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D Akullo

Department of Food Technology
and Nutrition, Makerere
University, Kampala, Uganda

J Kigozi

Department of Agricultural and
Biosystems Engineering,
Makerere University, Kampala,
Uganda

JH Muyonga

Department of Food Technology
and Nutrition, Makerere
University, Kampala, Uganda

Nutritional, sensory and shelf life quality of tilapia and Nile perch sausages enriched with fish bone soup

D Akullo, J Kigozi and JH Muyonga

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Abstract

Fish is well recognized as a healthy food of its high polyunsaturated fatty acids, including omega-3 fatty acids. Development of convenient fish products is one way to increase its consumption. Development of most of these products often entails the use of fish mince which is low in minerals, especially calcium. Fish bone powder is one of the most used by products in enriching fish products because it is rich in minerals. There is however limited information on utilization of fish bone soup to develop nutritious products. Fish sausage is one of the products from fish mince which can be used as a vehicle for incorporation of calcium from fish bones because it can easily be consumed as a snack. The aim of this study was to investigate the potential of fish bone soup in enriching fish based sausages. Design expert (version 11) was used to optimize fish mince and tilapia bone soup for sausage development. Commercially available fish sausage was used as a control. The nutritional value, functional properties and shelf life of the sausages were determined using standard methods. There were significant differences in the nutritional composition of the tilapia sausages (TS) and Nile perch sausages NPS ($p < 0.05$). TS contained more moisture (72.3%) compared to Nile perch sausages (71.2%), while NPS had calcium content (177.442 mg/100g) than TS (164.13 mg/100g). The highest value for protein, ash and carbohydrate was recorded in the control (19.21%, 1.19% and 6.56% respectively). NPS had a higher value for water holding capacity (17.6%) as compared to TS (12.3%). There were no significant differences in the sensory attributes of the sausages ($p > 0.05$). There were significant increases in the free fatty acids, peroxide value TBA values and microbial counts of the fish based sausages within the sample during six weeks of storage ($p < 0.05$ mince).

Keywords: Fish sausages, quality, fish bone soup

Introduction

Capture fisheries production (metric tons) in Uganda was reported at 396205^[6]. More than 70% of fish produced undergo processing. Fish and fishery products represent valuable sources of nutrients and contribute to diversified and healthy diets^[6]. Fish flesh is a source of high biological value proteins, polyunsaturated fatty acids, vitamins and minerals, with the minerals mainly concentrated in the bones^[18]. The recognition of fish as a healthy food by health conscious consumers has led to the development of convenient fish products such as fish sausages, fish balls, fish burgers, as a way to promote consumption. Nile tilapia, (*Oreochromis niloticus*) is a common fish mainly consumed worldwide and used in development of fish based sausages because of its flavored white meat and the absence of Y-shaped bones^[3]. On the other hand, industrial filleting of Nile perch is today the most profitable processing option for this fish^[7] However, there is limited information regarding the use of Nile perch in sausage production. It is recommended that mixing of minces from different fish species to produce sausages be reduced.

Unfortunately, in the development of most of these products, fish mince, which is low in calcium, is the main fish ingredient used. Fish bones or skeleton, which are by-products from production of fillets or mince, constitute approximately 10-15% of total fish biomass⁷ and are high in calcium, unlike the flesh^[12]. Of the several fishery products, fish sausage has attracted the attention of many researchers since it is a popular snack in different countries of the world. Fish sausages are made from fish mince which is low in calcium and could therefore act as an appropriate food vehicle for incorporation of calcium ingredients from fish bones. Fish bones are a potential source of calcium and other minerals for enrichment of other food materials such as sausages^[8, 10].

Corresponding Author:

D Akullo

Department of Food Technology
and Nutrition, Makerere
University, Kampala, Uganda

Although fish bones have been incorporated in other foods in powder form, little is known about its use as fish bone soup¹⁷. The main aim of this study was to investigate the potential of fish bone soup in enriching fish based sausages.

