

School of Public Health

**Using response surface methodology to model the impact of
extrusion on starch digestibility and expansion of sorghum
maize composite flour**

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**This thesis is presented for the Degree of
Master of Philosophy (Public Health)
of
Curtin University**

Nov 2012

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

ACKNOWLEDGEMENTS

I would like to thank the following people for the help and support they gave me during this Masters thesis:

The Australian Research Council (ARC) and Sanitarium Health & Wellbeing for their financial support

Dr Stuart K Johnson, my supervisor, for all the time and encouragement he gave me and for not letting me quit, Thank you. Also for the support of my co-supervisors Dr Hannah Williams, Dr Ranil Coorey, and Dr Yun Zhao

Jiayue (Rachel) Chu and Shilong (Tom) Wang for the great team we made running the extruder. It would have been so difficult alone and not half as much fun.

Haelee Fenton, Komal Tulsidas, Imran Khan, and ChemCentre, for assistance with analyses

Alan Cruickshank and the Queensland Department of Employment, Economic Development and Innovation (QDEEDI) for supply of the sorghum grain used in this study

Department of Agriculture and Food Western Australia (DAFWA) for the use of the extruder and milling facilities

To the Curtin University Food Science laboratory staff for their helpfulness and support whenever I was running analyses.

To my mother for always loving me and believing I could do this

And to my Saviour for giving me the strength to finish

Abstract

Sorghum (*Sorghum bicolor*) is a cereal grain, a member of the grass family and the fifth largest cereal crop grown worldwide after maize, rice, wheat and barley. Sorghum is grown in semi arid regions around the world. Sorghum is a heat and drought resistant crop which could make it important in light of the threat of climate change and the effects of global warming. This study consisted of the high temperature high pressure twin screw extrusion cooking and characterization of three sorghum varieties (white, red and brown) to determine if an expanded sorghum maize composite flour extrudate could exhibit high levels of slowly digestible starch (SDS). Firstly, the grains underwent proximate analysis and in-vitro starch digestion assays. Secondly, the extrusion of sorghum maize composite flour was performed using an incomplete factorial screening design to determine the formulation and processing parameters that had a statistically significant effect on the starch digestibility expansion and density of the extrudates. Then response surface methodology (RSM) was used to predict the optimal levels of formulation and processing parameters to achieve the desired high SDS levels and maximum expansion. Finally the three varieties of sorghum were extruded at the predicted optimal settings and the starch digestibility and expansion of the extrudates compared.

Three varieties of sorghum grain varieties being Liberty (white, tannin free), Alpha (red tannin free), and IS 1311 (brown tannin containing) underwent pre-cleaning, milling of wholegrain before flour characterization by proximate, dietary fibre analysis and in-vitro starch digestibility assays along with commercial maize polenta.

The red sorghum was then used for preliminary extrusion processing screening studies. An incomplete factorial experimental design was used to determine which of the formulation and extrusion processing parameters (independent variables) significantly affected the SDS and expansion of extruded maize composite flour. The five independent variables investigated were identified from the literature and were: the level of sorghum flour in the flour mix; the level of total moisture in the formulation; the final barrel zone temperature of the extruder; total feed rate of the material into the extruder; and the screw speed of the extruder. From the factorial analysis it was determined that the level of SDS in the extrudate increased significantly with an increase in sorghum flour level in the flour mix

($P < 0.01$) and decreased significantly ($P < 0.01$) with the increase in final barrel temperature. Extrudate density significantly increased ($P < 0.01$) with an increase in the total moisture level in the formulation. Therefore the three independent variables chosen for optimisation by RSM were: the level of sorghum in the flour mix; the total moisture in formulation; and the final barrel zone temperature.

An RSM central composite experimental design was generated using the three independent variables identified in the factorial study. The extrudates produced were analysed for their level of SDS, expansion ratio, density and instrumental hardness. Design Expert software was used to create mathematical models showing the predicted responses of each dependent variable across the range of independent variable levels. An overlay approach using the constraints of maximum level of SDS and maximum expansion ratio predicted optimal values of: 74.67% sorghum in flour mix; 22% total moisture in the formulation; and final barrel zone temperature of 115°C from the RSM model to give 12.57 g/100g dry starch SDS and 2.49 expansion ratio. To verify the RSM model the predicted optimized sample was then manufactured in duplicate and its SDS level expansion ratio and density were determined. The actual and predicted values were not significantly different validating the predictive model.

Finally, using the predicted optimized conditions for the RSM study sorghum maize composite flour using the three varieties of sorghum (white, red, and brown) were extruded along with a control extrudate of 100% maize polenta (no sorghum). Extrudates were analysed for their level of SDS, and their density, expansion ratio and instrumental hardness. The 100% maize extrudate had a significantly higher expansion ratio and significantly lower density than the white, red and brown sorghum maize composite flour extrudates ($P < 0.05$). No significant difference in SDS level was seen between the four extrudates run at the optimal setting.

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Chapter 1 Introduction

Sorghum is the world's fifth largest cereal grain crop (FAO, 2008). While sorghum is commonly used for breads and porridges in Africa and Asia, most consumers in the western world are not familiar with the grain (Rooney and Waniska, 2000). In 2010, 15,000 kilotonnes of sorghum was harvested in Australia; almost all of which was used for animal feed (DAFF, 2010).

Sorghum is a drought resistant crop that can withstand a hotter and dryer growing season than wheat or maize making it an ideal crop for dryer regions faced with the threat of climate change and the effects of global warming (IPCC, 2007; Srivastava et al., 2010). Sorghum also has great potential as a healthy food for human consumption: as a wheat replacement since it is gluten free; and through its characteristically low rate of starch digestion (Rooney and Serna-Saldivar, 2000). Evidence shows that diets high in slowly digestible starch reduce the risk of chronic disease, especially of type 2 diabetes mellitus (Buyken et al., 2010; Jenkins et al., 1981). The use of sorghum in potentially healthy foods could increase the consumption of sorghum based products which would increase the demand for sorghum flour and therefore would be a commercial benefit for Australian sorghum growers.

Sorghum has many varieties ranging in grain colour from white to red to black. Some sorghum grain varieties contain high amounts of phenolic compounds and tannins which can act as anti-nutrients and lower the starch and protein digestibility of sorghum in the human digestive system (Rooney and Serna-Saldivar, 2000). Research into sorghum has mainly focused on increasing the availability of energy in sorghum and improving starch and protein digestion rates both for human food use and animal feed (Rooney and Serna Saldivar, 2003; Taylor et al., 2006). However the potential for sorghum based products to deliver high levels of slowly digestible starch has been minimally researched and may provide grain foods with increased health benefits.

Extrusion is the process where ingredients are fed into a closed system and forced forward by a rotating screw, finally being pushed out through a hole or die (Riaz, 2000). Extrusion can be used to make a huge variety of food items including puffed snacks, textured vegetable protein, and breakfast cereals (Huber, 2000). High temperature, high pressure

extrusion cooking is a highly controllable and efficient single-unit food manufacturing process. In this process raw or preconditioned ingredients, such as dough mixtures, and water are fed into a sealed tube or barrel in which a rotating screw carries the material forward. As it moves forward heat is applied. The heat coupled with the shear of the rotating screw produces pressure. Heat, pressure, and water react with the material causing physical and chemical changes, such as gelatinization (the irreversible breakdown of starch granules), that cook the material. The screw mechanism finally forces the cooked material through an end plate (die) into the desired shape (Huber, 2000; Thomas and Atwell, 1999). For some products the pressure inside the barrel superheats the water within the material and when it leaves the barrel and comes out of the die, the sudden release of pressure generates steam that results in product expansion to give products with acceptable texture. End product physical characteristics such as expansion, density and hardness can be affected by the temperature, pressure, feed composition and rate, screw configuration, and moisture addition during processing (Ding et al., 2006). Chemical properties such as the rate of starch digestion can also be affected by these processing parameters (Mahasukhonthachat et al., 2010a). While there has been research done on how extrusion effects sorghum starch properties (Mahasukhonthachat, et al., 2010a), research looking at the optimization of extruded sorghum to achieve a slow rate of starch digestion is missing from the literature.

Response surface methodology (RSM) is a statistical modelling approach well suited to investigate the effect of independent formulation and processing variables on food product quality (dependant variables) and to optimise a small number of formulation and processing parameters (Buyken, et al., 2010). RSM generates mathematical and graphical models of the relationships between multiple formulation and processing parameters (eg. feed rate, moisture addition) and their combined effects on product quality (eg. rate of starch digestion and product expansion). From these models optimal levels of the independent variables can be predicted in order to deliver an end product of desired quality (Bas and Boyaci, 2007; Montgomery, 2001). Although an accepted approach for product and process optimisation of other foods, studies using RSM modelling to identify optimal formulations and processing conditions for sorghum foods appear absent from scientific literature.

When exposed to moisture, heat and pressure during food manufacturing processes such as extrusion, starch under goes gelatinization; a disruption of starch granule molecular

bonds that leads to granular swelling and starch solubilisation, resulting in greater susceptibility of starch to enzymatic digestion (Thomas and Atwell, 1999). The more degraded the starch granule in a food is when it is eaten, the more quickly it will be digested and absorbed into the human body. The measurement of the rate of starch digestion can be performed using in-vitro models of the human gastrointestinal tract (Englyst et al., 1999; Englyst and Englyst, 2005). It has been demonstrated that moisture level and screw speed in extrusion can be manipulated to maximise gelatinization and increase the rate of in-vitro starch digestibility of sorghum flour in order to maximise available energy in animal feed (Mahasukhonthachat, et al., 2010a). However it may also be possible to manipulate the extrusion parameters to minimize starch gelatinization and thereby maximise the levels of slowly digested starch. Studies investigating the development of an extrusion process to maximise the slowly digestible starch level in sorghum extrudates whilst having expansion for texturisation could not be found in the literature.

The aim of this project was to develop an optimal extrusion process for sorghum-maize composite flour where both product expansion and at the same time the level of slowly digestible starch (SDS) are maximised. The effect of different levels of sorghum, water, barrel temperature, screw speed, and feed rate during extrusion on the level of SDS in the resulting extrudates and their physical properties was determined. Factorial screening experimental design was used to identify the independent variables most significantly affecting the dependant variables. RSM using central composite experimental design was used to determine optimal levels of independent variables predicted to achieve the desired results of an extrudate with the highest possible level of SDS, whilst having expansion to ensure acceptable texture. A comparison of the SDS levels and physical properties of extrudates made with different varieties of sorghum (white, red and brown grain colour with differing levels of polyphenols which could potentially effect the rate of starch digestion) was performed. The optimised conditions for extrusion identified in this study could aid in the future commercial development of a sorghum based snack food with potential health-protective benefits such as reducing the risk of type 2 diabetes mellitus (Buyken, et al., 2010; Jenkins, et al., 1981).

References:

- Bas, D., Boyaci, I.H., 2007. Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering* 78, 836-845.
- Buyken, A.E., Mitchell, P., Ceriello, A., Brand-Miller, J., 2010. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* 53, 406-418.
- DAFF, 2010. Agricultural Food Production. In: Forestry, D.o.A.F.a. (Ed.) *Australian Food Statistics 2009-10*. Commonwealth of Australia 2011.
- Ding, Q.-B., Ainsworth, P., Plunkett, A., Tucker, G., Marson, H., 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. *Journal of Food Engineering* 73, 142-148.
- Englyst, K.N., Englyst, H.N., 2005. Carbohydrate bioavailability. *British Journal of Nutrition* 94, 1-11.
- Englyst, K.N., Englyst, H.N., Hudson, G.J., Cole, T.J., Cummings, J.H., 1999. Rapidly available glucose in foods: an in-vitro measurement that reflects the glycemic response. *American Journal of Clinical Nutrition* 69, 448-454.
- FAO, 2008. Food and Agricultural Commodities Production Food and Agriculture Organization of the United Nations
- Huber, G.R., 2000. Twin-Screw Extruders. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.
- IPCC, 2007. Climate Change 2007. In: M.L. Parry, O.F.C., J.P. Palutikof, P.J. van der Linden and C.E. Hanson (Ed.) *IPCC Fourth Assessment Report: Climate Change 2007*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jenkins, D., Wolever, T., Taylor, R., Barker, H., Fielden, H., Baldwin, J., Bowling, A., Newman, H., Jenkins, A., Goff, D., 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 34, 362-366.
- Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J., 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. *Journal of Cereal Science* 51, 392-401.
- Montgomery, D.C., 2001. *Design and analysis of experiments*, 5th ed. John Wiley, New York.
- Riaz, M.N., 2000. Introduction to Extruders and Their Principles. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.
- Rooney, L.W., Serna-Saldivar, S.O., 2000. Sorghum In *Handbook of Cereal Science and Technology*, ed. K. Kulp and J. G. Ponte Jr. Marcel Dekker, New York, pp. 149-175.
- Rooney, L.W., Serna Saldivar, S.O., 2003. Sorghum. In: Benjamin, C. (Ed.) *Encyclopedia of Food Sciences and Nutrition*. Academic Press, Oxford, pp. 5370-5375.
- Rooney, L.W., Waniska, R.D., 2000. Sorghum Food and Industrial Utilization. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons Inc, pp. 689-730.
- Srivastava, A., Naresh Kumar, S., Aggarwal, P.K., 2010. Assessment on vulnerability of sorghum to climate change in India. *Agriculture, Ecosystems and Environment* 138, 160-169.
- Taylor, J.R.N., Schober, T.J., Bean, S.R., 2006. Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science* 44, 252-271.
- Thomas, D.J., Atwell, W.A., 1999. *Starches* / David J. Thomas and William A. Atwell Eagan Press, St. Paul, Minn.

Chapter 2 Literature review

2.1 Sorghum

Sorghum (*Sorghum bicolor*) is a member of the grass family. It is the world's fifth largest cereal grain after maize, rice, wheat and barley (FAO, 2010). Some of the largest producers of sorghum include Nigeria, India, and The United States (FAO, 2010). Although Australia is not in the top producers worldwide (ranked below 20th), domestically, sorghum was ranked as the 4th largest crop for cereal grains produced in 2010 (DAFF, 2010). Sorghum has been cultivated for over a thousand years and the grain comes in many varieties and colours. Originating in Central Africa, sorghum is grown in semi-arid regions around the globe (De Wet, 1971; Pray and Nagarajan, 2009). Many traditional dishes and beverages are made with sorghum in India and Africa including porridge, breads, biscuits and beer. In the western world, sorghum is used almost exclusively for animal feed; however, as a gluten free grain, sorghum flour is used for some specialty foods (Rooney and Waniska, 2000; Taylor, et al., 2006).

This chapter focuses on sorghum, extrusion, starch digestion and Response Surface Methodology (RSM). It describes the composition, processing and future prospects of sorghum grain; explains extrusion cooking and its effect on sorghum and starch digestion; discusses in-vivo versus in-vitro starch digestion and compares in-vitro methods; and introduces RSM statistical modelling.

2.1.1 Chemical composition

The different varieties of sorghum grain can differ in colour from white through red to black. The grain is a small, single-celled seed (caryopsis) made up of three main parts: the outer layer (pericarp), the nutrient storage (endosperm) and the germ or embryo (Rooney and Serna-Saldivar, 2000) (Figure 2.1). The endosperm, where most of the nutrients are contained, has a unique protein matrix; the most abundant protein present is kafirin (Rooney and Serna-Saldivar, 2000).

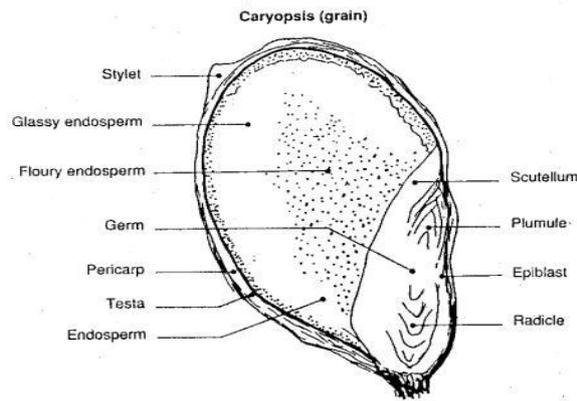


Figure 2.1 Diagram of Sorghum grain

(FCD, 1995)

The starch, like in maize and wheat, is present in granules and consist partially crystalline aggregates of amylose and amylopectin (Thomas and Atwell, 1999). Amylose and amylopectin are both anhydroglucose polymers (linked by the α -1, 4 bonds to form linear chains and linked by the α -1, 6 bonds to form branches). Amylose is mainly linear with only a few branches, while amylopectin is heavily branched and a much bigger polymer than amylose (Dona et al., 2010). Most sorghum varieties have about 75% branched amylopectin; however some sorghum starches are considered “waxy” due to their starch having little or no amylose (Rooney and Serna-Saldivar, 2000).

Starch can be described as having six levels of structure (Figure 2.2) that build on each other (Dona, et al., 2010). These basic levels of a starch granule structure apply to most grains including wheat, rice, maize and sorghum. The first level is chain length distribution or the measured length of the polymer branches; the second level is the whole molecule of starch (amylose or amylopectin) which is made up of the branched polymers (Dona, et al., 2010). The third level is the lamellar structure in which starch molecules are packed together to form double helix structures that are either crystalline (uniform structure) or amorphous (random or no structure); It is generally considered that the amylopectin is crystalline while the amylose is amorphous (Dona, et al., 2010). The next level of structure is the starch granule which itself is made up of the crystalline and amorphous layers alternating to create a concentric shell. The fifth level is the endosperm in which the starch granules are stored with the protein and lipids. It is in this way that sorghum differs from wheat or maize because sorghum does not have a proper hull. It has a matrix made of disulfide-bonded protein and starch particles; this matrix is harder to break down than a hull during processing and therefore has a lower digestion rate when eaten (Dendy, 1995;

Dona, et al., 2010). The sixth and final level is the whole seed made up of the endosperm, germ, and pericarp (Figure 2.2).

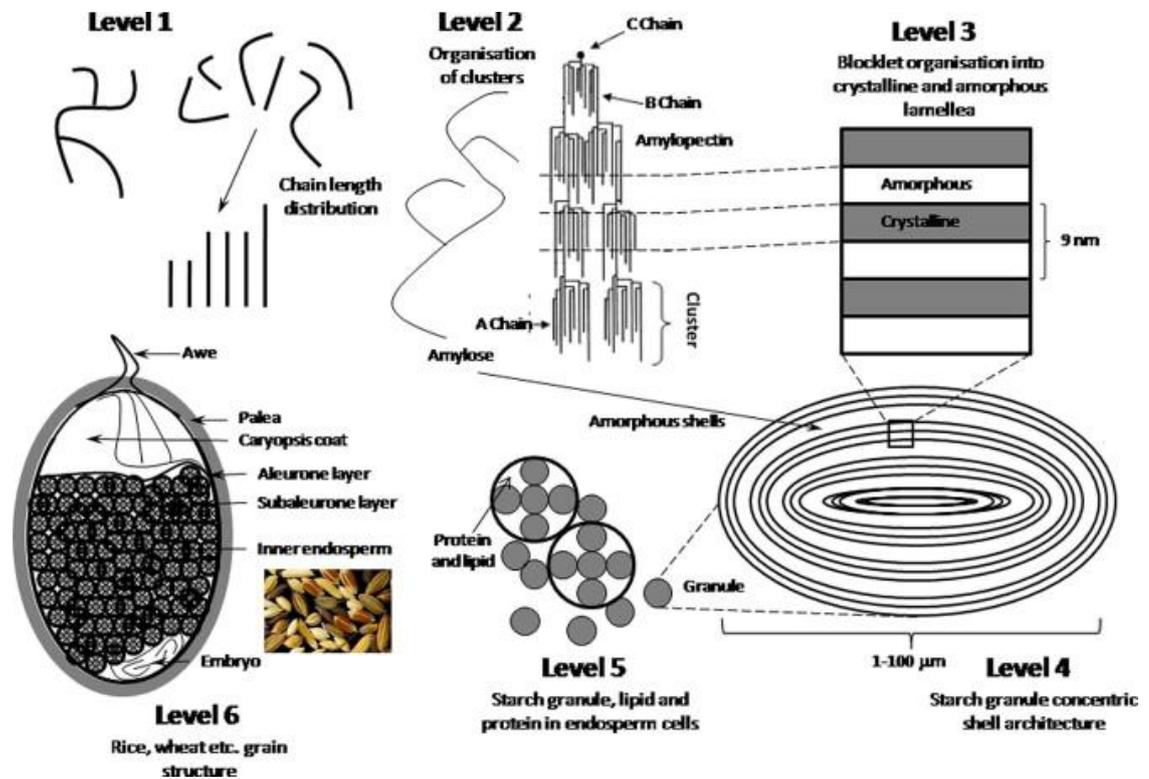


Figure 2.2 Six supramolecular levels of rice grain starch

(Dona, et al., 2010)

As stated above, starch granules are bound up in the protein matrix. During cooking, when water and heat cause the starch granules to gelatinize, the protein matrix may prevent water and heat from accessing starch; without heat and water, the granules will not swell and collapse (gelatinization) and therefore will not be readily available to digestive enzymes (Rooney and Serna-Saldivar, 2000; Rooney and Serna Saldivar, 2003; Taylor and Emmambux, 2010). In a recent review by Taylor and Emmambux (2010) it was concluded that it is the unique protein matrix in sorghum grain that inhibits the digestion of sorghum starch rather than the starch properties *per se*.

All sorghum grain varieties contain phenolic acids, the type and amount is related to the colour of the grain; brown (dark coloured) sorghum also contains polyphenolics tannin. Phenols, especially tannins, protect the grain from insects and disease threats. However,

they also act as an antinutrient when the grain is consumed lowering digestion and hence energy availability from the Sorghum grain (Rooney and Serna-Saldivar, 2000; Waniska and Rooney, 2000). In general, sorghum is very similar in proximate composition to maize and wheat; in particular they have similar amounts of starch and protein (Table 2. 1). However, the presence of the protein matrix and phenols in sorghum cause it to have lower energy availability than wheat or maize which creates possibilities for the use of sorghum in a low energy diet and health food products.

Many processes have been developed to increase the availability of energy from sorghum and improve its digestion rate for human food use in developing countries and for animal feed (Rooney and Serna Saldivar, 2003; Taylor, et al., 2006). White sorghum has been the focus of these studies as it has a lower phenol content and is more acceptable to humans when incorporated into foods, due to its light colour and mild flavour (Taylor, et al., 2006). However, sorghum varieties with darker grain colours and hence higher levels of phenols and tannins when used in foods have been shown to have reduced energy availability (Rooney and Serna-Saldivar, 2000).

Table 2.1 Comparison of nutritional composition of wholegrain sorghum, maize, and wholegrain wheat flours

Component	Wholegrain sorghum flour (g/100g Dry Wt)	Maize flour (g/100g Dry Wt)	Wholegrain wheat flour (g/100g Dry Wt)
% amylose in Starch	25	28	26
Protein	12	9	13
Fat	3	4	3
Ash	2	2	2
Dietary fibre	8	6	11
Total carbohydrate	74	76	72

(FAO, 1998; Rooney and Serna-Saldivar, 2000; USDA, 2009)

2.1.2 Milling and processing

After harvest, most sorghum in Australia is fumigated with phosphine, an insecticide gas. After fumigation the grain must be tested for residual gas levels (Thyer, 2008). According to the United States Food and Drug Administration's established tolerances for residue of phosphine; raw sorghum grain, having been treated postharvest by phosphine fumigation,

should have a residual phosphine reading of 0.1ppm or less (FDA, 1999). The Australian Pesticides and Veterinary Medicines Authority (APVMA) have also established maximum residue limits (MRLs) for pesticides and other chemicals with phosphine on cereal grains having an upper limit of 0.1ppm (APVMA, 2010).

Sorghum grain can be milled by either a wet or dry method. In the wet method, the grain can be steeped in SO₂ before it is milled and the pericarp (bran) and germ are removed by flotation (Rooney and Serna-Saldivar, 2000). In dry milling, abrasive disks or stones can first be used to remove the pericarp, this process is known as decortication or pearling. The pearling process usually removes 10-30% of the original grain weight (Rooney and Serna Saldivar, 2003). If whole meal sorghum is produced, roller mills can be used to mill the entire grain without decortication (Rooney and Serna-Saldivar, 2000). When sorghum grain is prepared for animal feed, steam flaking is the most common processing method employed prior to feeding (Rooney and Serna-Saldivar, 2000).

The particle size of the milled flour will have an effect on how the sorghum flour behaves during processing, as well as its nutrient availability. The smaller the particle size the more the starch granules are disrupted and broken (ie damaged starch) making them more susceptible to gelatinization (Al-Rabadi et al., 2011; Dona, et al., 2010; Garber et al., 1997). For example, a study investigating the effect of particle size of milled barley and sorghum on starch digestibility reported that the level of starch digestion decreased when the particle size of either flour increased (Al-Rabadi et al., 2009).

Most sorghum based food products are made with decorticated flour. Decortication or pearling removes the outer layer of the grain and reduces the percent of fibre and ash in the final flour; in the case of sorghum, decortication also removes some of the tannins and slightly increases nutrient digestion (Rooney and Serna-Saldivar, 2000). In addition to the traditional flat breads and porridges, sorghum traditionally has been mixed with wheat to make leavened bread and cakes. Sorghum can be mixed with maize to make tortillas. Some varieties of sorghum can be brewed into beer and others can be popped like popcorn (Rooney and Serna-Saldivar, 2000; Taylor, et al., 2006)

2.1.3 The future of sorghum

For the last several decades, research has been ongoing to improve sorghum as a crop and as an animal feed. India, starting in the 1960's, successfully developed sorghum hybrids to increase yield rates (Pray and Nagarajan, 2009). Milling studies have led to improved flour yield (Awika et al., 2002). Studies have also investigated processing methods to improve energy availability when used for animal feed as well as research into wider uses for human consumption (Mahasukhonthachat, et al., 2010a; Taylor, et al., 2006). The conventional research paradigm of breeding and processing sorghum has focused on increasing starch and protein digestibility and to increase the energy availability for animal feeds and foods for developing countries.

A new paradigm of research focusing on the reduction in the rate of starch digestion to create human foods low in glycemic index to promote good health has been reported only very recently (Yousif et al., 2012).

Sorghum may also play a major role in sustainable agriculture as global warming and climate change become prevalent. Farmers and growers face increased temperatures and decreased water availability. According to the Intergovernmental Panel on Climate Change (IPCC), projections for the next hundred years show a decrease in rainfall for the mid-latitude and dry tropic regions.

“Declines in water availability are therefore projected to affect some of the areas currently suitable for rain-fed crops (e.g., in the Mediterranean basin, Central America and sub-tropical regions of Africa and Australia)” (IPCC, 2007).

Sorghum is a drought resistant crop that requires less water than wheat or maize (Rooney and Waniska, 2000). Research into how sorghum will be affected by climate change was done by Srivastava, Naresh Kumar, and Aggarwal (2010) demonstrating how sorghum could best be grown under hotter drier conditions. It is important to investigate sorghum as a food source for human consumption so that in the event that conventional crops fail, sorghum may be utilized effectively.

As a gluten free grain, sorghum flour based foods are a challenge to produce a quality sensory texture and mouth feel (Yousif, et al., 2012). In order to utilize sorghum in human

food products, high temperature extrusion cooking may be a solution to provide products with acceptable texture.

2.2 Extrusion cooking

Extrusion is a manufacturing process in which ingredients are fed into a barrel and forced by a rotating screw inside a cylinder (or barrel) to be pushed finally out through a hole (or die) (Riaz, 2000) (see Figure 2.3). The stream of finished material is cut as it comes out of the die into discrete pieces that are known as collets or extrudates. Extrusion can be used to make a huge assortment of food items including puffed snacks, textured vegetable protein, and breakfast cereals (Huber, 2000).

Extrusion technology has been progressing for over a hundred years and has been an established process in the food industry since the 1950's. Today there are many kinds of extruders the two main classifications being single-screw and twin-screw (Riaz, 2000).

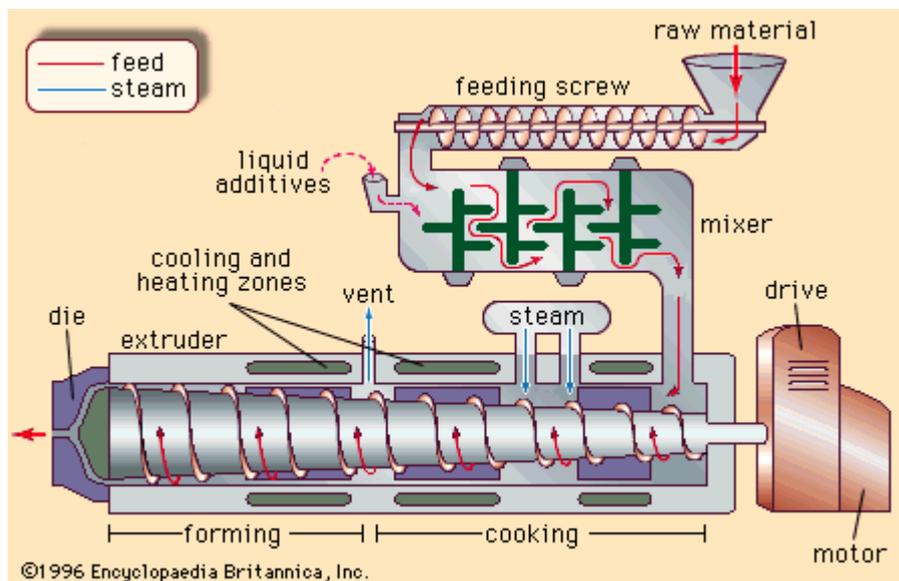


Figure 2.3 Diagram of single screw extruder cooker

(Britannica, 1996)

2.2.1 Extruder screw types

A single-screw extruder has one screw rotating through the barrel of the machine. These can be cold forming extruders that merely mix and press food through a die to form an uncooked shape, such as in pasta manufacturing, or high pressure/high temperature extruders that can cook and create expanded or “puffed” products (Riaz, 2000) (Figure 2.3).

