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Production of Corn (*Zea mays*) Starch and Cassava (*Manihot esculenta*) Starch and Their Application as Yogurt Stabilizer

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Abstract

The present study evaluated the stabilizing effect of starch produced from corns and cassava on the stability of cow milk yogurt. A sample of both corn and cassava starch was selected and used in the yogurt making as stabilizers. The yogurt samples have been analyzed for their WHC, syneresis and protein content. The yogurt with no added starch has found to have very low WHC and high syneresis compared to other samples. The yogurt made with the addition of corn starch as a stabilizer was highly accepted than the yogurt with cassava starch and the yogurt without starch. The findings from this study provide an alternative to add the value of local corns and cassava.

Keywords

Corn Starch, Cassava Starch, Cow Milk Yogurt, Stabilizer, Syneresis

1. Introduction

Starch is widely used in our daily life from centuries: Egyptians boiled wheat flour paste with diluted vinegar to cement strips of papyrus, while in ancient Chinese documents were first coated with a high fluidity starch to provide resistance to ink penetration, then covered with powdered starch to provide weight and thickness [1] [2].

From a labelling point of view, starches can be categorized mainly into two groups, as either native or modified. Native starches are produced through the extraction of naturally occurring starch from grain or root crops (such as tapioca, rice, corn and potato) and can be used directly in producing certain foods, such as noodles; modified starch is produced from the native starch through either chemical or physical modifications [3]. The modification of starch is carried out to improve its functionality (e.g. its ability to withstand low pH conditions and high temperatures during processing), as native starches are typically not process friendly [1] [3]-[9]. Native starches are considered clean label ingredients, whereas chemically modified starches carry an E number designation and are not recognized as natural [3]. The physical modification of native starches is used to make them as functional as their chemically modified counterparts, thus maintaining the label declaration "native" which gives a commercial advantage. In green leaves and plants in general, starch is formed by condensation polymerization of glucose with the aid of starch-synthesizing enzymes, the used glucose was formed during the process of photosynthesis as it is shown in below equation [3].

$$\underset{\text{water}}{6H_2O} + \underset{\text{carbon dioxide}}{6CO_2} \xrightarrow{\text{Light/Chlorophy11}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{oxygen}}{6O_2}$$

The conversion of glucose into starch can be written as follows:

$$\underset{\text{glucose}}{nC_6H_{12}O_6} \xrightarrow{\text{enzymes}} \underset{\text{starch}}{(C_6H_{12}O_5)n} + \underset{\text{water}}{nH_2O}$$

Starch consists two macromolecules: A highly branched Amylopectin and a linear Amylose (**Figure 1**). Amylopectin is a very large macromolecule with linear chains that contain an average of 20 - 25 linked *α*(1 - 4)D-glucopyranosyl residues, with 5% to 6% of *α*(1 - 6) bonds forming branch points. Amylose on the other hand is the lesser component by weight in normal starches which consists of a single or few long chains of *α*(1 - 4)D-glucose units making it linear [3] [10] [11].

Amylose forms inclusion complexes with iodine and various organic compounds such as butanol, fatty acids, various surfactants, phenols and hydrocarbons, these complexes are essentially insoluble in water, the complex of amylose with iodine gives a characteristic blue color, which is used to establish the presence of amylose-containing starch [3].

Starch functionality depends greatly on the molecular weight, size, and structure of its components, amylose and amylopectin, the greater the amylopectin

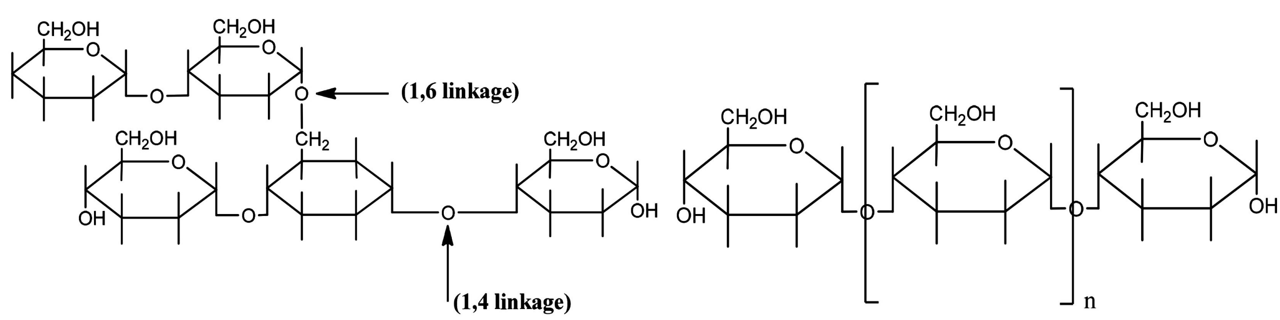

Figure 1. Highly branched Amylopectin (left) and linear structure of Amylose (right) [12].

