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Research article

Development of Chili Sauce from Pineapple and Banana Fortified with Eggshell Calcium

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mixture design

Abstract

Sauce is a popular condiment product. Thailand is a good source of pineapples and bananas, which are high in nutrients. Eggshell is also a source of calcium. This research developed a sauce from pineapple var. Phu Lae mixed with ripened banana var. Nam Wah and supplemented with calcium from eggshells. The suitable quantities of pineapple, banana and pineapple peel and core extract were studied based on a mixture design. The physical and chemical properties, sensory evaluation, three suitable calcium levels (0, 2, and 4%) and two pasteurization temperature levels (80-85ºC and 90-95ºC) were studied. Response surface methodology was applied and showed that all 3 components had significant effects on the sauce quality at the 0.05 level. The optimum product comprised 25.27% pineapple, 0.87% banana, 13.86% pineapple peel and core extract, 16% pickled red chili peppers, 11% pickled garlic bulbs, 26% sucrose, 2% salt, 1% vinegar, 2.12% water, and 1.88% calcium from the powdered eggshell. The product had an orange color, a pH of 4.20, and 1.61% acidity. This sauce was high in nutrients: 41.03% carbohydrate, 14.90% protein, 1.98% fat, 0.453% eggshell calcium, and 14.03 mg/kg total vitamin C. The antioxidant activity was 22.94 mg ascorbic acid/g. The optimum pasteurization temperature was 80-85ºC for 15 min. The sauce was moderately liked by panelists and has the potential to be processed into a low-cost commercial condiment.

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1. Introduction

Condiments are popular products that have shown considerable growth during the COVID-19 situation, particular over the past year. In the year 2020, the condiment industry was worth 47,977 million baht and grew 4.8%. Thai people consume an average of 5.8 kg of condiments per person per year [1]. According to the announcement of the Ministry of Public Health (No. 201) [2], chili sauce is a popular product that is a homogeneous thick liquid and is classified as a define 'Thai' quality and standard food. It is one of the five types of Thai sauces that contain chili peppers, vinegar, or other edible acids as their main ingredients. Chili sauce must have the following important qualities: the specific flavor of the sauce, not more than 10% acetic acid content, not less than 20% of total soluble solid (TSS), total bacterial count not more than $1x10^4$ cfu/g, *E. coli* less than 3 MPN/g (as determined by MPN method), yeast and mold not more than 10 cfu/g, no pathogenic microorganisms, no microbial toxins in amounts that may be harmful to health, etc.

Pineapple cv. Phu-Lae (*Ananas comosus* (L.) Merr) is a widely cultivated plant in Chiang Rai province that has been given a GI (geographical indication). The fruit is small (about 200-300 g) and both the pulp and core can be eaten fresh or cooked in various foods to provide a sweet, fragrant, crispy texture that does not burn the tongue. Pineapple is easy to grow, drought tolerant and produces fruit rapidly. However, the fruits from some harvests may be too large, of bad shape and be perishable. Such fruit requires processing to add value [3, 4]. The main substances found in pineapple are phytoestrogens, isoflavones, lignans, phenolics, citric acid, malic acid, vitamins, and enzyme bromelain. On average, 100 g Phu Lae pineapple provides 50 kcal of energy, 3.36 µg beta carotene, 1.68 g of total dietary fiber and 1.66 mg of bromelain [5]. Bromelain is a protease enzyme that has great potential in food industry [6]. Ketnawa and Rawdkuen [7] used bromelain to tenderize beef, chicken and squid. Mahrus *et al*. [8] prepared a sardine sauce using mixed pineapple fruit extracts. Promhhan *et al.* [9] reported that pineapple sauce consisted of 55.8% pineapple, 13.95% pickled chili and 23.25 % sucrose.

