fermentability and gut microbiota response. The following sections (1.2 through 1.7) explain a potentially valuable effect of whole grains on gut health, and is part of the published review mentioned above.

1.2. Whole grains and their influence on health

In the past decade, reports have indicated that dietary fiber derived from whole grains has the potential of influencing gut microbiota dynamics as well as improving overall human health (Roager et al., 2019; Christensen et al., 2019; Marlett et al., 2002; De Filippo et al., 2010). Some studies and reviews have described the importance of whole grains in the prevention and/or management of chronic diseases such as type II diabetes, colorectal cancer, weight gain and hypertension (Tang et al., 2015; Wang et al., 2020; Um et al., 2020; Malin et al., 2020; Slavin et al., 2001). Whereas some of these benefits could be related to their fiber content, these grains also have phytochemicals with health promoting potentials (Nystrom et al., 2008; Leoncini et al., 2012; Belobrajdic and Bird, 2013) and whole grain intake was shown to reduce inflammatory markers (Kopf et al., 2018).

Although whole grain foods contribute only 15% of overall dietary fiber intake in the US (Kranz et al., 2017), numerous groups and governments recommend much higher consumption levels. The value of whole grains in the diet is ascribed to fiber amount. While amount is a key attribute, the importance of *fiber type* in whole grains is generally overlooked. Whole grain fiber is principally composed of plant cell wall matrices and, with the exception of β-glucans, most are insoluble. The conventional thinking has been that these insoluble fibers are primarily important for their bulking and laxation properties in human health (Dhingra et al., 2012; Marlett et al., 2002; Slavin, 2013). However, with regards to the gut microbiota, whole grain fibers support an important group of beneficial colonic bacteria, the mucosal-associated commensal Clostridia (Roager et al., 2019; Martinez et al., 2013; Vanegas et al., 2017; Saa et al., 2014).

1.3. The abundance of gut microbiota

Millions of microbiota reside in the human gut and are involved in many physiological processes in the body. The dominant phyla include *Firmicutes*, *Bacteriodetes*, *Verrucomicrobia*, *Proteobacteria*, and *Actinobacteria*, (Pokusaeva et al., 2011; Andersson et al., 2008) with the fast two dominating in numbers. In Firmicutes, Clostridia class and the genera *Clostridium*, *Eubacterium* and *Ruminoccus* have shown frequent representation (Andersson et al., 2008). Bacteroidetes phylum in the human gut is comprised mainly of *Prevotella* and *Bacteroides* while the most abundant genus within the phylum Actinobacterium is *Bifidobacterium* (Ramakrishna, 2013). These microbiota produce short chain fatty acids mainly acetate, propionate and butyrate that have diverse health benefits (Andersson et al., 2008, Ramakrishna, 2013).

1.4. Role of commensal Clostridia in gut health

Commensal Clostridia, gram-positive bacteria in the *Firmicutes* phylum, are composed in the human gut of two main groups; *Clostridium cluster XIVa* – also called the *Clostridium Coccoides* group, and *Clostridium cluster IV* – also called the *Clostridium Leptum* group (Kurakawa et al., 2015; Shen et al., 2006). Some species that are members of commensal *Clostridium* include; *Faecalibacterium prausnitzii*, *Anaerostipes caccae*, *Eubacterium rectale* (now called *Agathobacter rectalis*), *Coprococcus eutactus*, and *Roseburia intestinalis* (Van den Abbeele et al., 2013; Lopetuso et al., 2013; Sheridan et al., 2016).

Commensal Clostridia play a critical role in helping colonocytes modulate immune responses, increase mucin production, and improve tight junctions. They have been reported to induce interleukin (IL)-6 and IL-10, which help regulatory T cells modulate immune responses and reduce inflammation (Lopetuso et al., 2013). These effects are related to the fermentation metabolite butyrate with the major butyrate producers in the gut belonging to the commensal Clostridia. These bacteria principally occupy the mucin layer (Van den Abbeele et al., 2013), enabling them to produce butyrate close to colonic epithelial cells that use it as an energy source and with effects related to the immune system (Chen et al., 2018; Ma et al., 2012). Butyrate-producing Clostridia ferment dietary fibers to release the fatty acid either directly or via cross-feeding, and some bacteria, such as *Anaerostipes caccae* and *Eubacterium hallii*, are capable of both fermenting dietary fibers directly to butyrate and by cross-feeding by converting lactate or

acetate to butyrate (Louis and Flint, 2009). Other functions of butyrate include apoptosis of cancerous cells in the colon, increase in mineral absorption, inhibition of activation of nuclear factor kappa B (NF-kβ) – a pro-inflammatory transcription factor, histone deacetylation, and promotion of growth of normal epithelial cells (Riviere et al., 2016; Hamer et al., 2008; Leonel and Alvarez-Leite, 2012; Dyson et al., 1992; Lee et al., 2017). Proof that butyrate improves barrier function was found through administration of sodium butyrate leading to increased expression of tight junction proteins (zonula occluden-1, occludin, and claudin-1) (Ma et al., 2012; Wang et al., 2012).

1.5. Insoluble cell wall dietary fibers and commensal Clostridia

Insoluble cell wall fibers matrices are generally less accessible to be metabolized by human gut bacteria and are often even considered non-fermentable. In actuality, they are always either partially or fully fermented in the gut, and especially by the commensal Clostridia. The structure of the grain cell wall matrix is composed of a heterogenous group of polysaccharides and lignin held together in a three-dimensional network (Figure 1.3). Consequently, their degradation requires numerous enzymes and binding and transport proteins to depolymerize and utilize them. While some bacteria have an array of gene clusters encoded to degrade different cell wall polysaccharide structures, most do not and it is likely done by consortia of bacteria. Cell wall matrices are more favorably degraded by Clostridia, while certain bacteria in the Bacteroidetes phylum are competitive to the soluble or exposed forms of cell wall matrix fibers such as solubilized arabinoxylans (Hamaker and Tuncil, 2014; Zhang et al., 2019). Accordingly, commensal Clostridia are typically promoted when insoluble dietary fibers, such as those in whole grains are, consumed.

The differences in preferences among bacteria for soluble versus insoluble matrix fibers are related to the different machinery and metabolic capabilities the bacteria have to access and bind, and then degrade and utilize dietary fibers. In the gram-negative Bacteroidetes, an assembly of proteins on the external periplasmic membrane are used to bind and cut large carbohydrate polymers into oligomers that are transported into the periplasm where they are further degraded to simple sugars for transport into the cytoplasm where they are used for energy (Tuson et al., 2018; Martens et al., 2011). This is called the starch utilization system (SUS), as it was first described

for starch, and is termed SUS-like for other polysaccharides. Bacteroidetes appear to be less adapted to utilize physically-bound and inaccessible cell wall matrix fibers, such as those found in whole grains. On the other hand, commensal Clostridia, within the gram-positive Firmicutes, have cellulosome extracellular appendages that are well suited to access insoluble fiber matrices typically found in plant cell walls of foods (Doi and Kosugi, 2004; Artzi et al., 2017). It has been suggested that extracellular enzymes penetrate micropore sizes while membrane-oriented enzyme complexes can extend to larger pore sizes in cell walls (Guillon et al., 1998), hence making the latter suitable for insoluble matrices.

Cellulosomes, which were first identified to degrade cellulose, are an assembly of enzymes that project out from the bacterial membrane surface. They are composed of an arm-like scaffoldin, the non-catalytic component, which houses subunits of enzymes via an integrated cohesion-dockerin interface; where enzymes can be switched out related to the fiber type to be degraded (Bule et al., 2018; Bayer et al., 2004). Scaffoldin has carbohydrate binding units used for aiming and binding to specific carbohydrate substrates. Among the catalytic subunits, there are enzymes that exhibit feruloyl esterase activity (Duerre, 2005) to enable the breaking apart of crosslinks in complex cell wall matrices. The cellulosome system can exist freely, presumably to enter insoluble matrices, or is bound to the bacterial cell surface. It initiates the degradation of insoluble polysaccharides to oligosaccharides, which are transported by proteins to the bacterial cell membrane for transport into the cytoplasm for further breakdown to yield energy (David et al., 2015; Krause et. al, 2003; Artzi el al., 2017). Walker et al. (2008) noted that the ability to degrade insoluble cell walls is conserved in the Firmicutes and they are well adapted to adhere to solid substrates for their degradation compared to Bacteroidetes. Thus, insoluble dietary fiber matrices as found in whole grains, as opposed to soluble refined fibers, preferentially promote Clostridia for gut health.

In support of this view, studies have shown fibers that are fabricated to have less accessible matrices disfavor gram-negative Bacteroidetes and are preferentially used by gram-positive Firmicutes. When starch was entrapped in porous alginate matrix microspheres and fed to mice for 2 weeks; gram-negative Bacteroidetes were disfavored, and gram-positive Firmicutes were favored – along with an increase in butyrate in the distal colon (Kaur et al., 2019). More recently, we showed that crosslinking of soluble arabinoxylans to make soluble polymer matrices led to a

shift in fermentation in favor of commensal Clostridial butyrate-producing bacteria (Zhang et al., 2019).

Additionally, large particle matrices favor butyrate-producing Clostridia. Tuncil et al. (2018a) observed that coarse wheat bran increased the family Lachnospiraceae, which contains many of the major butyrate-producing Clostridia, compared to fine bran particles which enriched members of Bacteroidaceae in *vitro*. In another *in vitro* fermentation study, while both fine and coarse oats increased Clostridial *Eubacteria spp.*, significant increase in butyrate was brought by oats with large particle size (0.85-1.0 mm) and increase in propionate in oats with smaller particle size (0.53-0.63 mm) (Connolly et al., 2010). Stewart and Slavin (2009) in an *in vitro* study observed that coarse wheat bran led to higher butyrate production after 24 h fermentation. Also, most butyrate-producing *Clostridium cluster XIVa* bacteria were abundant on insoluble wheat bran and starch, as revealed by FISH probes, compared to mucin (Leitch et al., 2007). The major butyrate producers stimulated were *Roseburia faecis*, and *Eubacterium rectale*. Similarly, insoluble wheat bran was recently shown to attach butyrate producing *Roseburia faecis, Roseburia hominis, Roseburia intestinalis, Clostridium xylanolyticum,* and genera of *Lachnospiraceae* including *Coprococcus* (De Paepe et al., 2018; 2019a)

1.6. *In vivo* and *in vitro* studies on whole grains and commensal Clostridia

Support of butyrogenic commensal Clostridia by whole grain fibers has been shown in human studies (Table 1). Roager et al. (2019), in an 8-week human crossover study, found that *Faecalibacterium prausnitzii*, a butyrate-producing Clostridia, was enriched during the high whole grain consuming period (179 g whole grain/d), while the bacteria declined in the low whole grain period (13 g whole grain/d) ($p < 0.05$). Also, gram-negative *Bacteroides thetaiotaomicron* was reduced with whole grain consumption ($p < 0.05$). In another study, intake of a whole grain diet (207 g of whole grain/d) compared to a more refined grain diet (0 g whole grain/d) increased Clostridial butyrate-producers *Roseburia* (Δ mean: 1.32), false discovery rate (FDR)-adjusted $p = 0.30$) and *Lachnospira* (Δ mean: 1.04, FDR-adjusted $p = 0.25$), and reduced proinflammatory Enterobacteriaceae (Δ mean: −0.07, FDR-adjusted $p = 0.25$) (Vanegas et al., 2017). Likewise, relative abundance of Clostridial species *Roseburia faecis* (Δ mean: 0.41, $p < 0.01$), *Roseburia intestinalis* (Δ mean: 0.21, $p < 0.05$), and *Eubacterium rectale* (Δ mean: 0.32–2.35, $p < 0.05$)

significantly increased with 60 g whole grain barley consumption in a 4-week study involving 28 humans relative to baseline levels (Martinez et al., 2013). In a recent study, whole wheat increased butyrate and abundance of Clostridia-assigned *Ruminococcus* in mice (Han et al., 2018). Furthermore, *in vitro* fermentation of wheat bran was shown to increase butyrate and butyrate-producing bacteria such as *Roseburia intestinalis/homis/faecis*, *Eubacterium rectale,* and *Coprococcus eutactus* (Duncan et al., 2016; Tuncil et al., 2018a; 2018b). Whole grains also increased fecal short chain fatty acid production in human studies. Consumption of whole grains at 5, 124, and 145 g whole grain/d in refined wheat, whole grain rye, and whole grain wheat diet groups reduced butyrate (−38%) in the refined wheat diet compared to the whole grain wheat (25%, $p = 0.014$) and whole grain rye (−1%, $p = 0.037$) groups, while not changing other short chain fatty acids (Vuholm et al., 2017) (Table 1.1). These treatments did not alter the gut microbiota significantly. In a randomized crossover trial where participants consumed a whole grain (32 g/d total dietary fiber) or refined grain diet (19 g/d of total dietary fiber), acetate and butyrate significantly increased in the whole grain compared to refined diet ($p = 0.02$ and $p = 0.05$, respectively) (Ross et al., 2013). In another human study (Vanegas et al., 2017), relative to the baseline, whole grain diets increased acetate and butyrate by 0.7 and 0.1 mmol/l, respectively, but reduced propionate by 1.9 mmol/l. These authors observed that refined grains reduced acetate, propionate, and butyrate by 1.4, 1.6, and 0.8 mmol/l, respectively, compared to the baseline. Vetrani et al. (2016) investigated the effects of a whole grain diet (40 g/d of dietary fiber) vs a refined cereal product diet (22 g/d of dietary fiber) in humans. The whole grain group significantly increased plasma propionate from 5.6 to 7.1 μmol/l, and reduced propionate in the refined grain group from 7.6 to 6.3 μmol/l ($p = 0.048$). Both treatments increased plasma butyrate levels with whole grain diet leading to greater increase by 0.4 μmol/l compared to 0.1 μmol/l in the refined control diet relative to baseline level. Plasma acetate reduced by 26 and 25 μmol/l in the refined and whole grain diets, respectively. In general, these studies show that whole grain diets increase short chain fatty acids production compared to refined diets

However, other studies did not show differences in butyrate or butyrogenic bacteria in refined versus whole grain diets. For example, Cooper et al. (2017) reported no differences in gut microbiota abundance in human subjects consuming either to a whole grain diet that supplied an of 13.7 g average daily fiber, or a refined grain diet that supplied an 4.2 g average daily fiber for 6 weeks. Moreover, Ross et al. (2011) did not observe substantial changes in human fecal microbiota

with either whole grain or refined grain treatments in a two-week crossover trial where whole grains and refined grains supplied 32 and 19 g of average total dietary fiber/d, respectively, though *Clostridium leptum* did increase in the whole grain group. A possible explanation for lack for changes in gut microbiota in these studies was that whole grain supplementation was not high enough, especially in the bran fraction, to observe a difference.

1.7. Health outcomes/implications with whole grain diets and the gut microbiome

Studies have reported an inverse association between whole grain intake and incidence of diseases and health conditions, such as colorectal cancers (Larsson et al., 2005; Egeberg et al., 2010; Kyro et al., 2013), weight gain, cardiovascular disease, and type 2 diabetes (Ye et al., 2012; Mellen et al., 2008). The impact of changing the human gut microbiota structure and function with whole grains could be a key mechanism of action in their health impact. For example, in a randomized parallel arm trial, Kopf et al. (2018) found that whole grain intake significantly reduced tumor necrosis factor alpha (TNF-α) and lipopolysaccharide binding protein (LBP), which are biomarkers of systemic inflammation and barrier function. These authors also found that persons who had lower Bacteroidetes but higher Firmicutes witnessed the largest decrease in LBP. Corroborating these results, Martinez et al. (2013) found that increase in butyrate producing *Eubacterium rectale* upon consumption of brown rice and whole grain barley blend was associated with a reduction in insulin as well as postprandial glucose response. Zhao and colleagues (2018) also observed that stimulation of butyrate production by gut microbiota in the presence of dietary fibers lead to alleviation of type 2 diabetes. These studies offer insights that changes in gut microbiota due to whole grains could be linked to these metabolic outcomes. Other microbiota and health outcomes of whole grains intake are summarized in Table 1.1.

1.8. Extrusion technology as a tool to modify whole grain fiber

Extrusion has been shown to increase the soluble fiber content, such as in the case of extrusion of soybean residue (Jing and Chi, 2013). Increasing temperature corresponded with an increase in soluble dietary fiber proportions. However, the authors cautioned that high extrusion temperatures can cause agglomeration of material within the barrel or cause unequal puffing, and these can cause a decrease in soluble dietary fiber amounts. Another parameter that has been shown

to have an influence on fiber solubility during extrusion is screw speed or shear rate. The same researchers investigated the effects of different shear rates (140, 160, 180, 200, 220 rpm) on fiber solubility, and found an increase in soluble fiber from 140 to 120 rpm, then a slight drop at 220 rpm (Jing and Chin, 2013). They explained that at high screw speed there is higher pressure in the barrel, which intensifies the pressure between the feed material and the screw leading increased transformation of insoluble to soluble fiber proportions.

Dietary fiber extractability in wheat bran and rye brans were shown to increase upon extrusion and the greatest fiber extraction was attained at lower feed moisture, higher temperatures and higher screw speeds (Andersson et al., 2017). Reduction of insoluble dietary fiber with extrusion occurred during extrusion of oat bran and rice bran and was less severe at high screw speeds (Gualberto et al., 1997). According to these authors, possible reasons for the decrease in the amount of insoluble fiber are, that at higher shear, strain is exerted on the insoluble fiber which results in the breaking of chemical bonds generating smaller particles with better solubility. At lower screw speed there is higher residence time and pressure attributed to better screw fill. Also, resistant starch can be formed by starch-lipid/protein/phytochemicals leading to increased dietary fiber. However, these researchers and others did not observe changes in the insoluble fiber in wheat bran upon extrusion. The amount of soluble fiber has generally been reported to increase during extrusion (Gualberto et al., 1997). In another study, Wang et al. (1993) extruded wheat bran and whole wheat at low shear (200 rpm), medium shear (300 rpm) and high shear (400 rpm) and moisture was added to the feed materials at 260 g/min per treatment. They reported an increase in amount of soluble fiber and a decrease in insoluble fiber. The greatest increase and decrease in soluble and insoluble fibers, respectively, were observed at high shear rate suggesting that higher shear favors conversion of insoluble to soluble fiber. In a separate study, Gajula et al. (2008) observed that extrusion of wheat flour supplemented with wheat bran at a screw speed of 200 rpm at 0, 10, 20, 30% incorporation levels, at barrel temperatures of 30, 32, 34, 38, 40°C resulted into increase in soluble fiber and decrease in insoluble dietary fiber. They proposed that during extrusion insoluble dietary fiber can be fragmented into smaller sugars or molecules that are still quantified as dietary fiber. Several other authors have observed a decline in insoluble dietary fiber during extrusion and this impact is always greater when temperature and screw speeds are higher (Sandrin et al., 2019; Arribas et al., 2019). Decreases in insoluble fiber and increase soluble fiber can be due to interruption noncovalent or covalent bonds present in carbohydrates upon exposure

to shear and temperatures during extrusion process (Wang et al., 1993). Alternatively, during extrusion the thermal and shear forces generated by the extruder can break down lignin and cellulose which form a large proportion of insoluble dietary fibers, and thereby liberate soluble fibers (Gajula et al., 2008). Upon extrusion of rice-bean flour blend with or without whole carob fruit, resistant starch was shown to reduce to non-detectable levels (Arribas et al., 2019; Arribas et al., 2017). Conclusively, extrusion can influence the fiber microstructure and influence the functionality of extruded foods.

1.9. Effects of extrusion on color and texture parameters of foods

Extrusion can influence the color of products. For instance, extrusion increased the darkness of tortillas and cookies made from instant extruded flours more than from non-extruded flour (Gajula et al., 2008). Extrusion was shown to increase the darkness in products by lowering the lightness (*L**) of the products, while redness (*a**) exhibited limited variability (Bakalov et al., 2016). These authors also observed that yellowness (*b**) was higher in extruded samples than in the non-extruded ones. In this study, low screw speed led to darker color due higher residence time in the extruder. However, at higher screw speeds there is increased temperature and shear, which may increase the Maillard browning reactions between proteins and reducing sugars (Bakalov et al., 2016; Nayak et al., 2011; Saldanha et al., 2019). Extrusion of composite flours at 130-140°C was shown to reduce hue, chroma compared to the non-extruded flour (Nayak et., 2011). Another factor that influences the color of extrudates is feed moisture content. It was observed that at lowest moisture conditions, extrudates had darker color (lowest L*-values) and higher *a** and *b**-values (Altan et al., 2008). It has been suggested that Maillard reactions occurring during extrusion increase *a* and *b* values (Altan et al., 2008; Kamau et al., 2020).

Extrusion has been shown to reduce peak, trough, final and setback viscosities of flours or starches compared to non-extruded ones (Zeng et al., 2011; Garcia-Valle et al., 2019; Hayes et al., 2020b). This could be due fragmentation of amylopectin and amylose during extrusion shear (Moussa et al., 2011; Kamau et al., 2020). It has been suggested that shear of amylopectin could be desirable by preventing amylopectin reassociation typical in bread staling and could be used to extend the shelf life of baked products (Hayes et al., 2020b). Fragmentation also was noted to improved consistency and smoothness of pearl millet thin porridge (Moussa et al., 2011). Other

advantages of extrusion include an increase in starch and protein digestibility due to inactivation of antinutritional factors that inhibit enzymes or by breaking starch to smaller molecules that are more digestible, production of expanded snacks with desired textural properties, and increase in antioxidant properties in products (Kamau et al., 2020; Nayak et al., 2011, Altan et al., 2009).

1.10. Consumers' willingness to pay (WTP) for nutritional attributes

Consumers' willingness to pay for novel, value-added, fortified, or safe food products has to be conducted in studies to assess consumers acceptance and interest to purchase (Lysak et al., 2019; Kimenju and De Groote, 2010; De Groote et al., 2016; Chege et al., 2019). Experimental auctions are typically used to determine WTP for products and they include the Vickery and Becker-DeGroot-Marshack (BDM) auction mechanisms (Becker et al., 1964; Chege et al., 2019). In the Vickery auction, consumers are requested to give a bid that is equivalent to the highest WTP for a product and they purchase the product at the second highest bid of all the bids placed in an auction (Chege et al., 2019). In the BDM auction, participants give a bid that is equated to a selling price that is randomly chosen from a pool of likely prices set by the researcher or seller. In the case that the bid submitted is equal or higher than the randomly selected vending price, the product is bought at that price (Becker et al., 1964).

There are several studies that have tried to determine WTP for food products with different characteristics such yoghurt with cholesterol-lowering potential in Paris (Europe) (Marette et al., 2010), organic foods in Greece (Europe) (Krystallis and Chryssohoidis, 2005), organic vs. natural chicken in the USA (Gifford and Bernard, 2011), and with different levels of food information on the label (Berning et al., 2010**;** My et al., 2018). In developing nations of Africa, there have been experiments on consumers' WTP for instant fortified pearl millet flours in Senegal (De Groote et al., 2018), maize meal (De Groote et al., 2011), and maize nutritional quality and color (De Groote and Kimenju, 2008) in Kenya, underutilized vegetables in South Africa (Senyolo et al., 2014), and in Ghana where the researchers assessed consumers' WTP for higher premiums for tilipaia and chicken meats that were certified to be free from antibiotics residues (Ragasa et al., 2019).

In some cases, interest in establishing the WTP for foods products with different characteristics could be because consumers are beginning to appreciate the central link between food products and health. Literature suggest that consumers are willing to pay more for products

with perceived health benefits such instant fortified flours with micronutrients sources compared to traditional non-instant pearl millet flours (De Groote et al., 2018), meats free from antibiotics traces (Ragasa et al., 2019), and ability of consumers to pay more when given nutrition information about food products (My et al., 2018, Chege et al., 2019). In Ghana, consumers who were aware of the cassava-wheat composite flour bread and liked the texture and taste were more likely to pay more compared to those that were unaware (Owusu et al., 2017).

The WTP of consumers for food products are influenced by demographic and socio-economic factors (Owusu et al., 2017; My et al., 2018; De Groote et al., 2018; 2020). For example, increase in age was inversely associated with WTP, while households with larger size had higher WTP for bread made from cassava-wheat composite flour (Owusu et al., 2017). Increase in family size has also been shown to be inversely proportional to WTP (Bett et al., 2013). Proponents of small family size increasing the WTP argues that small families have more disposable income to buy more products. Nutritional benefits or knowledge awareness are other variables shown to positively impact WTP by consumers (Owusu et al., 2017; Chege et al., 2019; Mabaya et al., 2010; Candance-Jackson et al., 2013). In Chege et al. (2019), consumers were willing to pay more for fortified or composite flours when given nutrition information. A similar observation was made by De Groote et al. (2018) as consumers were willing to pay more for instant pearl millet flours fortified with natural micronutrient sources such as carrot and mango powder. These studies suggest that when consumers are aware of or given nutrition information about a product, they are willing to pay more for the product. Taken together, consumers' WTP for food products depend on both demographic and socio-economic factors and can hence influence adoption of products.

1.11. Perspectives and future trends of extrusion technology

In conclusion, there is market potential in Africa for improved nutritious products, and potentially instant convenient products, and this partly depends on their adoption. Besides producing convenient foods, extrusion technology can spur product development by altering macronutrient structure such as making fiber more fermentable for gut health, improving composition and quality hence influencing product functionality, and utilization and metabolism in the body. This project addresses the questions of how to increase adoption of extruded instant pregelatinized fortified flours which are potential carriers for provitamin A and minerals such as

iron and zinc, how extrusion could be used to improve the value of whole grain fibers for gut health.

1.12. Experimental aims

According to the World Bank (2015), the population of Africans living in urban areas is expected to rise to 50% in 2030. This big shift in population from rural areas to cities is coupled with emerging food processing industries that make foods, often convenient ones, to feed the ever-growing population. The single-screw extruder developed by Purdue faculty and commercialized by Technochem, Inc. has been shown to make nutritious convenient instant fortified whole grain products that supply micronutrients and dietary fibers for growing urban populations (De Groote et al., 2020). Unlike most commercial extruders, this extruder is inexpensive, robust, and relatively easy to use. Bearing this in mind, this thesis research aimed to determine operating conditions for the single-screw extruder to make it more usable for African small- and medium-scale food processors to reduce extrusion cost and still produce quality instant flours, to assess the quality of extruded whole grain bran on its potential to improve colonic metabolic output and perhaps positively impact the gut microbiome, and to assess the acceptance and willingness to pay for instant fortified flours using food-to-food fortification for thin and thick porridges that can meet 25% of micronutrient sources for provitamin A, iron, and zinc.

Specific aims

1. To determine feed moisture conditions that lead to high quality instant flours and extrusion costs involved. We hypothesized that lowering the moisture content would produce extrudates that dry faster.
2. To determine the effects of different extrusion shear rates and feed moisture contents on colonic fermentation patterns of dietary fiber. We hypothesized that higher shear rates would increase the fermentability of the bran fibers.
3. To evaluate the effect of provision of nutrition information and demonstration on consumers acceptance and WTP for instant fortified porridge flour products using food-to-food fortification. We hypothesized that when consumers are given nutrition information alongside a demonstration of how to reconstitute the instant flours to make thin and thick porridges, they would be willing to pay more for these products.

1.13. References

Altan, A., McCarthy, K. L., and Maskan, M. (2009). Effect of extrusion cooking on functional properties and in vitro starch digestibility of barley-based extrudates from fruit and vegetable by-products. *Journal of Food Science,* 74(2), E77-86.

Altan, A., McCarthy, K. L., and Maskan, M. (2008). Twin screw extrusion of barley-grape pomace blends: extrudate characteristics and determination of optimum processing conditions. *Journal of Food Engineering*, 89, 24-32.

Ampatzoglou, A., Atwal, K.K., Maidens, C.M., Williams, C.L., et al. (2015). Increased whole grain composition does not affect blood biochemistry, body composition or gut microbiology in health, low habitual whole grain consumers. *Journal of Nutrition*, 145 (2), 215-21.

Andersson, A. A. M., Andersson, R., Jonsall, A., Andersson, J., and Fredriksson, H. (2017). Effect of Different Extrusion parameters on dietary fiber in Wheat Bran and Rye Bran. *Journal of Food Science*, 82 (6), 1344-1350.

Andersson, A.F.,Lindberg, M., Jakobsson, H.,backhed, F., and Neyren, P., et al. (2008). Comparative analysis of human gut microbiota by barcoded pyrosequencing. *PloS ONE 3(7),* e2836. doi: 10.1371/journal.pone.0002836.

Arcila, J. A., Weier, S. A., and Rose, D. J. (2015). Changes in dietary fiber fractions and gut microbial fermentation properties of wheat bran after extrusion and bread making. *Food Research International*, 74: 217-223.

Arribas, C., Cabellos, B., Cuadrado, C., Guillamon, E., et al. (2019). Extrusion effect on proximate composition, starch and dietary fiber of ready to eat products based on rice fortified with carob fruit and bean. *LWT-Food Science and Technology*, 111: 387-393.

Arribas, C., Cabellos, B., Sanchez, C., Cuadrado, C., Guillamon, E., & Pedrosa, M. M. (2017). The impact of extrusion on the nutritional composition, dietary fiber and in vitro digestibility of gluten-free snacks based on rice, pea and carob flour blends. *Food & Function, 8,* 3654–3663.

Artzi, L., Bayer, E. A., & Morais, S. (2017). Cellulosomes: bacterial nanomachines for dismantling plant polysaccharides. *Nature Reviews Microbiology*, 15,83-95.

Aune, D., Norat, T., Romundstad, P., & Vatten, L.J. (2013). Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis of cohort studies. *European Journal of Epidemiology*, 28, 845-858.

Bakalov, I. Y., Petrova, T. V., Petrova, T. V., Ruskova, M. M., Kalcheva-Karadzhova, K. D., and Penov, N. D. (2016). The effect of extrusion variables on the color of bean based extrudates. *Bulgarian Chemical Communications*, 48, 407-411.

Bayer, E. A., Belaich, J., Shoham, Y., & Lamed, R. (2004). The cellulosomes: multienzyme machines for degradation of plant cell wall polysaccharides. *Annual Review of Microbiology*, 58, 521-554.

Becker, G. M., Degroot, M. H., and Marschak, J. (1964). Measuring utility by single response sequential method. *Behavioral Science*, 9:226-232.

Belobrajdic, D., and Bird, A. R. (2013). The potential role of phytochemicals in whole grain cereals for the prevention of type 2 diabetes. *Nutrition Journal*, 12:62.

Berning, J.P., Chouinard, H. H., Manning, K. C., McCluskey, J. J., and Sprott, D. E. (2010). Identifying consumers preferences for nutrition information on grocery store shelf labels. *Food Policy*, 35, 429-436.

Bouvier, J. M., and Campanella, O. H. (2014). Extrusion processing technology-food and non-food biomaterials. *Wiley-Blackwell*. ISBN 13:9781444338119

Brennan, M. A., Derbyshire, E., Tiwari, B., and Charles, C. S. (2013). Ready to eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. *International Journal of Food Science and Technology*, 5: 893-902.

Brennan, C., Brennan, M., Derbyshire, E. & Tiwari, B.K. (2011). Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science & Technology*, 22, 570-575.

Bett, H.K., Peters, K.J., Nwankwo, U.M., and Bokelmann, W. (2013). Estimating consumer preferences and willingness to pay for the underutilized indigenous chicken products. *Food Policy*, 41, 218-225.

Bule, P., Pires, V.M.R., Alves, V.D. Carvalho, A. L., Jose, Prates, J. A. M., et al. (2018). Higher order scaffoldin assembly in *Ruminococcus flavefaciens* cellulosome is coordinated by a discrete cohesin-dockerin interaction. *Scientific Reports* 8, 6987. https://doi.org/10.1038/s41598-018-25171-8

Candance-Jackson, J., Weatherspoon, L., Nnyepi, M., Malete, L.,Mokgatlhe, L., et al. (2013). Sorghum bean composite porridge nutritional quality and acceptability. *Nutrition and Food Science*, 43, 453-461.

Chege, C.G.K., Sibiko, K. W., Wanyama, R., Jager, M., Birachi, E., et al. (2019). Are consumers at the base of the pyramid willing to pay for nutritious foods? *Food Policy*, 87, 101745.

Chen, G., Ran, X., Li, B., Li, Y., He, D., Hunag, B., et al. (2018). Sodium butyrate inhibits inflammation and maintains epithelial barrier integrity in a TNBS-induced inflammatory bowel disease mice model. *EBio Medicine*, 30, 317-325.

Christensen, L., Vuholm, S., Roager, H. M., Nielsen, D., Krych, L., et al. (2019). *Prevotella* abundance predicts weight loss success in healthy, overweight adults consuming a whole diet *ad libitum*: a post hoc analysis of a 6-week randomized controlled trial. *The Journal of Nutrition*, 149, 2174-2181.

Christensen, E.G., Licht, T.R., Kristensen, M., & Bahl, M.I. (2013). Bifidogenic effect of whole grain wheat during a 12-week energy – restricted dietary intervention in postmenopausal women. *European Journal of Clinical Nutrition*, 67,1316-1321.

Connolly, M. L., Lovegrove, J. A., and Tuohy, K. M. (2010). In vitro evaluation of the microbiota modulation abilities of different sized whole oats grains. *Anaerobe*, 16, 483-488.

Cooper, D.N., Kable, M. E., Marco, M. L., & De Leon, A. (2017). The effect of moderate whole grain consumption on fasting glucose and lipids, gastrointestinal symptoms, and gut microbiota. *Nutrients*, *9*, 173.

Costabile, A., Klindler, A., Fava, F., Napolitano, A., Fogliano, V., et al. (2008). Whole grain wheat breakfast cereal has prebiotic effect on the human gut microbiota: a double-blind placebo controlled, crossover study. *British Journal of Nutrition*, 99 (1),110-120.

David, Y.B., Dassa, B., Borovok, I., Lamed, R., Koropatkin, N.M., Martens, E.C., et al. (2015). Ruminococcal cellulosome systems from rumen to human. *Environmental Microbiology*, 17 (9), 3407-3426.

De Groote, H., Mugalavai, V., Ferruzzi, M., Onkware, A., Ayua, E., Duodu, K. G., et al. (2020). Consumer acceptance and willingness to pay for instant cereal products with food to food fortification in Eldoret, Kenya. *Food and Nutrition Bulletin*, 41(2), 224-243.

De Groote, H., Kariuki, S. W., Traore, D., Taylor, J. R., Ferruzzi, M. G., and Hamaker, B. (2018). Measuring consumers interest in instant fortified pearl millet products: a field experiment in Touba, Senegal. *Journal of the Science of Food and Agriculture*, 98, 2320-2331.

De Groote, H., Narrod, C., Kimenju, S. C., Bett, C., Scott, R. P. B., Tiongco, M. M., and Gitonga, Z. M. (2016). Measuring rural consumers' willingness to pay for quality labels using experimental auctions: the case of aflatoxin free maize in Kenya. *Agricultural Economics*, 47, 33-45.

De Groote, H., Kimenju, S. C., and Morawetz, U. B. (2011). Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. *Agricultural Economics*, 42, 1-16.

De Groote, H., & Kimenju, S. C. (2008). Comparing consumer preferences for color and nutritional quality in maize: Application of a semi-double-bound logistic model on urban consumers in Kenya. Food Policy, 33(4), 362–370. doi:10.1016/j.foodpol.2008.02.005

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S et al. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *National Academy of Sciences*, 107, 14691–14696.

De Paepe, K., Verspreet, J., Verbeke, K., Raes, J., Courtin, C. M., & Van de Wiele, T. (2018). Introducing insoluble wheat bran as a gut microbiota niche in an in vitro dynamic gut model stimulates propionate and butyrate production and induces colon region specific shifts in the luminal and mucosal microbial community. *Environmental Microbiology*, 20 (9), 3406-3426.

De Paepe, K., Verspreet, J., Courtin, C.M., & Van de Wiele, T. (2019a). Microbial succession during wheat bran fermentation and colonization by human faecal microbiota as a result of niche diversification. *The ISME Journal*, 14(2), 584-596. doi:10.1038/s41396-019-0550-5

Dhingra, D., Michael, M., Rajput, H., & Patil, R.T. (2012). Dietary fiber in foods: a review. *Journal of Food Science and Technology*, 49 (3), 255-266.

Doi, R.H., and Kosugi, A. (2004). Cellulosomes: plant cell wall degrading enzyme complexes. *Nature Reviews Microbiology*, 2,541-551.

Duncan, S.H., Russell, W.R., Quartieri, A., Ross, M., et al. (2016). Wheat bran promotes enrichment within the human colonic microbiota of butyrate producing bacteria that release ferulic acid. *Environmental Microbiology*, 18 (7), 2214-2225.

Duerre, P. (2005). Handbook on Clostridia. 1st Edition Boca Raton, CRC Press, p. 116. https://doi.org/10.1201/9780203489819

Dyson, J.E.D., Daniel, J., and Surrey, C.R. (1992). The effect of sodium butyrate on the growth characteristics of cervix tumor cells. *British Journal of Cancer*, 65, 803-808.

Garcia-Valle, D.E., Bello-Perez, L.A., Flores-Silva, P.C., Agama-Acevedo, E., and Tovar, J. (2019). Extruded Unripe Plantain Flour as an Indigestible Carbohydrate-Rich Ingredient. *Frontiers in Nutrition*, 6, 2. doi: 10.3389/fnut.2019.0000

Egeberg, R., Olsen, A., Loft, S., Christensen, J., Johnsen, N.F., Overvad, K., & Tjonneland, A. (2010). Intake of whole grain products and risk of colorectal cancers in the diet, cancer and health cohort study. *British Journal of Cancer*, 103,730-734.

Flint, H.J., Scott, K.P., Duncan, S.H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, 3(4), 289-306.

Gajula, H., Alavi, S., Adhikari, K., and Herald, T. (2008). Precooked bran-enriched wheat flour using extrusion: Dietary Fiber profile and sensory characteristics. *Journal of Food Science*, 73, S173-S179.

Gualberto, D.G., Bergman, C. J., Kazemzadeh, M., and Weber, C.W. (1997). Effect of extrusion processing on the soluble and insoluble fiber, and phytic acid contents of cereal brans. *Plant Foods for Human Nutrition*, 51, 187-198.

Guillon, F., Auffret, A., Robertson, J. A., Thibault, J.F., Barry, J.L. (1998). Relationships between physical characteristics of sugar-beet fiber and its fermentability by human fecal flora. *Carbohydrate Polymers*, 37(2), 185-197. DOI: 10.1016/s0144-8617(98)00053-8.

Hamaker, B.R., & Tuncil, Y.E. (2014). A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *Journal of Molecular Biology*, 426 (23), 3838-3850.

Hamer, H.M., Jonkers, D., Venema, K., Vanhoutvin, S., et al. (2008). The role of Butyrate on Colonic Function. *Alimentary Pharmacology and Therapeutics*, 27 (2),104-119.

Han, F., Wang, Y., Han, Y., Zhao, J et al. (2018). Effects of whole grain rice and wheat on composition of gut microbiota and short chain fatty acids. *Journal of Agricultural and Food Chemistry*, 66, 6326-6335.

Hayes, A. M. R., Gozzi, F., Diatta, A., Gorissen, T., Swackhamer, C., Bellman, S., and Hamaker, B. R. (2020a). Some pearl millet-based foods promote satiety or reduce glycaemic response in crossover trial. *British Journal of Nutrition*, https://doi.org/10.1017/S0007114520005036

Hayes, A. M. R., Okoniewska, M., Martinez, M.M., Zhao, B., and Hamaker, B. R. (2020b). Investigating the potential of slow retrograding starches to reduce staling in soft savory bread and sweet cake model systems. *Food Research International*, 138, 109745. https://doi.org/10.1016/j.foodres.2020.109745

Jing, Y., and Chi, Y. (2013). Effects of twin-screw extrusion on soluble dietary fiber and physicochemical properties of soybean residue. *Food Chemistry*, 138, 884-889.

Kamau, E. H., Smith, G. N., and Ayua, E. O. (2020). Extrusion and nixtamalization conditions influence the magnitude of changes in the nutrients and bioactive compounds of cereals and legumes. *Food Science and Nutrition*, 8, 1753-1765.

Kaur, A., Chen, T.,Green, S.J., Mutlu, E., et al. (2019). physical inaccessibility of a resistant starch shifts mouse gut microbiota to butyrogenic firmicutes. *Molecular Nutrition and Food Research*, 63, 1801012, DOI: 10.1002/mnfr.201801012

Kimenju, S. C., and De Groote, H. (2010). Consumer willingness to pay for genetically modified in Kenya. *Agricultural Economics*, 38, 35-46.

Kopf, J., Suhr, M.J., Clarke, J., Eyun, S., et al. (2018). Role of whole grains versus fruits and vegetables in reducing subclinical inflammation and promoting gastrointestinal health in individuals affected by overweight and obesity: a randomized controlled trial. *Nutrition Journal*, 17 (1), 72. https://doi.org/10.1186/s12937-018-0381-7.

Kranz, S., Dodd, K.W., Juan, W.Y., Johnson, L.K., et al. (2017). Whole grains contribute only a small proportion of Dietary Fiber to the U.S. Diet. *Nutrients*, 9 (2), 153.

