Synthesis of resistant starch in three Rwandese cassava varieties and its applicability in yoghurt processing

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Abstract

Starch is the most plentiful granular polysaccharide in plants where it is found in the chloroplast of leaves and the amyloplast. Based on digestibility starch is classified into three types: rapidly digestible starch, slowly digestible starch and resistant starch. Resistant starch (RS) is defined as the sum of starch and products of starch breakdown which are not absorbed in the small intestine of humans. In bowel, RS serve as a substrate for microbial fermentation that produce carbon dioxide, methane and short chain fatty acids known to prevent colon diseases. Cassava is a staple food crop in Rwanda where it is mainly utilized as flour for paste (Ugali) or consumed raw, boiled, fried or roasted and its leaves are consumed as vegetables. Differences in amounts of RS present in popular cassava varieties in Rwanda and effects of applying different heat treatments on cassava starch is not yet known. On the other hand, popular products such as yoghurt rely heavily on imported relatively expensive corn starch, pectin and gelatin for their production in Rwandan context. Application of cassava starch with good component of beneficial healthy RS in their processing as a thickening agent is yet to be tested. There is also a wide gap in cassava value addition in Rwanda where its industrialization is solely limited to manufacturing of packaged flour. The current proposed project seeks to determine the levels of RS in popular cassava varieties in Rwanda and to demonstrate opportunity in adding value to cassava starch by synthesizing RS and testing its applicability in processing acceptable yoghurt. This study will provide scientific information on which cassava variety and treatment can produce large amount of RS and will demonstrate the opportunity for cassava product diversification as well as providing yoghurt rich in probiotics and prebiotics (RS) as a preventive measure against colon cancer.

Key words: Resistant starch, Cassava, Yogurt
Abbreviations and Acronyms

AACC: American Association of Cereal Chemists
AMG: Amyloglucosidase
ANOVA: Analysis of Variance
AOAC: Association of Official Analytical Chemists
EFSA: The European Food Safety Authority
FAO: Food and Agriculture Organization
GOPOD: Glucose Oxidase–Peroxidase Reagent
HMT: Heat Moisture Treatment
ISO: International Organization for Standardization
M.C: Moisture Content
MINAGRI: Ministry of Agriculture and Animal Resources
MT: Metric Ton
NISR: National Institute of Statistics of Rwanda
RAB: Rwanda Agriculture Board
RDB: Rwanda Development Board
Rpm: Rotation per Minute
RS: Resistant Starch
SCFA: Short Chain Fatty Acids
USAID: United States Agency for International Development
WAI: Water Absorption Index
WSI: Water Solubility Index
**Terminology**

*Cassava* (*Manihot esculenta*) is a woody plant in the *Euphorbiaceae* family. It is widely cultivated as an annual crop in tropical and subtropical regions.

**Starch** is a natural polymer occurring in plant organisms as an indirect product of photosynthesis synthesized from glucose, which is formed from carbon dioxide and water and is the major component of most of plant-originated foodstuffs.

**Resistant starch** is the sum of starch and products of starch degradation which have not been absorbed in the small intestine of healthy humans. They are transported to the large intestine where they are fermented by microorganisms to yield SCFAs, carbon dioxide and methane.

**Starch Gelatinization** is a process of breaking down the intermolecular bonds of starch molecules in the presence of water and heat which irreversibly damages the starch granule in water.

**Starch retrogradation** is a process in which disaggregated amylose and amylopectin chains in gelatinized starch reassociate to form more ordered structures as the cooked starch cools.

**Hydrothermal modification of starch** refers to physical modification of starch without gelatinization or damage of the starch granules in terms of size, shape or birefringence through a controlled application of heat and moisture.
Starch annealing refers to alterations within the crystallites of the granules which consist of incubation of granular starch in excess water for a period of time at a temperature below the gelatinization temperature.

Heat-moisture treatment refers to hydrothermal treatment which consists of treatment of starch granules at restricted levels of moisture (<35%) during a given time (15 minutes–16 hours) and temperature (84–120°C) above the glass transition temperature but below the gelatinization temperature.
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CHAPTER ONE: INTRODUCTION

1.1. Background information

Starch is the most plentiful granular polysaccharide in plants where it is found in the chloroplast of leaves and the amyloplast (Ellis et al., 1998). It is the main source of energy for many people and in the diet it is contributed mainly by cereal grains, root crops and tubers (Vegh, 2009).

