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Research article

PAWPAW SAP AS ALTERNATIVE COAGULANT AND BOILING TIME IN THE PRODUCTION OF TRADITIONAL WARANKASHI (LOCAL CHEESE)

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ABSTRACT

Effect of concentration of unripe pawpaw sap as alternative coagulant and boiling time on chemical (moisture, protein, fat, ash and carbohydrate, Ca, Mg, Fe) composition of warankashi (local cheese) was investigated and optimized using response surface methodology. Unripe pawpaw sap concentration were used at various boiling times. The proximate compositions of the different concentrations were analyzed including the mineral compositions. The optimum concentration and boiling time recorded were 3.69 ml in 500 ml of raw fresh cow milk and boiled for 20 minutes to yield moisture content, protein, ash, fat, Ca, Mg and Fe of 38.32 %, 3.85 %, 2.75 %, 42.21 %, 7.02 ppm, 21.29 ppm and 27.21 ppm respectively. Investigated parameters (pH, protein, ash, magnesium) were significantly affected ($p<0.05$) by the process variables of concentration and boiling time while other investigated parameters (moisture, ether extract, calcium and iron) were insignificantly affected ($p>0.05$).The sensory result revealed the high acceptability and best combination for the production of warankashi which can be produced by adding 3.69 mls of unripe pawpaw sap into 500 mls of raw cow milk and boiled for 20 minutes.

Keywords: warankashi, pawpaw sap, optimization, coagulants,r esponse surface methodology

INTRODUCTION

Warankashi is a traditional soft cheese consumed in several parts of West Africa. It originates from the Fulani cattle herdsmen from northern Nigeria, who refer to the liquid cow's milk as "Wara" and the curd-like texture of the cheese as "Kashi" (Ogundiwin, 1978). Warankashi is an unripened soft cheese-like product made from fresh whole cow's milk by the application of a juice extract of sodom apple leaf (*Calotropis procera*) (Belewu and Aina, 2000; Fashakin and Unokiwedi, 1992). The preferred coagulant comes from sodom apple leaf extract (Calotropis procera) because the cheese made with this coagulant has a sweeter flavor and higher protein content (Omotosho *et al.,* 2011).

However, research work has been conducted on the use of certain plants as alternative coagulants. Adeniji *et al*., (2007) investigated the use of lemon and Belewu *et al*., (2005) worked on the use of pawpaw leaf in replacement of sodom apple leaf. The texture of white cheeses made with extract from *Carica papaya* leaves showed flavor defect, such as bitterness other vegetable coagulant from *Cynara cardunculus* and *Calotropis procera* have also been investigated in the manufacturing of wara. The flavor defect may be attributable to processing conditions such as the concentration of extract and boiling time. There exist no or scanty literature on the use of unripe pawpaw sap as alternative coagulant. The amount of rennet used in the different cheeses varies because of specific cheese requirements (Adeniji *et al.,* 2007)..

Papain, a proteolytic enzyme in *papaya*, has a wealth of industrial uses: it has milk-clotting (rennet) and protein-digesting properties. Papain, the proteolytic enzyme from *Carica papaya* is used in medicine for the treatment of dyspepsia, digestive disorders and reducing enlarged tonsils. The extract of unripe pawpaw has been reported to possess anti-sickling properties and it is being used as an anti-sickling agent by some sickle cell patients in Western Nigeria (Oduola *et. al*., 2007).

However, the use of unripe pawpaw sap could also be investigated to determine its suitability for the coagulation process in manufacturing of soft cheese and to determine appropriate boiling time which is a major factor that could affect the activity and quality of the enzyme responsible for the process of coagulation. Therefore, the use of response surface methodology is a veritable tool in designing experiment which may give the best results required in term of combinations of variables and levels (Akinoso and Adeyanju, 2010). Optimum conditions are those that produce the best, most favourable or most beneficial result from a system or process (Olaoye and Oyewole, 2012). The objective of this study was to determine the optimum concentration of pawpaw sap as alternative coagulant and boiling time for the manufacturing of traditional cheese.

MATERIALS AND METHODS

The fresh milk (cow) used for the experiment was sourced from a dairy farm in Ota, Ogun State. The unripe pawpaw was sourced from a garden where cattle are raised in the area. D- Optimal response surface methodology was used for the design of the experiment.

Design of the experiment and production of wara using unripe pawpaw sap

D-Optimal response surface methodology was used for the design of the experiment (Design-expert,version 8.0.1.0, StatEase Inc., Minneapolis, USA). The independent and levels of variables were decided mainly from literature (Belewu *et al*., 2005) and the result of the preliminary investigation carried out on the utilization of unripe pawpaw sap as coagulant and boiling time required for the manufacturing of traditional cheese. The unripe pawpaw concentrations were 1, 3, and 5ml in 500ml while 10, 15 and 20min were boiling time. The experimental design is presented (Table 1).

