



Effect of Resistant Cassava Starch on Quality Parameters and Sensory Attributes of Yoghurt

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Abstract

Resistant starch is known to impart a number of health benefits to consumers. It is therefore desirable to increase the content of resistant starch in popular foods such as yoghurt. The current research investigated the effect of cassava resistant starch synthesized by heat-moisture treatment of starch from I92/0057 cassava variety on physico-chemical properties and sensory attributes of yoghurt. Cassava starch rich in resistant starch was incorporated into yoghurt in the proportions of 0, 0.1%, 0.5% and 1%. Corn starch (0.6%) was used as control. Yoghurt was stored at 4 °C for 21 days and the effect of starch modification on resistant starch content, viscosity, syneresis, total solids, acidity, lactic acid bacteria count and sensory properties were determined on weekly basis. Applying cassava starch rich in resistant starch into yoghurt in the proportions of 0.5% and 1% had significantly higher ($p \leq 0.05$) resistant starch content of yoghurt 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. There was a significant correlation ($p \leq 0.05$) between resistant starch concentration and the physico-chemical properties of yoghurt. Yoghurt treated with 1% resistant starch enriched cassava starch had the highest viscosity during cold storage which was determined as 2721.5 mPa s, mPa s, 2650.0 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%). Addition of cassava starch rich in resistant starch significantly increased ($p \leq 0.05$) the total solids content of yoghurt but did not significantly ($P > 0.05$) change the sensory properties of yoghurt. The application of 1% of resistant starch enriched cassava starch as yoghurt thickener produces significant quantity of resistant starch in yoghurt with acceptable sensory and physico-chemical properties.



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Introduction

Resistant starch is a dietary carbohydrate which resists enzymatic digestion and is fermented in the colon by gut microflora into short chain fatty acids like acetic, propionic and butyric acids that are known to prevent colon diseases^{1,2}. The current technology of milling and food processing has strongly reduced dietary intake of resistant starch in both developed and developing countries³. Increase in prevalence of obesity, colorectal cancer, diabetes and other gastrointestinal diseases has led to the idea of incorporating resistant starch into different types of food especially those preferred by the modern society such as cheese, ice cream, yoghurt, milk, bread, corn flakes, cakes, muffins, pasta and batter⁴⁻⁷ 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^{8,9}. With the current controversy around corn gluten on its possible effect on gluten sensitive consumers due to the similarity between corn peptides and wheat gluten peptides¹⁰, cassava dietary starch appears to be the safe ingredient to fortify dairy products. Some technologies of starch modification like heat-moisture treatment have been reported to increase the resistant starch content¹¹, and this dietary starch is natural, flavourless, white, composed of small size particles and has high gelatinization temperature, good extrusion qualities and low water holding capacity^{4,12}.

Thickeners are normally applied in yoghurt in order to improve its texture which is an important yoghurt quality parameter¹³ and cassava dietary starch is preferred for this purpose due to its high purity, neutral flavour, high viscosity and good solubility and swelling capacity^{14,15}. Resistant starch has been used by Aryana *et al.*,¹⁶ to enrich yoghurt and they have reported that yoghurt enrichment with resistant starch produced an acceptable yoghurt and positive clinical response on weight loss was observed for adolescents. However, many other studies on incorporation of resistant starch into yoghurt have used industrially produced resistant starch which is quite expensive and rarely available in developing countries. In addition, these studies put emphasis on clinical aspects of resistant starch and not on its

effect on physico-chemical parameters of yoghurt. In the current study, natural resistant starch was synthesised from cassava, an abundantly available crop in Rwanda, and was incorporated in yoghurt as an alternative cost effective thickening agent. Yoghurt is a popular readily available product in Rwanda as a result of increased milk production due to government programs such as 'One cow per poor family', introduction of new cattle breeds, artificial insemination and active diseases eradication¹⁷, and milk production is projected to increase by 13% annually¹⁸. Yoghurt production in Rwanda relies on imported and expensive corn starch as a thickener. The use of locally produced cassava starch may cut down the production cost and increase cassava utilization. However, no studies on suitability of cassava varieties grown in Rwanda for production of resistant starch for use as a yoghurt thickener have been conducted so far. Therefore, the current study was designed to investigate the technological potential of increasing cassava resistant starch by hydrothermal treatments and to assess the effect of using cassava resistant starch as a thickening agent on yoghurt physico-chemical properties.

Materials and Methods

Raw Materials

Low fat, pasteurized and homogenized milk (Inyange industries, Rwanda) was purchased from a shop and transported at low temperature in a cool box to University of Rwanda laboratory for yoghurt processing. Cassava starch was extracted from variety I92/0057 collected from Rwanda Agriculture Board (RAB) research station of Muhanga. Variety I92/0057, introduced in Rwanda in 2006 and cultivated in the medium altitude of central plateau of Rwanda, was chosen due to its high dry matter content and resistance to disease^{19,20}. Corn starch (Tirupati, India) was purchased from a shop and used as the control sample.