Krause, D.O., Denman, S.E., Mackie, R.I., Morrison, M., Rae, A. L., Attwood, G.T., et al. (2003). Opportunities to improve fiber degradation in the rumen: microbiology, ecology and genomics. *FEMS Microbiology Reviews*, 27 (5), 663-693.

Krystallis, A., and Chryssochoidis, G. M. (2005). Consumers willingness to pay for organic food: factors that affect it and variation per organic food product type. *British Food Journal*, 107, 320-343.

Kurakawa, T., Ogata, K., Matsuda, K., Tsuji, H., Kubota, H., Takada, T., et al. (2015) Diversity of intestinal Clostridium Coccoides group in the Japanese population, as demonstrated by reverse transcription-quantitative PCR. *PLoS ONE* 10 (5), e0126226. doi: 10.1371/journal.pone.0126226.

Kyro, C., Skeie, G., Loft, S., Landberg, R., Christensen, J., Lund, E., et al. (2013). Intake of whole grains from different cereals and food sources and incidence of colorectal cancer in the Scandinavian HELGA cohort. *Cancer Causes and Control*, 24, 1363-1374.

Lappi, J., Salojarvi, J., Kolehmainen, M., Mykkanen, H., Poutanen, K., de Vos, W. M., et al. (2013). Intake of whole grains and fiber rich rye bread versus refined wheat bread doesn't differentiate intestinal microbiota composition in Finish adults with metabolic syndrome. *Journal of Nutrition*, 143, 648-55.

Larsson, S.C., Giovannucci, E., Bergkvist, L., & Wolk, A. (2005). Whole grain consumption and risk of colorectal cancer: a population-based cohort of 6000 women. *British Journal of Cancer*, 92,1803-1807.

Lee, C., Kim, B. G., Kim, J.H., Chun, J., Im, J.P., & Kim, J.S. (2017). Sodium butyrate inhibits the NF-kappa B signaling pathway and histone deacetylation, and attenuates experimental colitis in an IL-10 independent manner. *International Immunopharmacology*, 51,47-56.

Leitch, E.C.M., Walker, A.W., Duncan, S.H., Holtrop, G., and Flint, H.J. (2007). Selective colonization of insoluble substrates by human faecal bacteria. *Environmental Microbiology*, 9 (3), 667-679.

Leoncini, E., Prata, C., Malaguti, M., Marotti, I., Segura-Carretero, A., et al. (2012) Phytochemical Profile and Nutraceutical Value of Old and Modern Common Wheat Cultivars. *PLoS ONE* 7(9), e45997. doi:10.1371/journal.pone.0045997

Leonel, A.J., & Alvarez-Leite, J.I. (2012). Butyrate: implications for intestinal function. *Current Opinion on Clinical Nutrition and Metabolic Care*, 15, 474-479.

Lopetuso, L.R., Scaldaferri, F., Petito, V., & Gasbarrini, A. (2013). Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut Pathogens*, 5 (1), 23 http://doi.org/10.1186/1757-4749-5-23

Louis, P., & Flint, H.J. (2009). Diversity, metabolism and microbial ecology of butyrate producing bacteria from human large intestine. *FEMS Microbiological Letters*, 294 (1), 1-8.

Lysak, M., Ritz, C., and Henriksen, C. B. (2019). Assessing consumer acceptance and willingness to pay for novel value added products made from breadfruit in Hawaiian islands. *Sustainability*, *11*(11), 3135 http://dx.doi.org/10.3390/su11113135

Ma, X., Fan, P.X., Li, L.S., Qiao, S. Y., Zhang, G .L., & Li, D. F.. (2012). Butyrate promotes the recovering of intestinal wound healing through its positive effects on the tight junctions. *Journal of Animal Science*, 90 (suppl_4), 266-268.

Mabaya, E., Jordaan, D., Malope, P., Monkhei, M., and Jackson, J. (2010). Attribute preferences and willingness to pay for fortified cereal foods in Botswana. *Agrekon*, 49, 459-483.

Malin, S. K., Kullman, E. L., Scelsi, A. R., Godin, J., Ross, A. B., and Kirwan, J.P. (2020). A whole grain diet increases glucose stimulated insulin secretion independent of gut hormones in adults at risk for type 2 diabetes. *Molecular Nutrition and Food Research*, 63:1800967. https://doi.org/10.1002/mnfr.201800967

Marette, S., Roosen, J., Blanchemanche, S., Feinblatt-Meleze, E. (2010). Functional food, uncertainity and consumers choices: a lab experiment with enriched yorhurts for lowering cholesterol. *Food Policy*, 35, 419-428.

Marlett, J.A., McBurney, M.I., & Slavin, J.L. (2002). Position of the American Dietetic association: health implications of dietary fiber. *Journal of the American Dietetic Association*, 102 (7), 993-1000.

Martinez, I., Lattimer, J.M., Hubach, K.L., Case, J.A., Yang, J., Weber, C.G., et al. (2013). Gut microbiome composition is linked to whole grain induced immunological improvements. *International Society for Microbial Ecology*, 7, 269-280.

Martens, E.C., Lowe, E. C., Chiang, H., Pudlo, N.A., Wu, M., McNulty, N.P., et al. (2011). Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts. *PLoS Biology*, 9, e1001221, https://doi.org/10.1371/journal.pbio.1001221

Mellen, P.B., Walsh, T.F., Herrington, D.M. (2008). Whole grain intake and cardiovascular disease. A meta-analysis. *Nutrition, Metabolism and Cardiovascular Diseases*, 18 (4), 283-290.

Meng, X., Threinen, D., Hansen, M., and Driedger, D. (2010). Effects of extrusion conditions on systems parameters and physical properties of chickpea flour-based snack. *Food Research International*, 43, 650-658.

My, N.H.D., Demont, M., Loo, E. J. V., de Guia, A., Rutsaert, P., Tuan, T. H., and Verbeke, W. (2018). What is the value of sustainability produced rice? Consumer evidence from experimental auctions in Vietnam. *Food Policy*, 79:283-296.

Nayak, B., Berrios, J. J., Powers, J. R., and Tang, J. (2011). Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flour mixes. *Journal of Food Science*, 76, C874-C883.

Ndiaye, C., Martinez, M. M., Hamaker, B. R., Campanella, O. H., and Ferruzzi, M.G. (2020). Effect of edible plant materials on provitamin A stability and bioaccessibility of extruded whole pearl millet (*P. typhoides*) composite flours. *LWT Food Science and Technology*, 123, 109109.

Nystrom, L., Lampi, A., Andersson, A. A. M., Kamal-Eldin, A., Gebruers, K., Courtin, C. M., et al. (2008). Phytochemicals and dietary fiber components in Rye varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 21, 9758–9766

Plastic Processing Equipment and Machinery (2017). What does the advantages and shortcomings between twin screw extrusion machine? Retrieved January 2020 from https://ppemachine.weebly.com/twin-screw-extruder-03.html

Owusu, V., Owusu-Sekyere, E., Donkor, E., Darkwaah, N. A., and Adomako-Boateng Jr, D. (2017). Consumers perceptions and willingness to pay for cassava-wheat composite bread in Ghana. *Journal of Agribusiness in Developing and Emerging Economies*, 7, 115-134.

Pokusaeva, K., Fitzgerald, G.F., and van Sinderen, D. (2011). Carbohydrate metabolism in Bifidobacteria. *Genes Nutrition*, 6, 285-306.

Ponrajan, A., Tonner, T., Okos, M., Campanella, O., Narsihman, G. (2020). Comparing inline extrusion viscosity for differing operating conditions to offline capillary viscosity measurments. *Journal of Food Processing Engineering*, 43: e13199.

Ragasa, C., Andam, K. S., Amewu, S., and Asante, S. B. (2019). Consumer demand and willingness to pay for safe food in Accra,Ghana. Implications for public and private sectors

roles in food safety management. *International Food Policy Research Institute*, Discussion Paper 01795.

Ramakrishna, B.S. (2013). Role of the gut microbiota in human nutrition and metabolism. *Journal of Gastroenterology and Hepatology*, 28 (Suppl.4): 9-17.

Roager, H. M., Vogt, J. K., Kristensen, M., Hansen, L. B. S., Ibrugger, S., Maerkedahl, R.B., et al.

(2019). Whole grain rich diet reduces body weight and systematic low-grade inflammation without inducing major changes of the gut microbiome: a randomized cross over trial. *Gut*, 68, 83-93.

Rivière, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. (2016) Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Frontiers in Microbiology*, 7, 979. doi: 10.3389/fmicb.2016.00979

Ross, A.B., Pere-Trepat, E., Montoliu, I., Martin, F.J., et al. (2013). A whole grain rich diet reduces urinary excretion of markers of protein catabolism and gut microbiota metabolism in healthy men after one week. *Journal of Nutrition*, 143 (6), 766-773.

Ross, A., Bruce, S.J., Blondel-Lubrano, A., Oguey-Araymon, S., Beaumont, M., et al. (2011). A whole grain cereal rich diet increases plasma betaine and tends to decrease total and LDL-cholesterol compared with a refined diet in healthy adults. *British Journal of Nutrition*, 105, 1492-1502.

Saa, D.T., Turroni, S., Serrazanetti, D.I., Rampelli, S., Maccaferri, S., Candela, M., et al. (2014). Impact of Kamut Khorasan on gut microbiota and metabolome in healthy volunteers. *Food Research International*, 63, 227-232.

Sacchetti, G., Pinnavaia, G. G., Guidolin, E., and Rosa, M. D. (2004). Effects of extrusion temperature and feed composition on the functional, physical and sensory properties of chestnut and rice flour-based snack like products. *Food Research International*, 37, 527-534.

Salgado, N. Giraldo, G.I, and Orrego, C. E. (2017). Influence of extrusion operating conditions on the antioxidant, hardness and color properties of extruded mango. *LWT-Food Science and Technology*, 86, 209-218.

Saldanha do Carmo, C., Varela, P., Poudroux, C., Dessev, T., Myhrer, K., Rieder, A., et al. (2019). The impact of extrusion parameters on physicochemical, nutritional and sensorial properties of expanded snacks from pea and oat fractions. *LWT-Food Science and Technology*, 112, 108252.

Sandrin, R., Mejia, S. M. V., Caon, T., and de Francisco, A. (2019). Optimization of extrusion process parameters for preparing fiber rich oat flour. *Journal of Food Processing Engineering*, 42, e12943. https://doi.org/10.1111/jfpe.12943

Senyolo, G. M., Wale, E., and Ortmann, G. F. (2014). Consumers willingness to pay for underutilized vegetable crops: the case of African leafy vegetables in South Africa. Journal of Human Ecology, 47:219-227.

Shen, J., Zhang, B., Wei, G., Pang, X., Wei, H., Li, M., et al. (2006). Molecular Profiling of the *Costridium leptum* subgroup in the Human Fecal Microflora by PCR-Denaturing Gradient Gel Electrophoresis and clone library analysis. *Applied and Environmental Microbiology*, 72 (8), 5232-5238.

Sheridan, P. O., Martin, J. C., Lawley, T. D., Browne, H. P., Harris, H. M. B., Bernalier-Donadille, A., et al. (2016). Polysaccharide utilisation loci and nutritional specialisation in a dominant group of butyrate producing human colonic *Firmicutes*. *Microbial Genomics*, 2(2), e000043.

Singh, S., Gamlath, S., and Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. *International Journal of Food science and Technology*, 42, 916-929.

Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, 5, 1417-1435.

Slavin, J. L., Jacobs, D., Marquart, L., Wiemer, K. (2001). The role of whole grains in disease prevention. Journal of American Dietetic Association, 101: 780-785.

Stewart, M. L., and Slavin, J.L. (2009). Particle size and fraction of wheat bran influence short chain fatty acid production in *vitro*. *British Journal of Nutrition*, 102 (10), 1404-1407.

Tang, G., Wang, D., Long, J., Yang, F., and Si, L. (2015). Meta-Analysis of the Association between whole grain intake and coronary heart disease risk. *The American Journal of Cardiology*, 115, 625-629.

Tuncil, Y.E., Thakkar, R., Marcia, A. D.R., Hamaker, B.R., & Lindemann, S.R. (2018a). Divergent short chain fatty acid production and succession of colonic microbiota arise in fermentation of variously sized wheat bran fractions. *Scientific Reports*, 8, 16655.

Tuncil, Y.E., Thakkar, R.D., Arioglu-Tuncil, S., Hamaker, B.R., & Lindemann, S.R. (2018b). Fecal Microbiota responses to bran particles are specific to cereal type and *In vitro* Digestion methods that mimic upper gastrointestinal tract passage. *Journal of Agricultural and Food Chemistry*, 66, 12580-12593.

Tuson, H. H., Foley, M.H., Koropatkin, N.M., & Biteen, J.S. (2018). Starch utilization system assemblies around stationary starch binding proteins. *Biophysical Journal*, 115 (2), 242-250.

Um, C.Y., Campbell, P.T., Carter, B., Wang, Y., Gapstur, S. M., et al. (2020). Association between grains, gluten and risk of colorectal cancer in the cancer prevention sudy-II nutrition project. *European Journal of Nutrition*, 59, 1739-1749.

Van den Abbeele, P., Belzer, C., Goossens, M., Kleerebezem, M., De Vos, W.M., Thas, O., et al. (2013). Butyrate producing Clostridium cluster XIVa species specifically colonize mucins in in-vitro gut models. *International Society for Microbial Ecology*, 7, 949-961.

Vanegas, S. M., Meydani, M., Barnett, J. B., Goldin, B., Kane, A., Rasmussen, H., Brown, C., et al. (2017). Substituting whole grains for refined grains in a 6-week randomized trial has a modest effect on gut microbiota, and immune and inflammatory markers of healthy adults. *American Journal of Clinical Nutrition*, 105 (3), 635-650.

Vetrani, C., Costabile, G, Luongo, D., Naviglio, D., Rivellese, A. A., Riccardi, G., et al. (2016). Effects of whole grain cereal foods on plasma short chain fatty acid concentrations in individuals with metabolic syndrome. *Nutrition*, 32, 217-221.

Vuholm, S., Nielsen, D.S., Iversen, K.N., Suhr, J., et al. (2017). Whole grain rye and wheat affect some markers of gut health without altering the fecal Microbiota in Healthy Overweight adults: a week randomized trial. *Journal of Nutrition*, 147, 2067-75.

Walker, A.W., Duncan, S.H., Harmsen, H. J. M., Holtrop, G., Welling, G. W., & Flint, H. J. (2008). The species composition of the human intestinal microbiota differs between particle associated and liquid phase communities. *Environmental Microbiology*, 10 (12), 3275-3283.

Wang, W., Li, J., Chen, X., Yu, M., Pan, Q and Guo, L. (2020). Whole grain food diet slightly reduces cardiovascular risks in obese/overweight adults: a systematic review and meta-analysis. BMC Cardiovascular Disorders, 20: 82, https://doi.org/10.1186/s12872-020-01337-z

Wang, H., Wang, P., Wang, X., Wan, Y., & Liu, Y. (2012). Butyrate enhances intestinal epithelial barrier via up regulation of tight junction protein claudin-1 transcription. *Digestive Diseases and Sciences*, 57, 3126-3135.

Wang, M.W., Klopfenstein, C. F., and Ponte JR, J. G. (1993). Effects of twin-screw extrusion on the physical properties of dietary fiber and other components of whole wheat and wheat bran and on the baking quality of wheat bran. *Cereal Chemistry*, 70, 707-711.

World Bank (2015). Urbanization in Africa: Trends, Promises, and Challenges. Retrieved 20th December 2020 from https://www.worldbank.org/en/events/2015/06/01/urbanization-in-africa-trends-promises-and-challenges

Ye, E.Q., Chacko, S.A., Chou, E.L., Kugizaki, M., et al. (2012). Greater whole grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease and weight gain. *The Journal of Nutrition*, 142 (7), 1304-1313.

Zeng, J., Gao, H., Li, G., and Liang, X. (2011). Extruded corn flour changed functionality behavior of blends. *Czech Journal of Food science*, 29: 520-527.

Zhang, X., Chen, T., Lim, J., Xie, J., Zhang, B., Yao, T., et al. (2019). Fabrication of a soluble crosslinked corn arabinoxylan matrix supports a shift to butyrogenic gut bacteria. *Food and Function*, 10, 4497

Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y.Y., Wang, X., et al. (2018). Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science*, 359, 1151-1156.

Table 1.1 Studies on the effects of whole grains or isolated brans on human gut microbiota, metabolite, and health-related biomarker outcomes.

Design and period	Amount of fiber/whole grain used	Microbiota and/or health outcomes	References
Randomized crossover comparing whole grain vs refined (n=50) 8-week intervention separated with washout period greater than 6 weeks	179 g/d of whole grain in whole grain diet 13 g/d of whole grain in refined diet	Whole grain increased *Faecalibacterium prausnitzii* Whole grain reduced body weight, interleukin-6, and C-reactive protein compared to refined grain No major impacts on gut microbiota No changes in breath hydrogen, intestinal transit time, glucose homeostasis, and plasma short chain fatty acids	Roager et al., 2019
Randomized parallel arm trial (n=49)	3 servings/d of each of: 1) whole grain diet, 2) fruits and vegetables, 3) refined grain control diet	Reduced lipopolysaccharide binding protein and tumor necrosis factor-alpha (TNF-α) in whole grain intake compared to refined grains control diets No significant change in fecal SCFA in individuals No significant change in microbiota due to treatments	Kopf et al., 2018
Randomized parallel trial for 6 weeks (n=81), ages 40-65 years	207 g/d of whole grain in test diet 0 g/d of whole grain in refined diet	Trend towards increased *Lachnospira* and *Roseburia* in the whole grain group Whole grains increased stool frequency, total SCFAs, butyrate and acetate vs refined grain diet	Vanegas et al., 2017
Randomized parallel trial, 6 weeks (n=70), mean age 51 years, obese persons	145 g/d of whole grain wheat, 124 g/d of whole grain rye, 5 g/d of whole grain in refined wheat control	Fecal butyrate reduced in refined wheat compared to whole grain wheat/rye. Other SCFAs remained unchanged. No change in gut microbiota Frequency of flatulence was greater in whole grain groups than refined control	Vuholm et al., 2017
Dietary intervention (n=54), 12 weeks	40 g/d of dietary fiber in whole grain diet vs 22 g/d of dietary fiber in refined diet	Whole grain group significantly increased plasma propionate Both treatments increased butyrate levels with whole grain diet leading to greater increase relative to baseline level	Vetrani et al., 2016
Randomized control crossover study for 6 weeks separated with a washout period of 4 weeks (n=33) Ages 40-65 years BMI: 20-35 kg/m^2	At least 80 g/d of whole grain vs less than 16 g/d of whole grain in refined diet	No effect on the gut microbiota Trend of reduction of IL-10 and C-reactive protein in whole grain group Insulin was higher in refined group vs whole grain group	Ampatzoglou et al., 2015

Table 1.1 continued

Dietary intervention in overweight or obese persons (n=72) 12 weeks	105 g/d of whole wheat vs refined wheat (with no whole grain)	No significant differences in microbiota between whole wheat group vs refined group Whole wheat increased bifidobacteria	Christensen et al., 2013
Randomized parallel trial (n=51), mean age 60 years with metabolic syndrome 12 weeks	75 g/d of whole grain rye bread in diet 4 g/d of whole grain in refined white wheat bread in diet	Minor effect (no statistical difference) in fecal microbiota between the two groups	Lappi et al., 2013
Randomized crossover trial (n=28) 4 weeks	60 g/d of whole grain barley 60 g/d of brown rice + whole grain barley blend 60 g/d of brown rice	Whole grain barley increased *Eubacterium rectale*, *Roseburia faecis*, *Roseburia intestinalis*, with a reduction in *Bacteroides* No changes on fecal SCFAs among the groups Blend of brown rice and whole grain barley reduced postprandial glucose peak and interleukin-6 All groups increased microbiota diversity	Martinez et al., 2013
Randomized crossover trial for 2 weeks (n=17)	32 g/d mean fiber intake in whole grain diet 19 g/d of mean fiber intake in the refined diet	Whole grain intervention increased butyrate and acetate	Ross et al., 2013
Randomized placebo-controlled crossover trial for 3 weeks with a 2-week washout period (n=31). Ages 20-42 years	48 g/d of whole grain wheat cereal diet 48 g/d wheat bran cereal diet	Bifidobacteria as well as lactobacilli increased in whole wheat group vs wheat bran group No significant changes in gut microbiota, SCFAs, insulin, total cholesterol, fasting glucose, between the two treatments	Costabile et al., 2008

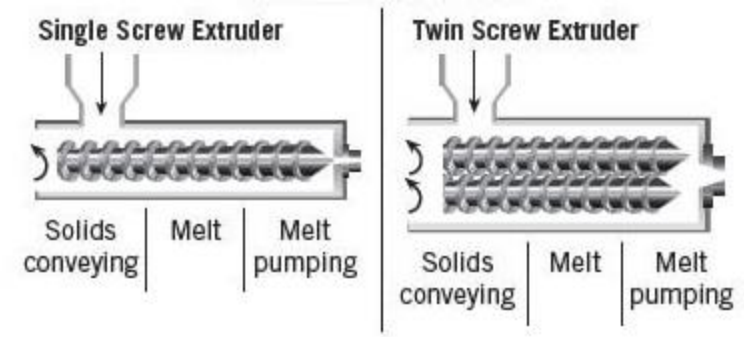


Figure 1.1. A diagrammatic illustration of the single- (left) and twin-screw extruder (right). Adapted from Plastic Processing Equipment and Machinery (2017).

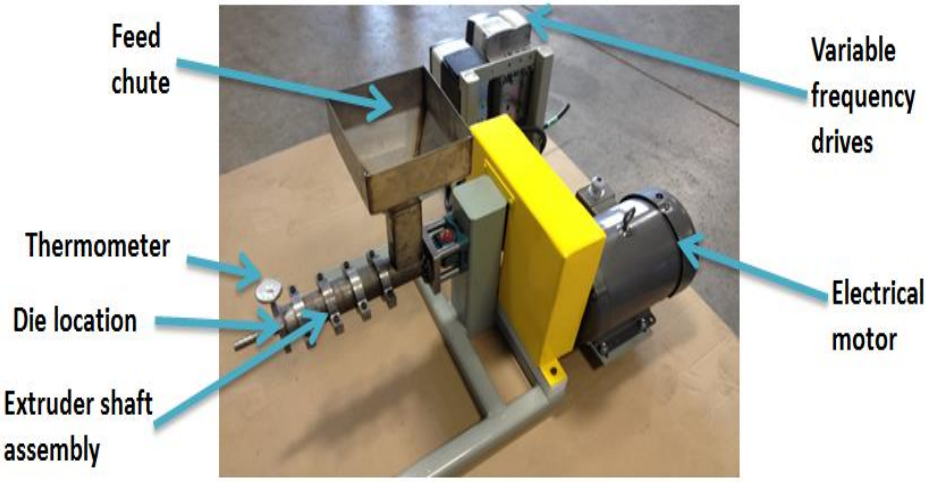


Figure 1.2**.** Single-screw extruder developed at Purdue and commercialized by Technochem Inc. (Boone, IA, USA) and is being installed in Incubation Centers to produce instant porridge flours.

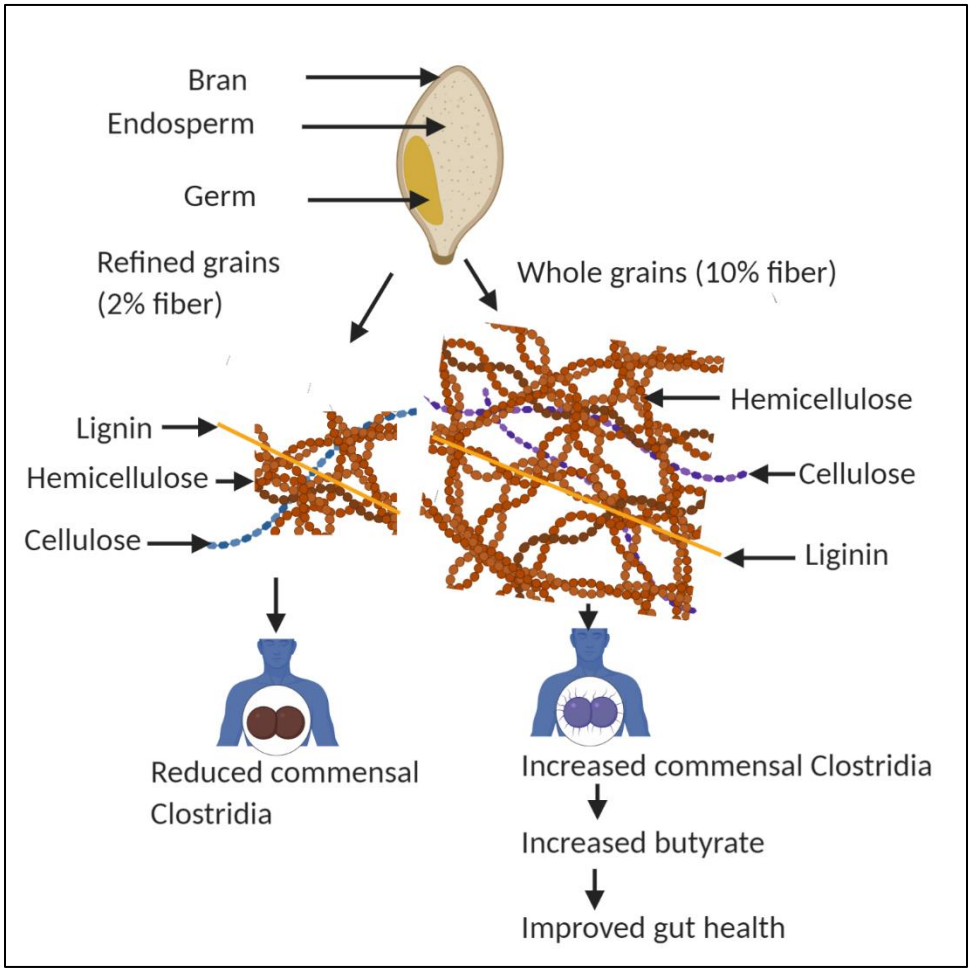


Figure 1.3. Illustration showing that the higher fiber content of whole grain flours, with cell wall matrix fibers, promotes butyrogenic commensal Clostridia, compared to refined grain flours with low fiber content. Cell wall diagram modified from Flint et al. (2012).

CHAPTER 2. FEED MOISTURE INFLUENCES THE PHYSICO-CHEMICAL PROPERTIES AND EXTRUSION ENERGY USED IN PROCESSING INSTANT PEARL MILLET (*PENNISETUM GLAUCUM*) PORRIDGE FLOURS

2.1. Abstract

This study aimed to optimize process conditions of a low-cost single-screw extruder for African small- to medium-scale entrepreneurs to produce good quality pregelatinized instant pearl millet porridge flours. Optimal feed moisture content was determined related to the amount of energy used in the extruder. Feed moisture content was varied (27, 29, 31, 33, and 35%), energy input measured for the extruder and drying of the extrudates, and physico-chemical properties analyzed. Extrusion at lower feed moisture contents more significantly reduced RVA peak, trough, final and setback viscosities compared to native flours. All flours exhibited viscoelasticity as the storage (G') modulus greater than loss (G'') modulus indication that the porridges exhibited solid-liquid like property. Further, extrusion significantly reduced the *L** color values in extruded flours compared to non-extruded flours with the greatest reduction being observed at the least feed moisture. The energy utilization during extrusion at 27, 29. 31, 33 and 35% feed moisture was 6.6±0.43, 5.8±0.19, 5.2±0.23, 3.9±0.25 and 3.4±0.39 kilowatt hour, respectively. Reduction of feed moisture content led to reduction of extrudate drying time. Nutrient profiles were kept constant. Total digestible starch was not statistically different in the extruded flours, and was higher than in the non-extruded flour, probably due to opening of cell walls by extrusion shear and shearing of gelatinized swollen starch granules. In conclusion, the single screw extruder can be efficiently operated at 27% feed moisture compared to currently used 35% feed moisture and obtain instant flours with desired quality.

2.2. Introduction

Pearl millet (*Pennisetum glaucum*) is the world's sixth most important cereal crop after maize, sorghum, wheat, rice and barley (Dias-Martins et al., 2018). Besides being used for food, pearl millet is used as feed and various industrial processes such as in the production of ethanol commercially (Gohel and Duan, 2012). The crop is widely domesticated in semi-arid regions of Africa and Asia because it is tolerable to harsh climatic conditions. This enhances its survival in

the dry regions which are prone to food insecurity (Rani et al., 2018). Nutritionally, whole grain pearl millet is comparable to other cereals rich in dietary fibers, phytochemicals, lipids, resistant starch, vitamins, and has been recommended as one of the whole grain foods that can be used to manage chronic degenerative diseases such cancers, type 2 diabetes, and cardiovascular disease (Arora et al, 2011; Sihag et al., 2015; Hithamani and Srinivasan, 2014; Hassan et al., 2020). Cisse et al. (2018) reported that foods made from millets and sorghum have a slower gastric emptying implying that they promote prolonged satiety among subjects, and this could be important in reducing food intake especially in persons that are overweight.

Conventionally, pearl millets are made to diverse food products such as thin and thick porridges, beers, and flat breads (Potter et al., 2013; Li et al., 2019). Traditional flours of both decorticated and whole grains of millets, sorghums, and corn are consumed in many regions of Africa either as thin or thick porridge (Hayes et al., 2020a; Moussa et al., 2011). Newer convenient instant flours that are precooked and require less time to prepare are being introduced to such populations, and it is an aim of the present study to test and extend a low-cost single-screw extruder to make instant flours to small- and medium-enterprise processors in Kenya. A study done in Niger revealed that consumers liked the taste, color, texture and overall liking of instant thin and thick porridges compared to conventional ones prepared from millet and sorghum grown locally (Moussa et al., 2011). These authors recommended that introduction of instant flours using low-cost extruders could offer untapped market opportunities for local farmers who produce cereal crops and entrepreneur processors. In another study, consumers highly ranked instant thin and thick porridges made from millets fortified with carrot and mango powders as well as mineral premixes (De Groote et al., 2018). The potential for adoption of instant fortified flours has been observed in Kenya (De Groote et al., 2020).

Extrusion technology is widely used for the manufacture of various snack and other foods using varied shear and moisture conditions. In the USAID Feed the Future Food Processing and Post-harvest Handling Innovation Lab (FPIL) and the Sorghum and Millet Innovation Lab (SMIL), a low-cost small-scale single-screw extruder developed at Purdue University was placed in different African Incubation Center laboratories formed by FPIL and SMIL to make instant flours for thin and thick porridges typically consumed. Local entrepreneurs are being trained on extrusion processing, and in some cases are obtaining the extruder. Conditions must be set for economical

use of the extruders and to produce high quality instant porridge flours. Important parameters for entrepreneurs to consider in the production of instant flours are the correct moisture condition for complete gelatinization of starch during extrusion cooking to obtain high-quality products and with acceptable energy costs (extrusion and drying of extrudates), and short production time. The goal of this study was to produce pre-gelatinized instant pearl millet porridge flours (would be similar for sorghum and maize) at different moisture contents (27, 29, 31, 33, and 35%) and to test energy usage, and their physicochemical properties related to quality.

2.3. Materials and methods

2.3.1. Materials

Whole grain pearl millet (*Pennisetum glaucum*) was purchased from Alif Group (Senegal, West Africa) and milled into flour using a pin mill (Alpine Augsburg 160Z, Augsburg, Germany) to particle size of between 300-500 µm.

2.3.2. Extrusion of flours

Milled whole grain flour moisture content was adjusted to 27, 29, 31, 33 and 35% moisture content by adding a determined amount of water and mixing at a moderate speed in a Hobart mixer (H600, Hobart Company, Troy, Ohio, USA) for 6 min. The different batches were allowed to equilibrate overnight at 7°C. The next day, the samples were removed from the cold room and let sit at room temperature for an hour and moisture content verified using moisture analyzer (HE53, Greifensee, Switzerland). Moistened flour was extruded using a single-screw extruder (Technochem International, Inc. 967 Quartz Ave. Boone, Iowa 50036, USA) at a feed rate of 30 kg/h using a Coperion K-Tron feeder (K2MLD5S60 IDF-1, Sewell, New Jersey, USA). Part of the obtained extrudates were put in Ziploc bags (S.C. Johnson & Son, Racine, Wisconsin, USA) and moisture content measured in an oven (VWR Oven, Gr Con 2.3 CF, VWR international, Pennsylvania, USA). Expansion ratio was determined by measuring the diameter of the die and the extrudates (8 measurements) (Zhu et al., 2010), and the other portion was dried at 50°C to below 12% moisture in an circulating air oven (Blue M146, Pennyslvania, USA). Dried extrudates were first milled in a hammer mill (Eberbach E3703 Cutting Mill, Belleville, Michigan, USA) at a speed of 900 rpm fitted with a 0.5 mm screen, and then a flour with particle size between 0.3-0.5

mm obtained from a sieve shaker (Rx-24, W. S. Tyler Inc. Mentor, Ohio, USA) and packaged in Ziploc bags and stored at -20°C for subsequent experiments.

2.3.3. Rapid Visco-Analyzer and rheometry

Pasting profiles for the extruded and non-extruded pearl millet flours were determined using a Rapid Visco-Analyzer (RVA) (Newport Scientific RVA-4, Australia). Pearl millet grains and their extrudates were milled to flour using a cyclone mill (Foss Tecator 1093 Cyclotec, Hoganas, Sweden) with a 0.5 mm screen. Flour (3.5 kg) was adjusted to 14% moisture content, and suspended in 25 ml of purified water in a RVA vessel. The heating and cooling regime was as follows: flours were heated to 50°C for 1 min, heated to 95°C at 6°C/min followed by holding for 2.7 min, cooling to 50°C at 6°C/min, and held at 50°C for another 2 min. Thermocline for Windows version 12.0 software was used for data analysis and management.

The effect of temperature on loss and storage modulus of thin porridges obtained from the RVA procedure was performed using an oscillatory rheometer (TA ARES-G2 rheometer, TA instruments, New Castle, DE, USA). A 4 mm parallel plate was fitted, and temperature measured from 25-90°C, 1% strain in the linear region, a frequency of 1 Hz, and approximately 1.3 mL of thin porridge used.

2.3.4. Differential scanning calorimetry

Starch gelatinization was done using differential scanning colorimetry (DSC, TA Instruments, Q2000, New Castle, Delaware, USA) as described by Parada et al. (2011). Briefly, 6 mg of instant flour was weighed into aluminum hermetic pans, and 18 µl of distilled water added, and sealed and left to equilibrate for 2 h. This was followed by heating at a temperature range of 20-120°C at a rate of 10°C/min. An empty reference pan was used.

2.3.5. Size exclusion chromatography

Molecular weight distributions of starch isolated from extruded and non-extruded flours was done using a size-exclusion liquid chromatograph equipped with multi-angle laser light scattering and refractive index detectors as described by Moussa et al. (2011) with modifications of Hayes et al. (2020b). About 9 mg of ground sample through a 0.5 mm sieve was transferred into

a 2 ml Eppendorf microcentrifuge tube and mixed with protease (P1236, Sigma-Aldrich, St. Louis, Missouri, USA) in tricine buffer 0.5 ml/ml (44.80 mg/L, pH 7.5), and vortexed and incubated for 30 min at 37°C. The resultant mixture was then centrifuged for 10 min at 4000×g and the supernatant discarded. To the precipitate, 0.5 mL sodium bisulfite solution (0.45%) was added, vortexed and further incubated for additional 30 min at 37°C. Next, this mixture was mixed by vortexing, centrifuged (10 min at 4000×g) and supernatant discarded. The obtained precipitate was suspended in 1.5 ml dimethyl sulfoxide (DMSO) and inverted manually by hand to ensure the mixture was homogenous, and was then heated in a thermomixer for 12 h overnight at 350 rpm and 80°C, with occasional inversion to ensure the mixture was homogenous. On the next day, purification was done by first centrifuging (10 min at 4000×g) and collecting the supernatant in a 15 ml tube, and then addition of 10 ml ethanol to the supernatant to precipitate the starch. The mixture was centrifuged for 10 min, 4000 g, supernatant discarded, and this procedure repeated thrice. The tube with the obtained residue was inverted on a paper towel to remove any traces of ethanol before vacuum-drying using a desiccator. Prior to the injection, the obtained starch samples were dissolved in boiling water (2 mg/ml) and heated in a thermomixer at 350 rpm, 95°C for 8 h, filtered through 5.0 µL syringe filter, and 100 µl injected on a Sephacryl S500-HR column (Amersham Bioscience, Piscataway, NJ, USA). Filtered water with 0.02% sodium azide was used as the eluent and pumped using a Shimadzu LC-10AP pump (Shimadzu Scientific Instruments, Kyoto, Japan), and monitored using a refractive index detector (Optilab 903 Interferometric Refractometer, Wyatt Technology Corp., Santa Barbara, CA, USA). The data was analyzed with Astra software v. 4.90.08 using the Berry plot.

2.3.6. Scanning electron micrographs of granules and expansion ratio

To determine the morphology of granules, a thin lay of extruded and non-extruded pearl millet flours were placed on a double-sided scotch tape and coated with gold for about 15 min to a thickness of 2 mbar. The carbon tapes were held in a scanning electron microscope (SEM) (S 4800, Hitachi, Japan) holder and observations made at 20 kV and × 1000 magnification.

2.3.7. Color measurements

Flour from the extrudates were placed in transparent petri-dishes. A Hunter colorimeter (XE, Lab Scan, New Jersey, USA) was calibrated using red and white tiles and color measured for lightness to darkness (L*), redness to green (a*) and yellowness to blue (b*). Five readings were recorded per sample.

2.3.8. Protein content, total digestible and resistant starch of the flours

Percentage protein content was analyzed by the Dumas method using a nitrogen analyzer (LECO TruMac, LECO Corporation, St. Joseph, MI, USA). The machine was calibrated using EDTA and the amount of nitrogen was multiplied by 6.25 to get percentage protein. Total digestible starch and resistant starch was analyzed using a Rapid Resistant Starch kit (K-RAPRS; Megazyme, Wicklow, Ireland) based on the manufacturer's procedures. Non-extruded samples were first cooked to gelatinize the starch before determination of total digestible and resistant starch fractions as described by Tuncil et al. (2018).

2.3.9. Power calculation using during extrusion

Power was calculated by reading the amperage on the extruder and fitting the data to an equation which related amperes to kilowatts (kW) (Equation 1). The equation was derived by fitting a performance curve of power (kW) and amperes using equations provided by the Techno-Chem Co. technical engineer. Amperes were read directly from the extruder during operation. The baseline amperage was determined by running the extruder with the screw on, but the barrel was removed to reduce added friction that may not be present when the extruder is under operation. Next, the barrel was fixed and the amperage determined when the flours at different moisture conditions were processed in the extruder. From the total power of the extruder, the baseline power was subtracted to get the net power. Power was then calculated using the following equation:

$$\text{Power (kW)} = 7.3497 * \ln(\text{Amps}) - 10.29 \qquad \text{(Equation 1)}$$

2.3.10. Data analysis

Analysis of variance was done using SAS version 9.4 to determine significant differences at different moisture conditions on pasting profiles, color measurements, expansion ratio, protein and starch proportions, and energy use during extrusion. Analysis of variance was done and differences among means were determined using Tukey's multiple comparison test, $p < 0.05$.

2.4. Results and discussion

For this model of a high-pressure, high temperature, single-screw extruder, extrusion is mostly done at 35% for processing of instant flours (De Groote et al., 2018; 2020). Using a continuous processor (i.e. a screw-type extruder, but which is not a high-pressure type), it was shown that lower feed moisture content could still attain full starch gelatinization (Moussa et al., 2011). Here, we demonstrated that we can reduce the moisture content to 27% feed moisture with improvements of lower extruder energy cost and extrudate drying time, and acceptable pasting profiles and instant porridge flour quality. This is relevant for small- and medium-scale enterprises in sub-Saharan Africa where markets for new convenient foods are growing.

2.4.1. Rheological properties and gelatinization of pearl millet flours

DSC thermograms of pearl millet flour extruded at the different feed moisture conditions had no apparent endotherms indicating complete gelatinization of starch in the flours, even when moisture content was reduced (Figure 2.2). Complete gelatinization of flours/starch cooked in a continuous processor (i.e. high temperature, low pressure screw-type processor) was previously observed at moisture content between 25-40% feed moisture (Moussa et al., 2011)

RVA pasting parameters of the extruded and non-extruded control flours are presented in Figure 2.3. Overall, the extruded flours had somewhat lower peak viscosity, and much lower trough, final and setback viscosities compared to the non-extruded control flour. This is to be expected as fully pre-gelatinized extruded flours have an initial high viscosity that carries to the region of the RVA peak, and then at high and cooled temperatures have comparably low viscosities as starch structures have been diminished during extrusion (Moussa et al., 2011). Still there were some minor differences among the samples extruded at different moisture contents. Using flour extruded at 35% feed moisture as the comparator, as it is the standard condition set up for the

extruder, peak viscosity (1800±32 cp) was statistically higher than for extruded flours at 27 (1706±44 cp) and 29% feed moisture content (1740±25 cp). Breakdown viscosity was slightly, though statistically, higher for the 35% extruded flour than flours extruded at lower moisture contents, and setback viscosity was similarly lower for the 31, 29, and 27% feed moisture content extruded flours.