Methods and Materials

Preparation of the fish bone soup

Fish bone soup was prepared according to methods described by) with slight modifications^[15]. The bone material was rinsed under running tap water for 15 minutes and mixed at room temperature with 0.25M acetic acid in a ratio of 1:50 (fish bone (g): acetic acid (ml) for 12 hours. The bones were boiled for 3 hours. The boiled material was stirred and filtered using a muslin cloth and the filtrate frozen for use in the fish sausages

Design of experiment for development of fish based sausages

Statistical software package Design-Expert (DX11, Stat Ease) was applied to construct as well as to analyze the design of the experiment. A central composite design with two factors and levels were used. The two independent variables used were fish type (tilapia or Nile perch) at two levels and the response included total calcium, extractable calcium, calcium extractability and overall acceptability for each of the fish mince. The range for the different variables was based on preliminary study suggest that fish based ingredients with 85% -90% for tilapia mince, 80%-90% for Nile perch mince and 10%-15% of fish bone soup have increased calcium levels from 65mg/100g to 170mg/100g and 70mg/100g to 173mg/100g respectively. The acceptability increased from means scores of and 6.1 to 7.4 and 6.3 to 7.1 for tilapia and Nile perch sausages, respectively. Therefore, 11 representative formulations from 14 trial runs (Table 1) were suggested by the software.

Table 1: Suggested combinations for development of calcium enriched tilapia sausages

Runs	TP mince (Ratio)	FBS (Ratio)	TP mince (%)	FBS (%)
1	87.88	12.20	70.30	9.76
2	85.00	13.08	68.00	10.46
3	88.20	14.00	70.56	11.20
4	90.00	10.03	72.00	8.02
5	85.00	14.75	68.00	11.80
6	90.00	14.38	72.00	11.50
7	90.00	12.23	72.00	9.78
8	86.13	11.73	68.90	9.38
9	87.90	10.09	70.32	8.08
10	85.78	10.00	68.62	8.00
11	86.65	15.00	69.32	12.00

Table 2: Suggested combinations for development of calcium enriched tilapia sausages

Runs	Suggested combinations		Actual amount in the sausage	
	NP mince (%)	FBS (%)	NP mince (%)	FBS (%)
1	80.00	11.78	64.00	14.72
2	80.00	10.00	64.00	12.50
3	85.00	15.00	68.00	18.75
4	82.88	12.88	66.30	16.09
5	82.85	15.00	66.28	18.75
6	81.68	11.70	65.34	14.63
7	80.73	15.00	64.58	18.75
8	81.85	10.00	65.48	12.50
9	83.28	11.00	66.62	13.75
10	85.00	12.88	68.00	16.09
11	85.00	10.75	68.00	13.44

Commercially available fish sausages purchased from Capital Shoppers Supermarket, Ntinda, Kampala was used as the control in this experiment. The fish mince and fish bone soup as factors in the design constituted 80% of the actual sausage formulation. The remaining 20% consisted of other ingredients such as spices.

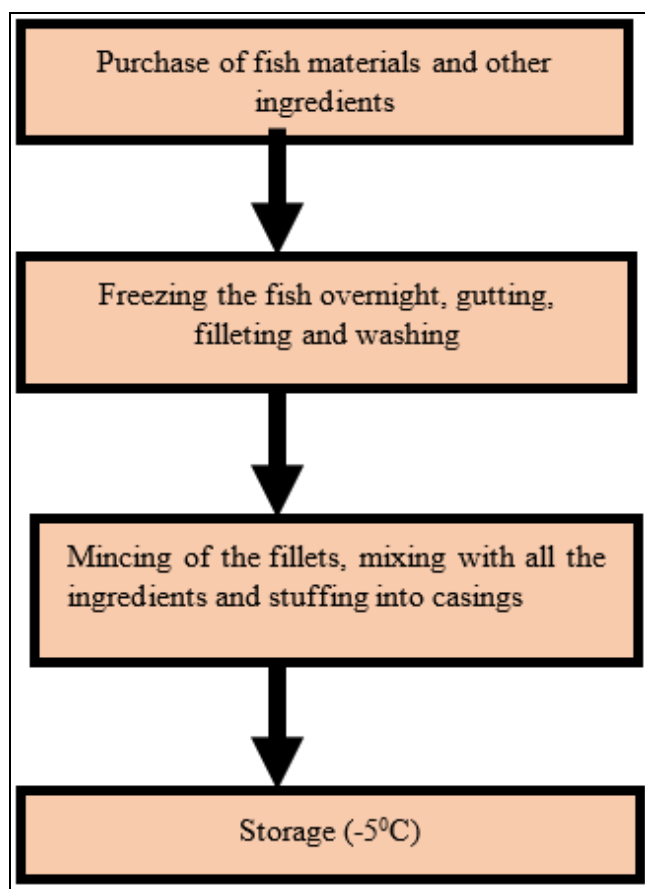


Fig 1: Procedure for development of the sausage

Determination of nutrition composition

Moisture, protein, ash and fat were determined^[1]. The carbohydrate content was determined by subtracting the sum of moisture, protein, ash and lipid contents from 100%. Calcium in the samples was extracted by shaking with 10 ml of 0.03 N HCl for 3 h at 37 °C and then filtered^[1]. The clear extract obtained was oven-dried at 100 °C and then acid-digested. Calcium was determined according to AOAC (2005). The amount of extractable calcium was determined. HCl extractability (%) was determined by dividing the total calcium extractable in 0.03N HCl by the total amount of calcium in the sausages then multiplying by 100.