Warankashi (Wara) cheese was prepared from fresh raw cow's milk in the laboratory using the traditional method as explained by O'Connor (1993) and modified by the levels of sap concentrations and boiling time as described in the experimental design. The raw fresh milk was filtered using a metal sieve to remove unwanted materials. The milk was then heated in a metal pot on a gas cooker and maintained at temperatures between 50^oC-55^oC for 15minutes. The coagulant (Unripe pawpaw sap) was extracted from unripe pawpaw fruit into a sterile bowl. The sap was quickly added to a portion of the warm milk after which it was transferred to the whole milk lot. The mixture was stirred and boiled for varied time (10 – 20 min) to inactivate the plant enzyme and facilitate whey expulsion. The loose curd pieces (very soft) were poured into a metal sieve and allowed to drain. The cheese was sufficiently cooled at ambient temperature after which was put into a portion of whey stored in a plastic container in the freezer at -18^oC.

Proximate Analysis

The Warankashi produced was analyzed for moisture content which was determined using oven method as described by Association of Official analytical chemist (AOAC, 2000); protein was determined using the klejdahl method (AOAC, 1990); ash according to method (AOAC, 1990). Crude fibre was determined using the method described by Pearson (1973). Carbohydrate was calculated by difference.

pH determination

The pH was determined using H1222pH meter (Hanna Instruments, Woonsocket, RI, USA). The electrode of the pH meter was dip into the sample and readings were obtained from the photo-detector on the pH meter.

Mineral Content Determination

The dry digestion (ashing) procedure was used for the preparation of the samples for mineral analysis. One (1) grams of the sample was accurately weighed into porcelain crucibles and pre-ashed until the sample was completely charred on a hot plate. The pre-ashed samples were thereafter ashed in the muffle furnace at 550^oC till the ash was white for about 2 hours. After ashing, the crucibles were transferred into the desiccator and cooled. The samples were transferred into 100 ml volumetric flasks and the crucibles were carefully washed with 0.1M of H_2SO_4. This was made up to 100 ml measurement of the volumetric flask with deionised water. The content of the minerals; calcium, magnesium, copper and iron were determined with the Atomic Absorption Spectrophotometer (Buck Scientific, Model 210).

Calculation

$$\%\text{Mineral Element Concentration} = \frac{\text{Machine reading(ppm)}}{\text{Weight of sample}} \text{ x dilution factor}(100)$$

Sensory evaluation

A panel of 12 judges was used for the sensory evaluation of the optimized product of Warankashi (511, 522, and 533) with respect to flavour, odour, colour, texture, appearance, aroma and overall acceptability Samples were coded with three digit random numbers and presented in random order using a 9-point hedonic scale where 9 was equivalent to like extremely and 1 meant dislike extremely(Lamond, 1977).

Data analysis

The software was used for the analysis of variance (ANOVA), mathematical modeling, regression analysis, and optimization. The response surface plots were generated for different interactions. The optimization of the wara production was aimed at finding the levels of unripe pawpaw sap concentrations and boiling time which could yield maximum protein, ash, fat, Ca, Mg contents and range pH (6.0 – 7.0), moderate carbohydrate and Fe.

Results and Discussion

Table 2: Result of chemical and mineral analysis of wara

Run	conc. ml/ml	Boiling, ^0C	pH	Moisture,%	Protein,%	Fat, %	Ash, %	Mg,mg/kg	Fe,mg/kg	Ca,mg/kg
1	1	15	7.57	41.35	3.29	48.62	2.95	16	31	3
2	1	20	7.46	49.33	3.5	30.81	2.85	18	6	3.6

3	5	10	7.11	35.89	3.31	36.29	2.45	30.5	28.5	2
4	3	20	7.08	34.74	3.85	34.67	2.15	16	24	12.8
5	5	20	7.06	41.67	3.43	47.41	2.85	24	30	2.4
6	1	20	7.44	41.69	3.79	42.86	2.25	25	37	9
7	3	10	7.25	39.99	3.86	36.83	2.44	11	24	11.6
8	2	12.5	7.62	50.32	3.4	34.5	3.34	21.5	16	5
9	5	20	7.08	41.67	3.44	47.49	2.86	24	31	2.8
10	4	15	7.34	40.42	2.54	36.98	5.25	39	40.5	12
11	1	10	6.82	46.21	3.32	40.67	4.39	15	58	3
12	5	10	7.12	41.69	3.3	36.38	2.5	31	25	2

Effect of unripe pawpaw sap concentration and boiling time on pH of wara

The result of effect of unripe pawpaw concentration and boiling time on pH of warankashi ranged from 6.82-7.62 as shown in Table 1. The highest amount of pH was recorded in the experiment at 1ml of unripe pawpaw sap in 500ml of fresh milk and boiled for 20minutes while the lowest was at 3ml of sap in 500ml of fresh milk at a boiling time of 10minutes.
There were significant differences ($p<0.05$) in pH obtained as a result of varied concentrations of sap and boiling time. The lack of fit test for the analysis was significant which is not good enough for the model. The R^2, Adjusted R^2, and predicted R^2 were 0.868, 0.758 and 0.451 respectively. The closer the R^2 value to one justified the degree of the fitness of the model. The standard deviation and the mean value were put 0.13 and 7.22 respectively. Figure 1 showed the response surface plot on the effect of sap concentrations and boiling time on pH.