Peak viscosity, also called maximum viscosity, is the highest viscosity attained when flour slurries undergo heating in an RVA, while final viscosity is the viscosity at the end of the heating cycle (Adedokun and Itiola, 2010). Peak viscosity can be used to determine the amount of additional flour that needs to be added to attain a suitable porridge thickness (Nkhata et al., 2021). Among the extruded flours, flour extruded at 35% feed moisture had significantly higher peak viscosity compared to that extruded at 27% feed moisture, suggesting it might have somewhat better porridge property though the difference was minor. Peak viscosity of the 29% feed moisture extruded flour was significantly higher than the 27% flour. The decrease in peak viscosity during extrusion could be due to greater starch fragmentation during extrusion at the lower feed moisture contents (Moussa et al., 2011, Roman et al., 2018). Martinez et al. (2014) also observed decreases in peak, trough, final viscosities from extrusion of rice flour. Kim et al. (2006) also showed decreasing feed moisture reduced peak viscosity. The non-extruded flour had a higher setback viscosity than extruded flours. A higher setback viscosity has been associated with less stable pastes (Moussa et al., 2011).

Size-exclusion chromatograms of molecular weight distributions of isolated starch from extruded and non-extruded samples showed starch fragmentation characterized by reduced amylopectin peaks (Figure 2.4) (note: fragmented amylopectin was too small to be recovered in the starch isolation procedure and does not appear at higher elution times; and, therefore the reduced peak indicates higher fragmentation). Degree of starch fragmentation was highly associated with feed moisture content, and likely explains the slightly lower viscosities of the extruded flours at lower feed moisture contents. Higher starch fragmentation was noted in the study by Moussa et al. (2011) to give instant thin porridge consistencies that were smoother and highly acceptable, though as noted more flour must be used to get a desired viscosity. Still, in the current study the differences in viscosity were quite small and this is not considered to be a quality problem with flours extruded at lower feed moisture contents.

Using oscillatory or small deformation rheometry, the storage modulus (G') was greater than loss modulus indicating that the extruded flours formed viscoelastic thin porridges (Figure 2.5). There was a reduction in the G' and G'' upon extrusion that has been observed by Klucinec and Thompson (1999). This is because the starch components are broken down to smaller fragments that can aggregate together and reduce the G' and G'' values (Roman et al., 2018).

2.4.2. Color measurements, expansion ratio, extrusion temperatures, and protein contents of extruded and non-extruded pearl millet flours

Changes in color of flours extruded at different feed moisture contents are presented in Table 2.1. Extruded samples had lower *L** and *a* *values, and higher *b** value, compared to the non-extruded control flour. At high feed moisture conditions, water minimizes the effect of browning due to lower temperatures and there is also reduced residence time (Gulati et al., 2016). In this study the screw speeds reduced from 813±7 to 780±5 rpm in flours extruded at 35% compared to 27% feed moisture content, respectively, and die extrudate temperatures were higher at lower feed moisture contents (Table 2.1). This indicates longer resident time and increased exposure of flours to higher temperatures in lower feed moisture content samples, leading to increase in the darkness value. Changes in *L**, *a** and *b** were observed in extruded samples due to Maillard reactions during extrusion at high temperatures (Peksa et al., 2016; Sharma et al., 2012).

Protein content of non-extruded pearl millet flour was 9.0%. This is within the range of 8.5-13.7% pearl millet protein content reported by Abdalla et al. (1998). There was a non-significant slight reduction in protein content during extrusion cooking (Table 2.1). Arcila et al. (2015) reported that extrusion did not bring changes in protein contents of wheat brans at 15 or 30 % feed moisture and 120 or 250 rpm shear rates. Additionally, these authors did not observe changes in total protein during extrusion of composite rice and pea flours at temperatures of 120-130^{o}C, and screw speeds of 900-950 rpm. No changes in protein content during extrusion was also observed by Guldiken et al. (2020).

Decreasing feed moisture content from 35 to 27% significantly ($P < 0.05$) increased the expansion ratio of the extrudates (Table 2.1). Extrusion at higher moisture conditions has been shown to decrease expansion ratio of extrudates. Chang and Ng (2011) observed that increasing

the feed moisture from 25 to 35%, at constant rpm and temperature, led to a decrease in the expansion ratio. Usually, expansion of extrudates is influenced by the differences in pressure between the die and the atmosphere. At lower feed moisture content, the viscosity of the melt is higher, leading to greater pressure difference, hence greater expansion compared to higher feed moisture flours. Other factors that influences the expansion ratio are rpm (i.e. screw speed) and temperature. When the rpm was increased from 200 to 300, but the moisture kept constant, the expansion ratio was significantly reduced (Chang and Ng, 2011). Reduced expansion at higher screw speeds has also been observed, because increased screw speed leads to lower melt viscosity which causes reduction in the die pressure and hence reduced expansion ratio (Blanche and Sun, 2004). On the other hand, at higher temperatures there is increased evaporation of water at the die leading to higher expansion ratio (Chang and Ng, 2011).

2.4.3. Total digestible starch, resistant starch, and scanning electron micrographs of extruded and non-extruded flours

Total digestible starch is the sum of rapidly and slowly digestible starch fractions. Extrusion significantly increased total digestible starch compared to the cooked non-extruded control (Figure 2.6A). The amount of resistant starch was low both in both extruded and non-extruded samples with no clear trend on the effect of feed moisture content for this variable (Figure 2.6B). Extrusion shear can break down starch to smaller fragments that are easily digested, and it was shown to be related to increased starch digestibility (170 mg/g in native barley to between 447-561 mg/g maltose equivalents as an indicator of total digestible starch) (Altan et al., 2009).

Figure 2.7 shows SEM images of extruded and non-extruded flours. Overall, extrusion led to swelling of starch due to gelatinization, but no differences could be observed due to feed moisture contents.

2.4.4. Moisture content of extrudates and power utilization during extrusion

The moisture contents of the extrudates before drying for the 35, 33, 31, 29 and 27% feed moisture flours were 31.17±0.82, 29.67±0.58, 27.5±0.49, 26.1±0.56 and 23.67±0.33%, respectively. It took 123±16, 118±14, 115±7, 104 ±11 and 98±8 min to dry the corresponding

extrudates to acceptable moisture contents of 11.3±1.3, 10.9±1.6, 12.2±0.6, 10.5±1.1 and 9.8±0.9%.

Power utilization during extrusion for the millet flours at 35, 33, 31, 29, and 27% feed moisture are presented in Figure 2.8. There was an inverse association between feed moisture and power consumed during extrusion with extrusion at the lowest feed moisture giving the highest power consumption. Power consumption for flours extruded at 35, 33, 31, 29, and 27% feed moisture was 3.4±0.39, 3.9±0.25, 5.2±0.23, 5.8±0.19, and 6.6±0.43 kwh, respectively. There was no significant difference in the amount of power used to extrude flours at 35 and 33% feed moisture, though power use rapidly increased with further reduction of feed moisture content. Water acts as a plasticizer in the extruder barrel, and reduction of water increases viscosity of the feed, hence increasing friction in the barrel and requiring more energy to push the material through it. Gulati et al. (2016) reported that higher feed moisture lowers energy use during extrusion by lowering the melt viscosity. Moreover, according to Meng et al. (2010), at high screw speeds the extruder barrel fill is reduced ultimately reducing the amount of energy required by the motor to push the material through during extrusion. The current expense for energy in Kenya is 30 cents per kwh (Stimatracker, 2020). Assuming the extruder is operated for 5 hours each day by a small- or medium-scale processor in Kenya to make instant porridge flours, to get the energy used during extrusion, the number of hours (5 h) is multiplied by the energy consumed (kwh). For 5 h, extrusion at 35 and 27% feed moisture would have an energy cost of Kshs 5.1 and Kshs 9.9, respectively.

2.5. Conclusion

Feed moisture content of flours used in the low-cost single-screw extruder was shown to influence quality parameters of the instant pearl millet porridge flours, but overall lowering feed moisture content to 27% was found acceptable for instant products as it still had a good pasting profile. Extrusion cost was inversely proportional to feed moisture content. Extrusion enhanced the color of the products compared to the non-extruded flour and the total digestible starch was higher in extruded samples. These findings can help entrepreneur processors in Africa, who are interested in buying the extruder, to set parameters for processing instant pearl millet flours for making high quality instant flours that can be reconstituted to thin and thick porridges at reasonable

energy (less than 6 kwh) and shorter drying times. An important aspect that should be considered is to design a dryer that is affordable and efficient in drying extrudates.

2.6. References

Abdalla, A. A., El Tinay, A. H., Mohamed, B. E., and Abdalla, A. H. (1998). Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. *Food Chemistry*, 2, 243-246.

Adedokun, M. O., and Itiola, O. (2010). Material properties and compaction characteristics of natural and pregelatinized forms of four starches. *Carbohydrate Polymers*, 79, 818-824.

Arcila, J. A., Weier, S. A., and Rose, D. J. (2015. Changes in dietary fibers fractions and gut microbial fermentation properties of wheatbran after extrusion and bread making. *Food Research International*, 74, 217-223.

Arora, S., Jood, S., and Khetarpaul, N. (2011). Effect of germination and probiotic fermentation on nutrient profile of pearl millet-based food blends. *British Food Journal*, 113, 470-481.

Arribas, C., Cabellos, B, Sanchez, C., Cuadrado, C., Guillamon, E., and Pedrosa, M. M. (2017). The impact of extrusion on the nutritional composition, dietary fiber and in vitro digestibility of gluten free snack based on rice, pea and carob flour blends. *Food and Function*, 8, 3654. DOI: 10.1039/c7fo00910k.

Altan, A., McCarthy, K. L., Maskan, M. (2009). Effect of extrusion cooking on functional properties and in vitro starch digestibility of barley-based extrudates from fruit and vegetable by-products. *Journal of Food Science*, 74, E77-E86.

Blanche, S. and Sun, X. (2004). Physical characterization of starch extrudates as a function of melting transitions and extrusion conditions. *Advances in Polymer Technology*, 23, 277–290.

Chang, Y.H., and Ng, P.K.W. (2011). Effects of extrusion process variables on quality properties of wheat-ginseng extrudates. *International Journal of Food Properties*, 14 (4), 914-925.

De Groote, H., Mugalavai, V., Ferruzzi, M., Onkware, A., Ayua, E., Duodu, K. G., et al. (2020). Consumer acceptance and willingness to pay for instant cereal products with food to food fortification in Eldoret, Kenya. *Food and Nutrition Bulletin*, 41(2), 224-243 https://doi.org/10.1177%2F0379572119876848

De Groote, H., Kariuki, S. W., Traore, D., Taylor, J. R. N., Ferruzzi, M. G., and Hamaker, B. R. (2018). Measuring consumers interests in instant fortified pearl millet products: a field experiment in Touba, Senegal. *Journal of the Science of Food and agriculture*, 98 (6), 2320-2331. doi:10.1002/jsfa.8722

Cisse, F., Erickson, D. P., Hayes, A. M. R., Opekun, A. R., et al. (2018). Traditional Malian solid foods made from sorghum and millets have markedly slower gastric emptying than rice, potato or pasta. *Nutrients*, 10, 124; doi:10.3390/nu10020124

Dias-Martins, A., Pessanha, K. L.F., Pacheco, S., Rodrigues, J.V., et al. (2018). Potential use of pearl millet (Pennisetum glaucum (L.) R. Br.) in Brazil: Food security, processing, health benefits and nutritional products. *Food Research International*, 109, 175-186.

Gohel, V., and Duan, G. (2012). No cook process for ethanol production using Indian broken rice and pearl millet. International Journal of Microbiology, ID 680232, https://doi.org/10.1155/2012/680232

Gulati, P., Weier, S. A., Santra, D., Subbiah, J., and Rose, D. J. (2016). Effects of feed moisture and extruder screw speed and temperature on physical characteristics and antioxidant activity of extruded proso millet (*Panicum miliaceum*) flour. *International Journal of Food science and Technology*, 51, 114-122.

Guldiken, B., Yovchev, A., Nosworthy, M. G., Stone, A. K.,et al. (2020). Effect of extrusion conditions on the physical properties of desi chickpea-barley extrudates and quality attributes of their resulting flours. *Journal of Texture Studies*,51, 300-307.

Hamaker, B. R., Siebenmorgen, T. J., and Dilday, R. H. (1993). Effect of disulfide bond containing protein on rice starch gelatinization and pasting. *Cereal Chemistry*, 70, 377-380.

Hassan, Z. H., Sebola, N. A., and Mabelebele, M. (2020). Assessment of the phenolics compounds of pearl and finger millets obtained from south Africa and Zimbabwe. *Food Science and Nutrition*, 8, 4888-4896.

Hayes, A. M. R., Gozzi, F., Diatta, A., Gorissen, T., Swackhamer, C., Bellman, S., and Hamaker, B. R. (2020a). Some pearl millet-based foods promote satiety or reduce glycaemic response in crossover trial. *British Journal of Nutrition*, https://doi.org/10.1017/S0007114520005036

Hayes, A. M. R., Okoniewska, M., Martinez, M.M., Zhao, B., and Hamaker, B. R. (2020b). Investigating the potential of slow retrograding starches to reduce staling in soft savory bread and sweet cake model systems. *Food Research International*, 138, 109745. https://doi.org/10.1016/j.foodres.2020.109745

Hithamani, G., and Srinivasan, K. (2014). Effect of domestic processing on the polyphenol content and bioaccessibility in finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*). Food chemistry, 164, 55-62.

Kamau, E. H., Smith , G. N., and Ayua, E. O. (2020). Extrusion and nixtamalization conditions influence the magnitude of changes in the nutrients and bioactive compounds of cereals and legumes. *Food Science and Nutrition*, 8 (4), 1753-1765.

Kim, J.H., Tanhehco, E. J., and Ng, P.K.W. (2006). Effect of extrusion conditions on resistant starch formation from pastry wheat flour. *Food Chemistry*, 99, 718-723.

Klucinec, J. D., and Thompson, D. B. (1999). Amylose and amylopectin interact in retrogradation of dispersed high amylose starches. *Cereal Chemistry*, 76, 282-291.

Li, H., Xiong, Z., and Li, X. (2019). Optimization of extrusion process for the development of extruded snacks using peanut, buckwheat and rice blend. *Journal of Food Processing and Preservation*, 43: e14264. https://doi.org/10.1111/jfpp.14264

Martinez, M. M., Calvino, A., Rossell, C., and Gomez, M. (2014). Effect of different extrusion treatments and particle size distribution on the physicochemical properties of rice flour. *Food Bioprocessing Technology*, 7, 2657-2665.

Meng, X., Threinen, D., Hansen, M., and Driedger, D. (2010). Effects of extrusion conditions on system parameters and physical properties of a chickpea flour-based snack. *Food Research International*, 43, 650-658.

Moussa, M., Qin, X., Chen, L., Campanella, O. H., and Hamaker, B. R. (2011). High quality instant sorghum porridge flours for the west African markets using continuous processor cooking. *International Journal of Food Science and Technology*, 46, 2344-2350.

Nkhata, S. G., Liceaga, A. M., Rocheford, T., Hamaker, B. R., and Ferruzzi, M. G. (2021). Storage of biofortified maize in Purdue Improved Crops Storage (PICS) bags reduces disulfide linkage driven decrease in porridge viscosity. *LWT-Food Science and Technology*, 136, 110262, https://doi.org/10.1016/j.lwt.2020.110262

Parada, J., Aguilera, J. M., & Brennan, C. (2011). Effect of guar gum content on some physical and nutritional properties of extruded products. *Journal of Food Engineering*, 103(3), 324-332.

Peksa, A., Kita, A., Carbonell-Barrachina, A. A., Miedzianka, J., Kolniak-Ostek, J., Tajner-Czopek, A., et al. (2016). Sensory attributes and physicochemical features of corn snacks as affected by different flour types and extrusion conditions. *LWT -Food Science and Technology*, 72, 26-36.

Potter, R., Stojceska, V., and Plunkett, A. (2013). The use of new fruit powders in extruded snacks suitable for children's diets. *LWT-Food Science and Technology*, 51, 537-544.

Rani, S., Singh, R., Sehrawat, R., and Kaur, B.P. (2018). Pearl millet processing: a review. *Nutrition and Food science*, 48 (1), 30-44.

Roman, L., Gomez, M., Hamaker, B. R., & Martinez, M. M. (2018). Shear scission through extrusion diminishes inter-molecular interactions of starch molecules during storage. *Journal of Food Engineering*, 238, 134–140

Sharma, P., Gujral, H. S., and Singh, B. (2012). Antioxidant activity of barley as affected by extrusion cooking. Food Chemistry, 131, 1406-1413.

Sihag, M. K., Sharma, V., Goyal, A., Arora, S., and Singh, A. K. (2015). Effect of domestic processing treatments on iron, beta-carotene, phytic acid and polyphenols of pearl millet. *Cogent Food and Agriculture*, 1, 1109171 http://dx.doi.org/10.1080/23311932.2015.1109171

Singh, S., Gamlath, S., and Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. *International Journal of Food Science and Technology*, 42, 916-929.

Solanas, E, Castrillo, C., Balcells, J., and Guada, J.A. (2005). In situ ruminal degradability and intestinal digestion of raw and extruded legume seeds and soya bean meal protein. *Journal of Animal Physiology Animal Nutrition*, 89(3-6), 166-71. doi: 10.1111/j.1439-0396.2005.00555.x. PMID: 15787989.

Stimatracker (2020). Electricity cost in Kenya. Retrieved 20th December 2020 from https://stimatracker.com/

Tuncil, Y.E., Thakkar, R.D., Arioglu-Tuncil, S., Hamaker, B.R., & Lindemann, S.R. (2018). Fecal Microbiota responses to bran particles are specific to cereal type and *In vitro* Digestion methods that mimic upper gastrointestinal tract passage. *Journal of Agricultural and Food Chemistry*, 66, 12580-12593.

Zhu, L., Shukri, R., de Mesa-Stonestreet, N.J., Alavi, S. (2010). Mechanical and microstructural properties of soy protein-high amylose corn starch extrudates in relation to physicochemical changes of starch during extrusion. *Journal of Food Engineering*, 100, 232-238.

Zhang, X., Chen, Y, Zhang, R., Zhong, Y., et al. (2016). Effects of extrusion treatment on the physical properties and in vitro digestion of pregelatinized high amylose maize flour. *Journal of Cereal Science*, 68, 108-115.

Table 2.1. Color measurements, expansion ratio (ER), extrusion temperatures, and protein contents of extruded and non-extruded pearl millet flours.

Samples	*l*	*a*	*b*	ER	Extrusion Temperature (°C)	Protein (%)
control	57.4±0.42a	2.0±0.04c	14.6±0.08d			9.0±0.10a
35% FM	54.3±0.04b	2.2±0.03c	18.1±0.05c	1.1±0.51b	115-121	8.3±0.15a
33% FM	53.5±0.02bc	2.4±0.01b	18.1±0.00c	1.2±0.59b	118-122	8.5±1.33a
31% FM	53.2±0.08c	2.5±0.01ab	18.4±0.02bc	1.3±0.35b	113-123	8.8±0.44a
29% FM	52.1±0.01d	2.6±0.11ab	18.9±0.25a	1.6±0.47a	115-124	8.6±0.39a
27% FM	51.7±0.31d	2.7±0.03a	18.8±0.31a	1.8±1.11a	118-126	8.2±0.11a

Letters with different letters in a column differ significantly at $p < 0.05$ using the Tukey multiple comparison test. Feed moisture (FM) is the extrusion moisture conditions.

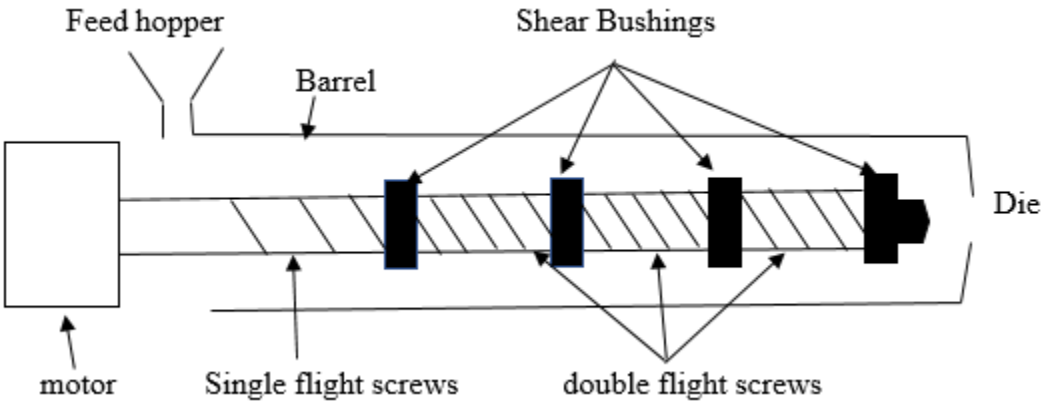


Figure 2.1. Schematic diagram of single screw extruder.

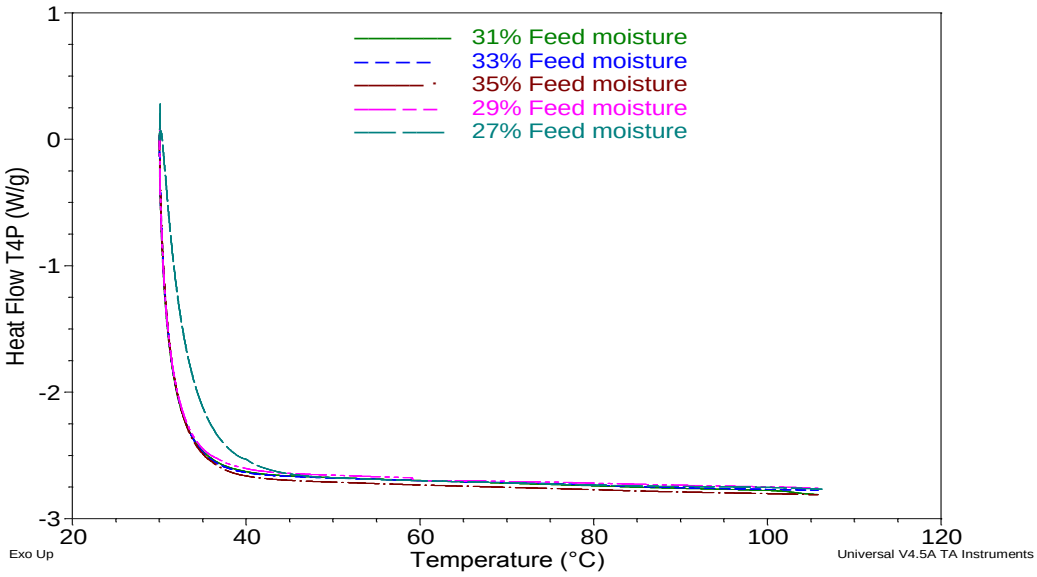


Figure 2.2. DSC thermograms for flours extruded at different moisture contents.

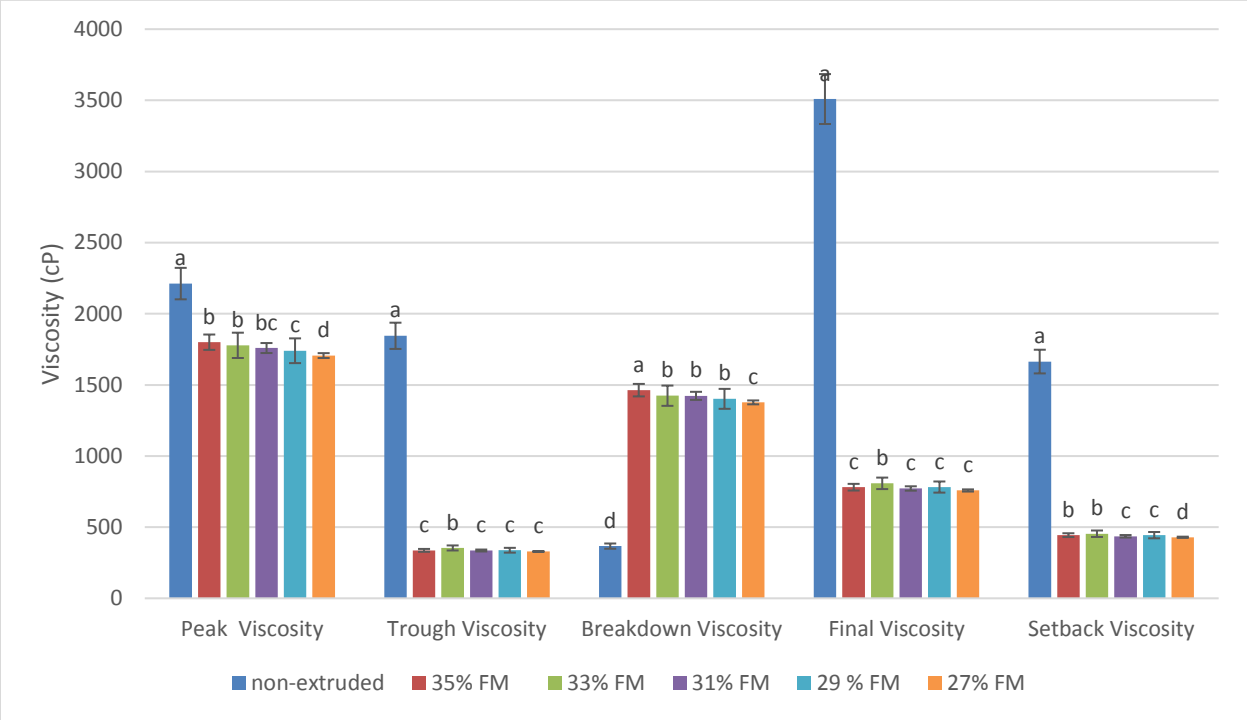


Figure 2.3. RVA pasting parameters of non-extruded and pearl millets flours extruded at different feed moistures (FM). Bars with different letters for each variable differed significantly using Tukey's multiple comparison test, $P < 0.05$.

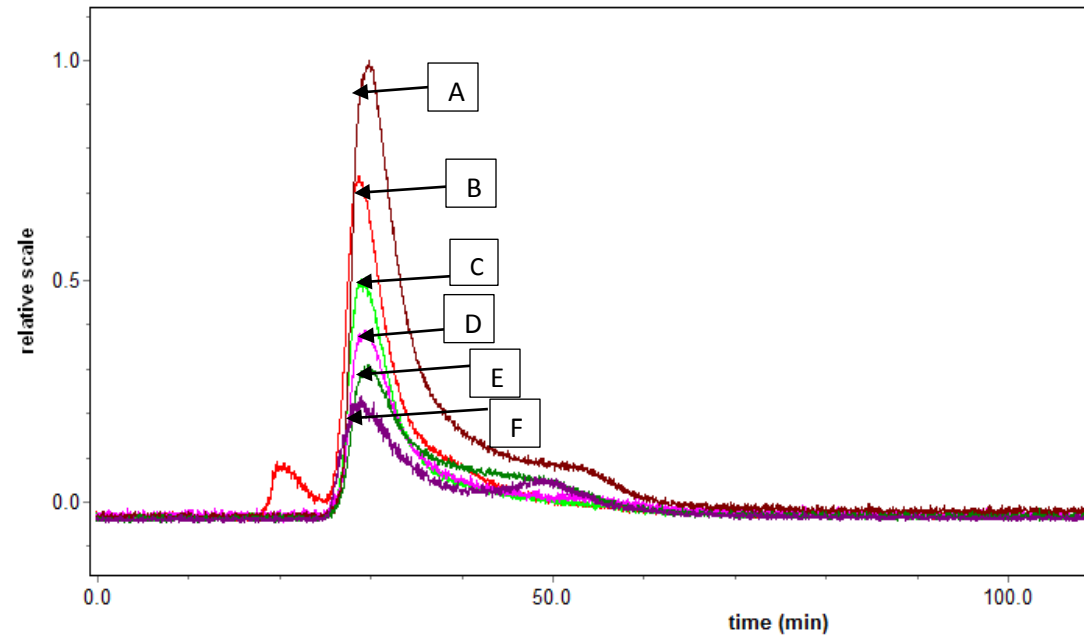


Figure 2.4. Size-exclusion chromatograms of the molecular weight distributions of starches isolated from millet flour extruded at 35 (B), 33 (C), 31 (D), 29 (E) 27% (F) feed moisture, and the non-extruded millet (A).

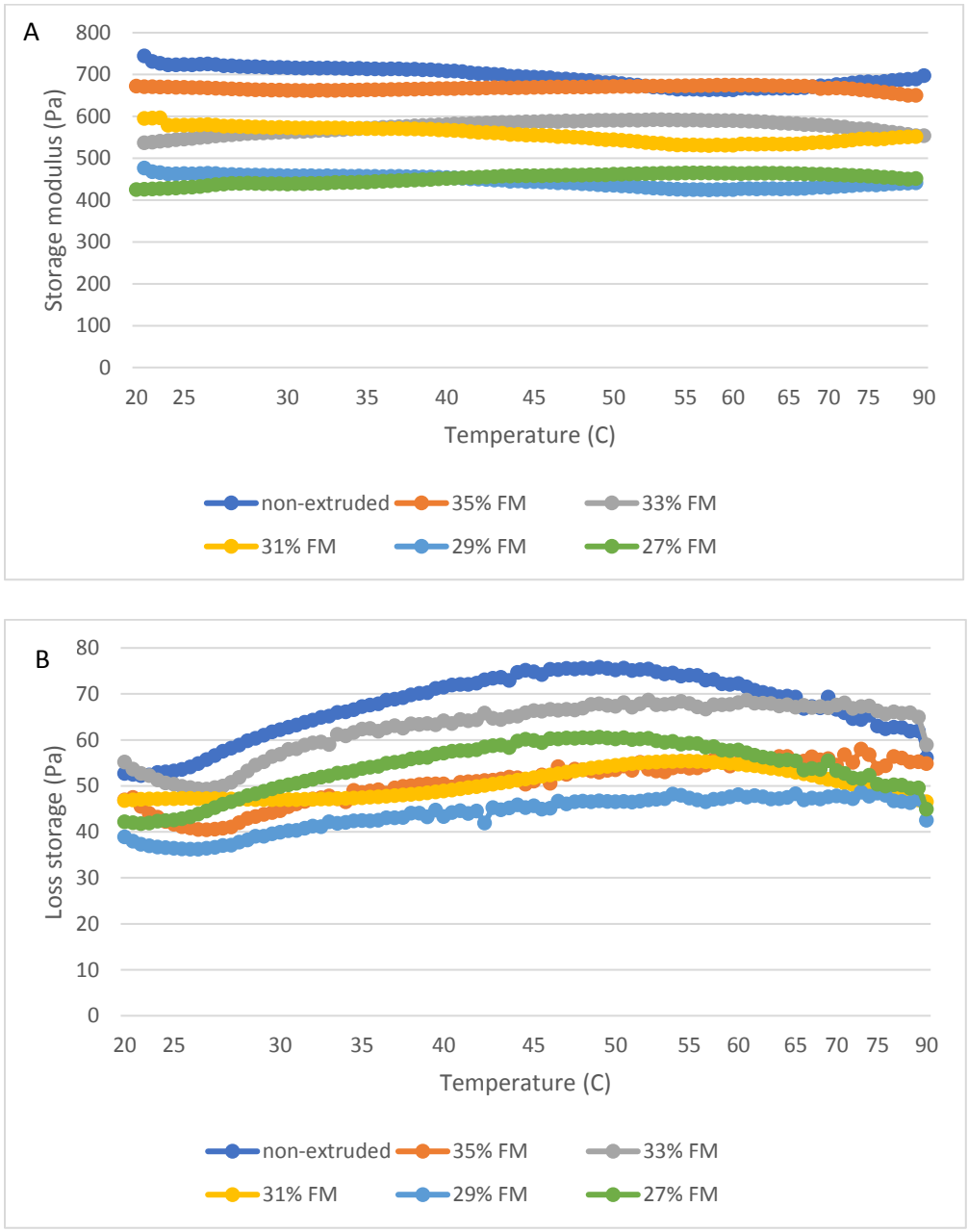
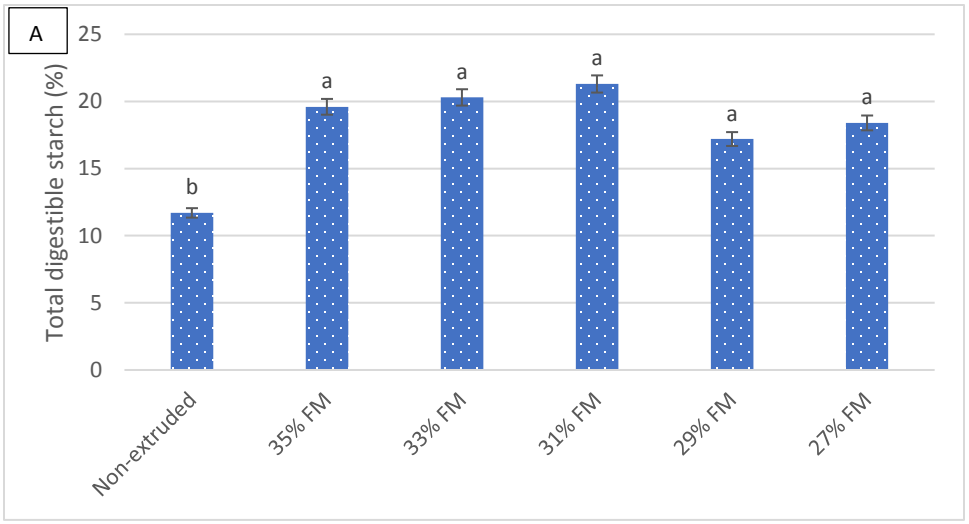


Figure 2.5. Storage moduli (G') (A) and loss moduli (G'') (B) of extruded and non-extruded (control) flours.



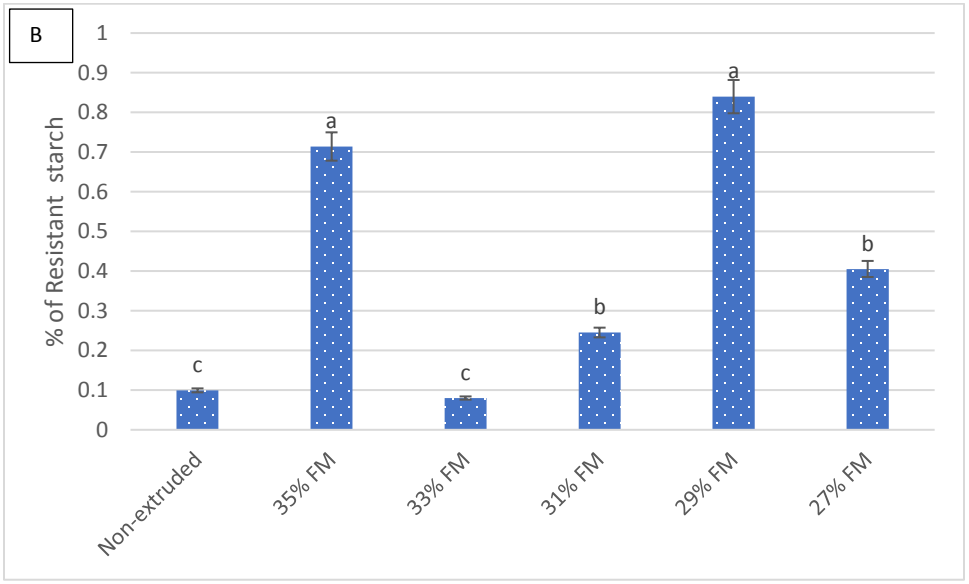


Figure 2.6. Total digestible starch (A) and resistant starch (B) of extruded at different feed moistures (FM) and non-extruded samples. Letters with different letters in a column differ significantly using the Tukey's multiple comparison test, $P < 0.05$.

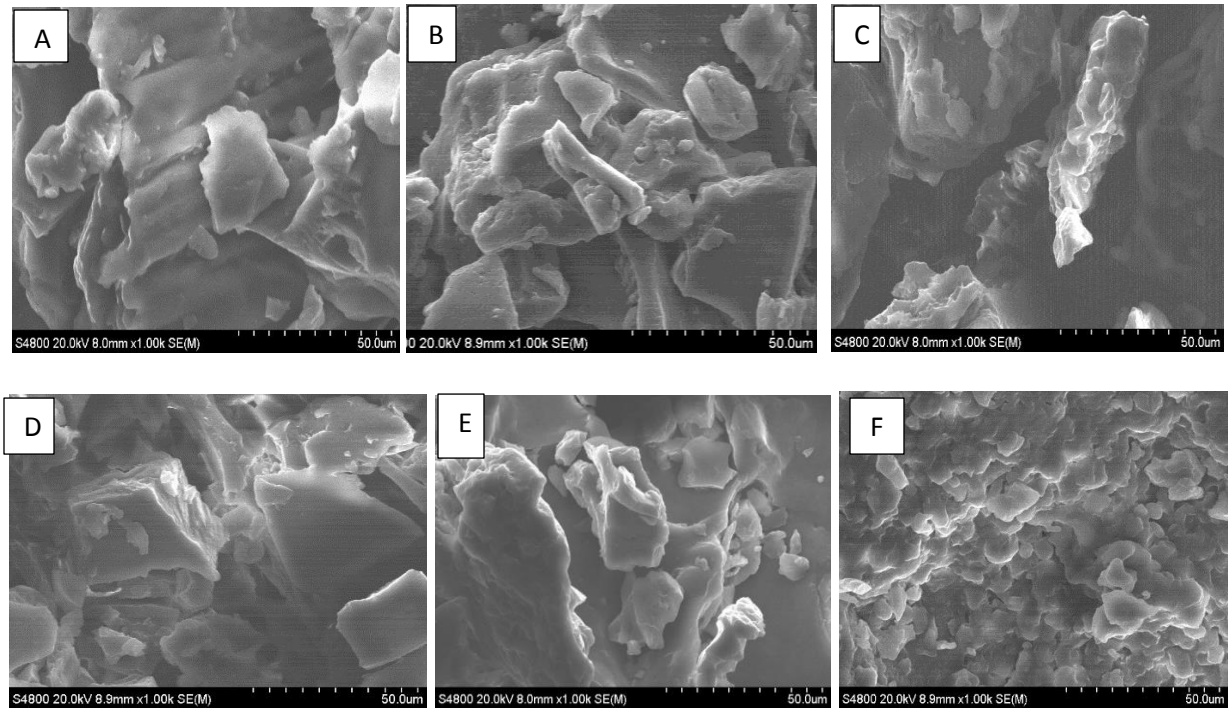


Figure 2.7. Scanning electron micrographs of pearl millet flours extruded at 35% (A), 33% (B), 31% (C), 29% (D), and 27% (E) feed moisture content, and the non-extruded control flour (F).

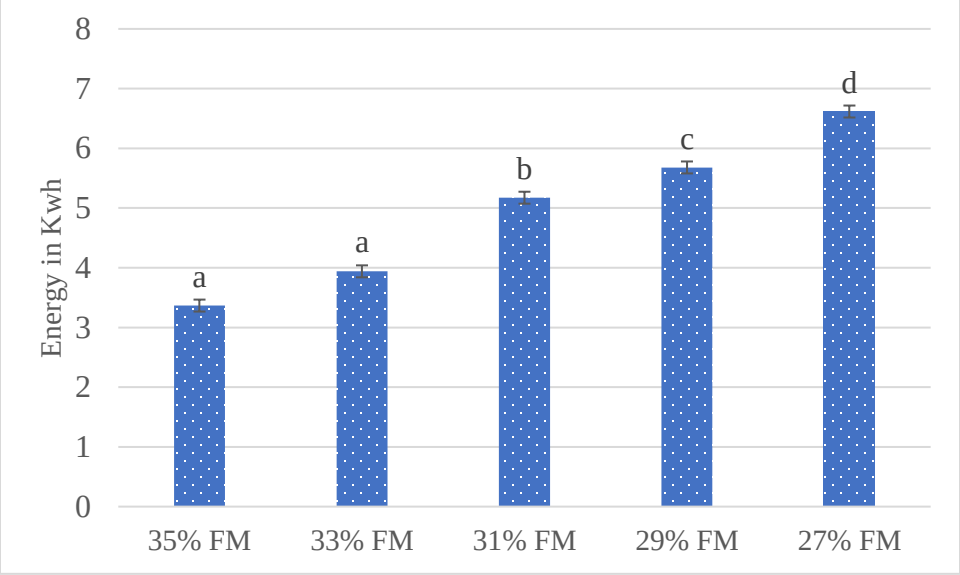


Figure 2.8. Power utilization during extrusion of pearl millet flours in three different runs for different feed moistures (FM). Bars with different letters are significantly different using Tukey's multiple comparison test, $P < 0.05$.