Banana *(Musa sapientum* Linn., ABB group) cv. Kluai Namwa is a common plant that is found in all regions of Thailand. It produces fruit throughout the year. Rat-a-pa *et al*. [10] reported that ripe banana fruit (100 g) contained high levels of carbohydrate (28.02 g), fiber (0.57 g), protein (0.73 g) and ash (0.95 g) and was low in fat (0.38 g). It also contained potassium (324 mg/100 g) and magnesium (27.08) [11], which are very important for the body. Boyd *et al.* [12] reported that low concentrations of potassium and magnesium in serum were implicated in the etiology of cardiac arrhythmias. Research has been carried out on chili sauce made from ripe banana fruits [13, 14] and raw banana flour fruits [15]. The high levels of carbohydrates found in banana help to prevent separation of the texture of the sauce and give it a sweet taste.

Eggshells constitute a large amount of the waste from the egg processing industry and incur a cost of disposal. Some eggshell is processed into fertilizers or animal feed, which are low-value products. Eggshells contain up to 98.2% calcium carbonate ($CaCO_3$) [16]. Rovenský *et al.* [17] reported that chicken eggshell powder (CP) improved the bone density in animal models of postmenopausal osteoporosis in ovariectomized female rats. It reduces pain and osteoresorption and increases mobility and bone density (or arrests its loss) in postmenopausal women and women with senile osteoporosis. The bioavailability of CP calcium was similar or better than that of purified calcium carbonate. Schaafsma *et al.* [18] reported that CP had about 39% Ca, with high level of Sr, and low levels of Al, Pb, Cd and Hg. A dairy-based CP supplement increased the bone mineral density of subjects with low bone mass and delayed bone demineralization [19]. Schaafsma and Beelen [20] found that CP was better than purified calcium carbonate. Hassan [21] reported on CP calcium supplement in biscuits of up to 6%. Panton and Suksamran [22] prepared CP for dietary supplements, and CP was supplemented in rotten beans [23]. CP was applied in extrusion cooking of rice [24]. Although calcium carbonate is insoluble in water, it can be solubilized in acid solution

to form a soluble salt that helps improve absorption [25]. Barbara *et al.* [26] studied the kinetics of calcium release in artificial gastric juice and confirmed that eggshell calcium in citrate tablets was more rapidly released than it was from tablets made with synthetic calcium carbonate. Extracted eggshell calcium with acid (EA) has been used in the food industry. EA with acetic acid or gluconic acid was used in soybean paste [27]; EA with lactic acid and gluconic acid was used in yogurt [28]; EA with hydrochloric acid was used as a firming agent in canned rambutan [29] and for maintaining the quality of fresh-cut fruits [30]. Its use in Thailand and China has been patented [31-35].

Therefore, this research aimed to make an innovative sauce from pineapple that had a sweet and sour taste suitable for making sauces. The carbohydrates from banana help the texture and can provide sweetness instead of sugar. Since there is no record of calcium-fortified sauces, the sauce developed in this study is fortified with calcium from eggshells. The sauce would be agreeable to consumers and would also support better utilization of pineapple, banana, and of waste eggshells.

2. Materials and Methods

Pineapple (cv. Phu Lae fruits, 80% mature), ripe bananas (white center pulp), long red chili peppers, garlic bulbs, white sucrose, salt, vinegar (5% acetic acid) and brown chicken eggshell were purchased from a local market in Thailand. Monohydrated citric acid (food grade) from China was purchased from the Thai Food and Chemical Co. Ltd. in Thailand.

2.1 Chili sauce materials preparation

Five pineapples were washed and measured for size, and the pulp was then chopped and ground using a 600 W electric grinder. This was followed by heating at 90ºC for 1 min and storage at -20ºC. The pineapple peels and cores were chopped in a blender with 50% added water and then filtered through a thin white cloth, followed by heating and storage as had been done for the pineapple pulp. The banana fruits were peeled and the seeds removed, followed by heating and storage as was done for the pineapple pulp. The peppers were washed and steamed at 100ºC for 3 min and left to cool. They were then placed in a jar, immersed in vinegar, and left for 20 days. The cloves of garlic were peeled and handled the same as the peppers. After 20 days, they were filtered through a colander, processed in an electric blender and stored at -20ºC.