content, the better the starch quality [11]. Both Amylose and Amylopectin constitute approximately 98% - 99% of the dry weight of starch, botanic source reports that starch chain generally consists of 20% water soluble amylose and up to 80% water insoluble amylopectin by mass depending on the starch source [5] [13]. Amylose contributes to the shear resistance, pasting and gel textural properties of cooked and cooled starches while amylopectin is responsible for the stability, thickening and firmness properties of starch preparations but it does not contribute to gel formation [3] [5] [11] [13].

Starch plays a major role in the food industry, and has been widely used as a thickener, stabilizer, gelling agent, water retention agent and as an adhesive due to its very adaptive physicochemical characteristics [5]. Starch is used in yogurt as thickening and gelling agents to increase its viscosity and to prevent syneresis [14]. The use of starch as stabilizer in dairy products, such as yogurt, is very important for appropriate viscosity, sensory properties, and inhibiting/reducing wheying-off during storage and transportation, as well as boosting the ratio of total solids [15]. Starch is preferred in the yogurt industry, because it is a good thickener and its ability to reduce yogurt flaws by improving texture and make the product more appealing to consumers [15]. Native starch as a powder acquired from plants comprising starch is the best thickening agent and a stabilizer than modified starches which is gotten from native starches as a result of physical, enzymatic or chemical processing methods where wet and dry chemical processes, drum drying and extrusion methods are all used [16]. The present study targeted to extract native starch from two crops identify with a high starch content.

2. Materials and Methods

Corn samples were collected from Byumba markets, Gicumbi District, Rwanda, while cassava samples were collected from Gaseke market, Gicumbi District; the milk used for yogurt making was obtained from Byumba farmers, and the starter culture used for yogurt making was obtained from Alpha Bread Company Ltd. The present study was carried out in the laboratory of Biology of the University of Technology and Arts of Byumba (UTAB), Rwanda.

2.1. Starch Extraction

2.1.1. Corn Starch

The corn starch extraction was carried out following the wet milling procedures [17]. After 48 hours of steeping in acidic solution, the corn samples were grinded in high speed blenders, then centrifuged at 2500 rpm for 10 minutes. The centrifugation was done twice for an efficient starch protein separation. The samples were dried in oven at 40˚C and allowed for further analysis (**Figure 2**). Three corn samples coded CO1, CO2 and CO3 were bought from Byumba market at different dates and from different sellers, the starch produced from these corns were coded COS1, COS2 and COS3.

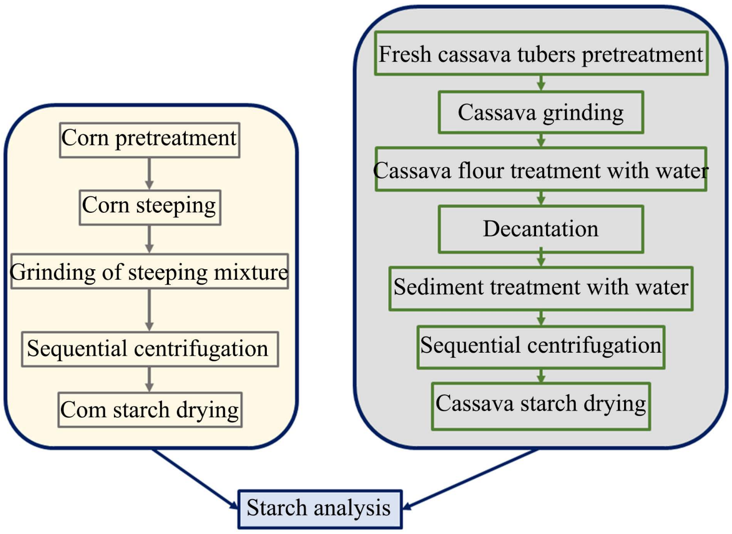

Figure 2. Flowchart corn and cassava starch making.