CHAPTER 3. EXTRUSION SHEAR AND FEED MOISTURE INFLUENCES MAIZE BRAN FERMENTATION AND MICROBIOTA OUTPUT IN *IN VITRO* FECAL FERMENTATION

3.1. Abstract

A compilation of recent reports indicates that dietary fibers derived from whole grains promote commensal bacteria in the gut with production of short chain fatty acids (acetate, propionate, and butyrate). Maize bran, as a component of whole grain, is a good source of fiber, but has been characterized by a poor gut microbiota fermentation property compared to other cereal brans from wheat, rice, oats, and sorghum. Its poor fermentation is related to a highly crosslinked and densely branched arabinoxylan chemical structure, which is tightly packed making it poorly available to the gut microbiota. We hypothesized that this dense cell wall matrix can be opened for better fermentation by applying extrusion, a high temperature and shear food processing technique that results in expanded, cooked products. Extrusion processing is being used in our USAID processing/nutrition projects as a way to make convenient food-to-food fortified whole grain instant maize porridge flours. A twin-screw extruder was used at low (200 rpm) and high (400 rpm) shear rates applied to a maize meal and bran mixture (60:40) at different feed moisture conditions (20, 25, 30%). *In vitro* fermentation was conducted independently on stools from three donors. The extrusion conditions only moderately increased short chain fatty acids fermentation metabolites, which was independent of shear rate but with variable effect at different feed moisture contents. The highest increase was at a feed moisture compared to the non-extruded blend). Extrusion did not change the bran chemical composition in extruded versus non-extruded brans. For the microbiota effect, extrusion did not alter α and β-diversity, however, there were individualized alterations in the microbiota structure. For example, extrusion at 30% FM and 400 RPM increased genera of *Subdoligranulum* and *Eubacterium hallii* and *Ruminococcus torques* groups in Donor 1 compared to non-extruded bran. In Donor 2, extruded brans increased genera in the family of Enterobacteriaceae compared to non-extruded bran. There was also an increasing trend of *Subdoligranulum* and *Blautia* in extruded compared to non-extruded bran in Donor 2. In Donor 3, *Lachnospiraceae NK4A136* group was increased at 20% and 25% FM at 200 RPM and 30% FM at 400 RPM compared to non-extruded bran. *Megamonas* was also increased at 25 and

30% FM both at 400 RPM compared to non-extruded bran in Donor 3. Generally, extrusion somewhat enhanced short chain fatty acids production with minor effects on the gut microbiota.

3.2. Introduction

Whole grains are recognized as one of the sources of dietary fibers with a large proportion being insoluble, which may have special value to the colonic microbiota and gut health (Ayua et al., 2020; Tuncil et al., 2018a). Corn bran as a fiber source is composed mainly of arabinoxylan, cellulose, β-glucans, lignin, and phenolic acids (Saulnier et al., 1995). Compared to other cereal brans, corn bran is poorly fermented after 24 or 48 hours *in vitro* human fecal fermentation (Rose et al., 2010; Tuncil et al., 2018b). Extraction of corn bran arabinoxylan increases its fermentation output *in vitro*, indicating that its poor fermentation property is related to its physical form in the corn bran cell wall (Rose et al., 2010).

Fermentation of fibers in the gut leads to formation of short chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate (Jha et al., 2011; Alexander et al., 2019). These metabolites have diverse roles including butyrate acting as a source of energy for the colonocytes and having localized anti-inflammatory effect (Donohoe et al., 2011), and propionate more directly affecting satiety hormones, such as glucagon-like peptide-1 and peptide YY, and thereby contributing to reduced energy intake (Chambers et al., 2015).

There have been approaches to increase the fermentation of fibers such as through enzyme hydrolysis (Karimi et al., 2020) and extrusion (Arcila et al., 2015; Brahma et al., 2017). Through the action of shear, and mixing at high temperature and pressure, extrusion processing has the potential to open the cell wall microstructure to both expand the insoluble fiber matrix and to solubilize some of the fiber. However, results have been inconclusive. Gualberto et al. (1997) reported modest increases in the soluble fiber of 3.5 to 5.5% and 3.1 to 3.5% in oat and wheat bran, respectively, with extrusion at 225 RPM and all barrel section temperatures set at 163^{o}C. The authors did not find significant changes in the insoluble fiber component of wheat bran. In another study, soluble dietary fiber in oat bran increased from 9 to 14 g/100g by extrusion at 10% feed moisture and barrel temperature of 140^{o}C (Zhang et al., 2011). Extrusion processing did not affect dietary fiber fractions of wheat bran (Arcila et al., 2015). Reyes-Perez et al. (2013) noted increase in dietary fiber during extrusion due to formation of resistant starch.

To date, there is evidence that extrusion can influence fermentation pattern (Arcila et al., 2015) and the microbiota (Smith et al., 2020; Brahma et al., 2017; Rubio et al., 2020). Arcila et al. (2015) used a 3×2 factorial design: feed moisture (15, 25, 30%) and shear rates (200, 400 rpm) in a twin-screw extruder. Extrusion did not alter the dietary fiber and protein content of wheat bran, and led to at least a 2-fold increase in the water-extractable (i.e., soluble) non-starch polysaccharides with the largest increase being observed at low feed moisture (15%) and low shear rate (120 rpm). In an *in vitro* fecal fermentation experiment, extrusion significantly increased production of SCFAs compared to non-extruded sample, particularly at 24 h. These authors did not evaluate the effect of extrusion on the gut microbiota composition. An *in vitro* fecal fermentation of whole grains oats extruded at 15% feed moisture lowered *Bifidobacterium* compared to that extruded at 18%, but increased *Lactobacillus* counts (Brahma et al., 2017). Mice fed with high fat diets containing extruded sorghum flours reduced Firmicutes and increased Bacteroidetes (de Sousa et al., 2019). In another study, extruded rice and oats increased absolute numbers of gut microbiota in piglets, while extruded barley led to a decrease (Torrallardona et al., 2012). In another study, extrusion of oats and barley was shown to reduce microbiota diversity and enrich *Streptococcus*, *Bulleidia* and *Blautia* genera (Moen et al., 2016). Extrusion has also been shown to increase lactobacilli numbers and reduce some bacteria (Rubio et al., 2020). Thus, the effects of extrusion of foods on microbiota is not conclusive. Corn bran is particularly hard for gut bacteria to ferment and, after a preliminary in vitro human fecal fermentation examination showed little effect of extrusion processing, it was hypothesized that a further increase in shear rate and feed moisture at moderately high temperatures would better open the bran microstructure and better increase fermentation and alter the microbiota profile. The following is the report of this study.

3.3. Materials and methods

Degermed cornmeal and corn bran were gifts from Bunge Company Ltd (St. Louis, MO, USA). Ethanol, hydrochloric acid, and sodium hydroxide were purchased from Fischer Scientific (Waltham, MA, USA) The enzymes used included pepsin, amyloglucosidase (Megazyme, E-AMGDF) (Megazyme, Wicklow, Ireland), and pancreatin (P7545, Sigma-Aldrich, St. Louis, MO, USA), pepsin (P7000, Sigma-Aldrich).

3.3.1. Extrusion using a twin-screw extruder

Corn bran was mixed with corn meal at the ratio of 40:60% w/w. Extrusion was done using a 3×2 factorial involving three moisture conditions (20, 25, 30%) and two shear rates (200 and 400 RPM). These extrusion conditions were chosen based on an initial study (Appendix 5). Corn bran-cornmeal mixtures were adjusted to the appropriate moisture conditions and stored 12 h at 4°C until the following day when the extrusion was done. Before extrusion, the moisture contents were confirmed using a Rapid Moisture Analyzer (HE53, Greifensee, Switzerland). The flour blends were extruded in a twin-screw extruder [Krupp Werner and Pfleiderer ZSK-25 (WP-25), Ramsey, NJ]. The screw arrangement consisted two sets of reverse screws, one located midway the screw and one close to the die. Two mixing disks were also fixed onto the screw. Feed came out of the extruder via two circular dies each having a diameter of 4 mm. A gravimetric feeder was used to feed the extruder at a rate of 3.5 and 7.0 kg/h at 200 and 400 rpm, respectively. Extruder barrel chamber temperatures were set at 60: 90: 100; 120; 140°C. Dried extrudates were first milled in a hammer mill (Eberbach E3703 Cutting Mill, Belleville, MI, USA) at a speed of 900 rpm fitted with 0.5 mm screen, and then particle size flour between 0.3-0.5 mm was obtained from a sieve shaker (Rx-24, W. S. Tyler Inc. Mentor, Ohio, USA). The obtained flour was packaged in Ziplock bags and then stored at -20°C for subsequent experiments.

3.3.2. *In vitro* upper gastrointestinal tract digestion

Simulation of upper gastrointestinal digestion was performed as described by Tuncil et al. (2018b). Prepared non-extruded and extruded flours (12.5 g) were weighed into a beaker and then 150 ml of purified water added and boiled for 20 min under constant stirring. The mixture was then cooled to 37°C and pH adjusted to 2.5 using 1 M HCl. Next, 5 ml of pepsin (P-7000, Sigma-Aldrich) dissolved in 50 mM HCl (100 mg pepsin/ml) were added and incubated at 37°C under constant stirring. Then, 25 ml of 0.1 M sodium maleate buffer (pH=6.0, 1 mM $CaCl_2$) were added, and the pH adjusted to 6.9 using 1M sodium bicarbonate. Twenty-five (25) ml of pancreatin (Sigma Aldrich P7545, Sigma-Aldrich) dissolved in 0.1 M sodium maleate buffer (125 mg pancreatin/ml buffer) and 1 ml of amyloglucosidase (Megazyme, E-AMGDF) were added to the bran digestion mix and incubated for 6 h at 37°C in a shaking water bath. After 6 h, the mixture

was boiled to kill the enzymes and dialyzed using dialysis bags (MWCO 6-8 kDa, 08-670F, Fisher Scientific) for 36 h, with water being changed every 6 h. The samples were then freeze-dried.

3.3.3. Protein and starch contents of bran after upper gastrointestinal tract digestion

Percentage protein content was analyzed by the Dumas method using a nitrogen analyzer (LECO TruMac, LECO Corporation, St. Joseph, MI, USA). The machine was calibrated using EDTA and the amount of nitrogen was multiplied by 6.25 to obtain percentage protein. Total starch that remained in the samples was analyzed using the Total Starch Kit (K-TSTA, Megazyme, Wicklow, Ireland) based on the manufacturer's procedure.

3.3.4. *In vitro* fecal fermentation

In vitro fecal fermentations were done in an anaerobic environment as described by Tuncil et al. (2018b). Ethical approval was obtained from the Institutional Review Board (IRB) at Purdue University (#1510016635). Briefly, stools were obtained from 3 healthy individuals without any gastrointestinal disorders, consuming normal diets, having not taken antibiotics for past 3 months, and with a BMI of 18.5-25.0. Fecal samples were obtained from individual donors and sealed in pre-weighed tubes, kept on ice, and then transferred into an anaerobic chamber.

Fifty mg of each of the fibers [fructooligosaccharide (FOS), non-extruded and extruded corn bran samples] were weighed into Balch tubes for sampling periods at 0, 6, 12, 24 and 48 h. Fermentations were done in triplicate and a blank without any substrate was included at each time point. Prepared carbonate phosphate buffer was sterilized by autoclaving at 121^{o}C for 60 min. After autoclaving, 0.25 mg/L of cysteine hydrochloride, a reducing agent was added. The buffer was then placed in an anaerobic chamber until it turned colorless. On the day of the experiment, 4 mL of the carbonate buffer was added to every Balch tube with the fibers.

Fecal material from each individual donor was homogenized in carbonate phosphate buffer (feces: buffer ratio 1:3 w/v). The resultant mixture was filtered through four layers of cheese cloth. Then, 1 mL of the fecal slurry was inoculated into each tube [with already added fibers (50 mg) and 4 mL buffer]. The tubes were then closed using rubber stoppers, followed by sealing using aluminum seals. Finally, the tubes were incubated at 37^{o}C in a water bath shaking at 150 RPM.

3.3.5. pH measurements

The pH was measured using a pH meter (Orion Star A211, Thermo Fischer Scientific, MA, USA).

3.3.6. Short chain fatty acid analysis and monosaccharide composition

Short chain fatty acids were analyzed as described by Tuncil et al. (2018b). The collected samples for SCFAs from fermentation were combined with 100 µl of already prepared internal standard. The internal standard composition was: 5-methylvaleric acid (157.5 µL), 85% phosphoric acid (1.47 ml), and copper sulfate pentahydrate (39 mg). The final volume was adjusted to 25 ml using purified water. A fecal slurry was centrifuged for 10 min at 13,000 rpm. Next, 4 µl of the obtained supernatant was analyzed by gas chromatography, with a silica capillary column (internal diameter 0.25 µm, 30 mm x 0.25 mm, NukonTM, Supelco, Bellefonte, PA, USA) and an Agilent ionization detector (GC-FID 7890A, Santa Clara, California, USA). Injection conditions were as follows: carrier gas, helium, 1.3 mL/min flow rate, and temperature of 185ºC. Quantification of SCFAs was done by measuring the peak areas of acetate, propionate, and butyrate relative to those of external standards using standard curves.

Monosaccharide composition of the brans was analyzed as alditol acetates using gas chromatography (SP2330, Supelco, Bellefonte, PA, USA) coupled with mass spectroscopy (GC-MS, 7890A, Agilent Technologies Inc, CA, USA) using helium as a carrier gas as outlined by Tuncil et al. (2018b). The scanning electron micrographs of the bran after upper gastrointestinal digestion was done as described in Chapter 2 section 2.3.6.

3.3.7. Extraction of DNA, amplification and sequencing

QIAamp PowerFecal Pro DNA Kit (Qiagen, Singapore Pte Ltd, Singapore) was used to extract DNA from 0 and 48 h ferments based on manufacturer's instructions. Briefly, fecal material was centrifuged for 10 min at 13,000 rpm, after which the supernatants were discarded. The obtained pellets were homogenized in lysis buffer. All other procedures were done as described by the manufacturer.

Amplification of the V4 region of 16S rRNA gene was done by polymerase chain reaction using the primers 515F: (ACACTGACGACATGGTTCTACAGTGCCAGCMGCCGCGGTAA) and 806-R (TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT) (Cantu-Jungles et al., 2019; Lamothe, 2014) at the University of Illinois Sequencing Core Chicago, USA. First, PCR was done in a reaction mix (20 µL) AccuPrime SuperMix II (No 12341-012, Life Technologies, New York, USA) with desalted primers (0.5 µM). The first denaturation was done for 5 min at 95°C, then another 28 cycles of denaturation for 30 sec at 95°C, annealing for 45 sec at 55°C, and elongation for 30 sec at 68°C. Final elongation was done for 7 min at 68°C after which the products of amplification were subjected to the second PCR reaction in 2X AccuPrime SuperMix II: product from 1st PCR (1 µL), barcorded primers (2 µL), SuperMix (5 µL) and water (2µL). The second cycle of PCR involved denaturation at 5 min at 95°C followed by another denaturation involving 8 cycles for 30 sec at 95°C, annealing for 30 sec at 60°C, elongation for 30 sec at 68°C. The final elongation was for 7 min at 68°C. Sequencing of amplicons were done using Illumina MiSeq runs of 2 × 250 cycles (Illumina, Inc., San Diego, CA) at the University of Illinois at Urbana Champaign Sequencing Core.

Sequences were processed using QIIME version 2 (Bolyen et al., 2019) by joining paired reads, filtering the sequences using a threshold Phred Quality score of 20 and the data denoised using Deblur and taxonomic classification was done using SILVA reference database (Bokulich et al., 2018). Sequences with 97% similarity were organized into operational taxonomic units. Alpha diversity was measured using Shannon, Simpson, and Chao indices while beta diversity was calculated using weighted Unifrac distances in QIIME. Data analysis was done using SAS v. 9.4 (SAS Institute Inc, NC, USA) and significant differences determined using Tukey's multiple comparison test ($P < 0.05$).

3.4. Results and discussion

3.4.1. Residual starch and protein after upper gastrointestinal tract digestion

The starch and protein proportions of the extruded corn bran-meal mixtures and non-extruded bran after upper gastrointestinal tract simulated digestion are presented in Table 3.1. All the samples had less than 2% starch. Corn bran extruded at 20% feed moisture had significantly higher residual starch content than the non-extruded flour. Residual starch may be a form of

resistant starch that was formed during extrusion at lower feed moisture content and was not statistically different at higher moisture contents from that of non-extruded bran-meal mixture. Among the extruded samples, screw speed did not have an impact on the amount of residual starch. It has been proposed that at lower screw speed, more resistant starch can be formed because of the increased residence time of the feed in the extruder which can increase the chances of amylose reassociation (Gonzalez-Soto et al., 2006). Increase in resistant starch upon extrusion has been reported previously upon extrusion (Kim et al., 2006) and is likely due to lipid/protein-starch interactions and starch retrogradation (Gonzalez-Soto et al., 2006; Sarawong et al., 2014). There was no clear trend among treatments for residual proteins after upper gastrointestinal tract digestion and were in the range of 4-9%. This is similar to that reported by Brahma et al. (2017) after upper gastrointestinal digestion of corn bran.

3.4.2. pH and short chain fatty acid production

Short chain fatty acids and pH were measured across the fermentation time points as an indicator of fermentation rates. Changes in pH were modest with FOS showing the greatest pH drop below 6.5 (Figure 3.1). All the pH measurements for bran treatments were above 7.3. Modest change in pH production upon fermentation of corn bran has been observed by Thakkar et al. (2020).

Corn bran has been reported to be poorly fermented (Rose et al., 2010). We hypothesized that extrusion would open up the corn bran cell wall and increase production of short fatty acids. Total SCFAs were significantly higher for FOS, but were higher than the blank without fiber. Extruded bran produced the highest total SCFAs (Figures 3.2A, 3.3B). In Donor 1, the total SCFAs increased by 13-41% in the extruded samples compared to non-extruded bran at 48 h. The increase in total SCFAs in Donor 2 in the extruded samples compared to non-extruded bran was between 13-40% at 48 h. In Donor 3, total SCFAs did not differ between non-extruded bran and bran extruded at 30% feed moisture and screw speed of 200 rpm, but was higher in the other extrusion treatments (0-39%) at 48 h. As such, extrusion enhanced the fermentation of the corn brans, though the increase was modest compared to the highly fermentable FOS control. Individual SCFAs over the 48-h fermentation period are presented in Tables 3.2-3.4. After 48 h fermentation, there was a significant ($P < 0.05$) increase in acetate in extruded samples compared to the non-extruded sample

with the largest increase observed for the low feed moisture and high screw speed treatment. Within the first 12 h, there were no clear differences in production of SCFAs among the donors for the extruded samples. The FOS positive control generated the highest total SCFAs, acetate, propionate, and butyrate. In Donors 2 and 3, propionate was somewhat greater than butyrate, while in Donor 1 butyrate was slightly higher than propionate. At 48 h of fermentation, the low feed moisture content (20%) and higher shear (400 RPM) combination led to a significantly ($P < 0.05$) higher amount of the individual SCFAs in all the donors (Figure 3.3). Overall, extrusion caused the largest increase in acetate and least increase in butyrate. These findings agree with those of Arcila et al. (2015) who found that extrusion significantly increased SCFA production in extruded brans. Thus, extrusion promoted fermentation and SCFA production and the amount produced depended on the donor microbiota.

As with the present work, Arcila et al. (2015) could not observe a clear trend between higher and lower extrusion shear on production of SCFAs. This could be because at lower shear, there is higher residence time upon which bran is expose to heat and mixing in the extruder, while a higher shear rate exposes bran to higher shear but for a shorter time.

3.4.3. Monosaccharide composition of the brans after upper gastrointestinal digestion

Table 3.5 shows the monosaccharide composition of extruded and non-extruded corn brans used in the fermentation experiment. Glucose and xylose were the most dominant in both extruded and non-extruded samples, while rhamnose and fucose were the least which each representing less than 1%. High glucose levels in extruded samples were likely from resistant starch forming during extrusion, when starch retrogrades or complexes with phenolic compounds or macronutrients (Kim et al., 2006; Neder-Suarez et al., 2016). Extrusion processing has previously been shown to increase resistant starch proportions (Agustiniano-Osornio et al., 2005; Hasjim and Jane, 2009). The high xylose proportion indicates the high arabinoxylan content of corn bran. The arabinose:xylose ratios did not significantly differ between extruded and non-extruded bran, suggesting that extrusion conditions were not severe enough to change arabinoxylan branched structures. Other studies have shown that extrusion reduces arabinose:xylose ratio, from induced structural and chemical changes (Liu et al., 2020; Liu et al., 2017). There also were some microstructural changes in the brans the extruded brans (Figure 3.4). Edges of the brans left after

upper gastrointestinal digestion showed minor microstructure changes suggesting that extrusion might have opened some cell wall components enhancing better fermentation.

3.4.4. Microbial profile of the initial inoculum at 0 h

To determine the major taxa changes in the donors, sequencing of the V4 region of the 16S rRNA gene was done. In all the three donors, Firmicutes was the predominant phyla. In Donor 1, Firmicutes (70±1.2%), Bacteroidetes (20.3±1.3%), and Actinobacteria (7.8±0.4%) were the prevalent phyla accounting for at least 98% of the total bacteria. In Donor 3, Firmicutes (69.8±1.9%), Bacteroidetes (20.2±0.9%), and Actinobacteria (9.4±0.36%) similarly represented the predominant phyla. Differently, Donor 2 had the lowest Firmicutes (61.4±0.3%) and highest Proteobacteria (10±0.2%) proportions. Further, Bacteroidetes and Actinobacteria in Donor 2 were 24.7±0.6 and 2.7±0.1%, respectively. Firmicutes and Bacteroidetes have been reported to be the most dominant phyla in the human gut (Tuncil et al., 2017; Rinninella et al., 2019), while Proteobacteria has been reported to be a sign of dysbiosis (Shin et al., 2015). Certain members of Firmicutes are known to produce butyrate, while propionate is predominantly produced by Bacteroidetes (Salonen et al 2014). These differences in gut bacteria can help explain the variations in SCFAs observed during fermentation, as Donor 2 had somewhat higher propionate compared to butyrate compared to Donor 1.

3.4.5. Alpha and beta diversity of the microbial communities in the donors

Alpha diversity represents richness (taxa numbers) and evenness (distribution of the predominant taxa) or a combination of the two (Willis, 2019). Alpha diversity changes were measured using Chao for richness (Figure 3.5), Shannon for both richness and evenness (Figure 3.6), and Simpson for evenness (Table 3.6) indices. Both richness and evenness did not change across extruded and on-extruded bran treatments. Corn bran has previously been reported not to alter alpha diversity, while FOS reduced these indices in *in vitro* fecal fermentations (Tuncil et al., 2018b). According to these authors, during batch fermentations a subgroup of microorganisms grow at the cost of those not growing, thereby reducing the evenness.

For beta diversity, community structure analysis across donors was done using weighted UniFrac on the relative abundances of relative taxonomic units. Donor had a substantial effect on the community structure as shown by the clusters (Figure 3.7). Similar observations have been reported by Tuncil et al. (2017) when they observed that donor's variation accounted for approximately 70% of the total variation in microbial composition. Extruded and non-extruded brans did not alter beta diversity (Figures 3.7-3.10), as there was no clustering due to different extrusion conditions. Processing methods such as extrusion, boiling, and sourdough fermentation of bread did not alter beta diversity when these samples were subjected to fermentation (Smith et al., 2020). On the other hand, Martinez et al. (2013) observed increase in microbial diversity during a 4-week feeding study in humans on brown rice, whole grain barley, and an equal mixture of the two. This could be because the gut bacteria may adapt to utilizing these carbohydrates during longer interventions.

3.4.6. Changes in microbiota across donors

Some differences were observed in the composition of the gut microbiota when comparing the extrusion treatments to the non-extruded bran (Figures 3.11-3.16). Different extrusion conditions, both related to feed moisture content and screw speed caused minor differences in growth of a number of bacteria in the Donors (Figures 3.14-3.16).

Minor trends in differences in specific bacteria operational taxonomic units (OTUs) by extrusion treatments follow. For Donor 1, the genus *Bifidobacterium* increased in bran that underwent extrusion at 30% FM and 400 RPM compared to the non-extruded bran (Figure 3.11). The genus *Blautia* was higher after extrusion at 20% FM at both screw speeds, and 25% FM and 200 RPM compared to the non-extruded bran. *Subdoligranulum* was also increased in extruded brans at 30% FM and 400 RPM compared to the non-extruded bran. Greater increase in *Alistipes* was observed in both non-extruded brans and brans extruded at 25% FM and 200 RPM. *Faecalibacterium* tended to increase both in extruded than non-extruded brans except at 30% FM and 400 RPM. For Donor 2, the genus *Prevotella* was somewhat increased in extruded samples compared to the non-extruded sample, except at 30% FM and 200 RPM of the screw (Figure 3.12). Further, all treatments increased *Desulfovibrio* in Donor 2. *Blautia* and *Subdoligranulum* were also increased by extrusion compared to the non-extruded bran. Increase in *Streptococcus*, *Bulleidia*

and *Blautia* have been in observed in extruded barley and oats (Moen et al., 2016). However, unlike in Donor 1, *Faecalibacterium* was not increased by extrusion but rather was reduced. In Donor 3, genera of *Blautia* was increased in extruded compared to non-extruded bran. *Faecalibacterium* was increased at 25 and 30% FM regardless of screw speeds (RPM) (Figure 3.13). Corn bran specifically has been shown to promote *Ruminococcaceae sp., Blautia*, and *Bifidobacterium sp*. (Thakkar et al., 2020). In a human study, whole grains have been shown to result in modest changes in gut microbiota composition and some of the species that were reported to increase are *Faecalibacterium prausnitzii* and *Prevotella copri* (Roager et al., 2019).

Costabile et al. (2008) did not observe significant changes in *Bacteroides sp.*, during a 3-week *in vivo* study of wheat grain or wheat bran, while Martinez et al. (2013) observed a reduction of the genus *Bacteroides* upon whole grain consumption in a human trial. Corn bran is predominantly composed of arabinoxylan (Rose et al., 2010; Roye et al., 2019) and *Bacteroides* genus have been reported to be capable of degrading such varied complex polysaccharides (Flint et al., 2012; Dodd et al., 2011). Members of *Bacteroides ssp.* are predominantly propionate producers and this could partly explain why Donor 2 produced more propionate than butyrate (Rios-Covian et al., 2017; Salonen et al., 2014). Increase in abundance of *Blautia* has been reported by Martinez et al. (2013) after intake of brown rice, whole grain barley, and a blend of whole grain barley and brown rice. *Blautia* genus is one of the commensal members of gut microbiota and does not make butyrate directly from dietary carbohydrates (Louis and Flint, 2017), however it has been implicated in health, as reduction of this genus has been shown in persons with insulin resistance and obesity (Benitez-Paez et al., 2020).

Inclusion of extruded legumes in cereal-based mice diets was reported to lower *Faecalibacterium prausnitzii, bifidobacteria, Clostridium leptum, Ruminococcus sp.*, but with an increase in *Lactobacilli* (Rubio et al., 2020). These authors also noted that *Prevotella*/*Bacteroides* were unaffected. In Donor 1, FOS significantly increased genera of *Blautia, Collinsella, Ruminococcus torques group, and Phascolarctobacterium*. In Donor 3, FOS increased *Megamonas* and *Eubacterium hallii* group. Reduction of families of *Lachnospiraceae* and *Ruminococcaceae* has been observed in whole grains processed via extrusion (Smith et al., 2020). Thus, donors' microbiota that can utilize a diverse dietary fiber responded to the whole grain fibers (Smith et al., 2020; Brahma et al., 2017). Smith et al. (2020) reported that extrusion can alter the

microstructure of fiber, thereby altering competition such that the microbiome can longer ferment the complex cell wall matrix, and consequently enabling fast growing microbes to outcompete others for the fibers created by extrusion. They observed increased growth of *Acinetobacter*, *Staphylococcaceae* and *Enterococcus*. Increases were also observed in putative harmful members of uncultured genera of *Succinivibrio* and *Desulfovibrio* (Donor 2) and the genera in the family of *Enterobacteriaceae* (Donor 1) (Figures 3.11 and 3.12). *Desulfovibrio* has been reported to play a role in sulfate reduction in the intestine (Kushkevych et al., 2019). This data suggests that in *in vitro* systems, when insoluble substrates are presented to gut microbiota, opportunistic pathogens may outcompete the beneficial bacteria.

3.5. Conclusions

In conclusion, this study shows that extrusion shear can be used to enhance the fermentation of corn bran fiber, however the fermentation outcome is dependent on the donor's microbiota. For example, Donor 1 produced overall higher amounts of butyrate than the other two donors, with Donor 2 producing the least amount of butyrate. Extrusion at lower feed moisture content seemed to have a better impact on fermentation profiles regardless of the screw speeds. Extrusion did not significantly alter the arabinose:xylose ratio nor the the alpha and beta diversities of microbial communities in the donors. Interestingly, extruder test conditions impacted certain bacteria. For example, extrusion at 30% FM and 400 RPM increased genera of *Subdoligranulum* and *Eubacterium hallii* and *Ruminococcus torques* groups in Donor 1 compared to non-extruded bran. In Donor 2, extruded brans increased genera in the family of Enterobacteriaceae compared to non-extruded bran. There was also an increasing trend of *Subdoligranulum* and *Blautia* in extruded compared to non-extruded bran in Donor 2. In Donor 3, *Lachnospiraceae NK4A136* group was increased at 20% and 25% FM at 200 RPM and 30% FM at 400 RPM compared to non-extruded bran. *Megamonas* was also increased at 25 and 30% FM both at 400 RPM compared to non-extruded bran in Donor 3. From this study, we learned that extrusion can be used to moderately enhance fermentation profile of corn bran substrates that are normally poorly fermented, but this is dependent on the fecal composition of the donors. This increased fermentation could positively impact the health of the host.

3.6. References

Adewole, D. (2020). Effect of dietary supplementation with coarse or extruded oat hulls on growth performance, blood biochemical changes parameters, cecal microbiota and short chain fatty acids in broiler chickens. *Animals*, 10, 1429.

Agustiniano-Osornio, J. C., Gonzalez-Soto, R. A., Flores-Huicochea, E., Manrique-Quevedo, N., sanchez-Hernandez, L., and Bello-Perez, L. A. (2005). *Journal of the Science of Food and Agriculture*, 85, 2105-2110.

Alexander, C., Swanson, K.S., Fahey, G. C., and Garleb, K. A. (2019). Perspective: physiologic importance of short chain fatty acids from non-digestible carbohydrates fermentation. *Advances in Nutrition*, 10, 576-589.

Arcila, J. A., Weier, S., and Rose, D. J. (2015). Changes in dietary fiber fractions and gut microbial fermentation properties of wheat bran after extrusion and bread making. *Food Research International*, 74: 217-223.

Ayua, E.O., Kazem, A.E., Hamaker, B.R. (2020). Whole grain cereal fibers and their support of the gut commensal Clostridia for health. *Bioactive Carbohydrates and Dietary Fibre* 24:100245.

Benítez-Páez A, Gómez del Pugar EM, López-Almela I, Moya-Pérez Á, Codoñer-Franch P, Sanz Y. 2020. Depletion of Blautia species in the microbiota of obese children relates to intestinal inflammation and metabolic phenotype worsening. *mSystems*, 5, e00857-19.

Bokulich, N., Kaehler, B. D., Rideout, J. R., Dillon, M., Boylen, E., Knight, R., et al. (2018). Optimizing taxonomic classification of marker gene amplicon sequences with QIIME 2s feature classifier plugin. *Microbiome*, 6:90.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N, A., Abnet, C. C., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37, 852-857.

Brahma, S., Martínez, I., Walter, J., Clarke, J., Gonzalez, T., Menon, R., Rose, D.J. (2017). Impact of dietary pattern of the fecal donor on in vitro fermentation properties of whole grains and brans. *Journal of Functional Foods*, 29, 281–289

Benitez-Paez, A., Del Pugar, E. M. G., Lopez-Almela, I., Moya-Perez, A., et al. (2020). Depletion of balutia Species in the microbiota of obese children relates to intestinal inflammation and metabolic phenotype worsening. *Msystems*, 24, 5, e00857.

Cantu-Jungles, T. M., do Nascimento, G. E., Zhang, X., Iacomini, M., Cordeiro, L. M. C., Hamaker, B. R. (2019). *Carbohydrates Polymers*, 206, 389-395.

Chambers, E.S., Viardot, A., Psichas, A., Morrison, D. J., Murphy, K.G., Zac-Varghese, S., et al. (2015). Effect of targeted delivery of propionate to the human colon on apetite regulation, body weight maintenance and adiposity in overweight individual. *Gut*, 64:1744–1754.

Costabile, A., Klindler, A., Fava, F., Napolitano, A., Fogliano, V., et al. (2008). Whole grain wheat breakfast cereal has prebiotic effect on the human gut microbiota: a double-blind placebo controlled, crossover study. *British Journal of Nutrition*, 99 (1),110-120.

de Sousa, A. R., Moreira, M.E.C., Grancieri, M., Toledo, R. C.L., Araujo, F. O., Mantovani, H. C., et al. (2019). Extruded sorghum (Sorghum bicolor L.,) improves gut microbiota, reduces inflammation, and oxidative stress in obese rats fed a high fat diet. *Journal of Functional Foods*, 58, 282-291.

Dodd, D., Mackie, R. I., and Cann, I. K. O. (2011). Xylan degradation, a metabolic property shared by rumen and human colonic Bacteroidetes. *Molecular Microbiology*, 79, 292-304.

Donohoe, D. R., Garge, N., Zhang, X., Sun, W., O'Connell, T. M., Bunger, M.K., and Bultman, S.C. (2011). The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabolism*, 4, 13: 517-526.

Flint, H.J., Scott, K.P., Duncan, S.H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, 3(4), 289-306.

Gonzalez-Soto, R. A., Sanchez-Hernandez, L., Solorza-Feria, J., Nunez-Santiago, C., Flores-Huicochea, E., and Bello-Perez, L. A. (2006). Resistant starch production from non-conventional starch by extrusion. *Food Science Technology International*, 12, 5-11.

Gualberto, D. G., Bergman, C. J., Kazemzadeh, M., and Weber, C. W. (1997). Effect of extrusion processing on the soluble and insoluble fiber and phytic acid contents of cereal brans. *Plant foods for Human Nutrition*, 51, 187-198.

Hasjim, J., and Jane, J. (2009). Production of resistant starch by extrusion cooking of acid modified normal maize starch. *The Journal of Food Science*, 74, C556-C562.

Jha, R., Bindelle, J., Kessel, A. V., and Leterme, P. (2011). In vitro fiber fermentation of feed ingredients with varying fermentable carbohydrates and protein levels and protein synthesis by colonic bacteria isolated from pigs. *Animal Feed Science and Technology*, 165,191-200.

Karimi, R., Azizi, M.H., Sahari, M. A.,Kazem, A. E. (2020). *In vitro* fermentation profile of soluble dietary fibers obtained by different enzymatic extractions from barley bran. *Bioactive Carbohydrates and Dietary Fiber*, 21, 100205.

Kim, J.H., Tanhehco, E. J., and Ng, P. K. W. (2006). Effect of extrusion conditions on resistant starch formation from pastry wheat flour. *Food Chemistry*, 99, 718-723.

Kushkevych, I., Dordevic, D., Kollar, P., Vitezova, M., and Drago, L. (2019). Hydrogen sulfide as a toxic product in the small-large intestine axis and its role in the IBD development. *Journal of Clinical Medicine*, 8, 1054.

Lamothe, L. (2014). Fermentable carbohydrates sources generated from cereals and pseudocereals insoluble dietary fibers and their in vitro fecal fermentation. Ph.D. Thesis at Purdue University.

Leitch, E.C.M., Walker, A.W., Duncan, S.H., Holtrop, G., and Flint, H.J. (2007). Selective colonization of insoluble substrates by human faecal bacteria. *Environmental Microbiology*, 9 (3), 667-679

Liu, H. H., Li, H. Y., and Wei, A. C. (2017). Enhanced polysaccharides yield obtained from hydrothermal treatment of corn bran via twin screw extrusion. *BioResources*, 12, 3933-3947.

Liu, H., Wei, Y., Li, H., Wei, A., and Wang, X. (2020). Structural changes of polysaccharides isolated from corn bran by hydrothermal treatment after twin screw extrusion. *Bioresources*, 15 (1), 1323-133.

Louis, P., and Flint, H.J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology*, 19, 29-41.

Martinez, I., Lattimer, J.M., Hubach, K.L., Case, J.A., Yang, J., Weber, C.G., et al. (2013). Gut microbiome composition is linked to whole grain induced immunological improvements. *International Society for Microbial Ecology*, 7:269-280.

Moen, B., Berget, I., Rud, I., Hole, A.S., Kjos, N. P., and sahlstrom, S. (2016). Extrusion of barley and oat influence the fecal microbiota and SCFA profile of growing piglets. *Food and Function*, 7, 1024.

Neder-Suarez, D., Amaya-Guerra, C. A., Quintero-Ramos, A., Perez-Carrillo, E., Alanis-Guzman, M. G., et al. (2016). Physicochemical changes and resistant starch content of extruded corn starch with and without storage at refrigerator temperatures. *Molecules*, 21, 1064.

Reyes-Pérez, F., Salazar-García, M. G., Romero-Baranzini, A. L., Islas-Rubio, A. R., and Ramírez-Wong, B. (2013). Estimated glycemic index and dietary fiber content of cookies elaborated with extruded wheat bran. *Plant Foods for Human Nutrition*, 68, 52–56.

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., et al. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet and diseases. *Microorganisms*, 7, 14.

Rios-Covian, D., Salazar, N., Gueimonde, M., de Los Reyes-Gavilan, C.G. (2017). Shaping the metabolism of intestinal *Bacteroides* population through diet to improve human health. *Front Microbiology*, 8:376.

Rivière, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. (2016) Bifidobacteria and Butyrate-Producing Colon Bacteria: Importance and Strategies for Their Stimulation in the Human Gut. *Frontiers in Microbiology*, 7,979.

Roager, H. M., Vogt, J. K., Kristensen, M., Hansen, L. B. S., Ibrugger, S., Maerkedahl, R.B., et al.

(2019). Whole grain rich diet reduces body weight and systematic low-grade inflammation without inducing major changes of the gut microbiome: a randomized cross over trial. *Gut,* 68, 83-93

Rose, D. J., Patterson, J. A., and Hamaker, B.R. (2010). Structural differences among alkali soluble arabinoxylans from Maize (Zea mays), Rice (Oryza sativa) and wheat (Triticum aestivum) brans influence human fecal fermention profiles. *Journal of Agricultural and Food Chemistry*, 58,493-499.

Roye, C., Bulkaen, K., De Bondt, Y., Liberloo, I., De Walle, D. V., et al., Side by side comparison of composition and structural properties of wheat, rye, oat and maize bran and their impact on in vitro fermentability. *Cereal Chemistry*, 97, 20-33.

Rubio, L. A., Aranda-Olmedo, I., Contreras, S., Gongora, T., Dominguez, G., Peralta, J. M., and Martin-Pedrosa, M. (2020). Inclusion of limited amounts of extruded legumes plus cereal mixes in normocaloric or obesogenic diets for rats: effects on intestinal microbiota composition. *Journal of the Science of Food and Agriculture*, 100: 5546-5557.

Rumpagaporn, P., Reuhs, B.L., Kaur, A., Patterson, J.A., Keshavarzian, A., Hamaker, B.R. (2015). Structural features of soluble cereal arabinoxylan fibers associated with a slow rate of in vitro fermentation by human fecal microbiota. *Carbohydrates Polymers*, 130:191-7.

Salonen, A., Lahti, L., Salojarvi, J., Holtrop, G., Korpela, K., Duncan, S. H.,Date, P., et al. (2014). Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *International Society for Microbial Ecology*, 8: 2218-2230.

Sarawong, C., Schoenlechner, R., Sekiguchi, K., Berghofer, E., and Ng, P. K. W. (2014). Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. *Food Chemistry*, 143, 33-39.

Saulnier, L., Vigouroux, J., and Thibault, J. (1995). Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydrate Research*, 272, 241-253.

Shin, N., Whon, T. W., and Bae, J. (2015). Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology*, 33, 496-501.

Smith, C., Van Haute, M. J., and Rose, D. J. (2020). Processing has differential effects on microbiota accessible carbohydrates in whole grains during *in vitro* fermentation. *Applied and Environmental Microbiology*, 86, e01705-20.

Thakkar, R. D., Tuncil, Y. E., Hamaker, B. R., and Lindemann, S. R. (2020). Maize bran particle size governs the community composition and metabolic output of human gut microbiota in in vitro fermentations. *Frontiers Microbiology*, 11,1009. doi: 10.3389/fmicb.2020.01009

Torrallardona, D., Andres-Elias, N., Lopez-Soria, S., Badiola, I., and Cerda-Cuellar, M. (2012). Effect of feeding piglets with different extruded and non-extruded cereals on the gut mucosa and microbiota during the first postweaning week. *Journal of Animal Science, 90: 7-9.*

Tuncil, *Y. E.*, Nakatsu, C. H., Kazem, A. E., Arioglu-Tuncil, S., Reuhs, B., Martens, E. C., and Hamaker, B. R. (2017). Delayed utilization of some fast fermenting soluble dietary fibers by human gut microbiota when presented in a mixture. *Journal of Functional Foods*, 32, 347-357.

Tuncil, Y.E., Thakkar, R., Marcia, A. D.R., Hamaker, B.R., & Lindemann, S.R. (2018a). Divergent short chain fatty acid production and succession of colonic microbiota arise in fermentation of variously sized wheat bran fractions. *Scientific Reports*, 8, 16655.