Single screws can be one solid screw that is narrower at the beginning of the barrel and steadily grows in diameter along the barrel so that the space available to the feed is less and less as it moves towards the die; this creates pressure as material builds behind the die. There are also segmented screws that have different configurations of screw type at changeable intervals. The first screw section may be a wide screw for ease of mixing and moving the feed forward, the centre screw might be a tighter wound corkscrew which will create more pressure or longer cooking time. The choice and placement of segments can have a significant effect on the finished product (Riaz, 2000). One of the main drawbacks of single screw extrusion is the lack of mixing ability. Premixing the materials becomes necessary to ensure a homogenous product (Moscicki et al., 2011).

Twin screw extruders have gained popularity over their single screw counterparts in the food industry as they are more versatile allowing a wider range of product options. With two screws instead of one, they also can have segments without impacting diameter consistency. The two screws can turn in the same direction (co-rotating) or the opposite direction (counter-rotating); they can be together as they turn (intermeshing) or have space between them (non-intermeshing). Counter-rotating screws can be used for making products that require a high level of mixing such as chewing gum. Whereas co-rotating screws are used for puffed snack products and cereal (Moscicki, et al., 2011).

The screw is configured using small segments of metal screws that slide onto the screw core and which are set tightly together. This allows for one extruder to be configured into many different kinds of screw settings. Each piece on the screw will affect the material as it moves through the barrel (Figure 2.4). Wide helical screw segments propel the material forward quickly; if they are closer together, the effect is greater mixing and shearing of the material. The selection and location of screw pieces set on the screw core will affect the final product characteristics. The nature of the raw material and final product determines which type of twin-screw configuration to use (Huber, 2000).



Figure 2.4 Twin screw extrusion screw arrangement

(Qingdao Friend Plastic Extrusion Technology Co., 2012)

2.2.2 High temperature high pressure extrusion

High temperature, high pressure extrusion cooking is an efficient and controllable food processing method. In this type of extrusion, the barrel of the extruder is heated through segmented temperature zones; as the material progresses through the system the increasing heat coupled with the shear of the rotating screw produces pressure. Heat, pressure, and water react with the material causing physical changes (eg. mixing of the materials, evaporation of water) and chemical changes (eg. denaturation of proteins, gelatinization of starch granules)(Huber, 2000).

In some cases the pressure inside the barrel superheats the water within the material and when this material exits the barrel through the die, the sudden release of pressure generates steam that results in product expansion and a 'puffed' product is achieved. There are multiple independent factors in extrusion processing and each has an effect on the quality characteristics, (dependent variables) of the end product:

Screw speed: is the revolutions per minute (rpm) that the screw turns in the barrel and will determine how long the material resides in the barrel. This in turn will affect the cooking time of the material and hence the length of time the starch and protein in the material are exposed to heat, shear, and pressure. It will also contribute to the amount of shear on the material and the pressure level at the die (Huber, 2000).

Final barrel zone temperature: is the last temperature zone that can be controlled and has the greatest effect on the temperature of the die and the material as it goes through the die. Some models of extruder also have a controllable die temperature (Huber, 2000). The final barrel temperature will have an effect on the pressure at the die, the evaporation of water (and therefore the density of the final product) as well as the chemical breakdown of the protein and starch in the material (Ding, et al., 2006; Huber, 2000).

Total feed rate: is the quantity (kg/hr) of the dry feed plus the added water that is added to the barrel. This rate has an effect on the pressure at the die and hence can affect the expansion and density of the final product (Huber, 2000; Moscicki, et al., 2011).

Total feed moisture: is the percent (%) moisture in the total feed. This is calculated by summing the moisture present in the dry feed and the added water. Percent moisture will have an effect on the rate of gelatinisation and denaturation of starch and protein in the barrel as well as the density and hardness of the final product (Huber, 2000; Moscicki, et al., 2011). Hardness is defined as the force needed for the initial penetration (or bite) into a piece of food (Ding, et al., 2006).

The other independent variable in extrusion processing is the formulation of the dry material. The levels and types of protein, starch, sugar and salt will all have an effect on the materials' reaction to the heat, pressure and shear that occur during extrusion (Ding, et al., 2006; Mahasukhonthachat, et al., 2010a).

A study performed on the puffing characteristics of yam flour processed on a single screw extruder showed that the low extrusion temperature and high total moisture introduced into the extrusion system had a negative effect on the expansion (puff) of the extrudates (Kpodo and Plahar, 1992). Another study investigating the addition of fish mince to rice flour snack food, extruded on a co-rotating twin screw extruder, found that an increase in barrel temperature increased the expansion of the products (Dileep et al., 2010).

2.2.3 Effects of extrusion on starch digestibility

The high temperature, moisture, and shear of extrusion all have an effect on the chemical structure of the material being extruded; in particular the starch in grain-based products.

When starch is exposed to moisture and heat it undergoes a process of gelatinization; the irreversible breakdown of starch granules (Thomas and Atwell, 1999). The heat and moisture during processing cause the starch granules to hydrate, swell, and lose their semi-crystalline structure making the starch more water soluble and thereby more viscous (Thomas and Atwell, 1999). After the starch granules have expanded they break apart releasing strands of amylose and amylopectin polymers and fragmented pieces of the granule (Dona, et al., 2010). It is the breakdown of the fourth structural starch level (starch granule) that occurs during gelatinization (Dona, et al., 2010). When ingested, gelatinized starch is more readily digested and absorbed by the human body since the digestive enzymes have greater access to the starch molecules than the native starch granule (Thomas and Atwell, 1999). The temperature at which a starch begins to gelatinize is the gelatinization temperature in excess water. for maize starch this is between 60-80°C and for sorghum 65-70°C (Taylor and Emmambux, 2010). Waxy sorghum starch (with only amylopectin polymers) has been shown to have a higher gelatinization temperature zone than normal sorghum starch (Sang et al., 2008a; Taylor and Emmambux, 2010).

When the starch fragments cool they can reform a somewhat ordered structure again. This process is known as retrogradation. A retrograded starch is less susceptible to digestive enzymes than its gelatinized counterpart (Thomas and Atwell, 1999).

Shear stress, on the material during extrusion, will also lead to the breakdown of the starch granules and may increase the extent of gelatinization of the starch (Thomas and Atwell, 1999). Screw speed, total feed rate and screw configuration, may have an effect on the amount of shear, whilst increased barrel temperature and moisture level may increase the rate of gelatinization and retrogradation of the starch (Moscicki, et al., 2011). The formulation of the dry feed and its starch structure will also affect how much the extrusion process will damage the starch.

While there has been research reported on how extrusion effects starch properties, research investigating the optimization of extruded sorghum to achieve extrudates with a high level of SDS is missing from the literature.

2.2.4 Sorghum and extrusion

The manufacture of many expanded snack-like food products, including those based on sorghum have been reported in the literature. One study examined sorghum mixed with corn or wheat in extruded snack products. The study showed that the addition of sorghum did not have adverse effects on the texture of the snack food and that sorghum could be used to make acceptable snack products (Youssef et al., 1990). However, this study did not look at the effect of extrusion of sorghum from a nutritional perspective. A recent study demonstrated that the particle size of the sorghum flour had an effect on the physical properties of the extrudate (Al-Rabadi, et al., 2011). Several studies have investigated the effect of extrusion of sorghum on its starch digestibility with the aim of increasing digestibility and hence energy availability for human food and animal feed (Davis and Arnold, 1995; Falcone and Phillips, 1988). In a study by Mahasukhonthachat et al (2010a) it was suggested that the same principles applied to increasing starch digestion could be applied to lower the starch digestibility in extruded sorghum for human consumption to reduce energy availability and provide health protective foods for the over nutritional population. No studies investigating this new paradigm are however found in the literature.

2.3 Starch digestion in humans

Starch is the most abundant macronutrient in most grains. In wheat, maize, and sorghum around 70% of the grain weight is starch (Taylor and Emmambux, 2010). The rate of starch digestion in the human system can be affected by intrinsic and extrinsic factors. Intrinsically the composition of the levels of the starch structure and the amount of gelatinization and retrogradation it goes through during processing will affect starch digestibility. Extrinsic factors are elements such as chewing, the level of enzymic activity in the gastrointestinal tract, and the presence of other foods during digestion (Englyst et al., 1992).

2.3.1 Physiologically important starch fractions as measured in-vitro

In an effort to categorise the complexity of the human starch digestion, an in-vitro classification of starch digestion was created based on the rate of glucose appearance after starch hydrolysis by digestion enzymes (Englyst, et al., 1992). The classifications of physiologically important starch fractions are: total starch (TS), rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS is determined by

measuring the rapidly available glucose (RAG) released between 0 and 20 minutes of in-vitro enzyme activity (excluding any free glucose (FG) or glucose from sucrose already present); SDS is determined by measuring the slowly available glucose (SAG) released between 20 and 120 minutes. Resistant starch is the difference between TS and RDS plus SDS (Englyst, et al., 1992). Although these in-vitro classifications cannot fully account for all the variability of human digestion, they give a clear and reproducible measurement that can be used to rank the potential in-vivo starch digestibility of foods (Dona, et al., 2010; Englyst, et al., 1992).

2.3.2 Englyst vs. Sopade and Gidley in-vitro starch digestion methods

The Englyst in-vitro starch digestion method uses colorimetry or high performance liquid chromatography (HPLC) to measure the glucose released from hydrolysed starch at 20 minutes and 120 minutes to determine starch fractions RDS, SDS, RS (resistant starch, i.e. starch that has not been hydrolysed after 120 minutes) and TS (total starch) (Englyst, et al., 1992). The Englyst method has several elements that help simulate human digestion including glass beads in the sample tubes, a shaking 37°C water bath to simulate in-vivo digestion motion and average body temperature, as well as a mixture of digestion enzymes to ensure a realistic breakdown of the sample food matrix (Englyst, et al., 1999). However, this method is time consuming and has many steps and several dilutions, each of which need to be carefully controlled to ensure a correct calculation of the starch value. In addition, a recent review stated that the Englyst method only took two readings at 20 and 120 minutes of digestion and therefore did not take into consideration the mechanism of the starch hydrolysis (as informed by the shape of the starch hydrolysis curve over time) (Dona, et al., 2010).

A more recent In-vitro starch digestion method developed by Sopade and Gidley also measures the release of glucose from starch hydrolysis (Sopade and Gidley, 2009a). It utilizes an enzyme mixture, along with motion and temperature, to simulate in-vivo digestion (Sopade and Gidley, 2009a). In contrast to the Englyst method, this is a more rapid method that measures the released glucose with the use of a glucometer. The quick and easy use of the glucometer allows for time-course measurement of glucose release as well as determining the starch fractions of RDS, SDS, and RS. However, the method relies on the accuracy of the glucometer unit; if high levels of other sugars, such as fructose or lactose, are present in the sample the glucometer reading may be affected (Sopade and

Gidley, 2009a). This method should therefore be most accurate for foods with little or no free sugars. A study investigating the time-course of starch digestion in extruded sorghum compared to non-extruded sorghum using this glucometer-based in-vitro method, found a significant ($P < 0.05$) correlation between extruder response and starch digestion (Mahasukhonthachat, et al., 2010a).

2.3.3 In-vivo vs. in-vitro assay

While in-vitro assays can never fully simulate in-vivo human digestion, they have many merits that make them a viable scientific approach. The purpose of an in-vitro digestion assay is to simulate the physiological and the physicochemical conditions of human digestion (Hur et al., 2011). There are many elements of human digestion that an in-vitro assay may not or cannot take into account, such as individual metabolic rate, age and health, type and amount of food eaten, as well as what hour of the day the food is eaten. However, by using a standard method that mimics in-vivo traits, the results for in-vitro assays can potentially be reproduced from lab to lab and allow researchers a standardized and relatively inexpensive means of measuring and ranking the rate of nutrient digestion and absorption (Dona, et al., 2010; Hur, et al., 2011). A recent review on in-vitro methods highlighted several of the elements that must be addressed in order for the method to mimic the in-vivo process. These include timing the assay to match human digestion and adding enzymes at the proper intervals and amounts to simulate the human digestive system. In some methods only the enzyme of the nutrient of interest is utilized; an example is amylase for starch. However, it is also important to consider the interactions of the different food components (Hur, et al., 2011). The review concludes by saying that,

“In vitro digestion systems are common and useful tools for the analysis of foods and drugs.”(Hur, et al., 2011)

However, it goes on to say that further work needs to be performed to verify the correlation between in-vitro and in-vivo assays. A study evaluating in-vitro vs in-vivo starch digestion found that the breakdown of amylopectin happened at different rates in-vitro than in-vivo, being more rapidly digested in-vitro; the study went on to say that the complexity of digesting a full meal in-vivo was not fully and accurately described by current in-vitro methods (Hasjim et al., 2010).

There have been studies that show significant association between in-vivo and in-vitro digestion. Englyst et al. (1999) demonstrated that a significant correlation ($p < 0.0001$) existed between the glycemic response and rapidly available glucose (RAG) intake when human test subjects were fed a range of meals containing different levels of RAG. This study by Englyst et al. is however one of the few studies that has directly addressed the association between an in-vitro defined measurement (RAG) and a human in-vivo response (Englyst, et al., 1999). RDS and SDS can be determined by measuring RAG and SAG respectively; glycemic response is used to calculate GI; this shows that an in-vitro method can potentially simulate in-vivo starch digestion (Englyst, et al., 1999). When running an in-vitro assay it is therefore important to use a robust method that accounts for as many of the human variables as possible (Hur, et al., 2011).

2.3.4 Slowly digestible starch health link

Slowly digested starches (SDS) typical of those found in sorghum have potential health benefits. As previously mentioned SDS and RDS are calculated, by measuring slowly available glucose (SAG) and rapidly available glucose (RAG) in-vitro respectively. The levels of RAG and SAG in a food, as measured in-vitro, are indicative of the in-vivo change in blood glucose levels (Englyst, et al., 1999). It was shown in a study by Englyst that the amount of RAG in a food matrix, as defined by in-vitro measurement, was significantly associated ($P < 0.01$) with the glycemic index (GI) of that food. An increase of RAG (and consequent decrease SAG) was associated with an increase in GI. This study was performed by determining the proximate analysis and starch fractions by an in-vitro method of a series of high starch foods (categorized by processing method) and running in-vivo assays to determine glycemic index on the same foods (Englyst et al., 2003). Conversely, an increase in SAG and therefore SDS was associated with a decrease on the GI. A similar in-vitro method was used to measure rapidly and slowly digesting starch in a study comparing postprandial starch metabolism in healthy subjects and type 2 diabetics (Seal et al., 2003). This study showed that the in-vitro starch digestion rate correlated with the blood glucose response in-vivo, suggesting that slowly digested starches (SDS) would be expected to produce a lower GI (Seal, et al., 2003).

Hence foods containing a high level of SDS have demonstrated a lower glycemic index (Jenkins, et al., 1981). A recent review concluded that a low glycemic index diet will reduce risk factors for type 2 diabetes such as elevated blood glucose and insulin levels (Buyken, et

al., 2010). The level of SDS in foods and hence the potential for the food to impact on risk factors for type 2 diabetes can be significantly influenced by the formulation and processing parameters used during manufacturing (Mahasukhonthachat, et al., 2010a). However, effect of formulation and food processing in particular on the levels of SDS in sorghum containing foods is little understood.

2.3.5 Effect of sorghum variety on starch digestibility

The structure of the starch itself will have an effect on the rate of starch digestion and the amount of the different physiologically important starch fractions. The ratio of amylose and amylopectin for instance may have an effect. Amylopectin made up of highly branched chains of glucose will tend to be more susceptible to enzyme activity than the less branched amylose. It is at the end point of a branch that susceptibility to enzymatic attack is greatest: amylopectin's many branches, each with an end point therefore make it much more available to enzymatic digestion (Taylor and Emmambux, 2010; Thomas and Atwell, 1999). In a study on rice starch digestibility, different types of rice with percentages of amylose ranging from high to very low, underwent in-vitro starch digestion. It was determined that rice with higher amylose content had greater amounts of resistant starch. It was also acknowledged that amylose:amylopectin ratio alone was not responsible for rate of starch digestion (Hu et al., 2004). Along with other factors, the intricate folding of the starch granule (see Figure 2.2) and the interaction with other elements in the grain, such as protein matrix, can be a determinant of digestion, particularly in sorghum (see Section 2.1.2) (Dona, et al., 2010). A study by Wong et al (2009) investigated two varieties of sorghum grain and found that the sorghum variety with a higher level of disulfide-cross linked protein had a lower level of digestion of both protein and starch. Other studies have shown that along with the protein, the polyphenolic compounds present in sorghum can lower starch digestibility (Waniska and Rooney, 2000). In a recent study, it was demonstrated that the gross energy of digestion (represented by the sum of in-vitro digestion of starch, protein and lipids) in sorghum grain was increased by irradiating sorghum seeds in order to reduce tannin (polyphenolics) and phytate (protein complexing compounds) content. Tannin and phytate reduction was shown to be the main reason that the energy availability from digestion increased significantly (Shawrang et al., 2011).

2.3.6 Effects of processing on starch digestion

Beyond grain structure, the chemical and physical changes that take place during food processing also have significant effects on the starch digestibility of grain (see Section 2.2.4). When a starch granule is exposed to heat, water, and shear the granule matrix is disrupted and undergoes changes such as granule swelling, gelatinization and retrogradation (Thomas and Atwell, 1999). It has been shown that increasing the cooking temperature and time of sorghum and maize flours significantly increased the digestion rate of starch, with breakdown of the protein matrix in the sorghum being an attributing factor (Ezeogu et al., 2005). The milling shear and final particle size of the flour can also be a factor influencing starch digestibility as the smaller particles have a greater surface area available to enzymatic attack. In a study on barley and sorghum it was found that as the particle size of the flour decreased, the in-vitro starch digestion increased. It should be noted that for all levels of particle size, sorghum had a lower digestion rate than the barley (Al-Rabadi, Gilbert et al., 2009) presumably due to the higher protein starch interaction in sorghum (Taylor and Emmambux, 2010). In a study evaluating the kinetics of extruded sorghum starch digestion, it was found that the independent processing variables (Section 2.2.3) of the extrusion process significantly affected the gelatinization of the starch in the sorghum extruded samples. By increasing the gelatinization, starch digestion also increased (Mahasukhonthachat, et al., 2010a). There are many documented studies on the effects of food processing on starch digestion of grain. However, the use of processing parameters to control and reduce the effects of extrusion on starch digestibility of sorghum, with the aim of maintaining slow starch digestibility while providing texture to final product through expansion, has not been reported in the literature.

2.4 Optimization of food processing using response surface methodology

Response surface methodology (RSM) is a statistical method used for optimization of processing systems. When a process is examined to see if it can be improved or optimized, the traditional technique is to keep all parameters (eg. formulation, time, temperature, speed) constant changing one at a time to alter the outcome (eg. product texture, flavour, size). This has two disadvantages: firstly the number of experimental trials needed to test every single combination of independent variables can be very expensive and time

consuming and secondly, this experimental design does not take into account the effects of interaction of process parameters (Anderson and Whitecomb, 2005; Bas and Boyaci, 2007).

RSM is a statistical predictive modelling approach that is well suited to optimizing processes of all kinds (Buyken, et al., 2010). It was first developed by Box and Wilson in the 1950's (Anderson and Whitecomb, 2005). RSM generates mathematical and graphical models of the relationships between multiple formulation and processing parameters (independent variables), and their combined effects on product quality (dependent variables or responses). From these models, optimal levels of the independent variables can be predicted in order to deliver an end product of desired quality (Bas and Boyaci, 2007; Montgomery, 2001).

2.4.1 Experimental design for RSM optimization

Assuming that all the independent and dependent variables are measurable and continuous the relationship between them is given in Equation 2.1:

$$\text{Equation 2.1} \quad y = f(x_1, x_2, \dots, x_n) + \varepsilon, \quad (\text{Bas and Boyaci, 2007})$$

where y is the dependent variable of interest and x_i the independent variables that can be manipulated in order to optimize it and ε is the statistical error (Bas and Boyaci, 2007).

There are four phases in the design that need to be considered.

Determine the independent variables in the process: Before the statistical experiment can be designed all the independent variables that exist in the process must be identified and their ranges determined. Running the machine or system and monitoring all elements of the process is performed to create the list of all the controllable processing parameters (Anderson and Whitecomb, 2005).

Screening studies on independent variables: Since the number of total independent variables in a process might be too numerous to realistically consider all of them in an RSM model; screening studies can be performed to determine which of the variables have the greatest impact on the dependent variables of interest (Anderson and Whitecomb, 2005).

Factorial 2^k designs are often used in screening studies where k is the number of independent variables in the model and $k-1$ denotes an incomplete design commonly used when $k \geq 5$ (Montgomery, 2001). In a 2^k factorial design only two levels of a variable are measured (usually the maximum and the minimum). Different variables may have different units and ranges; therefore the independent variables in a factorial design are coded, giving each variable a range of -1 to 1. A regression analysis can be performed and variables ranked according to their respective influences on the dependent variables. The two or three most influential independent variables can then be used in the RSM model (Bas and Boyaci, 2007; Montgomery, 2001).

Selecting the experimental design and generating models: A statistical design often used for RSM is the central composite design (CCD). It is based on the factorial 2^k maximum and minimum (-1 to 1) settings, but it adds a centre point setting (0) as well as axial or alpha ($-\alpha$, α) settings. The centre point is replicated several times to ensure an accurate model. The axial or α settings are important as they allow quadratic terms in the model; they are usually set at the square root of k . this allows for a robust, spherical CCD (See Figure 2.5) (Montgomery, 2001).

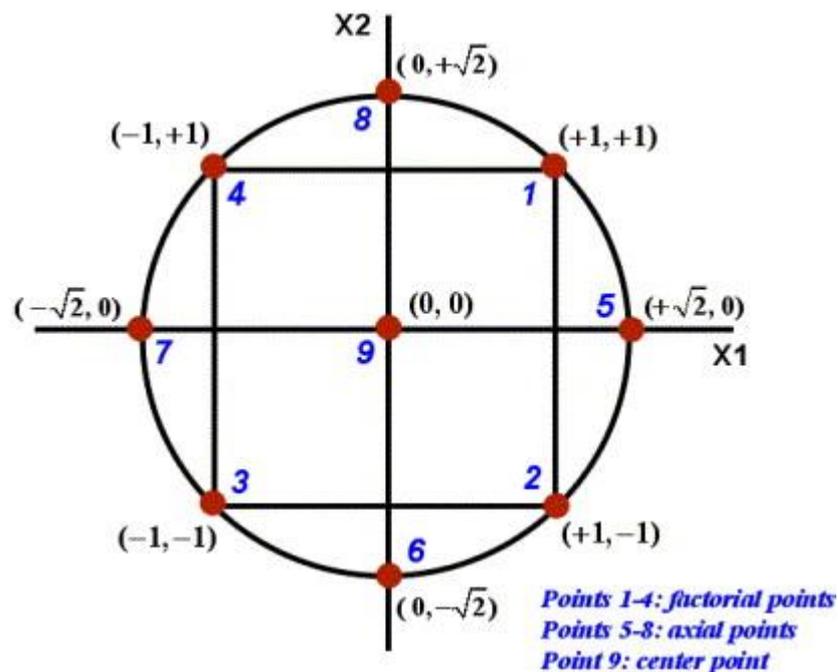


Figure 2.5 Example of a Central Composite design $k = 2$

(Lee et al., 2007)

The equation of regression for a CCD is given in Equation 2.2 (Montgomery, 2001):

Equation 2.2

$$y = \beta_0 + \sum_i \beta_i x_i + \sum_i \beta_{ii} x_i^2 + \sum_{i < j} \beta_{ij} x_i x_j + \varepsilon$$

Each independent variable is tested at five points: $-\alpha$, -1 , 0 , 1 , α ; in order to identify if/how the independent variables will have an effect on the dependent variables. With the use of statistical software a design for the experimental runs can be generated. Once the experimental runs have been completed and the results analysed the RSM mathematical and graphical models can be generated (Anderson and Whitecomb, 2005) (Figure 2.6).

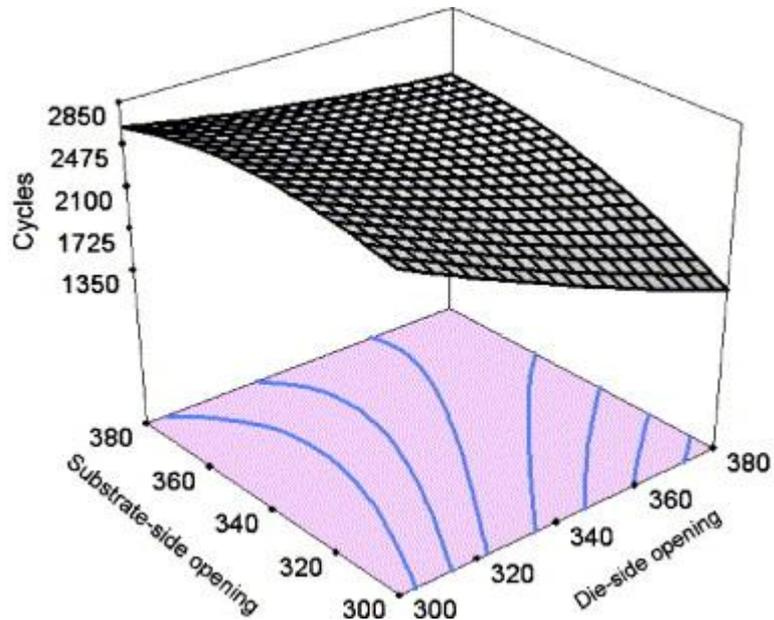


Figure 2.6 Example of a response surface graph

(Lee, et al., 2007)

Predicting and verifying of optimization: The response surface graph is helpful for visualizing the mathematical equation generated by the response surface model. By solving the regression equations and analysing the response surface contour and plot graphs, the

optimal levels of independent variables can be predicted to achieve the desired levels of the dependent variables (responses). By superimposing the RSM models of the different responses an optimal zone can be determined from where each dependant variable is as close to its ideal optimal as possible, until it is constrained by the other variables (Anderson and Whitecomb, 2005). From the optimal zone the predicted optimal settings can be determined. Validation processing runs, using these predicted optimal settings for independent variables, can be made and the dependent variables determined to verify the accuracy of the RSM model (Anderson and Whitecomb, 2005).

2.4.2 Response surface methodology in food processing systems

In a review by Nwabueze (2010), the use of RSM for food bioprocesses was evaluated. The author concluded that RSM is very valuable for food processing systems research and can be used to improve product quality by optimisation. RSM can evaluate the independent variables individually or in combination with the other independent variables (Bas and Boyaci, 2007). It is cost effective and efficient because it uses a small number of experimental runs as compared to traditional experimental designs (Montgomery, 2001).

A study, by Thakur and Saxena (2000), utilised RSM to optimize the formulation of a corn and green gram dhal flour-based extruded snack food. While the extruder settings were kept constant, the ingredients were varied, including the addition of several natural gums (e.g. guar gum, xanthan gum). By measuring expansion ratio and sensory responses, it was determined that the level of corn flour, green gram flour, and gaur gum had the greatest effect on the final product. By superimposing the RSM graphs, an optimal formulation was determined (Thakur and Saxena, 2000). A study on extruded sorghum flour used RSM to evaluate the physical appearance and nutritional changes that occurred with the addition of cowpea and groundnut flour to the formulation. It was shown that by increasing cowpeas and groundnut level, the protein, fat and ash of the extrudates were significantly increased; additionally a change in colour occurred with the increase in cowpea (Asare et al., 2010). RSM was used to optimize the increase of nutrients and appearance value. Although accepted as an approach for product and process optimisation of other foods, studies using RSM modelling to indentify optimal formulations and processing conditions for sorghum foods in order to maximize the amount of slowly digestible starch while maintaining expansion, appear absent from scientific literature.

2.5 Summary

Sorghum is a cereal grain crop that has great potential to be used as a health promoting food in the Western world. With its drought and high temperature tolerance, it may be grown more easily than wheat in the future due to of climate change. In addition its gluten free nature adds to its value as a useful alternative grain. The characteristic low starch digestibility and high polyphenolic antioxidant content of sorghum indicates its potential as an ingredient in health promoting foods, in particular to protect against type 2 diabetes and related chronic disease.

Extrusion can be used to manufacture many different kinds of foods, including puffed snack foods. The twin-screw, high temperature-high pressure extruder cooker is a versatile extruder that is well suited to making expanded snack products. Many extrusion processing parameters affect the product outcome including: ingredient formulation; moisture content; screw speed; barrel temperature; and total feed rate. The setting of the extrusion parameters will have an effect on the starch digestibility, as well as the expansion and density of the final extruded product. High moisture content and high temperature and shear levels will increase the gelatinization of the starch granules potentially increasing starch digestibility. However it may be possible to control the extrusion parameters to create an expanded extrudate with minimal gelatinization and hence slow starch digestibility, although this approach has not yet been reported in the literature.

Starch is an abundant nutrient in cereal grains including sorghum. Slowly digested starches (SDS) typical of those found in sorghum have potential health benefits. For instance, whole grains containing high levels of SDS, as measured using in-vitro models of the human gastrointestinal tract, have been shown to elicit lower post-prandial blood glucose response than food containing starches from refined grains that are more rapidly digested. Hence foods containing a high level of SDS demonstrate a lower glycemic index. A low glycemic index diet has been shown to reduce risk factors for type 2 diabetes such as elevated blood glucose and insulin levels. The level of SDS in an extruded sorghum food and hence the potential for the food to impact on risk factors for type 2 diabetes may be significantly influenced by the variety of sorghum grain used, as well as the formulation and processing parameters used during extruder manufacturing.