Based on digestibility starch is classified into three types: rapidly digestible starch, slowly digestible starch and resistant starch (Pereira and Magali, 2014). Resistant starch (RS) is defined as the sum of starch and products of starch breakdown which are not absorbed in the small intestine of healthy humans (Englyst et al., 1992). From there, it is conveyed to the large intestine and it is fermented by microorganisms into short chain fatty acids (SCFAs), methane and carbon dioxide. Starch’s resistance to digestion is contributed by different reasons which lead to description of four groups. These are: resistant starch I (RSI) that is unreachable to digestion by being surrounded in a indigestible medium; resistant starch II (RSII) which is un-gelatinized starch that is in a granular form and resistant to enzyme digestion; resistant starch III (RSIII) that is retrograded starch formed through cooling of gelatinized starch and resistant starch IV (RSIV) which is modified starch due to chemical cross-bonding with reagents, ethers and esters (Sajilata et al., 2006; Nugent, 2005).

In bowel, resistant starch serve as a substrate for microbial fermentation that produce carbon dioxide, methane and short chain fatty acids like acetic, propionic and butyric acid that are known to prevent colon diseases (Noor-Aziah et al., 2011, Nugent, 2005). As a result, resistant starch has got good impact on diabetes, cardiovascular diseases, colonic health and obesity (Lunn and Buttriss, 2007; Nugent, 2005; Sajilata et al., 2006; Morales-Medina et al., 2014). Researches in nutrition have demonstrated that RS
increases lipid and cholesterol breakdown, cut down the threat of ulcerative colitis, colorectal cancer, constipation, Type II diabetes and fixes toxins, bile acids, and carcinogens (Haralampu, 2000).

The level of RS in different crops and food products have been studied by Moongngarm (2013) in Thailand; Pereira and Leonel (2014) in Brazil; Ogbo and Okafor (2015) in Nigeria and these levels in cassava were found to range from 0.19% to 9.69% of total starch.

RS has also been perceived in different food like breads, breakfast cereals, biscuits, maize, mashed potatoes and legumes (Pereira and Magali, 2014). The main obstacle to the food industry is the production of foods with sufficient RS resulting in an important enhancement of consumer’s health (Chung et al., 2011), and cassava could be one of the important sources that can be incorporated in consumer friendly foods like yoghurt.

Cassava is a staple food crop in Rwanda with 3,161,470 MT of annual production on 195,910 ha of land in 2014 with an average annual increase of 7.28% since 2010 (FAOSTAT, 2014). Cassava is mainly utilized in Rwanda as flour for paste (Ugali) or consumed raw, boiled, fried or roasted and its leaves are consumed as vegetables (Adekunle, 2007; Umuhozariho et al., 2011). The current industrial utilization of cassava in Rwanda is solely for packaged flour and starch production is planned for the future. This indicates a gap in value addition to cassava which may not only promote industrial development in Rwanda but also increase incomes of both farmers and processors. Post-harvest losses for cassava are estimated at more than 30% in Rwanda (USAID, 2010), therefore, diversification of cassava products can be a solution to post-harvest losses that may arise from the increased production of this commodity.

The dairy sector in Rwanda has experienced an extreme revolution as a result of many programmes which have led to an increased milk production from 372,619 tons in 2006 to 706,030 tons in 2014 (NISR, 2015). The National Dairy Strategy envisages an increase of milk production of 13% per year and milk
exports is to reach 20 million US dollars per year by 2017 (RDB, 2012; MINAGRI, 2013). Value-added products like cheese and shelf-stable yoghurt, which is currently an emerging milk product, offer attractive market opportunities though impeded by import of packaging material and some ingredients (MINAGRI, 2013). Hence, sourcing ingredients within the country would lower the cost of production of value-added milk products. The current proposal is therefore designed to monitor the variations of resistant starch III content in popular Rwandese cassava varieties and assess its applicability in yoghurt processing.

1.2. Statement of the problem

Levels of RS vary with plant sources and technological treatment preceding their use (Sajilata et al., 2006). Differences in amounts of RS present in popular cassava varieties in Rwanda and effects of applying different heat treatments on cassava starch is not yet known. On the other hand, popular products such as yoghurt rely heavily on imported relatively expensive corn starch, pectin and gelatin for their production in Rwandan context. Application of cassava starch with good component of beneficial healthy RS in their processing as a thickening agent is yet to be tested. The rates of colorectal cancer are dramatically increasing in sub-Saharan Africa (Graham et al., 2012) and many epidemiological studies confirm the importance of probiotics against cancer (Kumar, 2010). Consequently, there is an increased demand for low cost prebiotics and probiotics especially among the middle class in Africa, Rwanda included (Reid et al., 2014, Monachese et al., 2011). Postharvest loss of cassava in Rwanda is estimated at more than 30% (USAID, 2010) and is due to availability of few appropriate post-harvest preservation facilities, perishability of the product and limited manufacture of cassava based products. There is also a wide gap in cassava value addition in Rwanda where its industrialization is solely limited to manufacturing of packaged flour. The current proposed project seeks to determine the levels of RS in popular cassava varieties in Rwanda and to demonstrate opportunity in adding value to cassava starch by synthesizing RS and testing its applicability in processing acceptable yoghurt.
1.3. Justification of the study

