

## DETERMINING THE EFFECT OF REPLACING COMPOSITE FLOUR MIXTURE WITH CASSAVA STARCH ON NUTRITIONAL AND PHYSICAL PROPERTIES OF CHICKEN SAUSAGES

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### ABSTRACT

Cassava starch extracted from cassava roots (*Manihot esculanta*) is a highly used ingredient for most commercial food products and has a high viscosity, water holding capacity, and binding abilities. The objective of this study was to determine the effect of replacing composite flour mixture with cassava starch on the nutritional and physical properties of chicken sausages. A scientific experiment was designed for the sausage formulation to incorporate 50% to 100% with cassava modified and native starch compared to the control sample. These samples were evaluated for sensory and textural properties. Nonparametric data obtained through sensory evaluation were analyzed using the Friedman ranking test. According to the test results, the best sample was analyzed for the nutritional toxicological composition and storage stability. According to the statistical analysis, the sample which was 50% replaced with native cassava starch showed the best sensory profile. The best sample showed average moisture, crude fat, crude protein, total solids, ash, and acid-insoluble ash content of 65.98, 7.19, 8.79, 34.02, 2.36, and 0.32 percent respectively. The starch content of the best-ranked sample was 3.12 and the cyanide content of that sample was 1.54 ppm on a dry basis. According to the microbial analysis results, the sausage sample was coliform negative and zero initial plate count was obtained. Besides, the best-ranked fresh chicken sausage was nutritionally acceptable and could keep for 14 days without adding preservatives.

**Keywords:** cassava starch; sausage; nutritional property; toxicological analysis; microbial analysis

### INTRODUCTION

Cassava (*Manihot esculanta*) is one of the most important food crops in tropical countries. Cassava has higher carbohydrate content and its tapioca starch and modified tapioca starch are highly used as an ingredient of many food products (Zhu et al., 2012). The Sri Lankan annual cassava production in 2015 was 324,097 million Tons and the area of land under the cultivation of cassava is 23,970 Hectares (Statistics, 2016). Over the last decades, cassava production in Sri Lanka grew by 45% between 2005 and 2015. According to the National Statistics of Household Income and Expenditure Survey, 2012/13 the average monthly consumption of cassava was 171.53g per person in Sri Lanka. Sausage is the most popular product among the oldest meat products in history about 1500 B.C. Sausage can be simply defined as a product manufactured from ground meat; mixed with salt, spices, and composite flour mixture; and shaped in some manner, using various sizes and types of casings. Raw sausages are produced without curing and are made from selected cuts of fresh meat. Fresh sausages must always be kept under refrigeration and also must be cooked thoroughly before serving.

The composite flour mixture is included with rice flour, maize powder, milk powder, and bread crumbs. Composite flour mixture has been used as binders in comminuted meat products due to their economic benefits. The amount of composite flour mixture used in processed meat varies depending upon the type of product, manufacturing procedure, and government regulations (Hedrick et al., 1994).

Nowadays, starches are used as binders in processed meat products to absorb moisture, which is released from meat protein during heating. A satisfactory binder should have the ability to hold more moisture throughout the processing, cooking, chilling, and storage of meat products. Today scientific principles are employed to improve production procedures, product quality, and product safety.

To maintain the water-holding ability the binder should also assist in binding the fat and maintaining its dispersion throughout the mix. Starches are commonly added to emulsion-type meat products and are popular not only for their functional properties but also to extend the more expensive lean meat portion of products (Hedrick et al., 1994). The effect is based on the ability of starch to

**Table 1** Ingredients content in sausage formulae.

Ingredient	aob0	aob1	C	aib0	aib1
	CFM 50% Starch 50%	CFM 0% Starch 100%	CFM 100% Starch 0%	CFM 50% Starch 50%	CFM 0% Starch 100%
Meat %	57.6	57.6	57.6	57.6	57.6
Emulsion %	33.3	33.3	33.3	33.3	33.3
Composite Flour Mixture (CFM) %	3.05	0	6.1	3.05	0
Cassava Native starch %	3.05	6.1	0	0	0
Cassava Modified starch %	0	0	0	3.05	3.05
Sugar & Salts %	2	2	2	2	2
Spices Mixture %	1	1	1	1	1

gelatinize when heated in a water-containing medium, thereby binding a relatively large amount of water (Hodge, Osman and Fennema, 1976).

