UNIVERSITY OF NAIROBI

SYNTHESIS OF RESISTANT STARCH FROM SELECTED RWANDESE CASSAVA
VARIETIES AND ITS APPLICABILITY IN YOGHURT PROCESSING

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Thesis submitted in partial fulfilment of the requirements for the award of the Degree of
Master of Science in Food Science and Technology of the University of Nairobi

Department of Food Science, Nutrition and Technology

2018
DECLARATION

I, Herve MWIZERWA declare that this thesis is my original work and has not been previously submitted for a similar award of a degree in any other University or Institution of higher learning.

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To the Almighty God,
To my beloved mother,
To my brothers and sisters,
To my grandparents,
To all my best friends
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ABSTRACT

Cassava is an important source of resistant starch since its starch displays a diffraction c-type associated with reduced digestibility. However, there is limited information on variation of resistant starch content of Rwandan cassava varieties and methods of increasing it. Moreover, its application of resistant starch in yoghurt is yet to be experimented in Rwanda where yoghurt production relies on imported and expensive corn starch as thickener. Therefore, the current study investigated the technological possibilities of increasing cassava resistant starch by hydrothermal treatment and its potential use as yoghurt thickener. Starch from varieties NASE14, I92/0057 and Rutanihisha were treated with hydrothermal treatments namely heat-moisture treatment at 18%, 24% and 30% moisture content for 16 hours at 100 °C as well as annealing at 45 °C and 55 °C with excess water for 24 hours. Modified cassava starch was added to yoghurt in the proportions 0, 0.1 %, 0.5 % and 1 % respectively and stored at 4°C for 21 days and the quality parameters were determined on weekly basis. Variety I92/0057 had the highest resistant starch content (5.98 g/100g) followed by NASE 14 (4.18 g/100g). Heat-moisture treatment of 24 % significantly (P≤0.05) increased resistant starch content. After heat moisture treatment, variety I92/0057 had the highest resistant starch (42.53 g/100g) while it was 31.56 g/100g after annealing at 45 °C. Water absorption index and water solubility index of cassava starch were both significantly (P≤0.05) decreased by hydrothermal treatment. Oil absorption index was significantly (P≤0.05) increased in all cassava varieties by heat-moisture treatments while it was significantly (P≤0.05) decreased by annealing methods. Application of modified cassava starch in yoghurt at 1 % proportion produced yoghurt with significantly (P≤0.05) higher resistant starch content of 5.58 g/100g on day one and 4.47 g/100g on day 21. This proportion also expressed the highest viscosity which varied from 2721.5 mPa s to 1034.5 mPa s between day 1 and day 21 of cold storage as well as the least syneresis. Adding modified cassava starch...
significantly (P≤0.05) increased the total solids and they were directly proportional to the quantity of the added starch. There was no significant difference (P>0.05) in colour and odour of yoghurt treated with different proportions of modified cassava starch. Heat-moisture treatment of 24% at 100 °C for 16 hours for starch from variety I92/0057 and its application in yoghurt at 1% proportion is recommended as the best method for scaling up and industrial application.

**Keywords:** Annealing, Cassava, Heat-moisture treatment, Resistant starch, Yoghurt
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ABBREVIATIONS AND ACRONYMS

AACC: American Association of Cereal Chemists
AMG: Amyloglucosidase
AN: Annealing
ANOVA: Analysis of Variance
AOAC: Association of Official Analytical Chemists
BHEARD: Borlaug Higher Education for Agricultural Research and Development
CIAT: Centro Internacional de Agricultura Tropical
CFU: Colony Forming Unit
EFSA: European Food Safety Authority
FAO: Food and Agriculture Organization
FAOSTAT: Food and Agriculture Organization Statistical Database
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
NAEB: National agricultural Export Development Board
NISR: National Institute of Statistics of Rwanda
OIA: Oil Absorption Index
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

USD: United State Dollar

WAI: Water Absorption Index

WHO: World Health Organization

WSI: Water Solubility Index
CHAPTER 1: 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 (Végh 2009). Based on digestibility starch is classified into three types: rapidly digestible starch, slowly digestible starch and resistant starch (Pereira and Leonel, 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) which 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 like ethers and esters (Nugent, 2005; Sajilata et al., 2006)

In human 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; Morales-Medina et al., 2014; Nugent, 2005; Sajilata et al.,
Researchers in nutrition have demonstrated that resistant starch 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 rate of colorectal cancer is increasing in developed and developing countries ((Graham, Adeloye, Grant, Theodoratou, & Campbell 2012) and its prevention by resistant starch consumption is extensively explored currently. Research with dietary components indicated resistant starch containing diets may prevent colon carcinogenesis by producing butyrate in the colon which binds to protein receptors responsible for regulating cancer development processes like inflammation and apoptosis (Malcomson, Willis, & Mathers 2015).

Many methods of increasing resistant starch in food have been experimented and they include genetic method (interbreeding), enzymatic, chemical, irradiation and physical methods (thermal treatment) (Dupuis, et al., 2014; Jacobs and Delcour, 1998). Resistant starch has also been perceived in different food like breads, breakfast cereals, biscuits, maize, mashed potatoes and legumes (Pereira and Leonel, 2014). The main obstacle to the food industry is the production of foods with sufficient resistant starch 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. The level of resistant starch of cassava and cassava 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.

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 in 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 2010b) 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 result of many programmes (MINAGRI 2013). 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). The national dairy strategy anticipates an increase of 13% every year (MINAGRI 2013) and milk exports is to reach 20 million US dollars per year by 2017(MINAGRI 2013; NISR 2015) hence an obvious need for milk products diversification. 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). Yoghurt is currently one of the most promising products that can be produced locally and cost-effectively if the ingredients are from inside the country. The present study was designed to determine the variations of resistant starch content in popular Rwandese cassava varieties and assess its applicability in yoghurt processing.
1.2. Statement of the problem

Differences in amounts of resistant starch 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, yoghurt production rely heavily on imported relatively expensive corn starch, pectin and gelatine as thickener in Rwandan context. Application of cassava starch with good component of beneficial healthy resistant starch 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 leading to significant postharvest loss of cassava in Rwanda. There is an increased demand for low cost prebiotics especially among the middle class in sub-Saharan Africa where the cases of colorectal cancer are increasing attaining 1.7/10000 persons and 7808 annual new case in adults (Graham et al., 2012). Many epidemiological studies confirm the importance of prebiotics like resistant starch to prevent this type of cancer (Kumar et al., 2010; Malcomson et al., 2015; Peres, 2014). The current project determined the levels of resistant starch in popular cassava varieties in Rwanda and demonstrated the opportunity in value addition of cassava starch by synthesizing resistant starch 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 annual production with 3,161,470 MT and third in terms of monetary value with USD. 307,969,520 in 2013 behind plantains and potatoes (FAOSTAT 2014). 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 2011). However among all these crops, cassava is scarcely involved in value addition. 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 (Ezeh et al., 2011; Saediman et al., 2015; Ukpongson 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. Currently in Rwanda, cassava is used mainly for flour and its utilization for starch production is still low. The present study shows the opportunity for developing other innovative high valued products such as resistant starch as a way of product diversification. This study provided scientific information on which cassava variety and treatment can produce large amount of resistant starch and its potential application in dairy industry as yoghurt thickener. This industry depend on imported and expensive corn starch as thickener hence a need to develop locally produced thickener such as cassava starch.

The dairy sector in Rwanda has experienced an extreme uprising as result of many programmes (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 it is cost-effective if the ingredients are from inside the country. Overreliance on imported corn starch, gelatin or pectin as thickening agent in yoghurt manufacturing can be reduced by using starch from local cassava in yoghurt processing as a thickener. Thickening agents are added to yoghurt to provide an acceptable texture, improve its viscosity and mouthfeel and reduce syneresis (Goncalvez et al., 2005). A similar effect may be achieved by adding cassava starch and the nutritional value of the added starch can be enhanced by using it in the form of resistant starch.

Resistant starch is a pre-biotic that when mixed with pro-biotic yoghurt culture may result into synbiotic product which has the advantage of providing health enhancing bacteria and promoting
the growth of naturally occurring microflora thus preventing the occurrence of some colon diseases especially colorectal cancer (Collins and Gibson, 1999).

1.4. Objectives

1.4.1. Main objective

The overall objective was to synthesize resistant starch from selected Rwandese cassava varieties and evaluate its applicability in yoghurt processing.

1.4.2. Specific objectives

1. To determine the levels of resistant starch in three Rwandese cassava varieties namely NASE14, THE I92/0057 and Rutanihisha.

2. To evaluate the effect of hydrothermal treatment on the resistant starch content of cassava starch extracted from Rwandese cassava varieties.

3. To produce yoghurt thickened with cassava starch high in resistant starch and test its quality and sensory acceptability.

1.5. Hypotheses

1. There is no significant difference in resistant starch content among the three popular Rwandese cassava varieties

2. Heat-moisture treatment and annealing treatment do not significantly increase resistant starch content in cassava starch.

3. Resistant starch synthesized from cassava starch does not significantly alter the texture, viscosity and sensory properties of yoghurt.
CHAPTER 2: LITERATURE REVIEW

2.1. Cassava utilization

Cassava (*Manihot esculenta*) is the major basis of energy for millions of human beings in third world. In 21st century, as a result of enlargement of international trade for cassava based food and its robust evolution in Africa, cassava has gained 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 is rich 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).

Cassava is currently used as raw material for the production of food and non-food products like starch, glycoe syrups, alcoholic beverages, industrial alcohol, confectionery, pharmaceutical products and adhesives (Akpa, 2014; Balagopalan, 2002; Falade and Akingbala, 2010; Marx and Nquma, 2013; Nwalo and Cynthia, 2014; Yu and Tao, 2008)

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 (Zhang et al., 2010; Jisha et al., 2010). In Rwanda, cassava serves as a revenue generating and food security produce (Mushiyimana 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 (Leszczynski 2004). It is an indirect product of photosynthesis as it is synthesized from glucose, which is formed from carbon dioxide and water (Leszczynski 2004). Starch is made up of chains of α-D-glucose linked by the activity of enzymes by 1-4 carbon atoms and 1-6 in some cases (Pierna et al., 2005). The linear chain, known as amylose, contains exclusively α -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 Leonel, 2014).

2.2.1. Rapidly digestible starch (RDS)

Rapidly digestible starch (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). Rapidly digestible starch is immediately broken into glucose, the latter is rapidly absorbed in the duodenum which contribute to the immediate increase of blood glucose (Zhang and Hamaker, 2009), insulin content and superoxide free radicals which results in blood glucose fluctuation and other health disturbances (Englyst et al., 1999; Monnier et al., 2006; Zhang et al., 2008). It is found in most processed starchy food like bread and moist heat treated starchy food like cooked potato (Zhang and Hamaker, 2009; Sajilata et al., 2006).

2.2.2. Slowly digestible starch (SDS)

Slowly digestible starch (SDS) is the amorphous starch which is physical inaccessible and due to different reasons is digested slowly (Sajilata et al. 2006). It is little by little digested in the small
intestine leading to slow release of glucose (Zhang and Hamaker, 2009) and it has the advantage of maintaining the stability of blood glucose and glycaemic index moderation (Lehmann and Robin, 2007).

Researchers have suggested that a delayed digestion and a resulted reduced absorption of carbohydrates is of paramount importance for people with diabetes type I and II but also to healthy humans (Guraya et al., 2001). Slowly digestible starch is also associated with glycaemic index control, low free fatty acid resulting from postprandial intake of starchy food (Zhang and Hamaker, 2009).

Slowly digestible starch is found in form of raw starch granules or in processed food where it covered by protein matrix (Zhang and Hamaker, 2009) and it can be produced in food by varying the moisture content, cooking time and temperature (Lehmann and Robin, 2007). It is measured as starch transformed to glucose units after 100 minutes of enzymatic treatment (Sajilata et al. 2006).

A method of generating slowly digestible starch was developed and it consists of debranching waxy and non-waxy starch suspensions with pullulanase enzyme followed by heating and cooling process at 1 °C. (Guraya et al. 2001).

2.2.3. **Resistant starch**

Resistant starch was introduced by Englyst in 1982 to designate minor portions of starch that was unaffected by *in vitro* hydrolysis by α-amylase and pullulanase (Sajilata et al. 2006). 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). Once in colon, resistant starch serve as a substrate for production of short chain fatty acid especially butyric acid which is the main source of energy for colon cell (Margareta et al., 2006).
Resistant starch has gained a lot of interest due to its health promoting potentialities similar to those of dietary fibres including but not limited to prevention of colon cancer, enhancement of satiety, prebiotic properties, control of glycaemic index (Gibson et al., 2004; Kovatcheva-Datchary et al., 2009; Maki et al., 2012; Ridlon and Hylemon, 2006).

Resistant starch is currently added to food intentionally or as a result of starch modification in order to withstand modern processing conditions (Khawas and Deka, 2017).

According to their natural structural and mode of formation, resistant starch has been subdivided into five types, resistant starch I, resistant starch II, resistant starch III, resistant starch IV and resistant starch V (Dupuis et al. 2014; Sajilata et al. 2006).

2.2.4. Types of resistant starch

2.2.4.1. 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).

2.2.4.2. Resistant starch II

Resistant starch II (RSII) is the natural starch that is shielded from digestion by the granules arrangement which make it 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 to hydrolysis (Birt et al., 2013).
2.2.4.3. Resistant starch III

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).

2.2.4.4. Resistant starch IV

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). This type of resistant starch is widely used due to its diversity in structure though it is not naturally found in the food crops (Moongngarm 2013).

2.2.4.5. Resistant starch V

Resistant starch V (RSV) results from the complexation of starch amylose with lipid compounds to form a structure that contain fatty acid within the centre of the formed helical pattern (Dupuis et al. 2014).