2.1.2. Cassava Starch

Fresh tubers were washed, peeled, chopped into smaller pieces and then ground into flour using a mortar and pestle. The flour was suspended in ten times its volume of water, stirred for 5 minutes and filtered. The filtrate was allowed to stand for 2 hours for the starch to settle and the top liquid was decanted and discarded. Water was added to the sediment and the mixture was centrifuged at 2500 rpm for 10 minutes. Centrifugation was repeated and after decanting the supernatant, the sediment (starch) was dried (**Figure 2**). Three cassava samples coded CA1, CA2 and CA3 were bought from Gaseke market, Gicumbi district, Rwanda, at different dates and from different sellers, the starch produced from these cassava were coded as CAS1, CAS2 and CAS3.

2.2. Yogurt Processing

The yogurt making was done in three main steps: 1) the preparation of the mix and all corresponding pre-treatment operations such as heat treatment and cooling, during this stage stabilizers are added to the mix, yoghurt is usually stabilized by using various agents including pectin, guargum, carboxymethyl cellulose (CMC), carrageenan, sodium alginate, cornstarch and gelatin [18]; 2) the fermentation process starting after inoculation with the starter culture (*Lactobacillus bulgaricus and Streptococcus thermophilus*); and 3) the yogurt harvesting, post-treatment, and packaging (**Figure 3**) [19]. There are numerous factors which affect the aromatic properties of yogurt including the microbial factors, processing parameters, source of milk, and chemicals and additives used [20].

Three samples of yogurt were made from the present study while the fourth yogurt sample was bought from local market to be used during analysis as a positive control. The samples were coded as follow: YCOS as yogurt with corn starch, YCAS as yogurt with cassava starch, YCT1 as yogurt without starch or negative control sample and YCT2 as yogurt from market or positive control sample.

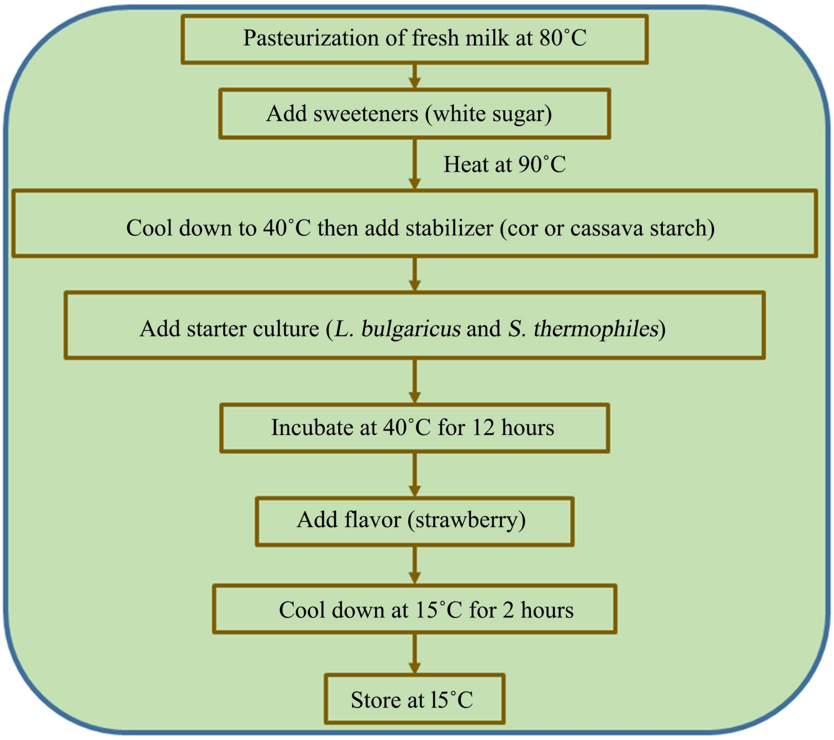

Figure 3. Flowchart for yogurt making.

2.3. Laboratory Analysis

2.3.1. Starch Content

The starch content of corn and cassava samples was determined using acid hydrolysis method [21]. The test samples were dispersed in water and heated in acidic solution to hydrolyze the starch and releasing the sugars. The resulting sugars were then determined by titration with Fehling solution according to the method of Lane Eynon [21].

$$\text{Starch (g/100g)} = \text{\% Total sugar} * 0.9$$

2.3.2. Protein Content

The protein content of the corn and cassava starches was determined by using Kjeldahl method [22]. This method is used to determine the nitrogen content of a given sample and from this the protein content can be obtained.

$$\text{N}(\text{g\%}) = \frac{(\text{Vol } 0.1 \text{ N HCl sample} - \text{Vol } 0.1 \text{ N HCl blank}) * 0.0014 \text{ N HCl} * 100}{\text{Weight sample}}$$

$$\text{Protein (g/100 g)} = \text{\% total nitrogen} \times \text{appropriate nitrogen conversion factor}$$