Cassava is a staple food crop in Rwanda ranking second after plantains in terms of production quantity with 3,161,470 MT and third in terms of monetary value with USD. 307,969,520 in 2013 behind plantains and potatoes (FAOSTAT, 2013). With an annual growth of 7.29% from 2010, cassava was put among the priority food crops in Rwanda together with maize, wheat, rice, Irish potato, soybean and bean (MINAGRI, 2012). In Rwanda, cassava is mainly utilized as flour for paste (Ugali) for household consumption or consumed raw, boiled, fried or roasted and its leaves are consumed as vegetables (Adekunle, 2007; Umuhozariho et al., 2011). Several studies indicated that manufacturing of cassava value added products is lucrative and has potential for food security achievement, improved revenue and decreased post-harvest losses (Saediman et al., 2015; Ukpongson et al., 2011; Ezeh et al., 2011). Cassava is highly perishable and production of resistant starch, a highly stable product, provides an opportunity to preserve the produce and add value. Furthermore, diversification of cassava products can be a solution to the increased production of this commodity and empowering the investment in industrial processing of cassava products hence enhancing the livelihood of population that rely on cassava farming as their income.

The dairy sector in Rwanda has experienced an extreme revolution as result of many programmes (RDB, 2012). Introduction of new cattle breeds, insemination program, active diseases eradication program and the “One Cow per Poor Family” program has increased ownership of dairy cows among Rwandans. This has led to an increased milk production from 372,619 tons in 2006 to 706,030 tons in 2014 (NISR, 2015) and the national dairy strategy anticipates an increase of 13% every year (MINAGRI, 2013) hence an obvious need for milk products diversification. Yoghurt is currently one of the most promising products that can be produced locally and cost-effectively if all raw materials are from inside the country. Overreliance on imported corn starch, gelatin or pectin as thickening agent in yoghurt manufacturing can be reduced by demonstrating possibility of using starch from local cassava in production of healthy starch.
Thickening agents are added to yoghurt to provide an acceptable texture, improve its viscosity and mouthfeel and reduce syneresis (Gonçalvez, 2005). Starch is the mostly used thickener, for it is not expensive, and if used at below 5%, it does not noticeably affect the taste (Saha and Battacharya, 2010). A similar effect may be achieved by adding cassava starch. The nutritional value of the added starch can be enhanced by using it in the form of RS.

Resistant starch is a pre-biotic that when mixed with pro-biotic yoghurt culture will result into symbiotic and healthy product which has the advantage of providing health enhancing bacteria and the promoting the growth of naturally occurring microflora thus preventing the occurrence of some gut diseases (Collins and Gibson, 1999). Those products are expensive and rarely found in developing countries (Monachese et al., 2011) like Rwanda; therefore the yoghurt enriched with resistant starch III may serve as an alternative source of prebiotics and probiotics as well as a cost-effective way to cut down the occurrence of such diseases.

Nutrition contributes to the incidences of colorectal cancer in world (Graham et al., 2012). Though the prevalence of colorectal cancer in Africa is very little compared to developed countries, there is a trend toward westernized lifestyle which increases the risk of Africans developing colorectal cancer (Graham et al., 2012). The treatment and management of this cancer imposes a large financial burden on both health-care systems and the families of diseased patients. The availability of yoghurt rich in probiotics and prebiotics (RS) may serve as a preventive measure against colon cancer as well as a relief for the government and family expenses on colon cancer and other gut diseases treatment.

Currently, cassava is used mainly for flour. Its utilization for starch and ethanol production is still low. There is an opportunity for development of other innovative high valued products such as resistant starch as a way of product diversification.
Consumers and processors are currently not aware of how local processing conditions lead to development of RS and its health importance. This study will provide scientific information on which cassava variety and treatment can produce large amount of RS for both processors and consumers.

1.4. Objectives

1.4.1. Main objective

The overall objective is to synthesize RS from three Rwandese cassava varieties and evaluate its applicability in yoghurt processing.