2.2.5. Resistant starch in food crops

Resistant starch content of crops depends on different factors including varietal variation, climate, postharvest handling, storage and processing conditions (Moongngarm 2013). Resistant starch content of different cultivars was studied by Moongngarm (2013) in Thailand and he found that it varies from 2.15 g/100g to 4.41 g/100g for rice, 1.16 g/100g for sweet corn, 3.19 g/100g for sweet potato, 3.69 g/100g to 23.25 g/100g for Yam, 4.12 g/100g for Taro, 9.69 g/100g for cassava, from 35.14 g/100g to 45.87 g/100g for green banana, from 4.59 g/100g to
8.18 g/100g for cowpea, from 9.54 g/100g to 10.63 g/100g for red bean and 2.23 g/100g for Mung bean. In a study in Slovakia by Mikulikova and Kraic (2006), they found resistant starch content to be 7.59 % in triticale (hybrid of wheat and rye), 7.01 % for rye, 5.64 % for wheat, 3.89 % to 3.97 % for barley and 0.87 % for oat. Remya and Jyothi (2015) surveyed the resistant starch content of different cultivars in India and found it to varying from crop to crop where it was 3.2 % for Barley, 1.3 % for oat, 2.7% for wheat, 2.2% for rice, 4.7% for maize, 3.3% for banana, 2.1 % for cassava, 3.3 % for sweet potato and 4.0 % for potato.

2.2.6. Importance of resistant starch

2.2.6.1. 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). Resistant starch 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 recognized as a prebiotic (Fuentes-Zaragoza et al. 2011). In the study by Mirzaei et al. (2012) to evaluate the survival of *Lactobacillus acidophilus* (La5) coated with calcium alginate and resistant starch in Iranian cheese, they found that this probiotic was kept above the minimum recommended by FAO/WHO and higher enough to have a therapeutic activity. It has also been reported by Zheng et al. (2016) that resistant starch produced by heat-moisture treatment has a greater impact on the growth of Bifidobacteria than glucose and high amylose corn.
2.2.6.2. 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 resistant starch III 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 et al., 2000). However, another study by Mathers et al. (2012) failed to confirm this effect in people with hereditary colon cancer.

2.2.6.3. Control of glycaemic response

Researchers have demonstrated the ability of resistant starch to cut down the blood glucose and insulin response and it was confirmed by the European Food Safety Authority (EFSA 2011). Since resistant starch escape small intestine digestion, it is expected to reduce glucose absorption and cut down the glycaemic index as a result (Brites et al., 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 resistant starch II was supplemented to the foods of pre-diabetics. Zenel and Stewart (2015) also reported that the consumption of rice rich in resistant starch reduce significantly the postprandial blood glucose and insulin response in comparison with rice containing traces of resistant starch.

2.2.6.4. Enhancement of satiety

Though this mechanism is not fully explained yet, many studies reported the importance of resistant starch to improve satiety (Anderson et al., 2010; Willis et al., 2009). It was reported to reduce the total food consumption by 15% when the food with 66% high amylose corn starch was ingested (Anderson et al., 2010).
2.2.6.5. Production of Short Chain Fatty Acids

Resistant starch is 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 Carbon dioxide, Hydrogen and Methane (Eliasson 2004).

2.2.7. Effect of processing on resistant starch

It has been proven that there is possibility to raise resistant starch 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 method like autoclaving, parboiling, baking, hydrothermal treatment, microwave irradiation, extrusion, fermentation and storage were testified to raise up the resistant starch content at different extents (Faraj et al., 2004; Hódsági et al., 2012; Yadav et al., 2010).

Extrusion has been reported to increase resistant starch of pearled barley flour form 46 mg/100g to 57 mg/100g where the screw speed was 60 rpm and the moisture content was 35% (Faraj, Vasanthan, & Hoover 2004b). Autoclaving followed by a number of cooling cycles has been reported to increase resistant starch levels of rice starch where it was 4.20 % after 4 cycles of autoclaving-cooling and it reached 17.90 % after twenty cycles (Zain 2016).

Storage has also been found to increase resistant starch levels of cereals and legumes by Yadav et al. (2010) who reported an increase of resistant starch content from 3.16% to 5.86% for pea seed, from 1.76 % to 2.49 % for wheat grain, from 2.57 % to 3.52 % for Barley and from 4.89 % to 6.50 % for lentil all stored at 4°C for 24 hours.
In the study by Kim et al. (2006) in Australia, parboiling has increased the resistant starch content of rice from 0.11 % to 0.81 %. Baking conditions have also been reported to impact resistant starch content like in the study by Amaral et al. (2016) where baking at 150 °C for 3 hours increased resistant starch content by 15% while baking at 120 °C for 4 hours increased it by 24 % in reference to the control conditions of 200 °C for 30 minutes.

Zeng et al. (2016) reported an increase in resistant starch due to microwave irradiation of lotus seed starch where it increased from 15.38 % to 30.42 % at microwave power of 8.0 W/g and the resistant starch increase was proportional to the increase of microwave power. Fermentation of sourdough and bulk fermentation were reported to increase the formation of resistant starch in baked products by Buddrick et al. (2015) who reported an increase from 0.67 % to 2.06 % for wheat dough and they attributed this increase to the production of lactic acid which has an impact on starch structure and is known to reduce gelatinization of starch.

Milling and germination are thought to reduce the formation of resistant starch (Buddrick et al., 2015; Devi et al., 2009; Elkhalifa et al., 2004; Nigudkar, 2014). Milling being a high shear processing method, it damages the starch granules which make them susceptible to enzymatic digestion (Alsaffar 2011; Devi et al. 2009). Dhital et al. (2010) reported an increase of damaged starch granules ranging from 1.0% to 24.7% for cryo-milled starches which in turn reduced resistant starch. Kavita et al. (1998) reported a 22 % decrease in resistant starch content for Bengalgram bean after 72 hours of germination and a reduction of 51% for cowpea resistant starch content after 48 hours of germination.
Hydrothermal treatment methods of annealing and heat moisture treatment have been reported to be the best physical methods to increase resistant starch and other starch properties without altering starch granules (Liu et al. 2016).

2.2.8. Heat moisture treatment of starch

Heat-moisture treatment of starch is a process of physically modifying starch at restricted moisture usually below 35% at high temperature ranging between 90 °C to 120 °C for an extended time of 15 minutes to 16 hours (Zavareze et al., 2011). There are evidences that native starches can be treated by different heat moisture conditions to increase their resistant starch content. In an attempt to prepare the low calorie cassava pearl by Asha et al. (2014), heat moisture treatment of cassava starch for 48 hours has raised the resistant starch content from 1.9 % to 27.1 %. Heat moisture treatment has also been applied to jackfruit seeds where their natural starch had 32 % resistant starch 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 resistant starch content from 33.38 % to 50.14 %.

2.2.9. Annealing of starch

Annealing of starch is referred to as a physical modification of starch with high moisture (above 60 %) at temperature below gelatinization temperature but above glass transition temperature (40 °C to 55°C (Tester and Debon, 2000). Asha et al. (2014) reported that annealing of cassava starch for 72 hours increased its resistant starch content up to 28.6 % while (Lertwanawatana et al. (2015) reported an increase from 2.4 % to 41.3 % of resistant starch III in cassava starch treated by pullulanase, a debranching enzyme and high pressure annealing.
Annealing was also reported to increase the resistant starch III in amylomaize, barley, pea and lentil starch from 9% to 19% (Vasanthan and Bhatti, 1998).

### 2.2.10. 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 (Leszczynski 2004). Resistant starch is currently added to food not only for its health benefits but also for its processing advantages like being natural, flavourless, 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. 2014).

Resistant starch has been included in many foods like cheese, ice cream, yoghurt, milk, bread, corn flakes, cakes, muffins, pasta and batter (Bi et al., 2016; Homayouni et al., 2014).

### 2.3. Yoghurt manufacturing

Yoghurt is a fermented milk product resulting from coagulation of milk protein by lactic acid produced by specific and viable bacteria (Sfakianakis and Tzia, 2014). Yoghurt is mostly liked for being highly nutritive and has good sensory properties (Sfakianakis and Tzia, 2014). Yoghurt is most of time produced from cow milk however, yoghurt from other mammalians like goat, sheep, camel and buffalo have been successfully manufactured (Sfakianakis and Tzia, 2014). During its manufacturing, some changes occur which lead to the development flavour and texture of yoghurt and it involves three main stages, homogenization, pasteurization and fermentation (Lee and Lucey, 2010). Currently, innovative methods of yoghurt processing have
been developed and they include high pressure treatment of milk prior to yoghurt processing, ultrasound treatment, micro-fluidization, pulsed electric field application and development of probiotic and prebiotics yoghurt where they enhance the conventional yoghurt quality characteristics like viscosity, water holding capacity, low fat and texture (Massoud et al., 2016; Naik et al., 2013; Sfakianakis and Tzia, 2014).

2.3.1. Application of thickeners in yoghurt

Thickeners are applied in yoghurt in order to improve the texture of yoghurt, which is an important factor to determine yoghurt quality (Goncalvez et al., 2005). Starch, gelatin and pectin are the most used thickeners in yoghurt 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 Bhattacharya, 2010). These thickeners interact with the casein present in yoghurt to make a strong structure which permits the development of rheological behaviour (Goncalvez et al., 2005). Goncalvez et al. (2005) reported that gelatin and starch applied in different proportions, positively affect the viscosity, creaminess and ropiness of yoghurt and reduced significantly the occurrence of syneresis. However it was reported that high concentration of pectin may affect the perception of aroma in yoghurt (Routray and Mishra, 2011). Pancar et al. (2016) indicated a correlation between viscosity of yoghurt and the amount of thickeners used.

Resistant starch has been used to enrich yoghurt by Aryana et al. (2015) and they have reported that this enrichment with resistant starch II produced an acceptable yoghurt and it was not affected by yoghurt heat treatment.
CHAPTER 3: RESISTANT STARCH CONTENTS OF THREE POPULAR RWANDESE CASSAVA VARIETIES

3.0. Abstract

Cassava (Manihot esculenta) is one of the most popular tropical root crops and is a staple food in Rwanda where it ranks second after plantains in terms of annual production. Cassava roots are rich in starch with good amounts of resistant starch, a fraction of starch which escapes the small intestine digestion. Interest in resistant starch has recently grown due to its properties of preventing colon cancer, enhancing satiety, glycaemic response control and having prebiotic properties. However, there is limited data on levels of resistant starch in Rwandan cassava varieties. The present study evaluated three cassava varieties, NASE 14, I92/0057 and Rutanihisha for their resistant starch content. Starch was extracted by wet method and was freeze dried and subjected to resistant starch, digestible and total starch content determination by enzymatic hydrolysis of starch to glucose. Resistant starch content was significantly (P≤0.05) higher in I92/0057 with 5.89 g/100g of total starch followed by NASE14 with 4.18 g/100g and lastly Rutanihisha with 3.88 g/100g. Varieties I92/0057 and NASE14 are the improved varieties and had significantly (P≤0.05)) higher starch yields compared to the local variety Rutanihisha with 81.15 %, 87.57 % and 71.31 % on dry weight basis, respectively. The dry matter content was 31.30%, 36.50% and 30.60% while ash content on dry weight basis was 2.08 %, 2.02 % and 3.14 % for NASE 14, I92/0057 and Rutanihisha respectively. Variety I92/0057 had the highest dry matter content, starch extraction yield and resistant starch content but lowest total starch and digestible starch. Variety Rutanihisha, however, has the highest ash content among the three varieties. Screening for resistant starch content of all cassava varieties and other starch sources
from Rwanda and investigating factors affecting variation in resistant starch content is recommended.

**Keyword: Cassava, Resistant starch, Starch**

### 3.1. Introduction

Cassava (*Manihot esculenta*) is one of the popular tropical root crops and is an important source of calorie in tropical countries (Li et al., 2016). Cassava is a staple food crop in Rwanda where it ranks second after plantains in terms of annual production with 3,159,551 tonnes and third in terms of monetary value with USD 308 millions in 2013 behind plantains and potatoes (FAOSTAT 2014). Rwanda has put a lot of effort in increasing cassava production and productivity where it tripled in a period of 4 years from 2007-2011 (MINAGRI 2011) and continue to increase gradually. Cassava roots are rich in starch which can go up to 90 % on dry weight basis (Zvinavashe et al., 2011).

Starch is subdivided into three types according to its digestibility: rapidly digestible starch, slowly digestible starch and resistant starch. The latter is defined as the starch which escape the small intestine digestion and is fermented in the colon into short chains fatty acids (SCFAs) by bacteria present in the colon (Englyst et al., 1992). Interest in resistant starch has recently grown due to its properties of preventing colon cancer, enhancing satiety, glycaemic response control and having prebiotic properties (Ahmed, et al., 2000; Eliasson, 2004; Johnston et al., 2010; Lunn and Buttriss, 2007; Maki et al., 2012; Nugent, 2005; Ridlon and Hylemon, 2006). Resistant starch content varies from species to species and within species of the same variety, climate and soil condition may affect the levels of resistant starch (Pereira and Leonel, 2014).
In this regard, the present study was designed in order to demonstrate the variation in resistant starch content among the popular cassava varieties in Rwanda. This will provide the basis of scientific information from which further use of resistant starch from cassava could rely on for its promotion, consumption and utilization. Three cassava varieties NASE 14, I92/0057 and Rutanihisha were evaluated for their resistant starch content. The samples were taken from two Rwanda Agriculture Board (RAB) research stations at Muhanga and Bugesera which are the two leading cassava producing districts.

3.2. Methodology

3.2.1. Study area

Cassava roots were collected from two research stations of the Rwanda Agriculture Board (RAB), Karama (Bugesera) and Muhanga in the eastern and southern province respectively. These regions are known to have a great share of cassava production in the country for both local and improved varieties (Nduwumuremyi et al., 2016a; Night et al., 2011; RAB, 2013).