Shelf life

The peroxide value, free fatty acids and thiobarbituric acid value was determined^[16]. Microbial load including total plate count, total coliforms, psychrotrophs, yeasts and moulds were determined using standard methods described^[14].

Results and Discussions

Both formulations used influenced dependent variables, for both tilapia and Nile perch sausages (Tables 3 and 4).

Table 3: D- Optimal design used to optimize the levels of tilapia mince and fish bone soup used in the tilapia based sausages

Runs	Component 1: Tilapia minceratio	Component 2: Fish bone soup ratio	Response 1: Total Ca (mg/100g)	Response 2: Extractable Ca (mg/100g)	Response 3: Ca extractability (%)	Response 4: Acceptability (Mean score)
1	87.88	12.20	151.30	91.91	60.74	6.77
2	85.00	13.08	141.30	81.17	57.45	7.43
3	88.20	14.00	166.50	87.73	52.69	7.20
4	90.00	10.03	136.99	75.03	54.77	6.90
5	85.00	14.75	168.31	101.01	60.01	6.86
6	90.00	14.38	172.08	96.23	55.92	7.09
7	90.00	12.23	151.30	89.91	59.42	7.03
8	86.13	11.73	141.30	81.23	57.48	6.92
9	87.90	10.09	131.22	79.98	60.95	6.83
10	85.78	10.00	127.49	76.68	60.15	6.83
11	86.65	15.00	166.50	101.01	60.67	7.32

Table 4: D- Optimal design used to optimize the levels of tilapia mince and fish bone soup used in the Nile perch based sausages

Runs	Component 1: Nile perch mince	Component 2: Fish bone soup	Response 1: Total Ca (mg/100g)	Response 2: Extractable Ca (mg/100g)	Response 3: Ca extractability (%)	Response 4: Acceptability (Mean score)
1	80.00	11.78	131.92	85.29	64.65	6.46
2	80.00	10.00	134.00	86.93	64.87	6.53
3	85.00	15.00	183.50	120.80	65.83	7.23
4	82.88	12.88	153.40	93.08	60.68	6.34
5	82.85	15.00	173.50	101.76	58.65	6.84
6	81.68	11.70	135.86	89.45	65.84	6.99
7	80.73	15.00	169.30	99.43	58.73	6.90
8	81.85	10.00	128.00	60.40	47.19	6.43
9	83.28	11.00	175.40	102.90	58.67	6.89
10	85.00	12.88	163.60	94.60	57.82	6.91
11	85.00	10.75	139.70	82.90	59.34	7.01

Predictive models for the dependent variables in the tilapia based sausages

Total calcium of tilapia sausages

Generally, there were significant differences ($p < 0.05$) in the calcium content of the tilapia based sausages produced using different formulations (Table 3). Addition of fish bone soup to the formulations increased the calcium content of the tilapia

sausages. Calcium content of the sausages ranged from 127.49 mg/100g to 166mg/100g. Addition of different levels of tilapia mince and fish bone soup to the tilapia sausages had a positive linear effect on the calcium content of the tilapia based sausages ($R^2=0.70$) (Equation 1.1).

$$Total\ Calcium\ \left(\frac{mg}{100g}\right) = 142.65 + 3.72X_1 + 17.76X_2 \tag{1.1}$$

Calcium extractability of tilapia sausages

There was a negative and positive effect of tilapia mince and fish bone soup, respectively, on the extractable calcium of the

tilapia based sausages (Equation 1.2). Both fish bone soup and tilapia mince significantly increased the extractable calcium in the sausages ($p=0.005$ and 0.008 respectively).

$$Extractable\ Ca\ \left(\frac{mg}{100g}\right) = 119.41 - 13753X_1 - 116.25X_2 + 53.36X_1X_2 - 62.62X_1^2 - 50.40X_2^2 - 43.11X_1^2X_2 - 20.79X_1X_2^2 + 170.05X_1^3 + 164.15X_2^3 + 19.94X_1^2X_2^2 \tag{1.2}$$