1.4.2. Specific objectives

1. To determine the levels of RS in three Rwandese cassava varieties namely NASE14, THE I92/0057 and GARUKUNSUBIRE.
2. To evaluate the effect of hydrothermal treatment on the RS content in cassava starch extracted from Rwandese cassava varieties.
3. To develop yoghurt with cassava starch high in RS and test its quality (Texture and viscosity) and sensory acceptability.

1.5. Hypotheses

1. There is no significant difference in RS content among the three Rwandese cassava varieties
2. Heat-moisture treatment and annealing treatment do not increase the RS content in cassava starch.
3. RS synthesized from cassava starch does not alter the texture, viscosity and sensory properties of yoghurt.
CHAPTER TWO: LITERATURE REVIEW

2.1. Cassava

Cassava (*Manihot esculenta*) is the major basis of energy for millions of human beings in third world (Fakir et al. 2012). In 21st century, as a result of enlargement of international trade of cassava based food and its robust evolution in Africa, cassava has gained an significant position in global agriculture where in 2012 it has reached to more than 280 million tons of harvest, a 60 % increase since 2000 (FAO, 2013).

Cassava root is reach in carbohydrate ranging between 32% and 35% on a fresh weight basis, and from 80% to 90% on a dry matter basis (Zvinavashe et al., 2011). Cassava contains very little fat (0.1%) and protein (2-3%) and it is relatively rich in vitamin C and calcium and it has considerable amounts of B-vitamins group (Ooye et al., 2014).

Variety of cassava is an important factor in production of diversified food products due to the fact that many parameters like amylase, starch, cyanide, whiteness and sweetness differ from variety to variety (Jisha et al., 2010; Zhang et al., 2010). In Rwanda, cassava serves as a revenue generating and food security produce (Mushimiyimana et al., 2011).

2.2. Starch

Starch is a natural macromolecule occurring in plant organisms and is the main make up of most plant-originated foods and numerous industrial raw materials (Leszczyński, 2004). It is an indirect product of photosynthesis as it is synthesized from glucose, which is formed from carbon dioxide and water (Leszczyński, 2004). Starch is made up of chains of $\alpha$-D-glucose linked by the activity of enzymes by 1-4 carbon atoms and 1 - 6 in some cases. The linear chain, known as amylose, contains exclusively $\alpha$ -1, 4 bonds. The branched polymer, amylopectin, contains branches of glucose molecules linked at carbons 1 and 6 (Pierna et al., 2005).
Based on digestibility, starch is grouped into three types: rapidly digestible starch, slowly digestible starch and resistant starch (Pereira and Magali, 2014).

2.2.1. Rapidly digestible starch (RDS)
RDS is the amorphous starch which is chemically determined as the starch converted to the integral glucose units in 20 minutes of breakdown by enzyme (Sajilata et al., 2006).

2.2.2. Slowly digestible starch (SDS)
SDS is the amorphous starch which is physical inaccessible and due to different reasons is digested slowly. It is measured as starch transformed to glucose units after 100 minutes of enzymatic treatment (Sajilata et al., 2006).

2.2.3. Resistant starch
Resistant starch was introduced by Englyst et al. (1982) to designate minor portions of starch that was unaffected by α-amylase and pullulanase hydrolysis in vitro. Currently, resistant starch is referred as the sum of starch and products of starch degradation which have not been absorbed in the small intestine of healthy humans (Englyst et al., 1992).

2.3. Types of resistant starch

2.3.1. Resistant starch I
Resistant starch I (RS I) is the grain starch which is protected by thick cell wall or protein matrix that prevent diffusion of water into the starch and this, inhibit gelatinization and swelling because of absence of moisture which makes it unsusceptible to enzymatic hydrolysis (Birt et al., 2013).

2.3.2. Resistant starch II
RSII is the natural starch that is shielded from digestion by the granules arrangement which make it is distinctive since it maintains its form and resistance during food preparation (Nugent, 2005). RS II has got
a gelatinization temperature exceeding 100°C and when cooked below this temperature, it remains unchanged and resistant hydrolysis (Birt et al., 2013).

2.3.3. Resistant starch III

RSIII is normally made during starch retrogradation (Nugent, 2005). This form has a gelatinization temperature as higher as 170°C and is not affected by cooking (Birt et al., 2013). Amylase enzyme is not able to hydrolyse RSIII due to the double helices structure formed when starchy foods are cooled and amylose and amylopectin lose the water-binding capacity which makes the starch molecules to not fit into the binding site of the enzyme (Birt et al., 2013).