Resistant Starch Production

Starch was extracted by the wet method described by Benesi *et al.*,²¹ with slight modification. Cassava roots were peeled, chopped and crushed in a blender (Aardee ARMG 550, India) for starch extraction. 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 two hours and the top fluid was

discarded. This process was repeated three times until clear water was observed. Sediment starch was freeze dried (Lyotrap, LTE scientific, Great Britain) for 4 days to attain a moisture content of 12.0%. In order to increase the resistant starch content, heat moisture treatment was used as per Franco *et al.*,²². Moisture content of the extracted starch was increased up to 24% by addition of the suitable quantity of distilled water, thoroughly mixing and keeping overnight for moisture stabilization. The starch was hermetically sealed in glass jars and heated in an oven (Mettler GmbH, Germany) at 100 °C for 16 hours. Resistant starch content determined after heat-moisture treatment was 42.53 g/100g of starch.

Research Design

Resistant starch enriched 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% and yoghurt physico-chemical parameters were determined on day 1, 7, 14 and 21 of storage at 4 °C. Sensory evaluation was conducted on day 1, 7 and 14. The effect of resistant starch enriched 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. Two replications were done and the mean values recorded.

Yoghurt Manufacturing

Yoghurt was processed using the method by Goncalvez *et al.*,¹³. Low fat milk (Inyange industries, Rwanda), 1.5% fat and 11.0% (w/w) total solids, was treated with resistant starch enriched cassava starch in three proportions: 0%, 0.5% and 1% of the initial milk, thoroughly mixed and pasteurized 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) then incubated at 42 °C for four hours. The fermented 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) was used as a control.

Determination of Resistant Starch Levels in Yoghurt

Levels of resistant starch in yoghurt treated with modified starch were determined as per AOAC Method 2002.0223. Yoghurt (30 g) was first centrifuged (NF1200R, Nuve, 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 (Mettler 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%) (Schaur, Spain) and resistant starch was collected as pellet by centrifugation (nuve, 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 measurements were taken at day 1, 7, 14 and 21 during storage at refrigeration temperature (4 °C).

Determination of Yoghurt Apparent Viscosity

Viscosity was measured as per Djurdjevic *et al.*,²⁴ 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 two minutes. The values were recorded after every 30 seconds and the mean value determined. The measurements were taken on day 1, 7, 14 and 21 of cold storage at 4 °C.

Determination of Syneresis in Yoghurt

The method by Goncalvez *et al.*,¹³ was used. Yoghurt (30 g) was placed in 50 ml corning test tube, centrifuged (nUve/NF 1200R, Turkey) at 400 xg 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.

Determination of Yoghurt Acidity and pH

Titrateable acidity of fermented yoghurt was determined according to Noh *et al.*,²⁵. Yoghurt (10g) was titrated against 0.1N NaOH using phenolphthalein as an indicator. The titrateable 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.

Determination of Yoghurt Total Solids

Total solids in yoghurt were determined as per ISO 13580²⁶. Yoghurt (10 g) was put in a 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.

Enumeration of Lactic Acid Bacteria in Yoghurt

The Lactic acid bacteria were enumerated on the deMan, Rogosa and Sharpe agar (MRS1.10661.0500, Merck KGaA, Germany) which was incubated anaerobically in an airtight plastic anaerobic incubation box (ThermoFisher Scientific, USA) at 30 °C for 72 hours as per the method described by Shori and Baba 27.

Determination of Texture Properties of Yoghurt

Yoghurt instrumental texture was analysed as per Joon *et al.*,²⁸. Texture analyser (TA.XT Plus, Stable Microsystem, Surry, UK) was used to analyse the firmness, cohesiveness, consistency and index of viscosity. The texture analyser with a 50 kg load cell was run in back extrusion and it was set as follows: Pre-test speed, 1.0 mm/s; test speed, 1.0 mm/s post-test speed 1.0 mm/s; trigger force, 10.0 g and the distance was 30 mm. The tests were carried out in the original containers of 150 ml at room temperature.

Sensory Evaluation of Yoghurt Treated With Modified Cassava Starch

Seventy five (75) panellists evaluated the sensory characteristics (colour, smell, 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. The five yoghurt samples were each coded with three digits (randomly assigned) and served to the panellists in similar colourless containers. The sensory evaluation was done only on day 1, day 7 and day 14 of cold storage because of the observed reduction in yoghurt quality on day 21 and also because most of the standards recommend 14 days as shelf life of yoghurt.