Figure 3.1: Map of Rwanda highlighting Bugesera and Muhanga districts.
(Source: ArcGIS 10.1, 2012)
Bugesera district is located in the eastern province of Rwanda and has an altered clay soil with an altitude of 1300-1500m, average precipitation of 900mm, and temperature of 18-30 °C which make it ideal for cassava cultivation (Nduwumuremyi et al., 2016b). Muhanga district is situated in the southern province with an average rainfall of 1100 mm and it is a part of the central plateau which is a high cassava producing region (Karamage et al., 2017; Night et al., 2011; USAID, 2010).

3.2.2. Sample collection

Cassava roots of one year old were randomly collected from the trial fields of the Rwanda Agricultural Board (RAB) at Karama Research Station in Bugesera and from the field of the satellite farmers partnering with the Research Station of Muhanga. Three cassava varieties were sampled namely NASE 14, I92/0057 and Rutanihisha. These varieties were chosen due to their availability and resistance to diseases and drought. Randomly chosen cassava trees were harvested from the field and the roots were transported in sample bags to University of Rwanda laboratory.

3.2.3. Study design

Each Cassava variety was sampled from individual trial field. Variety NASE 14 was sampled from Karama Research station while I92/0057 and Rutanihisha were sampled from Muhanga Research Station from different fields all arranged in a complete randomised design. Three replicates of sampling were conducted for each cassava variety and the parameters were analysed in duplicate.
3.2.4. Analytical methods

3.2.4.1. Starch Extraction

Wet method was applied for starch extraction as per Benesi et al. (2004). After peeling, washing and chopping, cassava roots (15 kg) were crushed in a blender (Aardee ARMG 550, India). The mash was put in 1:10 ratio of mash:water volume, agitated for 5 minutes followed by filtering with a cotton cloth. The suspended solids in the filtrate were allowed to settle for 2 hours and the top fluid was discarded. This process was repeated three times until clear water was observed. Sediment starch was kept at -20 °C and freeze dried (Lyotrap, LTE scientific, Great Britain) for 4 days in order to avoid the native starch being denatured by high temperature of oven drying as well as to eliminate the interference with the subsequent heat treatments.

3.2.4.2. Resistant starch determination

Resistant starch was determined using AOAC (2003) Method 2002.02 which first involved hydrolysing starch (100 mg) to glucose by the activity of pancreatic α-amylase (4.0 ml) and amylglucosidase (AMG) (Megazyme, Ireland). Glucose was washed by ethanol (Schauhl, Spain) and resistant starch was collected as pellets by centrifugation. Resistant starch pellets were dissolved in 2.0 ml of 2M KOH (Park Scientific LTD, UK), buffered by 1.2M Sodium acetate (8.0 ml) and hydrolysed to glucose by AMG (100 µl). The solution was then treated with 3.0 ml of glucose oxidase–peroxidase reagent (GOPOD) (Megazyme, Ireland) and the absorbance measured using a spectrophotometer (GYNESIS, Thermo Electron Corporation, USA) at 510 nm against the blank solution. The average of duplicate absorbance values was recorded.

3.2.5. Statistical analysis

The data were analysed in a complete randomized design. The mean values were recorded and compared by means of multiple comparison (Duncan test) at 5% significance level.
3.3. Results

3.3.1. Starch Extraction and quantification

Table 3.1 shows starch extraction yield and dry matter content of the three Rwandese cassava varieties. There was a significant difference (P≤0.05) among starch quantities extracted from the three varieties with I92/0057 having the highest starch extraction yield of 87.57% on dry weight basis followed by NASE14 (81.57%) and the lowest starch yield was observed in the local variety, Rutanihisha with 71.31%.

Table 3.1: Starch extraction yield and dry matter of three Rwandese cassava varieties

<table>
<thead>
<tr>
<th>Variety</th>
<th>Starch yield (%)</th>
<th>Dry Matter content (%)</th>
<th>Ash content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NASE14</td>
<td>81.15 ±3.95a</td>
<td>31.30 ±0.85g</td>
<td>2.08 ±0.11i</td>
</tr>
<tr>
<td>I92/0057</td>
<td>87.57 ±1.74b</td>
<td>36.50 ±1.15h</td>
<td>2.02 ±0.01i</td>
</tr>
<tr>
<td>Rutanihisha</td>
<td>71.31 ±1.63c</td>
<td>30.60 ±0.62g</td>
<td>3.14 ±0.09j</td>
</tr>
</tbody>
</table>

Values with different superscripts in a column are significantly different (P≤0.05)

The dry matter content was highest in I92/0057 variety with total dry matter of 36.50%. On the other hand, no significant difference (P>0.05) was observed between NASE14 and the local variety Rutanihisha. Ash content was highest in the local variety Rutanihisha at 3.14% of the dry matter and significantly different (P≤0.05) from the other two varieties. The ash contents of NASE14 and I92/0057 at 2.08% and 2.02% of the dry matter respectively were, however, not significantly (P>0.05) different.

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3.3.2. Resistant starch of three Rwandan cassava varieties

Table 3.2 shows resistant, digestible and total starch content of three Rwandese cassava varieties. Variety I92/0057 had higher resistant starch content with 5.89 g/100g of total starch and it was significantly different (P≤0.05) from the other two varieties. The other two varieties registered resistant starch contents of 4.18 g/100g for NASE14 and 3.88 g/100g for the local variety Rutanihisha and were not significantly different (P>0.05).

Table 3.2: Resistant, digestible and total starch of three Rwandese cassava varieties

<table>
<thead>
<tr>
<th>Variety</th>
<th>Resistant starch (g/100g)</th>
<th>Digestible Starch (g/100g)</th>
<th>Total Starch (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NASE14</td>
<td>4.18±0.46\textsuperscript{a}</td>
<td>89.13±0.75\textsuperscript{c}</td>
<td>93.31±0.38\textsuperscript{e}</td>
</tr>
<tr>
<td>I92/0057</td>
<td>5.89±0.56\textsuperscript{b}</td>
<td>86.79±1.11\textsuperscript{d}</td>
<td>92.67±0.60\textsuperscript{f}</td>
</tr>
<tr>
<td>Rutanihisha</td>
<td>3.88 ±0.11\textsuperscript{a}</td>
<td>89.32±0.92\textsuperscript{c}</td>
<td>93.20±0.85\textsuperscript{e}</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation, Values with different superscripts in a column are significantly different (P≤0.05)

There was no significant difference (P>0.05) between NASE14 and Rutanihisha for both total starch and digestible starch. I92/0057 had the lowest values, 92.67 g/100g for total starch and 86.79 g/100g for digestible starch and it was significantly different (P≤0.05) from the other two varieties.

3.4. Discussion

It was observed that the improved varieties had the highest starch yield and this can be explained by the fact that one of the objective among others of introducing new improved variety is to increase the yield. There was a direct correlation between high dry matter and high starch yield.
for all varieties indicating that the large amount of dry matter of cassava is made up of starch. The dry matter content of cassava depends on many factors including the age, location and variety (CIAT 1985). The present results are in accordance with the findings of Teye et al. (2011) who reported the dry matter content of cassava from Ghana to vary from 20 % to 47 % while Fakir et al. (2012) found the dry matter content of seven cassava accessions from Bangladesh to vary from 37.50 % to 56.26 %. The slight difference from the present findings could be attributed to genetic differences as well as the location of the cultivars.

The higher the dry matter content and starch yield, the higher was the resistant starch content. This could be related to the fact that native starch granules in roots are firmly stocked in a very compact and dehydrated pattern which make it inaccessible to digestive enzymes (BeMiller and Whistler, 2009; Sajilata et al., 2006). This may explain the presence of resistant starch in native cassava starch from the analysed three varieties. Variety, species or cultivars of crops display different granule structure as well as different amylose and amylopectin levels and their arrangement due to their genetic make-up and allelic change variation during starch biosynthesis (Birt et al., 2013; Shannon, Garwood, & Boyer, 2009). These factors influence the crystallinity of native starch which reduce the its digestibility (Alsaffar 2011; Jane et al. 1999). Other intrinsic factors also play an important role in resistant starch differences (Charles et al., 2005) including particle size, molecular weight, presence of amylose lipid complexes, level of sugar content, presence of protein matrix which surround the starch and prevent it from digestive enzymes (Alsaffar 2011; Charles et al. 2005). Extrinsic factors like temperature also impact the formation of resistant starch in crops through altering the starch biosynthetic enzymes activity (Birt et al., 2013) hence the difference in resistant starch content observed in the three cassava varieties analysed which were collected from two different regions. The current values are slightly lower
to the findings of Ogbo and Okafor, (2015) who reported the resistant starch content in six cassava varieties in Nigeria to range between 5.70 g/100 g to 7.075 g/100mg while Moongngarm (2013) reported the resistant starch content of 9.69 % in cassava roots from Thailand. This difference may be attributed to the fact that samples for the present study were freeze dried prior to analysis whereas the latter were oven dried before resistant starch determination. Oven drying has been reported to increase resistant starch content while the effect of freeze drying is minor or none (Mishra et al., 2008; Zhang et al., 2014). Mejia-Aguero et al. (2012) reported that cassava starch display a diffraction c-type which is known to have a negative correlation with thorough digestion both in *vitro* and in *vivo*. The presence of significant resistant starch in raw cassava indicate that it can be considered as an important source of resistant starch in Rwanda where cassava is highly consumed. Consumption of food rich in resistant starch has been associated with positive health advantages such as good colon health, low glycaemic index, body weight management (Birt et al., 2013).

3.5. Conclusion and recommendation

There is significant difference among resistant starch content of Rwandese cassava varieties. Variety I92/0057 has the highest resistant starch content, dry matter content and starch extraction yield but lowest total starch and digestible starch. Further studies are recommended to investigate the resistant starch content of many cassava varieties and other crop and explore the factors that affect the formation of resistant starch in cassava.
CHAPTER 4: EFFECT OF HYDROTHERMAL MODIFICATION ON THE
RESISTANT STARCH CONTENT AND FUNCTIONAL PROPERTIES OF STARCH
FROM THREE RWANDESE CASSAVA VARIETIES

4.0. Abstract

Interest in resistant starch consumption has been increasing in the recent years due to its health
advantages such as involvement in conditioning insulin resistance, overweight, diabetes,
cardiovascular disease and colorectal cancer. Subsequently, many methods of increasing resistant
starch in food have been experimented. Cassava, which contain a high content of starch that can
exceed 90 % on dry weight basis could be one of the important sources of resistant starch since
its starch displays a diffraction c-type which is associated with reduced digestibility. However,
there is limited information on variation of resistant content in cassava and ways of increasing it.
The current study evaluated the effect of hydrothermal treatment of cassava starch on resistant
starch content from three cassava varieties (NASE14, I92/0057 and Rutanihisha) from Rwanda
and explored its influence on starch functional properties. Heat moisture treatment at 18 %, 24 %
and 30 % moisture content for 16 hours at 100 °C as well as annealing at 45 °C and 55 °C with
excess water (2:1) for 24 hours were applied on the three cassava varieties. Hydrothermal
treatment significantly increased the resistant starch content in all cassava varieties. Among all
heat treatment, heat moisture treatment at 24 % moisture content of I92/0057 starch had the
highest increase, 5.89 g/100g to 42.53 g/100g followed by annealing method at 45 °C, which
increased it to 31.56 g/100g. Hydrothermal treatment significantly (P≤0.05) decreased the
digestible starch, water absorption index and water solubility in all cassava varieties while it had
no significant (P>0.05) effect on total starch. The oil absorption index of native starch was 5.60
g/g, 4.93 g/g and 5.13 g/g for NASE14, I92/0057 and Rutanihisha respectively and it was
significantly (P≤0.05) increased by heat moisture treatment of starch while it was significantly decreased (P≤0.05) by annealing method.

Keywords: Cassava, Resistant starch, Heat moisture treatment, Annealing

4.1. Introduction

Resistant starch (RS) is the portion of starch which are not broken down by the amylolytic enzymes in the intestine of humans (Englyst et al., 1992) and is then fermented in the colon into short chains fatty acids (SCFAs) and organic acids (Eliasson, 2004; Ferguson et al., 2000). Trends in resistant starch consumption have been increasing in the recent years due to its health advantages like involvement in conditioning insulin resistance, over weight, diabetes, cardiovascular disease and colorectal cancer (Anderson et al., 2010; EFSA, 2011; Jenkins et al., 1998; Ridlon and Hylemon, 2006). Due to these benefits many methods of increasing resistant starch in food have been experimented and they include genetic method (interbreeding), enzymatic, chemical, irradiation and physical methods (thermal treatment) (Dupuis et al., 2014; Faraj et al., 2004; Jacobs and Delcour, 1998; Marsono and Topping, 1999; Sajilata et al., 2006).

The main obstacle to the food industry is the production of foods with sufficient resistant starch resulting in an important enhancement of consumer’s health (Chung et al., 2011). Cassava could be one of the important sources of resistant starch since it is known to contain a high content of starch which can exceed 90% of dry basis (Zvinavashe et al., 2011). Cassava starch also displays a diffraction c-type which is known to have very reduced digestibility both in vitro and in vivo (Mejia-Aguero et al., 2012).
Though the resistant starch content of some cassava varieties from Rwanda was determined, the possible methods of increasing it have not been experimented. In Rwanda the industrial processing of cassava as well as the informal processing units across the country produce only cassava flour for home consumption though a starch processing line is proposed in the future. The objective of this chapter was to demonstrate the possibility of increasing resistant starch content of Rwandese cassava varieties and evaluate its effect on starch functional properties which may result in more diversified utilization, increased shelf life, facilitated transportation and trading and transform it into a cash crop.