2.3.4. Resistant starch IV

RSIV is a chemically modified starch by etherisation, esterification or cross-bonding with compounds in order to decline its digestibility (Nugent, 2005).

2.4. Starch content of Cassava

Starch content in mature cassava is around 30 % (Tonukari, 2004). However, this is dependent of cassava variety (Jisha et al., 2010). The study by Fakir et al. (2012) on starch content of seven cassava varieties indicated a significant difference in starch content of these varieties where it varied between 15.04% and 24.97% on fresh weight basis. In another study by Richardson (2013) on six varieties of cassava root from six different countries, reported their starch content to vary from 26.285to 31.9%.

In the food industry, cassava starch is mainly used for the following purposes: custard, thickener, filler, pharmacological products, binder and stabilizer (Yongfeng, 2013; Ozemoya et al., 2007, Oladunmoye et al., 2014, Da et al., 2008, Alarcón and Dufour 2012).

Cassava starch is preferred to corn starch due to the nonexistence of unwanted cereal flavor therefore it finds application in food industry for its unique flavor and its physicochemical performance when heated
in liquid medium since it produces better clarity and viscosity as well a low retro-gradation tendency and gelatinization temperature in comparison to other starches (Demiate and Kotovic, 2011). However it has got shortcomings including instability to acidic conditions and cooking (Takizawa et al., 2004).

2.5. Resistant starch in cassava

The level of RS in different crops and food products have been studied by Moongngarm (2013) in Thailand; Pereira and Leonel (2014) in Brazil; Ogbo and Okafor (2015) in Nigeria and these levels in cassava were found to range from 0.19% to 9.69g/100g of total starch.

Ogbo and Okafor (2015) found that RS in six improved cassava varieties namely, TMS30555, TMS 30572, TMS 693, TMS98/0505, TMS 4(2)1425 and TME varies from 5.7 to 7.07 g/100mg while Moongngarm (2012) found 9.69% as RS content in cassava root. Pereira and Leonel (2014) found a significant difference in RS content of 25 different cassava flours varying from 0.19% to 2.2% on dry weight basis.

Resistant starch type III is estimated to 2.4% in cassava starch and this value can be raised up to 41.3% by enzymatic treatment followed by Annealing (Lertwanawatana et al., 2015).

2.6. Importance of resistant starch

2.6.1. Prebiotic effect of resistant starch

A food ingredient is qualified as prebiotic when it can escape digestion and intestinal absorption attaining the large intestine where it changes the native microflora make-up and activity which results in evident health enhancement properties (Gibson et al., 2004).

RS has been demonstrated to regulate the bowel bacterial content through elevating bacteria that produce amylolytic and short chain fatty acids (Kovatcheva-Datchary et al., 2009), hence it is recognised as a prebiotic (Li, 2010).
2.6.2. Prevention of colon cancer

Nutrition researches reported a connection between food, colon bacteria and colon cancer especially in animal where feeds with high resistant starch have shown evidences of colon cancer prevention (Ridlon and Hylemon, 2006).

In South Africa, a research on people who eat good amount RSIII containing maize and few dietary fibre has demonstrated that they exhibited lower frequencies of colorectal cancer in comparison to others who consume higher amount of dietary fibre, but lower resistant starch content (Ahmed, 2000).

2.6.3. Control of glycemic response

Researches have demonstrated the ability of RS to cut down the insulin response and it was confirmed by the European Food Safety Authority (EFSA, 2011). The recent studies by Johnston et al. (2010) and Maki et al. (2012) indicated an enhancement in insulin sensitivity up to 72% after 30g of RSII was supplemented to the foods of pre-diabetics.

2.6.4. Enhancement of satiety

Though this mechanism is not fully explained yet, many studies reported the importance of RS to improve satiety (Willis et al., 2009, Anderson et al., 2010). It was reported to reduce the total food consumption by 15% when the food with 66% RSII HAMS was ingested (Anderson et al., 2010).

2.6.5. Production of Short Chain Fatty Acids

Resistant starch is thus initially broken down by bacterial amylases to produce glucose which is further broken down into organic acids especially short chain fatty acids (SCFA) like butyrate and lactic acid as well as CO₂, H₂ and CH₄ (Eliasson, 2004).

2.6.6. Effects on nutrients absorption

It has been demonstrated that the replacing some digestible starch by RS reduces blood glucose (Eliasson, 2004), and it is suggested that RS has the ability to increase calcium and magnesium uptake by improving
their solubility in the intestine through acidic condition created by fermentation of RS (Younes et al., 1993).