Regarding the calcium extractability of the tilapia sausages, there was a negative and positive effect of tilapia mince and fish bone soup on the calcium extractability ($R^2= 0.98$)

(Equation 1.3). Both fish bone soup and tilapia mince significantly increased the extractable calcium in the sausages ($p=0.02$).

$$Ca\ extractability\ (\%) = 70.57 - 40.57X_1 - 45.49X_2 + 16.40X_1X_2 - 19.40X_1^2 - 22.69X_2^2 - 18.42X_1^2X_2 + 8.90X_1X_2^2 + 50.98X_1^3 + 64.32X_2^3 + 9.12X_1^2X_2^2 \tag{1.3}$$

Calcium extractability in food products predicts the potential of bioavailability in the gastrointestinal tract. The high extractability of calcium in the sausages may be attributed to

the inclusion of acetic acid in the fish bone soup. Acetic acid is well known for its application in food products.

Acceptability of the tilapia based sausages

There was a negative and positive cubic effect of tilapia mince and fish bone soup on the acceptability of the tilapia based sausages ($R^2=0.99$) (Equation 1.4). Both fish bone soup

and tilapia mince significantly increased the extractable calcium in the sausages ($p<0.05$ respectively).

$$Acceptability = 7.08 - 0.9X1 - 0.11X2 + 0.04X1X2 - 0.146X1^2 - 0.27X2^2 + 0.03X1^2X2 + 0.23X1X2^2 + 0.89X1^3 + 0.26X2^3$$

1.4

Addition of fish bone soup to the Nile perch sausages had a significant positive effect on the extractable calcium in the Nile perch sausages ($p=0.002$, $R^2=0.62$) (Equation 1.5). Nile

perch mince did not have any significant effect on the extractable calcium in the Nile perch sausages.

$$Extractable Ca \left(\frac{mg}{100g} \right) N = 90.99 + 1.27X1 + 14.35X2$$

1.5

Increase in extractability of the fish sausages is probably due to the increase in the specific surface area by destruction of collagen matrix [18] during stirring and heating of fish bone soup in presence of acetic acid. In the present study, addition of acetic acid combined with boiling of the fish bones for 3

hours extracted calcium from the bones while highly enriching the soup. Therefore, when the soup was incorporated in the sausages, there was a great positive effect on the calcium content and extractability of the fish sausages