From the RS plot, an increase in sap concentration and boiling time increased the pH of wara. However, at a particular point a further increase in boiling time diminished the pH of wara. In the production of cheese using vegetable coagulant from cardoon *Cynara cardunculus,* the pH ranged between 5.00-5.66 (Galan *et al*., 2008). The variation could be as a result of the sources of the coagulants used for the experiments. The coagulant used by Galan *et al*., (2008) was sourced from animal while the coagulant used in this work was sourced from plant. Adetunji *et al*., (2008) obtained pH range of 5.37-6.40 which might have been as a result of the acidic (lemon juice) coagulant added during the production of warankashi. Therefore, the source of the rennet influenced the pH of the cheese.

The quadratic model equation for the experiment is given below:

$pH=+3.02+0.36*C+0.52*B-0.02*C^2-0.02*B^2-0.02*C*B$………………….. (1)

Note: C- concentration of sap, B- boiling time

Effect of unripe pawpaw sap concentrations and boiling time on moisture contents of wara

The moisture contents from the experiment ranged between 34.74-50.32 %. The maximum moisture content was recorded at treatment of 3ml sap concentration in 500 ml of fresh milk and boiling time of 15 minutes while the minimum value of moisture was recorded at 3 ml of sap and 20 minutes boiling time. The mean value was 41.46, there were no significant differences in values of moisture contents obtained at ($p>0.05$). The R^2 was

0.652 and the response surface plot for the interaction between unripe pawpaw sap concentrations and boiling time is shown in figure 2

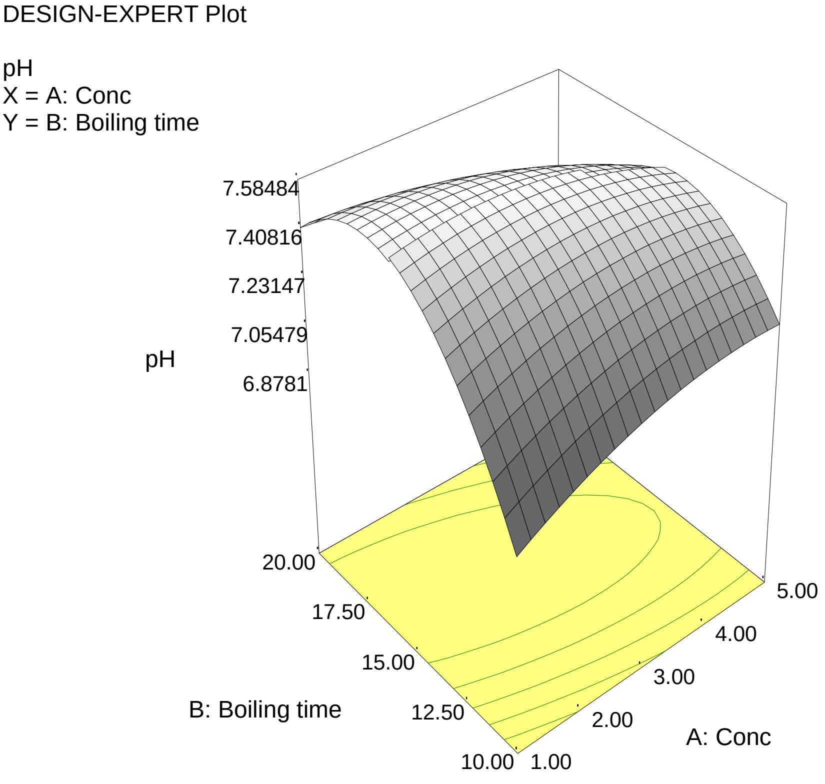

Figure 1: Effect of unripe pawpaw sap concentration and boiling time on the pH of wara