2.2 The eggshell calcium extraction

The eggshells were washed and dried in a hot-air oven at 100ºC for 10 min, after which they were ground thoroughly and filtered through a 2 mm sieve. The eggshell powder was placed in a jar, and vinegar (5%) at a ratio of 1:9 by weight was added and thn left for 20 days. Afterwards, it was passed through No. 4 (Whatman, England) filter paper and dried in a hot-air oven at 200ºC for 3 h. The dried eggshell calcium was ground using an electric blender, filtered through a 2 mm sieve, stored in a sealed jar, and the color, a_w and calcium content were measured.

2.3 The optimum basic sauce formula

Mixture design (extreme vertex design) [36] was used because there were constraints on the use of three main components. The proportion of pineapple had to be less than 100%, the peel and core extract in the sauce recipe were required to be less than 50%, while the proportion of banana was required to be less than 20%. Thus, the three components were: 1) 4-99% pineapple (X); 2) 0-50%

peel and core extract (Y); and 3) 0-20% banana (Z). The design had 13 sauce formula combinations (Table 1) and the simplex design was shown in Figure 1. The other ingredients were pickled pepper, pickled garlic, sucrose, salt, vinegar and water at 16, 11, 26, 2, 1 and 4%, respectively. The sauce production process is shown in Figure 2.

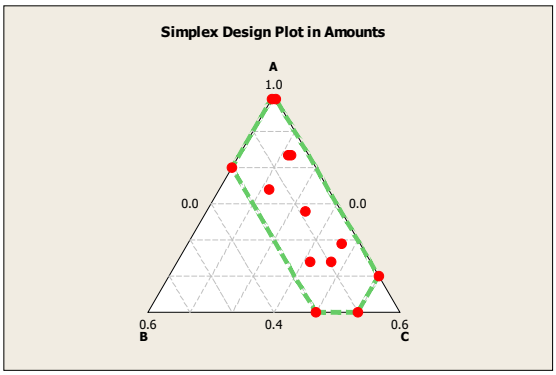


Figure 1. Simplex design plot of three ingredients: pineapple (A), banana (B), and pineapple peel and core extract (C), used for making sauce.

Table 1. Three major ingredients: pineapple (A), banana (B), pineapple peel and core extract (C), by weight (g) from mixture design of sauce processing

Formula	1	2	3	4	5	7	8	9	10	11	12	13
A	54.00	83.50	99.00	83.50	40.00	52.50	56.75	74.00	99.00	80.00	40	50
B	14.25	4.25	0	1.90	20.00	9.25	8.50	14.25	1.00	20.00	10	0
C	31.75	12.25	1.00	4.70	40	36.75	23.50	11.75	0	0	50	50

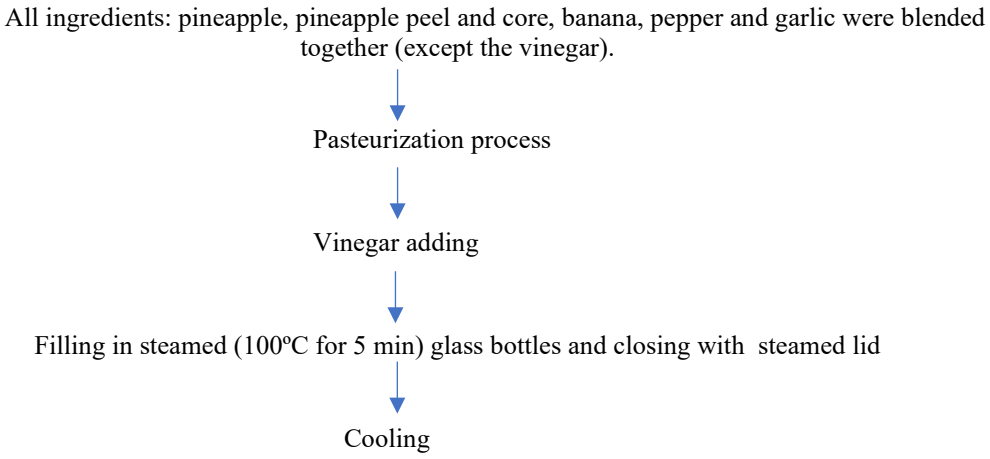


Figure 2. Sauce production process

2.4 Optimum pasteurization process

A completely randomized design (CRD) with 2 treatments was used consisting of 80-85ºC for 15 min or 90-95ºC for 1 min, with 2 replications.