Tuncil, Y.E., Thakkar, R.D., Arioglu-Tuncil, S., Hamaker, B.R., & Lindemann, S.R. (2018b). Fecal Microbiota responses to bran particles are specific to cereal type and *In vitro*

Digestion methods that mimic upper gastrointestinal tract passage. *Journal of Agricultural and Food Chemistry*, 66, 12580-12593.

Tuncil, Y. E., Thakkar, R. D., Arioglu-Tuncil, S., Hamaker, B. R., and Lindemann, S. R. (2020). Subtle variations in Dietary Fiber fine structure differentially influence the composition and metabolic *function of gut microbiota. mSphere 5, e00180-20.*

Willis, A.D. (2019). Rarefaction, alpha diversity, and statistics. *Frontiers in Microbiology*, 10, 2407. doi: 10.3389/fmicb.2019.0240

Zhang, M., Bai, X., and Zhang, Z. (2011). Extrusion process improves the functionality of soluble dietary fiber in oat bran. *Journal of Cereal Science*, 54, 98-103.

Zhao, J., Bai, Y., Tao, S., Zhang, G., Wang, J., Liu, L., and Zhang, S. (2019). Fiber rich foods affected gut microbial community and short chain fatty acids production in pig model. *Journal of Functional Foods*, 57, 266-274.

Table 3.1. Starch (%) and protein (%) in the brans after upper gastrointestinal tract simulated digestion.

Sample	Protein (%)	Starch (%)
20% FM 200 RPM	4.0±0.08c	1.38±0.76a
20% FM 400 RPM	5.7±1.2bc	1.25±0.91a
25% FM 200 RPM	6.8±0.72b	1.40±0.06a
25% FM 400 RPM	4.8±0.07c	0.61±0.10ab
30% FM 200 RPM	6.7±0.12b	0.64±0.01ab
30% FM 400 RPM	9.1±0.32a	0.54±0.01ab
Non-extruded flour	4.6±0.15c	0.13±0.02b

Values of mean ± SD that have different letters in a column do not differ (Tukey's multiple comparisons, $P < 0.05$). FM = feed moisture content, RPM = rotations per minute of the extruder screw.

Table 3.2. Acetate, propionate, and butyrate for Donor 1 across the 48-h fermentation period.

Donor1	Acetate	Propionate	Butyrate
0 h	1.2±0.87	0.5±0.14	1.0±0.10
6 h			
Blank	5.2±1.23c	0.9±0.19e	1.4±0.23b
FOS	34.7±3.69a	3.1±0.20a	10.74±0.98a
20% FM 200 RPM	9.0±0.74bc	1.6±0.07bc	2.0±0.08b
20% FM 400 RPM	8.8±0.63bc	1.5±0.11bcd	2.1±0.14b
25% FM 200 RPM	7.2±0.44bc	1.4±0.10bcd	1.6±0.0.20b
25% FM 400 RPM	7.6±0.74bc	1.2±0.08cde	1.7±0.06b
30% FM 200 RPM	8.8±0.34bc	1.6±0.05bc	2.1±0.06b
30% FM 400 RPM	9.6±0.94b	1.7±0.16b	2.1±0.19b
Non-extruded	6.57±0.99bc	1.2±0.10de	1.6±0.07b
12 h			
Blank	5.7±1.03c	1.2±0.36d	1.5±0.33c
FOS	37.6±1.28a	3.8±0.11a	12.6±0.70a
20% FM 200 RPM	10.0±0.93b	1.7±0.19c	2.30±0.24b
20% FM 400 RPM	9.8±1.43b	1.8±0.05c	2.4±0.15b
25% FM 200 RPM	9.5±0.91b	2.2±0.12b	2.4±0.10b
25% FM 400 RPM	10.1±1.32b	1.7±0.14c	2.1±0.53b
30% FM 200 RPM	9.8±1.40b	1.8±0.22c	2.3±0.21b
30% FM 400 RPM	12.1±0.69b	2.1±0.08b	2.5±0.16b
Non-extruded	9.1±1.25b	1.6±0.18c	2.1±0.22b
24 h			
Blank	6.18±0.15d	1.64±0.12d	1.59±0.20d
FOS	48.21±2.65a	6.13±0.35a	15.18±2.32a
20% FM 200 RPM	14.7±0.58b	2.4±0.12c	2.9±0.20b
20% FM 400 RPM	15.1±1.04b	2.5±0.19bc	2.8±0.39b
25% FM 200 RPM	15.01±0.11b	2.6±0.08b	3.0±0.22b
25% FM 400 RPM	14.6±0.23b	2.2±0.17c	2.9±0.35b
30% FM 200 RPM	13.5±0.82b	2.5±0.04bc	3.1±0.17b
30% FM 400 RPM	13.9±0.74b	2.6±0.21b	2.9±0.11b
Non-extruded	12.2±0.14c	2.1±0.82c	2.3±0.71c

Table 3.2 continued

48 h			
Blank	10.2±1.79d	2.4±0.47d	2.3±0.35d
FOS	55.9±2.79a	6.9±0.37a	15.6±1.10a
20% FM 200 RPM	18.6±0.72bc	3.2±0.12bc	3.4±0.16b
20% FM 400 RPM	20.1±1.83b	3.5±0.30b	3.7±0.24b
25% FM, 200 RPM	19.7±2.11b	3.4±0.15bc	3.5±0.18b
25% FM 400 RPM	18.7±2.19bc	3.6±0.58b	3.8±0.54b
30% FM 200 RPM	15.6±1.84bc	3.0±0.14bcd	3.2±0.22bc
30% FM 400 RPM	17.6±2.45bc	3.5±0.09b	3.3±0.18b
Non-extruded	13.9±0.86cd	2.7±10cd	2.8±0.21c

Values of mean ± SD that have different letters in a column do not differ (Tukey's multiple comparisons, $P < 0.05$). The blank is a negative control without fiber. FM = feed moisture content, RPM = rotations per minute of the extruder screw.

Table 3.3. Acetate, propionate, and butyrate for Donor 2 across the 48-h fermentation period.

Donor 2	Acetate	Propionate	Butyrate
0 h	2.1±0.15	1.2±0.05	0.4±0.01
6 h			
Blank	6.2±0.09c	2.0±0.04c	0.9±0.05c
FOS	28.0±2.59a	16.5±1.61a	6.7±0.41a
20% FM 200 RPM	8.1±0.26bc	2.9±0.05b	0.8±0.60c
20% FM 400 RPM	9.4±0.93bc	3.2±0.38b	1.2±0.15b
25% FM 200 RPM	9.1±1.08bc	3.2±0.60b	1.3±0.17b
25% FM 400 RPM	8.9±0.52bc	3.0±0.15b	1.1±0.17b
30% FM 200 RPM	8.7±0.25bc	3.1±0.05b	1.2±0.01b
30% FM 400 RPM	10.0±1.24b	3.5±0.25b	1.2±0.06b
Non-extruded	7.5±1.14bc	2.5±0.26b	1.0±0.56b
12 h			
Blank	9.1±0.69c	1.8±1.50c	1.1±0.05b
FOS	34.1±3.21a	17.6±0.18a	7.1±0.04a
20% FM 200 RPM	12.2±1.35bc	3.7±0.40bc	1.4±0.14b
20% FM 400 RPM	13.2±0.15b	4.1±0.05b	1.5±0.02b
25% FM 200 RPM	10.2±1.2bc	3.2±0.21bc	1.2±0.05b
25% FM 400 RPM	11.9±0.34bc	3.6±0.13bc	1.3±0.06b
30% FM 200 RPM	10.0±1.15bc	3.4±0.21bc	1.3±0.11b
30% FM 400 RPM	12.5±0.70bc	4.1±0.16b	1.4±0.06b
Non-extruded	9.5±0.18bc	2.8±0.08bc	1.1±0.04b
24 h			
Blank	9.4±0.79d	2.7±0.18c	1.2±0.06c
FOS	37.4±4.10a	17.5±1.59a	7.1±0.61a
20% FM 200 RPM	16.4±1.40b	4.5±0.38b	1.7±0.11b
20% FM 400 RPM	16.4±3.16b	4.4±0.21b	1.7±0.22b
25% FM 200 RPM	14.1±0.46b	3.7±0.23bc	1.4±0.04bc
25% FM 400 RPM	14.7±1.18b	4.0±0.21bc	1.6±0.10bc
30% FM 200 RPM	12.8±0.25c	3.6±0.10bc	1.4±0.04bc
30% FM 400 RPM	14.8±1.56b	3.9±0.14bc	1.4±0.08bc
Non-extruded	13.9±0.53b	3.6±0.15bc	1.5±0.07bc
48 h			
Blank	10.6±2.0d	2.8±0.53d	1.2±0.25d
FOS	48.7±3.38a	20.2±1.04a	8.5±0.42a
20% FM 200 RPM	17.9±1.44b	4.7±0.37b	1.9±0.14bc
20% FM 400 RPM	20.0±1.78b	5.2±0.46b	2.1±0.21b
25% FM 200 RPM	18.1±1.23b	4.9±0.69b	1.9±0.15bc
25% FM 400 RPM	18.4±0.43b	4.7±0.20b	1.9±0.11bc
30% FM 200 RPM	18.3±0.06b	5.0±0.06b	2.1±0.07b
30% FM 400 RPM	18.2±1.16b	5.1±0.24b	1.9±0.08bc
Non-extruded	14.2±1.66c	3.7±0.43c	1.6±0.17c

Values of mean ± SD that have different letters in a column do not differ (Tukey's multiple comparisons, $P < 0.05$). The blank is a negative control without fiber. FM = feed moisture content, RPM = rotations per minute of the extruder screw.

Table 3.4. Acetate, propionate, and butyrate for Donor 3 across the 48-h fermentation period.

Donor 3	Acetate	Propionate	Butyrate
0 h	1.1±0.31	0.5±0.08	0.3±0.05
6 h			
Blank	7.2±0.58c	2.0±0.19c	1.0±0.09c
FOS	37.9±2.92a	7.2±0.60a	8.1±0.73a
20% FM 200 RPM	11.7±0.56b	3.1±0.33bc	1.5±0.18bc
20% FM 400 RPM	13.7±1.17b	3.6±0.09b	1.7±0.02b
25% FM 200 RPM	10.1±1.82bc	3.2±0.47bc	1.4±0.01bc
25% FM, 400 RPM	11.1±0.82bc	2.9±0.12bc	1.5±0.06bc
30% FM 200 RPM	9.8±1.08bc	2.6±0.20bc	1.3±0.12bc
30% FM, 400 RPM	11.9±1.39b	3.4±0.35bc	1.6±0.12bc
Non-extruded	7.5±0.34c	2.0±0.11c	1.0±0.08c
12 h			
Blank	10.2±0.17d	2.6±0.02d	1.3±0.04d
FOS	46.0±1.57a	9.4±0.25a	10.3±0.12a
20% FM 200 RPM	14.4±0.73bcd	3.8±0.07bc	1.9±0.09bcd
20% FM 400 RPM	17.2±2.26b	4.3±0.28b	2.4±0.17b
25% FM 200 RPM	12.9±0.15bcd	3.4±0.36bcd	1.8±0.16cd
25% FM, 400 RPM	13.7±0.59bcd	3.5±0.13bcd	1.9±0.08bcd
30% FM 200 RPM	14.0±1.29bcd	3.4±0.14bcd	1.7±0.21cd
30% FM, 400 RPM	14.9±0.42bc	4.0±0.12bc	2.1±0.06bc
Non-extruded	12.2±3.14cd	3.1±0.70cd	1.6±0.32cd
24 h			
Blank	10.0±3.03d	2.4±0.71d	1.3±0.39d
FOS	41.0±0.87a	8.9±0.29a	9.6±0.21a
20% FM 200 RPM	17.7±1.08bc	3.9±0.19bc	2.2±0.10bc
20% FM 400 RPM	19.4±0.88b	4.5±0.19b	2.5±0.09b
25% FM, 200 RPM	17.3±0.95bc	3.4±0.26bcd	2.0±0.13bc
25% FM, 400 RPM	18.5±2.06bc	4.0±0.35bc	2.3±0.23bc
30% FM 200 RPM	19.3±1.70b	4.4±0.38bc	2.5±0.10b
30% FM, 400 RPM	16.1±0.55bc	3.8±0.21bc	2.1±0.14bc
Non-extruded	14.1±2.67cd	3.3±0.67cd	1.8±0.38cd
48 h			
Blank	12.0±1.02c	2.8±0.26b	1.6±0.14e
FOS	47.3±8.82a	10.8±2.06a	11.5±2.13a
20% FM 200 RPM	21.6±1.96bc	4.7±0.42b	2.8±0.10b
20% FM 400 RPM	24.3±0.60b	5.2±0.23b	3.0±0.17b
25% FM, 200 RPM	18.6±1.33bc	4.2±0.25b	2.6±0.15bc
25% FM, 400 RPM	20.8±2.11bc	4.6±0.42b	2.9±0.13b
30% FM 200 RPM	17.0±0.91bc	3.8±0.28b	2.3±0.13cd
30% FM, 400 RPM	18.1±0.77bc	4.5±0.14b	2.6±0.09bc
Non-extruded	17.2±0.44bc	3.8±0.26b	2.2±0.14d

Values of mean ± SD that have different letters in a column do not differ (Tukey's multiple comparisons, $P < 0.05$). The blank is a negative control without fiber. FM = feed moisture content, RPM = rotations per minute of the extruder screw.

Table 3.5. Monosaccharide composition (%) and arabinose:xylose ratio of the brans after upper gastrointestinal simulated digestion.

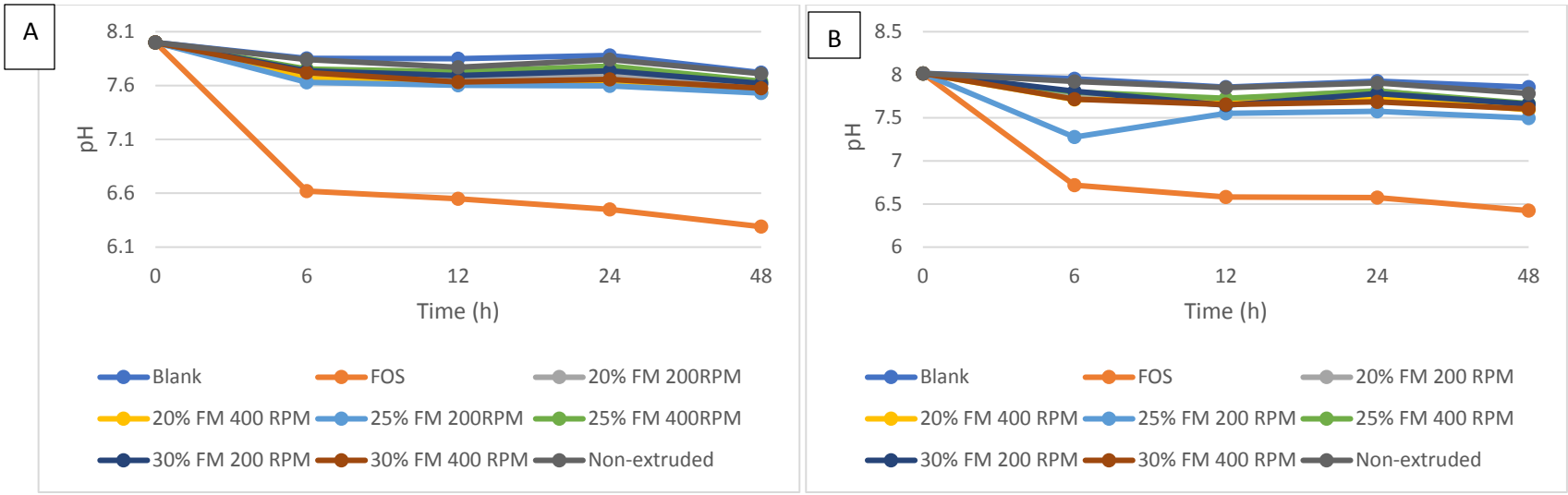
Codes	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Arabinose: Xylose
20% FM 200 RPM	0.54±0.17a	0.28±0.08a	17.41±2.28ab	28.71±3.40ab	1.30±0.22b	9.96±0.89ab	41.80±6.78ab	0.61a
20% FM 400 RPM	0.82±.24a	0.42±0.09a	17.76±0.18ab	27.80±1.38b	1.68±0.54ab	10.46±0.26a	39.81±1.20ab	0.64a
25% FM 200 RPM	0.82±0.46a	0.32±0.12a	16.93±1.94ab	27.51±2.37b	1.61±0.50ab	9.42±0.52b	43.40±3.76a	0.62a
25% FM 400 RPM	0.64±0.30a	0.42±0.08a	18.81±0.31ab	32.24±2.60ab	1.23±0.34b	9.55±0.64ab	37.11±1.60b	0.59a
30% FM 200 RPM	0.52±0.11a	0.26±0.03a	16.92±1.22b	26.77±2.65b	1.46±0.40ab	10.32±0.55ab	43.38±3.90a	0.63a
30% FM 400 RPM	0.68±0.23a	0.32±0.09a	18.37±0.80ab	28.12±1.94ab	2.01±0.44a	9.84±0.40ab	40.66±2.32ab	0.65a
NE	0.70±0.30a	0.41±0.16a	19.14±0.06a	30.11±2.23a	1.09±0.18b	8.08±0.01c	37.42±0.97b	0.64a

Values of mean ± SD that have different letters in a column do not differ (Tukey's multiple comparisons, $P < 0.05$). FM = feed moisture content, RPM = rotations per minute of the extruder screw.

Table 3.6. Simpson index for richness of the donors after 48-h fermentation period.

	Mean ± SD
Donor 1	
Initial	0.94±0.002a
Blank 48 h	0.94±0.01a
FOS	0.95±0.002a
20% FM 200 RPM	0.93±0.001a
20% FM 400 RPM	0.93±0.01a
25% FM 200 RPM	0.93±0.01a
25% FM 400 RPM	0.94±0.001a
30% FM 200 RPM	0.93±0.01a
30% FM 400 RPM	0.93±0.01a
Non-extruded	0.93±0.01a
Donor 2	
Initial	0.96±0.001a
Blank 48 h	0.95±0.001a
FOS	0.95±0.002a
20% FM 200 RPM	0.96±0.001a
20% FM 400 RPM	0.96±0.001a
25% FM 200 RPM	0.96±0.001a
25% FM 400 RPM	0.96±0.00a
30% FM 200 RPM	0.96±0.001a
30% FM 400 RPM	0.96±0.01a
Non-extruded	0.96±0.001a
Donor 3	
Initial	0.96±0.001a
Blank 48 h	0.93±0.001a
FOS	0.93±0.001a
20% FM 200 RPM	0.93±0.001a
20% FM 400 RPM	0.92±0.01a
25% FM 200 RPM	0.93±0.001a
25% FM 400 RPM	0.94±0.002a
30% FM 200 RPM	0.93±0.001a
30% FM 400 RPM	0.92±0.02a
Non-extruded	0.93±0.002a

Statistically significant differences calculated using Tukey's multiple comparison test, P <0 .05



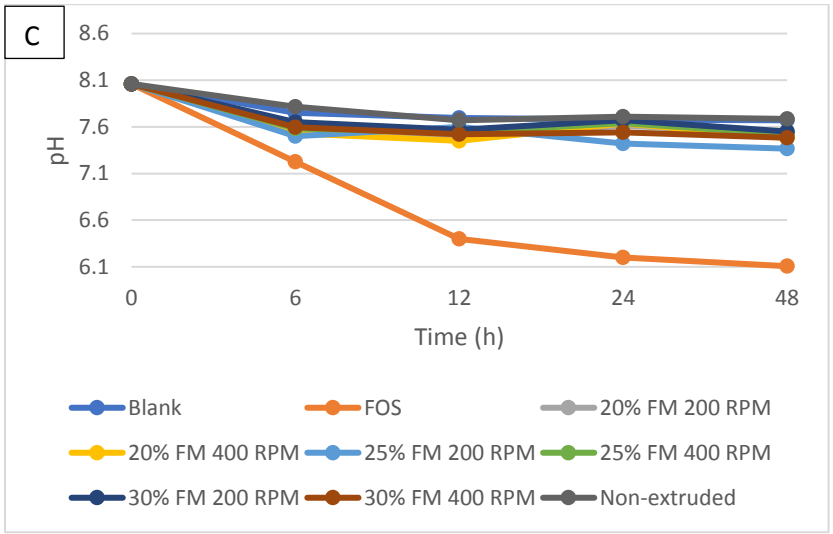


Figure 3.1. pH for Donor 1 (A), Donor 2 (B), and Donor (C) across the 48-h fermentation period. Feed moisture (FM) means moisture content at rotations per minute (RPM) under which extrusion was done, the blanks are negative control without fiber. FOS is the positive control.

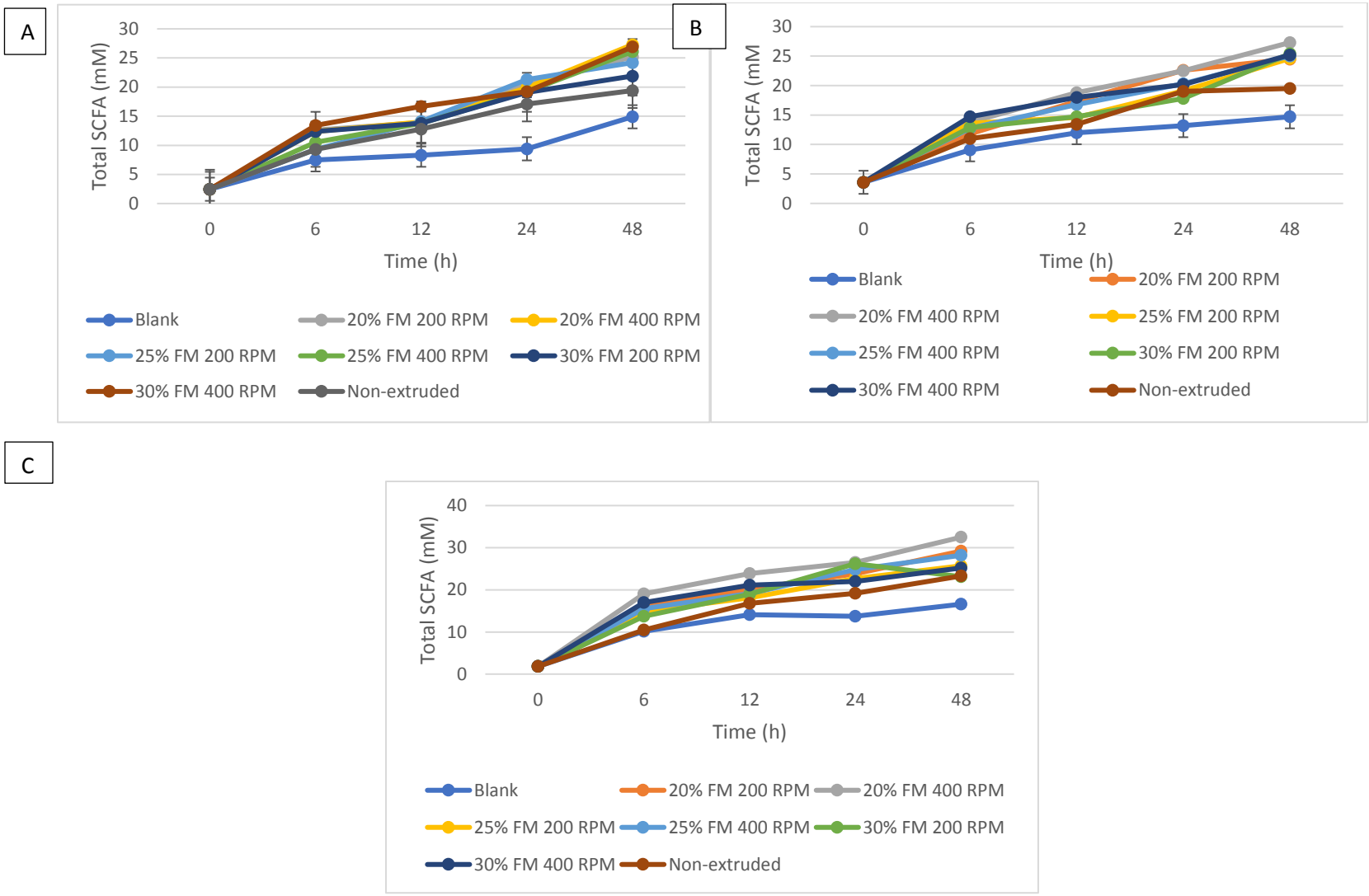


Figure 3.2. Total short chain fatty acids produced by Donors 1 (A), 2 (B), and 3 (C) across the 48-h fermentation period. The feed moisture (FM) and rotations per minute (RPM) describe extrusion conditions, the blank is the negative control without fiber.

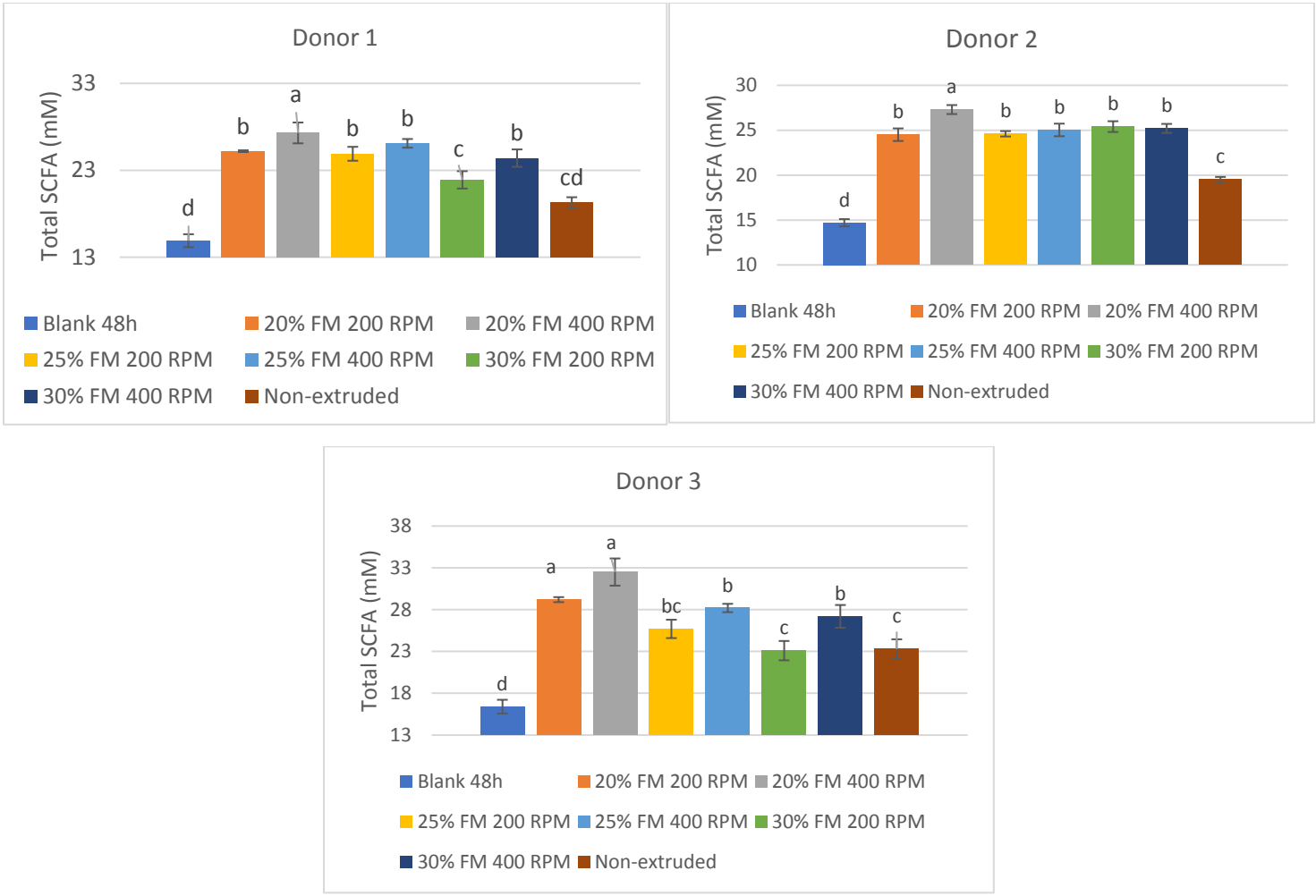


Figure 3.3. Total short chain fatty acids produced by Donors 1, 2 and 3 at 48-h fermentation time point. The feed moisture (FM) and rotations per minute (RPM) describe extrusion conditions, the blank is the negative control without fiber. Means with different letters differ using Tukey's multiple comparison test, $P < 0.05$.

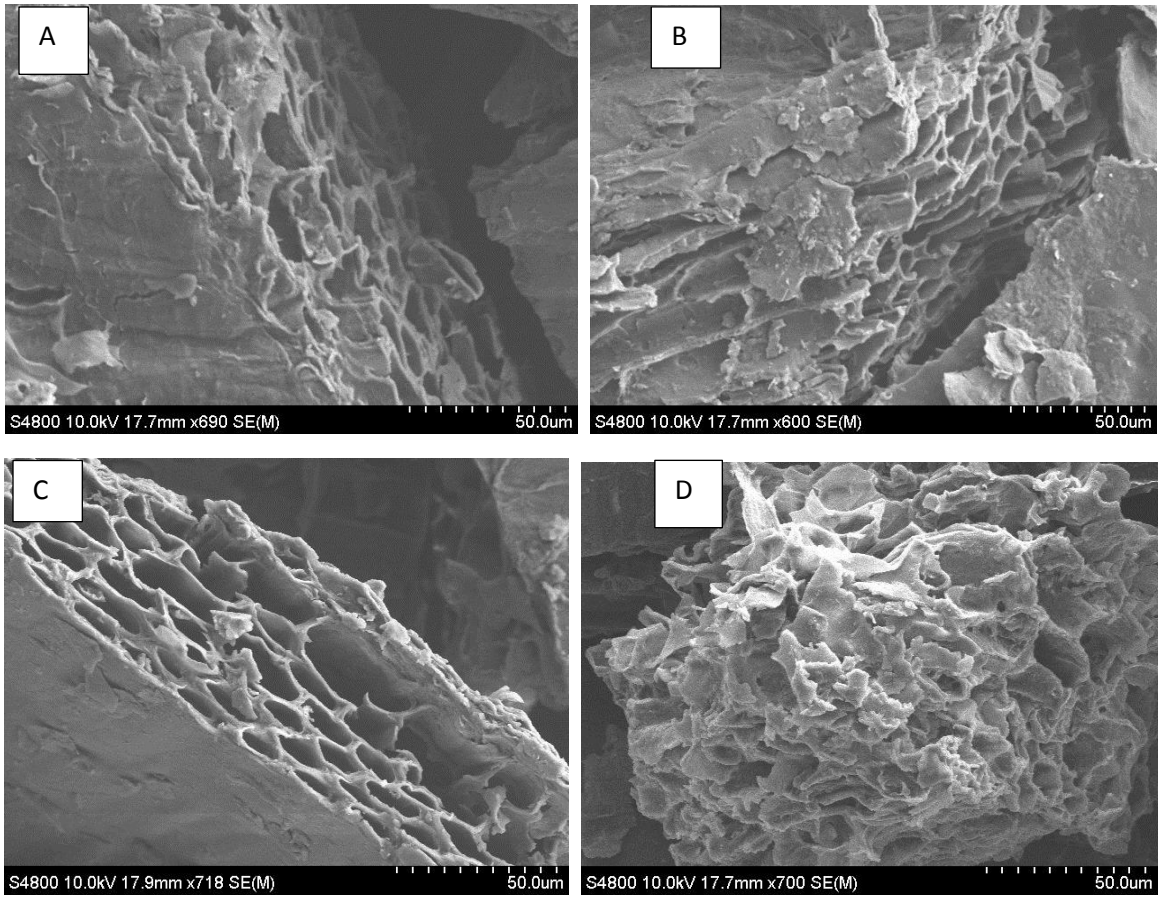
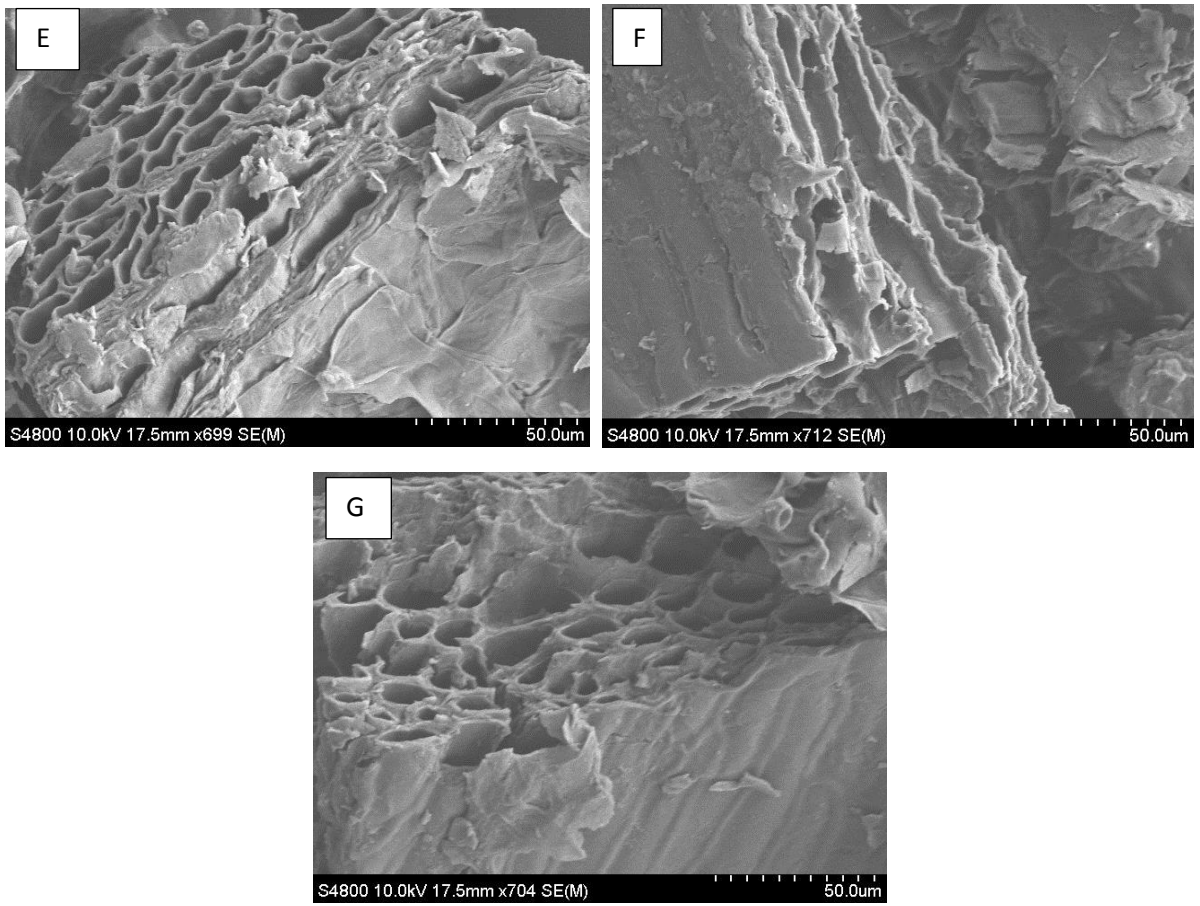


Figure 3.4. Scanning electron micrographs of the brans after upper gastrointestinal digestion. The pores left are the regions presumably occupied by starch and proteins that were removed during upper gastrointestinal digestion. The various treatments are A) 20% FM 200 RPM, B) 20% FM 400 RPM, C) 25% FM 200RPM, D) 25% FM 400 RPM, E) 30% FM 200% RPM, F) 30% FM 200 RPM, and G) non-extruded control.

Figure 3.4. continued



Scanning electron micrographs of the brans after upper gastrointestinal digestion. The pores left are the regions presumably occupied by starch and proteins that were removed during upper gastrointestinal digestion (E) 30% FM 200% RPM, (F) 30% FM 200 RPM, and (G) non-extruded control.

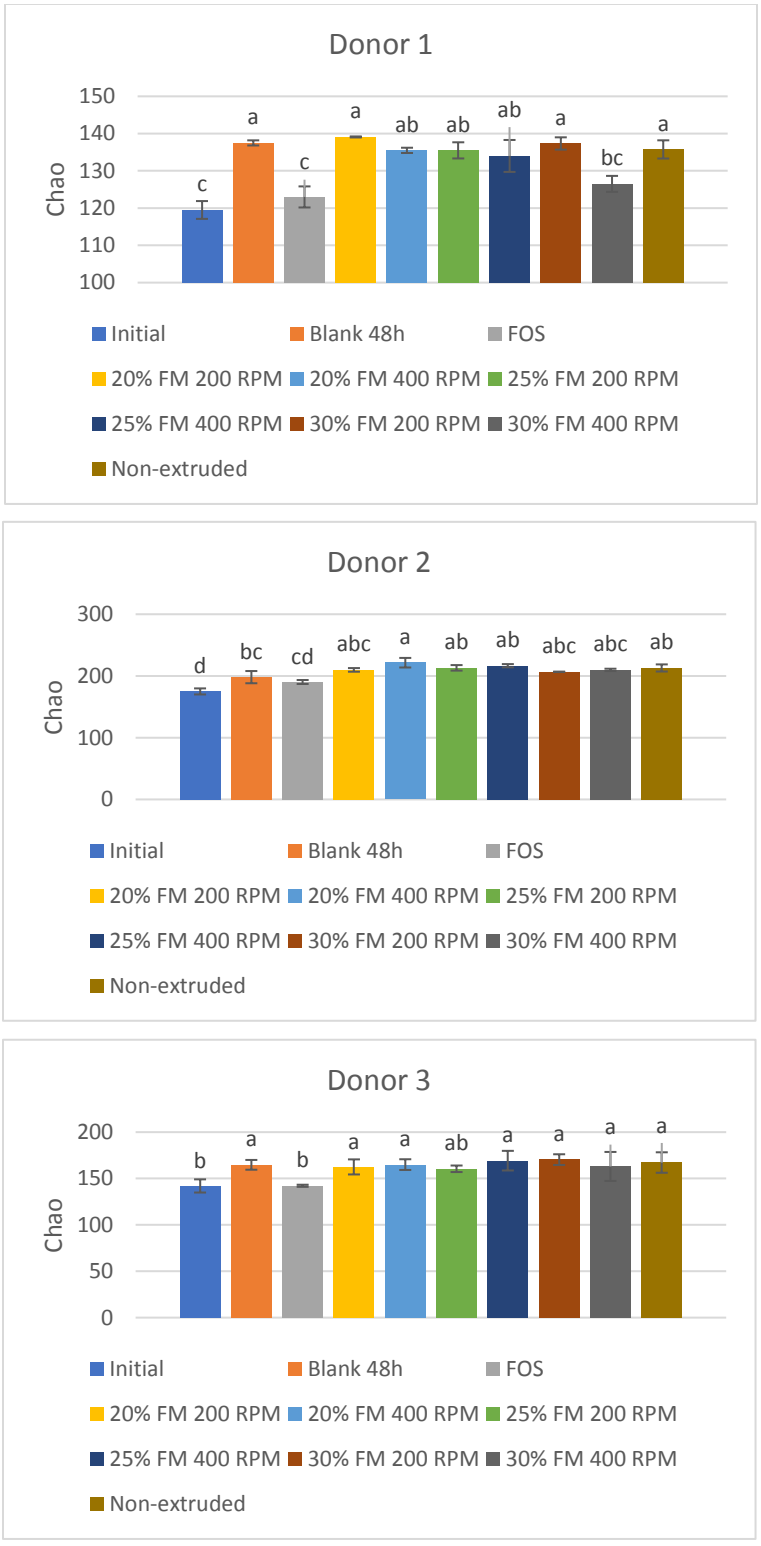


Figure 3.5. Chao alpha diversity score, a measure of richness, calculated based on sequencing of 16S rRNA gene. Statistically significant differences calculated using Tukey's multiple comparison test, $P < 0.05$.