In-vitro starch digestion assays to determine the levels of physiologically important starch fractions such as SDS, are reproducible laboratory methods that simulate in-vivo digestion. There are many in-vitro methods for determining physiologically important starch fractions. The method designed by Sopade and Gidley (2009a) offers a rapid method that has been shown to accurately and reproducibly measure starch digestibility.

Response surface methodology (RSM) is a statistical modelling approach well suited to investigate the effect of extrusion formulation and processing parameters on sorghum extruded food products. RSM generates mathematical and graphical models of the relationships between multiple formulation and processing parameters (independent variables) and their combined effects on product quality (dependant variables or response). From these models optimal levels of the independent variables can be predicted in order to deliver an end product of desired quality. RSM is efficient since fewer tests are required to develop a full understanding of the interaction of multiple independent variables on end product response of interest.

Therefore the objectives of the study described in this thesis were to test the hypotheses that:

- Processing and formulation variables affect the level of slowly digestible starch, expansion and density of extruded sorghum:maize composite flour.
- There are optimal levels of formulation and extrusion conditions for high levels of SDS and extrudate expansion.
- Sorghum variety (white vs. red vs. brown) has an effect on extruded sorghum SDS levels, density, expansion and hardness of sorghum:maize composite flour extruded under optimal conditions.

References:

- Al-Rabadi, G.J., Torley, P.J., Williams, B.A., Bryden, W.L., Gidley, M.J., 2011. Particle size of milled barley and sorghum and physico-chemical properties of grain following extrusion. *Journal of Food Engineering* 103, 464-472.
- Al-Rabadi, G.J.S., Gilbert, R.G., Gidley, M.J., 2009. Effect of particle size on kinetics of starch digestion in milled barley and sorghum grains by porcine alpha-amylase. *Journal of Cereal Science* 50, 198-204.

Anderson, M.J., Whitecomb, P.J., 2005. RSM Simplified: Optimizing Process Using Response Surface Methods For Design of Experiments Taylor & Francis Group, LLC, Boca Roton.

APVMA, 2010. Maximum residue limits in food and animal feedstuff. A.P.V.M.A (Ed.) Commonwealth of Australia

Asare, E.K., Sefa-Dedeh, S., Afoakwa, E.O., Sakyi-Dawson, E., Budu, A.S., 2010. Response surface methodology for studying the effects of feed moisture and ingredient variations on the chemical composition and appearance of extruded sorghum-groundnut-cowpea blends. International Journal of Food Engineering 6.

Awika, J.M., Suhendro, E.L., Rooney, L.W., 2002. Milling value of sorghums compared by adjusting yields to a constant product color. Cereal Chemistry 79, 249-251.

Bas, D., Boyaci, I.H., 2007. Modeling and optimization I: Usability of response surface methodology. Journal of Food Engineering 78, 836-845.

Britannica, E., 1996. Extruder Encyclopaedia Britannica Inc.

Buyken, A.E., Mitchell, P., Ceriello, A., Brand-Miller, J., 2010. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. Diabetologia 53, 406-418.

DAFF, 2010. Agricultural Food Production. In: Forestry, D.o.A.F.a. (Ed.) Australian Food Statistics 2009-10. Commonwealth of Australia 2011.

Davis, D.A., Arnold, C.R., 1995. Effects of two extrusion processing conditions on the digestibility of four cereal grains for *Penaeus vannamei*. Aquaculture 133, 287-294.

De Wet, J.M., 1971. The Origin and Domestication of *Sorghum bicolor*. Economic botany 25, 128.

Dendy, D.A.V., 1995. Sorghum and Millets : Chemistry and Technology American Association of Cereal Chemists St. Paul, Minn.

Dileep, A.O., Shamasundar, B.A., Binsi, P.K., Howell, N.K., 2010. Composition and quality of rice flour–fish mince based extruded products with emphasis on thermal properties of rice flour. Journal of Texture Studies 41, 190-207.

Ding, Q.-B., Ainsworth, P., Plunkett, A., Tucker, G., Marson, H., 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. Journal of Food Engineering 73, 142-148.

Dona, A.C., Pages, G., Gilbert, R.G., Kuchel, P.W., 2010. Digestion of starch: In vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release. Carbohydrate Polymers 80, 599-617.

Englyst, H.N., Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition 46, S33-S50.

Englyst, K., Vinoy, S., Englyst, H., Lang, V., 2003. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. The British Journal of Nutrition 89, 329.

Englyst, K.N., Englyst, H.N., Hudson, G.J., Cole, T.J., Cummings, J.H., 1999. Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. American Journal of Clinical Nutrition 69, 448-454.

Ezeogu, L.I., Duodu, K.G., Taylor, J.R.N., 2005. Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. Journal of Cereal Science 42, 33-44.

Falcone, R.G., Phillips, R.D., 1988. Effects of feed composition, feed moisture, and barrel temperature on the physical and rheological properties of snack-like products prepared from cowpea and sorghum flours by extrusion. Journal of Food Science 53, 1464-1469.

FAO, 1998. Carbohydrates in human nutrition. In: Repository, F.C.D. (Ed.) food and agriculture organization of the united nations.

FAO, 2010. Food and Agricultural Commodities Production Food and Agriculture Organization of the United Nations.

FCD, 1995. Chapter VII Sorghum: Post-harvest Operations. In: AGSI/FAO: Danilo Mejia (Technical), B.L.L.S., Carolin Bothe (HTML transfer) (Ed.) Food Security Department

FDA, 1999. Phosphine; tolerances for residues. In: Regulations, C.o.F. (Ed.) 40 CFR 180.225. U. S. Food and Drug Administration, United States of America, p. 1.

Garber, B.W., Hsieh, F., Huff, H.E., 1997. Influence of Particle Size on the Twin-Screw Extrusion of Corn Meal. *Cereal Chemistry* 74, 656-661.

Hasjim, J., Lavau, G.C., Gidley, M.J., Gilbert, R.G., 2010. In vivo and in vitro starch digestion: Are current in vitro techniques adequate? *Biomacromolecules* 11, 3600-3608.

Hu, P., Zhao, H., Duan, Z., Linlin, Z., Wu, D., 2004. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Science* 40, 231-237.

Huber, G.R., 2000. Twin-Screw Extruders. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.

Hur, S.J., Lim, B.O., Decker, E.A., McClements, D.J., 2011. In vitro human digestion models for food applications. *Food Chemistry* 125, 1-12.

IPCC, 2007. *Climate Change 2007*. In: M.L. Parry, O.F.C., J.P. Palutikof, P.J. van der Linden and C.E. Hanson (Ed.) *IPCC Fourth Assessment Report: Climate Change 2007*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jenkins, D., Wolever, T., Taylor, R., Barker, H., Fielden, H., Baldwin, J., Bowling, A., Newman, H., Jenkins, A., Goff, D., 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 34, 362-366.

Kpodo, K.A., Plahar, W.A., 1992. Effects of extrusion variables and starch modification on the puffing characteristics of yam (*Dioscorea alata*) flour. *Food Control* 3, 200-204.

Lee, C.-C., Lee, C.-C., Ku, H.-T., Chang, S.-M., Chiang, K.-N., 2007. Solder joints layout design and reliability enhancements of wafer level packaging using response surface methodology. *Microelectronics Reliability* 47, 196-204.

Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J., 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. *Journal of Cereal Science* 51, 392-401.

Montgomery, D.C., 2001. *Design and analysis of experiments*, 5th ed. John Wiley, New York.

Moscicki, L., Zuilichem, D.J.v., 2011. *Extrusion-Cooking and Related Techniques* In: Moscicki, L. (Ed.) *Extrusion-Cooking Techniques - Applications, Theory and Sustainability*. Wiley-VCH Verlag & Co, Weinheim, Germany.

Nwabueze, T.U., 2010. Review article: Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimisation in the food systems. *International Journal of Food Science & Technology* 45, 1768-1776.

Pray, C.E., Nagarajan, L., 2009. *Improving Crops for Arid Lands: Pearl Millet and Sorghum in India*. In: Institute, I.F.P.R. (Ed.) *Pearl Millet and Sorghum Improvement in India*. Douglas Smith, Washington DC, pp. 83-88.

Qingdao Friend Plastic Extrusion Technology Co., L., 2012. *Twin Screw Extruders Technology Support By: Cnsem.net* p. Picture of Extruder.

Riaz, M.N., 2000. Introduction to Extruders and Their Principles. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.

Rooney, L.W., Serna-Saldivar, S.O., 2000. Sorghum In *Handbook of Cereal Science and Technology*, ed. K. Kulp and J. G. Ponte Jr. Marcel Dekker, New York, pp. 149-175.

Rooney, L.W., Serna Saldivar, S.O., 2003. Sorghum. In: Benjamin, C. (Ed.) *Encyclopedia of Food Sciences and Nutrition*. Academic Press, Oxford, pp. 5370-5375.

Rooney, L.W., Waniska, R.D., 2000. Sorghum Food and Industrial Utilization. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons Inc, pp. 689-730.

Sang, Y., , Bean, S., Seib, P.A., Pedersen, J., Shi, Y.-C., 2008. Structure and functional properties of sorghum starches differing in amylose content. *J. Agric. Food Chem. Journal of Agricultural and Food Chemistry* 56, 6680-6685.

Seal, C.J., , Daly, M.E., Thomas, L.C., Bal, W., Birkett, A.M., Jeffcoat, R., Mathers, J.C., 2003. Postprandial carbohydrate metabolism in healthy subjects and those with type 2 diabetes fed starches with slow and rapid hydrolysis rates determined in vitro. *British Journal of Nutrition* 90, 853-864.

Shawrang, P., Behgar, M., Shahhoseini, G., Sadeghi, A.A., Zareshahi, H., 2011. Study of chemical compositions, anti-nutritional contents and digestibility of electron beam irradiated sorghum grains. *Food Chem. Food Chemistry* 125, 376-379.

Sopade, P.A., Gidley, M.J., 2009. A Rapid In-vitro Digestibility Assay Based on Glucometry for Investigating Kinetics of Starch Digestion. *Starch - Stärke* 61, 245-255.

Srivastava, A., Naresh Kumar, S., Aggarwal, P.K., 2010. Assessment on vulnerability of sorghum to climate change in India. *Agriculture, Ecosystems and Environment* 138, 160-169.

Taylor, J.R.N., , Schober, T.J., Bean, S.R., 2006. Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science* 44, 252-271.

Taylor, J.R.N., Emmambux, M.N., 2010. Developments in our understanding of sorghum polysaccharides and their health benefits. *Cereal Chemistry* 87, 263-271.

Thakur, S., Saxena, D.C., 2000. Formulation of extruded snack food (gum based cereal-pulse blend): optimization of ingredients levels using response surface methodology. *Lebensmittel-Wissenschaft und-Technologie* 33, 354-361.

Thomas, D.J., Atwell, W.A., 1999. *Starches / David J. Thomas and William A. Atwell* Eagan Press, St. Paul, Minn.

Thyer, R., 2008. *Pest Paradise Raises Grain Storage Stakes Ground Cover*. Grains Research and Development Corporation.

USDA, 2009. *Sorghum USDA National Nutrient Database for Standard Reference*. United States Department of Agriculture.

Waniska, R.D., Rooney, L.W., 2000. Structure and Chemistry of The Sorghum Caryopsis. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons Inc, pp. 649-688.

Wong, J.H., Lau, T., Cai, N., Singh, J., Pedersen, J.F., Vensel, W.H., Hurkman, W.J., Wilson, J.D., Lemaux, P.G., Buchanan, B.B., 2009. Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. *Journal of Cereal Science* 49, 73-82.

Yousif, A., , Nhepera, D., Johnson, S., 2012. Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability. *Food Chemistry* 134, 880-887.

Youssef, A.M.M., Moharram, Y.G., Moustaffa, E.K., Bolling, H., El-Baya, A., Harmuth, A.E., 1990. New extruded products from sorghum. *Food Chemistry* 37, 189-199.

Chapter 3: Grain and flour specifications of white, red, and brown varieties of sorghum and of maize

3.1 Abstract

Sorghum is a drought and high temperature tolerant grain, with a characteristically low rate of starch digestion, which has potential use in healthy snack foods with high amounts of slowly digestible starch (SDS). Evidence shows that diets high in SDS may reduce risk of chronic disease, especially of type 2 diabetes mellitus. Three sorghum grain varieties being Liberty (white), Alpha (red), and IS 1311 (brown) underwent pre-cleaning, milling, and along with commercial maize flour, underwent compositional and physical analysis and vitro starch digestibility analysis. The brown sorghum was shown to contain tannins while the white and red were tannin free. The maize flour was shown to have a significantly larger particle size than the three sorghum flours ($P < 0.01$). The proximate analyses of the four flours were shown to be similar to other published accounts. In-vitro starch digestibility demonstrated that the brown sorghum flour had significantly lower SDS ($P < 0.01$) and significantly higher RS ($P < 0.01$) content than the maize and the other sorghum flours. Maize was shown to have significantly higher starch damage than the three sorghum flours ($P < 0.01$).

3.2 Introduction

Sorghum (*Sorghum bicolor*) is a cereal grain, a member of the grass family and the fifth largest cereal crop grown worldwide after maize, rice, wheat and barley (FAO, 2010). Sorghum is grown in semi-arid regions around the world, and is a heat and drought resistant crop which could make it important in light of the threat of climate change and the effects of global warming (IPCC, 2007; Srivastava, et al., 2010). In this study, a composition of raw grains was performed to compare the key nutritional properties of the grains and to understand if any differences in the raw grain properties are reflected in those of the extruded materials produced in later studies

Sorghum starch, although present at a similar content to that found in other cereal grains such as wheat or maize, has a characteristically lower rate of starch digestion (Rooney and Waniska, 2000; Taylor and Emmambux, 2010). It has been suggested that this lack of

digestibility and hence energy availability from starch is due not to the starch itself, but the presence of antioxidant polyphenolics and a unique protein matrix surrounding the starch granules (Taylor and Emmambux, 2010). A grain that exhibits these low energy availability traits may be less than ideal in developing nations, where food may be in short supply, or for animal feed, where high energy availability from starch is desired, (Mahasukhonthachat, et al., 2010a). Evidence shows that diets high in slowly digestible starch (SDS) reduce the risk of chronic disease, especially of type 2 diabetes mellitus (Buyken, et al., 2010; Jenkins, et al., 1981), therefore in a developed country where obesity and related chronic diseases are a concern, sorghum may be ideal for the development of foods with potential health related benefits linked to SDS.

Sorghum is a grain that is not widely used for human consumption in developed nations (Rooney and Waniska, 2000), though it has limited commercial application in gluten free foods. In order to effectively utilise sorghum in the development of healthy human foods it is important to fully characterize the grains and determine their compositional and nutritional properties and physical attributes that may influence the nutritional quality and physical quality attributes of the final products.

In-vitro starch digestibility is a useful and reproducible method for understanding the rate of starch digestion of foods and the levels of physiologically important starch fractions under conditions simulating the physiological and physicochemical conditions of human digestion (Hur, et al., 2011). However there are aspects of human digestion that an in-vitro model may not simulate accurately. Examples include: individuals' metabolic rate, age and health; type and amount of food eaten; and time of day the food is eaten. However, by using a standard method that mimics in vivo conditions, the in-vitro assay can potentially be reproduced from lab to lab and allow researchers a standardized and relatively inexpensive means to compare the rate of nutrient digestion and hence potential in vivo glucose response to foods (Dona, et al., 2010; Hur, et al., 2011).

Two in-vitro methods were considered for this study. The Englyst method uses enzymatic hydrolysis of starches that is quantified by the release of glucose as measured by glucose oxidase peroxidase colorimetric assay (Englyst, et al., 1992). Englyst defined the physiologically important starch fractions of rapidly digested starch (RDS: starch digested in-vitro between 0 and 20 minutes), slowly digested starch (SDS: starch digested in-vitro between 20 and 120 minutes), resistant starch (RS: starch digested after 120 minutes), and

total starch (TS: the sum of RDS, SDS and RS). In this assay the RDS, SDS, and TS can all be determined using the same sample giving continuity to the measurements. Also the use of glass beads, 37°C temperature, and shaking of the sample vials helps simulate in vivo conditions. The use of a several enzymes found in the mouth, stomach and small intestine also demonstrates the robustness of this assay (Englyst, et al., 1992). However, this assay can be time consuming when many samples need testing. Also, the number of times dilutions need to be made makes for many critical points in the process that have to be carefully controlled to ensure accuracy and precision of measurements.

The Sopade and Gidley method uses enzymatic hydrolysis of starch, but glucose release is measured by a glucometer in a continuous manner (Sopade and Gidley, 2009a). This allows the relationship between time and starch digestion to be observed. Like the Englyst method, motion and temperature of the assay are designed to simulate in vivo digestion (Sopade and Gidley, 2009a). This method is rapid, the measurements being taken and recorded as the assay progresses with limited critical points that need to be controlled. However, the assay is only as accurate as the glucometer that is used. Other sugars present in the sample could potentially interfere with the glucose reading.

The aim of this study was to characterize the intact grain properties and wholegrain flour composition, physical properties and in-vitro starch digestion properties of a white, red and brown variety of sorghum flour and maize flour. This information will be used in later studies in this thesis to aid in understanding the relationship between the raw material properties and the properties of a high temperature high pressure extruded “puffed” snack-like food product manufactured using maize:sorghum composite flour.

3.3 Methods

3.3.1 Grain

Red sorghum grain (100 kg), a tannin free variety, “Alpha”, was supplied by Lochabar Enterprises Pty Ltd (Tara, Queensland, Australia) grown in the summer of 2009-2010. White Sorghum (100 kg), a commercial hybrid “Liberty” and Brown tannin sorghum (150kg), variety IS 1311 C were grown by the Queensland Department of Agriculture, fisheries, and Forestry (DAFF) in the summer of 2010-2011, at Hermitage Research Station (Warwick, Queensland, Australia). The sorghum grain was fumigated postharvest with phosphine gas insecticide to permit import into Western Australia. Prior to use in the present study, the phosphine residue on the sorghum flour was determined with a Dräger Multi Gas Detector by ChemCenter (Bentley, Perth WA). The phosphine residue on all three sorghum grains,

red (0.03 ppm), white (0.05 ppm), and brown (<0.01 ppm,) fell below the upper limit of 0.10 ppm (APVMA, 2010) for human food manufacture. The grain was stored at 15°C, at low humidity, at the Department of Agriculture and Food Western Australia (DAFWA) until analysis.

3.3.1.1 Sorghum grain characterization

The hardness, weight and diameter of the sorghum grains were determined by standard protocols using the Single Kernel Hardness Tester (Perten SKCS 4100).

The presence of tannins in the sorghum grains was determined by the bleach test based on Waniska, Hugo, and Rooney (1992). Approximately 15g of whole sorghum seed were added to a 250ml of 7.5g KOH and 70ml NaOCl (commercial bleach). Several glass balls (1.5cm) were added and the sample incubated in a shaking water bath at 60°C for 7 min. The bleach solution was decanted off and the sorghum grains rinsed in running distilled water for ~15 sec. The treated grains were examined by eye. Sorghum grains classified as containing tannins will turn black after treatment of bleach whereas sorghum classified as not containing tannin will be bleached to a white or pale yellow colour.

3.3.2 Milling and flour storage

The sorghum grains were milled at DAFWA with a ZM 200 Retch Mill (Retsch GmbH & Co, Haan, Germany) 100% through a 500 micron screen to match the particle size of commercial food-grade sorghum flour available in Australia. After milling, the sorghum flours were vacuum packed and stored at -20°C for two weeks, to inactivate any insects present in the flour, and then at 15°C at low humidity for up to 18 months until use. Maize flour (code 23805) was purchased in a single batch from Defiance Maize Products Pty Ltd (Toowoomba, Australia) and packed and stored as for the sorghum flour. The Maize flour was chosen as the appropriate particle size 4 grade after consultation with the Defiance Maize Products company who recommended it as the grade used for other extruded snack products within the food industry.

3.3.3 Particle size

The particle size distribution of the flours was determined in triplicate with laser light scattering by air dispersion using a Mastersizer 2000 (Malvern instruments Ltd, Malvern, UK). Data was calculated by the instrument software as d(0.1), d(0.5) and d(0.9) which

represents the maximum diameter of 10%, 50% and 90% of the particles respectively. In addition the volume weighted mean particle size, $D[4,3]$, was also calculated by the software.

3.3.4 Proximate analysis

Proximate analysis was performed on all the flours in triplicate. Dietary fibre analysis was performed in duplicate. All values were expressed as g/100g dry basis.

3.3.4.1 Moisture

Moisture was determined by drying the sample in a Contherm Digital Series Oven (Scientific LTD, New Zealand) at 130°C and weighed every hour until consistent weight was achieved (~2 hours); reported moisture was the difference in weight of sample before and after drying as a percentage of the original sample weight (AOAC International, 2008).

3.3.4.2 Protein

Protein content was determined by the Kjeldahl digestion distillation method (AOAC International, 2005a). The flour samples, without pre-drying, were digested with concentrated sulphuric acid using selenium tablets as a catalyst (Lab chem- Ajax finechem), neutralised with NaOH then distilled in a Foss Kjeltactm 2100 Distiller unit to produce free ammonia. This was titrated with 0.1 M HCl to determine, by visual colour change, the percent nitrogen in the sample. Percentage nitrogen was multiplied by a factor (f) of 5.7 to convert to percent protein for all flours.

3.3.4.3 Lipid content

Lipid content was determined by the Soxhlet extraction distillation method. Pre-dried sample prepared as in Section 3.3.4.1 was used. Lipid was extracted using a Buchi Soxhlet Extraction Unit e-816 (Switzerland) using petroleum ether as the solvent (AOAC International, 2006).

3.3.4.4 Ash

Ash content was determined by charring the sample before igniting it in a Thermolyne 48000 furnace oven at 550°C overnight, with the weight of ash residue recorded upon cooling to room temperature in a desiccator (AOAC International, 2005d).

3.3.5 Total soluble and insoluble dietary fibre

Total dietary fibre content was determined by enzymatic-gravimetric analysis using the Megazyme kit K-TDFR (Megazyme International Ireland Ltd, Co. Wicklow, Ireland). In this method the sample was treated with enzymes to hydrolyse the digestible starch and protein, then ethanol was added to precipitate any soluble dietary fibre. After filtering and washing with ethanol and acetone, the residue was dried and weighed. One of the duplicate residues was evaluated for protein (as in Section 3.3.4.2) and the other for ash (as in Section 3.3.4.4). The total dietary fibre was calculated as the weight of the dried residue minus the weight of the protein and ash in the residue (AOAC International, 2005c).

The insoluble dietary fibre content was determined as for total dietary fibre (section 3.3.4.5) but without the ethanol soluble fibre precipitation step. The soluble fibre content was calculated as total dietary fibre – insoluble dietary fibre (AOAC International, 2005c).

3.3.6 Total starch content

Total starch content was determined in duplicate using the enzymatic colorimetric method (K-TSTA 04/2009 kit; Megazyme International Ireland Ltd, Co. Wicklow, Ireland), based on AOAC method, Starch (Total) in Cereal Products, Amyloglucosidase - α -Amylase Method 996.11 (AOAC International, 2005b). This method entails solubilisation of total starch using dimethylsulfoxide and enzymatic treatment of starch to release glucose which is quantified by the glucose oxidase/peroxidase colorimetric assay.

3.3.7 Amylose content of starch

The amylose content of the starch was determined by the Megazyme kit K-AMYL (Megazyme International Ireland Ltd, Co. Wicklow, Ireland). This method is based on the lectin concanavalin A (Con A) protocol developed by Yun and Matheson (1990). The total starch is dissolved by heating in dimethylsulphoxide and the lipids precipitated using

ethanol. The amylopectin is then precipitated using the Con A and is hydrolysed to D-glucose and measured colorimetrically with glucose oxidase/peroxidase reagent.

3.3.8 Damaged starch

Starch damage (%) was determined in quadruplicate by the enzymatic colorimetric method (Gibson et al., 1991) using the K-SDAM 05/11, Starch Damage Kit (Megazyme International Ireland Ltd, Bray, Co. Wicklow, Ireland).

3.3.9 In-vitro starch digestibility

In-vitro starch digestion by a rapid glucometry method was determined in duplicate using a procedure based on that of Sopade & Gidley (2009). In summary, the procedure was as follows: An accurately weighed sample of approximately 250 mg was placed in a 150 ml glass jar (Ergo Flint Glass 70mm IM-106PK-1432-FL12, Plasdene Glass-Pak, Canning Vale, Australia) to which 1 ml of artificial saliva containing porcine α -amylase (Sigma A-3176 Type VI-B; 250 U / ml 0.2 M pH 7 carbonate buffer) was added. Fifteen - 20 seconds later 5 ml pepsin suspension (9mg of pepsin, [2500 units/mg, Chem-Supply, Gillman South Australia] and 5mL 0.02 M HCl [4500 U/ ml 0.02 M HCl]) was added. The mixture was incubated at 37 °C in a reticulating water bath (85 rev. / minute) for 30 minutes. It was then neutralized with 5 ml 0.02 M NaOH, and 50 ml 0.2 M pH 6 sodium acetate buffer was added. Followed by 5 ml pancreatin mixture (0.095g pancreatin [Chem-Supply PL378] added to 3.325ml Diaxame [DIAMZYME®X4 Genencor International Inc. Rochester NY, USA] and 44.175ml 0.2 M Acetate Buffer pH]. Incubation was continued at 37 °C in a reticulating water bath (85 rev / minute). Duplicate glucometer test strips (AccuCheck® Performa®, Roche Diagnostics Aust. Pty. Ltd, Castle Hill, Australia) were dipped into the digesta at time intervals of 0, 10, 20, 45, 60, 90 and 120 minutes and the glucose concentration read from the glucometer (AccuCheck® Performa®, Roche Diagnostics Aust. Pty. Ltd, Castle Hill, Australia). Starch digestibility was expressed as digested starch (DS) in g per 100g dry starch calculated for each time point using Equation 3.1.

$$\text{Equation 3.1} \quad \text{DS} = \frac{0.9 \times G_G \times 180 \times V}{W \times S [100-M]} \quad (\text{Sopade and Gidley, 2009})$$

Where G_G = glucometer reading (mM / L), V = volume of digesta (ml), 180 = molecular weight of glucose, W = weight of sample (g), S = starch content of sample (g per 100g dry sample), M = moisture content of sample (g per 100 g sample), and 0.9 = stoichiometric constant for starch from glucose contents (Sopade and Gidley, 2009).

The glucometer was calibrated by creating a calibration curve with known concentrations of glucose in digesta at 37°C and then measured in duplicate with the glucometer. The glucometer readings were corrected by using the regression equation of the calibration curve (Figure 3.1). In addition the glucometer readings were corrected for the readings at time zero, which represented the free glucose present in the enzyme preparations used in the study.

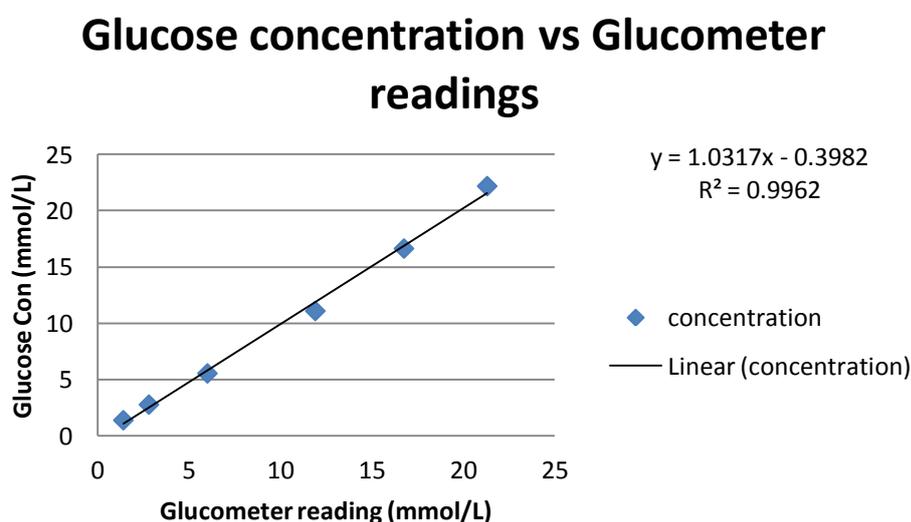


Figure 3.1 Calibration graph for glucometer readings

Digestograms of digested starch (g / 100 g dry starch) versus time of digestion with pancreatin / amyloglucosidase (min.) for each sample, corrected for the value at time zero (representing free glucose present in the enzyme preparations) were prepared.

Rapidly digestible starch (RDS) (g / 100 g dry starch) was calculated by replacing G_G in Equation 3.1 with $(G_{20} - G_0)$ representing the glucometer reading at 20 minutes minus the glucometer reading at 0 minutes. Similarly, slowly digested starch (SDS) (g / 100 g dry starch) was calculated by substituting G_G for $(G_{120} - G_{20})$ in Equation 3.1. Resistant starch

(RS) (g / 100 g dry starch) was calculated as 100 - RDS (g / 100g dry starch) – SDS (g / 100 g dry starch).

3.3.9.1 Free glucose (FG) analysis

Approximately 0.800g sample were weighed exactly into 50mL tubes and 25mL 0.1M sodium acetate buffer was added (8.203g sodium acetate anhydrous into 250mL saturated benzoic acid solution, made up to 1 litre with water). The pH was adjusted by adding 0.1M acetic acid until the pH reached 5.2, then 4mL 1M CaCl₂ was added). A blank tube with 25mL 0.1M sodium acetate buffer and duplicate glucose standard tubes with 25mL of 25g/L glucose standard were also made up. All tubes were treated in the same way for the rest of the assay (Englyst, et al., 1992).