4.2. Materials and methods

4.2.1. Raw materials

Cassava roots samples from three cassava varieties namely Rutanihisha, I92/0057 (Cyizere) and NASE 14 of 12 months old in the field were collected from Rwanda Agriculture Board field research stations of Muhanga (Rutanihisha and I92/0057) and Karama (NASE14). Randomly chosen cassava trees were harvested from the field by skipping equal interval of distance and 15 kg of each variety roots were transported in plastic bags to University of Rwanda laboratory. Cassava roots were then peeled, chopped and crushed in the blender (Aardee ARMG 550, India) for starch extraction. Starch was extracted from cassava roots by wet method (described in 3.2.4.1) and kept in the freezer at -80°C. The extracted starch was freeze-dried (Lyotrap, LTE scientific, Great Britain) for 4 days to attain the moisture content of less than 12% (EAS 742 2010) prior hydrothermal treatment.

4.2.2. Experimental Design

In order to evaluate the effect of hydrothermal treatment on resistant starch levels, three varieties of cassava were treated with five levels of hydrothermal modification. Heat-moisture treatment
(HMT) at 18%, 24% and 30% moisture content for 16 hours at 100°C were carried out as per Khunae et al. (2007) with slight modifications where moisture content levels were separated by 6% rather than 3%. Annealing (AN) at 45°C and 55°C for 24 hours as per Jacobs et al. (1995). Cassava starch with no heat treatment was used as a control. These treatments were analysed in a factorial arrangement of two factors (variety and heat treatment). Variety had three levels while heat treatment had 6 levels to form a 3x6 design. Two replications were done and the mean values were recorded.

4.2.2.1. Hydrothermal treatment

Heat-moisture treatment (HMT) was applied as per Franco et al. (1995). The moisture contents of the extracted starch (100 g) was increased up to 18%, 24% and 30% respectively by addition of the suitable quantity of distilled water. This step was followed by mixing and samples were kept overnight for moisture to stabilize. Then the samples of starch (100 g) were hermetically closed in glass jars and heated in an oven (Memmert GmbH, Germany) at 100°C for 16 hours. Starch samples were then cooled, air dried in tray dryer (CHITRA, India) to less than 12% moisture content (EAS 742 2010) and subjected to resistant starch measurement.

4.2.2.2. Annealing

The method of Jacobs et al. (1995) was applied for starch annealing. Starch (100 g) was heated in excess water (2:1) at 45°C and 55°C for 24 hours. Samples were then centrifuged (Nuve, Turkey) at 996 x g for 10 minutes to eliminate excess water, air-dried in tray dryer (CHITRA, India) and thereafter subjected to resistant starch determination.
4.2.3. Analytical methods

4.2.3.1. Determination of moisture content and dry matter content

To determine the moisture content and dry matter content, ISO 1660 (1996) method was applied which entailed drying the starch sample at 130 (± 3) °C for 90 minutes. The weight loss was expressed as percentage moisture content while the difference between the total weight and moisture content was expressed as the dry matter content.

4.2.3.2. Determination of resistant starch content

The hydrothermally treated starch was subjected to resistant starch content determination after being dried. Resistant starch was determined using AOAC (2003) Method 2002.02. Starch (100 mg) was hydrolysed to glucose by the 4.0 ml pancreatic α-amylase and amyloglucosidase (AMG) (Megazyme, Ireland) for 16 hours in shaking (200 strokes/minute) water bath (Memmert GmbH, Germany). Glucose was then washed by 99% ethanol (Schaurl, Spain) and resistant starch was collected as pellet by centrifugation (nőve, Turkey). Resistant starch pellets were dissolved in 2.0 ml of 2 M KOH (Park Scientific LTD, UK), buffered by 1.2 M Sodium acetate (8.0 ml) and hydrolysed to glucose by AMG (100 µl). The solution was then treated with 3.0 ml of glucose oxidase–peroxidase reagent (GOPOD) (Megazyme, Ireland) and measured with a spectrophotometer (GYNESIS, Thermo Electron Corporation, USA) at 510nm against the blank solution. The average of duplicate absorbance values was recorded. The digestible starch content was deduced from glucose obtained after 16 hours of incubation and total starch content obtained by adding the digestible and the resistant starch.

4.2.3.3. Determination of digestible starch and total starch

Digestible starch was determined by measuring glucose resulting from starch hydrolysis as per AOAC (2003) Method 2002.02. Total starch was obtained by adding resistant starch content to digestible starch content.
4.2.3.4. Determination of water absorption index (WAI) and water solubility index (WSI)

The two parameters were determined as per Anderson (1982) with minor adaptation. Starch (3.0 g) was added to 30 ml distilled water and kept at 60 °C for 1 hour then centrifuged (Nůve, Turkey) 4025 x g for 15 minutes. The supernatant was collected and dried for WSI measurement. The deposits drained off by leaving tubes containing samples to stand inverted for 10 minutes, weighed and WAI was expressed as a ratio of the initial starch as follows.

$$WAI (g/g) = \frac{\text{Weight of water uptake in hydrated residue}}{\text{Weight of starch sample}}$$ \hspace{1cm} (Equation 1)

$$WSI(\%) = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Weight of starch sample}} \times 100$$ \hspace{1cm} (Equation 2)

4.2.3.5. Determination of oil absorption index

The method by Adebowale et al. (2005) was used. Three grams of starch were mixed with 30 ml of oil, kept at 60°C for one hour and centrifuged (Nůve, Turkey) at 4025 x g for 15 minutes the supernatant was discarded and the pellets were collected and weighed. Oil absorption was expressed as follows:

$$OAI (g/g) = \frac{\text{Weight of pellets}}{\text{Weight of starch sample}}$$ \hspace{1cm} (Equation 3)

4.3. Data Analysis

Two replicates were conducted for each cassava variety and their mean were recorded in Excel and analysed in Genstat (15th edition). A two way ANOVA were performed and the means were compared at 95% confidence level using Duncan test.
4.4. Results

4.4.1. Effect of hydrothermal treatment on resistant starch content

Hydrothermal treatment of starch increased significantly (P≤0.05) the resistant starch content of cassava at different extents (Figure 4.1). Heat moisture treatment with 24 % moisture content (HMT-24) at 100 °C for 16 hours increased resistant starch content at the highest extent from 4.18 g/100g to 40.09 g/100g for NASE14, from 5.89 g/100g to 42.53 g/100g for I92/0057 and from 3.88 g/100g to 38.67 g/100g for the local variety Rutanihisha.

![Resistant Starch Content Graph](image)

**Figure 4.1:** Resistant starch content after hydrothermal treatments of three cassava varieties. The bars indicate standard error of the means, HMT-18: Heat moisture treatment at 18% moisture content, HMT-24: Heat moisture treatment at 24% moisture content, HMT-30: Heat moisture treatment at 30% moisture content at 100 °C for 16 hours, AN-45: Annealing at 45 °C, AN-55: Annealing at 55 °C for 24 hours.
Heat moisture treatment with 18% moisture (HMT-18) also increased the levels of resistant starch significantly (P≤0.05) in all three cassava varieties, it increased to 30.36 g/100g for NASE14 starch, 27.34 g/100g for I92/0057 and 25.47 g/100g for Rutanihisha.

The least increase in resistant starch content was due to heat moisture treatment with 30% moisture content at 100°C for 16 hours (HMT-30) with an increase to the level of 14.49 g/100g; 14.06 g/100g and 13.68 g/100g for NASE14, I90/0057 and Rutanihisha, respectively.

Annealing method at 45 °C (AN-45) with excess moisture (2:1) also produced a significant (P≤0.05) boost in resistant starch content where it significantly (P≤0.05) increased from 4.18 g/100g; 5.89 g/100g and 3.88 g/100g for NASE 14, I92/0057 and Rutanihisha respectively to above 31% in all three cassava varieties. Annealing at 55°C (AN-55) had the least, though significant (P≤0.05) effect on the resistant starch content. It significantly (P≤0.05) increased the resistant starch content up to 6.65 g/100g; 6.25 g/100g and 5.01 g/100g for NASE 14, I92/0057 and Rutanihisha, respectively.

4.4.2. Effect of hydrothermal treatment on starch parameters

4.4.2.1. Moisture content and dry matter of hydrothermal treated cassava starch

Table 4.1 shows the effect of hydrothermal treatment of starch on moisture and dry matter contents. The moisture contents of untreated samples of all varieties were not significant different between them. There was no significant difference (P>0.05) in moisture content among the samples to which heat moisture treatments (HMT-18, HMT-24 and HMT-30) were applied where it ranged from 12.0 % to 14.05 %. Annealing (AN) significantly (P≤0.05) increased the moisture content with annealing at 55°C (AN-55) increasing the moisture content from 13.80 %, 13.55 % and 12.3 % to 64.70 %, 68.10 % and 68.10 % respectively. AN-45 increased the
moisture content to 47.65\% for NASE 14, 46.05 \% for I92/0057and 54.45 \% for Rutanihisha. Dry matter was not significantly (P>0.05) affected by heat moisture treatments (HMT-18, HMT-24 and HMT-30). In native cassava starch the dry matter was 86.20\%, 86.45 and 87.70\% for NASE 14, I92/0057 and Rutanihisha. After heat moisture treatments, it ranged from 85.95\% to 88.00\%.

Table 4.1: Moisture and dry matter contents of three Rwandese cassava varieties under different hydrothermal treatments

<table>
<thead>
<tr>
<th>Varieties</th>
<th>NASE14</th>
<th>I92/0057</th>
<th>Rutanihisha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>MC (%)</td>
<td>DM (%)</td>
<td>MC (%)</td>
</tr>
<tr>
<td>Untreated</td>
<td>13.80±1.39abc</td>
<td>86.20±1.39efg</td>
<td>13.55±0.12abc</td>
</tr>
<tr>
<td>HMT-18</td>
<td>12.00±0.41a</td>
<td>88.00±0.41g</td>
<td>13.60±0.57abc</td>
</tr>
<tr>
<td>HMT-24</td>
<td>13.35±1.18abc</td>
<td>86.65±1.18efg</td>
<td>14.60±0.01c</td>
</tr>
<tr>
<td>HMT-30</td>
<td>14.05±0.94abc</td>
<td>85.95±0.94ef</td>
<td>13.35±0.61abc</td>
</tr>
<tr>
<td>AN-45</td>
<td>47.65±1.02d</td>
<td>52.35±1.02d</td>
<td>46.05±0.45d</td>
</tr>
<tr>
<td>AN-55</td>
<td>64.70±0.01f</td>
<td>35.30±0.01b</td>
<td>68.15±1.02g</td>
</tr>
</tbody>
</table>

MC: Moisture content, DM: Dry matter, HMT-18: Heat moisture treatment at 18 \% moisture content, HMT-24: Heat moisture treatment at 24 \% moisture content, HMT-30: Heat moisture treatment at 30 \% moisture content at 100\degree C for 16 hours, AN-45: Annealing at 45 \degree C, AN-55: Annealing at 55 \degree C for 24 hours. Values are mean ± standard deviation, Values with different superscripts are significantly different at P≤0.05 (for each parameter).

Annealing significantly (P≤0.05) reduced dry matter contents from 86.20 \%, 86.45 \% and 87.70\% for NASE 14, I92/0057 and Rutanihisha down to 47.65 \%, 46.05 \% and 45.55 \% through AN-45 and to 35.30 \%, 31.85 \% and 31.90 \% through AN-55 respectively.
4.4.2.2. Effect of hydrothermal treatment on Digestible and Total starch

Table 4.2 shows the contents of digestible and total starch in the hydrothermally treated cassava starches from the three cassava varieties. Both methods of hydrothermal treatment decreased the digestible starch but to different extents. A significant decrease (P≤0.05) in digestible starch was observed as a result of heat treatment of starch through annealing at 55 °C (AN-55) had the least effect on digestible starch. The digestible starch in NASE 14 native starch was 89.32% and it was reduced to 67.32%, 53.91%, and 78.92% by heat moisture treatment with 18%, 24% and 30% moisture content. Digestible starch of NASE 14 decreased to 53.86% and 86.66% after annealing at 45 °C and 55 °C.

Table 4.2: Digestible and Total starch in hydrothermally treated cassava varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Digestible starch (%)</th>
<th>Total starch (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS (%)</td>
<td>TS (%)</td>
</tr>
<tr>
<td>Untreated</td>
<td>89.13±0.75\textsuperscript{a}</td>
<td>93.31±0.38\textsuperscript{a}</td>
</tr>
<tr>
<td>HMT-18</td>
<td>62.12±0.51\textsuperscript{g}</td>
<td>92.48±0.59\textsuperscript{abcd}</td>
</tr>
<tr>
<td>HMT-24</td>
<td>52.31±0.70\textsuperscript{i}</td>
<td>92.40±0.37\textsuperscript{abcd}</td>
</tr>
<tr>
<td>HMT-30</td>
<td>77.91±0.83\textsuperscript{d}</td>
<td>92.01±1.10\textsuperscript{c}</td>
</tr>
<tr>
<td>AN-45</td>
<td>61.20±0.86\textsuperscript{g}</td>
<td>92.40±0.38\textsuperscript{abcd}</td>
</tr>
<tr>
<td>AN-55</td>
<td>84.88±0.70\textsuperscript{c}</td>
<td>91.53±0.69\textsuperscript{d}</td>
</tr>
</tbody>
</table>

DS: Digestible starch, TS: Total starch, HMT-18: Heat moisture treatment at 18 % moisture content, HMT-24: Heat moisture treatment at 24 % moisture content, HMT-30: Heat moisture treatment at 30 % moisture content at 100 °C for 16 hours, AN-45: Annealing at 45 °C, AN-55: Annealing at 55 °C for 24 hours. Values are mean ± standard deviation, Values with different superscripts are significantly different at P≤0.05 (for each parameter)
For variety I92/0057, the digestible starch was reduced from 86.79% to 66.10%, 50.34% and 77.88% by heat moisture treatment with 18%, 24% and 30% moisture content whereas annealing at 45 °C and 55 °C brought it down to 66.09% and 86.36% respectively.