2.5 Calcium fortification

CRD with 3 treatments of eggshell calcium powder (CP) as 0, 2 and 4% by weight of sauce, with 2 replications were performed. The optimum calcium content was calculated using response surface analysis [37].

2.6 Analysis of physico-chemical properties

The color analysis was conducted using a spectrophotometer (Spectraflash 600 plus, Datacolor International, USA) and recorded as L* = lightness (0 = black, 100 = white), a*(-a* = greenness, +a* = redness) and b* (-b* = blueness, b* = yellowness). The total soluble solids (TSS) were measured using a refractometer (MASTER series, Atago, Japan) and the pH using a pH meter (AMTAST, USA). The acidity was determined as citric acid [38]. Vitamin C (ascorbic acid) content was determined by titration against 2,6-dichlorophenol indophenol [39]. The water activity (a_w) was determined using a Novasina, EEJA, instrument (Switzerland), and analyzed for Ca using an in-house method (WI-TMC-19 based on 984.27 in AOAC, 2019) [40].

2.7 Determination of antioxidant activity

The antioxidant activity was assayed via DPPH assay [41]. In brief, the samples were blended with 95% ethanol solvent at a ratio of 10:75 weight (measured in grams) per volume (measured in milliliters). The extract sample obtained (0.15 mL) was mixed with 0.1 mM DPPH solution in methanol (0.9 mL) and left at 25°C for 20 min. Then, the absorbance was measured at a wavelength of 517 nm using a UV-spectrophotometer and compared with 100% methanol as a blank. The optical density (OD) was calculated as the percentage radical scavenging activity (%RSA) using equation (1):

$$\%RSA = (OD\ blank - OD\ of\ sample\ extract) \times 100 / OD\ blank \quad (1)$$

The %RSA value was used to calculate the antioxidant capacity of the sample from the standard graph of vitamin C and reported as vitamin C equivalents.

2.8 Sensory evaluation

The sensory evaluation (flavor and overall liking) based on a 7-point hedonic scale method was used, where 7 indicated like very much and 1 indicated dislike very much by untrained thirty volunteer panelists.

2.9 Statistical analysis

The data from the mixture design was analyzed with a Minitab 15 software**.** Data variance from CRD was analyzed using analysis of variance (ANOVA) and mean differences were compared by Duncan's new multiple range test method performed using the SPSS statistical software program (SPSS for windows Ver. 12.0, now a part of IBM Corp.; White Plains, NY, USA).

3. Results and Discussion

3.1 Materials for chili sauce preparation

The pineapple fruits weighed 363-376 g/fruit and on average were 26.41 cm wide and 11.48 cm long. The pulp (44.63% yield) was dark yellow color, of sweet taste and slightly acidic taste (pH 4.31), with 0.36% acid content as citric acid, and had a value of 25.36 ºB for the TSS. The peel and core extracts of the pineapple were slightly greenish yellow in color with less taste than the pineapple pulp, and had pH of 4.32, 0.35% acid content as citric acid and 24.61ºB for the TSS. For the banana fruit, there was 80-85 g/fruit. The pulp, 56.90% yield, was light yellow color, had a very sweet taste, pH of 4.38, 0.31% acid content as citric acid and 28.05ºB of TSS.

3.2 Eggshell calcium extraction

Because the eggshell does not dissolve in water and is not readily edible [42], it must be dissolved in an acid such as lactic acid, gluconic acid [28], hydrochloric acid [29] or acetic acid [31, 35]. In the current research, the eggshells were soaked in vinegar (acetic acid) that is commonly used in cooking, so the process is considered as a safe one in making edible products. When eggshells were immersed in vinegar, the pH of the vinegar solution continued to increase in the range 2.49-5.48 because the acetic acid (CH_3COOH) reacted with the eggshells (which contained $CaCO_3$) to form calcium acetate ($Ca(C_2H_3O_2)_2$). Carbonic acid was converted to water and carbon dioxide, the bubbles of which rose to the surface of the solution (see equation 2 for the chemical formula). This reaction reduced the thickness of the eggshells, and they became brittle [43].