Extracted cassava native starch and modified starch were used for the replacement of composite flour mixture as a source of dietary fiber content (Ruhee et al., 2020) and due to its specific physical properties as high viscosity, water holding capacity, and binding ability. Even though Sri Lanka has higher production of cassava the industrial utilization is limited (Somendrika et al., 2016). Also, cassava has lower postharvest life since it is a perishable food. Increasing the application of cassava starch will directly be beneficial to cassava farmers and for the Sri Lankan economy as it reduces the wastage of cassava. Therefore the objective of the study was to determine the effect of replacing composite flour mixture with cassava starch on the nutritional and physical properties of chicken sausages. Cassava MU51 cultivar was used for the extraction of cassava starch due to the higher availability and higher starch extraction yield (25% – 30%). Textural properties of cassava starch are also important to improve the texture of sausage. Also, the use of cassava starch as a composite flour mixture reduces the cost of sausage raw material.

**Scientific hypothesis**

The replacement of composite flour mixture with cassava starch decreases the cost of preparation, improves the organoleptic properties and nutritional composition of fresh chicken sausage.

**MATERIAL AND METHODOLOGY**

**Samples**

MU51 variety is one of the most cultivated and recommended cassava varieties in Sri Lanka. Thus, the pre-determined quantity of cassava MU51 variety was purchased from a cassava cultivator in Bandaragama, Sri Lanka.

**Chemicals**

Kjeldahl Tablets (Kjeldahl Tablets, Supleco, Sigma-Aldrich Chemie GmbH).

Conc. H<sub>2</sub>SO<sub>4</sub> (Concentrated Sulphuric acid, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH, 99.999%).

Conc. NaOH (Sodium Hydroxide, Sigald, Sigma-Aldrich Chemie GmbH, ≥98%)

95% Ethyl Alcohol (Etanol, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH, 95%).

6N HCl (Hydrochloric Acid, Sigma, Sigma-Aldrich Chemie GmbH, 37%).

Diethyl Ether (Diethyl Ether, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH, 99.7%).

Petroleum Ether (Petroleum Ether, SIGALD, Sigma-Aldrich Chemie GmbH, 99%).

Phenol (Phenol, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH, 99%).

Potassium cyanide (Potassium cyanide, Sigald, Sigma-Aldrich Chemie GmbH, 99%).

Picric Acid (2,4,6-Trinitrophenol, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH, 99%).

Methyl Red (Methyl Red, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH, 99.5%).

Methyl Blue (Methyl blue, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH, 70%).

Peptone (Peptone, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH).

Nutrient agar (Nutrient Agar, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH).

Potato Dextrose Agar (Potato Dextrose Agar, Sigma-Aldrich, Sigma-Aldrich Chemie GmbH).

**Instruments**

The Brookfield texture analysis (Model: CT3-4500, Brookfield Engineering, USA).

UV-Vis spectrophotometer (Model: UV mini-1240, SHIMADZU CORPORATION, Japan).

Muffle furnace (Model: SHIMADEN SR1, SHIMADEN CO., LTD, Tokyo).

Polarized Zeeman atomic absorption spectrophotometer (Model ZA3000, HITACHI, Japan).

**Laboratory Methods**

Analysis of storage stability – (SLS 516, 2013).

Pre-gelatinized modification (PG) – Rajapaksha et al. (2017).

Nutrition Analysis of sausage – AOAC (2012).

Toxicological Analysis – Spectrophotometric Determination of Cyanide (Picric acid method) by Wood (1965).