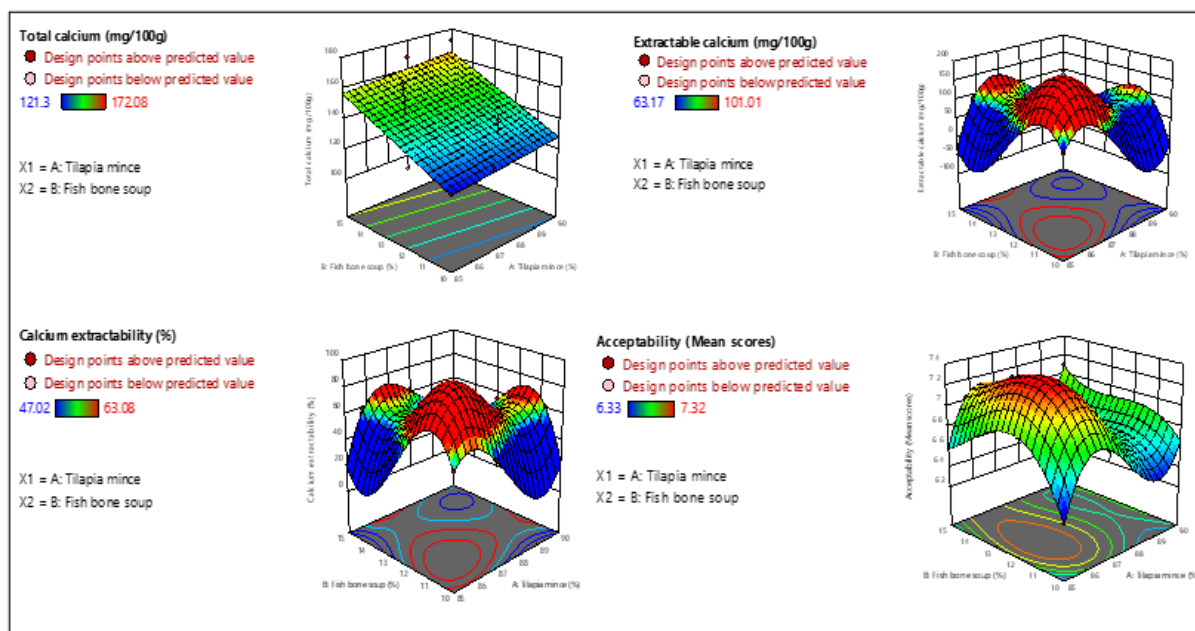


Fig 2: Response surface plots showing effect of varying different levels of tilapia mince and fish bone soup on the calcium content, extractable calcium, calcium extractability and acceptability of the sausages

Nile perch mince and fish bone soup had significant negative and positive effects, respectively, on the calcium extractability of the Nile perch sausages ($p=.0.023$ and 0.016

respectively, $R^2=1.0$). There was also a significant interactive effect of the mince and fish bone soup on the extractability of the sausages ($p=0.03$).

$$Ca extractability(\%) = 70.57 - 40.57X1 - 45.49X2 + 16.40X1X2 - 19.40X1^2 - 22.69X2^2 - 18.42X1^2X2 + 8.90X1X2^2 + 50.98X1^3 + 64.32X2^3 + 9.12X1^2X2^2$$

1.6

Extracted calcium activates the calcium-dependent enzyme, transglutaminase, suggesting the availability for biological reaction [8]. The contribution of the fish sausages towards meeting the recommended daily allowances of calcium was another important factor. Literature reports that 100g of three Ca salts (gluconate, lactate and citrate-malate) tested in sufficient amounts gave 20–40% of the Recommended Daily Allowance (RDA). In the present study sausages with highest amounts of fish bone soup in Nile perch sausages and tilapia

sausages gave 17.06% and 15.73% of the RDA respectively.

Acceptability of the Nile perch sausages

Acceptability of the Nile perch sausages significantly increased with increasing amount of fish bone soup in the formulations ($p=0.0003$, $R^2=0.99$) (Equation 1.7). However, the mince did not have any significant effect on the acceptability of the Nile perch based sausages ($p=0.2$).

$$Acceptability = 7.08 - 0.9X1 - 0.11X2 + 0.04X1X2 - 0.146X1^2 - 0.27X2^2 + 0.03X1^2X2 + 0.23X1X2^2 + 0.89X1^3 + 0.26X2^3 \quad 1.7$$

Effect of fish bone soup and Nile perch mince on calcium variables and acceptability

The calcium content, extractable calcium, calcium extractability and acceptability of Nile Perch sausages increased by increasing the amount of fish bone soup in the formulations (Figure 3). However, as the level of mince

increased, the extractable calcium reduced. The acceptability of the sausages decreased with decrease in the amount of fish bone soup and the mince. Reduction in the acceptability of the sausages could be attributed to strong fishy flavor when the mince was included in higher amount.