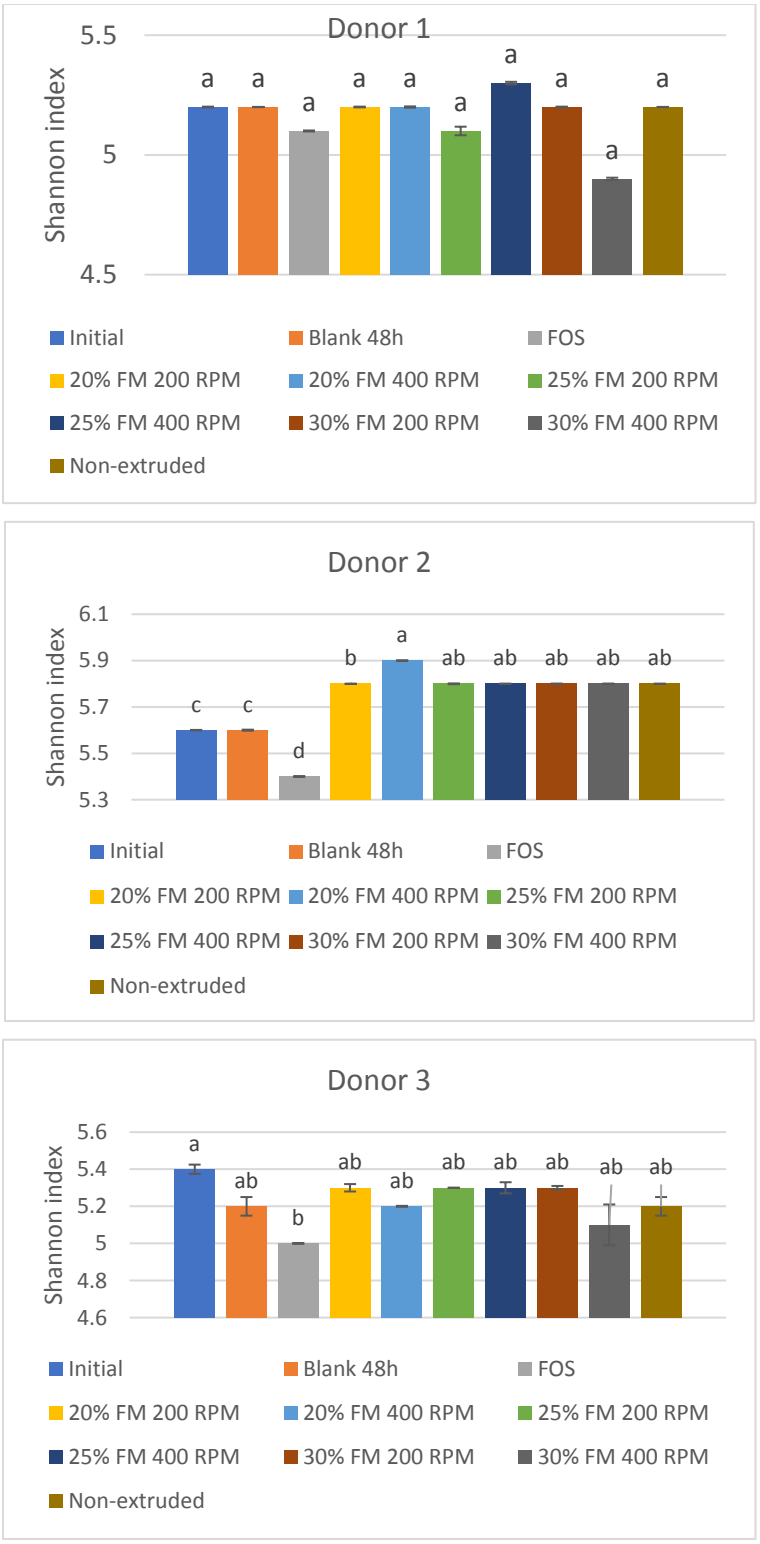


Figure 3.6 Shannon alpha diversity index, a measure combining richness and evenness, calculated based on sequencing of 16S rRNA gene. Statistically significant differences calculated using Tukey's multiple comparison test, $P < 0.05$.

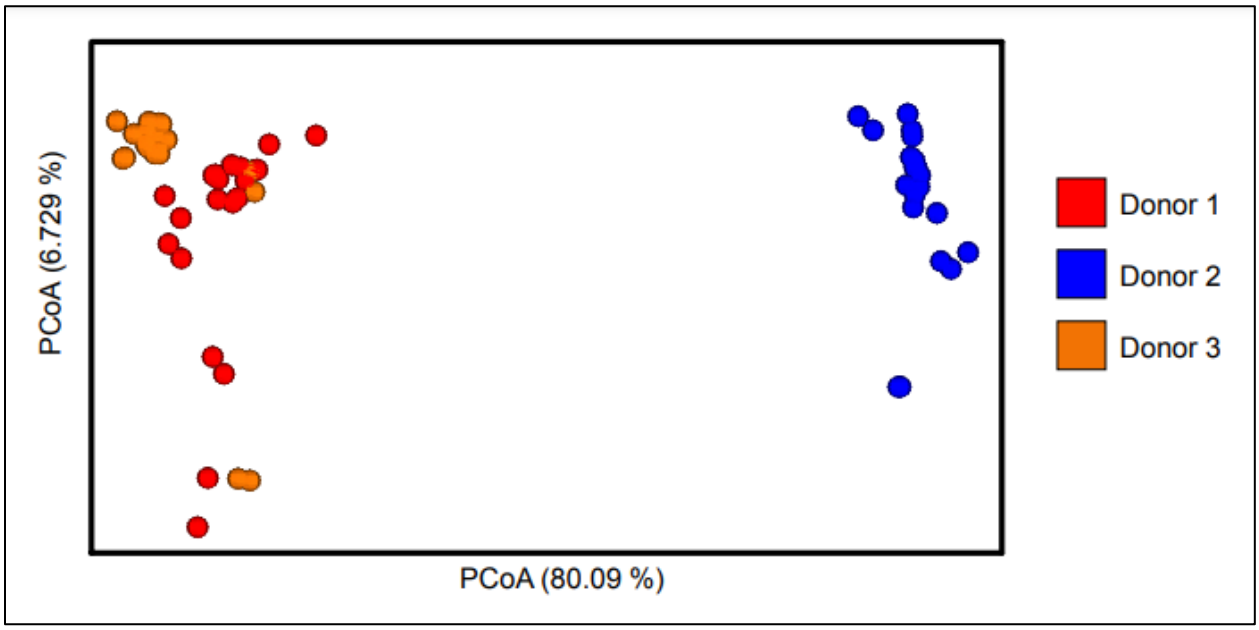


Figure 3.7 Weighted UniFrac beta diversity for microbial communities based on proportions of operation taxonomic units (OTU) at 97% resemblance at 48 h based on donors.

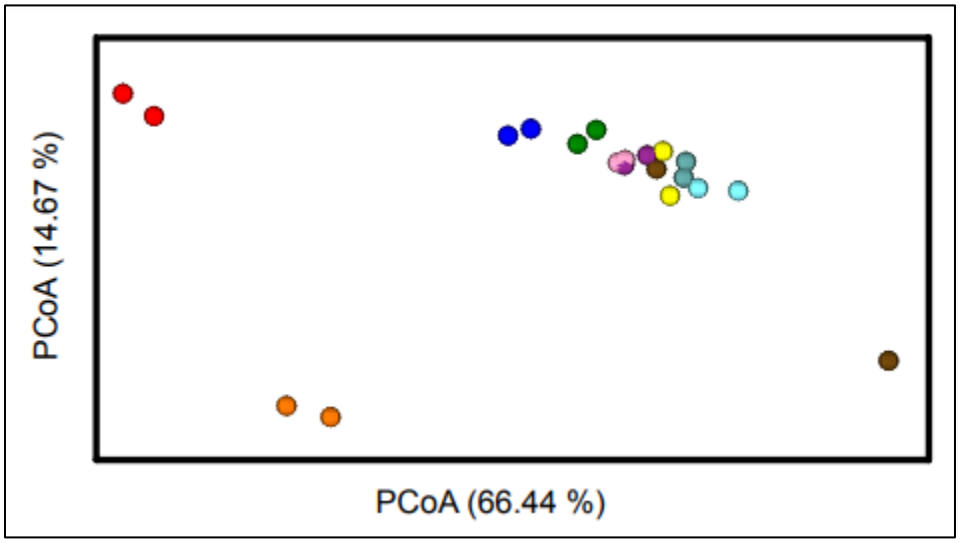


Figure 3.8. Weighted UniFrac beta diversity for microbial communities based on proportions of operation taxonomic units (OTU) at 97% resemblance at 0 and 48 h based on treatments Blank 0 h Blank 48 h FOS 20% FM 200 RPM 20% FM 400 RPM 25% FM 200 RPM 25% FM 400 RPM 30% FM 200 RPM 30% FM 400 RPM Non-extruded for Donor 1 (all treatments are at 48 h).

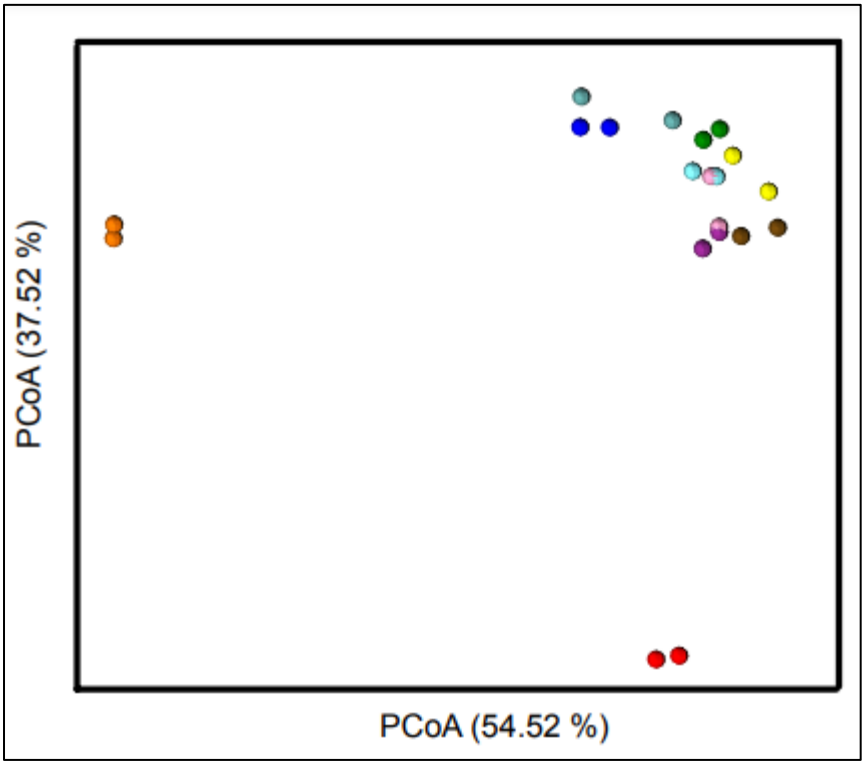


Figure 3.9. Weighted UniFrac beta diversity for microbial communities based on proportions of operation taxonomic units (OTU) at 97% resemblance at 0 and 48 h based on treatments Blank 0 h Blank 48 h FOS 20% FM 200 RPM 20% FM 400 RPM 25% FM 200 RPM 25% FM 400 RPM 30% FM 200 RPM 30% FM 400 RPM Non-extruded for Donor 2 (all treatments are at 48 h).

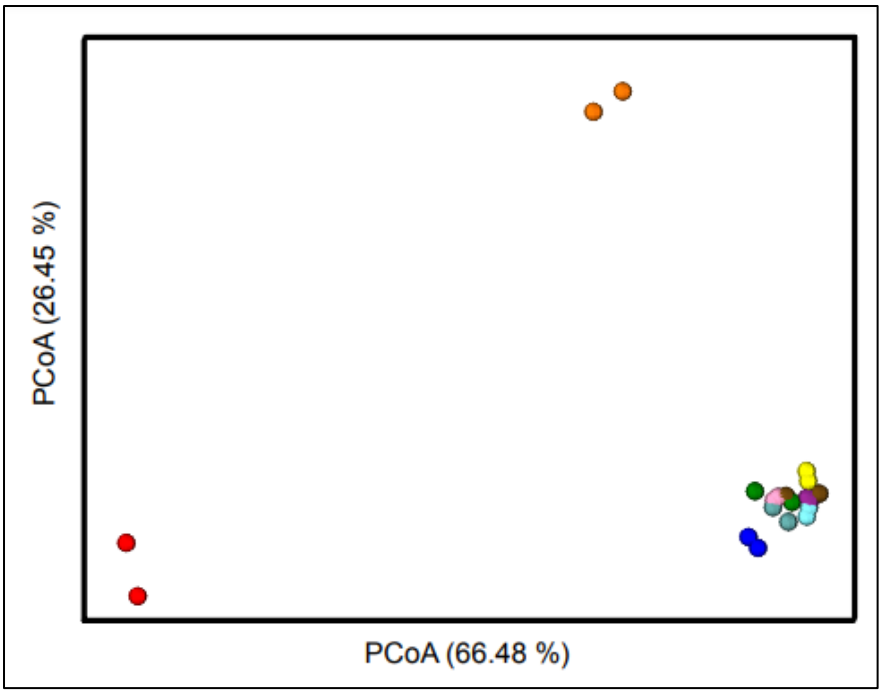


Figure 3.10. Weighted UniFrac for microbial communities based on proportions of operation taxonomic unit (OTU) at 97% resemblance at 0 and 48h based on treatments Blank 0h Blank 48h FOS 20% FM 200 RPM 20% FM 400 RPM 25% FM 200 RPM 25% FM 400 RPM 30% FM 200 RPM 30% FM 400 RPM Non-extruded for Donor 3 (all treatments are at 48 h).

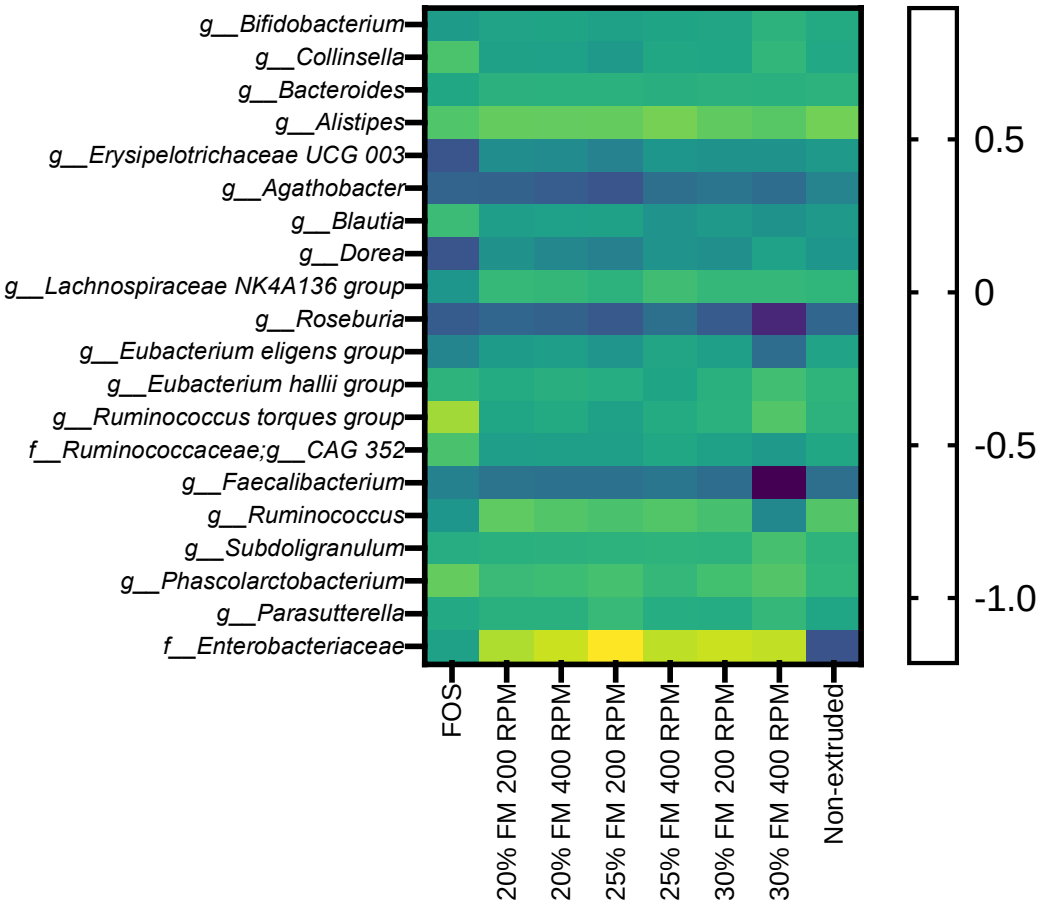


Figure 3.11. Heatmap of the relative abundance of the taxa greater than 1% after 48 h of *in vitro* fermentation of extruded and non-extruded brans for Donor 1. Extrusion was done at different feed moisture (FM) and rotations per minute (RPM). The letters g and f represent genus and family taxonomic units. Data used on the heat map was calculated by dividing the final relative abundance of bacteria by the initial one and then doing $\log_2$ transformation on the data.

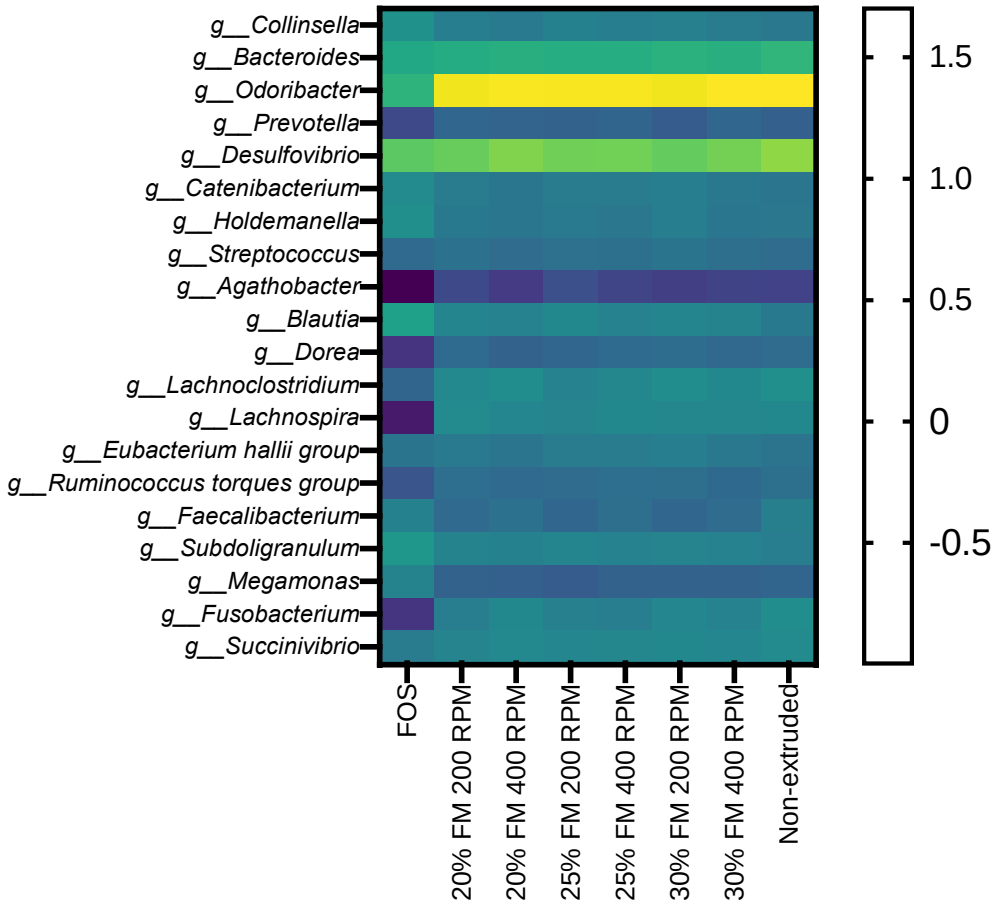


Figure 3.12. Heatmap of the relative abundance of the taxa greater than 1% after 48 h of *in vitro* fermentation of extruded and non-extruded brans for Donor 2. Extrusion was done at different feed moisture (FM) and rotations per minute (RPM). The letters g represents genus taxonomic unit. Data used on the heat map was calculated by dividing the final relative abundance of bacteria by the initial one and then doing $\log_2$ transformation on the data.

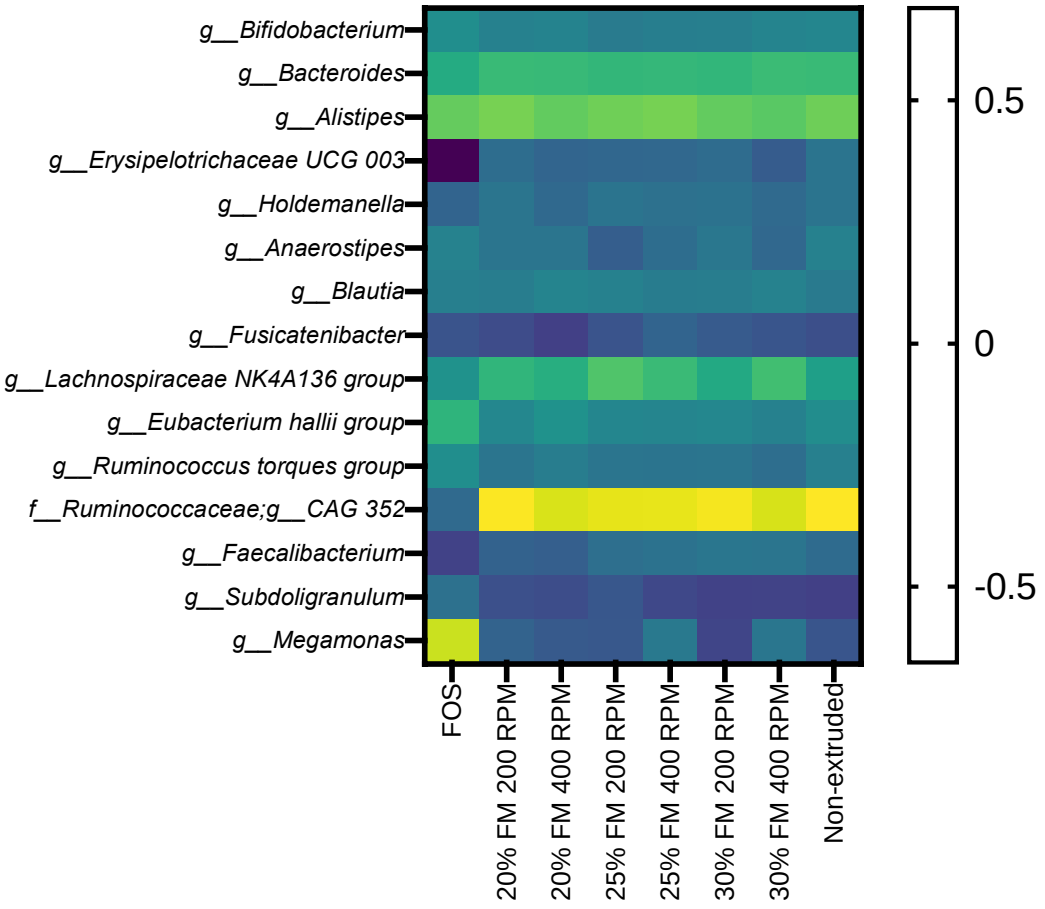


Figure 3.13. Heatmap of the relative abundance of the taxa greater than 1% after 48 h of *in vitro* fermentation of extruded and non-extruded brans for Donor 3. Extrusion was done at different feed moisture (FM) and rotations per minute (RPM). The letters g and f represent genus and family taxonomic units. Data used on the heat map was calculated by dividing the final relative abundance of bacteria by the initial one and then doing $\log_2$ transformation on the data.

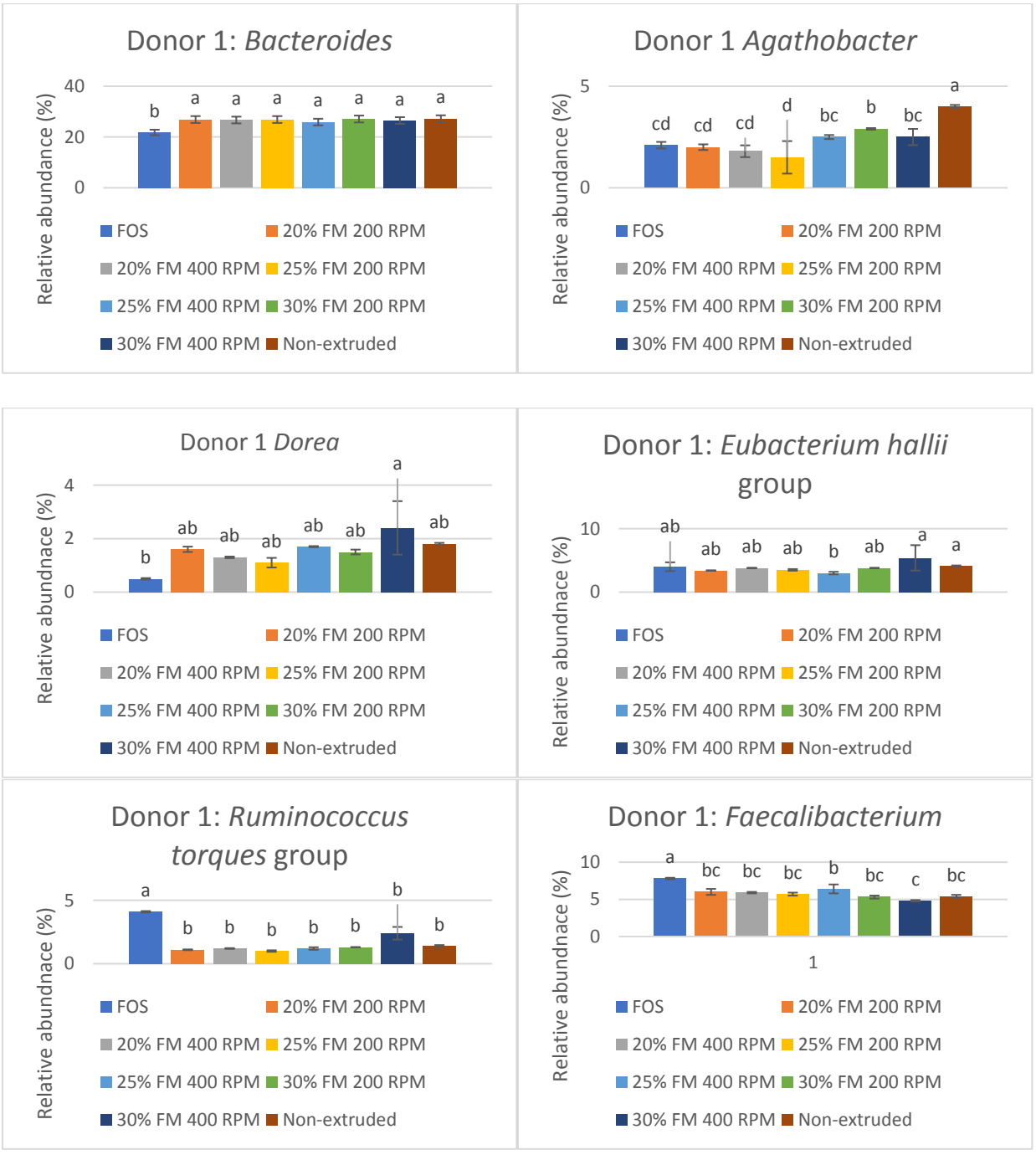
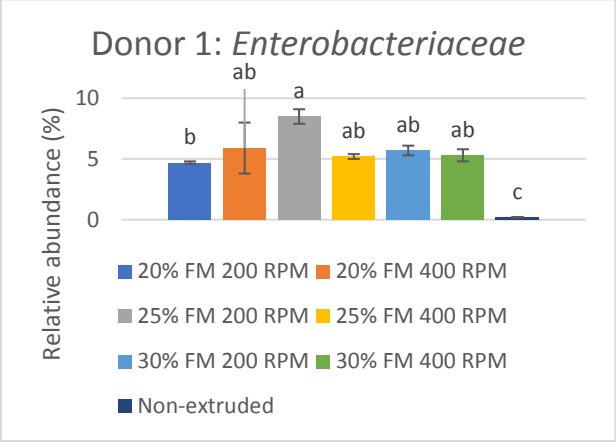


Figure 3.14. Graphs showing the genera that were impacted by the treatments at 48 h fermentation for Donor 1.

Figure 3.14. continued



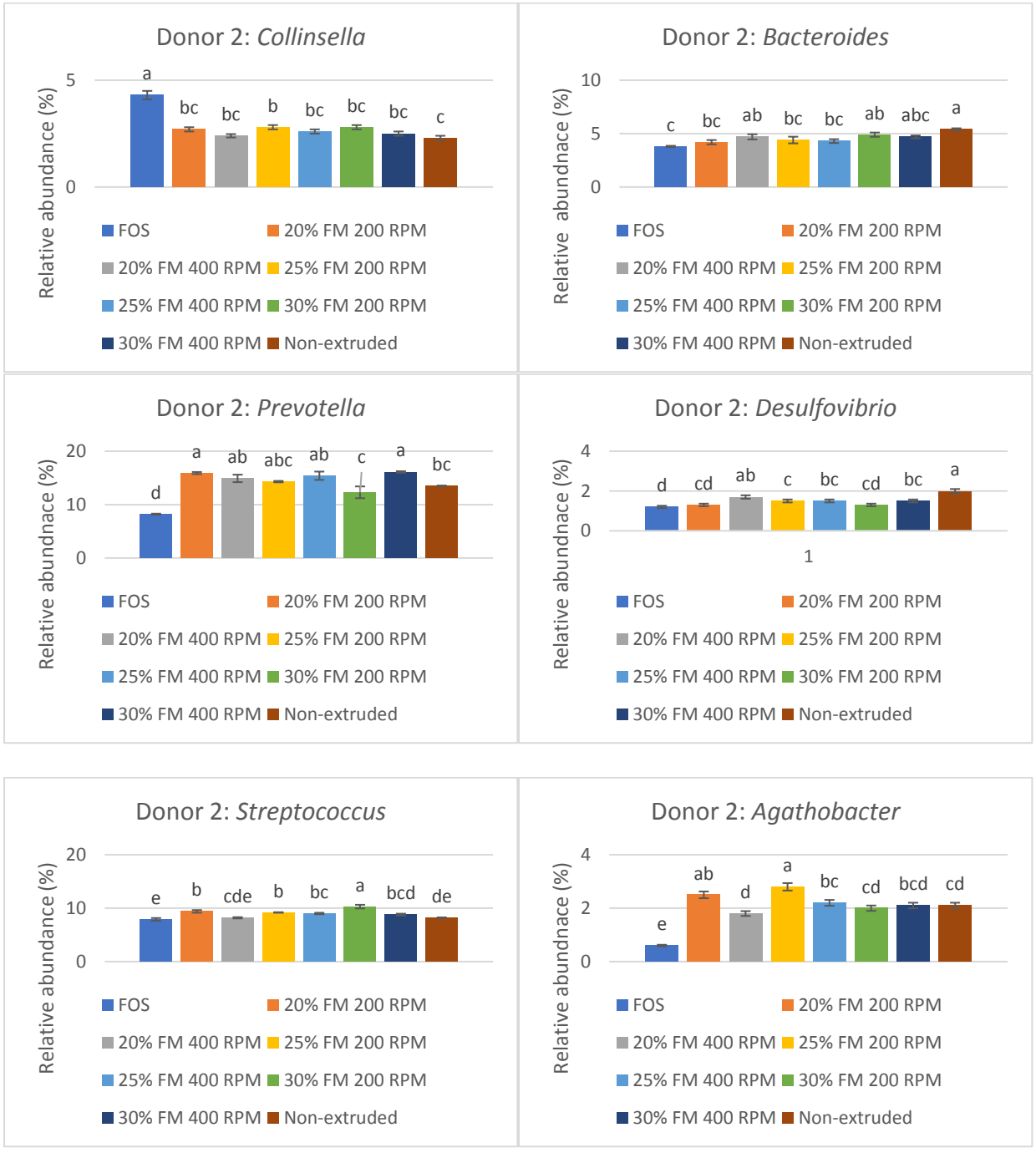
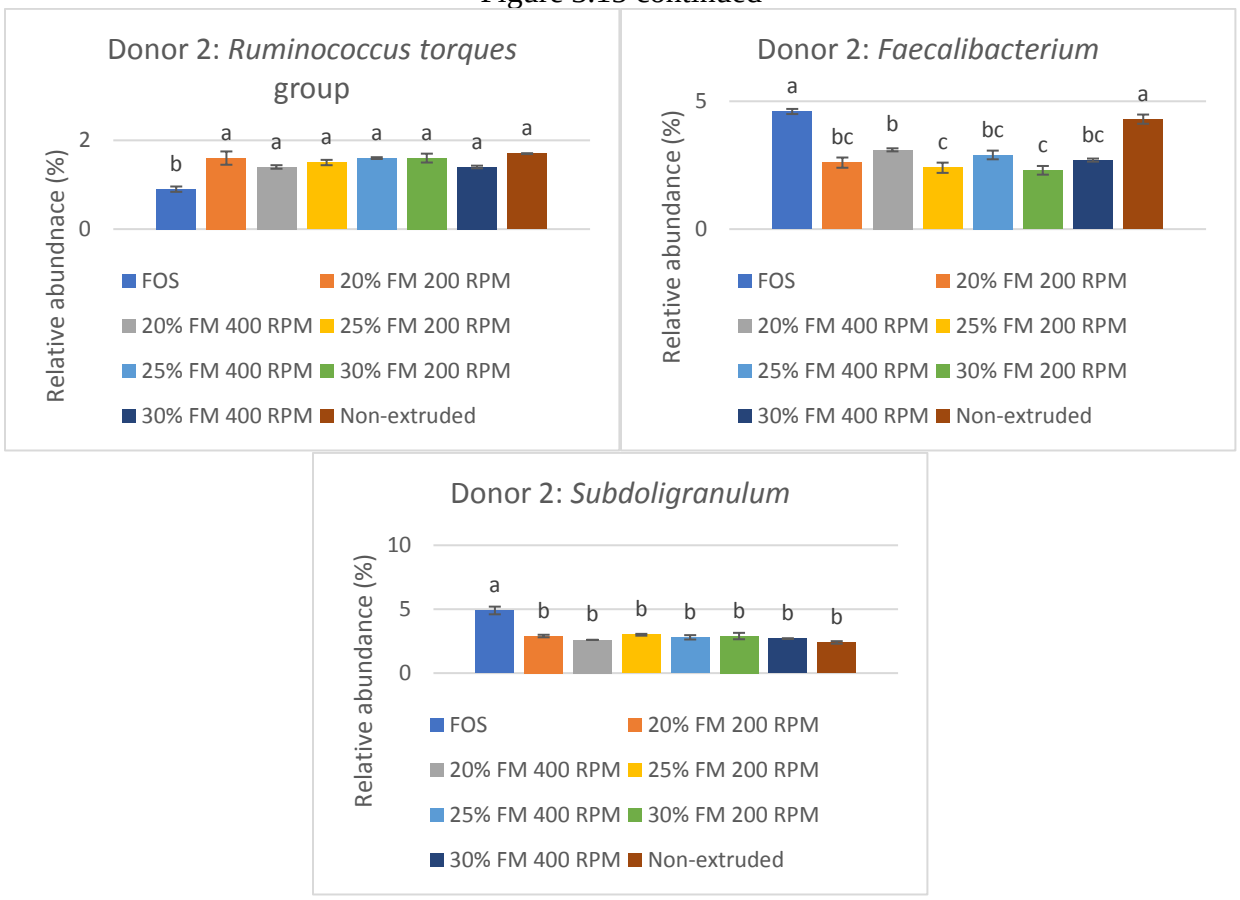


Figure 3.15. Graphs showing the genera that were impacted by the treatments at 48 h fermentation for Donor 2.

Figure 3.15 continued



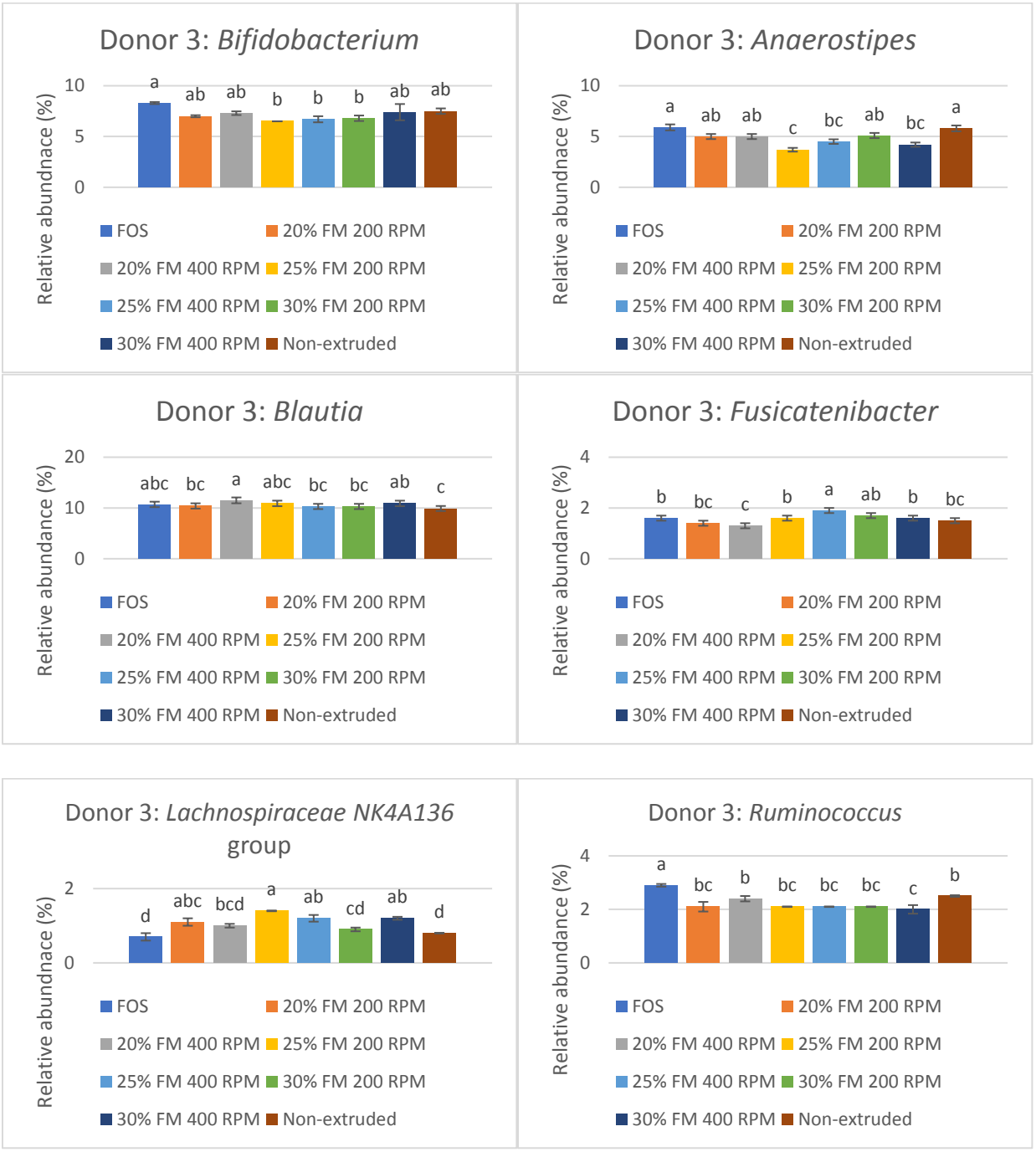
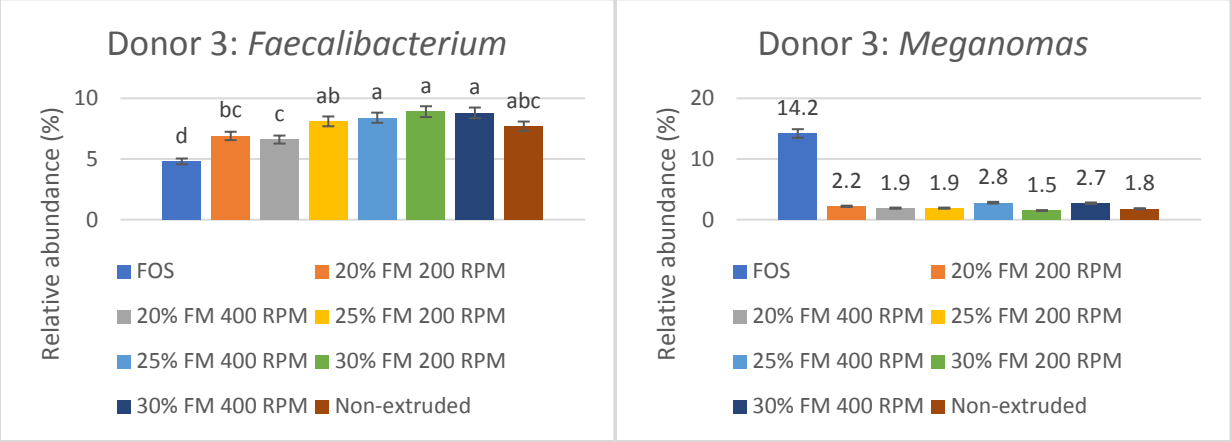


Figure 3.16. Graphs showing the genera that were impacted by the treatments at 48 h fermentation for Donor 3.

