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Optimization of Pre-Gelatinized Palmyrah Tuber Flour-Based Bread Formulation Using Response Surface Methodology

Sobini Nithiyananthan[1]*, Tharsika Ravindiran[2], Lasanthi Jayathunge[2]

1Palmyrah Research Institute, Kandy Road, Kaithady, Jaffna, Sri Lanka

2Department of Biosystems Technology, University of Sri Jayewardenepura, 10100, Sri Lanka

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Abstract

Palmyrah (*Borassus flabellifer* L.) tuber flour (PTF) is a locally available cheap raw material with many nutritional and medicinal properties. However, the PTF does not contain gluten which is essential to improve the texture of the bread. Hence, the pre-gelatinization technique is used in the development of PTF bread to improve the texture of the bread. This research aimed to develop a composite flour bread with a combination of pre-gelatinized PTF and wheat flour. Response surface methodology (Box-Behnken design) was used to analyze the responses of specific volume, crumb pH, moisture content, and hardness for the variables of PTF content (10, 25, 40 %), pregelatinized PTF content (2.5, 3.75, 5.0 %) and water content (25, 30, 35 %). The best formulation of 10 % PTF, 5 % pregelatinized PTF and 35% water content was identified as the optimized parameters for making bread. The results showed that the optimized PTF bread had significantly ($P<0.05$) high energy (1741.25±7.90 kJ/100 g), crude fiber (4.60±0.01 g/100 g), sodium (423.77±3.72 mg/100 g), calcium content (61.33±6.11 mg/100 g), potassium (243.9±22.00 mg/100 g) and total polyphenol (1.307±0.11 mgGAE/g) compared to control treatment. The physicochemical properties of PTF bread such as water activity, ash content, acid insoluble ash, crust and crumb colour differed significantly from the

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*Corresponding author
Postal address: Palmyrah Research Institute, Kandy Road, Kaithady, Jaffna, Sri Lanka
Email: sobinithi30@gmail.com

control. However, there is no significant difference in pH, moisture content and sensory properties between the two treatments.

Hence, the pre-gelatinized PTF can be used as a major ingredient to reduce the quantity of wheat flour in making bread.

Keywords: Box-Behnken design, Palmyrah tuber flour bread, pre-gelatinization, specific volume, wheat flour

Introduction

In recent decades, the demand for convenient foods made from wheat has increased with population increase, urbanization and changes in food habits. Since wheat cannot be grown in tropical countries, they are forced to depend on expensive wheat imports that must be paid with high foreign currency expenditure. Since bread is the major wheat-based food that is frequently consumed by the largest fraction of the global population, there is a need to introduce bread with partial substitutions of locally available flour types (Samuel, 1996).

Palmyrah products have significant demand in the local and export markets. There is a potential of producing about 5000 metric tons of Palmyrah tuber flour (PTF) per year in Sri Lanka. However, the amount that is used for value addition is insignificant (Krishanthy & Mahendran, 2008). PTF flour is rich in carbohydrates, protein and fiber, however it contains a limited amount of fat. The flour is a valuable source of micronutrients like iron, sodium, calcium, magnesium , *etc* (Mason & Henry, 1989).Therefore, the investigation of the possibility of using PTF as an ingredient in bread manufacturing without significant changes in baking and sensory properties to pure wheat bread is very promising in terms of an economical point of view. Furthermore, bread incorporated with PTF flour could enhance its nutritional as well as functional properties of bread.

Gluten is a specific protein present in wheat flour that causes the dough to be elastic and stretchy. Some studies on modifications have been done to improve the characteristics of cassava flour including enzymatic, microbial and physical treatment. Pre-gelatinization is a treatment that utilize heat below the temperature of starch gelatinization could modify the characteristics of cassava flour (Ratnaningsih *et al*, 2019). Hence this study was conducted to develop bread incorporated with pregelatinized palmyrah tuber flour by optimizing PTF % and moisture content % using the Response Surface Methodology.