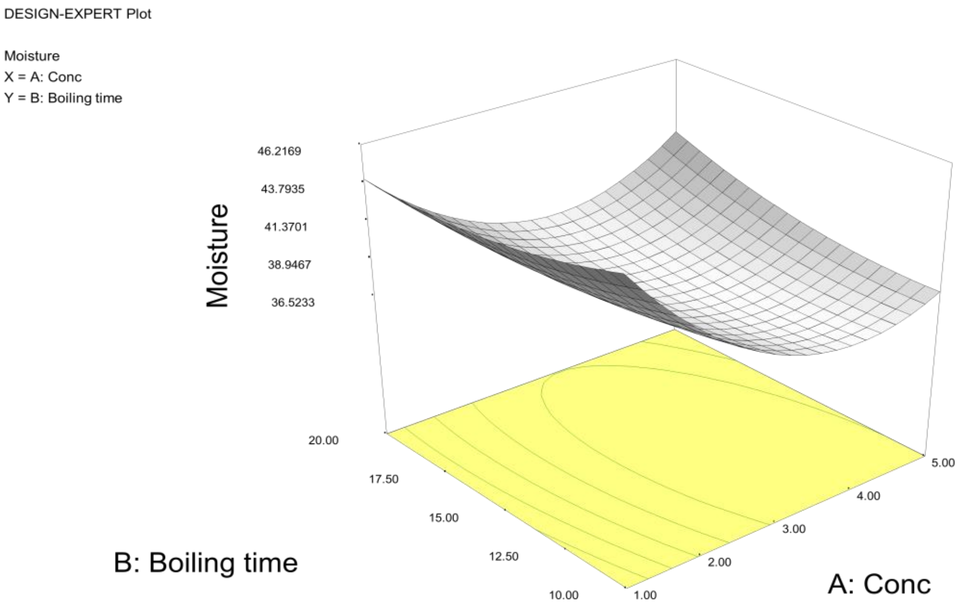

Figure 2: Effect of unripe pawpaw sap concentration and boiling time on the moisture contents of wara.

The moisture content increases as boiling time and concentration of the unripe pawpaw sap increases. Once there is a reduction in the concentration and boiling time, the moisture content decreases automatically. Galan *et al*., (2008) and Adetunji *et al*., *(2008)* recrded moisture range of 29.57-39.48 and 61.70-62.50 % respectively but with different coagulants. The variation may be due to the he moisture content varied from 61.70-62.50% in the production of cheese from *Calotropis procera* and *Carica papaya* (leaf) (Adetunji *et al*., 2008) but the moisture content of cheese produced from unripe pawpaw sap varied from 34.74-50.32 may be due to the concentration of unripe pawpaw sap and the time at which the cheese was boiled.

The linear equation that best described the model is presented below:

Moisture content=+64.73-10.03*C-1.47*B+1.11*C^2+0.4*B^2+0.13*C*B………… (2)

Effects of unripe pawpaw sap concentrations and boiling time on protein contents of wara

The protein contents from the experiment ranged between 2.54-3.86 %. The maximum protein content was recorded at treatment of 3ml sap concentration in 500 ml of fresh milk and boiling time of 10 minutes while the minimum value of moisture was recorded at 5 ml of sap and 15 minutes boiling time (Table 1). The mean value and standard deviation were 3.42 and 0.19 respectively and there were significant differences in values of protein contents obtained at ($p<0.05$). The lack of fit test was insignificant which is good for the fitness of the model. The R^2, Adjusted R^2, and Predicted R^2 were 0.832, 0.692, and 0.275 respectively. The response surface plot for the interactions between unripe pawpaw sap concentrations and boiling time is shown in figure 3.

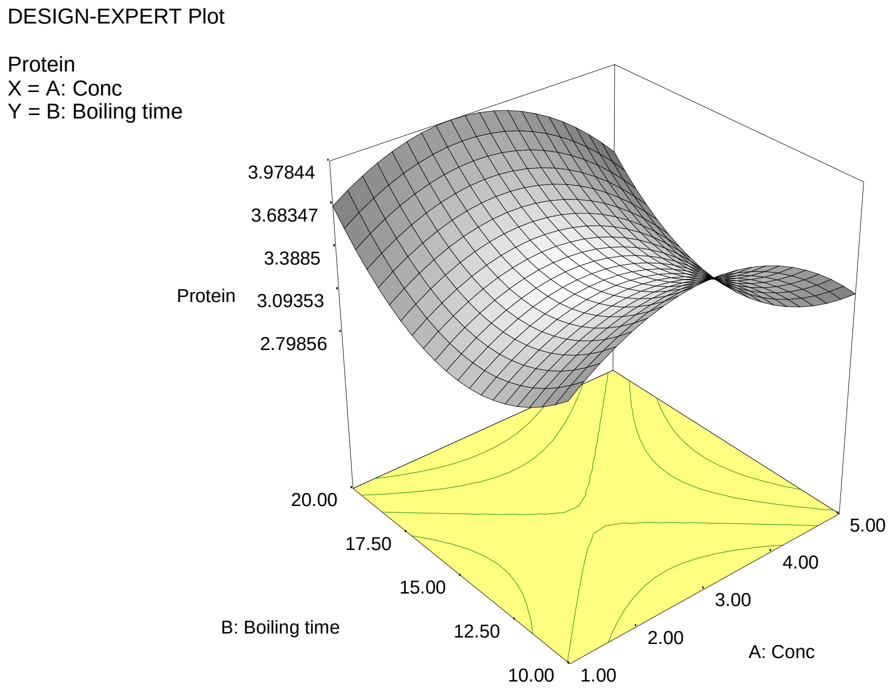

Figure 3: Effect of unripe pawpaw sap concentration and boiling time on the protein content of wara.