Five glass beads (1.5cm diameter) were added to the tubes and contents vortexed before being place in boiling water. After 30 minutes the tubes were cooled to 37°C. Invertase (0.2mL , Enzyme Solutions, Croydon South, Victoria, Australia; IUB 3.2.1.26) was added to each tube and placed horizontally into 37°C water bath (~70 rev. /min) for 30 minutes. 1mL of solution was removed for each tube and place into 10mL tubes with 2mL absolute ethanol and centrifuged at 1500g for 5 minutes. 1mL of the supernatant was taken and, for sample and blank was diluted with 5mL water, for glucose standard diluted with 20mL water. The glucose content of the diluted sample was measured using the glucose oxidase/peroxidase method (Thermo Fisher Scientific Inc. Worthing, West Sussex, UK), according to manufacturer's instructions. The absorbance was measured at 510nm and the glucose present was calculated using Equation 3.2:

$$\text{Equation 3.2} \quad \% \text{ glucose} = [(A_t \times V_t \times C \times D)/(A_s \times W)] \times 100$$

Where

A_t is absorbance of test solution,

V_t is total volume of test solution,

C is concentration of standard (g glucose/L),

A_s is absorbance of standard,

W_t is weight (mg) of sample analysed (corrected or moisture),

D is dilution factor

FG assay

$V_t = 25.2$ plus 1mL per gram wet weight of sample used,

$C = 0.394$,

$D = 18$

(Englyst, et al., 1992).

3.3.10 Statistical analysis

One-way analysis of variance with Bonferroni post hoc test was used to compare mean differences in chemical composition and physical properties between flours using SPSS Statistics V18 (SPSS Inc., an IBM Company Chicago, IL, USA), with $P < 0.05$ considered statistically significant.

3.4 Results and discussion

3.4.1 Grain characteristics

Table 3.1 shows the grain characteristics of the sorghum varieties. The bleach test confirms that the Alpha variety (red) has no tannins (defined as type I) while the Brown variety 1311C demonstrated tannins but only in ~ 40 % of the grains tested (eg. type II or III) (Waniska, Hugo et al., 1992). No intact grains were available from the batch of the white variety (Liberty) used in later extrusion studies in this thesis; however another source of white Liberty variety was tested and was shown to have no tannins.

Table 3.1 Grain characteristics

Variety	Pericarp colour	Bleach Test ^a	Kernel total moisture (%) ^b	Kernel hardness index (HI) ^b	Kernel weight (mg) ^b	Kernel diameter (mm) ^b
Liberty	White	n/a	n/a	n/a	n/a	n/a
Alpha	Red	0	10.8 ± 0.38	99.5 ± 16.0	18.9 ± 3.0	1.8 ± 0.23
IS 1311C	Brown	41	11.8 ± 0.50	42.8 ± 24.2	24.9 ± 6.25	2.2 ± 0.36

^a Number of kernels, out of a 100, with pigmented testa layer demonstrating the presence of tannins. ^b Mean ± standard deviation determined using the Single Kernel Hardness Tester (Perten SKCS 4100). n/a = not applicable

3.4.2 Particle size

The particle size distributions of the sorghum flours and maize flour are given in Table 3.2. The particle size of the maize flour was significantly larger ($P < 0.01$) than the all three sorghum flours at every level of volume and the weighted mean. The brown sorghum flour particle size was significantly smaller ($P > 0.05$) than all of the other flours and flour at the 10%, and 50% volume and the volume weighted mean, however all sorghum flours had similar particle size at the 90% volume ($P > 0.05$).

Table 3.2 Particle size distribution of flours^a

	d(0.1) ^b (μm)	d(0.5) ^b (μm)	d(0.9) ^b (μm)	D[4,3] ^b (μm)
Maize flour	424 \pm 8.2 ^c	706 \pm 18.8 ^c	1206 \pm 49.8 ^c	769 \pm 22.2 ^c
White sorghum flour	33 \pm 0.3 ^d	228 \pm 0.9 ^d	503 \pm 3.1 ^d	225 \pm 1.3 ^d
Red sorghum flour	48 \pm 0.6 ^e	237 \pm 5.3 ^d	514 \pm 4.0 ^d	267 \pm 1.2 ^d
Brown sorghum flour	17 \pm 0.3 ^f	172 \pm 3.5 ^e	466 \pm 4.9 ^d	213 \pm 3.0 ^e

d(0.1), d(0.5), d(0.9), are maximum diameters of 10%, 50% and 90% (of total volume) of particles; D[4,3] is the volume weighted mean particle size.

^a Means of triplicate analyses \pm standard deviation.

^b Values within the same column with a different superscript letter are significantly different ($P < 0.05$).

The particle size of sorghum flour has been shown to have a significant effect on its physical and chemical properties. In a recent study sorghum flour was fractionated by particle size (~ 120 - $560 \mu\text{m}$) and the fractions analysed for in-vitro starch digestibility; it was shown that the smaller the particle size, the greater the rate of digestion (Mahasukhonthachat et al., 2010b). Since the particle size of the sorghum flours in the present study was smaller than the maize flour, any effect of sorghum on slowing the rate of digestion of the extruded product in later sections of this thesis may be reduced. It is therefore recommended that in future studies, beyond the scope of this thesis; larger particle size sorghum is extruded, more closely matching that of the maize flour, to aid in lowering the rate of digestion of the resulting extrudate. Another study demonstrated that the smaller particle size caused

greater torque and energy output during extrusion and also increased water absorption of the extrudates (Al-Rabadi, et al., 2011).

3.4.3 Proximate and dietary fibre analysis

Table 3.3 gives the proximate and dietary fibre composition of the sorghum flours and maize flour.

Table 3.3 Proximate and dietary fibre content of maize and sorghum flours^a

Component (g/100g dry basis)	Maize flour ^b	White sorghum flour ^b	Red sorghum flour ^b	Brown sorghum flour ^b
Protein	*9.6 ± 0.29 ^c	*9.9 ± 0.56 ^c	*8.9 ± 0.39 ^c	***11.1 ± 0.32 ^d
Lipid	0.8 ± 0.06 ^c	1.3 ± 0.08 ^d	1.4 ± 0.09 ^d	***2.8 ± 0.04 ^e
Ash	0.4 ± 0.02 ^c	1.5 ± 0.03 ^d	1.3 ± 0.06 ^e	***1.8 ± 0.06 ^f
Total dietary fibre	3.6 ± 0.33 ^c	9.0 ± 0.46 ^d	9.6 ± 0.50 ^d	***15.8 ± 0.95 ^e
Soluble fibre	0.5 ± 0.43 ^c	1.4 ± 0.85 ^c	0.3 ± 0.60 ^c	***2.1 ± 0.08 ^d
Available carbohydrate ^x	69.3	47.2	53.7	50.8
Total starch	**83.75±1.36 ^c	**68.99 ± 3.95 ^d	**74.67 ± 1.13 ^e	**82.18±0.25 ^c

* data courtesy of Shilong Wang (Curtin University)

** data courtesy of Komal Tulsidas (Curtin University)

*** data courtesy of ChemCentre (Bentley, Australia)

X available carbohydrate calculated by difference

^a Means of triplicate analyses ± standard deviation.

^b Values within the same row with a different superscript letter are significantly different (P<0.05).

The brown sorghum flour had significantly higher (P<0.01) protein content on average than the other three flours. The maize flour had significantly lower (P<0.01) lipid content than the white, red and brown sorghum flours, whereas the brown sorghum flour had significantly higher (P<0.01) lipid content than the other three flours. All of the flours had significantly different ash content (P<0.01) with the ranking for highest to lowest being brown sorghum > white sorghum > red sorghum > maize. The higher levels for the sorghum

flour would be expected as they were whole grain flours and ash is present at higher levels in the outer bran layers. The maize flour had significantly lower ($P < 0.01$) total dietary fibre content than the white, red and brown sorghum flours, whereas the brown sorghum flour had significantly higher ($P < 0.01$) total dietary fibre content than the other three flours. For soluble fibre brown sorghum had significantly higher ($P < 0.01$) soluble fibre than the other three flours. White sorghum flour had significantly lower ($P < 0.01$) total starch content than the other three flours. Red sorghum flour had a significantly higher ($P < 0.01$) level of total starch content than the white sorghum flour, but a significantly lower ($P > 0.01$) level than the brown sorghum and maize flours. The available carbohydrate content for all the flours is much lower than the total starch content indicating a high level of fibre and potentially a high level of resistant starch (RS) in the flours, which would be expected as they are raw and ungelatinised.

Except for the total dietary fibre, which was higher than the published reports, the sorghum proximate analysis for this study was in the range of published sorghum nutrient compositions on a dry weight basis (Dendy, 1995). The sorghum flour in this study is whole grain and this could be the reason why fibre amount was higher. The published accounts did not specify if the sorghum flour was whole grain or from decorticated sorghum although decorticated sorghum is commonly used for food applications (USDA, 2009). The high protein content of the brown sorghum flour suggest potential for this variety to exhibit a greater resistance to starch digestion as the protein matrix in sorghum is known to encapsulate starch granules (Wong, et al., 2009). Despite the much larger particle size of the maize flour, the low total dietary fibre content compared to the three sorghum flours may suggest a faster rate of starch digestion in the maize as dietary fibre has the potential to inhibit digestion (Thomas and Atwell, 1999).

3.4.4 Damaged starch and amylose content

The damaged starch and amylose contents of the sorghum flours and maize flour are given in Table 3.5. The maize flour had significantly higher starch damage than the three sorghum flours ($P < 0.01$) which may lead to a higher rate of gelatinisation and hence higher starch digestibility of the maize flour during processing (Thomas and Atwell, 1999).

No differences in the amylose content of the flours were observed ($P > 0.01$). The levels of amylose were slightly lower than the range of 24-33% amylose reported for “normal” (non-waxy) starch (Beta et al., 2001; Sang et al., 2008b). Amylose content plays an important

role in starch digestibility, with waxy sorghum (low amylose) demonstrating higher starch digestibility than non-waxy (Wong, et al., 2009). However, the similarity of the amylose levels of the flours in this thesis suggests this factor will not be influential in the properties of the extruded products.

Table 3.4 Starch properties and in-vitro starch digestibility of maize and sorghum flours^a

	Starch Damage (g/100g dry basis) ^b	Amylose (g/100g of starch dry basis) ^b	Rapidly digested starch (g/100g of starch dry basis) ^b	Slowly digested starch (g/100g of starch dry basis) ^b	Resistant starch (g/100g of starch dry basis) ^b	Free Glucose (g/100g of starch dry basis)
Maize	*2.22 ± 0.36 ^c	**21 ± 3 ^c	10.31 ± 0.31 ^c	24.22 ± 1.16 ^c	65.47 ± 1.47 ^c	0.41 ± 0.12 ^c
White Sorghum	*1.69 ± 0.14 ^d	**16 ± 1 ^c	11.61 ± 0.31 ^c	21.69 ± 0.13 ^c	66.70 ± 0.18 ^c	0.66 ± 0.10 ^c
Red Sorghum	*1.81 ± 0.06 ^d	**20 ± 9 ^c	9.40 ± 0.06 ^c	22.57 ± 1.43 ^c	68.04 ± 1.49 ^c	0.51 ± 0.10 ^c
Brown Sorghum	*1.35±0.02 ^d	**18 ± 4 ^c	8.27 ± 5.07 ^c	4.45 ± 0.35 ^d	87.28 ± 5.43 ^d	0.44 ± 0.12 ^c

^a Means of duplicate analyses ± standard deviation.

^b Values within the same column with a different superscript letter are significantly different (P<0.05).

*data courtesy of Haelee Fenton (Curtin University)

** data courtesy of Jiayue Chu (Curtin University)

3.4.5 In-vitro starch digestibility

The RDS levels were similar (P > 0.05) for all flour samples. However, the brown sorghum flour had a significantly lower level of SDS (P < 0.01) and a significantly higher level of RS than all of the other flours (Table 3.4). The differences seen in the starch fractions of the brown sorghum flour could be due to the tannins present in the brown sorghum grain. It has been shown that the high levels of tannins and polyphenols present in sorghum along with the unique protein matrix may be the reason for its low starch digestion (Chu, 2011; Taylor and Emmambux, 2010). The high level of resistant starch in all the flours is expected as they are in their raw unprocessed form. It is anticipated that the RS levels will decrease and the RDS and SDS levels increase during the extrusion processing in later section of this thesis (Chapter 4, 5, 6). During extrusion under heat and moist conditions gelatinization is likely to occur, increasing the starch digestibility (Mahasukhonthachat, et al., 2010a).

The in-vitro method used in this study measures the release of glucose from starch digestion by a glucometer to track glucose release over time (Sopade and Gidley, 2009). This method is rapid, the measurements being taken and recorded as the assay progresses. The calibration curve of glucose to glucometer reading showed a linear relationship with an R^2 value of 0.9962. The presence of free glucose in the sample potentially interferes with a correct reading on the glucometer, however, in this study, the free glucose in the samples was negligible. The amyloglucosidase reagent used in this study did contain some glucose, a blank reading with the enzymes present in solution was used to correct for this.

3.5 Conclusion

The sorghum grains were milled and the grains and flours characterized along with commercial maize flour recommend for extrusion. The brown sorghum was shown to have tannins while the white and red were tannin free. The maize flour was shown to have a significantly higher particle size than the three sorghum flours. The proximate analyses of the four flours were shown to be similar to other published accounts. The brown sorghum had significantly higher levels of protein, lipid, and ash than the other flours as well as significantly higher total and soluble fibre. Maize was shown to have significantly higher starch damage than the three sorghum flours. The brown sorghum was shown to have significantly lower SDS and significantly higher RS than the maize and the other sorghums. Free glucose analysis showed the absence of free sugars in all four flours. Further experiments and analyses on sorghum and maize based products will be the next step in this study.

3.6 Acknowledgements

I would like to acknowledge the funding support for this project by The Australian Research Council (ARC) and Sanitarium Health & Wellbeing. I thank Alan Cruickshank and the Queensland Department of Agriculture, fisheries, and Forestry (DAFF) for supply of the sorghum grain.

I would also like to thank Jiayue Chu (Curtin University), Shilong Wang (Curtin University), Haelee Fenton (Curtin University), Komal Tulsidas (Curtin University) and the ChemCentre for assistance with the analyses as indicated in the text and for permission to use their data.

References:

- Al-Rabadi, G.J., Torley, P.J., Williams, B.A., Bryden, W.L., Gidley, M.J., 2011. Particle size of milled barley and sorghum and physico-chemical properties of grain following extrusion. *Journal of Food Engineering* 103, 464-472.
- AOAC International, 2005a. Official Method of analysis of AOAC International AOAC Official Method 920.87. AOAC International, [Gaithersburg, Md.].
- AOAC International, 2005b. Official Method of analysis of AOAC International AOAC Official Method 923.03. AOAC International, [Gaithersburg, Md.].
- AOAC International, 2005c. Official Method of analysis of AOAC International AOAC Official Method 985.29. AOAC International, [Gaithersburg, Md.].
- AOAC International, 2005d. Official Method of analysis of AOAC International AOAC Official Method 996.11. AOAC International, [Gaithersburg, Md.].
- AOAC International, 2006. Official Method of analysis of AOAC International AOAC Official Method 963.15. AOAC International, [Gaithersburg, Md.].
- AOAC International, 2008. Official Method of analysis of AOAC International AOAC Official method 925.10. AOAC International, [Gaithersburg, Md.].
- APVMA, 2010. Maximum residue limits in food and animal feedstuff. In: Authority, A.P.a.V.M. (Ed.) Commonwealth of Australia
- Beta, T., Obilana, A.B., Corke, H., 2001. Genetic diversity in properties of starch from Zimbabwean sorghum landraces. *Cereal Chemistry* 78, 583-589.
- Buyken, A.E., Mitchell, P., Ceriello, A., Brand-Miller, J., 2010. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* 53, 406-418.
- Chu, J., 2011. Modeling the effects of extrusion on the concentration of phenolic compounds and antioxidant capacity of red sorghum *Food Science & Technology*. Curtin University of Technology.
- Dendy, D.A.V., 1995. Sorghum and millets : Chemistry and Technology American Association of Cereal Chemists St. Paul, Minn.
- Dona, A.C., Pages, G., Gilbert, R.G., Kuchel, P.W., 2010. Digestion of starch: In vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release. *Carbohydrate Polymers* 80, 599-617.
- Englyst, H.N., Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* 46, S33-S50.
- FAO, 2010. Food and Agricultural Commodities Production Food and Agriculture Organization of the United Nations.
- Gibson, T., Al Qalla, H., McCleary, B., 1991. An improved enzymatic method for the measurement of starch damage in wheat flour. *Journal of Cereal Science* 15, 15-27.
- Hur, S.J., Lim, B.O., Decker, E.A., McClements, D.J., 2011. In vitro human digestion models for food applications. *Food Chemistry* 125, 1-12.
- IPCC, 2007. Climate Change 2007. In: M.L. Parry, O.F.C., J.P. Palutikof, P.J. van der Linden and C.E. Hanson (Ed.) IPCC Fourth Assessment Report: Climate Change 2007. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jenkins, D., Wolever, T., Taylor, R., Barker, H., Fielden, H., Baldwin, J., Bowling, A., Newman, H., Jenkins, A., Goff, D., 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 34, 362-366.
- Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J., 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. *Journal of Cereal Science* 51, 392-401.

- Rooney, L.W., Waniska, R.D., 2000. Sorghum Food and Industrial Utilization. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons Inc, pp. 689-730.
- Sang, Y., Seib, P.A., Shi, Y.C., Bean, S., Pedersen, J., 2008. Structure and functional properties of sorghum starches differing in amylose content. *Journal of Agricultural and Food Chemistry* 56, 6680-6685.
- Sopade, P.A., Gidley, M.J., 2009. A rapid in-vitro digestibility assay based on glucometry for investigating kinetics of starch digestion. *Starch - Stärke* 61, 245-255.
- Srivastava, A., Naresh Kumar, S., Aggarwal, P.K., 2010. Assessment on vulnerability of sorghum to climate change in India. *Agriculture, Ecosystems and Environment* 138, 160-169.
- Taylor, J.R.N., Emmambux, M.N., 2010. Developments in our understanding of sorghum polysaccharides and their health benefits. *Cereal Chemistry* 87, 263-271.
- Thomas, D.J., Atwell, W.A., 1999. *Starches* / David J. Thomas and William A. Atwell Eagan Press, St. Paul, Minn.
- USDA, 2009. Sorghum USDA National Nutrient Database for Standard Reference. United States Department of Agriculture.
- Waniska, R.D., Hugo, L.F., Rooney, L.W., 1992. Practical Methods to Determine the Presence of Tannins in Sorghum. *The Journal of Applied Poultry Research* 1, 122-128.
- Wong, J.H., Lau, T., Cai, N., Singh, J., Pedersen, J.F., Vensel, W.H., Hurkman, W.J., Wilson, J.D., Lemaux, P.G., Buchanan, B.B., 2009. Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. *Journal of Cereal Science* 49, 73-82.
- Yun, S.H., Matheson, N.K., 1990. Estimation of amylose content of starches after precipitation of amylopectin by concanavalin-A. *Starch-Starke* 42, 302-305.

Chapter 4 Determination of formulation and processing factors affecting health-related properties of extruded sorghum-maize composite flour

4.1 Abstract

Sorghum is a drought and high temperature tolerant grain with a characteristically low rate of starch digestion that has potential use in healthy snack foods with high amounts of slowly digestible starch (SDS). Evidence shows that diets high in SDS reduce risk of chronic disease, especially of type 2 diabetes mellitus. Blends of red sorghum flour and maize flour were processed using high-temperature, high pressure extrusion cooking to manufacture expanded snack-food like products. A 2^{k-1} factorial statistical design was used to predictively model which of the following formulation and processing parameters (independent variables),

- percent sorghum in flour (%)
- the final barrel zone temperature (°C)
- the total moisture of feed (%)
- the total feed rate (kg/hr) into the extruder
- the screw speed (rpm) of the extruder

would have a significant effect on the level SDS (g/100g starch, dry wt.), density (g/ml) and expansion ratio of the extrudates. The results of the factorial analysis predicted that the level of SDS decreased significantly ($P < 0.01$) with increasing final barrel zone temperature and significantly increased ($P < 0.01$) with increasing sorghum flour level. Extrudate density was predicted to significantly increase ($P < 0.01$) with increasing total moisture of feed and significantly decrease ($P < 0.01$) with increasing final barrel zone temperature. There were no significant relationships predicted between the dependent variables and expansion ratio. Based on these findings, further research is proposed to simultaneously optimise total moisture in feed, final barrel zone temperature, and sorghum level, to maximise the level of SDS in the extrudate and provide acceptable density for a snack-food like product using a response surface methodology predictive modelling approach.

4.2 Introduction

Sorghum is a cereal crop grown in semi arid regions around the world. Its resistance to drought and heat makes it an ideal crop for regions faced with the threat of climate change and global warming (IPCC, 2007; Srivastava, et al., 2010).

Sorghum has a characteristically low rate of starch digestion (Rooney and Waniska, 2000). While sorghum has a similar starch content to wheat and maize, the presence of polyphenolics and a unique protein matrix have been shown to lower the starch digestibility and the overall energy availability in sorghum (Taylor and Emmambux, 2010). This lack of energy availability may be a problem in developing nations or when sorghum is used for animal feed as in these situations maximum energy availability from food/feed is desirable (Mahasukhonthachat, et al., 2010a). However, evidence shows that diets high in slowly digestible starch (SDS) reduce risk of chronic disease, especially of type 2 diabetes mellitus (Buyken, et al., 2010; Jenkins, et al., 1981). Therefore sorghum has the potential to be used as a health-enhancing food for human consumption in developed nations where over-nutrition leading to obesity and type-2 diabetes mellitus is an issue.

Being gluten free, sorghum products can face the sensory problem of being dense and having a disagreeable mouth-feel. A process that can induce an expanded, crunchy agreeable texture is high temperature high pressure extrusion (HTHPE) cooking. HTHPE is a highly controllable and efficient single-unit food manufacturing process (Huber, 2000). In this process raw or preconditioned ingredients are fed into a barrel in which a rotating screw mixes and shears the material as it is carried forward and heat and pressure are applied. The screw mechanism finally forces the cooked material through an end plate (die) where the sudden release of pressure can generate steam from superheated water; that results in product expansion and texturization (Huber, 2000). When flour is extruded the presence of heat, water, pressure, and shear mean the starch will undergo gelatinization, the irreversible breakdown of the starch granules (Thomas and Atwell, 1999). Gelatinized starch is more susceptible to enzymatic digestion as well as creating a more viscous and smooth texture. In expanded extruded foods, consumer acceptable mouth feel and texture may be defined in terms of expansion ratio (diameter of die to diameter of finish product) and the density of the extrudate (Ding, et al., 2006). Mahasukhonthachat, et al (2010a) demonstrated that by adjusting the moisture level and screw speed during extrusion, the rate of digestion of sorghum flour could be maximized. They postulated that a minimisation of the rate of starch digestion could also be optimized using the extrusion process. Another study investigating the effect of particle size on the physico-chemical properties of extruded sorghum and barley demonstrated that the expansion ratio did not differ significantly in sorghum with different particle sizes; neither did the expansion ratio differ significantly with a change in extruder barrel temperature (Al-Rabadi, et al., 2011). Sorghum therefore has the potential to be used to create an extruded snack-like product

that combines acceptable textural properties and high levels of SDS with its potential health related benefits.

Factorial screening is a statistical approach that can be used to predict the impact of the formulation and processing variables during extrusion processing of an expanded snack-like extrudate on its level of SDS and its expansion ratio and density. This experimental approach can then identify the most significant few independent variables for further in-depth optimisation studies. In this approach, the first step is to identify all the independent variables that exist in the formulation and process and determine the limits of their operating ranges. By physically running the machine/system and monitoring all elements of the process, all controllable independent variables can be identified (Anderson and Whitecomb, 2005). Since the total number of independent variables in a process may be too numerous to realistically consider in-depth, a screening study can be done that determines which of the variables have the most significant effect on the dependant variable of interest (Anderson and Whitecomb, 2005). Factorial 2^{k-1} designs are often used in screening studies where k is the number of independent variables in the model and $k-1$ denotes an incomplete design commonly used when $k \geq 5$ (Montgomery, 2001). In a 2^{k-1} factorial only two levels (usually the maximum and the minimum) of the independent variable are assessed. Different independent variables may have different units and ranges; therefore the independent variables in a factorial are coded giving each variable a range of -1 to 1. A regression analysis is then performed on the values of the dependant variables, which are ranked according to their predicted influence on the dependent variables. The two or three most influential independent variables can be used for formulation and process optimisation using more robust experimental designs such as response surface methodology with central composite design (Bas and Boyaci, 2007; Montgomery, 2001).

Therefore the aim of this study was to determine, using a factorial screening experimental design, the most significant formulation and processing independent variables during HTHPE of sorghum:maize composite flour impacting on the level of SDS, the density, and the expansion ratio of the extrudate.

4.3 Material and methods

4.3.1 Raw materials

Red sorghum grain (100 kg), of a tannin free variety, “Alpha”, was supplied by Lochabar Enterprises Pty Ltd (Tara, Queensland) grown in the summer of 2009-2010. Maize flour (code 23805) was purchased in a single batch from Defiance Maize Products Pty Ltd (Toowoomba, QLD). The sorghum grain was milled to a wholemeal flour at the Department of Agriculture and Food Western Australia with a ZM 200 Retch Mill (Retsch GmbH & Co, Rheinische Strabe 36, Haan Germany). The sorghum flour and maize flour were vacuum packed and stored at -20°C for two weeks and then at 15°C, at low humidity, for up to 18 months until used.

The sorghum grain and sorghum flour and maize flour previously underwent characterization and proximate analysis which has been detailed in Chapter 3 as follows: presence of tannins, kernel hardness, diameter and weight (Section 3.3.1.1); particle size (Section 3.3.3); moisture, protein, lipid, and ash (Sections 3.3.4.1-4); total dietary fibre and soluble dietary fibre content (Sections 3.3.5); total starch content (Section 3.3.6); amylose content of the starch (Section 3.3.7); and the total starch damage (Section 3.3.8). For in-vitro starch digestion of flours see Section 3.3.9

4.3.2 Extruder specifications and operation

Extrusion was performed with a twin screw extruder model MPF 19:25 (APV Baker Ltd, Peterborough, England). The extruder is a small pilot plant size machine able to be run by one or two people. 19:25 denotes a barrel diameter of 19mm and a length to diameter ratio (L/D) of 25 (Anton et al., 2009). A peristaltic pump (504U Watson Marlow Ltd, Falmouth, England) was used to dispense water into the system and a twin screw volumetric feeder (K-MV-KT20, K-Tron, Schwiez, AG) was used for dry material feeding. For this study the screw configuration (Table 4.1) was based on one reported by Anton, Fulcher, and Arntfield (2009) for the extrusion of a puffed snack food. The screw configuration was modified to fit the extruder model used (Nguyen, 2008). In the Anton et al (2009) configuration only eight feed screws were used in position one and six in position 3, also a kneading paddle was used in position 4 instead of a signal lead screw. The die used in this study was a 3mm cylindrical one.

Table 4.1 Extruder screw configuration

Design of screw configuration			
Order	Quantity		Component parts
1	11	D	Feed screws
2	4	60°	Forwarding paddles
3	1	D	Feed screws
4	2	D	Single Lead screws
5	4	60°	Forwarding paddles
6	2	D	Single Lead screws
7	5	30°	Forwarding paddles
8	4	30°	Reversing paddles
9	1	30°	Single Lead screws
10	6	60°	Forwarding paddles
11	5	60°	Reversing paddles
12	1	D	Single Lead screws- Discharge

Where the order of different screw components are listed starting at the base at 1. Each individual screw component piece is counted and the length of the piece is coded as D where D= 19mm. Paddle pieces are set at an angle to each other, the degree of rotation between the paddles is given under quantity; one paddle length = 0.25 D

The extruder screw is divided into four temperature zones. A temperature profile was determined for the two final zone temperatures used in the factorial study (Table 4.2)

Table 4.2 Temperature Profile of extruder screw zones

Temperature profile for final temperature zone	Zone 1 (°C)	Zone 2 (°C)	Zone 3 (°C)	Zone 4 (°C)
150	90	105	125	150
120	90	95	100	120

The feed rate of the water and for each sorghum:maize composite flour were determined using a calibration curve (Appendix 1) for the water pump and a dry material feeder as follows: The water pump and material feeder settings, both operated by manual dials,

ranged from 1-60 and 0.0-10.0 respectively. By measuring the weight of water or dry feed dispensed at each whole number dial setting, a calibration curve of time (hr) verses feed weight (kg) was generated (the water pump was only calibrated up to 20 on the dial as experience showed only water levels below a dial setting of 15 could make an expanded extrudate). These calibration curves were then used to ensure accuracy of total feed rate from sample to sample as different sorghum:maize ratio flours had different dry feed rate flow.

4.3.3 Experimental design

4.3.3.1 Identifying limits of key formulation and processing independent variables

The base formulation of the extrudate (determined by experience by technician) was 100% maize run at a final zone barrel temperature of 140°C, 25% total moisture content, a total feed input rate of 5kg/hr, and a screw speed of 250 rpm. From this base, foundation extrusion runs were performed in order to identify all of the controllable formulation and processing parameters (independent variables) and their upper and lower operating limits. The 100% maize flour base formula was extruded and the processing parameters identified (Table 4.3). Then 100% sorghum and 20% sorghum: maize and a 80% sorghum:maize composite flour was extruded in order to determine the maximum and minimum settings of each identified processing parameter that the equipment could produce in stable conditions while still creating an extrudate that expanded (whose diameter was greater than the 3mm die outlet)(Table 4.3).