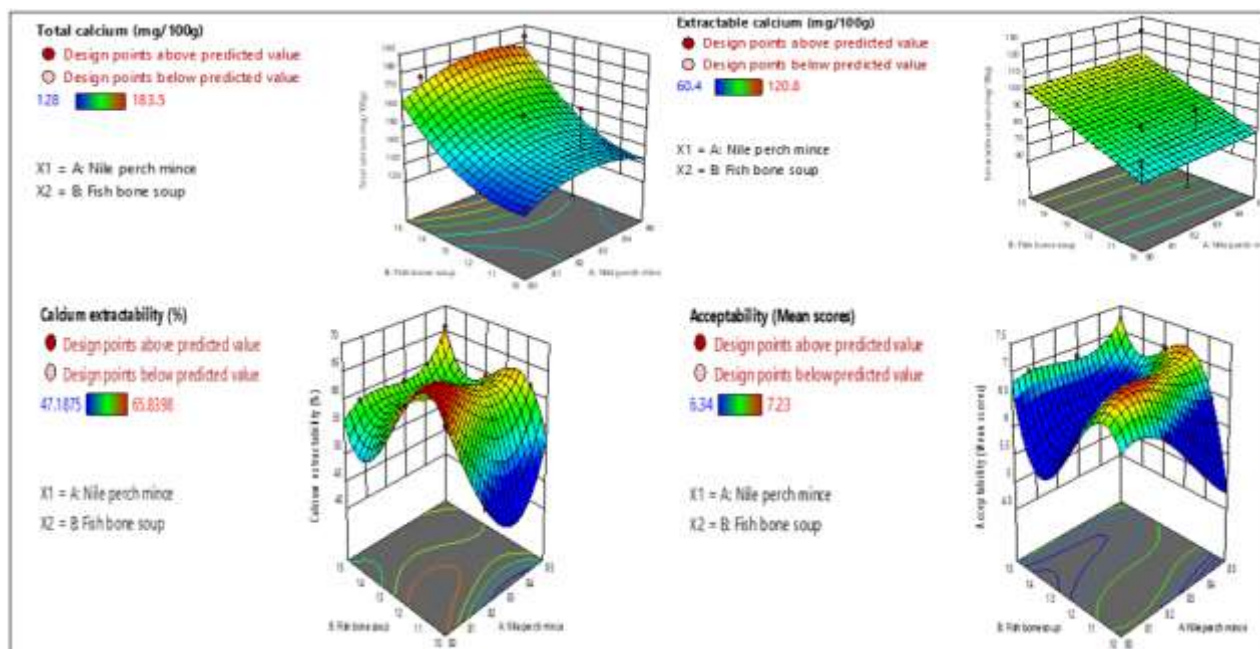


Fig 3: Response surface plots showing effect of varying different levels of Nile perch mince and fish bone soup on the calcium content, extractable calcium, calcium extractability and acceptability of the sausages

Table 4: Optimal solutions for acceptable calcium enriched tilapia sausages

NO	Tilapia mince	Fish bone soup	Total calcium (mg/100g)	Extractable Ca (mg/100g)	Ca Extractability (%)	Acceptability (Mean scores)	Desirability	
1	90.000	15.000	164.129	96.230	55.920	7.088	0.792	Selected
2	90.000	12.200	144.244	101.010	66.851	6.913	0.767	
3	87.583	15.000	160.532	113.347	65.585	6.931	0.753	
4	87.203	13.108	146.532	104.153	63.080	7.137	0.708	

Table 5: Optimal solutions for acceptable calcium enriched Nile perch sausages

NO	Nile perch mince	Fish bone soup	Total calcium (mg/100g)	Extractable Ca(mg/100g)	Ca extractability (%)	Acceptability (Mean scores)	Desirability	
1	85.000	15.000	177.442	106.610	65.831	7.261	0.938	Selected
2	84.973	15.000	177.565	106.596	65.599	7.245	0.936	
3	84.952	15.000	177.657	106.585	65.425	7.233	0.934	
4	84.928	15.000	177.763	106.573	65.225	7.219	0.930	

Table 6: Predicted and actual values of the Nile perch and tilapia sausages

Response	Prediction values		Actual values		P value (t test)
	TS	NPS	TS	NPS	
Total calcium (mg/100g)	164.13	177.44	161.6±1.68	175.3±1.09	<0.01
Extractable calcium (mg/100g)	96.23	106.61	86.10±1.82	103.36±0.82	<0.01
Calcium extractability (%)	55.92	65.83	53.29±1.66	58.95±0.59	0.005
Overall acceptability (mean)	7.09	7.26	7.06± 1.13	7.13 ±1.16	0.87

Sensory acceptability of the optimized calcium enriched tilapia and Nile perch fish sausages

There were no significant differences in the sensory attributes of the sausages from different formulations ($p>0.05$). Results

from the present study indicate that the mean score for the different sausage formulations was within the acceptable range (above average of 5) (Table 7).

Table 7: Sensory attributes of the optimized calcium enriched sausages

Fish type	Proportion (Ratio) Mince:FBS	Appearance	Aroma	Texture	Juiciness	Overall acceptability
Tilapia	90:15	6.96 ± 1.19 ^a	6.52±1.52 ^a	7.04±1.60 ^a	7.22±1.61 ^a	6.97 ± 1.72 ^a
Nile perch	85:15	7.13 ± 1.22 ^a	7.30±1.52 ^a	6.39±1.65 ^a	7.13±1.67 ^a	7.09 ± 1.71 ^a
Control		7.04 ± 1.39 ^a	7.04±1.33 ^a	7.26±1.09 ^a	6.96±1.89 ^a	6.04 ± 1.89 ^a

Results are means of triplicates with their standard deviation (\pm). Different superscripts across the columns show significant differences ($p < 0.05$) in the variable between the treatments; the control is the commercial fish sausages.