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 compare the mean values of quality parameters. Means were reported different when reported p value was less than or equal to 0.05 ($p \leq 0.05$). Correlation analysis was done in Excel spreadsheet.

Results

Effect of Resistant Starch Enriched Cassava Starch on Resistant Starch Content of Yoghurt

The addition of resistant starch enriched cassava starch and the storage time had a significant effect ($p \leq 0.05$) on the resistant starch content in yoghurt as shown in Table 1. Resistant starch content in yoghurt was found to significantly ($p \leq 0.05$) vary according to the quantity of resistant starch enriched cassava starch used. On day 1 yoghurt with 1% resistant starch enriched cassava starch had significantly higher resistant starch content (5.58 g/100g) compared to the other samples. The control had a resistant starch content of 1.93g/100g on dry weight basis. From day 14 onward, resistant starch concentrations significantly ($p \leq 0.05$) decreased in all treatments. On day 21, resistant starch was 0.32g/100g for yoghurt with 0.1% modified cassava starch, 1.92g/100g for yoghurt with 0.5% resistant starch enriched cassava starch and 4.47g/100 g for yoghurt with 1% resistant starch enriched cassava starch while it reached 1.22g/100g for the control.

Table 1: Resistant starch content of yoghurt treated with modified cassava starch

Treatments	Resistant starch (g/100g)			
	Day 1	Day 7	Day 14	Day 21
0%	-	-	-	-
0.1	0.70±0.06 ^a	0.66±0.01 ^a	0.63±0.08 ^a	0.32±0.08 ^a
0.5%	3.40±0.13 ^e	3.10±0.12 ^e	2.62±0.22 ^d	1.92±0.26 ^c
1%	5.58±0.23 ^g	5.44±0.22 ^g	4.88±0.28 ^f	4.47±0.44 ^f
Control	1.93±0.15 ^c	1.62±0.17 ^{bc}	1.41±0.05 ^b	1.22±0.08 ^b
		F pr. (9, 16, 5%)		
Treatments	<0.001			
Storage time	<0.001			
Treatment x Storage time	0.043			

Values are mean ± standard deviation, Values with different superscripts are significantly different ($p \leq 0.05$)

Effect of Resistant Starch Enriched Cassava Starch on Yoghurt Viscosity

Viscosity change in stored yoghurt treated with resistant starch enriched cassava starch is shown in Table 2. There were significant differences ($p \leq 0.05$) in viscosity among the yoghurt samples treated with different proportions of modified cassava starch. Storage time had also a significant effect ($p \leq 0.05$) on yoghurt apparent viscosity. A significantly higher ($p \leq 0.05$) viscosity was 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. The same trend was observed in yoghurt treated with 0.5% modified cassava starch. A significant decrease ($p \leq 0.05$) in viscosity for yoghurt treated with 1% and 0.5% resistant starch enriched cassava starch was observed from day 14 to day 21 of cold storage dropping from 2650 mPa s to 1138.5 mPa s.

Table 2: Change in viscosity of yoghurt treated with different proportions of modified cassava starch

Treatments	Apparent viscosity (mPa s.)			
	Day 1	Day 7	Day 14	Day 21
0%	1805.00±35.36 ^g	1716.00±50.91 ^f	707.50±24.74 ^{abc}	609.50±6.36 ^a
0.1	1866.25±37.12 ^{gh}	1839.50±43.13 ^g	738.50±36.06 ^{bc}	673.00±21.20 ^{ab}
0.50%	2077.50±88.38 ^l	2115.00±49.50 ^j	1947.00±9.89 ^h	781.50±33.23 ^c
1%	2721.50±17.67 ⁱ	2721.00±29.70 ⁱ	2650.00±28.28 ^k	1138.50±94.05 ^d
Control	2346.50±47.37 ^j	2337.50±61.51 ^j	1507.50±95.45 ^e	698.00±5.65 ^{abc}
		F pr. (12, 20, 5%)		
Treatments	<0.001			
Storage time	<0.001			
Treatment x Storage time	<0.001			

Values are mean ± standard deviation, Values with different superscripts are significantly different ($p \leq 0.05$)

During storage, the viscosity of yoghurt treated with 1% resistant starch enriched cassava starch was significantly higher ($p \leq 0.05$) than those of the other treatments, being 2721.5 mPa s, 2721 mPa s, 2650 mPa s and 1034.5 mPa s on day 1, 7, 14 and 21 respectively.

Effect Resistant Starch Enriched Cassava Starch on Yoghurt Syneresis

Table 3 shows the effect of cassava modified starch on yoghurt syneresis. The proportions of resistant

starch enriched cassava starch and the storage time had significant effect ($p \leq 0.05$) on yoghurt syneresis. Yoghurt treated with 1% resistant starch enriched cassava starch showed the lowest syneresis varying from 23.40% on day 1 to 27.18% on day 14 and then sharply increasing to 34.28% on day 21.