Table 4.3 Identified extrusion independent variables

Identified independent variable	Units	Minimum	Maximum
Sorghum in mix	%	15	60
Final barrel zone temperature	°C	120	150
Total moisture of total feed	%	21.4	25.8
Total input rate	kg/hr	2.3	6.8
Screw speed	rpm	250	450

4.3.3.2 Factorial design

A two level factorial experimental design 2^{5-1} (resolution V) was selected to investigate the effects of the independent variables: percent sorghum in mix; final barrel zone temperature; total moisture of feed; total input rate; and screw speed, on the level of SDS, density, and expansion ratio of the extrudates using Design-Expert Version 7 software (Anderson and Whitecomb, 2005) (Table 4.3).

Table 4.4 Factorial independent variables with coded values used to generate the factorial screening study experimental design

Factor	independent variable	Units	Type	Minimum	Maximum	Coded Values	
X1	Sorghum in mix	%	Numeric	15	60	-1	1
X2	Final barrel zone temperature	°C	Numeric	120	150	-1	1
X3	Total moisture of total feed	%	Numeric	21.4	25.8	-1	1
X4	Total input rate	Kg/hr	Numeric	2.3	6.8	-1	1
X5	Screw speed	rpm	Numeric	250	450	-1	1

4.3.4 Extrusion processing of samples

During each extrusion run the extruder was set at the desired temperatures (°C) and screw speed (rpm) and extrusion commenced with a high level of moisture (~75% of total feed) and a low level of dry feed (25%). Then the dry feed rate was increased and the moisture rate decreased in small increments until the desired settings were reached. After every change made, a wait of two minutes would elapse in order to maintain equilibrium of the machine. When the desired settings had been achieved, and after a two minute wait, a ~300g of sample of extrudate was taken as a representative sample and the pressure (psi), die temperature (°C), and torque (%) of the extruder recorded (Appendix 2). The extrudate as a single coil was allowed to cool, unpackaged at room temperature (~24°C) for one hour and then cut into 50mm long pieces. A representative sub-sample (~270g) of the extrudate was ground in a food processor wrapped in ice packs to counter any heat generated by the motor (Robot Coupe blixer®, Montceau-en-Bourgogne, France) to pass 100% through a 1mm sieve. The ground samples were vacuum sealed (VacPac Webomatic; Geprüfte, Sicherheit; Australia) in polypropylene bags (Vital Packaging, Myaree WA) and stored at room temperature for up to 6 months before analysis for total moisture content and in-vitro starch digestion. The remaining extrudate pieces were stored whole in zip seal polypropylene bags, within plastic bins, at room temperature for 24 to 72 hours before measurement of density and expansion.

4.3.5 Density measurement

Density of the extrudates was calculated according to Equation 4.1. Ten extrudates were weighed and the length and diameter measured with callipers to calculate density and the mean density (Alvarez-Martinez et al., 1988; Ding, et al., 2006).

Equation 4.1

$$\text{Density (g/cc)} = \frac{4 \times m}{\pi \times D^2 \times L}$$

Where m equals the mass (g) of an extrudate of length L (mm) with diameter D (mm) (Ding, et al., 2006)

4.3.6 Expansion ratio measurement

Expansion ratio was determined by the ratio of diameter of extrudate (mm) and the extruder die (3.0 mm). The extrudate diameter was measured with vernier callipers in two places. Ten extrudate samples were measured per extrusion run (Alvarez-Martinez, Kondury et al., 1988; Ding, et al., 2006).

4.3.7 Total moisture

Total moisture of the extrudates was determined by drying the ground samples in a Contherm Digital Series Oven (Scientific LTD, New Zealand) at 130°C for 1 hour; reported moisture was the differences in weight (g) of sample before and after drying as a percentage of original sample weight (g) (AOAC International, 2008).

4.3.8 In-vitro slowly digestible starch

Slowly digestible starch (SDS) defined as the starch digested to glucose between 20 and 120 minutes after pancreatin/amyloglucosidase addition (Englyst, et al., 1992), was determined by the in-vitro rapid glucometry method based on Sopade & Gidley (2009b). This procedure is reported in detail in Chapter 3 (Section 3.3.9).

4.3.9 Statistical analysis

Design Expert V8 software (Stat-Ease, Inc. Minneapolis MN, USA) was used to generate the factorial screening study sample run sequence and to generate a regression analysis on the results in order to rank their influence on the dependent variables. $P < 0.05$ was considered significant.

4.4 Results and Discussion

4.4.1 Raw Materials

The sorghum grain and sorghum and maize flour characterization and proximate analysis is reported in Chapter 3 of this study as follows: sorghum grain properties and presences of tannin (Table 3.1), particle size (Table 3.2), protein, lipid, ash, total starch and fibre content (Table 3.3), and amylose and starch damaged percentage and in-vitro starch digestion (Table 3.4).

4.4.2 Extrudates



Figure 4.1 Factorial design extrudates

The extrudates in this study (Figure 4.1) varied in physical appearance in term of darkness and brown/yellowness and had an expansion ratio ranging from 1.15 to 3.56. The SDS content, density, expansion ratio and moisture content of each extrudate is given in Table 4.5. The results of the regression analysis are given in Table 4.6.

Table 4.5 Screening study experimental design processing levels and test results

Run	Sample #	X1, Sorghum in mix (%)	X2, Final barrel temperature zone (°C)	X3, Total moisture (%)	X4, Total feed rate (kg/hr)	X5, Screw speed (RPM)	SDS g/100g of starch	Density (g/cc)	Expansion (ratio)	Total moisture (%)
1	30-5-11-40	15	150	21.4	6.8	450	0.09	0.14	2.76	9.64
2	30-5-11-41	15	150	25.8	2.3	450	3.08	0.44	1.30	12.18
3	30-5-11-42	15	120	21.4	2.3	450	3.84	0.31	2.37	13.92
4	3/6/11-44	60	120	25.8	6.8	250	15.22	0.39	2.26	14.08
5	3/6/11-46	60	150	21.4	2.3	450	3.52	0.14	3.56	10.01
6	3/6/11-45	60	120	21.4	6.8	450	10.82	0.25	1.79	10.15
7	13/6/11-47	15	120	25.8	6.8	450	5.63	0.32	2.47	14.46
8	13/6/11-48	15	150	25.8	6.8	250	-3.46	0.28	2.35	25.48
9	17/6/11-49	60	150	25.8	2.3	250	-0.53	0.51	1.15	12.58
10	17/6/11-50	15	120	21.4	6.8	250	5.11	0.27	3.38	12.38
11	17/6/11-52	15	120	25.8	2.3	250	2.79	0.43	2.15	12.93
12	27/6/11-56	60	150	25.8	6.8	450	5.40	0.23	2.13	12.77
13	30/6/11-57	15	150	21.4	2.3	250	1.64	0.21	1.82	11.06
14	30/6/11-58	60	150	21.4	6.8	250	5.79	0.18	2.21	10.36
15	13/7/11-59	60	120	25.8	2.3	450	8.51	0.41	1.70	13.02
16	13/7/11-60	60	120	21.4	2.3	250	15.05	0.43	2.07	13.01

Table 4.6 Independent variable influence on responses from factorial analysis^a

Factors	Expansion ratio			Density			Slowly digestion starch (SDS)		
	RE ^a	EC ^b	P value	RE ^a	EC ^b	P value	RE ^a	EC ^b	P value
X1, Sorghum in mix (%)	-0.19	3.06	0.51	0.00	0.69	0.58	126.97	31.57	0.01
X2, Final barrel zone temperature (°C)	-0.05	0.86	0.72	-0.03	13.71	0.03	-165.40	41.13	<0.001
X3, Total moisture of total feed (%)	-1.24	19.98	0.11	0.08	37.37	<0.001	5.31	1.32	0.49
X4, Total dry feed input rate (kg/hr)	0.65	10.52	0.23	-0.04	21.17	0.01	2.80	0.70	0.61
X5, Screw speed (rpm)	0.03	0.48	0.79	-0.01	6.35	0.11	0.03	0.01	0.96

^a Real Effect, difference in total score from low to high as each factor increases

^b% Effect Contribution, calculated by dividing each factors sum of squares by the total of all the term sum of squares and multiplying by 100. This ranks the magnitude of each factors effect

P value significant at P < 0.05

4.4.3 The influence of percent sorghum in mix on density, expansion ratio, and SDS level

Increased percent of sorghum in the mix was significantly associated with an increase in SDS level of the extrudates ($P < 0.01$) (Table 4.6). This effect of sorghum flour is consistent with another recent study investigating the addition of sorghum into flatbread (Yousif, et al., 2012). The percent of sorghum in the mix however was not significantly associated with the density or expansion ratio of the extrudate. This indicates that the inclusion of high levels of sorghum in an extruded snack food might not adversely affect the textural properties allowing consideration for higher sorghum incorporation levels in future studies than used in the present study.

4.4.4 The influence of final barrel zone temperature on density, expansion ratio, and SDS level

Increased final barrel zone temperature was significantly associated with decreased density ($P = 0.03$) and decreased SDS level of the extrudates ($P < 0.01$) (Table 4.6). This is consistent with the study done by Kpodo and Plahar (1992) on puffed extruded products. Elevated temperatures are likely to increase the rate and extent of gelatinisation which might explain the decreased SDS levels (Thomas and Atwell, 1999).

4.4.5 The influence of percent total moisture in feed on density, expansion ratio and SDS level

An increase in total moisture level of feed was significantly associated with an increase of extrudate density ($P < 0.001$) (Table 4.6). These findings were consistent with other studies investigating the physicochemical properties of extruded cereal grain flours and is logical as it would be expected that water would have a greater density than cereal grain flour (Chanlat et al., 2011; Ding, et al., 2006). No association was seen between total moisture level of feed and SDS level of extrudate. This is not consistent with a previous studies that show the moisture level was the main influential variables on SDS level in extruded sorghum products (Mahasukhonthachat, et al., 2010a).

4.4.6 The influence of total input rate on density, expansion ratio and SDS level

An increase in total input rate (water plus dry feed) was significantly associated with a decrease in the density of the extrudate ($P = 0.01$) this could have been because of the higher pressure at the die when a high amount of input was present. It, however it had no association with any other dependent variable (Table 4.6). In many extrusion studies where

extrusion processing parameters are tested at different levels, the feed rate is kept constant (Anton, Fulcher et al., 2009; Mahasukhonthachat, et al., 2010a; Méance et al., 1999).

4.4.7 The influence of screw speed on density, expansion ratio and SDS level

The screw speed of the extruder had no significant association ($P>0.05$) with any of the dependent variables in the study (Table 4.6). This is consistent with some studies investigating the physicochemical properties of extruded sorghum and other grains where little or no effect was found due to the screw speed (Mahasukhonthachat, et al., 2010a; Suksomboon et al., 2011).

4.4.8 Limitations of study

The extrusion process had some limitations as some of the independent parameters are not controllable. The pressure at the die as the extrudate exits the extruder is an uncontrollable variable. If there is a physical blockage or build up of material around the die the pressure will rapidly increase causing a change in expansion ratio when all controllable parameters are set and the sample is ready to be taken. In this study samples were only taken when the pressure was relatively stable. However, in some sample runs this was difficult as the independent variable settings were close to the physical limits of the machine. These unpredictable pressure changes may be the reason why the association between the independent variables and the expansion ratio were not significant. In future studies the process settings will be set further away for the machine's physical limits to ensure a more stable pressure.

4.5 Conclusion

This study successfully identified the most significant formulation and processing independent variables during HTHPE of sorghum:maize composite flour. The factorial analysis predicted that percent sorghum in mix, final barrel zone temperature, and total moisture of total feed were the three most influential independent variables on the extrudate density and SDS level. Since the factorial screening experimental design lacks robustness for full optimisation, the next step in the overall study was to perform a response surface methodology (RSM), central composite design experimental to model the effect of percent sorghum in mix, final barrel zone temperature, and total moisture of total feed on density, expansion ratio and SDS level of the sorghum:maize composite flour extrudates produced by HTHPE. This RSM model will then be used to propose optimal levels

of the independent variables to deliver an expanded extruded product with maximum levels of SDS.

4.6 Acknowledgments

I would like to acknowledge the funding support for this project by The Australian Research Council (ARC), Sanitarium Health & Wellbeing. I wish to thank Alan Cruickshank and the Queensland Department of Employment, Economic Development and Innovation (QDEEDI) for supply of sorghum grain.

I would also like to thank Jiayue Chu (Curtin University) and Shilong Wang (Curtin University) for their assistance in operating the extruder.

References:

- Al-Rabadi, G.J., Torley, P.J., Williams, B.A., Bryden, W.L., Gidley, M.J., 2011. Particle size of milled barley and sorghum and physico-chemical properties of grain following extrusion. *Journal of Food Engineering* 103, 464-472.
- Alvarez-Martinez, L., Kondury, K.P., Harper, J.M., 1988. A general model for expansion of extruded products. *Journal of Food Science* 53, 609-615.
- Anderson, M.J., Whitecomb, P.J., 2005. *RSM Simplified: Optimizing Process Using Response Surface Methods For Design of Experiments* Taylor & Francis Group, LLC, Boca Roton.
- Anton, A.A., Fulcher, G.R., Arntfield, S.D., 2009. Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus vulgaris* L.) flour: Effects of bean addition and extrusion cooking. *Food Chemistry* 113, 989-996.
- AOAC International, 2008. Official Method of analysis of AOAC International AOAC Official method 925.10. AOAC International, [Gaithersburg, Md.].
- Bas, D., Boyaci, I.H., 2007. Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering* 78, 836-845.
- Buyken, A.E., Mitchell, P., Ceriello, A., Brand-Miller, J., 2010. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* 53, 406-418.
- Chanlat, N., Songsermpong, S., Charunuch, C., Naivikul, O., 2011. Twin-screw extrusion of pre-germinated brown rice: physicochemical properties and γ -aminobutyric acid content (GABA) of extruded snacks. *ijfe International Journal of Food Engineering* 7.
- Ding, Q.-B., Ainsworth, P., Plunkett, A., Tucker, G., Marson, H., 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. *Journal of Food Engineering* 73, 142-148.
- Englyst, H.N., Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* 46, S33-S50.
- Huber, G.R., 2000. Twin-Screw Extruders. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.

IPCC, 2007. Climate Change 2007. In: M.L. Parry, O.F.C., J.P. Palutikof, P.J. van der Linden and C.E. Hanson (Ed.) IPCC Fourth Assessment Report: Climate Change 2007. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jenkins, D., , Wolever, T., Taylor, R., Barker, H., Fielden, H., Baldwin, J., Bowling, A., Newman, H., Jenkins, A., Goff, D., 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 34, 362-366.

Kpodo, K.A., Plahar, W.A., 1992. Effects of extrusion variables and starch modification on the puffing characteristics of yam (*Dioscorea alata*) flour. *Food Control* 3, 200-204.

Mahasukhonthachat, K., , Sopade, P.A., Gidley, M.J., 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. *Journal of Cereal Science* 51, 392-401.

Méance, S., Achour, L., Briend, A., 1999. Comparison of starch digestibility of a blended food prepared with and without extrusion cooking. *European Journal of Clinical Nutrition* 53, 844-848.

Montgomery, D.C., 2001. Design and analysis of experiments, 5th ed. John Wiley, New York.

Nguyen, T.H., 2008. The effects of process variables on the quality of extruded products of lupin School of Public Health. . Curtin University of Technology, Perth, pp. xvi, 274 leaves : col. ill. ; 31 cm.

Rooney, L.W., Waniska, R.D., 2000. Sorghum Food and Industrial Utilization. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons Inc, pp. 689-730.

Sopade, P.A., Gidley, M.J., 2009. A Rapid In-vitro Digestibility Assay Based on Glucometry for Investigating Kinetics of Starch Digestion. *Starch/Stärke* 61, 245-255.

Srivastava, A., , Naresh Kumar, S., Aggarwal, P.K., 2010. Assessment on vulnerability of sorghum to climate change in India. *Agriculture, Ecosystems and Environment* 138, 160-169.

Suksomboon, A., Limroongreungrat, K., Sangnark, A., Thititumjariya, K., Noomhorm, A., 2011. Effect of extrusion conditions on the physicochemical properties of a snack made from purple rice (Hom Nil) and soybean flour blend. *International Journal of Food Science and Technology* 46, 201-208.

Taylor, J.R.N., Emmambux, M.N., 2010. Developments in our understanding of sorghum polysaccharides and their health benefits. *Cereal Chemistry* 87, 263-271.

Thomas, D.J., Atwell, W.A., 1999. *Starches* / David J. Thomas and William A. Atwell Eagan Press, St. Paul, Minn.

Yousif, A., , Nhepera, D., Johnson, S., 2012. Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability. *Food Chemistry* 134, 880-887.

Chapter 5 Optimization of formulation and processing of sorghum:maize composite flour extrudates for maximum expansion and maximum levels of slowly digestible starch

5.1 Abstract

Sorghum is a drought and high temperature tolerant grain with a characteristically low rate of starch digestion that has potential use in the development of healthy snack foods with high amounts of slowly digestible starch (SDS). Evidence shows that diets high in SDS reduce risk of chronic disease, especially of type 2 diabetes mellitus. Results of the factorial analysis (Chapter 4) indicated that sorghum flour in the dry mix (%), total moisture in feed (%) and final barrel zone temperature ($^{\circ}\text{C}$) were the three formulation and processing factors that most significantly affected extrudate expansion and levels of SDS after high temperature high-pressure extrusion of red sorghum:maize composite flour. Therefore, in the present study, the interactive effects of these independent variables on SDS level, expansion and density of extrudates was studied using response surface methodology. (RSM) with central composite experimental design. Total moisture of feed (22-25%), final barrel zone temperature (115-140 $^{\circ}\text{C}$), and sorghum flour in the dry mix (30-80%) were chosen as the independent variables and their ranges. Multiple regression techniques were used to analyse the data. Linear and quadratic models were used to describe the effects of the independent variables on the level of SDS, density and expansion ratio of the extrudates. Based on constraints of maximum level of SDS, maximum expansion ratio and the lowest density, the RSM model predicted the optimal levels of 22% total moisture of feed, 115 $^{\circ}\text{C}$ final barrel temperature zone, and 74.67% sorghum in dry mix. The predicted model was verified by analysis of samples under optimal and sub-optimal predicted conditions.

5.2 Introduction

Sorghum is a cereal crop grown in semi arid regions around the world. Its resistance to drought and high temperatures makes it an ideal crop for regions faced with the threat of climate change and global warming (IPCC, 2007; Srivastava, et al., 2010).

While sorghum has a similar starch content to wheat and maize, the presence of polyphenolics and a unique protein matrix have been shown to lower the starch digestibility and the overall energy availability (Taylor and Emmambux, 2010). Therefore sorghum has a characteristically low rate of starch digestion (Rooney and Waniska, 2000)

and consequently is lower in available energy than some other cereals. This low energy availability may be a problem in developing nations where food is in short supply or when sorghum is used for animal feed as maximum energy availability is desirable (Mahasukhonthachat, et al., 2010a). However its slow starch digestibility gives sorghum the potential as a health-enhancing food for human consumption in developed nations where over-nutrition related chronic diseases are an issue. Evidence shows that diets high in slowly digestible starch (SDS) lower post-meal glycaemia and reduce risk of chronic disease, especially of type 2 diabetes mellitus (Buyken, et al., 2010; Jenkins, et al., 1981).

Sorghum lacks gluten, therefore sorghum food products can face the sensory problem of being dense and having a disagreeable mouth-feel. A process that can increase the desirability of texture of foods through expansion and lowering of product density is high temperature high pressure extrusion (HTHPE) cooking. HTHPE is a highly controllable and efficient single-unit food manufacturing process (Huber, 2000). In this process raw or preconditioned ingredients are fed into a barrel and mixed by a rotating screw mixes. The material is sheared as it is carried forward and heat is applied and pressure generated. The screw mechanism finally forces the cooked material through an end plate (die) where the sudden release of pressure can generate steam from superheated water that results in product expansion and texturization (Huber, 2000). When cereal flour is extruded the presence of heat, water, pressure, and shear can cause the starch to gelatinize, leading to the irreversible breakdown of the starch granules (Thomas and Atwell, 1999). This gelatinised starch is more susceptible to enzymatic digestion. Mahasukhonthachat, et al, (2010a) demonstrated that by adjusting the moisture level and screw speed during extrusion, increased starch digestibility of sorghum flour could be obtained. They postulated however that a product with decrease in starch digestibility with potential health benefits might also be produced by optimising the extrusion process. In expanded extruded foods, acceptable texture may be associated with product expansion ratio (diameter of die to diameter of finish product); density of the extrudate (Ding, et al., 2006); and its hardness. Hardness is indicative of the human sensory experience of initial bite down into a piece of food and the force required to fracture the food (Ding, et al., 2006). A study investigating the effect of particle size on the physio-chemical properties of extruded sorghum and barley demonstrated that the expansion ratio did not differ significantly in sorghum with different particle sizes; neither did the expansion ratio differ significantly with a change in extruder barrel temperature (Al-Rabadi, et al., 2011).

Sorghum has the potential to be used to create an extruded snack-like product that combines acceptable texture with high levels of SDS and thus provide potential health related benefits. Formulation and processing conditions such as dry feed composition, barrel temperature, and moisture content during extrusion are known to affect the starch digestion and the texture of extrudates (Ding, et al., 2006; Mahasukhonthachat, et al., 2010a). An increase in starch digestion of sorghum by extrusion has been reported (Mahasukhonthachat, et al., 2010a). However, no study was found in the literature that attempted to produce a sorghum extrudate that combined desirable textural traits with a high level of SDS.

Response surface methodology (RSM) is a statistical method that is used to optimize processes (Bas and Boyaci, 2007). Changes in process parameters (factor or independent variables) can affect the properties of end product (response or dependent variables eg. level of SDS and expansion ratio). RSM has the ability to show the relationship of multiple independent variables and their combined effects on a response in mathematical and graphical models. When several responses are of interest, a range of acceptability for each response can be specified (this is known as a constraint) and by overlaying the response models a region of optimized processing parameters fulfilling the constraints can be identified (Bas and Boyaci, 2007; Montgomery, 2001). RSM is also efficient because fewer tests are needed than when using traditional one-variable-at-a-time methods (Anderson and Whitecomb, 2005; Montgomery, 2001). A robust and commonly used experimental design in RSM is the central composite design (CCD); an efficient design that is well suited to a model with a small number of independent variables (Montgomery, 2001).

The aim of this study was to determine the effects of moisture content of total feed, temperature of final barrel zone, and sorghum content of dry mix on the level of SDS, and the expansion ratio and density of HTHPE extrudates. RSM with central composite experimental design was used to predict the optimal formulation and processing settings to achieve the highest level of SDS in the extrudate whilst still maintaining acceptable expansion and density and to verify the predicted optimal conditions. The instrumental hardness of the extrudates was also determined in comparison to commercial extruded snack products.

5.3 Material and methods

5.3.1 Raw materials

Red sorghum grain (100 kg), of a tannin free variety, “Alpha”, was supplied by Lochabar Enterprises Pty Ltd (Tara, Queensland) grown in the summer of 2009-2010. Maize flour (code 23805) was purchased in a single batch from Defiance Maize Products Pty Ltd (Toowoomba, QLD). The sorghum grain was milled to a wholemeal flour at the Department of Agriculture and Food Western Australia with a ZM 200 Retch Mill (Retsch GmbH & Co, Rheinische Strabe 36, Haan Germany). The sorghum flour and maize flour were vacuum packed and stored at -20°C for two weeks and then at 15°C, at low humidity, for up to 18 months until used.

The sorghum grain and sorghum flour and maize flour previously underwent characterization and proximate analysis which has been detailed in Chapter 3 as follows: presence of tannins, kernel hardness, diameter and weight (Section 3.3.1.1); particle size (Section 3.3.3); moisture, protein, lipid, and ash (Sections 3.3.4.1-4); total dietary fibre and soluble dietary fibre content (Sections 3.3.5); total starch content (Section 3.3.6); amylose content of the starch (Section 3.3.7); and the total starch damage (Section 3.3.8). For in-vitro starch digestion of flours see Section 3.3.9

5.3.2 Experimental design

The independent variables used in this study were the total moisture content (%) of the input material, the temperature (°C) of the final barrel zone, and the amount of sorghum (%) in the sorghum-maize composite flour used for the dry mix. The dependent variables of the extrudate investigated were the level of SDS (% of total dry starch), the expansion ratio (ratio of the final extrudate diameter to the die diameter) and the density (g/ml). Factorial screening studies (Chapter 4) had previously identified that these independent variables were the three most significant factors associated with the dependant variables of interest.

A central composite design was used in which each independent variable had a maximum and minimum values, (coded as -1 and 1), also a low and high value (coded $-\alpha$ and α), outside of the range of interest, to ensure accuracy of the response surface curves. In addition, multiply centre points (0) were used to ensure the robustness of the model (Table 5.1). Design-Expert Version 7 (Stat-Ease, Inc. Minneapolis, MN, USA) was used to generate the independent variables for the twenty runs; each run consisting of a combination of independent variables at different levels (Table 5.2) (Chauhan et al., 2011). It was determined in the screening studies in Chapter 4, that screw speed and feed rate had no

significant effect on the SDS of the extrudate and were therefore kept constant at a medium setting of 350 rpm and 4.55 kg/hr respectively, throughout all experimental runs. These medium settings were used to avoid extremes in extrusion operation which had previously led to fluctuations in pressure during runs and inconsistent expansion.

The equation for the experimental design using RSM where n = mathematical functions of f_k (where $k = 1, 2, \dots, n$) and Y_k in terms of m independent variables X_i (where $i = 1, 2, \dots, m$) existed for each dependent variable was:

$$Y_k = f_k (X_1, X_2, \dots, X_m)$$

The second order polynomial equation was as follows:

$$Y_k = \beta_{k0} + \sum_{i=1}^3 \beta_{ki} X_i + \sum_{i=1}^3 \beta_{kii} X_i^2 + \sum_{i=1, j=i+1}^3 \beta_{kij} X_i X_j$$

(Montgomery, 2001)

Where β_{k0} is the centre point (0,0), β_{ki} is the linear regression term, β_{kii} the quadratic, and β_{kij} the interactive regression term (Chauhan, et al., 2011).

A multiple regression model was used to analyse the data. F ratio, correlation coefficient (R^2) and lack of fit were used to determine if the model was adequate (Chauhan, et al., 2011; Montgomery, 2001).

Table 5.1 Independent variables and their actual values and coded values for the central composite design

Factor	Independent variables	Units	$-\alpha$	-1	0	1	$+\alpha$
X_1	Total moisture in total feed	%	21	22	23.5	25	26
X_2	Final barrel zone temperature	°C	106	115	128	140	149
X_3	Sorghum in dry feed	%	12.96	30	55	80	97.04

5.3.3 Extrusion

The extrusion was performed with an APV Baker Ltd (Peterborough, England) twin screw extruder MPF 19:25. The screw configuration is outlined in Table 4.1 of Chapter 4 of this thesis.

After manufacture, the extrudate coil was allowed to cool to room temperature ($\sim 24^{\circ}\text{C}$) for one hour and then cut with scissors into $\sim 50\text{mm}$ long pieces. A representative sub-sample ($\sim 270\text{g}$) of each extrudate was ground in a food processor wrapped in ice packs to avoid excess heat from the food processor motor (Robot Coupe blixer[®], Montceau-en-Bourgogne, France) to pass 100% through a 1mm sieve. The food processor bowl was wrapped with ice packs to minimize any heat generated by the processor motor affecting the product. The ground samples were vacuum sealed (VacPac Webomatic, Australia) in polypropylene bags (Vital Packaging, Myaree WA) and stored at room temperature for up to 6 months before analysis for total moisture content and in-vitro starch digestion. The remaining unmilled extrudate pieces were stored whole in zip seal polypropylene bags, at room temperature, for 24 to 72 hours before measurement of density, expansion ratio and hardness.

5.3.4 Optimization verification

Duplicate extrusion runs were made using the predicted optimal conditions generated by the RSM model. Also duplicate extrusion runs were made at non-optimal settings to verify the model. The extrudate samples from these runs underwent all the analyses described for RSM model extrudates.

5.3.5 Density and expansion

Ten samples of $\sim 50\text{mm}$ long extrudates of each sample were weighed and measured to determine density (g/ml) and expansion ratio as outlined in Section 4.3.5-6 (Ding, et al., 2006).

5.3.6 Hardness analysis

Hardness was determined on ten, $\sim 50\text{mm}$ long, extrudate pieces for each sample run (Anton, Fulcher et al., 2009; Ding, et al., 2006). Hardness testing was performed using a Stable Micro System TA-XT2i Texture analyser (Godalming, England) using a 2 mm thick cylinder probe and a 5 kg load cell with a cross head speed was 0.4 mm per second. The probe was set to penetrate 40% of the diameter of the sample. Texture Experts (Godalming, England) software was used to generate a force (g) by time (seconds) curve

and the height of the first peak (peak force, g) recorded (Ding, et al., 2006). The mean value of the peak force for each sample run was calculated.

In order to compare the experimental sorghum extrudates with commercially available extruded snack products, a second hardness analysis was performed after the extrudates were dried in a 46°C oven until the moisture level was ~5%, a level consistent with the commercial products. The moisture levels were monitored by loss of weight using an automatic moisture detector (Mettler & Toledo, HB43-s halogen, Switzerland). The dried samples were allowed to cool on racks at room temperature (~24 °C) for one hour and then ten samples from each extrusion run were tested for hardness following the method described above. A range of commercially available extruded snack products namely: Twisties[®] cheese (Smith's Snackfood Co., Chatswood, NSW.); Cheetos[®] brand cheese balls (Smith's Snackfood Co., Chatswood, NSW); Cheezls[®] original cheese (Snack Brand Australia, Bella Vista, NSW); and Grain Waves[®] original flavour (Smith's Snackfood Co., Chatswood NSW) were purchased. Ten pieces from one bag of each commercial product were tested for hardness using the method described above.

5.3.7 Moisture content and water activity

Total moisture of the extrudates was determined by drying the ground samples in a Contherm Digital Series Oven (Scientific LTD, New Zealand) at 130°C for 1 hour; reported moisture was the differences in weight of sample before and after drying (AOAC International, 2008). Water activity (a_w) was determined using an automated water activity apparatus (Decagon Devices Inc, Pullman WA, USA).