The Response surface plot has a hyperbolic effect on the concentration; the RS graph is concave upward which showed that mid concentration of sap favoured high protein content in wara while the minimum and maximum concentrations recorded low protein. With boiling time the plot is concave downward with the extremes boiling time favourable to high protein in wara.

In the production of cheese using vegetable coagulant from cardoon *Cynara cardunculus*, the protein content ranged between 20.99-26.94% (Galan *et al*., 2008) because the coagulant used was sourced from animal while the coagulant used in this work of study was sourced from plant. In the production of cheese using *Carica papaya* (leaf) and *Calotropis procera*, the protein content ranged from 31.60-33.84% (Adetunji *et al*., 2008) as compared to 2.54-3.86% obtained in this work. The quality of protein in raw milk may be affected by many factors such as nutrition, specie and biological value of the protein in the milk.

The quadratic equation for the model is presented below:

Protein content=+6.48+0.67*C-0.56*B-0.11*C^2+0.02*B^2-4.02E-003*C*B……… (3)

Effect of unripe pawpaw sap concentration and boiling time on ash contents of wara

The Ash content from the experiment ranged between 2.15-5.25 %. The maximum Ash content was recorded at treatment of 5ml sap concentration in 500 ml of fresh milk and boiling time of 15 minutes while the minimum value of Ash was recorded at 3 ml of sap concentration in 500 ml of fresh milk and boiling time of 20 minutes. The mean value was 3.01 and there were significant differences in values of ash contents obtained at ($p<0.05$). The R^2, Adjusted R^2, and predicted R^2 were 0.687, 0.570, and 0.4276 respectively.

The RS plot for the interaction between unripe pawpaw sap concentrations and boiling time is shown in figure 4.

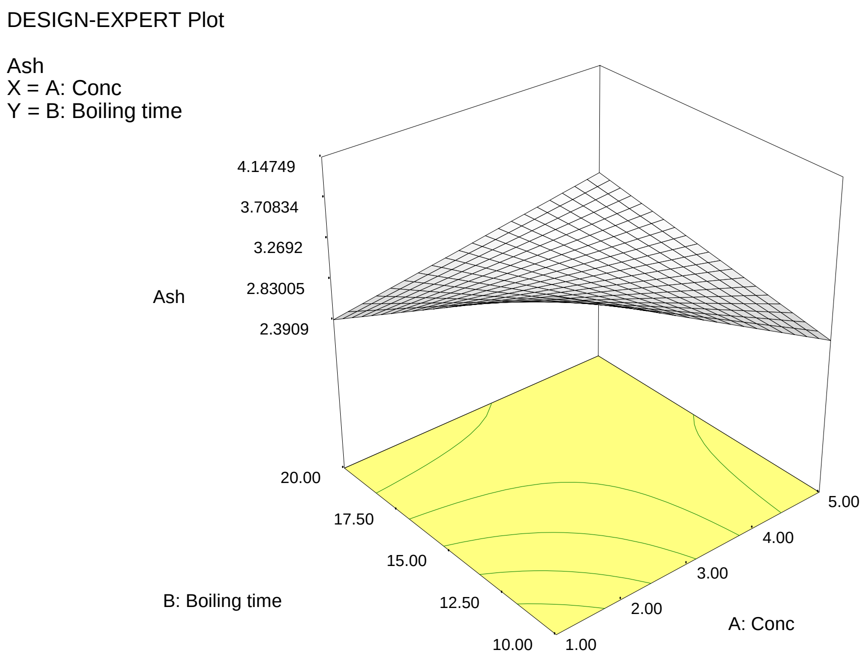

Figure 4: Effect of unripe pawpaw sap concentration and boiling time on the ash contents of wara.

The response surface plot indicates that the ash content steadily decreases with increase in concentrations of the unripe pawpaw sap and boiling time, therefore as the boiling time and concentration increases there is decrease in the ash content.

The quadratic equation for the model is presented below:

Ash content= +6.91-1.00*C- 0.23*B + 0.06*C*B…………….. (4)

Effect of unripe pawpaw sap concentration and boiling time on ether extracts of wara

The ether extract ranged between 34.5-48.62%. The maximum ether extract was recorded at treatment of 1ml sap concentration in 500 ml of fresh milk and boiling time of 15 minutes while the minimum value of ether extract was recorded at 3 ml of sap and 15 minutes boiling time

The mean value was 39.82 and there were no significant differences in the values of ether extract obtained at ($p > 0.05$).The R^2 and Adjusted R^2 were 0.328 and 0.073 respectively. The RS plot for the interaction between unripe pawpaw sap concentrations and boiling time is shown in figure 5