For Rutanihisha, the digestible starch was reduced from 89.13 % to 66.12%, 52.31 % and 77.91 % by HMT-18, HMT-24 and HMT-30% and to 61.24 % and 84.88 % by annealing at 45 °C and 55 °C respectively. There was no significant change (P>0.05) in total starch content as a result of any heat treatment where it varied between 91.53 % before heat treatment and 93.44 % after heat treatment.

**4.4.2.3. Effect of hydrothermal treatment on Water absorption index and Water Solubility Index**

Table 4.3 shows the effect of hydrothermal treatment on water absorption index and water solubility. Water absorption index (WAI) decreased significantly (P≤0.05) due to hydrothermal treatment by both heat moisture treatment and annealing. Native starch from the local variety (Rutanihisha) had lower values of water absorption index with 3.08 g/g and consequently registered the least water absorption index (0.73 g/g) as result of annealing at 55 °C (AN-55).

The improved varieties (NASE14 and I92/0057) had the WAI of 6.78 g/g and 6.66 g/g, respectively and it was reduced to 3.23 g/g and 3.13 g/g after annealing at 55 °C (AN-55).

Water solubility index (WSI) was significantly (P≤0.05) decreased by hydrothermal treatments. Heat-moisture treatment showed significantly (P≤0.05) greater reduction in water solubility index with HMT-24 registering the lowest values where in decreasing order Rutanihisha had 0.93 % followed by NASE14 with 0.86 %. Variety I92/0057 had the lowest values of water solubility index with 0.50 %.
Table 4.3: Water absorption index and Water Solubility Index of hydrothermally treated cassava starch

<table>
<thead>
<tr>
<th>Varieties</th>
<th>NASE14</th>
<th>I92/0057</th>
<th>Rutanhisha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>WAI (g/g)</td>
<td>WSI (%)</td>
<td>WAI (g/g)</td>
</tr>
<tr>
<td>Untreated</td>
<td>6.78±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.66±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HMT-18</td>
<td>4.89±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98±0.06&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>3.95±0.13&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>HMT-24</td>
<td>4.65±024&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.86±0.04&lt;sup&gt;k&lt;/sup&gt;</td>
<td>3.99±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>HMT-30</td>
<td>3.71±0.24&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.89±0.07&lt;sup&gt;ijk&lt;/sup&gt;</td>
<td>3.18±0.14&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>AN-45</td>
<td>3.83±0.02&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.99±0.05&lt;sup&gt;fgih&lt;/sup&gt;</td>
<td>3.04±0.13&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>AN-55</td>
<td>3.23±0.13&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.06±0.03&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>3.13±0.03&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

WAI: Water absorption index, WSI: Water solubility index, HMT-18: Heat moisture treatment at 18 % moisture content, HMT-24: Heat moisture treatment at 24 % moisture content, HMT-30: Heat moisture treatment at 30 % moisture content at 100 °C for 16 hours, AN-45: Annealing at 45 °C, AN-55: Annealing at 55 °C for 24 hours. Values are mean ± standard deviation, Values with different superscripts are significantly different at P≤0.05 (for each parameter)

The least effect on WSI was observed on Rutanhisha treated with AN-55 (1.53 %) while the native starch had 3.16 %.

**4.4.2.4. Effect of hydrothermal treatment on oil absorption index on cassava starch**

Table 4.4 shows the effect of hydrothermal treatment on oil absorption index of cassava starch. Heat-moisture treatment increased the starch oil absorption index (OAI) significantly (P≤0.05) for all the three cassava varieties. The native starch, the oil absorption index was 5.60 g/g for NASE14, 4.93 g/g for I92/0057 and 5.13 g/g for Rutanhisha. The highest oil absorption index
was observed for NASE14 (5.60 %) and it significantly increased (P≤0.05) to 5.97 % when treated with HMT-24.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cassava varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NASE14</td>
</tr>
<tr>
<td>untreated</td>
<td>5.60±0.07c</td>
</tr>
<tr>
<td>HMT-18</td>
<td>5.87±0.06ab</td>
</tr>
<tr>
<td>HMT-24</td>
<td>5.97±0.03a</td>
</tr>
<tr>
<td>HMT-30</td>
<td>5.67±0.11bc</td>
</tr>
<tr>
<td>AN-45</td>
<td>5.40±0.04cd</td>
</tr>
<tr>
<td>AN-55</td>
<td>5.00±0.08efg</td>
</tr>
</tbody>
</table>

OAI: Oil absorption index, HMT-18: Heat moisture treatment at 18% moisture content, HMT-24: Heat moisture treatment at 24% moisture content, HMT-30: Heat moisture treatment at 30% moisture content at 100°C for 16 hours, AN-45: Annealing at 45°C, AN-5: Annealing at 55°C for 24 hours. Values are mean ± standard deviation, Values with different superscripts are significantly different at P≤0.05 (for each parameter)

In contrast with HMT, annealing decreased the oil absorption index significantly (P≤0.05) for all treated cassava starch samples. The lowest value was observed in the local variety (Rutanihisha) where it was significantly (P≤0.05) reduced from 5.13 g/g to 4.23 g/g. Native starch from I92/0057 registered the lowest value of oil absorption index (4.93 g/g).
4.5. Discussion

4.5.1. Effect of hydrothermal treatment on resistant starch content

The present results indicate that moisture content prior to hydrothermal treatment plays an important role in resistant starch content formation. Most importantly moisture content played a great impact on resistant synthesis because it was the only varying factor while temperature and time were made constant for heat moisture treatments. Temperature also played a role in synthesizing resistant starch through annealing where moisture and time were constant and temperature only varied. The results indicated a large difference in resistant starch content of samples annealed at 45 °C and those treated at 55 °C (Figure 4.1.). These findings show that there is a minimum temperature and moisture content needed for starch granules to undergo transition that lead to resistant starch formation depending on their size, crystallinity, shape and arrangement. Moisture and heat combination have been reported as important factors influencing resistant starch formation (Sajilata et al. 2006) where they lead to the formation of strong hydrogen bonds among compounds in starch granule (Chung et al., 2009; Kurakake et al., 1997; Sankhon et al., 2014a). It has also been reported that heat-moisture treatment increases resistant starch levels in starchy food through modifying the starch crystals and by increasing amylose and amyllopectin ratio as well as forming double helices (Dupuis et al., 2014; Hoover and Vasanthan, 1994; Kawabata et al., 1994). The present results substantiate the findings of Asha et al. (2014) who found an increase of resistant starch content from1.9% to 27.1% for heat-moisture treated cassava while they differ from findings of Sankhon et al. (2014) who found heat-moisture treatment of African locust bean starches to significantly increase resistant starch content from 33.38 % to 39.64 % for heat-moisture treatment at 30 % moisture content, 46.63 % for heat-moisture treatment at 25 % moisture content; 50.14 % for heat-moisture treatment at 20 %
moisture content and 37.79 % for heat-moisture treatment at 15 % moisture content, respectively. Li et al. (2011) found a smaller increase in resistant starch content of mung beans treated with heat-moisture treatment at 15 % moisture content (30.89 %) compared to the content observed with heat-moisture treatment at 25 % moisture content (36.49 %). The difference observed may attributed to other factors like variety, age and methodology used for resistant starch analysis.

The difference between the effects of annealing of starch at 45 °C (AN-45) annealing at 55 °C (AN-55) could be attributed to the difference in temperature at which these treatments were applied. It has been reported that when starch is stored at temperature below its gelatinization temperature; its granules are not disrupted, instead their crystallinity is increased (Leszczynski 2004). AN-45 being a treatment carried out in excess water (1:2) for a long period of time between glass transitional temperature and gelatinization temperature, it causes the rearrangement of starch components by forming amylopectin double helices which is a much more complex structure (Hoover and Vasanthan, 1994). However when starch is kept at temperature closer or above its gelatinization temperature with a great amount of water, the latter diffuse into starch granules and increase their size which destroy the hydrogen bond and this may lead to increased digestion susceptibility (Leszczynski 2004). Annealing at 55 °C (AN-55) could have led to less resistant starch formation because starch was kept at 55 °C where partial gelatinization may occur and exposed it for enzymatic digestion. The present findings substantiate the results reported by Asha et al. (2014) who reported the increase of resistant starch content for annealed cassava starch from 1.9 % to 28.6 % while they are higher to the findings of Chung et al. (2009) who reported the increase of resistant starch by annealing of corn, pea and lentil from 4.6 %, 10.0 % and 9.1 % to 8.7 %, 11.2 % and 11.4 % respectively and this was attributed to the enhanced interaction between amylose and amylopectin chains of starch.
The observed dissimilarities with these findings may be related to the differences in the starch botanical sources as well as to the different annealing conditions.

4.5.2. Effect of hydrothermal treatment on digestible and total starch

The significant reduction of digestible starch could be attributed to the same conditions that increase the resistant starch level including granules crystallinity due to starch retrogradation, interaction between amylose and amylopectin chain of starch and development of double helices pattern during retrogradation (Hoover and Vasanthan, 1994; Liu et al., 2016; Wang et al., 2016; Zeng et al., 2015). Liu et al. (2016) found the same trend for heat-moisture treatment and annealing of maize starch where they found that HMT-35 decreased the digestible starch by 28.9 % and annealing had reduced it by 5.9 %. The results also indicates that total starch does not change as result of heat moisture treatment or annealing and could be attributed to the fact that starch is only physically modified which could not reduce it. The present findings correlate with the findings of de la Rosa-Millan et al. (2014) who found total starch of native and annealed banana starch not changing as a result of annealing and remained above 75 %.

4.5.3. Effect of hydrothermal treatment on Moisture content and dry matter

The increase of moisture content and reduced dry matter as a result of annealing could be attributed to the diffusion of water into starch granules facilitated by temperature closer to its gelatinization temperature. The reduction of total solid could be attributed to the solubilisation of solids into excess water used for annealing process. It has been reported that heating of starch in excess water cracks the semi-crystalline pattern which favour the association of water molecules to hydroxyl groups of amylose and amylopectin chains (Alcázar-Alay and Meireles, 2015). This might have contributed the increased moisture content observed in annealed samples.
4.5.4. **Effect of hydrothermal treatment on Water absorption index and Water Solubility Index**

Water absorption index is the capacity of starch to absorb water and swell which results in adequate evenness and body in food (Choi et al., 2012). The observed reduction in water absorption ability is attributed to reorganization of molecular structure throughout the heat-moisture treatment process during which, starch gelatinize and retrograde, which strengthens their maintenance force and produces a much more firm starch matrix consequently restricting water for being absorbed (Eerlingen and Delcour, 1995; Franco et al., 1995). The current finding are in accordance with the results reported by Adebowale et al. (2005) in finger millet and Sankhon et al. (2014) in African locust bean who attributed the reduction of water absorption to the rearrangement of molecular chain in heat moisture treated starch. Low water absorption index due to annealing was also observed in annealed sorghum starch (Ali and Hasnain, 2014), corn and lentil starch (Chung et al., 2009) and cassava starch (Gomes et al., 2005).

Water solubility index (WSI) is the function of amylose leaching from starch granules (Choi et al., 2012). The reduction of water solubility index due to heat moisture treatment may be due to the increased crystallinity and strong interaction between chains of amylose in starch granules as a result of retrograded starch (Chung et al., 2009) which further reduced amylose leaching. The loss of solubility in annealed cassava starch indicate that the temperature used was not enough to weaken the bonds that prevent amylose from leaching due to the unaltered firm structure of starch granules. These findings are in accordance with the report by Dias et al. (2010); Gomes et al. (2005); Olayinka et al. (2008) and Sharma et al. (2015) who observed a decrease in solubility due to the annealing of starch from rice, white sorghum, unfermented cassava and pearl millet respectively and they attribute it to crystallinity and strong interaction between amylose and amylopectin chains. However, the current results are opposite to the findings of Adebowale et al. (2005) in annealed red sorghum starch, Sankhon et al. (2014) in African locust bean starch.
and by Kurakake et al. (1997) in maize starch. They have suggested that the increase in solubility was a result of temperature increase which raises starch molecules mobility and facilitate their dispersion in water. This contradictory findings could be attributed to the techniques of hydrothermal treatments, handleings of starch prior to analysis and starch sources.

4.5.5. Effect of hydrothermal treatment on oil absorption index on cassava starch

Oil absorption index is an indicator of starch potentiality to serve as an emulsifier (Sharma et al. 2015). The increase of oil absorption index due to heat-moisture treatment is related to the loss of water during this process due to high temperatures used (100 °C) which can increase the hydrophobic nature of starch while the decrease of oil absorption index in annealed cassava starch could be attributed to the excess water used during this process. The current findings corroborate with the findings of Sharma et al. (2015) who reported a significant increase in oil absorption capacity of heat-moisture treated pearl millet starch from 1.31 g/g to 2.66 g/g by heat-moisture treated at 30% moisture content as well as to the results obtained by Adebowale et al. (2005). Oil absorption index of starch plays an important role in flavour retention, palatability and extension of shelf life of foods (Sharma et al. 2015).