5.3.8 In-vitro slowly digested starch determination

SDS was determined on each of the ground extrudate samples in duplicate by the in-vitro rapid glucometry method based on Sopade & Gidley (2009b). This procedure is reported in Section 3.3.9.

5.3.9 Statistical analysis

RSM modelling, including prediction of optimal levels of independent variables, was performed using Design-Expert Version 7 (Stat-Ease, Inc. Minneapolis, MN, USA). $P < 0.05$ was considered significant for these analyses.

SPSS Statistics V17 (SPSS Inc., Chicago, IL, USA) was used to perform a one-sample t-test to compare levels of the predicted (single value from RSM model) and the experimental mean value of SDS, expansion ratio and density of the validation extrudates. Due to the small number of replicates a more conservative of $P < 0.01$ rather than the conventional $P < 0.05$ was considered statistically significant.

5.4 Results and Discussion

5.4.1 Raw materials characterisation

The sorghum grain and sorghum and maize flour characterization and proximate analysis is reported in Chapter 3 of this study as follows: sorghum grain properties and presences of tannin (Table 3.1), particle size (Table 3.2), protein, lipid, ash, total starch and fibre content (Table 3.3), and amylose and starch damaged percentage and in-vitro starch digestion (Table 3.4).

5.4.2 Effect of sorghum level, moisture level and barrel temperature on level of SDS in extrudates

The level of SDS in the RSM experimental samples ranged from 7.36-25.85% of dry starch (Table 5.2). It was found that a linear model was the best fit for describing the effects of the independent variables on the level of SDS ($R^2 = 68.23\%$), with an insignificant lack of fit indicating the model was adequate (Appendix 3).

Equation 5.1 is the regression equation:

$$\text{Equation 5.1} \quad Y_{\text{SDS}} = 16.10 - 0.19X_1 - 2.77X_2 + 5.72X_3$$

Where X_1 = Total moisture in total feed (%); X_2 = Final barrel zone temperature ($^{\circ}\text{C}$); X_3 = Sorghum in dry feed (%)

Table 5.2 Levels of independent variables and resulting dependant variables of experimental samples of Sorghum:Maize Extrudates

Run	X1, Sorghum in mix (%)	X2, Final barrel zone temperature (°C)	X3, Total moisture in feed (%)	SDS g/100g of dry starch	Expansion ratio	Density (g/ml)	Moisture content of extrudate (%)	Water activity (a_w)	Hardness (g)
1	30.0	140	22.0	10.5	2.4	0.24	12.1	0.61	-*
2	80.0	140	22.0	21.5	2.4	0.24	12.0	0.64	-*
3	55.0	128	23.5	20.6	2.5	0.30	12.2	0.68	1959
4	30.0	115	22.0	7.4	2.9	0.30	13.3	0.70	2139
5	55.0	128	23.5	15.1	2.5	0.31	12.8	0.66	1868
6	55.0	128	23.5	11.8	2.6	0.29	12.7	0.68	1239
7	55.0	128	21.0	20.8	2.7	0.24	11.1	0.57	1485
8	30.0	115	25.0	13.5	2.4	0.37	13.8	0.75	2411
9	80.0	115	25.0	25.8	2.4	0.33	12.4	0.67	2187
10	80.0	140	25.0	18.9	2.2	0.28	11.5	0.58	1237
11	55.0	128	23.5	15.6	2.5	0.31	12.7	0.69	1859
12	55.0	128	23.5	16.7	2.5	0.29	12.7	0.70	2134
13	55.0	149	23.5	9.0	2.3	0.22	10.9	0.55	1082
14	55.0	128	26.0	16.1	2.4	0.31	13.2	0.73	1878
15	80.0	115	22.0	21.2	2.4	0.34	12.5	0.68	2729
16	30.0	140	25.0	7.4	2.1	0.29	12.8	0.65	2584
17	13.0	128	23.5	-1.4	2.5	0.27	12.8	0.67	2954
18	55.0	128	23.5	13.6	2.7	0.23	11.2	0.57	1890
19	55.0	106	23.5	25.9	2.5	0.39	12.4	0.71	2823
20	97.0	128	23.5	24.1	2.2	0.37	13.0	0.73	2838

*Samples runs 1&2 were accidently discarded before hardness was measured

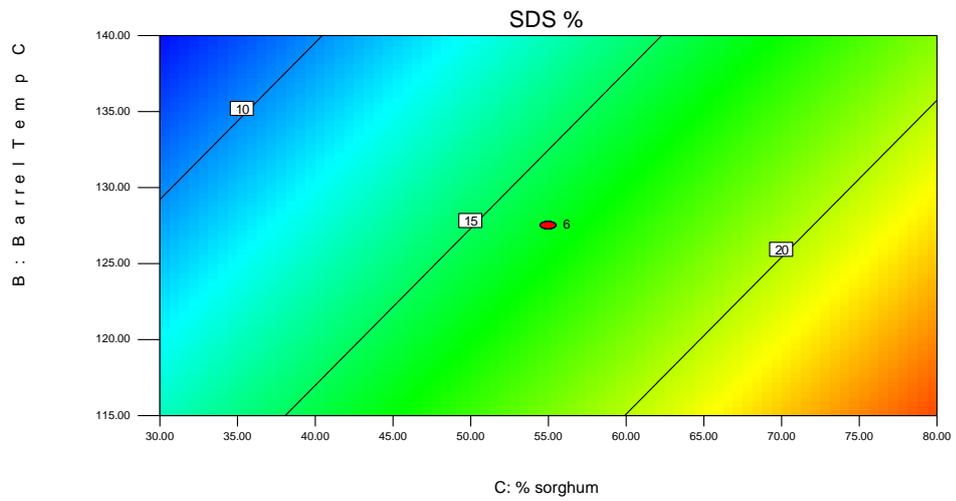
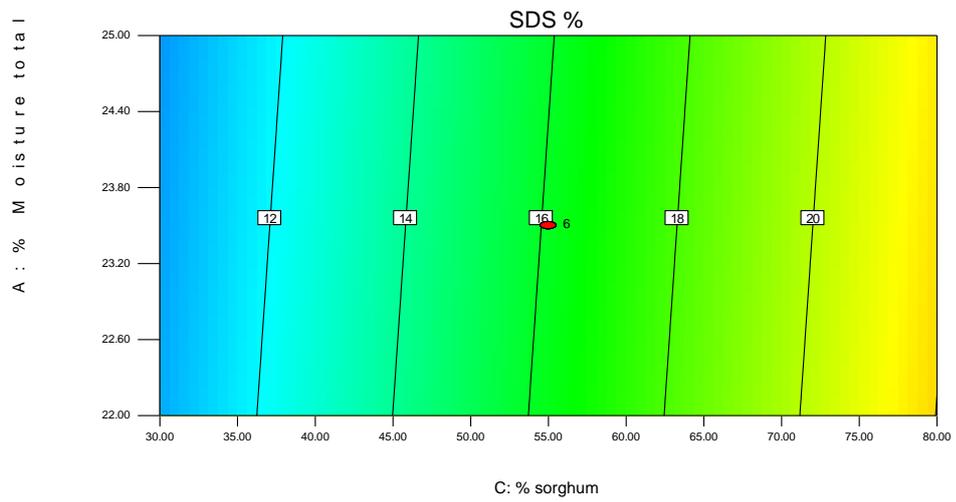
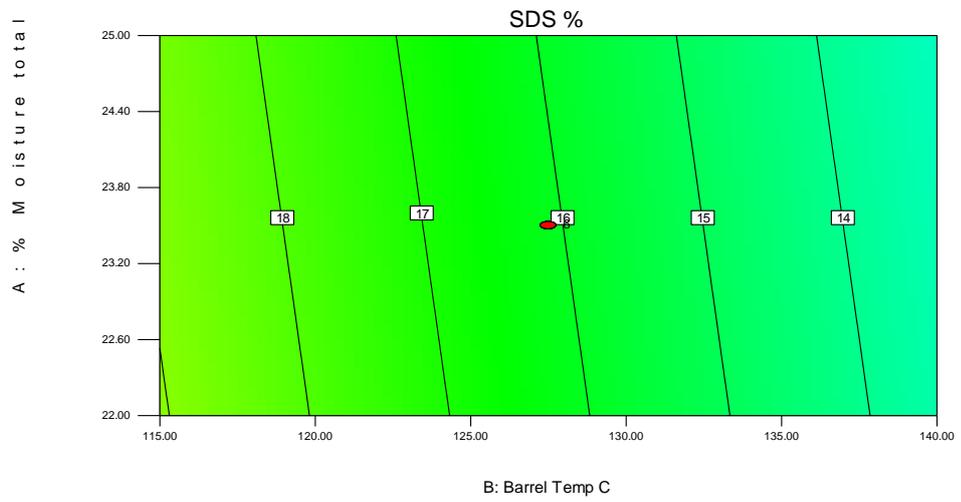


Figure 5.1 Contour plots illustrating the interactive effects of sorghum content, moisture content and barrel temperature on SDS level in extrudates

The sorghum level of dry feed had a positive association ($P < 0.001$) with the level of SDS in the extrudates and the final barrel zone temperature had a negative association ($P = 0.007$). The level of moisture in the total feed did not significantly affect the SDS level ($P > 0.05$). The positive effect of sorghum on SDS could be because the sorghum was less broken down by the extrusion process than the maize flour. This negative effect of temperature on SDS is consistent with current knowledge of starch gelatinisation under moist heating, higher temperatures resulting in increased granule breakdown making the starch susceptible to enzymatic digestion (Thomas and Atwell, 1999). However unlike in the present study it has been previously reported that increased moisture content had an effect on the level of SDS (Mahasukhonthachat, et al., 2010a).

5.4.3 Effect of sorghum level, moisture level and barrel temperature on the expansion ratio of extrudates

The expansion ratio of the extrudates in the experimental samples ranged from 2.07-2.89 (Table 5.2). It was found that a quadratic model was the best fit for describing the effects of the independent variables on the expansion ($R^2 = 75.24\%$). This model had an insignificant lack of fit indicating the model was adequate (Appendix 2).

Equation 5.2 is the regression equation:

Equation 5.2

$$Y_{\text{expansion}} = 2.54 - 0.11X_1 - 0.11X_2 - 0.049X_3 + 4.7E^{-003}X_1X_2 + 0.074X_1X_3 + 0.085X_2X_3 + 2.887E^{-003}X_1^2 - 0.06X_2^2 - 0.074X_3^2$$

Where X_1 = Total moisture in total feed (%); X_2 = Final barrel zone temperature ($^{\circ}\text{C}$); X_3 = Sorghum in dry feed (%)

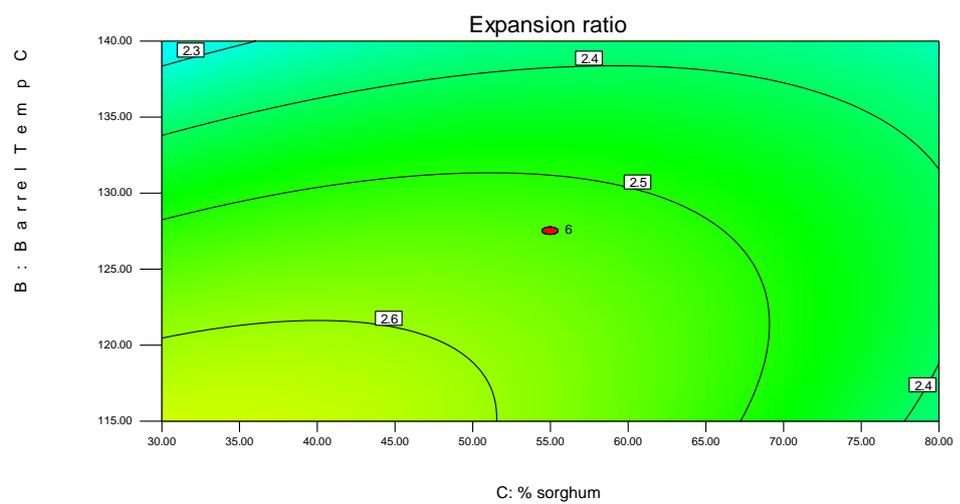
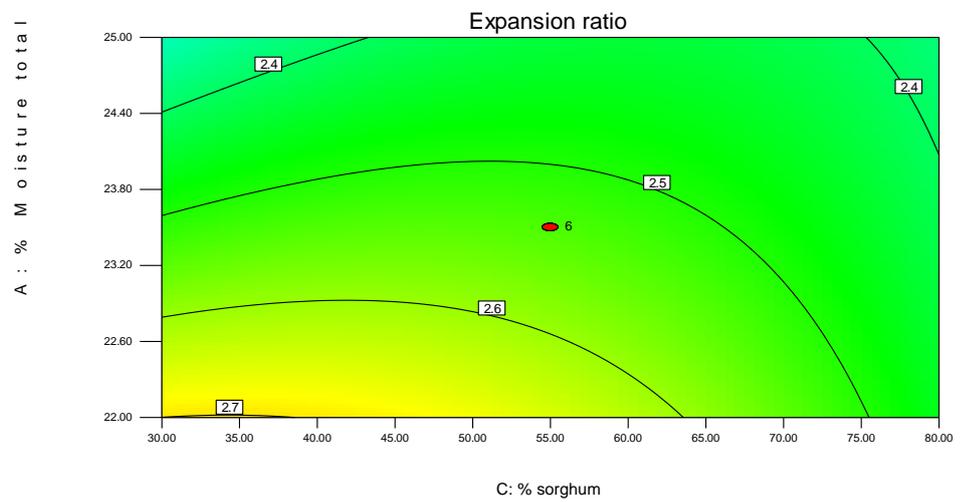
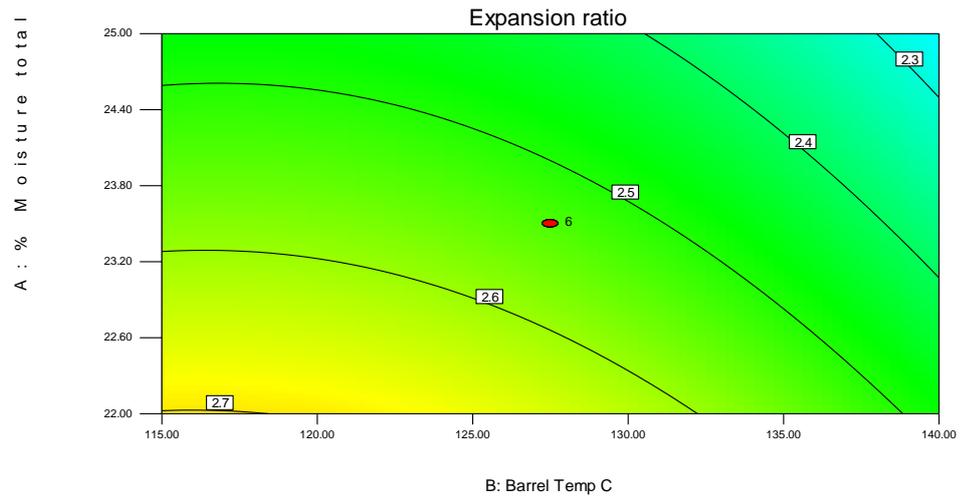


Figure 5.2 Contour plots illustrating the interactive effects of sorghum level, moisture level, and barrel temperature on extrudate expansion ratio

The increased moisture level of total feed had a negative association with the expansion ratio ($P < 0.001$). This could possibly be because the water, with a higher density than the flour, weighed down the product causing less expansion. The final barrel zone temperature showed a curved quadratic model association with expansion ratio ($P = 0.002$); with lower temperature giving a higher expansion ratio. This is consistent with other studies investigating extrusion of sorghum and other grains (Asare, et al., 2010; Ding, et al., 2006). These studies investigated the textural aspects of extruded snack foods, but did not incorporate the issue of SDS levels in the extrudates. Sorghum level however was not significantly associated with expansion ratio ($P > 0.05$). This suggests the potential for the addition of high levels of sorghum to extruded snack-like products without sacrificing expansion.

5.4.4 Effect of sorghum level, moisture level and barrel temperature on extrudate density

The density of the extrudates in the experimental samples ranged from 0.22-0.39 g/ml (Table 5.2). It was found that a linear model was the best fit for describing the effects of the independent variables on the density ($R^2 = 66.94\%$). This model had an insignificant lack of fit indicating the model was adequate (Appendix 3).

Equation 5.3 is the regression equation:

$$\text{Equation 5.3} \quad Y_{\text{Density}} = 0.30 + 0.02X_1 - 0.043X_2 + 0.013X_3$$

Where X_1 = Total moisture in total feed (%); X_2 = Final barrel zone temperature ($^{\circ}\text{C}$); X_3 = Sorghum in dry feed (%)

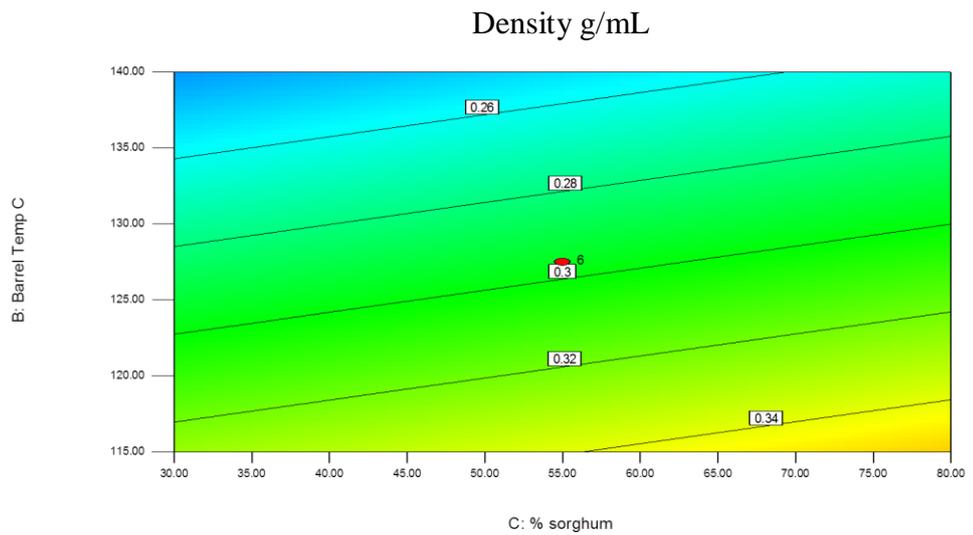
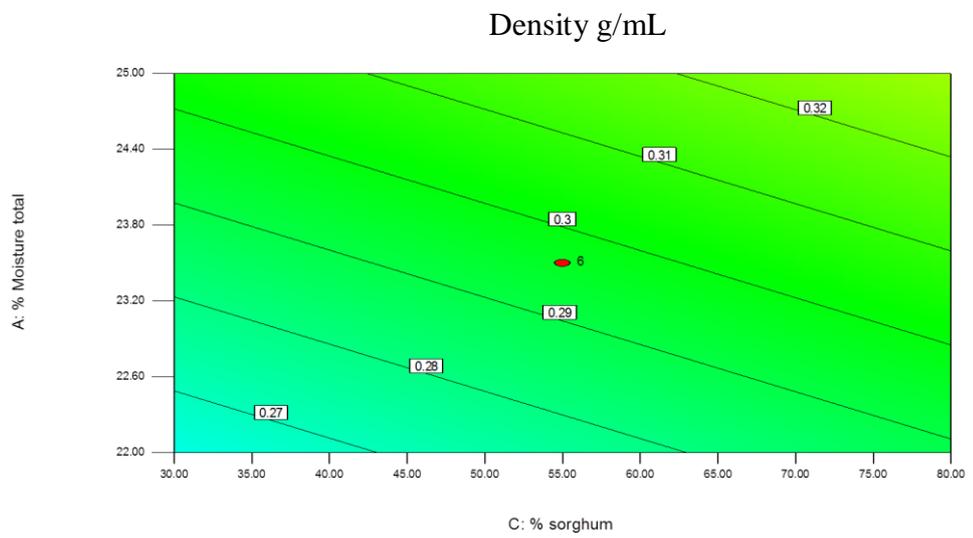
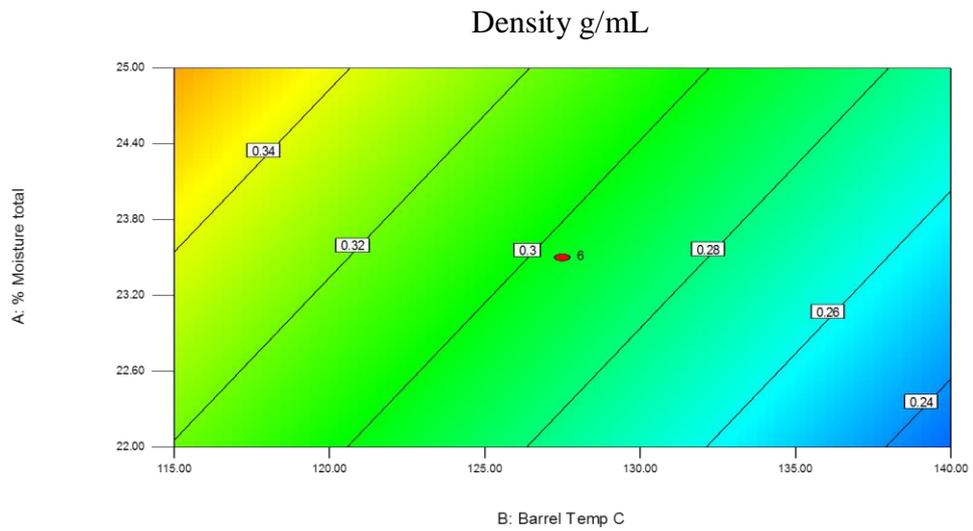


Figure 5.3 Contour plots illustrating the interactive effect of sorghum level, moisture level and barrel temperature on extrudate density

The final barrel zone temperature had a negative association with density ($P < 0.001$), whereas moisture level had a positive association ($P = 0.02$). This effect is consistent with a study investigating density of extruded wheat flour into a snack-like food (Ding, et al., 2006) as temperature increases, more water has the opportunity to turn to steam increasing expansion and lowering density. In the present study, sorghum level was not significantly associated with extrudate density ($P > 0.05$); this lack of effect also suggests the possibility of inclusion of high levels of sorghum in extruded snack like foods without adversely affecting texture.

5.4.5 Hardness

5.4.5.1 Extrudates prior to drying

The hardness as measured by peak force of the extrudates in the experimental samples ranged from 1082-2954 g (Table 5.2). It was found that a quadratic model was the best fit for describing the effects of the independent variables on hardness ($R^2 = 72.89\%$). This model had an insignificant lack of fit indicating the model was adequate (Appendix 4).

Increasing barrel zone temperature was negatively associated with the hardness of the extrudate ($P = 0.003$). The sorghum level affected the hardness in a quadratic model ($P = 0.003$). High and low levels of sorghum had a positive association with hardness whilst middle levels having a negative association. In the present study hardness was not included as a constraint in predicting the optimal condition for sorghum extrusion, however in future studies this dependent variable could be included in the optimisation.

5.4.5.2 Comparison of hardness of commercial extruded snack foods with the experimental extrudates after drying

The commercial products had a mean peak force range of 446-1139g while the dried experimental extrudates had a range of 852-2513 g (Appendix 5). This demonstrates that only four of the dried sorghum experimental extrudates have hardness equivalent to that of the commercial products whereas sixteen were harder. The inclusion of sugars and other additives in the commercial products could explain the lower range of hardness. Further studies using human taste panel sensory evaluation are now required to better understand the acceptability of the texture of sorghum extrudates. However these were outside the scope and timeframe for this thesis.

5.4.6 Moisture content and water activity

Increase in barrel temperature was negatively associated with both moisture content and water activity of the extrudates ($P=0.015$ and $P=0.002$, respectively). Interestingly, the level of total moisture added to the feed during extrusion did not have a significant effect on the moisture content or the water activity of the extrudates. This is most likely due to the small range of total moisture levels used. All extrudates had a water activity below 0.73aw indicating that they are shelf stable (Afoakwa et al., 2010), however, all extrudates had a moisture content above 10% which is higher than recommended for dry food systems to be considered shelf stable (Afoakwa, Aidoo et al., 2010; Asare, et al., 2010). Further drying of extrudates and shelf-life studies should be considered in future research.

5.4.7 Optimization

Using Design Expert software, the independent variables were optimized to find the levels that were predicted to maximize both the SDS levels and the expansion ratio of the resulting extrudate. Expansion ratio was chosen as the texture-related dependant variable as it had a more robust predictive model than for density. The optimal levels of the independent variable and the predicted values for SDS level and expansion ratio are given in Table 5.3. In addition a non-optimal set of independent variable values was also chosen to verify the predictive model away from the optimal zone. The levels of the independent variables and the predicted values for SDS level and expansion ratio for both the optimal and non-optimal settings are given in Table 5.3.

Table 5.3 Verification of the RSM model^a

		Optimal independent variable settings ^b		Non-optimal independent variable settings ^b	
Assay	Units	Predicted	Actual ^a	Predicted	Actual ^a
SDS	g/100g dry starch	23.57 ^a	21.67 ± 9.28 ^a	16 ^a	15.58 ± 0.86 ^a
Expansion ratio	-	2.49 ^a	2.21 ± 0.11 ^a	2.6 ^a	2.27 ± 0.02 ^a

^a Means of duplicate analyses ± standard deviation.

^b Values within the same row and category with a different superscript letter are significantly different ($P<0.01$). One-sample t-test.

For both the optimal and non-optimal settings there was no significant difference ($P > 0.01$) between the predicted value and the actual value of level of neither SDS nor expansion ratio. This verifies the robustness of their RSM model for predicting these dependant variables.

5.5 Conclusion

This study successfully predicted the effect of sorghum level in dry feed, moisture level in total feed and final barrel zone temperature on the SDS level and the expansion ratio of sorghum:maize composite flour extrudates. Using RSM, a robust model was developed and verified. Optimal settings for the independent variables predicted to give maximum SDS level and expansion ratio were identified.

5.5.1 Limitations

Some limitations of this study included: the relatively low levels of SDS found in the optimal samples indicating that this may be very difficult to develop an expanded product with very high levels of SDS; that fact that only one variety of sorghum was evaluated though other varieties of sorghum may have generated different models and the optimal extrudates may have different properties.

Further studies are now required to compare different varieties of sorghum under the optimized extrusion settings found here to determine if higher levels of SDS could be achieved.

5.6 Acknowledgments

I would like to acknowledge the funding support for this project by The Australian Research Council (ARC), Sanitarium Health & Wellbeing. I would like to thank Alan Cruickshank and the Queensland Department of Agriculture, fisheries, and Forestry (DAFF) for supply of the sorghum.

I would also like to thank Jiayue Chu (Curtin University), Bonny Rawson (Curtin University), and Asteria Hendrianto (Curtin University) for their assistance with operating the extruder.

References:

- Afoakwa, E.O., Aidoo, P.R., Adjonu, R., 2010. Effect of spontaneous fermentation and amylase-rich flour on the nutritive value, functional and viscoelastic properties of cowpea-fortified nixtamalized maize. *International Journal of Food Sciences and Nutrition* 61, 256-271.
- Al-Rabadi, G.J., Torley, P.J., Williams, B.A., Bryden, W.L., Gidley, M.J., 2011. Particle size of milled barley and sorghum and physico-chemical properties of grain following extrusion. *Journal of Food Engineering* 103, 464-472.
- Anderson, M.J., Whitecomb, P.J., 2005. *RSM Simplified: Optimizing Process Using Response Surface Methods For Design of Experiments* Taylor & Francis Group, LLC, Boca Roton.
- Anton, A.A., Fulcher, G.R., Arntfield, S.D., 2009. Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus vulgaris* L.) flour: effects of bean addition and extrusion cooking. *Food Chemistry* 113, 989-996.
- AOAC International, 2008. Official Method of analysis of AOAC International AOAC Official method 925.10. AOAC International, [Gaithersburg, Md.].
- Asare, E.K., Sefa-Dedeh, S., Afoakwa, E.O., Sakyi-Dawson, E., Budu, A.S., 2010. Response surface methodology for studying the effects of feed moisture and ingredient variations on the chemical composition and appearance of extruded sorghum-groundnut-cowpea blends. *International Journal of Food Engineering*, 6, article no. 12.
- Bas, D., Boyaci, I.H., 2007. Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering* 78, 836-845.
- Buyken, A.E., Mitchell, P., Ceriello, A., Brand-Miller, J., 2010. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* 53, 406-418.
- Chauhan, O.P., Raju, P.S., Ravi, N., Roopa, N., Bawa, A.S., 2011. Studies on retention of antioxidant activity, phenolics and flavonoids in high pressure processed black grape juice and their modelling. *International Journal of Food Science & Technology* 46, 2562-2568.
- Ding, Q.-B., Ainsworth, P., Plunkett, A., Tucker, G., Marson, H., 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. *Journal of Food Engineering* 73, 142-148.
- Huber, G.R., 2000. Twin-Screw Extruders. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.
- IPCC, 2007. *Climate Change 2007*. In: M.L. Parry, O.F.C., J.P. Palutikof, P.J. van der Linden and C.E. Hanson (Ed.) *IPCC Fourth Assessment Report: Climate Change 2007*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jenkins, D., Wolever, T., Taylor, R., Barker, H., Fielden, H., Baldwin, J., Bowling, A., Newman, H., Jenkins, A., Goff, D., 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 34, 362-366.
- Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J., 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. *Journal of Cereal Science* 51, 392-401.
- Montgomery, D.C., 2001. *Design and analysis of experiments*, 5th ed. John Wiley, New York.
- Rooney, L.W., Waniska, R.D., 2000. Sorghum Food and Industrial Utilization. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons Inc, pp. 689-730.
- Sopade, P.A., Gidley, M.J., 2009. A rapid In-vitro digestibility assay based on glucometry for investigating kinetics of starch digestion. *Starch/Stärke* 61, 245-255.