2.3.3. Moisture Content

The samples were dried in oven to remove all contained water, the moisture content of the samples were obtained as the mass difference of sample before drying and after drying. In this method moisture is referred to the amount of free water and volatile substances that are lost by drying the food under vacuum and controlled temperature. It was expressed in g per 100 g sample [21].

$$\text{Moisture content}(\%) = \frac{(\text{Weight of sample before drying} - \text{Weight of sample after drying}) * 100}{\text{Weight of sample before drying}}$$

2.3.4. Determination of Syneresis

The wheying-off or syneresis of the yogurt was measured using centrifugation (2500 rpm, 25˚C for 10 minutes) method and the results was calculated as percentages [23].

$$\text{Syneresis}(\%) = \frac{\text{Weight of supernatant}}{\text{Weight of sample}} * 100$$

2.3.5. Determination of Yogurt's Water Holding Capacity

Yogurt's ability to hold all or a part of its own water (water holding capacity: WHC) was measured using centrifugation (2500 rpm, 25˚C for 10 minutes) method [23].

$$\text{WHC}(\%) = \frac{\text{Weight of sample} - \text{Weight of supernatant}}{\text{Weight of sample}} * 100$$

2.3.6. Total Microbial Content Determination

The enumeration of total bacteria content in samples followed the procedures of counting the colonies growing in a solid medium after aerobic incubation at 30˚C as identified in ISO4833-2003 [24]. After incubation, the colonies were counted using the colony counting machine.

2.3.7. Sensory Evaluation

A total numbers of 10 panelists were selected to evaluate the quality of the yoghurt samples through sensory evaluation. The qualities assessed were, color, texture, taste and overall acceptability. The yoghurt samples were rated successively on a scale 1 - 9: like extremely = 9, like very much = 8, like moderately = 7, like slightly = 6, neither like nor dislike = 5, dislike slightly = 4, dislike moderately = 3, dislike very much = 2, dislike extremely = 1.

2.4. Statistical Analysis

All laboratory tests were done in triplicates, the statistical analysis was done and the results were expressed as means and standard deviations. We used one-way analysis of variance (ANOVA) to compare means from three replications at the significant level $\alpha = 0.05$. All analysis was performed using MiniTab software version 17.

3. Results and Discussion

This project was based on the production of starch from cassava and corns and studying their effect on the stability of yogurt. Three samples of corn starch and three of cassava starch were produced and analyzed for their starch content, moisture content and protein content. Three samples of yogurt were produced and one sample of yogurt from local market was used as a positive control, the WHC, syneresis and protein contents of all four yogurt samples were analyzed. Each parameter was analyzed in triplicate for each sample.

3.1. Starch Produced from Cassava and Corn

A total of six starch samples were produced and analyzed for their proximate compositions, the samples were coded as follow: COS1, COS2 and COS3 as corn starches, CAS1, CAS2 and CAS3 as cassava starches. All six samples were analyzed for their moisture, protein and starch contents, the results of the analysis are recorded in the **Table 1** below. The moisture content of all six samples are in accordance with findings reported by [3] that the moisture content of starch should range from 10% to 20%. The highest moisture content was found to be 13.83% for CAS1 while the lowest found was 12.33% for CAS2. McDonagh, 2012 further reported that cereal starches should contain 0.2% - 0.4% proteins, the findings from this study align with the reported data where the lowest protein content of three corn starch samples was 0.26% and the highest was 0.34%; while cassava samples have the lowest protein content in the range of 0.05%. The starch content of all six samples are in accordance with findings reported by different research groups that starch content of cereals and tubers are lower than 95% - 98% [5] [13], the findings from this study revealed that the lowest starch content was found in corn, 83.16% for COS1 whereas the highest starch content was found in cassava, 85.91% for CAS3. This variation might be due to the different climatic conditions where crops were grown, the variety of crops as well as analysis methods and conditions.

For all six samples statistically have the same moisture content which also falls in the acceptable range of starch granule's moisture content as previously described by McDonagh, 2012. The samples COS1 and COS3 were said to have the same protein content while the sample COS2 is different from other corn

Table 1. Chemical composition of cassava and corn starches.