Proximate composition

There were significant differences in the proximate composition of the sausages ($p < 0.05$). Nile perch sausages had the highest values for moisture (72.3±1.69) while Nile

perch sausages had the highest value for fat (3.76±1.3). The highest values for protein, ash and carbohydrate were recorded in the control (19.21±1.10, 1.19±0.44 and 6.56±1.70 respectively).

Table 3: Proximate composition of the optimized tilapia and Nile perch sausages

Fish mince	Proportion Mince: FBS (Ratio)	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Carbohydrates (%)
Tilapia	90:15	72.3±1.69 ^a	18.10±1.20 ^a	2.13±1.3 ^a	1.11±0.21 ^a	6.36±1.57 ^a
Nile perch	85:15	71.2± 1.50 ^a	19.16±0.15 ^b	3.76±1.3 ^b	1.12± 0.30 ^a	5.71±1.61 ^b
Control		69.7± 1.32 ^b	19.21±1.10 ^b	3.72±1.2 ^b	1.19±0.44 ^b	6.56±1.70 ^a

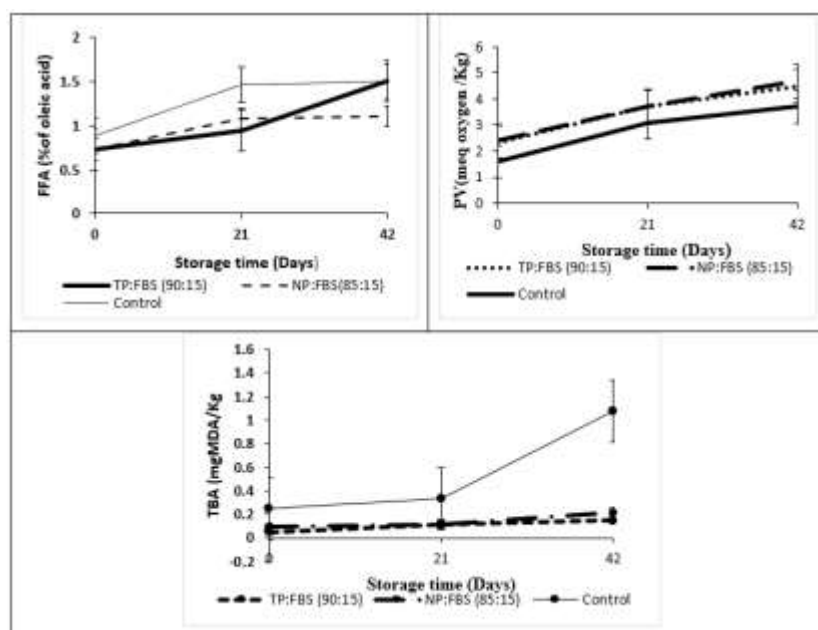
Results are means of triplicates with their standard deviation (\pm). Different superscripts across the columns show significant differences ($p < 0.05$) in the variable between the treatments; the control is the commercial fish sausages.

The proximate composition of freshly prepared sausage had moisture 72.62%, protein 19.02%, total lipids 5.62% and 2.67% of ash². These results are comparable to the results from the present study and those obtained by Hugo *et al.* (2015) with respect to moisture (66.5 – 73.3%), fat (0.9–6.5%) and ash (1.7–2.6%). The higher moisture content for this study could be due to the excess amount of water from the soup added to the sausages. The higher protein, ash and lipid could also be due to their presence in the fish bone soup as extracted from the bones during boiling. In addition, according to the Uganda National Bureau of Standards (UNBS, 2015) sausages elaborated with different ingredients must have the following composition: minimum moisture of 60%, minimum protein of 12% and maximum lipids of 30%. All the sausage formulations were within the acceptable limit. Regarding carbohydrates, fish is usually less than 1% but 2–5% can be obtained in fatty species and striated muscle where carbohydrate occurs as glycogen and as part of the chemical constituents of nucleotides [14]. Carbohydrates was added to

sausages as wheat flour hence their increased value in the sausages.

Shelf life of the sausages

There were significant increases in the free fatty acids, peroxide value and TBA values of the fish based sausages of the fish based sausages within the sample during six weeks of storage ($p < 0.05$ mince (90%) and fish bone soup (10%) on day 42 (1.59% of oleic acid) and the lowest was sausages with tilapia mince (85%) and fish bone soup (15%) (0.02% of oleic acid)(Fig 4). The values of thiobarbituric acid reactive substance (TBARS) were less than 1 mgMDA/Kg for all the days of storage with no significant differences for all the storage time ($P > 0.05$). The control which was the commercially purchased fish sausages had the highest level of thiobarbituric acid reactive substances throughout the storage period (0.25 mgMDA/Kg) on day 0 and 0.38 (mgMDA/Kg) on day 42). Therefore, results from the present study indicated that the fish sausages had excellent quality.