Figure 3.16 continued



CHAPTER 4. CONSUMERS' WILLINGNESS TO PAY MORE FOR FOOD-TO-FOOD FORTIFIED INSTANT PRODUCTS IN ELDORET, KENYA WITH NUTRITION INFORMATION AND DEMONSTRATION

4.1. Abstract

People of most developing nations consume cereal-based products that are low in micronutrients such as pro-vitamin A, iron, and zinc that are critical for human health. Solving these deficiencies include diet-based strategies such as fortification of cereal diets with plant-based micronutrient rich sources such as baobab, orange-fleshed sweet potato, moringa, and amaranth. In this study, we assessed the acceptance and willingness to pay (WTP) of instant maize/sorghum porridge flours with food-to-food fortification from locally-sourced micronutrient plant sources are rich in pro-vitamin A, iron, and zinc when consumers were given different degrees of nutrition information and explanation and demonstration of how to reconstitute the instant flours. Firstly, 378 participants evaluated thin (*ugi*) and thick (*ugali*) porridges made from two flour blends varying in whole grain maize and sorghum contents. Secondly, those who met who bid at least 0.5 USD participated in an experimental auction where they were asked for their WTP for the products after given the following information: product name and nutrient composition, procedure for reconstituting the instant flours, and a demonstration how to reconstitute the instant flour. Participants preferred the fortified thick porridge higher in maize content than fortified thin porridge prepared from the same blend. Contrarily, thin porridge made from fortified flour with higher sorghum content was ranked highly than for the corresponding thick porridge. Participants were willing to pay more for instant fortified products higher in sorghum when given product name and nutrient composition, even without a practical demonstration of how to reconstitute the flours. For the instant product higher in maize, consumers needed demonstration of how to reconstitute the instant flour for them pay a higher premium. These findings suggest that food-to-food fortified instant porridge flours have the potential to be adopted and can be used as a vehicle to deliver micronutrients to these populations.

4.2. Introduction

One of the agendas of the sustainable development goals of the United Nations is to reduce hunger levels and improve nutrition of people in the world (United Nations, 2015). Globally, it is estimated that 2 billion people are food insecure and unable to meet their nutrition needs (FAO/IFAD/UNICEF/WFP/WHO, 2020). Critical micronutrient deficiencies of vitamin A, iron, zinc, folate, and iodine are still witnessed in sub-Saharan countries like South Africa, Nigeria, Ethiopia, and Kenya (Harika et al., 2017; Muthayya et al., 2013). In Kenya specifically, vitamin A, iron, and zinc deficiencies are high at 84.4, 34.5, and 35.8%, respectively (Muthayya et al., 2013).

Worldwide, several strategies have been proposed to combat micronutrient deficiencies including biofortification (Nestel et al., 2006; Nkhata et al., 2020), food fortification (Das et al., 2013), dietary diversification (Gibson and Hotz, 2001; Nair et al., 2016), and food supplementation (Harrison et al., 2010). Of these, food fortification which is addition of nutrient sources to foods with an aim of boosting micronutrient levels is common and achieved by incorporation of micronutrient-rich synthetic powders in processed foods (De Groote et al., 2018), or through plant sources rich in micronutrients that are used as fortificants in staple grain and tuber products, \referred to as "food-to-food fortification" (Kruger et al., 2020). Some plant sources with potential to be used in food fortification include moringa (*Moringa oleifera*) which is rich in iron, zinc, calcium, magnesium, potassium, and β-carotene (Glover-Amengor et al., 2017; Teixeira et al., 2014); orange-fleshed sweet potato, rich in carotenoids (Alam et al., 2016); and baobab, a good source of calcium, iron, potassium, magnesium, and zinc (Chadare et al., 2008; Braca et al., 2018). In general, fortification has been useful in tackling multiple micronutrient deficiencies (Adams et al., 2017; Awasthi et al., 2020; Ara et al., 2019). Yet, it does not reach everyone, particularly more vulnerable nutritionally-deficient populations, and ways to get low-cost desirable-to-eat fortified products that more consumers will buy is needed.

Most people of the countries in sub-Saharan African consume diets high in starchy staple foods that are low in micronutrients, likely predisposing such populations to micronutrient deficiencies. This is exacerbated for those consuming cereals as most cereal foods consumed, either prepared in the home or purchased as products, are refined are not in whole grain form, so that micronutrients are lost. To improve nutrition value of cereal-based products desirable for

consumers, the Feed the Future Food Processing and Post-harvest Handling Innovation Lab (FPIL) (USAID), led by Purdue University, introduced small-scale, low-cost extrusion technology for developing stabilized food-to-food fortified instant/pre-gelatinized whole grain porridge flours, that supply pro-vitamin A, iron, and zinc (FPIL, 2019). Goals of the project were that the products be convenient and with good shelf life and overall acceptability to garner market demand for nutritious products in the target countries of the project, Kenya and Senegal. Previous reports from the group have some shown that extrusion technology can be used to produce instant flour products with minimal degradation of pro-vitamin A carotenoids (N'Diaye et al., 2020; Ortiz et al., 2018) and consumer acceptance sensory tests have shown that the food-to-food fortified instant porridge flour products or just the plain instant porridge flours are well accepted by consumers (De Groote et al., 2018; Moussa et al., 2011).

In addition to sensory attributes, success of the food-to-food fortified instant porridge flour products depends on market pricing for small- to medium-size processing enterprises to be profitable. There are several studies that have tried to determine willingness to pay (WTP) for food products with added attributes, such yogurt with cholesterol-lowering potential in Paris (Europe) (Marette et al., 2010), organic foods in Greece (Europe) (Krystallis and Chryssohoidis, 2005), and whether provision of nutrition information influences the WTP for food products (My et al., 2018; De Groote et al., 2018). In developing nations of Africa, there have been experiments on consumers' WTP for instant fortified pearl millet flours in Senegal (De Groote et al., 2018), maize meal (De Groote et al., 2011), and maize nutritional quality (De Groote and Kimenju, 2008; De Groote et al., 2014) in Kenya, underutilized vegetables in South Africa (Senyolo et al., 2014), and foods free of antibiotics in Ghana (Ragasa et al., 2019).

Provision of nutrition information to consumers can positively impact purchase of food products (My et al., 2018, Chege et al., 2019; De Groote et al., 2018). My et al. (2018) found that consumers had WTP more for rice in Vietnam when they were given information about its origin and production practices. Recently, Chege et al. (2019) and Wanyama and Godecke (2019) reported that consumers were willing to pay more for fortified flours (non-instant) compared to conventional flours in Nairobi and Kampala, which are the major urban centers in Kenya and Uganda. Chege et al. (2019) further observed that provision of nutrition information about the product significantly increased the WTP for fortified flours. In a study from our project, consumers

were willing to pay more for instant flours with natural micronutrients/fortificants rather than micronutrient additives (De Groote et al., 2018). Fortification would be helpful in Kenya where iron deficiency and vitamin A deficiency prevalence is estimated to be 29 and 14%, respectively, in persons between 0 and 19 years old (Harika et al., 2017).

In the current study, we developed food-to-food fortified whole grain instant porridge products with natural fortificants [orange-fleshed sweet potatoes (OFSP), baobab, amaranth flour] rich in vitamin A, iron, and zinc chosen to give the products nutritional and sensory attributes that would make consumers want to purchase the products. Hypothetically, the instant property of the fortified foods would make them even more desirable to consumers, as they would be convenient to use to make popular instant *uji* and *ugali* products. An urban market study was conducted in Eldoret, Kenya to test whether consumers respond to these cues and improvements, and if the fortified instant porridge flour products have potential to be successful in the marketplace. Furthermore, the study sought to understand how addition of baobab and sweet potato powders in instant whole grain composite flour formulations, which provides pro-vitamin A, iron, and zinc, would influence sensory and nutritional perception and adoption of these products by consumers when given four levels of nutrition information and instructions and/or demonstration on how to reconstitute the instant fortified flours.

4.3. Materials and Methods

4.3.1. Overview

In this study, consumers conducted affective tests after which they participated in experimental auctions to determine their WTP for food-to-food fortified instant porridge flours. During the affective test, participants were given a food product that they evaluated for sensory characteristics (i.e. texture, flavor, taste, aroma) (De Groote et al., 2018). For the experimental auction, the Becker-DeGroot-Marshak (BDM) approach was used (Becker et al., 1964). According to this model, consumers are willing to pay for a selling price that is equivalent to the value of a good or service, which accords them satisfaction for the value they derive from the good or service. Consumers give a bid that is compared to a random product price that is chosen from a range of probable prices set by the investigator. If the bid is greater than the set price, the consumers buy the product, and otherwise if the bid is lower they lose and do not buy the product. For the

experimental auction, participants are given some money to buy the products or food stuffs and are presented with actual products being sold so that they can purchase them (De Groote et al., 2011). Experimentation auctions have been used previously to determine WTP for flour products (De Groote et al., 2018; 2020; Chege et al., 2019).

4.3.2. Selection of participants

Eldoret city in Uasin Gishu county of Kenya was selected for the study as it rapidly growing city and acts as the headquarters for county. Uasin Gishu has 41% poverty level (KNBS, 2018) . The Feed the Future Food Processing and Post-harvest Handling Innovation Lab (FPIL) project previously established a Food Processing, Technology and Incubation Center (FPTIC) at University of Eldoret to process, train, and facilitate adoption of extruded, fortified instant cereal flours in the region to reduce poverty levels. A list of all supermarkets in Eldoret metropolitan was obtained from the county government of Uasin Gishu. The participants were recruited from points of sale (supermarkets) within Eldoret towns. The major supermarkets in Eldoret town included Tuskys, Naivas, Khetias, Ukwala, Eldomatt, Uzuri, and Yako. The choice of using supermarkets to perform the study was based on the fact that most flours are sold in the supermarkets making it easier to recruit consumers of commercially purchased thin and thick porridge flours. Moreover, it was previously reported that most of the consumers who bought maize meal in Kenyan supermarkets were aware of existence of fortified maize flours (De Groote et al. 2008). Additional participants were recruited from government offices and all went through a pre-screening checklist: they had to be at least 24 years of age, had some form of employment, and consumed thin or thick porridges at least 5 times a week. Upon recruitment, they were invited to come for participation on a specific date and time to the Palm Travellers Guest House in Eldoret city. Four enumerators administered the affective tests, and another four did the experimental auctions. There were two other enumerators who demonstrated to the participants how to reconstitute the instant flour.

4.3.3. Products

Food-to-Food fortified instant porridge flours (formulated by the FPIL project) had the following ingredients: whole grain maize, whole grain sorghum, OFSP, baobab, and amaranth

grain. Two fortified instant flour products were processed in the FPTIC at University of Eldoret; one having more maize than sorghum (42.5% whole grain maize, 22.5% whole grain sorghum, 5% amaranth, and 15% each of OFSP and baobab), and the other having more sorghum than maize (22.5% whole grain maize, 42.5% whole grain sorghum, 5% amaranth, and 15% each of OFSP and baobab). Traditionally, sorghum is added to composite flours because it is thought to have low glycemic index and is one of the foods recommended for persons with chronic diseases such as type 2 diabetes. Maize is a rich source of carbohydrates, proteins, lipids, and phenolic compounds (Xiong et al., 2019; Theobald et al., 2004), while amaranth flour is a rich source of dietary fiber, proteins, and minerals such as iron, zinc, calcium and magnesium (Venskutonis and Kraujalis, 2013).

In terms of micronutrients, OFSP and baobab are relatively rich in iron, zinc, calcium, and pro-vitamin A (Laurie et al., 2012; Osman, 2004; Islam et al., 2016). Finally, amaranth grain is a rich source of zinc, copper, manganese, and iron (Nascimento et al., 2014). These products were designed to provide 25% recommended daily allowances for provitamin A, iron, and zinc (FPIL, 2019). Maize is the major staple food in Kenya used to make thin (*ugi*) and thick (*ugali*) porridges. Thick porridge is consumed at lunch or dinner while thin porridge is consumed as a snack and/or for breakfast (De Groote et al., 2008). A study by De Groote et al. (2008) in Kenya revealed that white maize, which typically has low amounts of pro-vitamin A carotenoids is more preferred than yellow maize. Consequently, fortification with foods rich in such micronutrient sources can enhance their nutritional value for pro-vitamin A, iron, and zinc, which are micronutrient identified to have major deficiencies in the developing world (Bhutta et al., 2013; Underwood, 2000). The two fortified instant porridge flour products were put into 0.5 kg packets that were each used to make thin and thick porridges, giving a total of four products given to each participant.

4.3.4. Informed consent and affective tests

Upon arrival, participants were welcomed and registered at the registration desk and given an outline of the process of the study. They were informed that participation is voluntary and the information they provide would be treated with confidentiality. They were also told that they could stop participating at any stage of the study, without any feeling of victimization. Those who were comfortable participating in the study, were asked to sign an informed consent form and

questionnaire (Appendix 2). They were then given equivalent of $6.25 which they were told to use for participation in the WTP experiments, for transport, and as an appreciation for their time for participating in the study. It has been suggested that the amount given to participants during bidding experiments should be approximately twice the mean product price (De Groote et al., 2018). The mean price of instant fortified flour used in this study was estimated to be Kenya Shillings (Kshs) 200 or ~$2 ($1 = ~Kshs 100 during the time at which the study was conducted), which was determined based on the prevailing market prices of 500 g of instant flour products in the local supermarkets in the study area. Prior to participation in the affective tests, participants were asked socio-demographic information including age, gender, family size income, and education. All data were collected using electronic tablets programmed with CSPro software (United States Census Bureau, 2016) as previously been described by De Groote el. (2020).

Thereafter, the affective test was conducted where participants evaluated the products made from the fortified instant flours. The instant porridges were reconstituted by gradual addition of boiling water in a bowl with instant flour to obtain a homogenous mixture of the porridge. The porridges were presented to the participants in a random order and evaluated by the participants using a 5-point hedonic scale (1=dislike very much, 5=like very much) on texture in hand, texture in mouth, taste, flavor, appearance, and overall acceptability. Symbols (circle, rectangle, triangle, and diamond) were used to represent products which were presented to consumers in a random order (Table 4.1). Hence, the study was single-blinded.

4.3.5. Determination of willingness to pay using experimental auctions

Following completion of affective tests, consumers' WTP for instant fortified products was done using experimental auctions (De Groote et al., 2018; My et al., 2018). The two fortified instant porridge flour products were packaged in white paper with symbols or with printed information based on the four treatment groups. For Group 1, the participants did the experimental auction without product information. Groups 2, 3, and 4 participated in affective tests then proceeded to experimental auctions with increasing levels of information (Table 1). Depending on the group, participants received information on the composition of the products, nutrient information, the procedure how to make it, micronutrient sources incorporated in the product, benefits of instant flour products, and demonstration of how to reconstitute the instant product.

Before the actual bidding, a trial round of the bidding experiment was done using two products - a small packet of biscuits and a lollipop sweet. These simulations were intended to help the study participants to understand the bidding process prior to the actual experimental auctions of the food-to-food fortified instant porridge flours.

The actual experimental auction was done as described by My et al. (2018). Study participants were first given a chance to evaluate the two products after which they gave their bids. Next, consumers randomly drew a price slip from an envelope which acted as the prevailing market price for the product. The lowest price was Kshs 10 and the highest price was twice the price of instant flours found in the market at increments of Kshs 5. In instances where a participant's bid was equal to or greater than the price drawn from the envelope, a product purchase was made (My et al., 2018).

4.3.6. Modelling of willingness to pay

Modelling was done as described by (My et al., 2018), where WTP_i is the value of flour placed by the n^{th} participant for corresponding explanatory variables (X_i), coefficient of the explanatory variables (β_i), constant/intercept (β_0), and residuals (ε_i):

$WTP_i = \beta_0 + \beta_1 + \ldots\ldots + \beta_n + \varepsilon_i$ where *i* denotes participants 1, 2, 3……..n

The difference between the four groups, i.e. treatments based on information provided, was predicted using dummy variables based on the group. Group 1, which was considered the control, was coded zero for the dummy variables (the product name and nutrition information, procedure how to make the instant flour porridge, and demonstration of how to reconstitute the instant flour) indicating that these variables were absent. For Group 2, product name and nutrition information were coded 1, while the procedure how to reconstitute the flour to make thin/thick porridge, and demonstration on how to reconstitute the flour to make thin/thick porridge were coded 0. For Group 3, product name and nutrition information, and a procedure of how to make instant flour porridge were coded 1, while demonstration of how to reconstitute the flour to make thin/thick porridge was coded zero indicating they did not observe a demonstration on how to reconstitute the instant flour. For Group 4, all the dummy variables were coded 1 indicating the participants were given information on the product name and nutrition information, procedure on how to

prepare the instant flour porridges, and a demonstration of how to reconstitute the flour to make the porridges. Data was analyzed using SAS version 9.4.

4.3.7. Data analysis

Data were analyzed for ANOVA using SAS v. 9.4 (SAS Institute Inc, NC) to determine significant differences for texture in hand, texture in mouth, taste, flavor, appearance, and overall acceptability. Scores (liked, disliked, neither liked or disliked, liked very much or disliked very much) of the four porridges made from the two flour formulations were represented as percentages. Robust regression was used to determine how different explanatory variables influenced WTP for the products. For the regression, explanatory data such as male gender, income level, and received information were considered as binary variables. The premiums were determined as the change in WTP price when no information was given to the participants and when information was given depending on the treatments/groups.

4.4. Results and Discussion

4.4.1. Demographics

Table 4.2 represents the demographic profile of the participants of the study. The Kenyan population demographic structure is skewed toward the young with 75.6% of the population of age below 34 years (KNBS, 2015). The participants included in this study were young with a mean age of 32 years and age range of 25 to 63 years. In terms of age brackets, 50, 22, and 28% were 25-29, 30-35, and above 35 years, respectively. The majority of the respondents were women (59.5%), with men comprising 40.5% of the respondents.

High income participants had the highest mean years (17) of completed education, while the low-income group was 13 years (Table 4.1). The distribution of married persons among the income groups were 50, 48, and 71% in the low, middle- and high-income groups, respectively. On average, household size was small with the low-income group having 3 persons, and the middle/high income groups had 4 persons. At the national level, the KNBS (2015) report gives average households sizes as 3.2 and 4.4 in the urban and rural, respectively.

4.4.2. Sensory evaluation of the food-to-food fortified instant porridge flours

Generally, the sensory parameters of the two food-to-food fortified instant porridge flour products show that they were well appreciated (Table 4.3). Regardless of the formulation, the mean sensory scores for appearance, texture, flavor, taste and overall acceptability were above 3 on the 5-point Likert scale. Sensory scores for the "more sorghum than maize" thin porridge were significantly higher than for "more maize than sorghum" thin porridge, and categorized in the 'like' for appearance, flavor, texture and overall acceptability. Overall, fortified instant porridge having more sorghum than maize was significantly preferred for thin porridge (*uji*), while flour having more maize than sorghum was preferred for thick porridge (*ugali*). These findings agree with De Groote et al. (2020) who found that consumers preferred products darker in color for thin porridge, as is with the case of higher sorghum content, and lighter ones for thick porridges in Kenya, as with more maize. In agreement with our study, Bangu, Mtebe and Nzallawahe (1994) found that incorporating of sorghum flour (10%) to maize flour caused a reduction in consumer acceptability, because of the change to darker color and textural properties that were undesired for thick porridges in Tanzania, East Africa. They observed that consumers prefer thick porridges from maize composite flours having lower amounts of sorghum. In our study, there were significant differences in the texture of the porridges, but the appearance/color of thick porridge made from flour having less sorghum was ranked higher for thick porridges, supporting this previous work.

The difference in sensory characteristics as influenced by overall acceptance of the thin and thick porridges was assessed using regression. Regardless of the product, the major factor that influenced the overall assessment of the porridges was taste and this was highest in the "more sorghum than maize" (Product 2) thin porridge (Table 4.4). The second most important sensory characteristic was texture in the mouth for products, except the Product 2 thin porridge. The least contributing sensory variables influencing acceptance were aroma and appearance of porridges made from Product 1 and Product 2 thick porridge. Appearance and texture in hand were insignificant variables influencing acceptance for Product 2 thin porridge.

Regardless of the product, at least 49% of the participants liked the thin or thick porridges made from the two products (Figure 4.1). Less than 10% of the participants disliked the product.

This data suggest that the instant fortified porridge flours have good and acceptable sensory attributes.

After completion of the experimental auction, participants judging each treatment were asked as to what preparations they would use the instant products. Most participants (50%) were willing to use Product 2 which had more sorghum than maize to make thin porridge. On the other hand, a large proportion (44.4%) of participants preferred Product 1 to make the thick porridge (Figure 4.2). The use of either product to make both thin and thick porridges was about the same at 25.6 and 28.4% for Products 1 and 2, respectively. These findings suggest that product formulations dictate what form they will be consumed.

After evaluating the sensory attributes, participants were asked about their knowledge of minerals, proteins, and vitamins (Figure 4.3). On average, nutritional knowledge was higher for proteins and vitamins than for minerals. Approximately 97% of the participants said they knew of protein, while the those who knew sources of proteins were 95%. Some of the protein sources they gave were milk, beans, and meats. In terms of vitamins, the majority (91%) said that they heard of vitamins, of which 81% knew of vitamin A, 67% vitamin C, and 62% the B-vitamins. Approximately 44% were aware of vitamin A deficiency. Finally, participants were asked about minerals, with 69% saying they had heard of them. However, knowledge of specific minerals was low with 1, 4, and 6% saying they had heard of zinc, iron, and calcium, respectively. A previous study supports the view that consumers have basic information of the major nutrient sources (De Groote et al., 2020).

4.4.3. Willingness to pay for instant fortified flours

Provision of nutritional information, by way of nutrient composition, increased the mean WTP for the two products, but a larger increase was found for Product 2. For Group 1, when there was no information given, consumers were willing to pay a mean price of Kshs 74 and 79 for Products 1 and 2 (Table 4.5). In Group 2, when consumers were given information on the nutrient composition of the product, they were willing to pay a premium of Kshs 10 (or 13.5%) increase in price for Product 1, and a premium of Kshs 20 for Product 2, representing a significant 25% increase in price. In Group 3, consumers were given additional information on the procedure of how to reconstitute the instant product into thin and thick porridges, however this alone did not

increase WTP of either product compared to Group 2. When the participants were additionally given a demonstration of how to reconstitute the instant flour, the WTP price for high-maize Product 1 increased significantly by Kshs 25 or 31% compared to the Group 3 price, and did not further increase the WTP price of Product 2. Overall, information on product composition was the main driver for increased WTP for Product 2, while demonstration of how to reconstitute the instant flour was the the driver for increase in WTP price for Product 1. My et al. (2018) also observed that consumers were willing to pay more when consumers were given nutrient composition information about the product.

4.4.4. Willingness to pay pooled data

The determinants of WTP by participants are represented in Table 4.6. These independent explanatory variables were chosen from previous studies on determinants of WTP (My et al., 2018; De Groote et al., 2008; 2018; 2020). The factors that influenced consumers' WTP for the instant products were analyzed using robust regression as constant variance assumption was not met for Product 1 (Breusch Pagan=0.0163, Appendix 3). There was no multicollinearity as all the variance inflation factors were below 10 for the explanatory variables (Appendix 4).

First, pooled data are presented for all the treatments combined. Effect of provision of nutritional information somewhat depended on the product type. Giving the participants' information on product name and nutrient composition, the coefficient for Product 1 was negative, but insignificant, while for Product 2 was positive and significant; suggesting that the WTP increased with information for Product 2, but not for Product 1. Since Product 2 had 22.5% more sorghum, we speculate that the participants associated increased sorghum with improvement to their health. These results are in line with Chege et al. (2019) who found that consumers were willing to pay more for multi-composite flours compared to their non-composite conventional counterparts in Kenya and Uganda. Interestingly, provision of information on the procedure for reconstituting the instant flours did not increase WTP for either products, but when participants were further provided a demonstration on how to reconstitute the instant flour to thin and thick porridges, Product 1 WTP price significantly increased. This suggests that the information given on reconstitution did not register well with the participants, but demonstrating the value of the instant flour was effective.

We also assessed how factors such as hours after meal, time of the day when the auction was done, and other demographic information influenced WTP. Binary variables were used for the following: time of the day, gender, income, and marital status. Time of the day was positive and significant for the two products suggesting that those who did the WTP exercise in the morning were willing to pay more for the products. Contrarily, Chege et al. (2019) found that whether experimental auctions were done in the morning or evening did not influence the WTP. Unexpectedly, male gender did not influence consumers WTP. This could be because in Kenya, most decisions regarding purchase and preparation of foods are done by women. Surprisingly, income and marital status did not significantly increase the WTP. It would be anticipated that, as income levels increase, people would be willing to pay more for valued food products. Our results differ from Chege et al. (2019) who found that persons with higher incomes were willing to pay more for composite flours with food-to-food fortification.

Family size had a negative and insignificant coefficient for the WTP for a packet of Product 1 indicating that as family size increased, their WTP for Product 1 decreased. On the contrary, for Product 2, the coefficient for family size was positive, indicating that as family size increased, their WTP for Product 2 increased. This implies that families with larger households were less willing to pay for the instant fortified products for a packet of Product 1. Bett et al. (2013) found that households with small family size were willing to pay more for indigenous chicken products compared to large family sizes, likely because in large families the amount of disposable or available income that can be used to buy premium products like instant flour is less leading to lower bids.

Years of completed education influenced WTP depending on the product type with positive and negative coefficients for Products 1 and 2, respectively. This means that as years of education increased, the WTP for Product 1 increased while that for Product 2 decreased. De Groote and Kimenju (2008) also found that education did not influence WTP for fortified maize flours in Kenya. A possible explanation is that consumers already consume fortified flours, and hence have knowledge about their nutrient profile (Chege et al. (2019).

4.4.5. Willingness to pay by treatments/groups

Because different information was provided at each group level, we also did an analysis of WTP by groups and treatments. Table 4.7 shows the results of robust regression analysis. For the treatment that received no information (i.e. Group 1), time of the day and income had a positive and significant coefficient indicating that they positively influenced WTP; while hours after meals, education in years, and family size had negative but significant coefficients indicating that as these variables increased, WTP decreased for Product 1. For Product 2, time of the day and hours after last meal had positive and significant coefficients suggesting as these variables increased, WTP increased. Income, years of completed education, and gender were significant and inversely related with WTP.

For Group 2 receiving information on product name and nutrient composition, time of the day and gender had a positive coefficients and significant effect on WTP for Product 1. Hours after last meal was the only significant variable with a positive coefficient for Product 2. Regardless of the product, variables such as marital status, age, years of completed education, family size, and income did not positively impact the WTP.

Finally, for participants who received nutritional information and demonstration on reconstituting the instant fortified porridge flours; age, years of completed education, and time of the day positively impacted WTP for Product 1. For Product 2, time of the day and marital status positively impacted WTP, while hours after last meal, gender, and education did not significantly increase WTP.

4.4.6. Cost analysis

The cost of processing a kilogram of the food-to-food instant fortified porridge flours are outlined in Table 4.8. The ingredient costs were the prevailing market prices for whole grain ingredients, namely sorghum and maize, and fortificants (baobab and carrots) as published recently (De Groote et al., 2020). Amaranth flour (Kshs 120/kg) and OFSP (Kshs 150/kg) were additional fortificants used in the products. The extra cost of fortificants per kg was Kshs 51/kg. Extrusion cost was calculated to be Kshs 12/kg. Assuming that the retail price for each of the fortified products is increased by a 20% margin, the prices per 500 g thus became Kshs 57.4 and Kshs 57.9 for Products 1 and 2, respectively.

The premium for the instant fortified porridge flour products was based on the Group 4 information: nutritional information, procedure how to reconstitute the products, and demonstration on how to reconstitute the instant flours. Participants were willing to pay higher premium for Product 2 (Kshs 33/ 500g) than Product 1 (Kshs 48/ 500 g) (Table 4.8)

4.5. Conclusions

Consumer preferences for the two food-to-food fortified instant porridge flours differed based on their formulation such that the one with higher sorghum was preferred for thin porridge (*uji*), while the one having a higher proportion of maize was preferred for thick porridge (*ugali*). Overall, the sensory characteristics of the thin and thick porridges were judged as good by the consumers indicating that these fortified products were accepted. Wanyama et al. (2019) previously found that fortified products made from maize, millet, beans, and micronutrient sources such as OFSP, amaranth flour, vitamin A, iron and zinc had minor effects on how consumers perceived sensory characteristics, namely texture, appearance, and taste of the composite products in Kenya and Uganda..

Significant factors of provision of name, nutrient composition and demonstration of how to reconstitute the instant flours were found to influence WTP for the fortified instant porridge flours. Interestingly, WTP for the higher maize-based fortified instant flour (Product 1), with consumer interest to use for the thick porridge (*ugali*) was substantially increased by 42% when participants were shown by demonstration how to reconstitute the instant flours, while for the higher sorghum-based fortified instant flour to use for thin porridge (*uji*) was increased by 25% when name and nutritional composition was given. These results support other studies that found that consumers were willing to pay more for fortified products (Chege et al., 2019; Wanyama et al., 2019), but here show the added WTP for the convenience of instant ugali flour.

4.6. References

Awasthi, S., Reddy, N.U., Mitra, M., Singh, S., et al. (2020). Micronutrient fortified infant cereal improves Hb status and reduces iron deficiency anemia in Indian infants: an effective study. *British Journal of Nutrition*, 123 (7), 780-791.

Adams, A.M., Ahmed, R., Latif, A.H.M.M., Rasheed, S., Das, S.K., et al. (2017). Impact of fortified biscuits on micronutrients deficiencies among primary school children in Bangladesh. *PloS One*, 12(4), e0174673. doi: 10.1371/journal.pone.0174673

Alam, M. K., Rana, Z. H., and Islam, S. Z. (2016). Comparison of the proximate composition, total carotenoids and total polyphenol content of nine orange fleshed sweet potato varieties grown in Bangladesh. *Foods*, 5, 64.

Ara, G., Khanam, M., Rahman, A. S., Islam, Z., Farhad, S., Sanin, K. I., Khan, S. S., Rahman, M. M., Majoor, H., & Ahmed, T. (2019). Effectiveness of micronutrient-fortified rice consumption on anaemia and zinc status among vulnerable women in Bangladesh. *PloS one*, *14*(1), e0210501. https://doi.org/10.1371/journal.pone.0210501

Bangu, N.T.A., Mtebe, K., and Nzallawahe, T.S. (1994). Consumer acceptability of stiff porridges based on various proportions of sorghum, maize and cassava. *Plant Foods for Human Nutrition*, 46, 299-303.

Becker, G. M., Degroot, M. H., and Marschak, J. (1964). Measuring utility by single response sequential method. *Behavioural Science*, 9:226-232.

Bett, H.K., Peters, K.J., Nwankwo, U.M., and Bokelmann, W. (2013). Estimating consumer preferences and willingness to pay for the underutilized indigenous chicken products. *Food Policy*, 41, 218-225.

Bhutta, Z.A., Salam, R.A., and Das, J.K. (2013). Meeting the challenges of micronutrient malnutrition in the developing world. *British Medical Bulletin*, 106 (1), 7-17.

Braca, A., Sinisgalli, C., De Leo, M., Muscatello, B., Cioni, P. L., Milella, L., Ostuni, A., et al. (2018). Phytochemical Profile, Antioxidant and Antidiabetic Activities of *Adansonia digitata* L. (Baobab) from Mali, as a Source of Health-Promoting Compounds. *Molecules*, 23, 3104.

Chadare, F.J., Linnemann, A. R., Hounhouigan, J. D., Nout, M. J. R., Van Boekel, M. A. J. S. (2008). Baobab Food Products: A review on their composition and nutritional value. *Critical Reviews in Food Science and Nutrition*, 49, 254 - 274.

Chege, C. G. K., Sibiko, K. W., Wanyama, R., Jager, M., and Birachi, E. (2019). Are consumers at the base of the pyramid willing to pay for nutritious foods? *Food Policy*, 87, 101745.

Das, J.K., Salam, R.A., Kumar, R. *et al.* (2013). Micronutrient fortification of food and its impact on woman and child health: a systematic review. *Systematic Reviews* 2, 67.

De Groote, H., Mugalavai, V., Ferruzzi, M., Onkware, A., Ayua, E., Duodu, K. G., Ndegwa, M., et al. (2020). Consumer acceptance and willingness to pay for instant cereal products with food to food fortification in Eldoret, Kenya. *Food and Nutrition Bulletin*, 41, 224-243.

De Groote, H., Kariuki, S. W., Traore, D., Taylor, J. R., Ferruzzi, M. G., and Hamaker, B. (2018). Measuring consumers interest in instant fortified pearl millet products: a field experiment in Touba, Senegal. *Journal of the Science of Food and Agriculture*, 98, 2320-2331.

De Groote, H., Gunaratna, N.S., Okuro, J.O., Wondimu, A., Chege, C.K., and Tomlins, K. (2014). Consumer acceptance of quality protein maize (QPM) in East Africa. *Journal of the Science of Food and Agriculture*, 94: 3201-3212.

De Groote, H., Kimenju, S. C., and Morawetz, U. B. (2011). Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. *Agricultural Economics*, 42, 1-16.

De Groote, H., & Kimenju, S. C. (2008). Comparing consumer preferences for color and nutritional quality in maize: Application of a semi-double-bound logistic model on urban consumers in Kenya. *Food Policy*, 33(4), 362–370. doi:10.1016/j.foodpol.2008.02.005

FAO, IFAD, UNICEF, WFP and WHO. (2020). In Brief to The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets. Rome, FAO. https://doi.org/10.4060/ca9699en

FPIL (2019). Feed the Future Innovation Lab for Food Processing and Post-Harvest Handling Five-Year Final Report. Food Processing Innovation Lab, Purdue University, West Lafayette, IN, 51 pp.

Gibson, R. S., and Hotz, C. (2001). Dietary diversification / modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *British Journal of Nutrition*, 85, S159-S166.

Gifford, K., and Benard, J. C. (2011). The effect of information on consumers willingness to pay for natural and organic chicken. *International Journal of Consumer Studies*, 35, 282-289.

Glover-Amengor, M., Aryeetey, R., Afari, E., and Nyarko, A. (2016). Micronutrient composition and acceptability of Moringa oleifera leaf-fortified dishes by children in Ada-East district, Ghana. *Food Science and Nutrition*, 5, 317-323.

Harika, R., Faber, M., Samuel, F., Kimiywe, J., Mulugeta, A., Eilander, A. (2017). Micronutrients status and dietary intake of iron, vitamin A, iodine, folate and zinc in women of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria and South Africa: A systematic review of data from 2005 to 2015. *Nutrients*, 9: 1096.

Harrison, G.G. (2010). Public health interventions to combat micronutrient Deficiencies. *Public Health Reviews*, 32, 356-266.

Islam, S. N., Nusrat, T., Begum, P., and Ahsan, M. (2016). Carotenoids and β-carotene in orange fleshed sweet potato: A possible solution to vitamin A deficiency. *Food Chemistry*, 199 (15), 628-631.

Kenya National Bureau of Statistics (2014/15). Kenya Demographic and Health Survey 2014. Nairobi, Kenya: Kenya National Bureau of Statistics

Kenya National Bureau of Statistics (2018). Basic Report on Well-Being in Kenya, Based on the 2015/16 Kenya Integrated Household Budget Survey (KHBS). Nairobi, Kenya: Kenya National Bureau of Statistics.

Kruger, M., Taylor, J. R. N., Ferruzzi, M. G., and Debelo, H. (2020). What is food-to-food fortification? A working definition and framework for evaluation of efficiency and efficacy and implementation of best practices. *Comprehensive Reviews in Food Science and Food Safety*, 19, 3618-3658.

Krystallis, A., and Chryssohoidis,G. (2005). Consumers willingness to pay for organic food: Factors that affect it and variation per organic product type. *British Food Journal*, 107, 320-343.

Laurie, S.M., van Jaarsveld, P.J. Faber, M., Philpott, M.F., and Labuschagne, M.T. (2012). *Trans*-β-Carotene, selected mineral content and potential nutritional contribution of 12 sweet potato varieties. *Journal of Food Composition and Analysis*, 27, 151-159.

Marette, S., Roosen, J., Blanchemanche, S., and Feinblatt-Meleze, E. (2010). Functional food, uncertainity and consumers choices: A lab experiment with enriched yoghurts for lowering cholesterol. *Food Policy*, 35, 419-428.

Muthayya, S., Rah, J. H., Sugimoto, J. D., Roos, F. F., Kraemer, K., & Black, R. E. (2013). The Global Hidden Hunger Indices and Maps: An Advocacy Tool for Action. *PLoS ONE*, 8(6), e67860. doi:10.1371/journal.pone.0067860

Moussa, M., Qin, Xu, Chen, L., Campanella, O.H., Hamaker, B. R. (2011). High quality instant sorghum porridge flours for the West African market using continuous processor cooking. *International Journal of Food Science and Technology*, 46,2344-2350.

My, N. H. D., Demont, M., Loo, E. J. V., de Guia, A., Rutsaert, P., Tuan, T. H., and Verbeke, W. (2018). What is the value of sustainability produced rice? Consumer evidence from experimental auctions in Vietnam. *Food Policy*, 79, 283-296.

Nair, M. K., Augustine, L. F., and Konapur, A. (2016). Food based interventions to modify diet quality and diversity to address multiple micronutrient deficiency. *Frontiers in Public Health*, 3:277. doi: 10.3389/fpubh.2015.00277

Nascimento, A. C., Mota, C., Coelho, I., Gueifao, S., Santos, M., Matos, A. S., et al. (2014). Characterisation of nutrient profile of quionoa (Chenopodium quinoa), amaranth (Amaranthus caudatus), and purple corn (Zea mays L.) consumed in the North of Argentina: Proximates , minerals and trace elements. *Food Chemistry*, 148, 420-426.

N'diaye, C., Martinez, M. M., Hamaker, B. R., Campanella, O. H., Ferruzzi, M. G. (2020). Effects of edible plant materials on provitamin A stability and bioaccessibility from extruded whole pearl millet (*P. typhoides*) composite blends. *LWT Food Science and Technology*, 123, 109109.

Nestel, P., Bouis, H., Meenakshi, J. V., Pfeiffer, W. (2006). Biofortification of staple crops. *The Journal of Nutrition*, 136: 1064-1067.

Nkhata, S. G., Chilungo, S., Memba, A., Mponela, P. (2020). Biofortification of maize and sweet potatoes with provitamin A carotenoids and implication on eradicating vitamin A deficiency in developing. *Journal of Agriculture and Food Research*, 2, 100068.

Ortiz, D., Ponrajan, A., Bonnet, J. P., Rocheford, T., Ferruzzi, M. G. (2018). Caretenoid stability during milling, storage, and extrusion processing of biofortified maize genotypes. *Journal of Agricultural and Food Chemistry*, 66, 4683-4691.

Osman, M. A. (2004). Chemical and Nutrient Analysis of Baobab (Adansonia digitate) Fruit and Seed Protein Solubility. *Plant Foods for Human Nutrition*, 59,29-33.

Ragasa, C., Andam, K. S., Amewu, S., and Asante, S. B. (2019). Consumer demand and willingness to pay for safe food in Accra, Ghana. Implications for public and private sectors roles in food safety management. *International Food Policy Research Institute*, Discussion Paper 01795.

Rastogi, A., and Shukla, S. (2013). Amaranth: A new millennium crop of nutraceutical values. *Critical Reviews in Food Science and Nutrition*, 53, 109-125.

Senyolo, G. M., Wale, E., and Ortmann, G. F. (2014). Consumers willingness to pay for underutilized vegetable crops: the case of African leafy vegetables in South Africa. Journal of Human Ecology, 47:219-227.

Teixeira, E. M. B., Carvalho, M. R. B., Neves, V. A., Silva, M. A., and Arantes-Pereira, L. (2014). Chemical characteristics and fractionation of proteins from *Moringa oleifera* Lam. leaves. *Food Chemistry, 147*, 51-54.

Theobald C. E. Mosha & Mary M. Vicent (2004). Nutritional value and acceptability of homemade maize/sorghum-based weaning mixtures supplemented with *rojo* bean flour, ground sardines and peanut paste. *International Journal of Food Sciences and Nutrition*, 55(4), 301-315, DOI: 10.1080/09637480410001225184

Underwood, B.A. (2000). Overcoming micronutrient deficiencies in developing countries: Is there a role for agriculture. *Food and Nutrition Bulletin*, 21 (4), 356-360.

United Nations (2015). Sustainable development goals. Retrieved June 2020 from https://www.un.org/sustainabledevelopment/sustainable-development-goals/

United States Census Bureau (2016). *Census and Survey Processing System (CSPro)*. United States Census Bureau, Washington.

Venskutonis, V.R., and Kraujalis, P. (2013). Nutritional components of amaranth seeds and vegetables: A review on composition, Properties and Uses. *Comprehensive Reviews in Food Science and Food Safety*, 12, 381-412.

Wanyama, R., and Godecke, T. (2019). Poor consumers preferences for nutritionally enhanced foods. *British Food Journal*, 121(3), 755-770.

Xiong, Y., Zhang, P., Warner, R.D., and Fang, Z. (2019). Sorghum grain: from genotype, nutrition, and phenolic profile to its health benefits and food applications. *Comprehensive Reviews in Food Science and Food Safety*, 18, 2025-20146.

Table 4.1. Experimental auction groups defined by incrementally increased amounts of information on the two food-to-food fortified instant porridge flours.

Groups	Description
Group 1	No information provided on the label Flour package in white plain packets with symbols to indicate that it can be used to make thin and thick porridges (see)  Diamond (thick porridge) and triangle (thin porridge) for product one  Rectangle (thick porridge) and circle (thin porridge) for product two
Group 2	Instant flour, nutrient composition of the product
Group 3	Instant flour, nutrient composition of the product, procedure how to reconstitute the instant flour to make thin/thick porridge)
Group 4	Instant flour, nutrient composition of the product, procedure how to reconstitute the instant flour to make thin/thick porridge, a demonstration of how to reconstitute the instant flour to make instant porridge

Table 4.2. Summary of demographic characteristics of the study participants.