**Description of the Experiment**

**Sample preparation:**

**Extraction of cassava starch**

Cassava starch was extracted according to the method described by Rajapaksha et al. (2017). Roots of the best quality were washed and peeled manually and grated into small pieces. They were ground with normal tap water in a 1:4 ratio. The slurry was filtrated through a muslin cloth and the filtrate was allowed to sediment for 4 – 5 hours. The sediment starch was separated by decanting and starch was dried by keeping in the mechanical dryer at 60 °C for 5 hours until the moisture content reduced up to around

10%. The dried starch was ground and passed through a 300-micron sieve.

Finally, ground starch was packed in a polyethylene package and was stored (-5 °C) in an airtight container.

#### **Pre-gelatinization modification of cassava starch**

Pre-gelatinized modification (PG) was done with slight modifications of the method described by **Rajapaksha et al. (2017)**. As 1:10 starch solution (100 g of starch in 1000 mL deionized water) was incubated at 65 °C for 10 minutes. The starch slurry was allowed to sediment for 4 – 5 hours. Then starch sediment was dried at 45 °C till the moisture level dropped to 10% – 13%.

The pre-gelatinized cassava starch was packed in a polyethylene package and stored under cold room conditions at -5 °C.

#### **Formulation of sausage**

The basic ingredients used for the chicken sausage were lean meat (minced chicken) fat, ice, isolated soy protein, composite flour mixture, rice flour, maize powder, bread crumbs, milk powder, cassava starch (native and modified), sugar, salt, garlic, black pepper, and chili powder. The amount of ingredients added to sausage preparation is summarized in Table 1.

**Number of samples analyzed:** Sensory and color analysis – five samples, Nutritional composition, and microbial quality analysis – One sample with three replications for the final sample.

**Number of repeated analyses:** Three times.

#### **Design of the experiment**

Two cassava starch types (i.e., native cassava starch (a<sub>0</sub>) and modified cassava starch (a<sub>1</sub>)) were used in the experimental design. In addition, two replacement levels (i.e., 50% (b<sub>0</sub>) and 100% (b<sub>1</sub>)) were included.

Four different sausage batters were prepared by replacing composite flour mixture with cassava native starch in 50% (a<sub>0</sub>b<sub>0</sub>) and 100% (a<sub>0</sub>b<sub>1</sub>) levels and with cassava modified starch in 50% (a<sub>1</sub>b<sub>0</sub>) and 100% (a<sub>1</sub>b<sub>1</sub>) levels. A sausage was prepared without replacing the composite flour mixture as a control. Other ingredients and additives were incorporated in the formulated batters (Table 1).

The batter was filled into cellulose casings and cooked in steam for 15 minutes until the core temperature becomes 72 °C. Then the prepared sausage was stored at -5 °C in a freezer for further analysis.

#### **Analyzing the effect of sensory and physical properties of replacements (developed formulae)**

##### **Analysis of sensory attributes of sausages**

Six sensory attributes (i.e., Appearance, Colour, Aroma, Flavour, Hardness, Overall acceptability) were analyzed by using the five-point hedonic scale (5 = Like extremely, 1 = Extremely dislike) and 30 untrained panelists.

##### **Texture profile analysis of sausages**

The texture profile (TPA) was analyzed according to the method described by **Bourne (2002)** with slight modifications by using the Brookfield texture analysis (Model: CT3-4500, Brookfield engineering, USA) at a constant crosshead velocity of 1 mm.s<sup>-1</sup>, Trigger point of 10.0 g, and cylindrical sausage samples with a diameter of about 17 mm and a height of about 10 mm were used for TPA evaluation. Each test was performed with four replications. Hardness, Springiness, Gumminess, and Chewiness factors were evaluated in the test.

#### **The Color analysis of sausages**

Values for the color dimensions L\*, a\*, b\*, C\*, and h\* were measured by using the Colorimeter (Lovibond).