$$CaCO_3(solid) + 2CH_3COOH\ (aqueous) \rightarrow Ca(CH_3COO)_2\ (aqueous) + H_2O\ (liquid) + CO_2\ (gas) \quad (2)$$

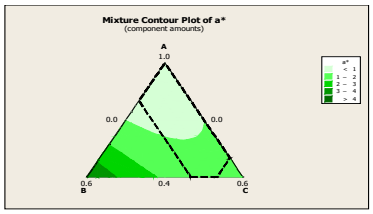
The texture of eggshells became soft after 20 days of soaking which indicated that the calcium carbonate in the eggshells were probably completely dissolved; therefore, the liquid extract was then filtered through a thin white cloth and dried to obtain calcium acetate powder extract (ECP) resulting in 50.51% yield based on raw eggshell weight with 0.61 of a_w and 24.10% of calcium content. The ECP was white with color of 94.87L*, 0.33a* and 4.50b*. The part of the membrane that had not been dissolved in the vinegar had a high protein content, which was 41.90% of the ECP weight [44].

3.3 The optimum basic sauce formula

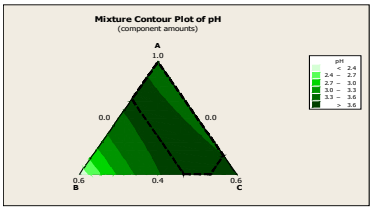
The results in Table 2 show that all formulas had different red a* values in shades of orange to red orange, which were mainly derived from the color of the peppers. The pH (3.40-3.81) was in the acidic range, which met the Thai standard for a pH of less than 4.5. In addition, flavor and overall liking were in the slight-to-moderate range. The mixture design analysis contour plots shown in Figure 3 indicate that the curves of all response values changed according to the banana content; the red value a* decreased when the banana content was reduced. The flavor and overall liking by the panelists improved with reduced amount of banana, perhaps because the bananas produced a yellow color and sweet taste that reduced the concentration of the sauce flavor.

Table 2. The pH value, a* value, and sensory scores of 13 sauce formulas

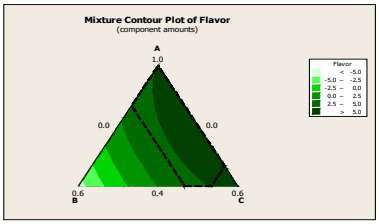
Item/Formula	pH Value	a* Color	Sensory Evaluation	
			Flavor	Overall Liking
1	3.56±0.04	1.45±0.04	5.17±0.95	5.67±0.88
2	3.63±0.02	0.63±0.01	5.60±0.72	5.73±0.64
3	3.40±0.04	0.75±0.01	5.10±0.88	5.23±0.86
4	3.58±0.02	0.62±0.01	5.50±1.14	5.93±0.58
5	3.80±0.0	0.55±0.00	5.60±0.62	5.83±0.53
6	3.70±0.01	1.01±0.01	5.47±0.68	5.37±0.72
7	3.56±0.04	0.82±0.01	5.33±0.92	6.20±0.66
8	3.77±0.0	1.00±0.00	5.33±0.73	5.50±0.68
9	3.59±0.02	0.50±0.00	4.80±0.71	5.20±0.55
10	3.41±0.01	0.78±0.01	5.15±0.97	5.67±0.92
11	3.69±0.04	0.41±0.01	5.27±0.83	5.67±0.71
12	3.81±0.01	0.56±0.33	5.13±5.70	5.30±0.65
13	3.59±0.02	1.45±0.06	5.33±0.80	5.40±0.56



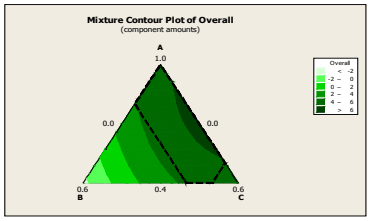
(a) Contour plot of a* color



(b) Contour plot of pH



(c) Contour plot of flavor



(d) Contour plot of overall liking

Figure 3. Contour plots of three sauce components (pineapple (A), banana (B) and pineapple peel and core extract (C)) of a* color (a), pH (b) and sensory evaluation of flavor (c) and sensory evaluation of overall liking (d)