Samples	Moisture content (%)	Protein content (%)	Starch content (%)
COS1	13.16 ± 0.28^{a}	0.26 ± 0.01^{b}	83.16 ± 0.05^{ab}
COS2	13.90 ± 0.85^{a}	0.34 ± 0.02^{a}	84.44 ± 0.40^{bc}
COS3	13.40 ± 0.53^{a}	0.30 ± 0.03^{b}	85.16 ± 0.07^{ab}
CAS1	13.83 ± 0.76^{a}	0.05 ± 0.01^{c}	84.81 ± 0.27^{ab}
CAS2	12.33 ± 0.57^{a}	0.04 ± 0.02^{c}	84.59 ± 1.15^{ab}
CAS3	13.40 ± 0.69^{a}	0.05 ± 0.02^{c}	85.91 ± 0.19^{a}

Values are means ± SD of 3 replications. Treatment means followed by different letters in the same column have a significant difference according to Tukey's test ($\alpha = 0.05$). The sample codes mean: COS1, corn starch sample 1; COS2, corn starch sample 2; COS3, corn starch sample 3; CAS1, cassava starch sample 1; CAS2, cassava starch sample 2; and CAS3, cassava starch sample 3.

starches in protein content, this might be due to the corn steeping conditions, protein-starch separation by centrifugation or due to the variety of corn samples. The samples CAS1, CAS2 and CAS3 have the same amount of proteins, however, they have the smaller amount of proteins compared to samples COS1, COS2 and COS3, this difference is from the fact that corns normally have the higher protein content than cassavas. The starch content of samples COS1, COS3, CAS1, CAS2 is the same, the increased amount of starch content in samples COS2 and CAS3 might be caused by the variety of samples and efficiency of starch extraction.

3.2. Yogurt Analysis

The results of analysis of all four yogurt samples are recorded in the **Table 2** below. The protein content of the samples YCOS, YCAS, YCT1 and YCT2 have been found to in the range from 4.18% to 4.77%. The results showed that sample YCAS had the lowest protein content of 4.18% while the sample YCT2 had the highest with 4.77%. According to Codex standards, the yoghurt sample should contain not less than 2.70% protein content, in addition, the study of [25] has reported the protein content of yogurt to be 3.52%. Furthermore, the percentage of protein content of cow milk yoghurt sample has previously been reported to be 4.83% from previous studies [26]. Thus, the results from the present study are in accordance with previous researches. This in turn revealed that the addition of a little amount of a stabilizer does not affect the nutritional profile of the yogurt.

The findings revealed that the lowest WHC was 65.37% for YCT1 whereas the highest was 74.92% for YCT2. The lowest syneresis was 25.08% for YCT2 and the highest was 34.63% for YCT1. The syneresis for YCOS and YCAS showed no significancy difference with sample YCT2, this confirmed the quality of corn and cassava starch extracted from the present study as stabilizing agents. Both WHC and syneresis are indicators of yogurt stability, the more the WHC of yogurt the

Table 2. Protein content, bacterial load and stability of cow milk yogurt supplemented with cassava and corn starches.

Samples	WHC (%)	Syneresis (%)	Protein (%)	Total Bacteria content (log cfu/ml)
YCOS	74.53 ± 0.91^{a}	25.39 ± 0.79^{b}	4.19 ± 0.02^{b}	4.46 ± 1.53^{a}
YCAS	73.12 ± 2.45^{a}	26.88 ± 2.45^{b}	4.18 ± 0.015^{b}	4.45 ± 1.53^{a}
YCT1	65.37 ± 1.24^{b}	34.63 ± 1.24^{a}	4.18 ± 0.015^{b}	4.45 ± 2.52^{a}
YCT2	74.92 ± 0.42^{a}	25.07 ± 0.41^{b}	4.77 ± 0.02^{a}	4.37 ± 1.53^{b}

Values are means ± SD of 3 replications. Treatment means followed by different letters in the same column have a significant difference according to Tukey's test (α = 0.05). The sample codes mean: YCOS, yogurt supplemented with corn starch; YCAS, yogurt supplemented with cassava starch; YCT1, yogurt with no starch; and YCT2, yogurt from market.

lower its syneresis rate. The sample YCT1 had been found to have the lowest WHC of 65.37% and the highest syneresis of 34.63%, upon transportation and other kinds of disturbance, this yogurt tends to have an increased wheying off rate. The total plate count technique was used to enumerate the total number of bacteria in yogurt samples, the lowest number of bacteria was 4.37 logcfu/ml for YCT2 whereas the highest was 4.46 logcfu/ml for YCOS. The findings are in line with results from previous studies where the where yogurt have been found to have the microbial content of 4.43 logcfu/ml [26].