#### **Analyzing the nutritional and toxicological composition of the developed formulae**

##### **Nutritional composition analysis of sausage**

Moisture content, crude protein content, crude fat content ash content, and acid insoluble ash contents were analyzed for the top-ranked sausage samples (a<sub>0</sub>b<sub>0</sub> - 50% replacement with Cassava Native starch) following the **AOAC (2012)** standard methods.

The total solids content was analyzed according to the arithmetic calculation.

The top-ranked sausage sample was subjected to dry ashing (using muffle furnace at 500 °C) and analyzed for the presence/quantity of Na, K, Ca and Mg minerals using Atomic Absorption Spectrophotometric (AAS) method.

Starch content was measured by the method of **Dubois (Dubois and Cotter, 1955)** with slight modifications.

##### **Toxicological composition analysis (Cyanide content)**

The cyanide content of the top-ranked sausage sample (a<sub>0</sub>b<sub>0</sub> - 50% replacement with Cassava Native starch) was analyzed according to Spectrophotometric Determination of Cyanide (Picric acid method) by **Wood (1965)**.

About 10 – 20g of the grounded top-ranked sausage sample was weighed and the weight was recorded. Then the starch sample was introduced into a triple-necked round bottom flask containing 100 mL of deionized water. About 50 mL of 2N H<sub>2</sub>SO<sub>4</sub> was added to the round bottom flask. The flask was immediately connected to the steam generator and the distillate was collected in 50 mL of Na<sub>2</sub>CO<sub>3</sub> solution till it becomes 200 ml. The solution was transferred into a 250 mL volumetric flask and the volume was made up to 250 mL using deionized water.

Then 10.00 mL of the distillate (final solution should be contained 3 mL of 5% Na<sub>2</sub>CO<sub>3</sub>), and 4 mL of 1% Picric acid were added into a boiling tube and the boiling tube was immersed in a boiling water bath for 12 – 15 minutes. Then the solution was left to cool to room temperature and volume was made up to 25 mL.

Finally, the absorbance was measured at 530 nm in UV-Vis spectrophotometer (VU mini – 1240) for the developed color of CN<sup>-</sup> with Picric acid. Reading of absorbance was noted down and CN<sup>-</sup> concentration was calculated based on the equation (Cyanide Content = (absorbance+0.03)/0.0048) which was been first developed using standard concentration series of KCN solution.

##### **Analysis of storage stability**

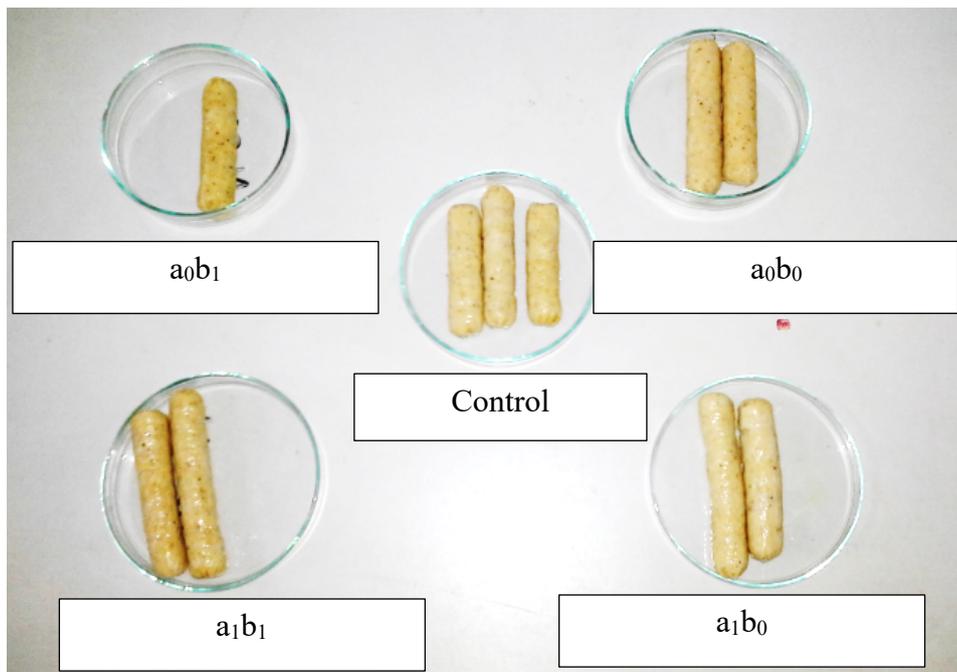
The total plate count and coliforms of the prepared sausage samples were determined during the storage using the methods described by **SLS 516 (2013)**.