**Fig 4:** Free fatty acids, peroxide value and Thiobarbituric acid value TBA values for the fish sausages (Ratios are fish mince to fish bone soup)

Microbial load in the sausage

There were significant differences in the total plate count, psychrotrophs and total coliforms in the different fish sausages during the storage period in a freezer ($P < 0.05$) (Fig 5). Generally, the total plate count in the sausages did not

change significantly from day 0 to day 21 but was lower at day 42. Nile perch sausages mince was also observed to have higher total plate counts compared to tilapia sausages and the control.

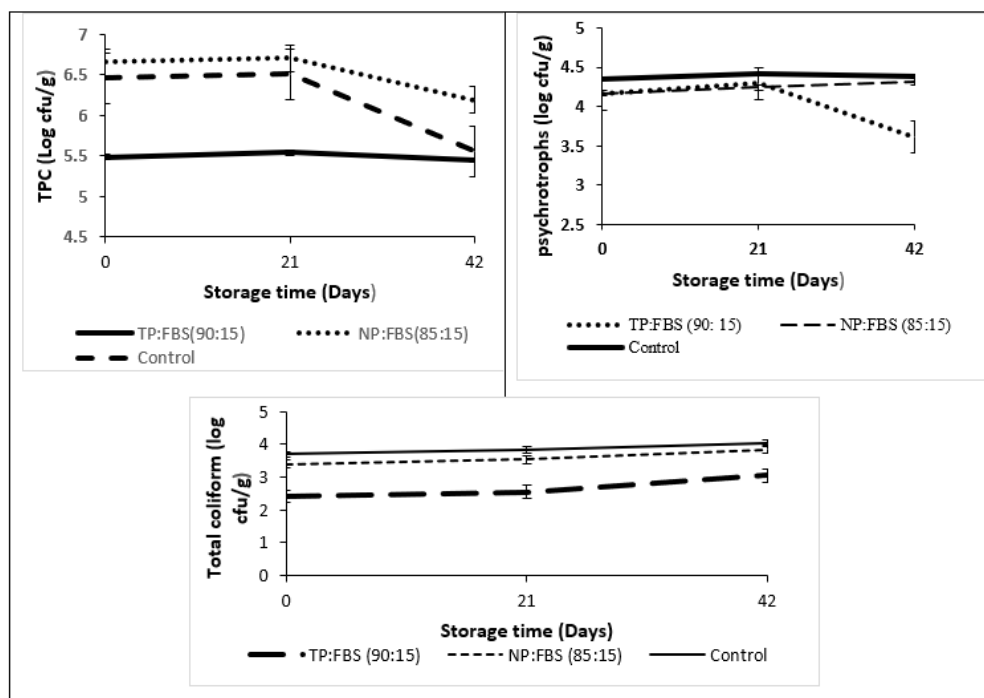


Fig 5: Microbiological load in the fish sausages

The differences in the microbial load could be due to the different species of fish used. Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors [5].

The observed total plate counts were all within acceptable limits; since 10^6 cfu/g is the maximum permissible level for aerobic plate counts in meat products (UNBS, 2015; ICMFS 2002). Therefore, all the fish sausages analysed over the six weeks of frozen storage can be considered acceptable for human consumption.

Conclusion

The nutritional composition of calcium enriched sausages from the different formulations shows that it has the highest content of protein (19.16%) and fat (3.76%) in Nile perch sausages when 85% fish mince and 15% of fish bone soup was added. The moisture and ash content varied between 70.1% and 76.16%, and 1.11% and 1.19%, respectively. Calcium content was highest in Nile perch sausages (166.50mg/100g) when 15% of fish bone soup was added. The increase in the nutritional composition in the calcium enriched fish based sausages from the different fish mince also suggests its suitability for inclusion in the diet of many consumers. Incorporation of fish bone soup into food products may contribute to providing a potential calcium enrichment ingredient without any undesirable effects on the qualities of the food products. The shelf life of the different fish sausage formulations met the recommended allowable limit for the different oxidative and microbial parameters. The sausages remained shelf stable for all the 6 weeks of storage (0 °C) indicating their safety for human consumption.

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