Yoghurt with no stabilizer treatment (0%) and yoghurt treated with 0.1% resistant starch enriched cassava starch had significantly higher ($p \leq 0.05$) syneresis compared to the control sample.

Table 3: Syneresis of yoghurt treated with modified cassava starch

Treatments	Syneresis (%)			
	Day 1	Day 7	Day 14	Day 21
0%	37.56±2.07 ^{de}	42.32±3.25 ^{fg}	44.97±0.31 ^g	48.15±0.92 ^h
0.1	36.76±3.05 ^{de}	40.17±1.47 ^{ef}	44.00±1.11 ^g	44.73±0.40 ^g
0.50%	24.76±1.23 ^{ab}	30.02±0.11 ^c	37.35±0.84 ^{de}	42.39±0.53 ^{fg}
1%	22.25±0.77 ^a	25.14±0.96 ^{ab}	27.18±0.45 ^{bc}	34.28±0.93 ^d
Control	23.40±1.76 ^a	35.06±2.61 ^d	39.77±1.19 ^{ef}	42.14±0.67 ^{fg}
	F pr. (12, 20, 5%)			
Treatments	<0.001			
Storage time	<0.001			
Treatment x Storage time	<0.001			

Values are mean ± standard deviation, Values with different superscripts are significantly different ($p \leq 0.05$)

Effect of Resistant Starch Enriched Cassava Starch on Yoghurt Acidity and pH

Variation in pH and titratable acidity of yoghurt treated with resistant starch enriched cassava starch is shown in Table 4. Both modified starch content and storage time had significant ($p \leq 0.05$) effects on yoghurt pH and titratable acidity. However, their interaction did not significantly affect the titratable acidity ($p > 0.005$). On day 1, yoghurt with no starch incorporation had the highest titratable acidity (0.66%). However, this value was lower than that of the control sample (0.89%). During storage, a gradual increase in yoghurt acidity was observed in yoghurt treated with resistant starch enriched cassava starch in different proportions. On day 21,

yoghurt with 1% treatment had the highest titratable acidity value (0.90%) but this value was significantly ($P \leq 0.05$) lower than that of the control sample (1.13%). The lowest values of titratable acidity (0.84%) were observed in yoghurt with no thickener (0%) and in yoghurt with 0.1% thickener. During storage the pH of yoghurt 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 for all treatments (4.06-4.09), however they were all significantly higher ($P \leq 0.05$) than the control sample pH (3.95).

Table 4: Titratable acidity and pH of yoghurt treated with modified cassava starch

Treat-ments	Day 1		Day 7		Day 14		Day 21	
	pH	TA (%)	pH	TA(%)	pH	TA (%)	pH	TA(%)
0%	4.74±0.04g	0.66±0.04a	4.33±0.01d	0.73±0.02b	4.26±0.01c	0.83±0.01cde	4.09±0.01b	0.84±0.04cdef
0.1%	4.82±0.01h	0.64±0.01a	4.37±0.01d	0.72±0.01b	4.34±0.01d	0.82±0.01cd	4.09±0.02b	0.84±0.02cdef
0.5%	4.83±0.06h	0.64±0.03a	4.39±0.01de	0.67±0.02ab	4.37±0.06d	0.80±0.01c	4.07±0.01b	0.87±0.02def
1%	4.85±0.02h	0.62±0.02a	4.43±0.05e	0.65±0.01a	4.38±0.02de	0.79±0.02c	4.06±0.01b	0.90±0.01f
Control	4.54±0.01f	0.89±0.03ef	4.37±0.03de	1.08±0.04g	4.25±0.01c	1.13±0.02g	3.95±0.01a	1.13±0.01g

T.A: Titratable acidity, Values are mean ± standard deviation, Values with different superscripts in the same column are significantly different (p≤0.05)

Effect Resistant Starch Enriched Cassava Starch on Total Solids Content of Yoghurt

Total solids content change during storage is shown in Table 5. The amount of resistant starch enriched cassava starch and storage time had significant (p≤0.05) effects on total solids content of yoghurt. Addition of resistant starch enriched cassava starch significantly (p≤0.05) increased the total solids in yoghurt with the application of 1% resistant starch

enriched cassava starch as a thickener having the highest value (19.26%).

Total solids were found to decrease during storage dropping from 17.08% on day 1 to 13.51% on day 21 in yoghurt with no thickener. The decrease was not significant (p>0.05) for yoghurt treated with 1% thickener.