##### **Total plate count-(SLS 516: part 1 Section 2:2013)**

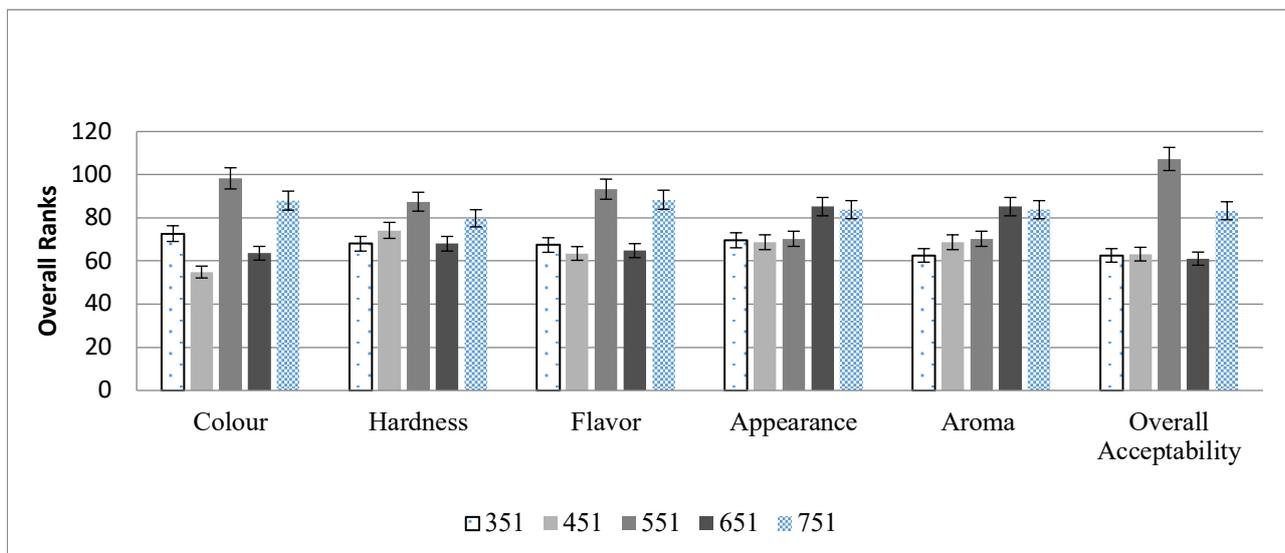
The total plate count of the sausage sample was performed using a Colony count at 30 °C by the surface plating technique.

##### **Detection and enumeration of Coliform (SLS 516: Part 3 Section 1: 2013)**

Coliform detection and enumeration of frozen fries samples were carried by the Most Probable Number (MPN) method.



**Figure 1** Photographs of sausages with different treatment types and levels. Note: a<sub>0</sub>b<sub>0</sub> – Native 50%, a<sub>0</sub>b<sub>1</sub>– Native 100%, a<sub>1</sub>b<sub>0</sub> – Modified 50%, a<sub>1</sub>b<sub>1</sub> – Modified 100%, C- 0% Replacement.



**Figure 2** Bar Chart of Friedman overall of Ranks 751 – 50% replacement with Cassava Native starch, 351 – 100% replacement with Cassava Native starch, 451 – 50% replacement with Cassava Modified starch, 651 – 100% replacement with Cassava Modified starch, 551 – Control.