2.7. Effect of processing on resistant starch

It has been proven that there is possibility to raise RS up to 20% by varying a number of input process variables like water content, pH, temperature and time, heating and cooling rounds, freezing and drying (Englyst et al., 1987). Cooking, autoclaving, parboiling, baking, microwave irradiation, extrusion cooking and storage were testified to rise up the RS content at different extents (Hódsági et al., 2012; Faraj et al., 2004; Yadav et al, 2010). Milling, fermentation and germination are thought to reduce the formation of resistant starch (Devi et al., 2009; Nigudkar, 2014; Abd-Elmoneim et al., 2004; Buddrick et al., 2015).

2.7.1. Heat moisture treatment of starch

There are evidences that native starches can treated by different heat moisture conditions to increase their RS content as in the attempt to prepare the low calorie cassava pearl by Vijayakumari et al. (2014), heat moisture treatment of cassava starch for 48 hours has raised the RS content from 1.9% to 27.1% . Heat moisture treatment has also been applied to jackfruit seeds where their natural starch had 32% RS and it was increased up to 52% by heating these seeds at 80°C for 16 hours and the moisture content was 25% (Kittipongpatana and Kittipongpatana, 2015). Sankhon et al. (2014) reported that heat moisture treatment of African locust bean starch increased its RS content from 33.38% to 50.14%.

2.7.2. Annealing of starch

Vijayakumari et al. (2014) reported that annealing of cassava starch for 72 hours increased its RS content up to 28.6% while Lertwanawatana et al. (2015) reported an increase from 2.4% to 41.3% of Resistant starch type III in cassava starch treated by pullulanase, a debranching enzyme and high pressure annealing.

Annealing was also reported to increase the RS type III in amyloaize, barley, pea and lentil starch from 9% to 19% (Vasantahn and Bhatt, 1998).
2.8. **Applications of resistant starch in food industry**

A high consumption of ultra-processed plant products may cause a decrease in dietary fibre intake which makes the production and application of resistant starch in food to become inevitable (Leszczyński, 2004). In contrast to the conventional dietary fibers which can change the general food organoleptic qualities, RS is natural, flavorless, white, small size particle, high gelatinization temperature, good extrusion qualities and low water binding capacity (Sajilata et al., 2006). This permits the production of food products with appreciable texture, appearance and mouth feel and can serve in designing of new functional foods or probiotics encapsulation (Homayouni et al., 2013).

RS has been included in many foods like cheese, ice cream, yoghurt, milk, bread, corn flakes, cakes, muffins, pasta and batter (Homayouni et al., 2013).

2.9. **Yoghurt manufacturing**

Yogurt is mostly liked for being highly nutritive good sensory properties (Sfakianakis and Tzia, 2014). During its manufacturing, some changes occur which lead to the development flavor and texture of yogurt and it involves three main stages, homogenization, pasteurization and fermentation (Lee and Lucey, 2010, 2014).

2.9.1. **Application of thickeners in yoghurt**

Thickeners are applied in yogurt in order to improve the texture of yogurt, which is an important factor to determine yogurt quality (Gonçalvez et al., 2005). Starch, gelatin and pectin are the most used thickeners in yogurt manufacturing with starch being more preferred due to a number of factor including being cheap, simplicity of processing and it does not affect the sensory properties (Saha and Battacharya, 2010). These thickeners interact with the casein present in yogurt to make a strong structure which permits the development of rheological behavior (Gámbaro, 2002).

The study by Gonçalvez et al. (2005) reported that gelatin and starch applied in different proportions, positively affect the viscosity, creaminess and ropiness of yogurt and reduced significantly the occurrence
of syneresis. However it was reported that high concentration of pectin may affect the perception of aroma in yogurt (Routray and Mishra, 2011). Pancar et al. (2015) indicated a correlation between viscosity of yogurt and the amount of thickeners used.

Resistant starch has been used to enrich yogurt by Aryana et al. (2015) and they have reported that this enrichment with RSII produced an acceptable yogurt and RSII was not affected by yogurt heat treatment.

### 2.10. Identified gaps

- Researches on cassava in Rwanda have emphasized on productivity and resistance to diseases leaving out the postharvest utilization of cassava.
- The improvement of cassava resistant starch by hydrothermal treatment has not been tested in Africa despite it producing half of the world production.
- The cross link of two value chains; cassava and dairy products, has not been tested.
CHAPTER THREE: MATERIALS AND METHODS

3.1. Determination of the levels of RS in three Rwandese cassava varieties

3.1.1. Raw material acquisition

Cassava roots samples from three different cassava varieties (NASE14, THE I92/0057 and GARUKUNSUBIRE) of 10 months in the field will be collected from Rwanda Agriculture Board field research stations of Rubona and Rilima. The samples will be brought to laboratory for analysis which will be consisted of first extracting starch followed by isolating and measurement of RS.