Table 5: ANOVA table of the effect of treatment and storage time on pH and titratable acidity of yoghurt treated with modified cassava starch

Source of variation	d.f.	s.s.		m.s.		v.r.		F pr.	
		pH	TTA	pH	TTA	pH	TA	pH	TTA
Treatments	4	0.12	0.6	0.03	0.15	49.79	204.2	<.001	<.001
storage time	3	2.54	0.31	0.84	0.1	1403.25	143.1	<.001	<.001
Treatments x storage time	12	0.07	0.03	0.005	0.003	9.66	3.57	<.001	0.006
Residual	20	0.01	0.01	0.0006	0.0007				
Total	39	2.74	0.97						

T.A: Titratable acidity, d.f: Degree of freedom, s.s: Sum of Square, m.s: Mean sum of Square, v.r: Variance Ratio

Effect of Resistant Starch Enriched Cassava Starch on Lactic Acid Bacteria Count of Yoghurt

Lactic acid bacteria counts in stored yoghurt treated with resistant starch enriched cassava starch are presented in Table 6. After pasteurization, prior to inoculation, the lactic acid bacteria count was less than 3.00 log cfu/g for all treatments.

Lactic acid bacteria count significantly increased (p≤0.05) during storage period till day 14 with the lowest final count being 8.41 logcfu/g (for 1%) and the highest final count being 8.79log cfu/g (control). There was no significant difference (p>0.05) in lactic acid bacteria count among the treatments on day 21(7.71-8.07 logcfu/g) but they were all higher than that of the control (7.66 logcfu/g).

Table 6: Total solids of yoghurt treated with modified cassava starch

Total solids (%)				
Treatments	Day 1	Day 7	Day 14	Day 21
0%	17.08 ±0.17 ^{cd}	16.89±0.01 ^c	15.48±0.08 ^b	13.51±0.14 ^a
0.1	18.43±0.29 ^{fg}	18.08±0.09 ^{ef}	17.06±0.11 ^{cd}	13.67±0.19 ^a
0.5%	18.75±0.06 ^{gh}	18.44±0.09 ^{fg}	17.80±0.16 ^e	17.37±0.17 ^d
1%	19.26±0.09 ⁱ	19.10±0.04 ^{hi}	19.03±0.05 ^{hi}	18.87±0.12 ^{ghi}
Control	18.77±0.04 ^{gh}	18.51±0.02 ^{fg}	18.15±0.24 ^{ef}	16.77±0.27 ^c
		F pr. (12, 20, 5%)		
Treatments	<0.001			
Storage time	<0.001			
Treatment x Storage time	<0.001			

Values are means ± standard deviation, Values with different superscripts are significantly different ($p \leq 0.05$).

Table 7: Lactic acid bacteria count in stored yoghurt treated with modified cassava starch

Lactic acid bacteria count (Logcfu/g)				
Treatments	Day 1	Day 7	Day 14	Day 21
0%	7.94 ±0.19 ^{cd}	8.34±0.19 ^{def}	8.58±1.30 ^{fghi}	8.03±1.30 ^{cd}
0.1	7.71±0.06 ^{abc}	8.22±0.19 ^{cde}	8.58±0.10 ^{fghi}	8.07±1.3 ^{cd}
0.50%	7.48±0.09 ^{ab}	7.77±0.47 ^{abc}	8.52±0.10 ^{fgh}	7.93±1.34 ^{bc}
1%	7.38±0.15 ^a	7.76±0.58 ^{abc}	8.41±0.94 ^{efg}	7.71±1.85 ^{abc}
Control	7.84±0.23 ^{bc}	8.00±0.55 ^{cd}	8.79±1.16 ^{ghi}	7.66±1.82 ^{ab}
		F pr. (12, 20, 5%)		
Treatments	0.002			
Storage time	<0.001			
Treatment x Storage time	<0.001			

Values are means ± standard deviation, Values with different superscripts are significantly different ($p \leq 0.05$).

Effect of Resistant Starch Enriched Cassava Starch on Yoghurt Textural Attributes

Yoghurt textural attributes are presented in Table 8. There was a significant difference ($p \leq 0.05$) among the firmness values for all treatments and it increased with the amount of resistant starch enriched cassava starch used with yoghurt incorporating 1% resistant starch enriched cassava starch having higher value of firmness, 0.42 N than the control sample, 0.37 N. The same trends were observed for yoghurt consistency. The absolute value of cohesiveness was highest for yoghurt containing 1% resistant starch

enriched cassava starch (0.30 N) while it was lowest for yoghurt with no thickener (0.19 N).

There was no significant difference in cohesiveness for yoghurt with 0.1% and 0.5% resistant starch enriched cassava starch ($p \leq 0.05$). The index of viscosity was significantly different ($p \leq 0.05$) among all yoghurt samples, with values of 0.09 N s and 0.61 Ns for yoghurt with no thickener and yogurt with 1% resistant starch enriched cassava starch respectively.