4.6. Conclusions and recommendation

Hydrothermal treatments has a significant influence on the synthesis of resistant starch in cassava starch. Hydrothermal treatments has a significant influence on the synthesis of resistant starch in cassava starch. Heat moisture treatment of starch from I92/0057 of 24% moisture content at 100°C for 16 hours gives the highest increase resistant starch content. This methods can be indorsed for industrial production of resistant starch and further studies on application of the resulting cassava starch rich in resistant starch is highly recommended.
5.0. Abstract

The current technologies of food processing have strongly reduced the intake of resistant starch. In an attempt to promote the consumption of resistant starch in Rwanda, resistant starch was physically synthesized from 192/0057 cassava variety and applied as a thickener of yoghurt, a preferred dairy product in Rwanda. Incorporation of resistant starch into yoghurt in the past have emphasized on clinical aspect of resistant starch and the technological effect of it on physico-chemical parameters of yoghurt being left out. The current research investigated the effect of incorporating cassava resistant starch from Rwanda synthesized by heat moisture treatment on physico-chemical properties yoghurt. Modified cassava starch was incorporated into yoghurt in the following proportions: 0, 0.1%, 0.5% and 1%, respectively. Corn starch (0.6%) was applied as a control. Yoghurt was stored at 4°C for 21 days and the effect of the modified starch on resistant starch content, viscosity, syneresis, total solids, acidity, lactic acid bacteria count and sensory properties was determined on weekly basis. The modified cassava starch proportions of 0.5% and 1% increased resistant starch content significantly (P≤0.05) reaching 3.40 g/100 g and 5.58 g/100 g on day one and 1.92 g/100 g and 4.47 g/100 g on day 21, respectively. Yoghurt treated with 1% modified cassava starch expressed the highest viscosity during cold storage (2721.5 mPa s, 2721 mPa s, 2650 mPa s and 1034.5 mPa s at day 1, day 7, day 14 and day 21 respectively) and it had the least syneresis (22.25%) followed by 0.5% treatment (24.76%) which had no significant difference (P>0.05) with the control (23.40%). The addition of modified cassava starch significantly increased (P≤0.05) the total solids and did not significantly (P>0.05) change the sensory properties of yoghurt. The application of 1% of modified cassava starch is
recommended as it produces yoghurt with acceptable sensory and physico-chemical properties that is also rich in resistant starch.

**Keywords:** *Heat moisture treatment, Resistant starch, Yoghurt.*

### 5.1. Introduction

Resistant starch is a dietary carbohydrate which resists enzymatic digestion and it is fermented in the colon by gut microflora into short chain fatty acids like acetic, propionic and butyric acid that are known to prevent colon diseases (Aryana et al., 2015; Noor-Aziah et al., 2011; Nugent, 2005; Ridlon and Hylemon, 2006). The current technology of milling and food processing has strongly reduced the intake of resistant starch in both developed and developing countries (Sweatt et al., 2016; Baghurst et al., 2001). Increase in prevalence of obesity, colorectal cancer, diabetes and other gastrointestinal diseases has led to the rise of resistant starch incorporation into different types of food especially those liked by the modern society including but not limited to cheese, ice cream, yoghurt, milk, bread, corn flakes, cakes, muffins, pasta and butter (Homayouni et al., 2014; Noronhan et al., 2007; Ozturk et al., 2009; Sanz et al., 2009) without changing the inherent quality of these products. The use of native starch in food industry is limited by the fact that they are not able to withstand extreme temperatures and pressures and different pH levels and also because they have poor pasting properties and a tendency to retrograde (Gunorubon and Kekpugile, 2012; Singh et al., 2007). Resistant starch has been used to enrich yoghurt by Aryana et al. (2015) and they have reported that this enrichment with resistant starch produced an acceptable yoghurt and resistant starch was not affected by yoghurt heat treatment. Resistant starch appears to be a good thickening agent since is natural, flavourless, white, composed of small size particles, and has high gelatinization temperature,
good extrusion qualities and low water holding capacity (Homayouni et al., 2014; Sajilata et al., 2006). Thickeners are normally applied in yoghurt in order to improve its texture which is an important yoghurt quality parameter (Goncalvez et al., 2005) and cassava starch is preferred for this purpose due to its high purity, neutral flavour, high viscosity, good solubility and swelling capacity (Collares et al., 2012; Demiate and Kotovicz, 2011). Milk production in Rwanda has sprouted as a result of government programs such as ‘One cow per poor family’ introduction of new cattle breeds, insemination programmes and active diseases eradication program which increased the milk production from 372,619 tonnes in 2006 to 706,030 tonnes in 2014 (Karenzi et al., 2013; NISR, 2015) with a projected annual milk production increase of 13% up to 2018 (MINAGRI 2013). The dairy products in Rwanda include cheese, whole and low fat milk, traditionally fermented milk (Ikivuguto) and yoghurt which is, together with Ikivuguto, the most popular amongst the aforementioned products (Karenzi et al., 2013; Olok-asobasi and Sserunjogi, 2001). Yoghurt production in Rwanda relies on imported and expensive corn starch, gelatine or pectin as thickeners. The use of these enzyme and proteins for texture improvement in yoghurt processing has got limitation due to the fact that they don’t withstand heat treatment and their activity is temperature dependent product as opposed to native starch (Chandan & Kilara 2013). Moreover they produce yoghurt with lumps when they are used alone which makes imperative to combine them with starch in order to avoid such technological failure (Chandan & Kilara 2013). The use of locally produced and value added native cassava starch will be an advantage for farmers, producers and consumers and its adoption may contribute to improved cassava utilization and hence livelihoods to the actors along the value chain. The current research was designed to demonstrate the technological possibility of increasing cassava resistant starch by hydrothermal treatment and showcase its potential use in yoghurt processing as a thickening agent especially in Rwanda.
5.2. Materials and methods

5.2.1. Raw material acquisition

Low fat milk (Inyange industries, Rwanda) was purchased in the local shop and brought in ice box to University of Rwanda laboratory for yoghurt processing. Starch was extracted from cassava variety I92/0057 collected from Rwanda Agriculture Board (RAB) research station of Muhanga. Cassava Starch was subjected to heat moisture treatment by increasing its moisture content to 24 % and keeping it at 100 °C for 16 hours and air drying to 12.3 % moisture content. Corn starch (Tirupati, India) was purchased from the shop and used as the control sample.

5.2.2. Research Design

Modified Cassava starch was incorporated into milk used to make yoghurt in the following proportions: 0%, 0.1%, 0.5% and 1%, respectively. Corn starch was applied as a control at 0.6% as it normally used in the yoghurt processing industry (Goncalvez et al., 2005) and the yoghurt quality parameters were determined on day 1, 7, 14 and 21 of storage at refrigeration temperature (4°C). The effect of modified cassava starch application on yoghurt quality was analysed in a factorial arrangement of two factors; modified starch proportions and storage time. Modified starch had five levels while storage time had four levels. Sensory evaluation data were analysed in a factorial design of two factors where modified cassava starch proportions had five levels while storage time had three levels.

5.2.3. Yoghurt manufacturing

Yoghurt was processed as described by Goncalvez et al. (2005). Five litres of low fat milk (Inyange industries, Rwanda) (1.5 % fat) with 11 % (w/w) total solids was treated with modified cassava starch in three proportions: 0%, 0.1% 0.5% and 1% of the initial milk, well mixed and heated at 90 °C for 15 minutes. The samples were cooled to 42 °C and inoculated with a
commercial thermophilic (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) starter culture (CRH HANSEN/CH-1 Yo-Flex® Freeze-dried 50u, Denmark) at 3% and incubated at 42 °C for 4 hours. Yoghurt was cooled to 4 °C and kept at the same temperature for 21 days with weekly testing of yoghurt quality changes. Yoghurt with 0.6% corn starch (Tirupati, India) as per Goncalvez et al. (2005) was used as a control.

5.2.4. **Analytical methods**

5.2.4.1. **Determination of resistant starch levels in yoghurt**

Levels of resistant starch in yoghurt treated with modified starch were determined as per AOAC (2003) method 2002.02. Yoghurt (30 g) was first centrifuged (NF1200R, Nüve, Turkey) at 3992 x g for 15 minutes, supernatants were discarded off and the pellets were air dried for 24 hours. Dried pellets (100 mg) were put in a screw tube and 4.0 ml of pancreatic α-amylase containing amyloglucosidase (AMG) (Megazyme, Ireland) were added and incubated in shaking (200 strokes/minutes) water bath (Memmert GmbH, Germany) at 37˚C for 16 hours in accordance with the manufacturer’s instructions. Glucose was then washed by 2.0 ml of ethanol (99.9%) (Schaurl, Spain) and resistant starch was collected as pellet by centrifugation (nüve, Turkey) at 412.5 x g. Resistant starch pellets were dissolved in 2.0 ml of 2M KOH (Park Scientific LTD, UK), buffered by 8.0 ml of sodium acetate and hydrolysed to glucose by 0.1 ml AMG (Megazyme, Ireland). The obtained glucose was treated with 3.0 ml of glucose oxidase–peroxidase reagent (GOPOD) (Megazyme, Ireland) and quantified with the use of a UV- Vis spectrophotometer (GYNESIS, Thermo Electron Corporation, USA) at 510nm against the blank solution made of 0.1 ml of sodium acetate buffer and 3.0 ml of GOPOD. The average of duplicate absorbance values was recorded. The records were taken at day 1, 7, 14 and 21 during storage at refrigeration temperature (4 °C).
5.2.4.2. **Determination of yoghurt viscosity**

Viscosity was measured as per Djurdjević et al. (2002). A viscometer (Haake Viscometer 6 plus, Thermo Scientific, USA) was used for viscosity measurement. Spindle number 4 was inserted and it was allowed to rotate at 100 rpm in yoghurt (200ml) contained in a glass beaker for 2 minutes. The values were recorded after every 30 seconds and the mean value determined. The measurements were taken at 8°C on day 1, 7, 14 and 21 of cold storage.

5.2.4.3. **Determination of Syneresis in yoghurt**

A method by Goncalvez et al. (2005) was used to determine the syneresis. Yoghurt (30 g) was placed in 50 ml corning test tube centrifuged (nůve/NF 1200R, Turkey) at 400 x g for 10 minutes and the supernatant liquid removed, weighed and expressed as a percentage of the initial yoghurt weight. The measurements were taken on day 1, 7, 14 and 21, respectively.

5.2.4.4. **Determination of yoghurt acidity and pH**

Titratable acidity of fermented yoghurt was determined according to Noh et al. (2013). Yoghurt (10 g) was titrated with 0.1N NaOH using phenolphthalein as an indicator. The titratable acidity was recorded as percentage lactic acid. The pH was determined using a pre-calibrated pH meter (Hanna pH 211 Microprocessor, USA). The measurements were taken on day 1, 7, 14 and 21 respectively.

5.2.4.5. **Determination of yoghurt total solids**

Total solids in yoghurt were determined as per ISO 13580:(2005). Yoghurt (10 g) was put in dry crucible, weighed and kept in the oven (Memmert GmbH, Germany) at 105 °C for 3 hours. The crucibles were again weighed and the total solid expressed as a percentage of the initial yoghurt weight. The measurements were taken on day 1, 7, 14 and 21, respectively.
5.2.4.6. **Enumeration of lactic acid bacteria in yoghurt**

The Lactic Acid Bacteria (LAB) were enumerated on the deMan, Rogosa and Sharpe agar (MRS1.10661.0500, Merck KGaA, Germany) which was incubated anaerobically in gas pack jar at 30°C for 72 hours as per the method described by Shori and Baba (2012).

5.2.4.7. **Sensory evaluation of yoghurt treated with modified cassava starch**

Seventy two (72) panellists evaluated the quality characteristics (colour, odour, taste, mouth feel and overall acceptability) of all yoghurt samples treated with different thickeners using a 9-point hedonic scale where 9= extremely like 8= like very much, 7= like moderately, 6= like slightly, 5= neither like nor dislike, 4= dislike slightly, 3= dislike moderately, 2= dislike very much and 1= dislike extremely. Five yoghurt samples were coded with three digits each and served to the panellists in equal colourless containers. The sensory evaluation was done on day 1, day 7 and day 14 of cold storage only because of the observed reduction in yoghurt quality on day 21 and most of the standards recommend 14 days as shelf life of yoghurt.

5.2.5. **Statistical analysis**

Yoghurt quality parameters were measured in duplicate and the mean value was obtained. Using Gensat 14th Edition software, a two way ANOVA was performed and a multiple comparison test (Duncan test) was done to group the mean values of yoghurt quality parameters. Means were reported different when reported P value was less than 0.05 (P≤0.05).

5.3. **Results**

5.3.1. **Effect of modified cassava starch on resistant starch content of yoghurt**

The proportions of modified cassava starch and the storage time had a significant effect (P≤0.05) on the resistant starch content in yoghurt as shown in Figure 5.1. Resistant starch content in
yoghurt was found to significantly (P≤0.05) vary with the quantity of modified cassava starch used. Yoghurt treated with 1% and 0.5% modified cassava starch had a significantly higher (P≤0.05) resistant starch content; 5.58 g/100g for yoghurt with 1% modified cassava starch and 3.40 g/100g for yoghurt with 0.5% modified cassava starch compared to the control with 1.93 g/100g on dry weight basis. Resistant starch content of stored yoghurt did not significantly (P>0.05) change up to day 7. However, it significantly (P≤0.05) decreased afterward to reach, on day 21, 0.32 g/100g for yoghurt incorporated with 0.1% modified cassava starch, 1.92 g/100g for yoghurt incorporated with 0.5% modified cassava starch and 4.47 g/100g for yoghurt incorporated with 1% modified cassava starch while it reached 1.22 g/100g for the control.

![Resistant starch content of yoghurt treated with modified cassava starch](image)

**Figure 5.1: Resistant starch content of yoghurt treated with modified cassava starch, The bars indicate standard error of the means**

Yoghurt samples to which, 0.1% of modified cassava starch was added had the lowest levels of resistant starch content compared to the other treatments.
5.3.2. Effect of modified cassava starch on yoghurt viscosity

There were significant differences (P≤0.05) in viscosity among yoghurt samples treated with different proportions of modified cassava starch. Viscosity change in stored yoghurt treated with modified cassava starch is illustrated in the Figure 5.2. Significantly higher (P≤0.05) values of yoghurt viscosity were observed as a result of addition of 0.5% and 1% modified cassava starch. There was no significant difference (P>0.05) in viscosity from day 1 to day 14 in yoghurt sample treated with 1% modified cassava starch. Only 1% and 0.5% starch proportions performed better than the control.