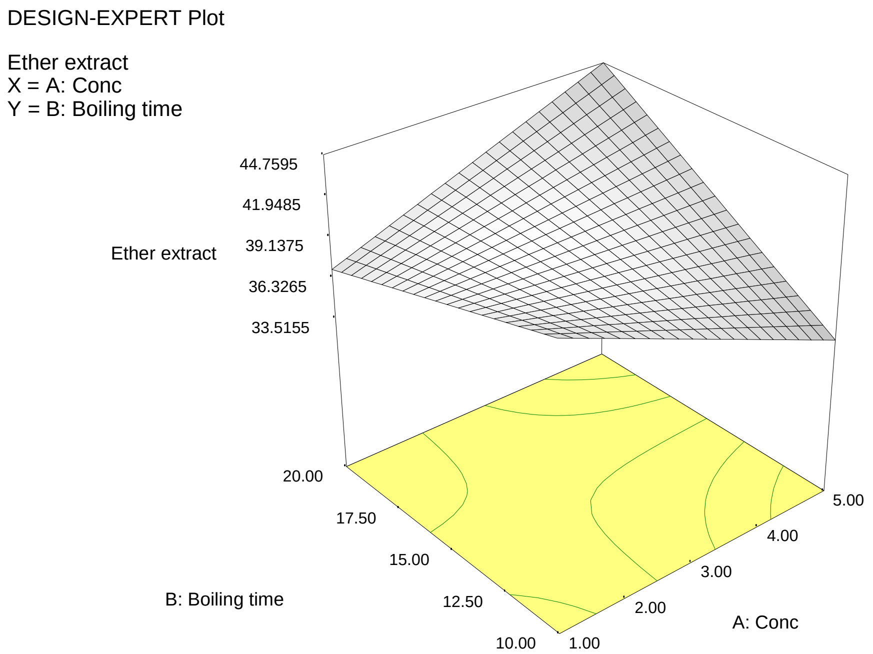

Figure 5: Effect of unripe pawpaw sap concentration and boiling time on the ether extract of wara

The response surface plots indicates an increase in the fat content of the wara as the concentration of the unripe pawpaw sap increases and a decrease in the fat content as the boiling time increases.The fat content of cheese produced from Carica papaya (leaf) and Calotropis procera ranged between 22.33-31.45% as compared to cheese produced from unripe pawpaw sap which ranged from 34.5-48.62% due to the acidity of the sap during the production of the warankashi. Therefore, the source of the rennet influenced the fat content of the cheese

The equation for the model is presented below:

Ether extract=+53.47-6.24*C-0.92*B+0.41*C*B………….. (5).

Effect of unripe pawpaw sap concentration and boiling time on calcium of wara

The calcium content from the experiment ranged between 2-12.8ppm. The maximum calcium level was recorded at treatment of 3ml sap concentration in 500 ml of fresh milk and boiling time of 20 minutes while the minimum value of calcium level was recorded at 5ml of sap and 10 minutes boiling time. The mean value was 6.68 and there was no significant differences in values of calcium level obtained at ($p>0.05$). The lack of fit test was insignificant which is good for the fitness of the model. The R^2, Adjusted R^2, and predicted R^2 were 0.308, 0.049, and -1.1912 respectively. The negative predicted R^2 implied that overall mean is the best predictor of calcium in the interaction between sap concentration and boiling time in traditional production of warankashi.

The RS plot for the interaction between unripe pawpaw sap concentrations and boiling time is shown in figure 4.6

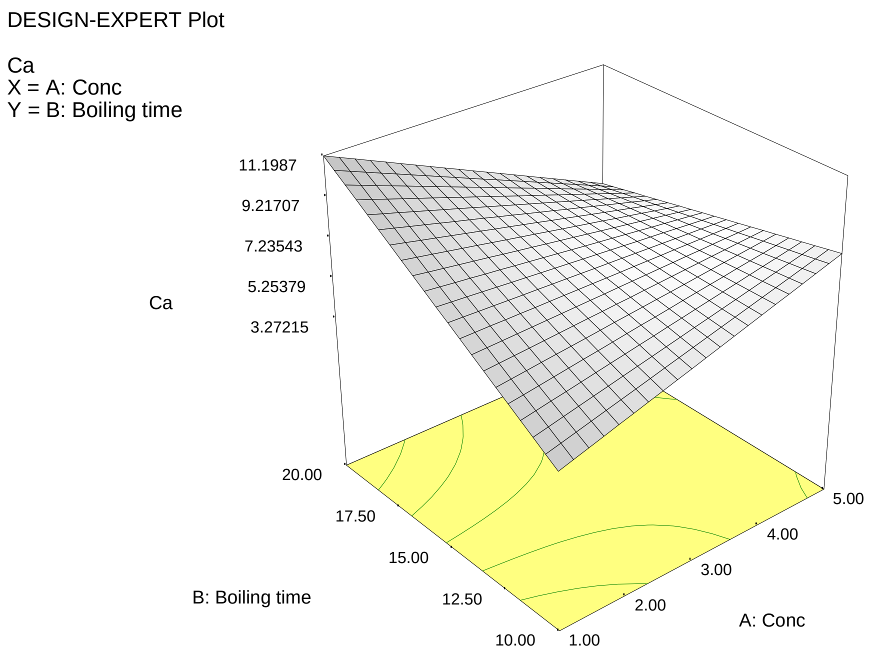

Figure 6: Effect of unripe pawpaw sap concentration and boiling time on the calcium content of wara.