Table 8: Textural attributes of yoghurt incorporated with modified cassava starch

Percentage of starch	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Index of viscosity (N.s)
0%	0.26±0.01a	5.89±0.12a	-0.19±0.01d	-0.09±0.01e
0.1%	0.30±0.01b	6.91±0.13b	-0.21±0.01c	-0.21±0.01d
0.5%	0.32±0.01c	7.28±0.12c	-0.22±0.01c	-0.25±0.01c
1%	0.42±0.01e	9.87±0.16e	-0.30±0.01a	-0.61±0.02a
Control	0.37±0.01d	8.59±0.12d	-0.27±0.01b	-0.48±0.03b
F Pr. (15, 4, 5%)	<0.001	<0.001	<0.001	<0.001

Values are mean ± standard deviation, Values with different superscripts in the same column are significantly different ($p \leq 0.05$)

Correlation Between Resistant Starch Content And Physico-Chemical Properties of Yoghurt

The correlation coefficients between resistant starch concentration and physico-chemical parameters of yoghurt are listed in Table 9. There was a significant correlation ($p \leq 0.05$) between resistant starch levels

and high viscosity ($r=0.64$), low syneresis ($r=.081$), high titratable acidity ($r=0.35$) and high total solids ($r=0.67$). There was no significant correlation ($p > 0.05$) between resistant starch content and the pH of yoghurt and lactic acid bacteria count.

Table 9: Correlation between resistant starch content and physical-chemical parameters of yoghurt

	Viscosity	Syneresis	pH	TA	TS	LAB
Resistant starch						
r(38)	0.64	0.81	0.30	0.35	0.67	0.29
p-value	<0.001	<0.001	0.06	0.02	<0.001	0.06

T.A: Titratable acidity, T.S: Total Solids, LAB: Lactic acid Bacteria

Effect of Application of Resistant Starch Enriched Cassava Starch on Yoghurt Sensory Attributes

The scores of sensory properties of yoghurt samples incorporating modified cassava starch, after day 1, day 7 and day 14 of storage at 4 °C, are shown in Table 10. There was no significant difference

($p > 0.05$) in scores of colour in all yoghurt samples.

There was no significant statistical difference ($p > 0.05$) among scores observed after 14 days of storage.

Table 10: Sensory attribute scores of yoghurt treated with different levels of modified cassava starch

Storage time	Treatments	Sensory attributes				
		Colour	Smell	Taste	Mouthfeel	Overall acceptability
Day 1	0%	7.47±1.30 ^a	6.33±1.80 ^a	7.47±0.74 ^{de}	6.53±1.36 ^{ab}	7.13±0.99 ^b
	0.1%	7.33±0.98 ^a	7.73±1.22 ^c	7.53±0.99 ^e	6.73±1.39 ^{ab}	7.47±0.64 ^{ab}
	0.5%	7.60±1.12 ^a	6.53±2.03 ^{abc}	6.93±1.16 ^{bcd}	7.33±1.23 ^{ab}	6.93±1.10 ^{ab}

	1%	7.73±0.88 ^a	6.93±2.05 ^{abc}	6.93±1.39 ^{bcd}	7.20±1.42 ^{ab}	6.87±1.06 ^{ab}
	Control	7.27±1.39 ^a	6.73±1.79 ^{abc}	7.27±0.80 ^{cde}	7.07±1.10 ^{ab}	6.87±1.06 ^{ab}
Day 7	0%	7.17±1.03 ^a	6.83±0.80 ^{abc}	7.27±0.89 ^{de}	6.80±1.92 ^{ab}	7.13±1.41 ^b
	0.1%	7.43±0.92 ^a	7.43±1.2 ^{bc}	6.93±1.25 ^{bcd}	6.40±1.60 ^a	7.17±1.00 ^b
	0.5%	7.33±0.99 ^a	7.10±1.10 ^{abc}	6.97±1.22 ^{bcd}	7.03±1.31 ^{ab}	6.93±1.37 ^b
	1%	7.40±1.19 ^a	6.90±1.08 ^{abc}	6.90±1.13 ^{bcd}	7.67±0.98 ^{bc}	6.63±1.2 ^{ab}
	Control	7.03±1.25 ^a	6.98±1.53 ^{abc}	7.27±1.06 ^{de}	7.33±1.06 ^{ab}	7.23±1.39 ^b
Day 14	0%	7.07±1.71 ^a	6.80±1.21 ^{abc}	6.60±0.99 ^{abcd}	6.67±1.45 ^{ab}	7.47±0.83 ^b
	0.1%	7.60±0.74 ^a	6.93±1.53 ^{abc}	6.33±0.72 ^{ab}	6.27±1.39 ^a	6.87±1.30 ^{ab}
	0.5%	7.53±0.90 ^a	7.07±1.36 ^{abc}	6.33±0.72 ^{bcd}	7.13±1.45 ^{ab}	6.47±1.18 ^b
	1%	7.40±0.90 ^a	6.40±1.37 ^{ab}	6.00±0.85 ^a	7.33±1.05 ^{ab}	6.07±1.10 ^a
	Control	7.27±1.22 ^a	6.87±1.36 ^{abc}	6.47±0.74 ^{abc}	6.67±1.42 ^{ab}	6.67±1.18 ^{ab}
	Storage time	0.435	0.411	<.001	0.672	0.711
F Pr.	Treatments	0.360	0.072	0.717	0.292	0.146
	Interaction	0.980	0.677	0.145	0.093	0.038