The optimization tool in the design of experiment was utilized to find the best proportion of each component by setting the sensory test values for flavor and overall liking. The resulting optimal plot is shown in Figure 4, which indicates that when the amounts of pineapple (A), banana (B) and peel and core extract (C) were 63.19%, 2.17% and 34.6%, respectively, the rated flavor and overall liking of the developed sauce were 6.05 and 6.20, respectively, which were at the moderately like level that was considered satisfactory for this project. The main effect and 2-way interactions were included in the regression model as shown in Table 3.

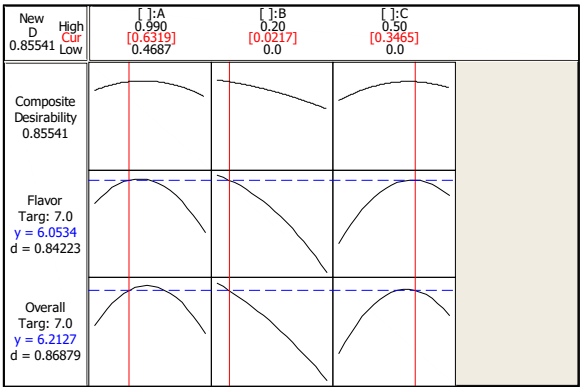


Figure 4. Optimal three sauce components: A= pineapple, B= banana and C = pineapple peel and core extract

Table 3. R^2 values and regression model

Item	R^2 values	Regression model
pH value	0.6735	3.424A –1.901B +4.055C + 8.151 A*B -0.578 A*C+7.190 B*C
a* color	0.8703	3.424A –1.901B +4.055C + 8.151 A*B -0.578 A*C+7.190 B*C
Flavor	0.7222	5.02A –24.83B + 1.13 C +28.71 A*B + 10.94 A*C+ 32.09 B*C
Overall liking	0.6892	5.36A – 13.35B – 0.09C + 13.70A*B + 12.77A*C + 21.11B*C

The R^2 values of all responses were satisfactory, indicating that the predictive equations could be used when there were changes to the mixture components of pineapple, banana, or pineapple peel extracts.

3.4 The optimum pasteurization process

The analysis results for color and pH values are shown in Table 4. Only the red a* values were significantly different at the high temperature treatment (90-95ºC), which gave a more intense red color. This may have been due to the Maillard reaction of the amino acids and monosaccharide molecules [45]. The sensory results are shown in Table 5; the panelists liked all characteristics in the medium range, with the color, odor and texture not being different but the flavor and overall liking were different. The panelists preferred the sauces with a low pasteurization temperature because they had a better flavor. This temperature corresponded to the pasteurization of ripe banana sauce and raw banana flour sauce [13-15]. This product was highly acidic (below pH 4.6), so it could be commercially pasteurized at 100°C to destroy heat-labile spoilage organisms such as nonspore-forming bacteria, yeasts, and molds [46]. Siripanaporn *et al*. [13] found that the shelf life of banana sauce (pH < 4) that had been stored in hot-filled containers was ≥ 6 months in a sealed pasteurized jar.

Table 4. Color and pH of the pasteurized sauce using two different conditions

Temperature	L* value	a* value	b* value	pH value
80-85ºC	0.36±0.49^a	0.86±0.50^b	0.86±0.01^a	3.91±0.01^a
90-95ºC	0.38±0.00^a	1.85±0.57^a	0.19±0.07^a	3.93±0.01^a

Means in each column with same lowercase superscript are not significantly ($p > 0.05$) different.

Table 5. Sensory score of the pasteurized sauce using two different conditions

Temperature	Color	Odor	Flavor	Texture	Overall
80-85ºC	5.67±0.64[a]	5.73±0.41[a]	6.23±0.53[a]	5.53±0.39[a]	6.28±0.55[a]
90-95ºC	5.77±1.01[a]	5.50±0.67[a]	5.70±0.67[b]	5.63±0.38[a]	5.77±0.60[b]

Means in each column with same lowercase superscript are not significantly ($p > 0.05$) different.