Srivastava, A., Naresh Kumar, S., Aggarwal, P.K., 2010. Assessment on vulnerability of sorghum to climate change in India. *Agriculture, Ecosystems and Environment* 138, 160-169.

Taylor, J.R.N., Emmambux, M.N., 2010. Developments in our understanding of sorghum polysaccharides and their health benefits. *Journal of Cereal Chemistry* 87, 263-271.

Thomas, D.J., Atwell, W.A., 1999. *Starches* Eagan Press, St. Paul, Minn.

Chapter 6 Comparison of starch digestibility, expansion and density of maize and white, red, and brown sorghum:maize extrudates

6.1 Abstract

Sorghum is a drought and high temperature tolerant grain with a characteristically low rate of starch digestion that has potential use in healthy snack foods with high amounts of slowly digestible starch (SDS). Evidence shows that diets high in slowly digested starch reduce risk of chronic disease, especially of type 2 diabetes mellitus. Different varieties of sorghum of differing grain colours are known to differ in starch properties. Using the optimized high temperature high pressure extrusion (HTHPE) formulation and processing conditions determined in Chapter 5, sorghum:maize composite flour of sorghum with white, red, and brown grain colour, with maize, were extruded and compared to an extrudate of 100% maize polenta. The in-vitro slowly digested starch (SDS), density, expansion ratio and instrumental hardness of the extrudates was determined. No significant differences in SDS or textural aspects were found between the three sorghum varieties.

6.2 Introduction

Sorghum is a cereal crop grown in semi arid regions around the world. Its resistance to drought and heat makes it an ideal crop for regions faced with the threat of climate change and the effects of global warming (IPCC, 2007; Srivastava, et al., 2010). Sorghum grain comes in many varieties. These varieties may differ in grain colour from white to red to black, as well as differing in the amount and types of polyphenolic compounds and the presents or absences of tannins (Rooney and Serna-Saldivar, 2000).

Sorghum has a characteristically low rate of starch digestion (Rooney and Waniska, 2000). While sorghum has a similar starch content of wheat and maize, the presence of polyphenolics and a unique protein matrix have been shown to lower the starch digestibility and the overall energy availability (Taylor and Emmambux, 2010). This lack of energy availability may be a problem in developing nations or when sorghum is used for animal feed as maximum energy is desirable (Mahasukhonthachat, et al., 2010a). However, sorghum has the potential to replace wheat and to be used as a health-enhancing food for human consumption in developed nations where over-nutrition and obesity is an issue. Evidence shows that diets high in slowly digestible starch (SDS) reduce risk of chronic disease, especially of type 2 diabetes mellitus (Buyken, et al., 2010; Jenkins, et al., 1981).

The differences in polyphenolic composition of different sorghum varieties may impact on starch digestibility through their potential to inhibit starch digestion enzymes (Rooney and Serna Saldivar, 2003) and their interaction with the protein matrix (Taylor and Emmambux, 2010). Therefore food products incorporating flours from different sorghum varieties may differ in their levels of physiologically important starch fractions (RDS, SDS, RS) in sorghum products and hence the potential health benefits of these products.

Being gluten free formulating sorghum products with desirable texture and mouth feel can be difficult. A process that can generate crunching desirable expanded and crunchy texture in gluten free formulations is high temperature high pressure extrusion (HTHPE) cooking. HTHPE is a highly controllable and efficient single-unit food manufacturing process (Huber, 2000). In this process raw or preconditioned ingredients are fed into a barrel in which a rotating screw mixes and shears the wet ingredients as it carries the material forward. During this process heat is applied and there is an increase in pressure within the barrel resulting in gelatinisation of starch denaturation of protein and development of colours and flavours. The screw mechanism finally forces the cooked material through an end plate (die) where the sudden release of pressure generates steam from the superheated water in the product that results in product expansion and texturization through “puffing” (Huber, 2000). When flour is extruded in the presence of heat, water, pressure, and shear the starch present will undergo gelatinization, the irreversible breakdown of the starch granules (Thomas and Atwell, 1999). This gelatinized starch is more susceptible to enzymatic digestion and is important for the texturisation of the product. In expanded extruded foods consumer acceptable mouth feel and texture may be defined in terms of expansion (ratio of diameter of die to diameter of finished product) and the density (mass per unit volume of finished product) (Ding, et al., 2006). Instrumental hardness is also a good indicator of acceptable sensory texture. The instrumental hardness is indicative of the human sensory experience of initial bite down into a piece of food and the force required to break the product (Ding, et al., 2006).

In Chapter 4 of this thesis factorial analysis was used to identify the independent variables of level of sorghum in the dry mix, level of water in total feed and the final barrel zone temperature that most significantly affected levels of SDS, density and expansion ratio of red sorghum:maize composite flour extrudates manufactured by HTHPE. Using these independent variables the optimum conditions to maximise levels of SDS, maximise

expansion ratio and minimise density of red sorghum:maize composite flour extrudates was identified using response surface methodology (RSM) predicative modelling (Chapter 5).

The main aim of this study is to determine and compare the levels of SDS, the density, expansion ratio and hardness of extrudates made with sorghum:maize composite flours using sorghum with red, white and brown grain colour; manufactured under the optimised conditions predicted by RSM for red sorghum:maize composite flour in Chapter 5.

6.3 Methods

6.3.1 Raw materials

White Sorghum (100 kg), a commercial hybrid “Liberty” and brown tannin sorghum (150kg), variety IS 1311 C were grown by the Queensland Department of Employment Economic Development and Innovation (QDEEDI) in summer 2010-2011 at Hermitage Research Station (Warwick, Queensland). Red sorghum grain (100 kg), of a tannin free variety, “Alpha”, was supplied by Lochabar Enterprises Pty Ltd (Tara, Queensland) grown in the summer of 2009-2010. Maize polenta (code 23805) was purchased in a single batch from Defiance Maize Products Pty Ltd (Toowoomba, QLD). The sorghum grain was milled as whole meal flour at the Department of Agriculture and Food Western Australia with a ZM 200 Retch Mill (Retsch GmbH & Co, Haan, Germany). The sorghum flour and maize polenta were vacuum packed and stored at -20°C for two weeks and then at 15°C at, low humidity, for up to 18 months until used.

6.3.2 High temperature high pressure extrusion

The extruder specifications (Section 4.3.2) and screw configuration (see Table 4.1) were those detailed in Chapter 4. The formulation and extruder conditions for the manufacture of the sorghum:maize composite flour extrudates were those optimized conditions determined by RSM in Chapter 5 (Section 5.4.7). (Table 6.1) The maize polenta was also run at optimum levels without any sorghum. However the red sorghum and maize extrudates were manufactured in one set of extrusion runs (they are the validation samples reported in Chapter 5), whilst the white and brown sorghum containing extrudates were manufactured in a separate set of extrusion runs. Within each run, each extrudate was manufactured in a randomised design. Each of the four flours (100% maize, white

sorghum:maize, red sorghum:maize, and brown sorghum:maize) was extruded in duplicate and each sample was analysed in duplicate.

Table 6.1 Optimal extrusion settings for manufacture of sorghum:maize expanded extrudates

Independent variable	Value
Sorghum flour in dry mix (%)	74.67 ^a
Total moisture in feed (%)	22 ^a
Final barrel zone temperature (°C)	115 ^a
Screw speed (rpm)	350 ^b
Total feed rate (kg/hr)	4.55 ^b

^a Optimal value predicted from RSM optimisation (Chapter 5)

^b Mid range setting as this independent variable does not significantly influence the dependant variables (Chapter 4 & 5)

The extrusion was performed with an APV Baker Ltd (Peterborough, England) twin screw extruder MPF 19:25. The screw configuration is outlined in Table 4.1 of this thesis.

After manufacture the extrudate coil was allowed to cool to room temperature (~24°C) for one hour and then cut with scissors into ~50mm long pieces. A representative sub-sample (~270g) of each extrudate was ground in a food processor wrapped in ice packs to avoid excess heat from the food processor motor (Robot Coupe blixer®, Montceau-en-Bourgogne, France) to pass 100% through a 1mm sieve. The food processor bowl was wrapped with ice packs to minimize any heat generated by the processor motor affecting the product. The ground samples were vacuum sealed (VacPac Webomatic, Australia) in polypropylene bags (Vital Packaging, Myaree WA) and stored at room temperature for up to 6 months before analysis for total moisture content and in-vitro starch digestion. The remaining unmilled extrudate pieces were stored whole, in zip seal polypropylene bags, at room temperature for 24 to 72 hours before measurement of density, expansion ratio and hardness.

6.3.3 Proximate and dietary fibre analysis

The sorghum flour and maize polenta underwent characterization and proximate analysis, which has been previously detailed in Chapter 3 as follows: presence of tannins, kernel hardness, diameter and weight see Section 3.3.1.1; particle size see Section 3.3.3; moisture,

protein, lipid, ash, total fibre and soluble fibre content see Sections 3.3.4-5; total starch content see section 3.3.6; amylose content of the starch see Section 3.3.7; and the total starch damage see section 3.3.8. In-vitro determination of SDS was performed by the method of Sopade and Gidley (Sopade and Gidley, 2009a) as described in detail in Chapter 3 (see Section 3.3.9).

6.3.4 Expansion and hardness analysis

The density and expansion ratio were measured as described in Chapter 4 (Sections 4.3.5-6). The instrumental hardness of the extrudates was measured as described in Chapter 5 (Section 5.3.6).

6.3.5 Statistics

A one-way ANOVA [Bonferroni post-hoc test] using SPSS Statistics V17 (SPSS Inc., Chicago, USA) was used to compare means of SDS, and the expansion ratio, density, and hardness of the different extrudates. $P < 0.05$ was considered statistically significant for all analysis for this study.

6.4 Results and discussion

6.4.1 Raw material properties

The sorghum and maize flour characterization and proximate analysis were previously reported in Chapter 3 of this study as follows: sorghum grain properties and presences of tannin (Table 3.2), particle size (Table 3.2), protein, lipid, ash, total starch and fibre content (Table 3.3), and amylose and starch damaged percentage (Table 3.4). However for clarity of comparison with data on the final extrudates some of the data is repeated here in Table 6.2 and will be discussed along with the results of the extrudates.

6.4.2 Extrudates

A photograph of samples of the duplicate extrusion runs of 100% maize and sorghum:maize composite flour of the three varieties of sorghum is given in Figure 6.1.

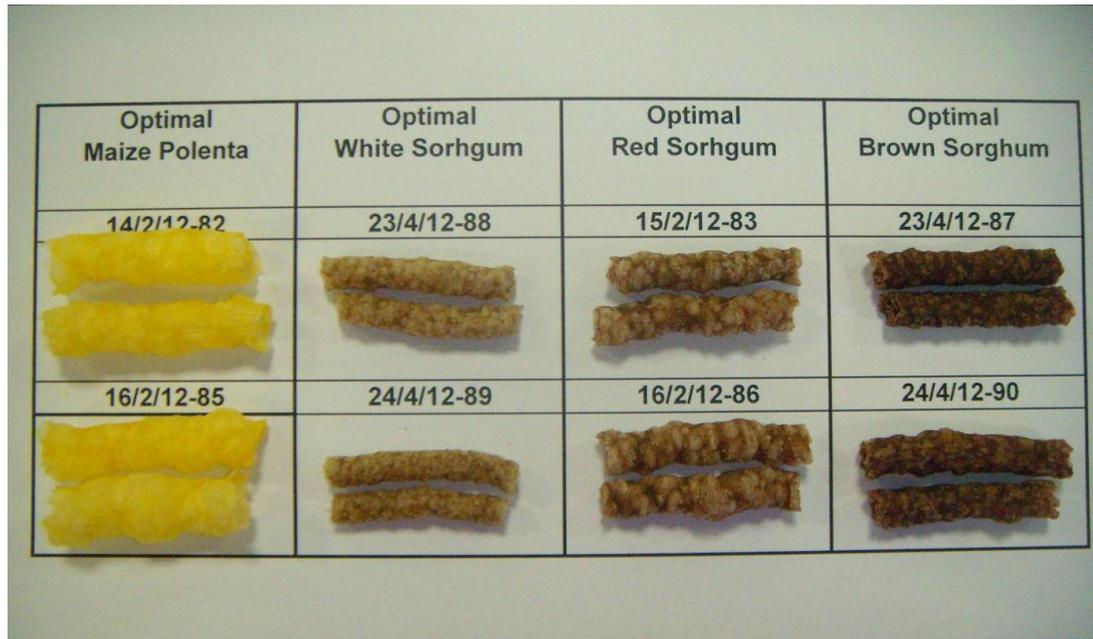


Figure 6.1 Extrudates of 100% maize (“Optimal Maize Polenta”) and sorghum: maize composite flour using white, red and brown sorghum

As can be seen from Figure 6.1, under the same processing conditions the four flours result in extrudates with very different appearances. The 100% maize polenta control is bright yellow in colour and highly expanded, whereas all of the sorghum:maize samples appear darker in colour (even from the white sorghum flour) and less expanded. It is interesting to note that the red and brown sorghum flour appear similar in colour, but after processing, the brown sorghum extrudate appears darker in colour. This could be on account of the tannins in the brown flour.

6.4.3 Chemical characterization of the extrudates

Table 6.2 gives the proximate dietary fibre and chemical composition of the 100% maize, white sorghum, red sorghum, and brown sorghum:maize composite flour extrudates along with those of the raw materials (previous reported in Chapter 3 but presented again here for clarity of comparison and discussion).

Table 6.2 Proximate and fibre analysis on raw flours and optimized extrudates¹

Component (g/100g dry basis)	Maize polenta ²	100% Maize extrudate ³	White sorghum flour ²	White Sorghum-containing extrudate ³	Red sorghum flour ²	Red sorghum-containing extrudate ³	Brown sorghum flour ²	Brown sorghum-containing extrudate ³
Protein*	^x 9.62 ± 0.29	7.26 ± 0.16 ^b	^x 9.92 ± 0.56	10.05 ± 0.17 ^c	^x 8.94 ± 0.39	8.58 ± 0.14 ^b	^x 11.08 ± 0.32	10.17 ± 0.27 ^c
Lipid*	0.81 ± 0.06	0.23 ± 0.12 ^b	1.34 ± 0.08	0.51 ± 0.17 ^b	1.36 ± 0.09	0.53 ± 0.17 ^b	2.80 ± 0.04	0.63 ± 0.56 ^b
Ash*	0.38 ± 0.02	0.19 ± 0.07 ^b	1.50 ± 0.03	1.19 ± 0.05 ^c	1.25 ± 0.06	0.97 ± 0.03 ^c	1.75 ± 0.06	1.30 ± 0.15 ^c
Total dietary fibre*	3.57 ± 0.33	5.50 ± 0.47 ^b	9.00 ± 0.46	11.98 ± 3.72 ^b	9.56 ± 0.50	13.27 ± 1.14 ^b	15.78 ± 0.95	10.25 ± 2.37 ^b

¹ Means of duplicate analyses ± standard deviation.

^x data courtesy of Shilong Wang (Curtin University)

^b Data presented previously in Chapter 3

* data courtesy of ChemCentre, Bentley, WA

^c Values for extrudates within the same row with a different superscript letter are significantly different (P<0.05).

The protein content of the 100% maize extrudate was significantly lower than the white and brown sorghum:maize extrudates ($P=0.014$). The white and brown sorghum:maize extrudates had similar protein content to each other that was significantly lower than the red sorghum:maize extrudate ($P<0.05$). The rankings of the protein content of the extrudates are consistent with those of the raw materials. All extrudates had similar lipid content ($P > 0.05$). The brown sorghum flour had significantly higher lipid content than the other flours ($P<0.05$) but this was not reflected in the extrudates. The 100% maize extrudate had significantly less ash than the three sorghum:maize extrudates ($P=0.006$). This is consistent with the ash content of the flours. No differences were observed in the total dietary fibre of the extrudates ($P>0.05$). This was not consistent with the differences observed in the flours (see chapter 3).

Table 6.3 gives the results of the in-vitro starch digestion of the four extrudates. The SDS level of the 100% maize extrudate was not different from the sorghum:maize composite flour extrudates ($P>0.05$). This is not consistent with other studies which showed the presence of sorghum to have an effect on starch digestion (Ezeogu, et al., 2005; Mahasukhonthachat, et al., 2010a). Surprisingly given the presence of tannins known to be digestion enzyme inhibitors, the brown sorghum composite flour extrudate had lower SDS levels than the red sorghum composite flour extrudate. The reason for this finding is unclear. It is recommended that the extrudates be reanalysed for their SDS levels after being only coarsely ground to more closely mimic the particle size after chewing than the more finely milled sample used in the present study which may have negated any effects of sorghum variety.

Table 6.3 Starch fractions form in-vitro starch digestibility, density, expansion ratio and instrumental hardness of extrudates^a

Extrudate	Slowly digested starch* (g/100g dry starch) ^b	Density (g/cm ³) ^b	Expansion ratio ^b	Instrumental hardness ^b (peak force, g)
100% Maize White	55.1 ± 3.2 ^{cd}	0.27 ± 0.02 ^c	3.19 ± 0.12 ^c	2415 ± 43.1 ^c
Sorghum:maize Red	63.2 ± 0.1 ^{cd}	0.46 ± 0.02 ^d	1.83 ± 0.06 ^d	2556 ± 75.0 ^c
Sorghum:maize Brown	65.2 ± 3.5 ^d	0.43 ± 0.01 ^d	2.13 ± 0.11 ^d	2837 ± 771 ^c
Sorghum:maize	53.6 ± 2.0 ^c	0.44 ± 0.04 ^d	2.16 ± 0.09 ^d	3832 ± 571.5 ^c

^a Means of duplicate analyses ± standard deviation.

^b Values within the same column with a different superscript letter are significantly different (P<0.05).

*Data courtesy of Jiayue Chu (Curtin University)

6.4.4 Expansion ratio, density and hardness of extrudates

Table 6.3 presents the expansion ratio, density and hardness of extrudates. The expansion ratio of the 100% maize extrudate was significantly greater than the three sorghum extrudates (P< 0.05). The protein-starch matrix in sorghum which has been shown to lower the starch gelatinization of sorghum flour could have had been a contributor to the lowering of expansion ratio in the sorghum-containing extrudates (Wong, et al., 2009). The density of the 100% maize extrudate was significantly lower than the three sorghum extrudates (P< 0.05). No difference was seen in the instrumental hardness of the extrudates (P > 0.05). For extruded samples, the level of RDS was greater than 25% and the level of RS was less than 10% (full data not presented), indicating the total digestible starch was higher in the extruded samples than the raw materials.

6.5 Conclusion

Extrudates using 100% maize flour or white, red or brown sorghum:maize composite flours were successfully manufactured under optimal conditions as specified by RSM in Chapter 5. The sorghum-containing extrudates did not demonstrate higher levels of SDS than the 100% maize extrudates that might be expected, given the reports of the slow starch digestibility of sorghum. A more in depth analysis of the in-vitro kinetics of starch digestion, at a larger extrudate particle size more closely resembling that after chewing of the

extrudates is now required. In addition, in vivo studies on their effect on post-meal blood glucose are now required to determine any potential health benefits.

6.5.1 Limitations

Some limitations of this study included: the lack of analysis done on the flours after they had been blended into composites of sorghum and maize which limits the comparison to the extruded samples: the fact that the extrudates were only prepared in duplicate do to time constraints. Samples in triplicate would have contributed to making the data more robust.

6.6 Acknowledgements

I would like to acknowledge the funding support for this project by The Australian Research Council (ARC) and Sanitarium Health & Wellbeing. I wish to thanks Alan Cruickshank and the Queensland Department of Agriculture, fisheries, and Forestry (DAFF) for supply of the sorghum.

I would like to thank Jiayue Chu (Curtin University), Shilong Wang (Curtin University), Haelee Fenton (Curtin University), Komal Tulsidas (Curtin University), Bonny Rawson (Curtin University) and Asteria Hendrianto (Curtin University)and ChemCentre for assistance with extrusion and some of their chemical analyses.

References:

- Buyken, A.E., , Mitchell, P., Ceriello, A., Brand-Miller, J., 2010. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* 53, 406-418.
- Ding, Q.-B., , Ainsworth, P., Plunkett, A., Tucker, G., Marson, H., 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. *Journal of Food Engineering* 73, 142-148.
- Ezeogu, L.I., , Duodu, K.G., Taylor, J.R.N., 2005. Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. *Journal of Cereal Science* 42, 33-44.
- Huber, G.R., 2000. Twin-Screw Extruders. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.
- IPCC, 2007. *Climate Change 2007*. In: M.L. Parry, O.F.C., J.P. Palutikof, P.J. van der Linden and C.E. Hanson (Ed.) *IPCC Fourth Assessment Report: Climate Change 2007*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

- Jenkins, D., Wolever, T., Taylor, R., Barker, H., Fielden, H., Baldwin, J., Bowling, A., Newman, H., Jenkins, A., Goff, D., 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 34, 362-366.
- Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J., 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. *Journal of Cereal Science* 51, 392-401.
- Rooney, L.W., Serna-Saldivar, S.O., 2000. Sorghum In *Handbook of Cereal Science and Technology*, ed. K. Kulp and J. G. Ponte Jr. Marcel Dekker, New York, pp. 149-175.
- Rooney, L.W., Serna Saldivar, S.O., 2003. Sorghum. In: Benjamin, C. (Ed.) *Encyclopedia of Food Sciences and Nutrition*. Academic Press, Oxford, pp. 5370-5375.
- Rooney, L.W., Waniska, R.D., 2000. Sorghum Food and Industrial Utilization. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons Inc, pp. 689-730.
- Sopade, P.A., Gidley, M.J., 2009. A rapid in-vitro digestibility assay based on glucometry for investigating kinetics of starch digestion. *Starch - Stärke* 61, 245-255.
- Srivastava, A., Naresh Kumar, S., Aggarwal, P.K., 2010. Assessment on vulnerability of sorghum to climate change in India. *Agriculture, Ecosystems and Environment* 138, 160-169.
- Taylor, J.R.N., Emmambux, M.N., 2010. Developments in our understanding of sorghum polysaccharides and their health benefits. *Cereal Chemistry* 87, 263-271.
- Thomas, D.J., Atwell, W.A., 1999. *Starches* / David J. Thomas and William A. Atwell Eagan Press, St. Paul, Minn.
- Wong, J.H., Lau, T., Cai, N., Singh, J., Pedersen, J.F., Vensel, W.H., Hurkman, W.J., Wilson, J.D., Lemaux, P.G., Buchanan, B.B., 2009. Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. *Journal of Cereal Science* 49, 73-82.

Chapter 7 Conclusion and future work

7.1 Thesis conclusion

The main objectives of this study was to test the hypotheses that: (1) variations in ingredient proportions and processing conditions will affect starch digestibility, expansion, density and instrumental hardness of extruded sorghum:maize composite flour ; (2) product expansion in combination with the level of SDS in extruded sorghum:maize composite flour could be maximised and (3) sorghum variety will affect the SDS level, expansion, density and instrumental hardness of sorghum:maize composite flour extrudates.

It was determined by factorial screening that the level of SDS, expansion and density of red sorghum:maize composite flour extrudates was significantly associated with levels of some independent ingredient and processing variables namely % moisture content in total feed, % sorghum in dry feed, and final zone temperature. Using response surface methodology (RSM), optimal levels of the independent variables selected from the factorial screening to deliver an extrudate with maximum levels of SDS and maximal expansion were predicted and verified. Using these optimal conditions developed for red-sorghum:maize composite flour extrusion, the SDS levels and expansion, density and instrumental hardness of extrudates made with 100% maize flour and white, red and brown sorghum:maize composite flours were compared.

The SDS levels did not differ significantly between the 100% maize and the sorghum-containing extrudates. This was unexpected given the reports of the slow starch digestibility of sorghum. A more in depth analysis of the in-vitro kinetics of starch digestion at a larger extrudate particle size more closely resembling that after chewing of the extrudates is now required. In addition, in vivo studies on their effect on post-meal blood glucose are now required to determine any potential health benefits.

7.1.5 Final statement

This thesis successfully determined that sorghum:maize composite flour can be used to make an extruded snack-like product with expansion and that, through controlling formulation and processing parameters, the level of SDS in the extrudate can be maximized.

7.2 Future work

This thesis is a part of a larger project investigating the utilization of sorghum in the development of foods to assist with type 2 diabetes mellitus prevention, being performed by a large team of researchers. In parallel to the work presented in this thesis, other researchers have also studied (a) the polyphenolic and antioxidant properties and (b) the protein digestibility properties of the raw materials and extrudates described in this thesis. This data will be integrated into several manuscripts for submission to leading international journals that will describe formulations and processing to deliver a sorghum:maize composite flour prototype expanded snack food with a combination of high levels of SDS, high polyphenolic content and high antioxidant capacity for maximum potential health benefits. In addition other researchers are applying the experimental approaches described in this thesis to develop other sorghum containing foods such as biscuits, pasta, flat bread and flaked breakfast cereal.

Based on the findings from this thesis the optimized extrusion conditions identified in Chapter 5 will now be used to develop a consumer acceptable sorghum-based snack food. The interaction of various levels of salt and sugar added to the dry mix on consumer acceptability and instrumental textural properties will be evaluated. This future study will aim to identify a more commercially relevant extruded product based on the simple model food system prototype reported in this thesis.

Once a consumer acceptable extruded snack product maintaining high levels of SDS and high antioxidant properties has been developed its potential to reduce the risk of type 2 diabetes mellitus and other related chronic disease such as obesity and cardiovascular disease will be investigated in human participants. Firstly, post-prandial studies investigating the effect of the sorghum extruded snack on satiety, blood glucose, insulin and oxidative stress biomarkers will be performed. Secondly, a longer term dietary intervention study will be performed to evaluate the effect of a diet high in sorghum containing foods, compared to those made from conventional grain foods, on chronic disease risk biomarkers.

This suite of ongoing and future studies will ultimately provide formulations and processes for the commercialisation of sorghum foods with substantiated health benefits.

7.3 Global relevance

The results of this study and future work will help provide food processing researchers and companies with new information to assist future development of extruded sorghum foods such as snack foods with potential protective benefits to those at risk of type 2 diabetes mellitus and related chronic diseases such as obesity and cardiovascular disease (Buyken et al., 2010; Seal et al., 2003).

Sorghum is a viable crop in climate change, when conventional grain crops are at risk of failing. As a drought resistant grain, with low levels of SDS and high levels of antioxidants, polyphenolics and phytochemicals, sorghum has great potential to assist with food security and consumer health (Rooney and Waniska, 2000). Developing food uses of sorghum that may result from this project will lead to greater demand and value for this grain and hence also has the potential to increase financial returns to sorghum growers through opportunity for production of higher-value sorghum for specific end-use (Morell, 2012). Food processors will benefit through new value-addition options for both the domestic and export markets. Through greater use of health based sorghum snack foods consumers and government will benefit by reducing burden of diseases such as type two diabetes and heart disease. Health professionals as well will benefit through availability of new products to recommend for health maintenance. Finally the methodological approach utilized in this thesis and future work can also be applied to maximizing the protein digestibility and nutritional quality of sorghum foods for the developing world, to help ensure food security and help combat malnutrition.

References:

- Buyken, A.E., Mitchell, P., Ceriello, A., Brand-Miller, J., 2010. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* 53, 406-418.
- Morell, M.K., 2012. New Cereal Value Chain: From Seed to Sewage. *CEREAL FOODS WORLD* 57, 44-49.
- Rooney, L.W., Waniska, R.D., 2000. Sorghum Food and Industrial Utilization. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons Inc, pp. 689-730.
- Seal, C.J., Daly, M.E., Thomas, L.C., Bal, W., Birkett, A.M., Jeffcoat, R., Mathers, J.C., 2003. Postprandial carbohydrate metabolism in healthy subjects and those with type 2 diabetes fed starches with slow and rapid hydrolysis rates determined in vitro. *British Journal of Nutrition* 90, 853-864.

Publications and presentations

Oral presentations:

R Licata, J Chu, S Wang, R Coorey, A James, H Williams, S Johnson. 2012 Determination of formulation and processing factors affecting health-related properties of extruded sorghum-maize composite flour. *Food Structures, Digestion and Health Conference*, Palmerston North, NZ, 7-9 March 2012.

R Licata, S. Johnson, 2011. Sorghum: A Sustainable Grain Crop for the Future?. *Global Food: Issues and Solutions, Royal Australian Chemical Institute Seminar*, Curtin University, Perth, Western Australia.