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

Discussion

Effect of Resistant Starch Enriched Cassava Starch on Resistant Starch Content of Yoghurt

These results show that incorporation of resistant starch enriched cassava starch increased the content of resistant starch. The resistant starch concentration was proportional to the quantity of resistant starch enriched cassava starch used. A decrease in resistant starch concentration of stored yoghurt could be attributed to the breakdown of resistant starch by lactic acid bacteria. In fact lactic acid bacteria are known to ferment resistant starch²⁹⁻³¹ producing mainly short chain fatty acids under *in vitro* and *in vivo* conditions³². These short chain fatty acids are essential for bowel bacteria growth, prevention of colon cancer and reduction of glycaemic index¹². The resistant starch content found in the present study are lower than those of Aryana *et al.*,¹⁶ who found the resistant starch content in yoghurt supplemented with High Amylo Maize (HAM-RS2) to vary from 45% to 46% on dry weight basis. This difference may be attributed to the fact that the resistant starch content of resistant starch enriched cassava starch was lower compared to that of High Amylo Maize (HAM-RS2) as well as to the difference in the botanical origin of starch.

Effect of Resistant Starch Enriched Cassava Starch on Yoghurt Viscosity

The current results demonstrate the ability of resistant starch enriched 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 indicating there is a threshold of resistant starch enriched cassava starch necessary to maintain the viscosity of yoghurt. Moreover, the higher the resistant starch content the higher was the viscosity, this may indicate that resistant starch enhances the viscosity of yoghurt. Increased yoghurt viscosity during storage is attributed to the rearrangement of proteins and interaction of casein micelles and modified starch^{33,34} and to the total solids in yoghurt as 1% was the highest percentage of modified starch used. High viscosity is an important technological parameter of yoghurt quality since it enhances its mouthfeel and reduces whey separation³⁵. Starch increases yoghurt viscosity through absorption of water by its granules which considerably increases their size³⁶. The decrease in viscosity on day 21 may be attributed to the breakdown of yoghurt components due to increased acidity which can lead to increased water flow and therefore affecting the viscosity²⁴. Noh *et al.*,²⁵ found a significant increase in viscosity during storage up to 15 days. They attributed this increase to protein rearrangement. Domagała *et al.*,³⁷ found a decrease in viscosity at day 21 of storage of yoghurt treated with oat-maltodextrin which was attributed to the long time of storage.

Effect of Resistant Starch Enriched Cassava Starch on Yoghurt Syneresis

Syneresis is the phenomenon of whey separation from yoghurt gel and it is considered as a technological failure^{38,39}. A linear decrease of syneresis with regards to the quantity of resistant starch enriched cassava starch used could be attributed to the added starch which increased the total solids and hence reduced the water flow in yoghurt. There was a correlation between high resistant starch and reduced syneresis which indicates the ability of resistant starch to reduce whey separation in yoghurt. In fact resistant starch has a high water binding capacity hence reducing free water in yoghurt by trapping it within its matrix^{12,35}. Mani-López *et al.*,⁴⁰ reported syneresis of 32.65% and 34.62% in two commercial yoghurts which corroborate the present findings and they attributed this trend to the formation of a three dimensional 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.*,¹³ who reported the reduction of yoghurt syneresis by 18% as a result of starch addition as a thickener. An increase in syneresis observed on day 21 of the present study could be attributed to the reduction of total solids in yoghurt as a result of macromolecules breakdown due to the high acidity observed on day 21. It has been reported that modified starch loses its water holding capacity when it is kept at low temperature for a long time⁴¹.