**Statistical Analysis**

All the parametric and non-parametric data were analyzed by using MINITAB 17 statistical tool. Non-parametric data which are results obtained from the sensory analysis were analyzed using the Friedman ranking test. One-way ANOVA was used to analyze the significant differences of mean values of each sample, followed by Tukey Pairwise comparison test to analyze the samples that had a significant difference in color and texture. Two sample t-test was used to analyze the significant difference in nutritional composition by referring to the reference values obtained from SLS and USDA standards.

**RESULTS AND DISCUSSION**

**Sensory analysis**

As the sensory profile is the most critical factor results of the sensory analysis were used for the selection of the best sample. The top-ranked sample was 551(Control sausage-0% replacement) according to the average ranking of Friedman analysis. The second best-ranked sample was 751 (a<sub>0</sub>b<sub>0</sub> – 50% replacement with cassava native starch) according to the results of the Friedman ranking analysis. Results further suggest that the color, appearance, and overall acceptability of all the treatment levels are significantly different from the control sausage sample, (*p* < 0.05) caused by the treatment on that parameters. Since the *p*-value for hardness, flavor, and aroma of all the

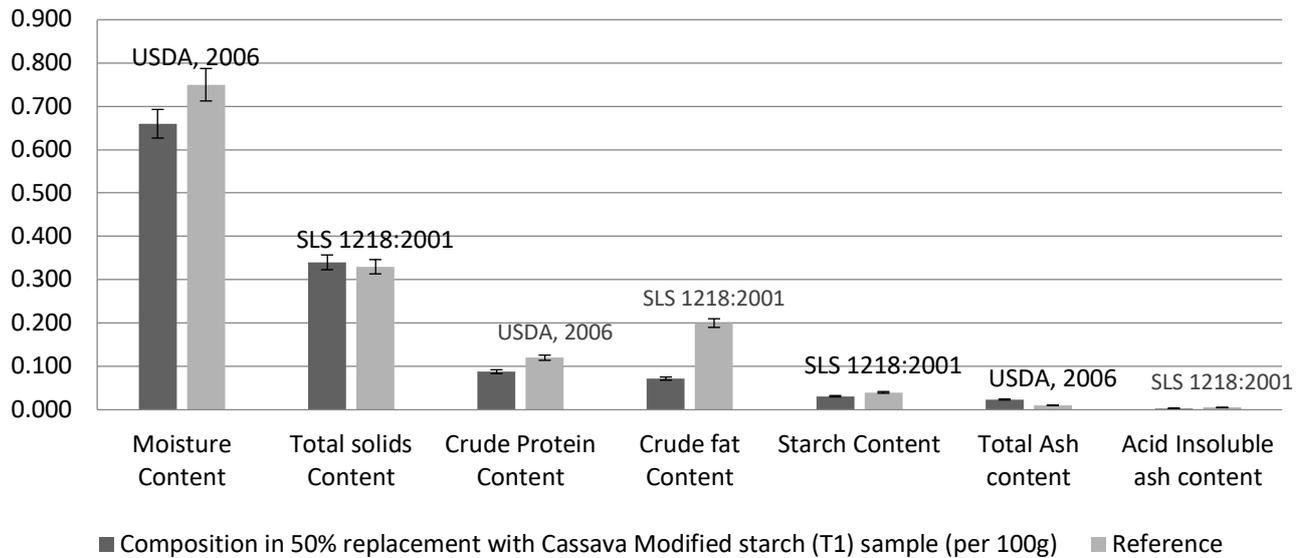


Figure 3 Bar chart of compares nutritional composition of sausage with referred SLS and USDA standards.

treatment levels was 0.05, there was no significant effect caused by the treatment on those parameters.