A significant decrease (P≤0.05) in viscosity for the yoghurt treated with 1% and 0.5% modified cassava starch was observed from day 14 to day 21 of cold storage dropping from 2650 mPa s to
1138.5 mPa s and from 1997 mPa s to 698 mPa s respectively. Yoghurt treated with 1% cassava starch expressed significantly ($P\leq 0.05$) highest values of viscosity; 2721.5 mPa s, 2721 mPa s, 2650 mPa s and 1034.5 mPa s on days 1, 7, 14 and 21 respectively. Yoghurt treated with 0.5% cassava starch had significantly ($P\leq 0.05$) lower viscosity value (2077.5 mPa s) on day 1 compared to the control (2346.5 mPa s) however on day 14, it was significantly ($P\leq 0.05$) higher (1997.0 mPa s) compared to the control whose viscosity was reduced to 1507.5 mPa s. There was no significant difference ($P>0.05$) observed between the yoghurt with no stabilizer treatment (0%) and the one treated with 0.1% modified cassava starch. A sharp decrease in viscosity was observed from day 7 for yoghurt treated with 0.1% modified cassava starch and yoghurt with no thickener. There was no significant difference ($P>0.05$) in the apparent viscosity of yoghurt at day 21 except for the yoghurt treated with 1 % cassava starch which was significantly ($P\leq 0.05$) higher compared to the rests of yoghurt samples.

5.3.3. Effect of application of modified cassava starch on yoghurt syneresis

The proportions of modified cassava starch and the storage time had significant effect ($P\leq 0.05$) on yoghurt syneresis. Figure 5.3 illustrates the effect of cassava modified starch on yoghurt syneresis. Yoghurt treated with 1% modified cassava starch showed the lowest syneresis where it varied from 23.40% on day 1 to 27.18% on day 14 and then sharply increased up to 34.28% on day 21 while it was 42.14% for the control sample. Yoghurt sample with 0.5% modified cassava starch had comparable ($P>0.05$) trends as the control sample on day 1 and day 21, however, on day 7 and 14, the syneresis was lower ($P\leq 0.05$) for yoghurt with 0.5% modified cassava starch.
Syneresis was significantly higher (P≤0.05) for yoghurt with no thickener (0%) where it ranged from 37.56% to 48.15% while the control sample’s syneresis varied from 23.40% to 42.14%. Yoghurt treated with 1% modified cassava starch had the least syneresis.

5.3.4. Effect of modified cassava starch on yoghurt acidity and pH

Variation in pH of yoghurt treated with modified cassava starch is shown in Table 5.1. There was significant difference (P≤0.05) in yoghurt pH values on day 1 for all yoghurt samples which were all significantly higher than the pH of the control sample. Yoghurt with no thickener had lower pH value (4.74), however, it was significantly higher than that of the control yoghurt sample (4.55). During storage, pH was found to decrease in all yoghurt samples treated with modified cassava starch. The decrease in pH was sharp on day 7 and a slow decrease was observed thereafter. On day 21, there was no significant difference (P>0.05) in pH values for all
samples (4.06-4.09) but all were significantly higher (P≤0.05) than the control sample which had the lowest pH (3.95).

Table 5.1: pH of yoghurt treated with modified cassava starch

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>4.74±0.04^g</td>
<td>4.33±0.01^d</td>
<td>4.26±0.01^c</td>
<td>4.09±0.01^b</td>
</tr>
<tr>
<td>0.1%</td>
<td>4.82±0.01^h</td>
<td>4.37±0.01^d</td>
<td>4.34±0.01^d</td>
<td>4.09±0.02^b</td>
</tr>
<tr>
<td>0.5%</td>
<td>4.83±0.06^h</td>
<td>4.39±0.01^de</td>
<td>4.37±0.06^d</td>
<td>4.07±0.01^b</td>
</tr>
<tr>
<td>1%</td>
<td>4.85±0.02^h</td>
<td>4.43±0.05^e</td>
<td>4.38±0.02^de</td>
<td>4.06±0.01^b</td>
</tr>
<tr>
<td>Control</td>
<td>4.70±0.01^f</td>
<td>4.37±0.03^de</td>
<td>4.25±0.01^c</td>
<td>3.95±0.01^a</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation, Values with different superscripts are significantly different (P≤0.05).

Variation in Titratable acidity of yoghurt treated with modified cassava starch is shown in Figure 5.4. There was no significant difference (P>0.05) for yoghurt samples treated with 1%, 0.5%, 0.1% of modified cassava starch and the yoghurt with no thickener but they were all significantly (P≤0.05) lower than that of the control.
Figure 5.4: Titratable acidity variation of yoghurt incorporated with modified cassava starch. The bars indicate standard error of the means.

During storage period, a gradual increase in yoghurt acidity was observed in yoghurt treated with modified cassava starch in different proportions with the 1% treatment having higher values (0.90%, on day 21) but this value was significantly (P≤0.05) lower to the control sample which had the highest titratable acidity (1.13%) on day 21. The lowest values (0.84%) were observed in yoghurt with no thickener (0%).

5.3.5. Effect of application of modified cassava starch on total solids of yoghurt

Total solids change during storage is shown in Table 5.2. The amount of modified cassava starch and storage time had a significant (P≤0.05) effect on total solids of yoghurt. Addition of modified cassava starch significantly (P≤0.05) increased the total solids in yoghurt with the application of 1% modified cassava starch as a thickener having the highest value (19.26%).
Table 5.2: Total solids of yoghurt treated with modified cassava starch

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total solids (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
</tr>
<tr>
<td>0%</td>
<td>17.08 ±0.17&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>16.89±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.48±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.51±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1</td>
<td>18.43±0.29&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>18.08±0.09&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>17.06±0.11&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>13.67±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5%</td>
<td>18.75±0.06&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>18.44±0.09&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>17.80±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.37±0.17&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>1%</td>
<td>19.26±0.09&lt;sup&gt;i&lt;/sup&gt;</td>
<td>19.10±0.04&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>19.03±0.05&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>18.87±0.12&lt;sup&gt;ghi&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>18.77±0.04&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>18.51±0.02&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>18.15±0.24&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>16.77±0.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation, Values with different superscripts are significantly different (P≤0.05).

Total solids were found to decrease during storage dropping from 17.08% on day 1 to 13.51% on day 21 for yoghurt with no thickener while there was no significant decrease (P>0.05) in the yoghurt treated with 1% modified cassava starch. These values are higher than the values for the control (18.77% to 16.77%). On day 21 of storage, the total solids were not significantly different (P>0.05) in yoghurt with no thickener and the yoghurt with 0.1% of modified cassava starch. However, the values were significantly (P≤0.05) lower compared to the control sample. Yoghurt treated with 1% and 0.5% modified cassava starch had significantly (P≤0.05) higher total solids and were maintained higher during storage compared to the control sample. On day 21, the total solids of yoghurt treated with 1% modified starch was 18.87% and 17.37% for yoghurt treated with 0.5% modified starch while it was 16.77% for the control sample.

5.3.6. Effect of modified cassava starch on Lactic Acid Bacteria count in yoghurt

Lactic acid bacteria counts in stored yoghurt treated with modified cassava starch are presented in Table 5.3. After pasteurization, prior to inoculation, the lactic acid bacteria count was < 3.00
log CFU/g and there was no significant difference (P>0.05) observed among all treatments. On day 1, the lactic acid bacteria count varied from 7.38 log CFU/g for yoghurt treated with 1% modified cassava starch to 7.94 log CFU/g for yoghurt with no thickener. The control sample had a lactic acid bacteria count of 7.84 log CFU/g.

**Table 5.3: Lactic acid bacteria count in stored yoghurt treated with modified cassava starch**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Lactic acid bacteria count (Log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 1</td>
</tr>
<tr>
<td>0%</td>
<td>7.94 ±0.19&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1%</td>
<td>7.71±0.06&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.50%</td>
<td>7.48±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>1%</td>
<td>7.38±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>7.84±0.23&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values are means ± standard deviation, Values with different superscripts are significantly different (P≤0.05).*

Lactic acid bacteria count significantly increased (P≤0.05) during storage period till day 14 reaching 8.41 log CFU/g for yoghurt treated with 1% modified cassava starch to 8.79 log CFU/g for the control. Lactic acid bacteria count decreased on day 21. Yoghurt samples with 1% and 0.5% modified cassava starch showed no significant difference (P>0.05) in lactic acid bacteria count when compared to the control sample with values of 7.66 log CFU/g (control), 7.71 log CFU/g (1%) and 7.93 log CFU/g (0.5%). Yoghurt with no thickener and yoghurt with 0.1% modified cassava starch had a significantly higher (P≤0.05) lactic acid bacteria count; 8.03 log CFU/g and 8.07 log CFU/g respectively on day 21.
5.3.7. Effect of application of modified cassava starch on yoghurt sensory attributes

The scores of sensory properties of yoghurt samples incorporated with modified cassava starch, after day 1, day 7 and day 14 of storage at 4°C, are shown in Table 5.4. There was no significant difference (P>0.05) in scores of colour for all yoghurt samples from day 1 to day 14 where the score varied from 7.03 to 7.73 observed for control on day 7 and for yoghurt treated with 1% modified cassava starch on day 1, respectively. Also there was no significant difference (P>0.05) among the scores of odour which varied from 6.33 to 7.73. The score for taste varied from 6.00 to 7.53 observed for yoghurt containing 1% modified cassava starch on day 14 and yoghurt containing 0.1% proportion of modified cassava starch on day 1 respectively. The score of mouthfeel varied from 6.40 to 7.67 while the score for the overall acceptability varied from 6.07 to 7.47 observed for yoghurt containing 1% proportion of modified cassava starch on day 14 and for yoghurt containing 0.1% cassava starch on day 1 respectively.

Though there was no significant statistical difference (P>0.05) in scores observed after 14 days of storage for colour, odour and mouthfeel, a significantly lower (P≤0.05) scores of taste and overall acceptability was observed for yoghurt sample with 1% proportion of modified cassava starch.
Table 5.4: Sensory attribute scores of yoghurt treated with different levels of modified cassava starch

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Treatments</th>
<th>Colour</th>
<th>Odour</th>
<th>Taste</th>
<th>Mouthfeel</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>0%</td>
<td>7.47±1.30\textsuperscript{a}</td>
<td>6.33±1.80\textsuperscript{a}</td>
<td>7.47±0.74\textsuperscript{de}</td>
<td>6.53±1.36\textsuperscript{ab}</td>
<td>7.13±0.99\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>7.33±0.98 \textsuperscript{a}</td>
<td>7.73±1.22\textsuperscript{c}</td>
<td>7.53±0.99\textsuperscript{e}</td>
<td>6.73±1.39\textsuperscript{ab}</td>
<td>7.47±0.64\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>7.60±1.12\textsuperscript{a}</td>
<td>6.53±2.03\textsuperscript{abc}</td>
<td>6.93±1.16\textsuperscript{bcde}</td>
<td>7.33±1.23\textsuperscript{ab}</td>
<td>6.93±1.10\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>7.73±0.88\textsuperscript{a}</td>
<td>6.93±2.05\textsuperscript{abc}</td>
<td>6.93±1.39\textsuperscript{bcde}</td>
<td>7.20±1.42\textsuperscript{ab}</td>
<td>6.87±1.06\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.27±1.39\textsuperscript{a}</td>
<td>6.73±1.79\textsuperscript{abc}</td>
<td>7.27±0.80\textsuperscript{cde}</td>
<td>7.07±1.10\textsuperscript{ab}</td>
<td>6.87±1.06\textsuperscript{ab}</td>
</tr>
<tr>
<td>Day 7</td>
<td>0%</td>
<td>7.17±1.03\textsuperscript{a}</td>
<td>6.83±0.80\textsuperscript{abc}</td>
<td>7.27±0.89\textsuperscript{de}</td>
<td>6.80±1.92\textsuperscript{ab}</td>
<td>7.13±1.41\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>7.43±0.92\textsuperscript{a}</td>
<td>7.43±1.2\textsuperscript{bc}</td>
<td>6.93±1.25\textsuperscript{bcde}</td>
<td>6.40±1.60\textsuperscript{a}</td>
<td>7.17±1.00\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>7.33±0.99\textsuperscript{a}</td>
<td>7.10±1.10\textsuperscript{abc}</td>
<td>6.97±1.22\textsuperscript{bcde}</td>
<td>7.03±1.31\textsuperscript{ab}</td>
<td>6.93±1.37\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>7.40±1.19\textsuperscript{a}</td>
<td>6.90±1.08\textsuperscript{abc}</td>
<td>6.90±1.13\textsuperscript{bcde}</td>
<td>7.67±0.98\textsuperscript{bc}</td>
<td>6.63±1.2\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.03±1.25\textsuperscript{a}</td>
<td>6.98±1.53\textsuperscript{abc}</td>
<td>7.27±1.06\textsuperscript{de}</td>
<td>7.33±1.06\textsuperscript{ab}</td>
<td>7.23±1.39\textsuperscript{b}</td>
</tr>
<tr>
<td>Day 14</td>
<td>0%</td>
<td>7.07±1.71\textsuperscript{a}</td>
<td>6.80±1.21\textsuperscript{abc}</td>
<td>6.60±0.99\textsuperscript{abcd}</td>
<td>6.67±1.45\textsuperscript{ab}</td>
<td>7.47±0.83\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>7.60±0.74\textsuperscript{a}</td>
<td>6.93±1.53\textsuperscript{abc}</td>
<td>6.33±0.72\textsuperscript{ab}</td>
<td>6.27±1.39\textsuperscript{a}</td>
<td>6.87±1.30\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>7.53±0.90\textsuperscript{a}</td>
<td>7.07±1.36\textsuperscript{abc}</td>
<td>6.33±0.72\textsuperscript{bcde}</td>
<td>7.13±1.45\textsuperscript{ab}</td>
<td>6.47±1.18\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>7.40±0.90\textsuperscript{a}</td>
<td>6.40±1.37\textsuperscript{ab}</td>
<td>6.00±0.85\textsuperscript{a}</td>
<td>7.33±1.05\textsuperscript{ab}</td>
<td>6.07±1.10\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.27±1.22\textsuperscript{a}</td>
<td>6.87±1.36\textsuperscript{abc}</td>
<td>6.47±0.74\textsuperscript{abc}</td>
<td>6.67±1.42\textsuperscript{ab}</td>
<td>6.67±1.18\textsuperscript{ab}</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation. Values with same superscripts in the same column are not significantly different (P>0.05).
5.4. Discussion