The response surface plot indicates that the value of the calcium slightly increase as concentration of unripe pawpaw sap and boiling time increases.

The equation for the model is presented below:

Calcium=-8.34+3.68*C+1.05*B-0.26*C*B…………. (6)

Effect of unripe pawpaw sap concentration and boiling time on magnesium of wara

The magnesium ranged between 11-39 ppm. The maximum magnesium level was recorded at treatment of 1ml sap concentration in 500 ml of fresh milk and boiling time of 10 minutes while the minimum value of magnesium level was recorded at 1 ml of sap and 20 minutes boiling time.

The mean value was 6.61 and there were significant differences in values of magnesium level obtained at ($p<0.05$). The R^2 and Adjusted R^2 were 0.017 and 0.596 respectively.

The RS plot for the interaction between unripe pawpaw sap concentrations and boiling time is shown in figure 7

The response surface plot indicates that as the concentration of the unripe pawpaw sap is increase, there is increase in the magnesium level of the warankashi and as the boiling time also increases there is decrease in the magnesium level of the warankashi.

The quadratic equation for the model is presented below:

Magnesium=+13.14+4.65*C-0.34*B…………….. (7)

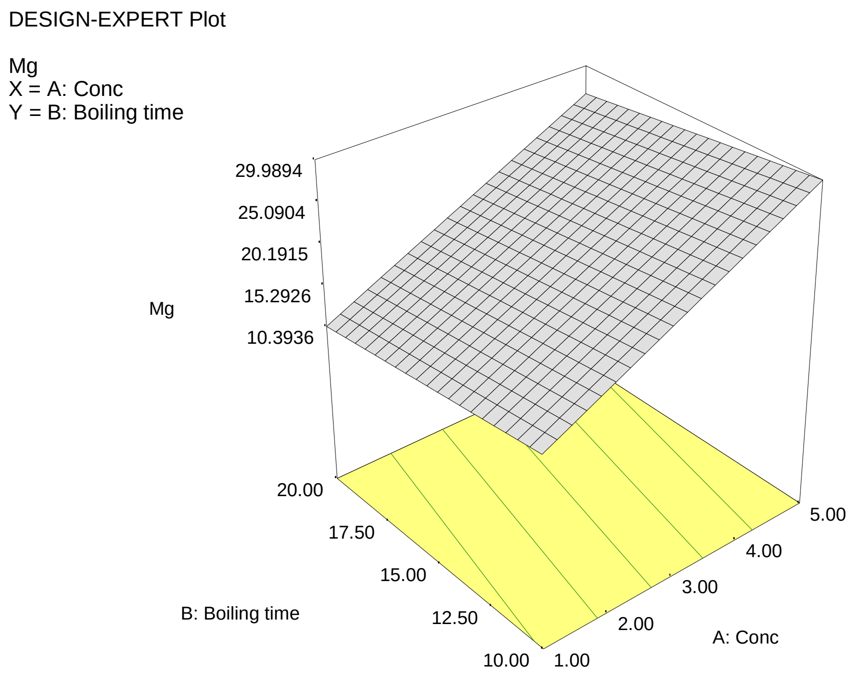

Figure 7: Effect of unripe pawpaw sap concentration and boiling time on the magnesium content of wara.

Effect of unripe pawpaw sap concentration and boiling time on iron of wara

The Iron level from the experiment ranged between 6-58 ppm. The maximum Iron level was recorded at treatment of 3ml sap concentration in 500 ml of fresh milk and boiling time of 10 minutes while the minimum value of Iron level was recorded at 5 ml of sap and 10 minutes boiling time. The mean value was 32.00 and there was no significant differences in values of magnesium level obtained at ($p>0.05$). The lack of fit test was insignificant which is good for the fitness of the model. The R^2, Adjusted R^2, and predicted R^2 were 0.521, 0.341, and -0.077 respectively. The negative predicted R^2 implied that overall mean is the best predictor of Iron level in the interaction between sap concentration and boiling time in traditional production of wara. The RS plot for the interaction between unripe pawpaw sap concentrations and boiling time is shown in figure 8.

The response surface plot indicates that an increase in the concentration of the unripe pawpaw sap and increase in the boiling time reduces the Iron level in the warankashi produced. The iron level gotten from the variation of *Carica papaya* (leaf) and *Calotropis procera* varied from 4.77-4.88ppm (Adetunji *et al*., 2008) as compared to the cheese produced from the unripe pawpaw sap which varied from 6-40.5ppm as a result of the concentration of the unripe pawpaw sap and the boiling time during the preparation of the warankashi.