Garcia-Santos et al. (2019) showed that no effect on the sensory parameters including appearance, color, flavor, texture, aroma, and overall acceptance attributes of the Beef and Chicken mix sausages with the treatment of cassava starch. Also, Grizzotto et al. (2012) carried out a study with the addition of two types of okara flour in Frankfurter type sausages with partial (1.5%) and total (4.0%) replacement of texturized soy protein and concluded that the addition of okara flour did not influence sensory acceptance.

### Texture profile analysis

According to the texture profile analysis data  $a_0b_0$  sample has been given  $1475 \pm 278$  (g) for gumminess,  $99.5 \pm 19.7$  (mJ) for Chewiness,  $0.24 \pm 0.01$  for cohesiveness, and  $6.88 \pm 0.06$  (mm) for springiness values.

Since the  $p$  values were higher than 0.01 (at 1% significant level) there was no relationship between the treatment level of each treatment type with gumminess, chewiness, cohesiveness, and springiness. However, the addition of wheat fiber had a significant effect on the hardness of sausages (Choe et al., 2013).

According to Chang et al. (2012), the textural characteristic of sausages with modified starch were better than sausage with native starch. But in their research, the starch modifications used were starch acetate, cross-linked esterified starch, and phosphate starch, wherein current research heat moisture treated starch was used for the experiment (Chang et al., 2012).

### Colour analysis

In the colorimetric values analysis  $L^*$ ,  $a^*$ ,  $b^*$ ,  $c^*$ ,  $h_0$  values refer to lightness, redness, yellowness, saturation, and hue, and those dimensions were considered. According to the color values analysis results, there was no significant effect (at 0.05 significant level) by the 50% replacement by cassava native starch ( $a_0b_0$ ) on lightness and hue. However,

that treatment ( $a_0b_0$ ) affected the redness, yellowness, and saturation of sausage.

The 100% replacement by the cassava native starch ( $a_0b_1$ ) significantly affected (at 0.05 significant level) lightness, redness, and hue. However, it was not significantly affected by yellowness and saturation. A similar result was observed by Hughes, Mullen and Troy (1998). According to Hughes, Mullen and Troy (1998), incorporation of tapioca starch decreased the redness and lightness of the frankfurters, whereas yellowness was not significantly altered.

The 50% replacement with Cassava Modified starch ( $a_1b_0$ ) was significantly influenced (at 0.05 significant level) on the lightness and redness. Also, it was not significantly affected by yellowness and saturation.

But the treatment of 100% replacement with Cassava Modified starch ( $a_1b_1$ ) was significantly affected (at 0.05 significant level) on the lightness, redness, yellowness, and saturation and significantly not affected on hue according to Tukey's pairwise comparison results.

Since the  $p$  values are less than 0.01 (at 1% significant level) for the correlation of  $a_0$  vs.  $L^*$ ,  $a_1$  vs.  $a^*$ ,  $a_1$  vs.  $L^*$ ,  $a_1$  vs.  $a^*$ ,  $a_1$  vs.  $b^*$ ,  $a_1$  vs.  $C^*$  and  $a_1$  vs.  $h_0$  ( $p < 0.01$ ) the treatment with cassava starch was significantly affected on color.

According to the Pearson correlation analysis results, there was a strong negative relationship between  $a_0$  vs.  $L^*$ ,  $a_1$  vs.  $L^*$ , and  $a_0$  vs.  $h_0$ . Also, there was a strong positive relationship between  $a_1$  vs.  $a^*$ ,  $a_1$  vs.  $b^*$ , and  $a_1$  vs.  $C^*$ .

### Nutritional Composition analysis

The top-ranked sample showed average moisture, crude fat, crude protein, total solids, ash, and acid-insoluble ash content of 65.98%, 7.19%, 8.79%, 34.02%, 2.36%, and 0.32% respectively. The starch content of the top-ranked sample was 3.12%. According to Bozhko et al. (2020), the protein content in meat containing semi-smoked sausages

with different ratios of duck and fish meat varied within 17.90%-21.34%.