3.5 Calcium fortification

Calcium extract obtained from eggshells provides calcium in the form of an acetate salt which is water soluble [47], so it mixes well in sauces. The addition of calcium increased the brightness of the sauce, lowered the red value, and increased the yellow value, as shown in Table 6, because calcium acetate has a white color. The soluble solids content increased with increasing amount of calcium acetate because the calcium acetate powder had a weak acidity of about 5.48, causing the pH to increase and acidity to decrease (1.81-1.50%). The sensory evaluation (Table 7) showed that all the panelists liked the color of the formula without calcium the most because it was less red in color; however, the values for odor, flavor, texture, and overall liking indicated that the panelists liked the formula with 2% calcium the most (at the moderate level) because it was not too sour or too pungent. The panelists did not like the formula with 4% calcium because it was bitter, and the color was too light. As this was a quantitative study, the optimal response for overall liking can be used for analysis, as shown in equation 3. The most suitable calcium (N) content was 1.88. When this N value was substituted in this equation, the overall liking was 6.74 (moderately favorable). The amount of calcium in 1.88 g of calcium acetate powder contains 0.453 g or 453 mg of calcium. The recommended daily calcium intake in the United States for healthy adults is 1000 mg [48]. If 20 g of sauce is consumed, this provides 90.60 mg of calcium, or 11.03% of the recommended daily calcium intake.

$$\bar{y} = -2.0202N^2 + 7.605N - 0.424 \quad (3)$$

Table 6. Color, TSS and pH of sauce supplemented with eggshell calcium powder

Calcium (%)	L*	a*	b*	TSS (ºB)	pH
0	0.12±0.05[c]	1.45±0.06[a]	0.06±0.01[c]	35.10±0.14[a]	3.84±0.02[c]
2	0.24±0.01[b]	0.94±0.01[b]	0.25±0.01[b]	42.70±0.57[b]	4.52±0.01[b]
4	0.36±0.02[a]	0.42±0.04[c]	0.51±0.01[a]	44.10±0.14[c]	4.81±0.01[a]

Means in each column with same lowercase superscript are not significantly ($p > 0.05$) different.

Table 7. Sensory evaluation of sauce supplemented with eggshell calcium powder

Calcium (%)	Color	Odor	Flavor	Texture	Overall
0	6.43±0.02[a]	5.50±1.04[b]	5.33±0.66[b]	5.40±0.93[b]	5.63±0.61[b]
2	5.80±1.04[b]	6.27±0.74[a]	6.67±0.61[a]	6.30±0.70[a]	6.70±0.53[a]
4	3.87±1.22[c]	5.10±1.47[c]	3.47±1.33[c]	4.23±1.50[c]	3.73±1.28[c]

Means in each column with same lowercase superscript are not significantly ($p > 0.05$) different.

For our developed sauce, 100 g contained 14.03 mg of vitamin C which likely came mainly from pineapple because raw pineapple contains 56.8 mg vitamin C/100 g [49]. The vitamin C in this sauce was reduced (23.29%) from the raw material because it is a heat-sensitive degradation [50]. The antioxidant activity was 22.99 mg of ascorbic acid/g by the DPPH assay method, a commonly used antioxidant assay, which is convenient, quick, and easy to analyze and can be run in the most rudimentary laboratories with the provision of high accuracy and precision [51-53]. An amount of 100 g of sauce provided 138.31 kcal, 31.94 g, 1.22 g, 0.63 g and 0.98 g of energy, carbohydrates, protein, fat, and dietary fiber, respectively. The highest content was the carbohydrates which was probably sucrose. Sugar in the diet is associated with increased risk of obesity and other chronic diseases [54]. Therefore, there should be ways to reduce carbohydrates in the sauce for health benefit.

4. Conclusions

A new chili sauce, which contained pineapple and banana, and was fortified with calcium from eggshells and had considerable nutritional value, was successfully developed. Its sensory attributes were deemed acceptable by sensory evaluation panelists. Production involves a simple pasteurization process that can be run commercially. However, its shelf life and marketing should be further studied.

5. Acknowledgements

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