Effect of Resistant Starch Enriched Cassava Starch on Yoghurt Acidity and pH

A gradual development of acidity in yoghurt during storage period could be linked to the activity of lactic acid bacteria in yoghurt which break down lactose into lactic acid⁴² which further breaks down the resistant starch enriched cassava starch into small molecules which in turn can be fermented into acid. Menzel⁴³ reported the breakdown of starch into small molecules at low pH values. The post-fermentation acidification of yoghurt is attributed to the activity of starter culture during low temperature storage⁴⁴. Acidity development is important in yoghurt manufacturing since it plays important roles including formation of its structure, enhancement of Lactobacilli bacteria growth and flavour development⁴⁵. A decreased pH and a corresponding increased titratable acidity during

yoghurt storage was also observed by Singh and Byars⁴². The current results fall in the appropriate pH range for yoghurt which is 4.6-4.0³⁶. Behrad *et al.*,⁴⁶ reported an increase in titratable acidity of up to 1.23% and a pH of 4.1 after 21 days of cold storage of yoghurt. They attributed this to the continuous production of organic acids by lactic acid bacteria during refrigeration storage and to the activity of β -galactosidase at low temperatures.

Effect of Resistant Starch Enriched Cassava Starch on Total Solids of Yoghurt

High total solids in yoghurt correlate positively with high resistant starch content. This increase was due to the addition of dry modified starch rich in resistant starch. A decrease in total solids during cold storage period could be attributed to the depletion of lactose as well as starch degradation⁴⁷. However, samples with high resistant starch maintained high total solids during storage which may indicate the possible ability of resistant starch to withstand rapid degradation. Total solids is the most paramount technological property which determines the stability of yoghurt gel structure by preventing poor body and whey off⁴⁸. The current results corroborate the findings of Muhammad *et al.*,⁴⁹ who reported 18.87% as total solid of yoghurt stored at refrigeration temperature on first day of refrigeration. They observed 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 resistant starch enriched cassava starch which maintained high total solids content due to the slow breakdown of resistant starch¹³.

Effect of Resistant Starch Enriched Cassava Starch on Lactic Acid Bacteria Count of Yoghurt

The current results indicate that incorporating 1% resistant starch enriched cassava starch to yoghurt slightly affected the growth of lactic acid bacteria. This can be attributed to increased restriction of water necessary for proper growth of lactic acid bacteria⁴⁵ and it could also be evidenced by low activity of lactic acid bacteria as the same sample had low titratable acidity. A decrease in lactic acid bacteria count observed on day 21 may be related to the high acidity observed in yoghurt on the same day restricting their growth. It has been reported that

the production of hydrogen peroxide by *Lactobacillus delbrueckii* subsp. *Bulgaricus* can reduce the survival of lactic acid bacteria in yoghurt⁴⁴. The same microorganism was used in the present 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 product⁵⁰. Gustaw *et al.*,⁵¹ reported an increase in *Bifidobacterium* sp count of yoghurt treated with 1% resistant starch from 7.1 logcfu/g to 7.5 logcfu/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 107 cfu/g⁵².

Effect of Resistant Starch Enriched Cassava Starch on Yoghurt Textural Attributes

The texture attributes were enhanced by the amount of resistant starch enriched cassava starch used. This is related to the fact that starch granules are able to absorb water and swell which can increase the texture of yoghurt³⁵. The appropriate water holding capacity of resistant starch present in the cassava starch used could have also contributed to the enhancement of yoghurt texture. The improved texture of yoghurt could also be related to the production of exopolysaccharide by lactic acid bacteria throughout the storage. Yang *et al.*,⁵³ reported that the exopolysaccharides influence the water holding capacity and rheological behaviour of yoghurt. These exopolysaccharides interact with milk protein which improves viscosity hence producing a preferred yogurt texture⁵⁴. Sajilata *et al.*,¹² reported that the water holding property of resistant starch provides improved texture to food products. Texture of yoghurt is of importance in enhancing flavour retention and influencing the sensory preferences of yoghurt^{45,55}. The values in the present study are higher compared to the findings of other researchers⁵⁶ who found the firmness of yoghurt treated with modified wheat starch to be

0.14 N. However, they are lower than those of other researchers⁵⁷ who reported the firmness of yoghurt incorporated with starch to be 3.89 N. The observed difference could be attributed to the difference in starch sources and the method of its modification.]

Effect of Resistant Starch Enriched Cassava Starch on Yoghurt Sensory Attributes

Resistant starch enriched cassava starch did not influence the colour, smell, taste, mouthfeel and hence the overall acceptability since it was applied in small amounts (less than 1%). The results point out that the addition of more than 1% of resistant starch enriched cassava starch may adversely affect the sensory properties of yoghurt. Goncalvez *et al.*,¹³ 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.*,⁵⁸ 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 is possible to produce an acceptable yoghurt with modified cassava starch without using any additional stabilizer.

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Conflict interest

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