3.1.2. Design

Three replicates of analysis will be conducted for each cassava variety and the mean value will be recorded.

3.1.3. Methodology

i. Starch Extraction

Wet method will be applied for starch extraction as per Benesi et al. (2004). After washing, peeling and chopping, cassava roots will be pulverized for 5 minutes in a blender. The mash will be put in 1:10 ratio of water volume, agitated for 5 minutes followed by filtering with a cotton cloth. The sediment will be allowed to settle for 2 hours and the top fluid will be discarded. The process will be repeated until clear water is observed. Sediment starch will be dried for 24 hours and kept.

ii. Resistant starch determination

RS will be determined using AOAC Method 2002.02 (2003). Briefly described, non-resistant starch will be hydrolyzed to glucose by the collective activity of pancreatic α-amylase (4mL) and amyloglucosidase (AMG) (3U/mL) for 16 hours at 37°C. This reaction will be ended by adding ethanol (4mL) and RS will be recuperated as pellet by centrifugation. RS in the pellet will be dissolved in 2M KOH (2mL) by strongly stirring in an ice–water bath. This solution will be buffered with acetate buffer and the starch will
be hydrolyzed to glucose with AMG. The aliquot (0.1 mL) will be put in duplicate into glass test tubes (16 × 100 mm), and then treated with 3.0 mL of glucose oxidase–peroxidase reagent (GOPOD) and the tube contents will be shaken on a Vortex mixer and incubated at 50°C for 20 minutes. The spectrophotometer will be set to 0 with the blank solution and the absorbance of every solution will be measured at 510nm against the blank solution. The average of duplicate absorbance values will be recorded.

3.1.4. Data analysis
The sample will be analysed in triplicate and the mean value will be considered. The mean values of RS in three cassava varieties will be compared by means of T-test at 5% significance level.

3.2. Evaluation of the effect of hydrothermal modification on the RS content in starch extracted from Rwandese cassava varieties

3.2.1. Design
In order to evaluate its effect on RS levels, three varieties of cassava will be treated with two methods of hydrothermal modification; annealing and Heat-Moisture treatment (HMT) respectively. Three HMT (18%, 24% and 30% moisture content) and two annealing treatments (45°C and 55°C) will be applied to starch from each of the three cassava varieties. This will be treated in 3x5 factorial design of 3 cassava varieties and 5 hydrothermal treatments.

3.2.2. Methodology

i. Heat-moisture treatment (HMT)
HMT will be applied as per the method by Franco et al. (1995). The 45°C and 55°C moisture contents of the samples will be increased up to 18% and 24% respectively by the addition of the suitable quantity of distilled water and mixed; the samples will be hermetically closed in glass jars and heated in an oven at 100°C for 16 hours. Starch samples will be cooled, air dried and subjected to RS measurement.
ii. Annealing

The method of Knutson (1990) will be applied for starch annealing with slight modification. Starch (100g) will be heated in excess water at 45°C and 55°C for 24 hours. Samples will be centrifuged to eliminate excess water and air-dried and thereafter subjected to RS determination.

iii. Starch profiling

a. Moisture content and dry matter content determination

To determine the moisture content and dry matter content, the ISO 1660 (1996) method will be applied which consists of drying the sample at 130 (± 3) °C for a period of 90 minutes.

b. Total starch determination

The total starch content will be determined as per AOAC (1996) Official Method 996.11 Starch (Total) in Cereal Products Amyloglucosidase – α-Amylase Method.

c. Water absorption index (WAI) and Water Solubility Index (WSI)

(WAI) and (WSI) will be determined as per Anderson (1982) with minor adaptation. Starch (3.0 g) will be mixed with 30 ml distilled water and heated at 60°C for 1 hour in a water bath, then centrifuged at 3000×g for 15 minutes and the supernatant will be recuperated in a pre-weighed dry aluminum bowls. The remaining deposits will be drained off by leaving tubes containing samples to stand inverted for 10 minutes. The supernatant will be dried in an air oven at 105°C for 12 hours for WSI measurement.