The equation for the model is presented below:

Iron=+101.95-17.06*C-4.35*B+1.02*C*B……………… (8)

The 2F1 model best describes the concentration of unripe pawpaw sap and boiling point on the iron of wara.

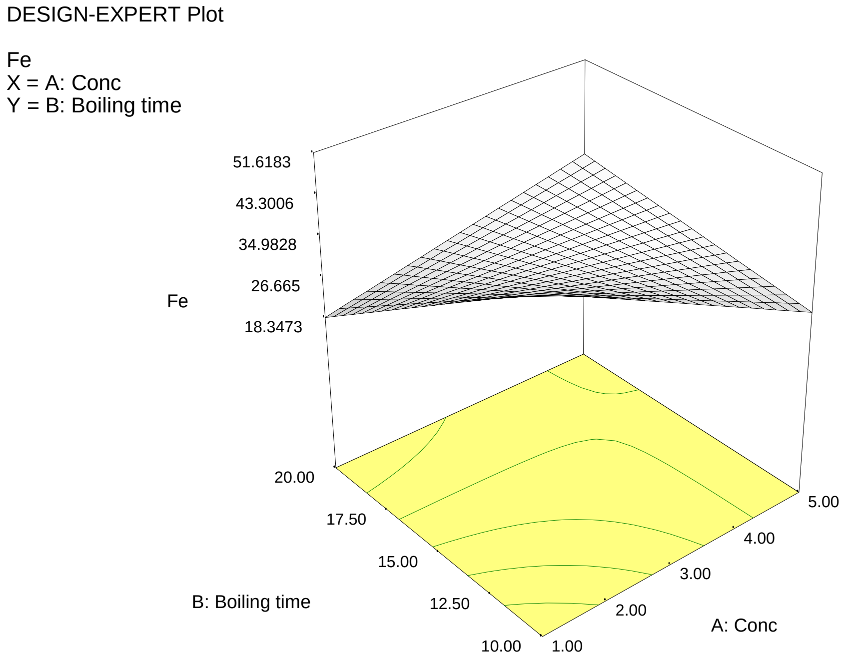

Figure 8: Effect of unripe pawpaw sap concentration and boiling time on the iron content of wara

Optimization of the use of unripe pawpaw sap concentrations and boiling time on manufacturing of wara

The result showing appropriate combinations for the production of wara with high protein content, high fat, high mineral content, maximum Ca, Mg, and Fe as generated by the optimization process is presented in Table 2.

Table 2: **Result of optimisation for wara production**

No	Concentration (ml/ml)	Boiling time (minutes)	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Desirability
421	3.75	10	36.84	3.67	3	36.2	6.2	24.9	32.7	0.62
521	3.73	20	38.35	3.84	2.8	42.3	6.97	21.4	27.3	0.575
568	3.69	20	38.32	3.85	2.8	42.2	7.02	21.3	27.2	0.575

Sensory assessment of warankashi

The sensory evaluation showed significant difference ($p<0.05$) between samples 502 and 421 and insignificant difference ($p>0.05$) between samples 502 and 568, there was also a significant difference ($p<0.05$) between samples 568 and 421. From the sensory evaluation carried out, it showed that 41.6% preferred sample 568, 33.3% preferred sample 502 and 25% preferred sample 421.Therefore, sample 568 was the most preferred though the panelist liked all the samples but there was still variance in their judgments. The sensory evaluation also showed that the colour of sample 568 was more acceptable than samples 502 and 421; the texture of sample

421 was more acceptable than samples 502 and 568; the aroma of sample 568 was more acceptable than samples 502 and 421; the overall acceptability was for sample 568.

Conclusion

This study has shown that the unripe pawpaw sap concentrations and boiling time have influence on chemical and mineral composition of wara. It is revealed that the variation the boiling time and the concentration of the unripe pawpaw sap in the production of warankashi have significant effects ($p<0.05$) on the pH, protein, ash and magnesium while no significant effect were found ($p>0.05$) on the moisture, ether extract, calcium, iron of the wara. The quadratic models best described the effect of unripe pawpaw sap and boiling time on pH, protein, ash and magnesium of wara.2F1 factorial best described ether extract and iron, linear model for moisture description while calcium was best described by the mean. Sensory evaluation conducted on optimized products revealed combination of 3.69mls in 500mls for 20mins was the best(568) and gave a yield of 38.32 % moisture, 3.85 % protein, 2.75 % ash, 42.21 % fat, 7.02 ppm calcium, 21.29 ppm magnesium, 27.21 ppm iron and 0.575 desirability. The study also revealed that the use of unripe pawpaw for the production of warankashi is a suitable alternative coagulant and can be recommended for use at cottage level and wara manufacturing industries.

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