References

- Afoakwa, E.O., Aidoo, P.R., Adjonu, R., 2010. Effect of spontaneous fermentation and amylase-rich flour on the nutritive value, functional and viscoelastic properties of cowpea-fortified nixtamalized maize. *International Journal of Food Sciences and Nutrition* 61, 256-271.
- Al-Rabadi, G.J., Torley, P.J., Williams, B.A., Bryden, W.L., Gidley, M.J., 2011. Particle size of milled barley and sorghum and physico-chemical properties of grain following extrusion. *Journal of Food Engineering* 103, 464-472.
- Al-Rabadi, G.J.S., Gilbert, R.G., Gidley, M.J., 2009. Effect of particle size on kinetics of starch digestion in milled barley and sorghum grains by porcine alpha-amylase. *Journal of Cereal Science* 50, 198-204.
- Alvarez-Martinez, L., Kondury, K.P., Harper, J.M., 1988. A general model for expansion of extruded products. *Journal of Food Science* 53, 609-615.
- Anderson, M.J., Whitecomb, P.J., 2005. *RSM Simplified: Optimizing Process Using Response Surface Methods For Design of Experiments* Taylor & Francis Group, LLC, Boca Roton.
- Anton, A.A., Fulcher, G.R., Arntfield, S.D., 2009. Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus vulgaris* L.) flour: Effects of bean addition and extrusion cooking. *Food Chemistry* 113, 989-996.
- AOAC International, 2005a. Official Method of analysis of AOAC International AOAC Official Method 920.87. AOAC International, [Gaithersburg, Md].
- AOAC International, 2005b. Official Method of analysis of AOAC International AOAC Official Method 996.11. AOAC International, [Gaithersburg, Md].
- AOAC International, 2005c. Official Method of analysis of AOAC International AOAC Official Method 985.29. AOAC International, [Gaithersburg, Md].
- AOAC International, 2005d. Official Method of analysis of AOAC International AOAC Official Method 923.03. AOAC International, [Gaithersburg, Md].
- AOAC International, 2006. Official Method of analysis of AOAC International AOAC Official Method 963.15. AOAC International, [Gaithersburg, Md].
- AOAC International, 2008. Official Method of analysis of AOAC International AOAC Official method 925.10. AOAC International, [Gaithersburg, Md].
- APVMA, 2010. Maximum residue limits in food and animal feedstuff. A.P..V.M.A. (Ed.) Commonwealth of Australia
- Asare, E.K., Sefa-Dedeh, S., Afoakwa, E.O., Sakyi-Dawson, E., Budu, A.S., 2010. Response surface methodology for studying the effects of feed moisture and ingredient variations on the chemical composition and appearance of extruded sorghum-groundnut-cowpea blends. *International Journal of Food Engineering* 6.
- Awika, J.M., Suhendro, E.L., Rooney, L.W., 2002. Milling value of sorghums compared by adjusting yields to a constant product color. *Cereal Chemistry* 79, 249-251.
- Bas, D., Boyaci, I.H., 2007. Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering* 78, 836-845.
- Beta, T., Obilana, A.B., Corke, H., 2001. Genetic diversity in properties of starch from Zimbabwean sorghum landraces. *Cereal Chemistry* 78, 583-589.
- Britannica, E., 1996. *Extruder Encyclopaedia* Britannica Inc.
- Buyken, A.E., Mitchell, P., Ceriello, A., Brand-Miller, J., 2010. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* 53, 406-418.
- Chanlat, N., Songsermpong, S., Charunuch, C., Naivikul, O., 2011. Twin-screw extrusion of pre-germinated brown rice: physicochemical properties and γ -aminobutyric acid content (GABA) of extruded snacks. *International Journal of Food Engineering* 7.

- Chauhan, O.P., , Raju, P.S., Ravi, N., Roopa, N., Bawa, A.S., 2011. Studies on retention of antioxidant activity, phenolics and flavonoids in high pressure processed black grape juice and their modelling. *International Journal of Food Science & Technology* 46, 2562-2568.
- Chu, J., 2011. Modeling the effects of extrusion on the concentration of phenolic compounds and antioxidant capacity of red sorghum Food Science & Technology. Curtin University of Technology.
- DAFF, 2010. Agricultural Food Production. In: Forestry, D.O.A.F.A. (Ed.) Australian Food Statistics 2009-10. Commonwealth of Australia 2011.
- Davis, D.A., Arnold, C.R., 1995. Effects of two extrusion processing conditions on the digestibility of four cereal grains for *Penaeus vannamei*. *Aquaculture* 133, 287-294.
- De Wet, J.M., 1971. The Origin and Domestication of *Sorghum bicolor*. *Economic Botany* 25, 128.
- Dendy, D.A.V., 1995. Sorghum and millets : chemistry and technology American Association of Cereal Chemists St. Paul, Minn.
- Dileep, A.O., Shamasundar, B.A., Binsi, P.K., Howell, N.K., 2010. Composition and quality of rice flour–fish mince based extruded products with emphasis on thermal properties of rice flour. *Journal of Texture Studies* 41, 190-207.
- Ding, Q.-B., , Ainsworth, P., Plunkett, A., Tucker, G., Marson, H., 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. *Journal of Food Engineering* 73, 142-148.
- Dona, A.C., , Pages, G., Gilbert, R.G., Kuchel, P.W., 2010. Digestion of starch: In vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release. *Carbohydrate Polymers* 80, 599-617.
- Englyst, H.N., , Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* 46, S33-S50.
- Englyst, K., Vinoy, S., Englyst, H., Lang, V., 2003. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *The British Journal of Nutrition* 89, 329.
- Englyst, K.N., , Englyst, H.N., Hudson, G.J., Cole, T.J., Cummings, J.H., 1999. Rapidly available glucose in foods: an in vitro measurement that reflects the glycaemic response. *American Journal of Clinical Nutrition* 69, 448-454.
- Englyst, K.N., Englyst, H.N., 2005. Carbohydrate bioavailability. *British Journal of Nutrition* 94, 1-11.
- Ezeogu, L.I., , Duodu, K.G., Taylor, J.R.N., 2005. Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. *Journal of Cereal Science* 42, 33-44.
- Falcone, R.G., Phillips, R.D., 1988. Effects of feed composition, feed moisture, and barrel temperature on the physical and rheological properties of snack-like products prepared from cowpea and sorghum flours by extrusion. *Journal of Food Science* 53, 1464-1469.
- FAO, 1998. Carbohydrates in Human Nutrition. In: Repository, F.C.D. (Ed.) Food and Agriculture Organization of the United Nations.
- FAO, 2008. Food and Agricultural Commodities Production Food and Agriculture Organization of the United Nations
- FAO, 2010. Food and Agricultural Commodities Production Food and Agriculture Organization of the United Nations
- FCD, 1995. Chapter VII Sorghum: Post-harvest Operations. In: AGSI/FAO: Danilo Mejia (Technical), B.L.L.S., Carolin Bothe (HTML transfer) (Ed.) Food Security Department
- FDA, 1999. Phosphine; tolerances for residues. In: Regulations, C.o.F. (Ed.) 40 CFR 180.225. U. S. Food and Drug Administration, United States of America, p. 1.
- Garber, B.W., Hsieh, F., Huff, H.E., 1997. Influence of particle size on the twin-screw extrusion of corn meal. *Cereal Chemistry* 74, 656-661.

Gibson, T., Al Qalla, H., McCleary, B., 1991. An improved enzymatic method for the measurement of starch damage in wheat flour. *Journal of Cereal Science* 15, 15-27.

Hasjim, J., Lavau, G.C., Gidley, M.J., Gilbert, R.G., 2010. In vivo and in vitro starch digestion: Are current in vitro techniques adequate? *Biomacromolecules* 11, 3600-3608.

Hu, P., Zhao, H., Duan, Z., Linlin, Z., Wu, D., 2004. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Science* 40, 231-237.

Huber, G.R., 2000. Twin-Screw Extruders. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.

Hur, S.J., Lim, B.O., Decker, E.A., McClements, D.J., 2011. In vitro human digestion models for food applications. *Food Chemistry* 125, 1-12.

IPCC, 2007. *Climate Change 2007*. In: M.L. Parry, O.F.C., J.P. Palutikof, P.J. van der Linden and C.E. Hanson (Ed.) *IPCC Fourth Assessment Report: Climate Change 2007*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jenkins, D., Wolever, T., Taylor, R., Barker, H., Fielden, H., Baldwin, J., Bowling, A., Newman, H., Jenkins, A., Goff, D., 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 34, 362-366.

Kpodo, K.A., Plahar, W.A., 1992. Effects of extrusion variables and starch modification on the puffing characteristics of yam (*Dioscorea alata*) flour. *Food Control* 3, 200-204.

Lee, C.-C., Lee, C.-C., Ku, H.-T., Chang, S.-M., Chiang, K.-N., 2007. Solder joints layout design and reliability enhancements of wafer level packaging using response surface methodology. *Microelectronics Reliability* 47, 196-204.

Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J., 2010a. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. *Journal of Cereal Science* 51, 392-401.

Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J., 2010b. Kinetics of starch digestion in sorghum as affected by particle size. *Journal of Food Engineering* 96, 18-28.

Méance, S., Achour, L., Briend, A., 1999. Comparison of starch digestibility of a blended food prepared with and without extrusion cooking. *European Journal of Clinical Nutrition* 53, 844-848.

Montgomery, D.C., 2001. *Design and Analysis of Experiments*, 5th ed. John Wiley, New York.

Morell, M.K., 2012. New cereal value chain: From seed to sewage. *Cereal Foods World* 57, 44-49.

Moscicki, L., Zuilichem, D.J.v., 2011. *Extrusion-Cooking and Related Techniques* In: Moscicki, L. (Ed.) *Extrusion-Cooking Techniques - Applications, Theory and Sustainability*. Wiley-VCH Verlag & Co, Weinheim, Germany.

Nguyen, T.H., 2008. The effects of process variables on the quality of extruded products of lupin. School of Public Health. Curtin University of Technology, Perth, pp. xvi, 274 leaves : col. ill. ; 31 cm.

Nwabueze, T.U., 2010. Review article: Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimisation in the food systems. *International Journal of Food Science & Technology* 45, 1768-1776.

Pray, C.E., Nagarajan, L., 2009. Improving Crops for Arid Lands: Pearl Millet and Sorghum in India. In: Institute, I.F.P.R. (Ed.) *Pearl Millet and Sorghum Improvement in India*. Douglas Smith, Washington DC, pp. 83-88.

Qingdao Friend Plastic Extrusion Technology Co., L., 2012. *Twin Screw Extruders*.

Riaz, M.N., 2000. Introduction to Extruders and Their Principles. In: Riaz, M.N. (Ed.) *Extruders in food applications*. CRC Press, Boca Raton, FL, pp. 81-114.

Rooney, L.W., Serna-Saldivar, S.O., 2000. Sorghum In *Handbook of Cereal Science and Technology*, ed. K. Kulp and J. G. Ponte Jr. Marcel Dekker, New York, pp. 149-175.

Rooney, L.W., Serna Saldivar, S.O., 2003. SORGHUM. In: Benjamin, C. (Ed.) *Encyclopedia of Food Sciences and Nutrition*. Academic Press, Oxford, pp. 5370-5375.

Rooney, L.W., Waniska, R.D., 2000. Sorghum Food and Industrial Utilization. In: Smith, C.W., Frederiksen, R.A. (Eds.), Sorghum: Origin, History, Technology, and Production. John Wiley & Sons Inc, pp. 689-730.

Sang, Y., Bean, S., Seib, P.A., Pedersen, J., Shi, Y.-C., 2008a. Structure and Functional Properties of Sorghum Starches Differing in Amylose Content. *Journal of Agricultural and Food Chemistry* 56, 6680-6685.

Sang, Y., Seib, P.A., Shi, Y.C., Bean, S., Pedersen, J., 2008b. Structure and functional properties of sorghum starches differing in amylose content. *Journal of Agricultural and Food Chemistry* 56, 6680-6685.

Seal, C.J., Daly, M.E., Thomas, L.C., Bal, W., Birkett, A.M., Jeffcoat, R., Mathers, J.C., 2003. Postprandial carbohydrate metabolism in healthy subjects and those with type 2 diabetes fed starches with slow and rapid hydrolysis rates determined in vitro. *British Journal of Nutrition* 90, 853-864.

Shawrang, P., Behgar, M., Shahhoseini, G., Sadeghi, A.A., Zareshahi, H., 2011. Study of chemical compositions, anti-nutritional contents and digestibility of electron beam irradiated sorghum grains. *Food Chemistry* 125, 376-379.

Sopade, P.A., Gidley, M.J., 2009a. A rapid in-vitro digestibility assay based on glucometry for investigating kinetics of starch digestion. *Starch - Stärke* 61, 245-255.

Srivastava, A., Naresh Kumar, S., Aggarwal, P.K., 2010. Assessment on vulnerability of sorghum to climate change in India. *Agriculture, Ecosystems and Environment* 138, 160-169.

Suksomboon, A., Limroongreungrat, K., Sangnark, A., Thititumjariya, K., Noomhorm, A., 2011. Effect of extrusion conditions on the physicochemical properties of a snack made from purple rice (Hom Nil) and soybean flour blend. *International Journal of Food Science and Technology* 46, 201-208.

Taylor, J.R.N., Schober, T.J., Bean, S.R., 2006. Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science* 44, 252-271.

Taylor, J.R.N., Emmambux, M.N., 2010. Developments in our understanding of sorghum polysaccharides and their health benefits. *Cereal Chemistry* 87, 263-271.

Thakur, S., Saxena, D.C., 2000. Formulation of extruded snack food (gum based cereal-pulse blend): optimization of ingredients levels using response surface methodology. *Lebensmittel-Wissenschaft und-Technologie* 33, 354-361.

Thomas, D.J., Atwell, W.A., 1999. *Starches* / David J. Thomas and William A. Atwell Eagan Press, St. Paul, Minn.

Thyer, R., 2008. Pest Paradise Raises Grain Storage Stakes Ground Cover. Grains Research and Development Corporation.

USDA, 2009. Sorghum USDA National Nutrient Database for Standard Reference. United States Department of Agriculture.

Waniska, R.D., Hugo, L.F., Rooney, L.W., 1992. Practical methods to determine the presence of tannins in sorghum. *The Journal of Applied Poultry Research* 1, 122-128.

Waniska, R.D., Rooney, L.W., 2000. Structure and Chemistry of The Sorghum Caryopsis. In: Smith, C.W., Frederiksen, R.A. (Eds.), Sorghum: Origin, History, Technology, and Production. John Wiley & Sons Inc, pp. 649-688.

Wong, J.H., Lau, T., Cai, N., Singh, J., Pedersen, J.F., Vensel, W.H., Hurkman, W.J., Wilson, J.D., Lemaux, P.G., Buchanan, B.B., 2009. Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. *Journal of Cereal Science* 49, 73-82.

Yousif, A., Nhepera, D., Johnson, S., 2012. Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability. *Food Chemistry* 134, 880-887.

Youssef, A.M.M., Moharram, Y.G., Moustaffa, E.K., Bolling, H., El-Baya, A., Harmuth, A.E., 1990. New extruded products from sorghum. *Food Chemistry* 37, 189-199.

Yun, S.H., Matheson, N.K., 1990. Estimation of amylose content of starches after precipitation of amylopectin by concanavalin-A. *Starch-Starke* 42, 302-305.

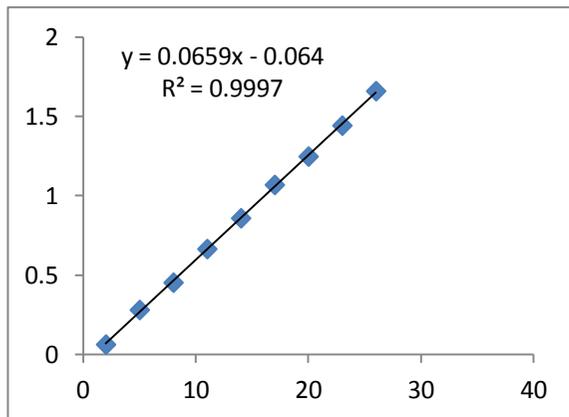
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Appendices

Appendix 1 Calibration curve of water flow rate and sample feed flow rate

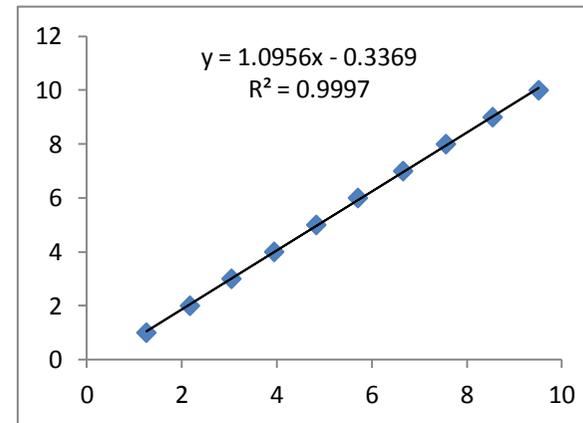
Water flow rate

Dial (%water)	Flow (kg/36s)	Flow (Kg/hr)
2	0.00062	0.062
5	0.0028	0.28
8	0.00452	0.452
11	0.00664	0.664
14	0.00858	0.858
17	0.01068	1.068
20	0.01247	1.247
23	0.01442	1.442
26	0.01659	1.659



Feed flow rate 15% Red Sorghum

Dial setting	Flow (Kg/36s)	Flow (Kg/hr)
1	0.01258	1.258
2	0.02175	2.175
3	0.0305	3.05
4	0.03949	3.949
5	0.04835	4.835
6	0.05712	5.712
7	0.06663	6.663
8	0.07565	7.565
9	0.08549	8.549
10	0.09518	9.518



Appendix 2 Factorial Design extrusion sample runs

Date	Sample	Flour	% sorghum	% moisture in flour	Feed Rate Dial #	% dry feed in total input	Moisture Dial #	Total material input kg/hr	% added moisture in total material	total % moisture of total material	Final barrel temp (°C)	Screw speed	Recorded die temp (°C)	Recorded Pressure at time of sample taking [psi]	Recorded Torque at time of sample taking [%]	temperature of water source for extruder (°C)
5/30/2011	30-5-11-40	red sorghum: maize	15	13.8	6.4	91.1	10	6.8	8.9	21.46	150	450	154	290	55	n/a
5/30/2011	30-5-11-41	red sorghum: maize	15	13.8	1.8	86	6	2.3	14	25.86	150	450	154	120	38	n/a
5/30/2011	30-5-11-42	red sorghum: maize	15	13.8	2	91.1	4	2.3	8.9	21.46	120	450	125	230	50	n/a
6/3/2011	3/6/11-44	red sorghum: maize	60	12.7	7.4	85	16	6.8	15.1	25.84	120	250	128	340	90	n/a
6/6/2011	3/6/11-46	red sorghum: maize	60	12.7	7.8	90	11	6.8	10	21.44	120	450	128	425	90	n/a
6/6/2011	3/6/11-45	red sorghum: maize	60	12.7	2.4	90	4	2.3	10	21.44	150	450	156	220	36	n/a
6/13/2011	13/6/11-47	red sorghum: maize	15	13.8	6	86	15	6.8	14	25.86	120	450	129	260-290	52	n/a
6/13/2011	13/6/11-48	red sorghum: maize	15	13.8	6	86	15	6.8	14	25.86	150	250	155	250	51	n/a

6/17/201 1	17/6/11-49	red sorghum: maize	60	12.7	2.2	85	6	2.3	15.1	25.84	150	250	150	125	28	n/a
6/17/201 1	17/6/11-50	red sorghum: maize	15	13.8	6.4	91.1	10	6.8	8.9	21.46	120	250	132	425	90	n/a
6/17/201 1	17/6/11-52	red sorghum: maize	15	13.8	1.8	86	6	2.3	14	25.86	120	250	131	170	42	n/a
6/27/201 1	27/6/11-56	red sorghum: maize	60	12.7	7.4	85	16	6.8	15.1	25.84	150	450	158	205-230	39	24
6/30/201 1	30/6/11-57	red sorghum: maize	15	13.8	2	91.1	4	2.3	8.9	21.46	150	250	158	176	50	21.5
6/30/201 1	30/6/11-58	red sorghum: maize	60	12.7	7.8	90	11	6.8	10	21.44	150	250	160	260	100	21.5
7/13/201 1	13/7/11-59	red sorghum: maize	60	12.7	2.2	85	6	2.3	15.1	25.84	120	450	129	180-200	27	21
7/13/201 1	13/7/11-60	red sorghum: maize	60	12.7	2.4	90	4	2.3	10	21.44	120	250	133	297	39	21

Temperature profile	zone 1 (°C)	zone 2 (°C)	zone 3 (°C)	zone 4 (°C)
150	90	105	125	150
120	90	95	100	120

Appendix 3

Multiple regression analysis statistical output for response slowly digestible starch

ANOVA for Response Surface Linear Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value	Prob > F	
Model	455.4613	3	151.8204	13.88573	0.0001		significant
A-Moisture total	0.497833	1	0.497833	0.045533	0.8339		
B-Barrel Temp	104.9811	1	104.9811	9.601741	0.0073		
C-% sorghum	349.9823	1	349.9823	32.00993	< 0.0001		
Residual	164.0033	15	10.93355				
Lack of Fit	118.7027	10	11.87027	1.310165	0.4036		not significant
Pure Error	45.30064	5	9.060129				
Cor Total	619.4646	18					

Std. Dev.	3.306592478	R-Squared	0.735249906
Mean	16.61111433	Adj R-Squared	0.682299888
C.V. %	19.90590404	Pred R-Squared	0.547465995
PRESS	280.3287905	Adeq Precision	12.07229959

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	16.104398	1	0.7638531	14.476283	17.732512	
A-Moisture total	0.1909266	1	0.8947575	-2.098057	1.7162038	1
B-Barrel Temp	-2.772556	1	0.8947575	4.6796864	0.8654256	1
C-% sorghum	5.7246153	1	1.0118216	3.5679686	7.881262	1

Final Equation in Terms of Coded Factors:

$$\begin{aligned} \text{SDS} &= \\ &16.1044 \\ &-0.19093 * A \\ &-2.77256 * B \\ &5.724615 * C \end{aligned}$$

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{SDS} &= \\ &34.7815 \\ &-0.12728 * \text{Moisture total} \\ &-0.2218 * \text{Barrel Temp} \\ &0.228985 * \% \text{ sorghum} \end{aligned}$$

Appendix 4

Multiple regression analysis statistical output for expansion ratio

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.5822817	9	0.064698	7.4137321	0.0022	significant
A-Moisture total	0.1692743	1	0.1692743	19.39712	0.0013	
B-Barrel Temp	0.1555799	1	0.1555799	17.827884	0.0018	
C-% sorghum	0.0327281	1	0.0327281	3.7503145	0.0815	
AB	0.0001837	1	0.0001837	0.0210479	0.8875	
AC	0.0442531	1	0.0442531	5.0709602	0.0480	
BC	0.0569531	1	0.0569531	6.5262516	0.0286	
A ²	0.0001201	1	0.0001201	0.0137618	0.9089	
B ²	0.0526761	1	0.0526761	6.036145	0.0339	
C ²	0.0789399	1	0.0789399	9.0457133	0.0132	
Residual	0.0872677	10	0.0087268			
Lack of Fit	0.0447988	5	0.0089598	1.0548584	0.4773	not significant
Pure Error	0.042469	5	0.0084938			
Cor Total	0.6695494	19				

Std. Dev.	0.093417205	R-Squared	0.869661989
Mean	2.446833333	Adj R-Squared	0.752357779
C.V. %	3.817881839	Pred R-Squared	0.362749179
PRESS	0.426670933	Adeq Precision	11.40898788

Factor	Coefficient		Standard Error	95% CI		VIF
	Estimate	df		Low	High	
Intercept	2.5366835	1	0.0381	2.4517913	2.6215757	
A-Moisture total	-0.111332	1	0.0252785	-0.167656	-0.055008	1
B-Barrel Temp	-0.106734	1	0.0252785	-0.163058	-0.05041	1
C-% sorghum	-0.048954	1	0.0252785	-0.105278	0.0073704	1
AB	0.0047917	1	0.033028	-0.068799	0.0783826	1
AC	0.074375	1	0.033028	0.0007841	0.1479659	1
BC	0.084375	1	0.033028	0.0107841	0.1579659	1
A^2	0.0028868	1	0.0246079	-0.051943	0.0577167	1.0182652
B^2	-0.060458	1	0.0246079	-0.115288	-0.005628	1.0182652
C^2	-0.074011	1	0.024608	-0.128841	-0.019181	1.0182652

Final Equation in Terms of Coded Factors:

$$\begin{aligned}
 \text{Expansion} &= \\
 &2.536684 \\
 &-0.11133 * A \\
 &-0.10673 * B \\
 &-0.04895 * C \\
 &0.004792 * A * B \\
 &0.074375 * A * C \\
 &0.084375 * B * C \\
 &0.002887 * A^2 \\
 &-0.06046 * B^2 \\
 &-0.07401 * C^2
 \end{aligned}$$

Final Equation in Terms of Actual Factors:

$$\begin{aligned}
 \text{Expansion} &= \\
 &4.760066 \\
 &-0.27619 * \text{Moisture total} \\
 &0.069274 * \text{Barrel Temp} \\
 &-0.06997 * \% \text{ sorghum} \\
 &0.000256 * \text{Moisture total} * \text{Barrel Temp} \\
 &0.001983 * \text{Moisture total} * \% \text{ sorghum} \\
 &0.00027 * \text{Barrel Temp} * \% \text{ sorghum} \\
 &0.001283 * \text{Moisture total}^2 \\
 &-0.00039 * \text{Barrel Temp}^2 \\
 &-0.00012 * \% \text{ sorghum}^2
 \end{aligned}$$

Appendix 5

Multiple regression analysis statistical output for density

ANOVA for Response Surface Linear Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.03327	3	0.01109	13.82491	0.0001	significant
A-Moisture total	0.00555	1	0.00555	6.918688	0.0182	
B-Barrel Temp	0.025567	1	0.025567	31.87177	< 0.0001	
C-% sorghum	0.002153	1	0.002153	2.684265	0.1209	
Residual	0.012835	16	0.000802			
Lack of Fit	0.008688	11	0.00079	0.952435	0.5631	not significant
Pure Error	0.004146	5	0.000829			
Cor Total	0.046105	19				

Std. Dev.	0.028322657	R-Squared	0.721616735
Mean	0.296129003	Adj R-Squared	0.669419872
C.V. %	9.564296781	Pred R-Squared	0.55972642
PRESS	0.020298664	Adeq Precision	11.99770738

Factor	Coefficient		Standard Error	95% CI		VIF
	Estimate	df		Low	High	
Intercept	0.296129	1	0.00633314	0.28270335	0.30955466	
A-Moisture total	0.02015907	1	0.00766406	0.003912	0.03640614	1
B-Barrel Temp	-0.0432675	1	0.00766406	0.0595146	-0.0270204	1
C-% sorghum	0.01255658	1	0.00766406	0.0036905	0.02880365	1

Final Equation in Terms of Coded Factors:

$$\begin{aligned} \text{density} &= \\ &0.296129 \\ &0.020159 * A \\ &-0.04327 * B \\ &0.012557 * C \end{aligned}$$

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{density} &= \\ &0.394007 \\ &0.013439 * \text{Moisture total} \\ &-0.00346 * \text{Barrel Temp} \\ &0.000502 * \% \text{ sorghum} \end{aligned}$$

Appendix 6

Multiple regression analysis statistical output for hardness (peak force)

These Rows Were Ignored for this Analysis.

1, 2

ANOVA for Response Surface Quadratic Model
Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4925045.6	9	547227.289	6.07812535	0.0092	significant
A-Moisture total	100325.88	1	100325.88	1.11433272	0.3220	
B-Barrel Temp	1658705.7	1	1658705.7	18.423462	0.0026	
C-% sorghum	93653.704	1	93653.704	1.04022399	0.3376	
AB	116670.693	1	116670.693	1.29587671	0.2879	
AC	290447.996	1	290447.996	3.22604403	0.1102	
BC	259769.361	1	259769.361	2.88529239	0.1278	
A^2	110668.224	1	110668.224	1.22920649	0.2998	
B^2	389.638471	1	389.638471	0.00432777	0.9492	
C^2	1554254.37	1	1554254.37	17.2633073	0.0032	
Residual	720257.985	8	90032.2481			
Lack of Fit	256513.158	3	85504.3858	0.92189045	0.4942	not significant
Pure Error	463744.828	5	92748.9655			
Cor Total	5645303.59	17				

Std. Dev.	300.0537421	R-Squared	0.872414659
Mean	2072.084833	Adj R-Squared	0.72888115
C.V. %	14.48076533	Pred R-Squared	-0.01001696
PRESS	5701852.372	Adeq Precision	8.144682034

Factor	Coefficient		Standard Error	95% CI		VIF
	Estimate	df		Low	High	
Intercept	1830.957042	1	122.4154	1548.667	2113.248	
A-Moisture total	104.170657	1	98.682	-123.39	331.7318	1.236802
B-Barrel Temp	-423.568429	1	98.682	-651.13	-196.007	1.236802
C-% sorghum	-98.4792435	1	96.55645	-321.139	124.1803	1.207107
AB	162.6756682	1	142.9028	-166.859	492.2101	1.310522
AC	-248.954253	1	138.6068	-568.582	70.6735	1.28033
BC	-235.439497	1	138.6068	-555.067	84.18826	1.28033
A^2	-90.4492402	1	81.58165	-278.577	97.67839	1.068278
B^2	5.366910681	1	81.58165	-182.761	193.4945	1.068278
C^2	338.9648693	1	81.58169	150.8372	527.0926	1.068278

Final Equation in Terms of Coded Factors:

$$\begin{aligned}
 \text{Peak Force} &= \\
 &1830.957 \\
 &104.1707 * A \\
 &-423.568 * B \\
 &-98.4792 * C \\
 &162.6757 * A * B \\
 &-248.954 * A * C \\
 &-235.439 * B * C \\
 &-90.4492 * A^2 \\
 &5.366911 * B^2 \\
 &338.9649 * C^2
 \end{aligned}$$

Final Equation in Terms of Actual Factors:

$$\begin{aligned}
 \text{Peak Force} &= \\
 &-3133.61 \\
 &1217.77 * \text{Moisture total} \\
 &-205.094 * \text{Barrel Temp} \\
 &188.4737 * \% \text{ sorghum} \\
 &\quad * \text{Moisture total} * \text{Barrel} \\
 &8.676036 \text{Temp} \\
 &-6.63878 * \text{Moisture total} * \% \text{ sorghum} \\
 &-0.75341 * \text{Barrel Temp} * \% \text{ sorghum} \\
 &-40.1997 * \text{Moisture total}^2 \\
 &0.034348 * \text{Barrel Temp}^2 \\
 &0.542344 * \% \text{ sorghum}^2
 \end{aligned}$$

Appendix 7

Peak force (hardness) of commercial extruded products means

Commercial extruded product	Peak force mean (g)
Twisties®	943.11
Cheezels®	445.93
Cheetoes®	594.56
Grain Waves®	1139.19