According to the SLS and USDA standards moisture, crude fat, crude protein, total solids, ash, and acid-insoluble ash contents were 74.6%, 33%, 12.1%, 20%, 4 %, 1%, and 0.5% respectively (SLS 1218:2001; USDA, 2006). According to the two-sample t-test results, the p-value for total solids content, there was not a significant effect on the total solids content by the 50% replacement with cassava native starch. Also since the p values for other nutritional properties are less than 0.05 the replacement was significantly affected on that nutritional property.

Previous research on the effect of substitution of fat with Sorgam and finger-millet showed protein, fat, ash, crude fiber, and carbohydrates contents as 10.4%, 3.1%, 1.6%, 2.0%, and 70.7% respectively (Das et al., 2013).

High fat consumption has been associated with cardiovascular diseases, obesity, cancer, and hypertension, among other illnesses (Mapiye et al., 2012; Hygreeva, Pandey and Radhakrishna, 2014).

According to the AAS analysis results, there was a high Na content in the sausage sample which was 1348.39 mg.100g<sup>-1</sup>. Also, the K and Mg contents were 232.15 mg.100g<sup>-1</sup> and 73.21 mg.100g<sup>-1</sup> respectively. According to the analysis the absorbance value for the Fe content was not observed in the standard range. Calculation suggests that the Fe content of the sausage sample should be less than 0.012 mg.100g<sup>-1</sup>.

#### Toxicological composition

Cassava starch has a cyanide content of 7.05 mg.kg<sup>-1</sup> on a dry basis (Rajapaksha et al., 2017). The cyanide content in the sausage sample was around 1.54 ±0.02 mg.kg<sup>-1</sup> on a dry basis. Around 0.0528 mg amount of cyanide contained in added 7.5g of starch sample. The cyanide content of the product has been further reduced due to the processing steps in sausage processing such as steaming. (Akingbala, Oguntimein and Abass (1991).

#### pH analysis

The detected average pH value of the sausage was 5.64 ±0.11. The standard level for pH 6.6 has been shown by Venturini et al., (2011). According to Garcia-Santos et al. (2019) addition of resistant starch in sausages increased pH values where the average varied from 6.10 to 6.21.

#### Analysis of storage stability

Microbial analysis was conducted by TPC and coliform test. Total plate count was analyzed with the purpose of shelf life analysis of the best-ranked sausage and coliform analysis was conducted to determine whether the coliform bacteria are present or absent in the sausage sample.

Sausage is usually exposed to high temperatures by heating till the middle temperature becomes greater than 74 °C by using the steaming process. But this temperature might not be enough to inactivate all the microorganisms.

Sausages are also re-contaminated with spoilage bacteria during the processing stages followed after cooking. Since that the initial plate count was around 10 C.F.U.g<sup>-1</sup>.

The excessive proliferation of present microflora in the sausage content or on the surface during storage causes economic losses because of spoilage and deterioration.

Especially, lactic acid bacteria are considered to be a major component of the microbial population found on vacuum-packaged sausages (Özdemir, 1997). Lactic acid bacterial growth on the surface of the sausages produces undesirable sensory attributes (Korkeala and Björkroth, 1997).

Findings from research by Onishi. 1957 has shown that the growth of osmophilic yeast in the presence of 18% NaCl was limited to the range pH 4.0 – 5.0 and according to the findings in current research the pH was higher than this limited range and because of that, the analysis was not done for yeasts and molds.

#### CONCLUSION

The best composite flour mixture replacement formula was a 50% composite flour mixture with cassava native starch. There was no significant effect on the total solids content by the 50% replacement with cassava native starch and it was significantly affected on moisture content, protein content, fat content, starch content, ash content, acid insoluble ash content, and pH value of the final product. Shelf life analysis of developed sausage suggests that the product was safer to consume up to 14 days of manufacturing under frozen conditions (-5 °C).

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#### Conflict of Interest:

No conflicts of interest to declare regarding the present study.

#### Ethical Statement:

Not applicable.

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