\[
\text{WAI(g/g)} = \frac{\text{Weight of water uptake in hydrated residue}}{\text{Weight of starch sample}}
\]

\[
\text{WSI(\%)} = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Weight of starch sample}} \times 100
\]

d. Pasting profile of starch

Pasting properties of the flours will be measured with a Brabender Viscograph using AACC Approved Method 61.02. Flour (40 g) and water (420 ml) will be weighed and mixed to make slurry.
The slurry will be heated from 30°C and temperature increased at a constant rate of 1.5°C per minute until 93°C where it will be held for 15 minutes, after the temperature will be decreased at a constant rate of 1.5°C per minutes until 30°C and then held at this temperature for 15 minutes. The resistance to stirring as a viscosity curve will be recorded. The pasting temperature, peak viscosity, hot paste viscosity and cold paste viscosity will be determined from the pasting curve. The breakdown viscosity which is the peak viscosity minus trough viscosity, setback which is equivalent to cold paste viscosity minus trough viscosity and consistency which equals to cold paste viscosity minus peak viscosity will be also calculated.

**e. Texture analysis**

Texture Analyser will be used to objectively evaluate the texture of starch according to the method by Kaszab et al. (2002).

**3.2.3. Data Analysis**

Three varieties will be treated with 4different hydrothermal modification which will make a 3x4 factorial experiment. Each experiment will be done in triplicate which will make and be analysed in a factorial design of 36 treatments and the analysis of variance (ANOVA) will be performed at P ≤ 0.05 using GenStat application.

**3.3. Effect of addition of RS on the quality of yoghurt**

**3.3.1. Design**

Yogurt will be processed as per figure 1 below. Cassava starch will be applied in three different proportions, 0.1%, 0.5% and 1% respectively. Corn starch will be applied as a control at 0.6%.

The processed yogurt will be analysed for its quality and sensory parameters. Sensory evaluation data will be analysed in a complete randomised design.
3.3.2. Methodology

i. Yogurt manufacturing

The yoghurt will be processed as per Gonçalvez (2005). Commercial skim UHT milk will be standardized at 11.1 % (w/w) total solids using 8 % white commercial sugar and dried skimmed milk. Thickeners will be added in the following proportions: 0, 0.1%, 0.5% and 1% of cassava starch. All the components will be mixed and heated at 90°C for 15 minutes. The solution will be cooled at 42 °C and inoculated with a commercial starter culture (Streptococcus thermophilus and Lactobacillus bulgaricus) then incubated at 42°C awaiting the pH to reach 4.5. The yogurt will be cooled to 5°C and kept at the same temperature for 5 days before testing. Sample without thickener will be used as control.

The RS contents in yoghurts (Treated with cassava starch high in RS and ordinary yoghurt) will be measured as per AOAC (2003): Official Method Number: 2002.02.

![Generalized scheme for Yoghurt manufacturing](image)

**Figure 1: Generalized scheme for Yoghurt manufacturing**
ii. Analysis of yoghurt quality

a. Texture analysis
Texture Analyser will be used to objectively evaluate the texture of modified yoghurt as per Kaszab et al. (2002).

b. Viscosity analysis
Viscosity will be measured by Viscometer as per Djurdjević et al. (2002) which consist of comparing the apparent viscosity of the yogurt to the apparent viscosity of the reference liquid (Water).

c. Syneresis
The method by Gonçalvez et al. (2005) will be used. Yogurt will be centrifuged and the supernatant will be removed and measured as percentage syneresis.

d. Acidity and pH
Titratable acidity of Fermenting yogurt will be determined according to AOAC method 942.15 (2000). Yogurt (10 ml) will be titrated with 0.1 N NaOH using phenolphthalein as an indicator. The titratable acidity will be recorded as percent lactic acid. pH will recorded using a pH meter.

e. Total solids
Total solids in yogurt will be determined as per ISO 13580: 2005 method.

f. Levels of RS
Levels of RS in yogurt treated with modified starch will be determined as per AOAC Method 2002.02 (2003).

iii. Sensory evaluation
A panel of 12 people will be tasked to evaluate the quality characteristics (taste, colour, flavour, mouthfeel and overall acceptability) of all yoghurt samples treated with different thickeners using a 7-point hedonic scale where 7= like very much, 6= like moderately, 5= like slightly, 4= dislike slightly, 3= dislike moderately, 2= dislike very much and 1= dislike extremely.
3.3.3. Statistical analysis

Sensory evaluation data will be analysed in a complete randomised design when evaluating the overall acceptability of the yogurt treated with cassava starch containing high RS content. Yoghurt quality parameters will be measured in triplicate and the mean value will be considered and the ANOVA will be performed at 5% significance level.
## 4.0 Work plan

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6.0 References


30. FAOSTAT. (2014). Food and Agricultural Commodities Production; Available online: http://faostat3.fao.org/browse/rankings/commodities_by_country/E