Variables	**Lower income (0-10,000 Kshs**	**Middle income (11,000-50,000 Kshs)**	**High income (Above 50, 000 Kshs)**
Female (%)	100	62.8	50
Male (%)	0	37.2	50
Mean age (years)	33.5	31.1	37.1
Mean years of completed education	13	14.5	16.6
Married (%)	50	47.7	71.4
Average family size	3.3	3.9	4.5
Average hours after last meal	4.0	4.4	4.4
N, (%) *	4, (1.1%)	304, (80.4%)	70, (18.5%)

N represents the number of participants per income group and percentages are given in bracket.

Table 4.3. Sensory characteristics of the two food to food fortified flours assessed using a hedonic scale (1=dislike very much, 5=like very much).

	Appearance	Texture (hand)	Flavor	Texture (mouth)	Taste	Overall acceptability
More maize than sorghum						
Thick porridge (diamond)	3.9±0.86b	3.8±0.74b	3.9±0.77ab	3.8±0.87b	3.7±0.23b	4.0±0.82b
Thin porridge (triangle)	3.5 ±0.92c	3.9±0.71ab	3.8±0.75b	3.9±0.81ab	3.6±0.32b	3.90±0.81b
More sorghum than maize						
Thick porridge (horizontal)	3.4±0.90c	3.7±0.76c	3.8±0.78b	3.8±0.83b	3.7±0.18b	3.9±0.83b
Thin porridge (circle)	4.2±0.69a	4±0.69a	4.0±0.73a	4.0±0.74a	3.9±0.11a	4.1±0.76a

Means±SD with different letters in a column are significantly different, Tukey' s multiple comparison test, $p < 0.05$, n=378.

Table 4.4. Factors that influenced acceptance of fortified instant thin (*uji*) and thick (*ugali*) porridges as determined by robust regression (n=378). The errors represent robust standard errors (n=378).

	Product 1 (more maize than sorghum)				Product 2 (more sorghum than maize)			
	Thin porridge		Thick porridge		Thin porridge		Thick porridge	
	Mean SE	p-value	Mean SE	p-value	Mean SE	p-value	Mean SE	p-value
Intercept	0.71±0.21	0.0010	0.45±0.18	0.0108	0.29±0.18	0.1106	0.29±0.19	0.1281
Appearance	0.13±0.04	0.0017	0.06±0.03	0.0860	0.05±0.04	0.2243	0.11±0.03	0.0015
Texture in hand	0.21±0.05	<.0001	0.08±0.04	0.0395	0.01±0.03	0.7312	0.16±0.04	0.0002
Aroma	0.13±0.05	0.0078	0.10±0.04	0.0107	0.20±0.04	<.0001	0.15±0.04	0.0003
Taste	0.25±0.04	<.0001	0.46±0.03	<.0001	0.59±0.03	<.0001	0.34±0.02	<.0001
Texture in mouth	0.14±0.04	0.0011	0.21±0.04	<.0001	0.10±0.04	0.0096	0.19±0.05	<.0001

SE means standard error.

Table 4.5. Effect of provision of nutrition and product preparation information, and instant product demonstration, on the mean willingness to pay in Kenyan shillings (Kshs) for the instant products.

Treatments	Product 1 (Higher maize than sorghum)	Product 2 (Higher sorghum than maize)
Group 1 (no information about the product)	74±28b	79±37b
Group 2 (name and nutrient composition)	84±50b	99±55a
Group 3 (Group 2 information plus procedure for reconstituting the instant flour)	80±36b	94±45ab
Group 4 (Group 3 information plus demonstration of how to reconstitute it)	105±52a	91±38ab
p-value	0.0003	0.0621

Means with different letters in a column are significantly different as determined by Tukey's multiple comparison test at $p < 0.05$. Values are means ± SD. Sample sizes per treatments: Group 1 (n=63), Group 2 (n=64), Group 3 (n=57), Group 4 (n=69). 1 USD is equivalent to 100 Kshs.

Table 4.6. Pooled data on the determinants of WTP (n=253).

	Product 1		Product 2	
	Estimate ±SE	Pr > ChiSq	Estimate ±SE	Pr>ChiSq
Intercept	57.35±18.32	0.0017	62.88±20.57	0.0022
Product name and composition	-4.54±6.22	0.4650	12.45±6.99	0.0746
Procedure of making the instant flour	6.03±6.41	0.3469	-1.04±7.19	0.8853
Demonstration of how to make the Instant flour	18.66±6.20	0.0023	-0.57±6.96	0.9347
Time of the day (1=morning, otherwise=0)	15.88±4.76	0.0009	15.06±5.35	0.0049
Hours after last meal	-0.54±1.15	0.6405	-0.19±1.29	0.8808
Gender (Male=1, otherwise zero)	2.59±4.54	0.5677	-0.51±5.09	0.9200
Age	0.24±0.35	0.4919	0.40±0.39	0.2977
Completed years of education	0.28±0.85	0.7374	-0.16±0.95	0.8698
Marital status (1=married, otherwise=0)	-3.11±5.12	0.5432	-5.30±5.52	0.3370
Family size	-0.01±1.08	0.9956	0.56±1.17	0.6348
Income (from Kshs. 50, 000=1, otherwise zero)	-3.14±5.31	0.5540	-1.64±5.67	0.7728
R-square	0.0937		0.0468	

Abbreviations: WTP, willingness to pay; SE, robust standard error.

Table 4.7. Determinants of WTP by treatments.

	Product 1		Product 2	
Group 1 (n=63)	Estimate ±SE	Pr > ChiSq	Estimate ±SE	Pr>ChiSq
Intercept	1.07±0.60	0.0749	3.27±0.83	<.0001
Time of the day	0.62±0.01	<.0001	0.05±0.02	0.0105
Hours after last meal	-0.12±0.01	<.0001	0.48±0.02	<.0001
Gender/sex	-0.01±0.02	0.7205	-0.12±0.03	<.0001
Age	0.002±0.01	0.8695	-0.02±0.02	0.2981
Education in years	-0.05±0.02	0.0036	-0.16±0.02	<.0001
Marital status	0.02±0.01	0.2406	-0.02±0.02	0.2996
Family size	-0.03±0.01	0.0019	-0.01±0.01	0.4745
Income	0.04±0.01	<.0001	- 0.02±0.01	0.0623
Group 2 (n=64)				
Intercept	0.98±0.24	<.0001	3.80±0.89	<.0001
Time of the day	0.70±0.00	<.0001	-0.02±0.01	0.2303
Hours after last meal	-0.04±0.01	<.0001	0.78±0.02	<.0001
Sex /gender	0.02±0.004	<.0001	-0.200±0.02	<.0001
Age	-0.004±0.01	0.4629	0.001±0.02	0.9613
Education	0.001±0.01	0.9295	0.01±0.02	0.7878
Marital status	-0.01±0.01	0.2131	-0.01±0.02	0.5973
Family size	-0.003±0.00	0.5215	-0.03±0.02	0.1309
Income	0.004±0.01	0.9445	-0.03±0.02	0.1218
Group 3 (n=57)				
Intercept	0.59±0.37	0.1128	-0.05±0.56	0.0627
Time of the day	-0.02±0.00	<.0001	0.02±0.004	0.0001
Hours after meal	0.002±0.01	0.8119	0.07±0.01	<.0001
Gender	-0.02±0.01	0.0175	-0.04±0.01	0.0008
Age	0.01±0.01	0.4413	0.02±0.01	0.0491
Education	0.99±0.01	<.0001	-0.02±0.01	0.1966
Marital status	-0.0009±0.01	0.8887	0.75±0.01	<.0001
Family size	-0.0051±0.01	0.4381	0.0048±0.01	0.6317
Income	-0.002±0.01	0.7590	0.01±0.01	0.2772
Group 4 (n=69)				
Intercept	-0.01±0.02	0.5603	-1.13±0.46	0.0148
Time of the day	0.003±0.00	<.0001	0.08±0.01	<.0001
Hours after last meal	-0.01±0.00	<.0001	0.0001±0.01	0.9919
Gender	-0.001±0.00	0.0067	0.01±0.01	0.1447
Age	0.001±0.0005	0.0020	-0.0004±0.01	0.9726
Education	1.00±0.00	<.0001	0.01±0.01	0.2119
Marital status	0.0001±0.0004	0.7095	0.67±0.01	<.0001
Family size	0.00±0.0003	0.9096	-0.0002±0.01	0.9730
Income	0.00±0.00	0.9740	0.002±0.01	0.6430

Abbreviations: WTP, willingness to pay; SE, standard error.

Table 4.8. Cost of production of instant fortified flours used in the study.

Variable	Explanation	Unit	Unit cost (Kshs)	Product 1 (More maize than sorghum)	Product 2 (More sorghum than maize)
Raw material	Whole sorghum flour	Kg	44	0.225	0.425
	Whole maize flour	Kg	40	0.425	0.225
	Baobab dried flour	Kg	150	0.15	0.15
	Orange fleshed sweet potato	Kg	150	0.15	0.15
	Amaranth flour	Kg	120	0.05	0.05
	Subtotal ingredients (Kshs)			**77.9**	**78.7**
Extrusion	Electricity (3.4 Kwh/35kg	Kwh	13.5	0.4	0.4
	Extruder (US $ 18 000; 10 years, 250 days, 6 h/d)	Kshs/hour	120	3.4	3.4
	Skilled labor (1 hour/35 kg)	hour	125	3.6	3.6
	Unskilled labor (1 hour/35 kg)	hour	50	1.4	1.4
Drying	Electricity (5 Kw coil 0.7 kw fan, 10 years, 250 days, 6 hrs/day)	kwh	13.5	1.6	1.6
	Unskilled labor	hour	60	1.4	1.4
	Dryer (US $ 600, 3 years, 250 days, 10 h/d)	Kshs/h	3.3	0.1	0.1
	Subtotal extrusion			12	12
Packaging	Package (kshs 500 for 100 bags)	5	5	5	5
	Labor (1 hour for 35 kg)		50	0.7	0.7
	Subtotal for packaging			**5.7**	**5.7**
Total cost		Kshs/kg		95.6	96.4
Retail	(with 20% increase)	Kshs/kg		114.72	115.7
Retail price		Kshs/500 g		57.36	57.85
WTP*		Kshs/500 g		105	91
Difference		Kshs/500 g		48	33
Profit margin		%		84	57

WTP* is the willingness to pay for 500 g of the fortified products when nutrition information, procedure for preparing the instant, and practical demonstration of how to reconstitute the products is given about the product. The cost of extrusion, drying and packaging have been published by De Groote et al. (2020).

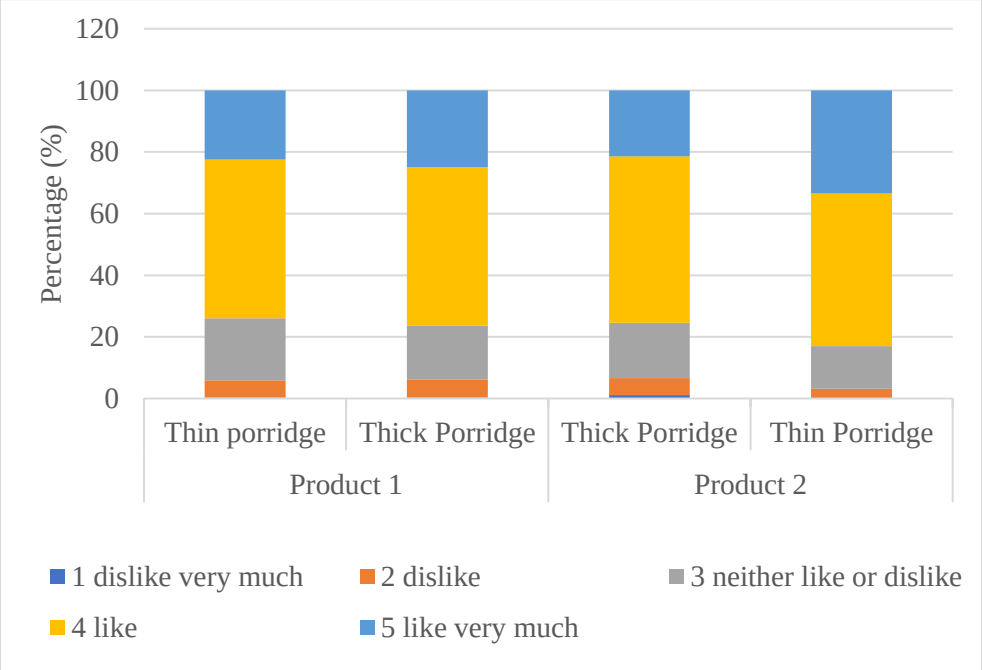


Figure 4.1. Distribution of sensory characteristics by product.

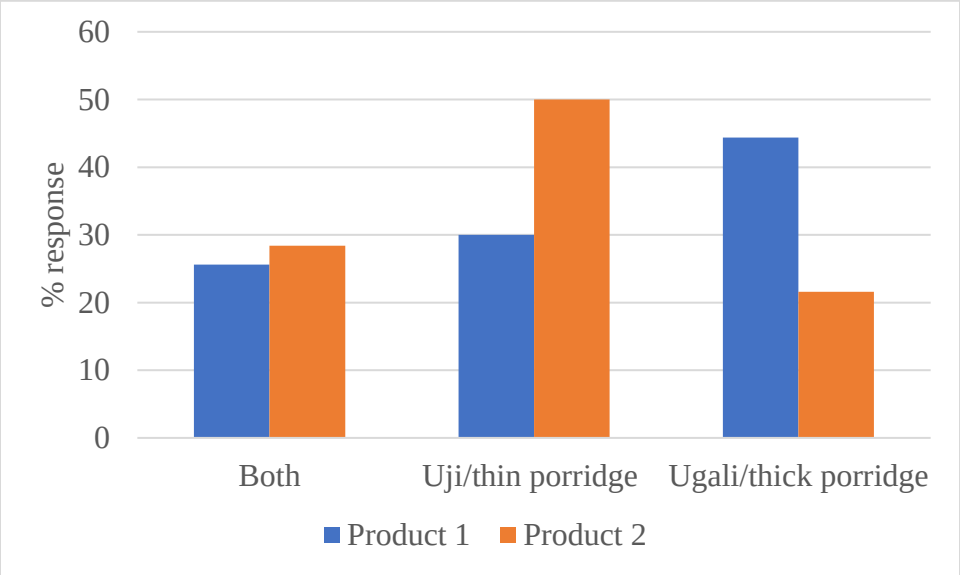


Figure 4.2. Food preparations that would be made from the two instant fortified porridge flours. This information was gathered after completion of the experimental auctions.

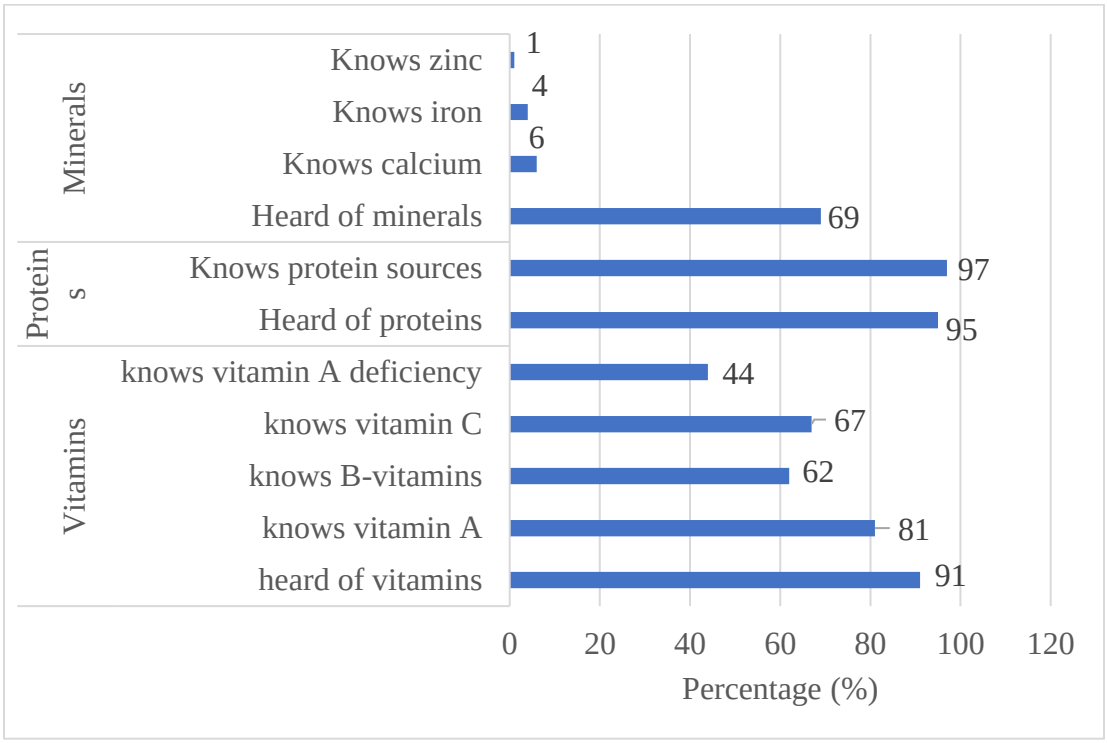


Figure 4.3. Nutritional knowledge of participants about minerals, protein, and vitamins.

CHAPTER 5. CONCLUSIONS AND FUTURE WORK

In this study, a low-cost single-screw extrusion technology was assessed for use by small- and medium-scale processors in Africa to process instant whole grain porridge flours with and without natural fortificants ("food-to-food fortification"), consumers' sensory acceptability willingness to pay for formulated food-to-food fortified instant porridge flour products to reach 25% RDA of pro-vitamin A, iron, and zinc, and how extrusion might be used to increase fermentability of fiber and thus its value for the gut microbiome.

In the first study, while extrusion of whole grains is typically done at 35% feed moisture content, it was found that lowering the feed moisture content to 27% still produced instant products with good pasting profile and expansion ratio, but with a darker color, and with reduced extrudate moisture content and drying time. Since the drying unit used was a small oven that is not typically designed for mass drying, future experiments should test drying times using an appropriate drying oven that can handle mass drying and calculate the energy used during drying.

In the second study, we observed that extrusion increased short chain fatty acids production in *in vitro* fecal fermentation experiments. Especially, extrusion at low feed moisture led to the greatest increase in SCFAs. Further, extrusion had modest impact on the chemical composition by reducing the arabinose and xylose proportions in extruded compared to non-extruded brans. Extrusion conditions tested in this study didn't alter alpha and beta diversity but showed some trend to promote genera of of *Subdoligranulum, Eubacterium hallii* and *Ruminococcus torques* groups extrusion at 30% FM and 400 RPM Donor 1 compared to non-extruded bran. Further, in Donor 1, extruded brans increased genera in the family of Enterobacteriaceae compared to non-extruded bran. There was also a trend of increase in *Subdoligranulum* and *Blautia* in extruded compared to non-extruded bran in Donor 2, and *Lachnospiraceae NK4A136* group was increased at 20% and 25% FM at 200 RPM and 30% FM at 400 RPM compared to non-extruded bran in Donor 3. In the last study, instant whole grain porridge flour blends varying in sorghum and maize were fortified with natural micronutrient sources (baobab, amaranth, orange fleshed sweet potatoes) and were assessed in terms of their acceptance, and willingness to pay by consumers when given incrementally increased information on nutrient composition, label information on preparation of instant flours, and demonstration of preparation of instant flours. Products were

ranked highly in terms of their sensory characteristics and Product 1 that was higher in maize was preferred to make thick porridge (*ugali*), while Product 2 that was higher in sorghum was preferred for making thin porridge (*uji*). Taste, texture in the mouth, and aroma were the major factors that influenced the overall acceptability of the porridges. In terms of the willingness to pay, the premium for Product 1 was higher when participants were demonstrated how to prepare the instant porridges, while Product 2 had a higher premium placed when participants were given nutrient composition information. Apparently, the instant quality of the flour was not important to consumers of the high sorghum instant porridge formulation, as premiums for Product 2 reduced when additional information of how to reconstitute the instant flours and a practical demonstration of how to reconstitute the flour. Time of the day at which the participant did the willingness to pay experiments is the only factor that positively influenced willingness to pay for both products. Generally demographic characteristics had little influence on the willingness to pay for both products perhaps because these population are already consuming their non-instant versions. Since participants were exposed to the products for a short time during the auction, an in-home use study where participants were given the product to use for an extended time would make them more familiar with the products and probably increase their willingness to pay for them. Additionally, since the participants were given nutrition information on product composition and nutrients present, further work should include additional information on the functions of the nutrients. This is because some participants may not be aware of the functions of these nutrients in the body, and buying habits may be influenced by this knowledge.

APPENDIX A. INFORMED CONSENT FORM AND QUESTIONNAIRE USED DURING MARKET STUDY EXPERIMENTS

Informed consent, consumer evaluation and determination of WTP for fortified maize products in Eldoret

Questionnaire and tools

1. Introduction

Show-up fee

Dear Sir, Madam, we are glad you came for this experiment. As a token of our appreciation, we are providing you with 625 Kshs.

Informed consent

- We work for Eldoret University, in collaboration with Purdue University and CIMMYT.
- We would like to ask you some questions on cereal production and utilization, and will invite you to taste and evaluate some new cereal products, so see if it is worthwhile bringing them to the market.
- Your name will be kept confidential.
- We will explain all procedures step-by-step, do not hesitate to ask any questions.
- Taking part in this study is voluntary; you do not have to participate. If you choose to take part, you have the right to stop at any time and there will be no consequences. We would like to thank you for your full cooperation in advance.

Participant code……signature……Date……

For the enumerator

I have described to the respondent this research study, its purpose, risks and benefits of participation, steps that will be taken to protect the privacy of the respondent and his/her household, and the voluntary nature of participation. The respondent was given the opportunity to ask questions regarding the study, and I have provided all answers to the respondent's satisfaction. I confirm that the respondent has freely consented to participate in this study.

Enumerator's name: _____signature _____Date_____/_____/ 2018

2. Identification of the participant

1. Date of evaluation (dd/mm/yy) _____/_____/_____
2. Place _____
3. Participant code_____
4. Participant name_____
5. Sex _____age _____
6. Completed years of education _____ (years)
7. Highest education level attained:_____ (none, some primary, primary, some secondary, secondary, some tertiary, tertiary)
8. Marital status *(check one)* 1. Unmarried 2. Married 3. Divorced 4. Widow 5. Other, specify : _____
9. Family size (members)_____
10. Occupation? *(check one)* 1. Farming 2.Employed 3.Self-employed off-farm 4. Casual labor 5.Student 6.others, specify: _____
11. Annual cash income last (in Kshs/month)

Live stock sale	Crop sale	Salary	Business (non-agriculture)	other sources	Total annual income

3. Affective tests

Please evaluate each product/food preparation in the order that it is presented to you, for five attributes (appearance, texture in hand, aroma, texture in mouth, taste) and for its overall quality. Use a five-point likert scale (dislike very much, dislike, neither like nor dislike, like, like very much). Please completely finish your evaluation of one food preparation before moving to the next food preparation. For each attribute, please indicate your score with tick marks **(X)** in the tables. To identify each product, use one of the four symbols (triangle, rectangle, circle, diamond), as indicated on their label.

Table for consumer affective test with five-point Likert scale, for five products

Product number and Food Code	Attribute	Dislike very much 	Dislike 	Neither like nor dislike 	Like 	Like very much 
1	Appearance					
	Texture in hand					
	Aroma					
	Texture in mouth					
	Taste					
	Overall					
2	Appearance					
	Texture in hand					
	Aroma					
	Texture in mouth					
	Taste					
	Overall					
3	Appearance					
	Texture in hand					
	Aroma					
	Texture in mouth					
	Taste					
	Overall					
5	Appearance					
	Texture in hand					
	Aroma					
	Texture in mouth					
	Taste					
	Overall					
3	Appearance					
	Texture in hand					

	Aroma					
	Texture in mouth					
	Taste					
	Overall					

4. Experimental auction

1. Experimental auction test round

- *Do tests round with another product that does not affect the cash-in-hand much Biscuit (2 types).*
- *First explain the procedure*
- I will show you two biscuits, one at a time, and I will ask you how much you are willing to pay for each .
- I will ask you to make bid for each of the biscuits and I will write your two bids down.
- Afterwards we will determine the binding product by drawing a number from this bag.
- Then you will draw a number from a random distribution. If your bid is higher than the random number, you will buy the product at the price equal to the number you drew. If your bid is lower than the random number, you will not buy the product.
- *Explain that it is in the best interest of the participant to bid his or her true WTP, give a numerical example.*
- Kindly note that it will be to your own benefit that your bid is the true amount that you are willing to pay for the product. In this kind of auction, if you give a lower bid than your true willingness to pay (for example you bid 20 KSHS when your WTP is 100 Kshs), you might lose an opportunity to buy when you draw a number of 40. If your bid is too high, for example 200 KSHS and you draw the number 150, you have to buy at that price. At your true WTP when the number /higher bid than your true value, you are the one who ends up losing. Do you have any questions?

- Now let start our test round, please make bids for the two products:

Biscuit	Bid in KSHS
Bid for: biscuit _____	
Bid for : Lollipop Sweet _____	
Randomly assign binding product : _____ (number between 1 and 2) or symbols Random number drawn:_____ Is the bid higher than the random number? _____ 1.Yes 2.no	

If yes, the participant buys the biscuit at the random price/number

If no, the participant does not buy any biscuit.

2 Experimental auctions without information

- ❖ I will show you two packages of 0.5 kg cereal product, one of each of the products you just tasted, labeled with the same symbol, one at a time, and I will ask you how much you are willing to pay for each .
- ❖ I will ask you to make bid for each of the two packets and I will write your two bids down.
- ❖ Afterwards we will determine the binding product by drawing a number from this bag/box.
- ❖ Then you will draw a number from a random distribution. If your bid is higher than the random number, you will buy the product at the price equal to the number you drew. If your bid is lower than the random number, you will not buy the product.
- ❖ (*Invite the participant to make bids for the five products labeled with symbols, without information)*

Product	Symbol	Bid in 0.5 kg
Bid for product 1		
Bid for product 2		

- ❖ Now these are the same products, now with the label of what they contain (labels have both

the symbol and the content)

- Please make a bid for each of the five products

Product	Symbol	Content	Bid in Kshs
Bid for product 1			
Bid for product 2			

- Now please draw a number from 1 to 4 to determine the binding product with the binding bid.
  - Randomly assign binding product: _____ (number between 1 and 2), or use the symbols:

- Now, you will pick a random number from a distribution to determine the winning price for the binding product.
- If the bid you offered is higher than or equal to the randomly drawn price, you win the auction and you have to buy the product at the price of the random number you picked. Otherwise you lose the auction and you do not purchase the product.
- Kindly note that it will be to your own benefit that your bid is the true amount that you are willing to pay for the product. In this kind of auction, if you give a lower bid than your true willingness to pay (for example you bid 100 Kshs when your WTP is 400 Kshs), you might lose an opportunity to buy when you draw a number of 300. If your bid is too high, for example 500 Kshs. and you draw the number 500, you have to buy at that price. At your true WTP when the number /higher bid than your true value, you are the one who ends up losing.
- Now let start our bidding

Random number drawn: _____ (from the set of random numbers)

Is the bid higher than the random number? _____ 1.Yes 2.no

If yes, the participant buys the product at the random price/number

If no, the participant does not buy any product.

3 Experimental auctions with information

- *(The two products are labeled with symbols and text)*

❖ I will show you two bags of 0.5 kg cereal product, one of each of the products you just tasted, in the same order, now labeled with the same symbol, and with additional information on the content of the product : fortified with micronutrients, having instructions on how to reconstitute it or not, being shown a practical demonstration on how to reconstitute it or not. Please take the time to read the labels and let me know if you have any questions.

❖ Questions asked:

  ❖ 1. ……..

  ❖ 2. ……

  ❖ 3. …….

❖ Now I will ask you how much you are willing to pay for each.

❖ I will ask you to make bid for each of the five bags and I will write your five bids down.

❖ Afterwards we will determine the binding product by drawing a number from this bag.

❖ Then you will draw a number from a random distribution. If your bid is higher than the random number, you will buy the product at the price equal to the number you drew. If your bid is lower than the random number, you will not buy the product.

❖ winning price for the binding product.

❖ If the bid you offered is higher than or equal to the randomly drawn price, you win the auction and you have to buy the product at the price of the random number you picked. Otherwise you lose the auction and you do not purchase the product.

❖ Kindly note that it will be to your own benefit that your bid is the true amount that you are willing to pay for the product. In this kind of auction, if you give a lower bid than your true willingness to pay (for example you bid 100 Kshs when your WTP is 400 Kshs), you might lose an opportunity to buy when you draw a number of 300. If your bid is too high, for example 500 Kshs and you draw the number 500, you have to buy at that price. At your true WTP when the number /higher bid than your true value, you are the one who ends up losing.

❖ Now please draw a number from 1 to 2 to determine the binding product with the binding bid.

  ❖ Randomly assign binding product: _____ (number between 1 and 2), or use the symbols:

- (*Invite the participant to make bids for the five products labeled with symbols, without information)*
- Now let's start the bidding.

Product	Symbol	Content	Bid in Kshs
Bid for product 1			
Bid for product 2			

- Now, you will pick a random number from a distribution to determine if you will purchase the product

Random number drawn: _____ (from the set of random numbers)

Is the bid higher than the random number? _____ 1.Yes 2.no

If yes, the participant buys the product at the random price/number

If no, the participant does not buy any product.

APPENDIX B. HETEROSCEDASTICITY TEST FOR WTP FOR THE TWO PRODUCTS.

Response variable	Heteroscedasticity test	statistics	Pr>ChSq
WTP for product 1	White's test	79	0.1622
	Breusch-Pagan	11	0.0163
WTP for product 2	White's test	62	0.6535
	Breusch-Pagan	11	0.4112

APPENDIX C. VARIANCE INFLATION FACTOR (VIF) AND TOLERANCE FOR THE EXPLANATORY VARIABLES THAT INFLUENCED WTP

Variables	Tolerance	VIF
Product name	0.65396	1.52913
Procedure of making the instant flour	0.46066	2.17081
Demonstration of how to make the instant flour	0.61964	1.61384
Time of the day (1=morning, otherwise=0)	0.92831	1.07723
Hours after last meal	0.91853	1.08869
Gender	0.95408	1.04813
Age	0.63747	1.56869
Completed years of education	0.83445	1.19839
Marital status	0.78522	1.27353
Family size	0.86543	1.15550
Income	0.75630	1.32222

APPENDIX D. SHORT CHAIN FATTY ACIDS AND GAS PRODUCTION AT 24 HOURS FOR THE PRELIMINARY STUDY

	acetate	butyrate	propionate	Gas
Blank	6.70±0.32d	1.43±0.08e	2.44±0.13d	1.17±0.06d
FOS	49.79±1.72a	17.58±0.32a	9.12±0.18a	15.19±0.27a
25% FM 150 RPM	12.60±0.38b	1.98±0.08d	4.54±0.15b	2.10±0.22c
25% FM 300 RPM	13.73±0.65b	2.88±0.10b	4.93±0.21b	2.87±0.06b
30% FM 150 RPM	13.05±0.52b	2.52±0.09c	4.69±0.31b	2.13±0.43c
30% FM 300 RPM	13.20±0.04b	3.04±0.06b	4.95±0.15b	2.93±0.12b
Non extruded	10.92±0.31c	1.92±0.07d	3.94±0.24c	1.93±0.13d

FM= feed moisture conditions used during extrusion, RPM= rotations per minute

CURRICULUM VITAE

EMMANUEL OWINO AYUA

1. PERSONAL INFORMATION

Name: Emmanuel Owino Ayua
Address: Tel: +254 725-029914
Email: emmanuel.ayua@gmail.com

2. EDUCATIONAL QUALIFICATIONS

2016 to date: Doctor of philosophy in Food science and technology, Purdue University, West Lafayette, Indiana.

Thesis: Studies on extrusion processing of instant porridge flours for african processor optimization, acceptance, marketability for consumers, and improvement in *in vitro* fecal fiber fermentation

.

2015: M.Sc. Community Nutrition, University of University, P.O Box 1125- 3010, Eldoret, Kenya.

Thesis: Postharvest handling and value addition of African Indigenous Vegetables in western Kenya.

2007-2011: B.Sc. Food Science and Nutrition, Moi University, P.O. Box 3900 (30100), Eldoret, Kenya. **First clas**

Project: Assessment of processing methods and preservation of African Leafy Vegetables in Siaya county

2002 - 2005: Kenya Certificate of Secondary Education (K.C.S.E.), St. Mary's School Yala, P.O. Box 701 (40610), Yala, Kenya
Grade: A-(minus)

1994 - 2001 Kenya Certificate of Primary Education (K.C.P.E), Bar Sauri Primary School, P.O. Box 7 (40610), Yala, Kenya
Grade: 391 out of 500 marks

Computer Skills: MS-Word, MS-Excel, MS-PowerPoint, Internet/E-mail

3. WORK EXPERIENCE

2014 August to date: Tutorial Fellow at the Department of Food science and Nutrition, University of Eldoret, Box 1125 – 30100, Eldoret, Kenya.

- Undergraduate Courses taught: FSN 410 Dairy Products Manufacture and Marketing, FSN 412 Food and Nutrient Analysis
- Diploma Courses taught: DFN 031: Basic Food science, CHN 010: Introduction to community health, ZOO: 035: Introduction to Human Anatomy and Physiology.

2013: Part time lecturer at Mount Kenya University, Box 2591-30100, Eldoret, Kenya, Department of Nutrition and Dietetics.

Lecturing Diploma students in Food science and technology I & II, Clinical Nutrition I& II, Food Microbiology.

2011-2012: Lecturer of Food Science and Nutrition at African Institute of Research and development studies, Nairobi city campus

- Concerned with lecturing Food microbiology, Food chemistry, Specific Food Technologies, Diet therapy

2011: Internship at Kenya Agricultural and Livestock Research Institute – Box 147 – 60500, Marsabit, Kenya.

- Carrying out surveys on milk quality, milk postharvest handling technologies, platform test, microbial analysis of milk (Total plate count of bacteria).
- Value addition of milk, honey, meats and hides.
- Carrying out anthropometric measures, general food distribution and nutrition education in Laisamis, Marsabit County.

May-August 2010: Industrial attachment at Corn Products International, P.O. Box 1012 (30100),

Eldoret, Kenya.

- Was involved in corn products processing (wet milling and refining) of modified corn starches, glucose syrups, dextrose, heavy steep liquor and quality assurance procedures (proximate analysis, microbial analysis), Aflatoxin analysis, Instrumentation on (High performance liquid chromatography, UV VIS instrumentation, Atomic absorption spectroscopy).

4.0 RESEARCH EXPERIENCE

- **2016 to date:** Research Assistant at the Whistler Centre for Carbohydrate Research, Department of Food Science, Purdue University, West Lafayette, USA.
  - enhancement of colonic short chain fatty acids products by changing fiber microstructure using extrusion technology
  - economic modelling of consumer acceptance and willingness to pay for instant fortified flours with food to food fortification using the robust and tobit models.
- **2012- 2014** Research/Graduate Assistant in the Horticulture Innovation Lab – USAID that was led by University of California, Davis and implemented by Purdue University and Rutgers University in Kenya**.**

  -Carrying baseline surveys on the production and postharvest handling practices of African Indigenous Vegetables (AIVs) in western Kenya

  - Assessing the performance of Horticultural Innovation Lab mixed modes solar dryer for processing AIVs.

  -Assessing the effect of various postharvest handling practices on the nutrient composition of AIVs.

  -Assessing consumer acceptance of improved AIVs (released by the World Vegetable Centre) in western Kenya.

Voluntary activities: Participated in Eldoret town clean up during environmental day; Sports (football) during Ramogi sports day

5.0 PERSONAL VALUES

Diligence, ability to multi-task and work with minimal supervision, integrity, huge respect for diversity

Moral values: Self-identity, Power of vision.

7.0 HOBBIES

Making friends, community service, reading inspirational and current affairs literature, travelling

8.0 CAREER OBJECTIVE

To diligently contribute towards addressing the problems of food and nutrition security in Kenya by upholding professionalism, team spirit, transparency and accountability

9.0 TRAININGS, WORKSHOPS AND CONFERENCES

2020

2019: Kevin Aduol**, Emmanuel Ayua,** Elijah Kamau. Salmonella typhimurium Impairs Absorption of Nutrients and Induces Inflammation via Type 3 Secretory System. International Food safety Conference, 20-24th May, 2019, Kenyatta University, Nairobi ,Kenya,

2018: Feed Moisture influences the pasting and visco-elastic properties of pre-gelatinized flours, ACCI international, London, UK.

2018: Feed moisture optimization of single screw extruder, in sorghum in the 21st century, April 19-21st, Pretoria, South Africa.

4th- 6th October 2016: Short course on Whistler Center for Carbohydrate Research (on Starch modification and functionality, Food hydrocolloids and dietary fibers utilization by the gut microbiome), Purdue University, West Lafayette, USA

.

2015: USAID/Save the Children TOPS Postharvest refresher workshop organized by World Food Logistics Organization (WFLO), 19th to 20th February 2015, Arusha, Tanzania.

2014 Jan-2014 December: E-learning on postharvest technology of horticultural crops, Postharvest Education Foundation, Oregon area, USA**.**

2014: Ayua, E., Mugalavai, V., Simon, J., Weller, S., Obura, P. A poster presentation on the design and performance of mixed modes solar dyer for vegetable processing at Horticulture Innovation Lab – USAID symposium, 22nd -23rd July 2014, Kisumu, Kenya.

2014: **Ayua, E.,** Mugalavai, V., Simon, J., Weller, S., Obura, P. Oral presentation on effect of sun preservation on the nutrient composition of Amaranthus hybridus at Horticulture Innovation Lab – USAID symposium, 22nd -23rd July 2014, Kisumu, Kenya.

2013: Training on HIV and Nutrition by National AIDS & STI Control Program (NASCOP) 11th to 16th November 2013 in Eldoret, Kenya.

2010: Training on peer counselling (introduction to counselling, counselling techniques, drug and substance abuse), Moi University, Kenya.

2010: Phase three peer counselling at chepkoilel university college/University of Eldoret, Eldoret, Kenya.

10.0 SELECTED PUBLICATIONS

Hugo De Groote, Violet Mugalavai, **Emmanuel Ayua**, Michael Ndegwa, Bruce Hamaker et al. (2020). Consumer acceptance and willingness to pay for instant products with food to food fortification in western Kenya. Food and Nutrition Bulletin. https://doi.org/10.1177%2F0379572119876848

Elijah Kamau, Smith Nkhata, and **Emmanuel Ayua** (2020) Nixtamalization and extrusion conditions influence the magnitude of change in cereals and Legumes. Journal of Food science and Nutrition https://doi.org/10.1002/fsn3.1473

Violet Mugalavai, Josiah Koyalo, **Emmanuel Ayua**, Onkware Augustino (2019). Measuring consumer interest in sorghum composite flours in western Kenya. DOI: 10.29322/IJSRP.9.07.2019.p9149

Smith G. Nkhata and **Emmanuel Ayua** (2019). Limitation of dietary and β-Carotene in Prostate and Breast cancer chemoprevention based on their mechanism of action. CPQ Cancer, 1:5

Smith Nkhata, **Emmanuel Ayua**, Elijah Kamau, Bosco Shingiro (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Journal of Food and Nutrition

Smith G Nkhata and **Emmanuel Ayua** (2018). Quality attributes of homemade tomato sauce stored at different temperatures. African journal of Food science, 12 (5): 97-103.

Ayua, E., Mugalavai, V., Simon, J., Weller, S., Obura, P et al. (2016). Ascorbic acid content in leaves of Nightshade (*Solanum spp*.) and spider plant (*Cleome gynandra*) varieties grown under different fertilizer regimes in western Kenya. African journal of biotechnology, 15 (7):199-206

11.0. CLUB MEMBERSHIP

American Association of Cereal chemist international
Moi University Food Science, Nutrition and Technology Club
Kenya Nutrition Dietetics' Institute

12.0. REFEREES

Dr. Florence Wamunga
Head of Department, Family and Consumer Sciences,
University of Eldoret.
Box 1125, Eldoret, Kenya.
Tel: 0733 581471 Email: florencewakhu@yahoo.com

Dr. Gertrude Were
Senior Lecturer Department of Family and Consumer sciences,
University of Eldoret.
Box 1125, Eldoret, Kenya.
Tel: 0722780952 Email: gmwere@yahoo.com

Prof. Violet Mugalavai,
Department of Family and Consumer sciences,
University of Eldoret.
P.O. Box 1125 (30100),
Eldoret, Kenya.
Tel: 0720384145;
Email: violet.mugalavai@gmail.com

PUBLICATIONS

Ayua, E.O., Kazem, A.E., Hamaker, B.R. 2020. Whole grain cereal fibers and their support of the gut commensal Clostridia for health. *Bioactive Carbohydrates and Dietary Fibre* 24:100245