In samples YCOS, YCAS and YCT2, the WHC and syneresis are the same while the sample YCT1 had the smaller WHC and the higher syneresis, this is the result of using stabilizers in yogurt making. The sample YCT1 will lose its water upon transportation due to the fact that it was made with no added stabilizer. These results showed that the corn starch and cassava starch produced from this research increases the stability of yogurt, where the corn starch showed promising result without significance different when using commercial stabilizers (positive control yogurt, YCT2).

The protein content of samples YCOS, YCAS and YCT1 were the same, this proved that the addition of a little amount of a stabilizer do not affect the nutritional profile of the yogurt, however, the protein content of the sample YCT2 is higher, this was believed to be caused by the pretreatment operations made at the dairy and the area of raw milk production. Thus, the corn starch from this study could be tried at industrial level in different dairy and we believe that with high pretreatment level found in dairy this corn starch could provide promising result and help reducing the dairy stabilizer cost by using local starch.

Statistically, the samples YCOS, YCAS and YCT1 have the same bacterial content while the sample YCT2 had the lowest bacteria content compared to other three samples, this variation might be caused by the milk treatment prior to yogurt production, and from the fact that in the dairy the hygiene and sanitation of the processing area is given high priority and the aseptic packaging in dairy help in reducing the bacteria count.

3.3. Sensory Evaluation

The result of the organoleptic test conducted by a total number of ten panelists comprising of both male and female is as shown in **Table 3**. Yoghurt sample from market was rated the best in terms of over-all acceptability followed by yoghurt sample made by addition of corn starch, cassava starch and no starch; which was expected according to the results showed by analysis of these yogurt samples. All samples were served at 10˚C - 15˚C using plastic cups. The panelists compared the samples on the basis of color, texture, taste, and over-all acceptability using the 1 - 9 hedonic scale. Water was also available for the panelist to rinse their mouths after tasting each sample.

The difference in colors among the samples may be caused by the panelist's preferences, both samples YCOS, YCAS and YCT2 were highly appreciated by the panelists for their texture while the sample YCT1 was given the lowest score

Table 3. Sensory evaluation of the cow milk yogurt supplemented with corn and cassava starches.

Samples	Color	Texture	Taste	Overall acceptability
YCOS	7.50 ± 1.08[ab]	8.10 ± 0.74[a]	8.10 ± 0.734[a]	8.00 ± 0.82[a]
YCAS	7.90 ± 0.99[a]	7.50 ± 1.08[a]	7.60 ± 0.69[a]	6.80 ± 0.92[b]
YCT1	6.30 ± 1.16[b]	4.60 ± 1.17[b]	7.10 ± 1.28[a]	5.80 ± 1.03[b]
YCT2	7.60 ± 1.71[ab]	7.90 ± 0.99[a]	8.10 ± 1.28[a]	8.30 ± 0.82[a]

Values are means ± SD of 3 replications. Treatment means followed by different letters in the same column have a significant difference according to Tukey's test ($\alpha = 0.05$). The sample codes mean: YCOS, yogurt supplemented with corn starch; YCAS, yogurt supplemented with cassava starch; YCT1, yogurt with no starch; and YCT2, yogurt from market.

for its texture, this is believed to be caused by the fact that it was made without the addition of any stabilizer. The results of the sensory evaluation has shown that all samples were of the same taste, this means that the addition of stabilizers do not have an impact on the taste of the yogurt. The sample YCT1 had the lowest score on overall acceptance while the sample YCT2 had the highest overall score, followed by YCOS which continue emphasize the acceptability of the corn starch from the present study.

4. Conclusion

It has been observed that the addition of stabilizers during yogurt manufacturing affect its stability and acceptability on the market. The addition of starch as a stabilizer in yogurt do not affect its nutritional values, it only prevent the demixing of milk components which improve the yogurt's ability to withstand transportation damage which is indicated by wheying off. There has been no significance difference in the syneresis and WHC of yogurts made by addition of corn starch and cassava starch, however, corn starch has been found to increase the ability of yogurt to hold its water. From the results of sensory evaluation, it has been found that yogurt made with the addition of corn starch as a stabilizer was highly accepted than the yogurt with cassava starch and the yogurt without starch. Thus, the starch produced during this study has shown a significance impact on the yogurt stability and the corn starch could be tried at industrial level in Rwanda in order to reduce the cost when using imported stabilizers.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

The article does not contain any studies with human participants performed by any of the authors.

Data Availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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