5.4.1. Effect of modified cassava starch on resistant starch content of yoghurt

The results show that incorporation of cassava starch enriched with resistant starch increased the content of resistant starch. The resistant starch concentration was proportional to the quantity of resistant starch enriched cassava starch used which explain why yoghurt with 1% modified cassava starch had higher resistant starch content. The current results show that resistant starch in stored yoghurt was slowly fermented throughout the storage time and this could be attributed to the breakdown of resistant starch by lactic acid bacteria and high acidity resulted from lactose breakdown. This indicates that the modified cassava starch could have potentiality to be used as a prebiotic. Prebiotic is food component that escapes 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). Lactic acid bacteria are known to ferment resistant starch (Elkhalifa et al., 2004; Kavita et al., 1998; Ogbo and Okafor, 2015) producing mainly short chain fatty acids even under in vitro conditions (Martín-Bernabé et al., 2011). These short chain fatty acid are essential for bowel bacteria growth, prevention of the colon cancer and reduction of glycaemic index (Sajilata et al. 2006). The current results are lower than those of Aryana et al. (2015) who found the resistant starch content in yoghurt supplemented with High Amylo Maize (HAM-RS2) to vary from 45% to 46% on dry basis. This difference may be attributed to the fact that the resistant starch content of modified cassava starch was lower compared to that of High Amylo Maize (HAM-RS2) as well as to the difference in the botanical origin of the starch.
5.4.2. Effect of modified cassava starch on yoghurt viscosity

The results indicate the ability of modified cassava starch to maintain yoghurt structure during storage. There was a direct correlation between the proportions of modified starch used and the apparent viscosity of yoghurt (Figure 5.2.) indicating that there is a threshold of modified cassava starch necessary to maintain the viscosity of yoghurt. Moreover, the higher the resistant starch (Figure 5.1.) the higher was the viscosity, this may indicate that resistant starch can enhance the viscosity of yoghurt. The high viscosity during yoghurt storage is also associated with rearrangement of protein (Sahan et al., 2008). A significant increase in viscosity was observed as a result of addition of high proportions (1%) of modified cassava starch. This can be attributed to the increase in water retention as well as to the increase of total solids in yoghurt (Table 5.2) as 1% was the highest percentage of modified starch used indicating a relationship between the amount of modified cassava starch used and the yoghurt viscosity. High viscosity is an important technological parameter of yoghurt quality since it enhances its mouthfeel and reduces whey separation (Abdelmoneim and Sherif, 2016). Starch enhance yoghurt viscosity by absorbing water through its granules, in presence of moisture, which considerably increases their size leading to an increased viscosity (Temesgen 2015). It has been also reported that functional properties of carbohydrate dietary fibres like resistant starch can alter the functional properties of foods by absorbing free water which in return increases the viscosity (Elleuch et al. 2011). A decrease in viscosity observed on day 21 (Figure 5.2.) may be attributed to the breakdown of yoghurt components by high acidity observed on day 21 (Figure 5.4) which can further release water and leading to increased water flow and therefore affecting the viscosity (Djurdjević et al. 2002). The increase of yoghurt apparent viscosity as a result of resistant starch addition was also observed by Gustaw et al. (2011) and Rezaei et al. (2015). Noh et al. (2013) found a significant increase in viscosity during storage up to 15 days. They attributed this increase to protein
rearrangement. Domagała et al. (2006) found a decrease in viscosity at day 21 of storage of yoghurt treated with oat-maltodextrin which was attributed to long time of storage.

5.4.3. Effect of modified cassava starch on yoghurt syneresis

Syneresis is the phenomenon of whey separation from the yoghurt gel and it is considered as a technological failure (Amatayakul et al., 2006; Dönmez et al., 2017). A decreasing syneresis with regards to the quantity of modified cassava starch could be attributed to the added starch which increased the total solids (Table 5.2) and hence reduced the water flow in yoghurt. There was a relationship between high resistant starch (Figure 5.1) and reduced syneresis (Figure 5.3.) which indicate the ability of resistant starch to reduce whey separation in yoghurt. This can be related to the fact that resistant starch has a high water binding capacity hence reducing free water in yoghurt by trapping it within its matrix (Abdelmoneim and Sherif, 2016; Sajilata et al., 2006). An increased syneresis observed on day 21 of cold storage (Figure 5.3) could be attributed to the reduction of total solids (Table 5.2) in yoghurt as a result of macromolecule breakdown due to high acidity observed on day 21 (Figure 5.4). Mani-López et al. (2014) reported syneresis of 32.65% and 34.62% in two commercial yoghurts and they support the present findings. Mani-López et al. (2014) attributed this trend to the formation of a three dimension structure as a result of interaction between proteins and stabilizers which increases the firmness hence reducing the syneresis. The current results agree with the findings of Goncalvez et al. (2005) who reported the reduction of yoghurt syneresis by 18% as a result of starch addition as a thickener. The increased syneresis during storage was reported by Küçüköner and Tarakçı (2003); Salvador and Fiszman (2004); Singh and Byars (2009) and Temesgen (2015) and it was attributed to acidic conditions in yoghurt and loose casein micelle network. It has been
reported that modified starch loses its water holding capacity when is kept at low temperature for long time (Abbas et al., 2010).

5.4.4. Effect of modified cassava starch on yoghurt acidity and pH

A gradual development of acidity in yoghurt during storage period as shown in Table 5.4 and Figure 5.2 could be linked to the activity of lactic acid bacteria in yoghurt (Table 5.3) which break down lactose into lactic acid (Singh and Byars, 2009). Furthermore, this acidic conditions breakdown the modified cassava starch into small molecules which in turn can be fermented into organic acids. Yoghurt with corn starch (control) had higher acidity while yoghurt with 1% modified cassava starch had low acidity (Table 5.4 and Figure 5.2) indicating that starch rich n resistant starch is less prone to breakdown than native starch. Menzel (2014) reported that starch is broken-down into small molecules at low pH values. A decreased pH and a corresponding increased titratable acidity during yoghurt storage was also observed by Singh and Byars (2009). Acidity development is important in yoghurt manufacturing since it plays important roles including formation of its structure, enhancement of Lactobacilli bacteria growth and flavour development (Routray and Mishra, 2011). The current results fall in the appropriate pH range for yoghurt which varies from 4.6-4.0 (Temesgen 2015). Similar findings were reported by Ibrahim and Khalifa (2015) who reported the titratable acidity of 0.79% after 21 days of storage of yoghurt incorporated with 1% modified starch and 0.81% for yoghurt incorporated with 0.5% modified corn starch. They attributed this increased acidity to the continuous production of organic acids by lactic acid bacteria during refrigeration storage and to the activity of β-galactosidase at low temperatures as well as to acidity contributed by added thickeners.
5.4.5. **Effect of modified cassava starch on total solids of yoghurt**

The observed increased total solids (Table 5.2) could be attributed to the addition of dry modified cassava starch. High total solids in yoghurt correlated positively with high resistant starch content (Figure 5.1). This increase was due to the addition of dry modified starch rich in resistant starch. Total solids is the most paramount technological property which determine the stability of yoghurt gel structure by preventing poor body and whey off (Yildiz 2010). A decreased in total solids during cold storage period may be attributed to the depletion of lactose and to starch degradation (Ibarhim and Khalifa, 2015). However samples with high resistant starch (Figure 5.1) maintained high total solids during storage (Table 5.2) which may indicate the possible ability of resistant starch to withstand rapid degradation. The currents results substantiate the findings of Muhammad et al. (2009) who reported 18.87% as total solid of yoghurt stored at refrigeration temperature on first day followed by a gradual decrease to 9.96% on day 21 of refrigeration storage. This value on day 21 is lower compared to the findings of the present study and this may be due to the effect of the added modified cassava starch which maintained high the total solids due to its slow breakdown (Goncalvez et al., 2005).

5.4.6. **Effect of modified cassava starch on Lactic acid bacteria count in yoghurt**

The current results indicate that incorporating 1% modified cassava starch to yoghurt affected slightly the growth of lactic acid bacteria (Table 5.3). This can be attributed to increased restriction of water activity necessary for proper growth of lactic acid bacteria (Routray & Mishra 2011) and it could also be evidenced by the fact that the same sample had low titratable acidity (Figure 5.4). The growth of lactic acid bacteria in other samples could be attributed to the continued multiplication of lactic acid bacteria during storage with lactose and added starch serving as substrates as well as to the slow acidification (Figure 5.4) during this period due to the
low temperature at which yoghurt was kept. This temperature (4 °C) does not stop the lactic acid bacteria growth (Freitas et al., 2015). A decrease in lactic acid bacteria count observed on day 21 (Table 5.3) may be related to the high acidity observed in yoghurt on the same day (Figure 5.4) restricting their growth. Other literature reports also indicate that the production of hydrogen peroxide by \textit{Lactobacillus delbrueckii} subsp. \textit{Bulgaricus} can reduce the survival of lactic acid bacteria in yoghurt (Saccaro et al., 2009). The same microorganism was used in the current study as part of starter culture. The survival of lactic acid bacteria in yoghurt at low pH is one of the indicators of potentiality of being a probiotic product (Sharifi, et al., 2017). Gustaw et al. (2011) reported an increase in \textit{Bifidobacterium} sp count of yoghurt treated with 1% resistant starch from 7.1 log CFU/g to 7.5 log CFU/g from day 1 to 14 and a subsequent decrease to 6.9 on day 21 of cold storage and they attributed this to the importance of resistant starch on the growth of lactic acid bacteria when it is applied in the range of 1-3%. The values obtained in the present study are within the range stipulated by FAO/WHO standard which requires the living microorganisms in yoghurt to be greater than $10^7$ CFU/g (FAO/WHO 2002).

5.4.7. \textit{Effect of modified cassava starch on yoghurt sensory attributes}

The current results indicate that modified cassava starch did not influence the colour, odour, and mouthfeel (Table 5.4) since it was applied in small amounts (less than 1%). Yoghurt treated with 1% modified cassava starch had the lowest score for taste and overall acceptability (Table 5.4). This may point out that the addition of more than 1% of modified cassava starch as yoghurt thickener may adversely affect the sensory properties of yoghurt. Goncalvez et al. (2005) reported that there is a correlation between the amount of starch used as a thickener and the organoleptic properties of yoghurt. These findings corroborate those of Okoth et al. (2011) who reported that there was no significant difference in sensory properties among the yoghurt
samples treated with 0%, 0.3% and 0.5% modified corn starch. This confirms that it possible to produce an acceptable yoghurt with modified cassava without using any other stabilizer.

5.5. Conclusions

It is possible to produce acceptable resistant starch enriched yoghurt by incorporating cassava starch rich in resistant starch. Adding of 1% modified cassava starch produces yoghurt rich in resistant starch and it increases the viscosity and total solids of yoghurt and it reduces syneresis and acidity while it does not affect lactic acid bacteria count. Though it affect the taste and acceptability, the 1% proportion of modified cassava starch is recommended for use in processing of resistant starch enriched yoghurt because it favours all other yoghurt quality parameters which increase the yoghurt shelf life.
CHAPTER SIX: GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. General conclusions

There is significant difference among resistant starch content of Rwandese cassava varieties. Variety I92/0057 has the highest dry matter content, starch extraction yield and resistant starch content but lowest total starch and digestible starch

Hydrothermal treatments has a significant influence on the synthesis of resistant starch in cassava starch. Heat moisture treatment of starch from I92/0057 of 24% moisture content at 100°C for 16 hours gives the highest increase resistant starch content.

It is possible to produce acceptable resistant starch enriched yoghurt by incorporating cassava starch rich in resistant starch. Adding of 1% modified cassava starch produces yoghurt rich in resistant starch and it increases the viscosity and total solids of yoghurt and it reduces syneresis and acidity while it does not affect lactic acid bacteria count.

6.2. Recommendations

Interest in resistant starch has grown globally as a result of significant health related advantages. Therefore the following recommendations were elaborated in order to catch up with this growing attention toward resistant starch:

Screening many cassava varieties as well as the other starch sources available in the country for their resistant starch content and to investigate factors affecting the variation of resistant starch in crops
Promoting cassava as source of resistant starch and hydrothermal treatment as a method of boosting resistant starch especially the heat moisture treatment of native starch of 24% moisture content at 100 °C for 16 hours since it showed the ability to increase resistant starch at highest amount.

To local research institutions, continue researches on various methods other than heat treatment of increasing resistant starch in different starch bearing crops and also to explore the possibilities of applying resistant starch in many consumer friendly diets.

Facilitate and encourage researches on health benefits especially in vivo conditions as well as to carry out researches that can demonstrate the actual intake and sources of resistant starch in all categories of populations.
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