Methodology

Raw materials

Wheat flour, Palmyrah tuber flour (PTF), salt, sugar, margarine and yeast (*Saccharomyces cerevisiae*) were purchased from the local market in Jaffna, Sri Lanka.

Debittering of PTF

PTF was sieved through a 250 µm screen to remove fibers and bigger particles to obtain fine flour with a uniform particle size. Flour was soaked in distilled water for one hour at room temperature and free water was decanted from the pellet completely using a muslin cloth. This process was repeated. After that, debittered flour was dried in an oven at 60°C for six hours (Sharaniya & Navaratne, 2015) and stored in an air- tight container for further use.

Preparation of pre-gelatinized PTF flour

Debittered PTF was mixed with water (flour: water =1:5). After that, the slurries were submitted to the heating process. The pre-gelatinization was conducted by heating the slurries up to 80°C with continuous stirring using a magnetic stirrer. The slurries were then cooled down and allowed to stand for one hour at room temperature.

Experimental design for optimization of best bread formulation

A box-Behnken design with three factors at three levels (Incorporated PTF% (10, 25, 40), Pre-gelatinized PTF % (2.5, 3.75, 5), water content % (25, 30, 35)) was designed using the Design-Expert 13 software to analyze the response of texture, and specific volume, crumb pH and moisture content.

Process of bread making

The active dry yeast (1.6%) was pre-hydrated using lukewarm water and sugar (1%). All other ingredients such as wheat flour, pre-gelatinized PTF, rest of PTF, sugar (5%), salt (1.6%), and margarine (4%) were mixed homogeneously. The amount of all other ingredients except flour was calculated for 100% of the total flour (PTF and wheat). After that, pre-hydrated yeast was mixed and the mixture was kneaded to prepare the dough for 14 minutes. The prepared dough was allowed for fermentation for 30 minutes in an incubator at 37ºC. The risen dough was degassed, shaped, and kept for another 30 minutes in an incubator for the fermentation in individual aluminum bread pan. Finally, prepared dough was baked at 210°C in an electric oven. The prepared breads were cooled for 50 minutes, sealed in polythene bag and kept under ambient conditions for further analysis.

Proximate composition and physiochemical analysis of bread

The optimized bread formulation in the previous experiment was compared with the bread made of 100 % wheat flour. Proximate composition analysis was carried out for total sugar, salt, fat, sodium, potassium, calcium, magnesium, ash, and acid-insoluble ash. All physio-chemical properties including pH, water activity, and moisture content, baking loss, total polyphenol content and microbial analysis were also compared using AOAC 2019; SLS 141:1992 for the two bread types. Texture profiles were analysed using a texture profile analyser (FRTS201803E, IMADA, Japan) according to the method described by Rathnayake et al., (2021) with slight modification. The colour of the bread crumb and crust was determined using a precision colorimeter (NR20XE, China). Total phenolic content of the sample was determined using the method described by Ambikaipahan et al., (2020).

Sensory evaluation

Sensory evaluation was conducted with a fifteen-member trained panelists at the Palmyrah Research Institute, Jaffna. Paired comparison test was used to compare the samples. A 9-point hedonic scale (dislike extremely-1, dislike very much-2, dislike moderately-3, dislike slightly-4, neither like nor dislike-5, like slightly-6, like moderately-7, like very much-8 and like extremely-9) was used. The data was analyzed through the Friedman non parametric test in Minitab 17.

Statistical analysis

Physiochemical properties of the samples were analysed through one way ANOVA. Tukey's test was used to analyse the mean separation of various properties. The sensory evaluation data were analysed using the Friedman non-parametric test in the Minitab 17 software package with a 95 % confidence interval.

Results and discussion

Optimization of the best bread formulation through surface response methodology

Specific volume, moisture content, pH of the crumb, and hardness of different formulations were used as the responses to optimize the best formulation of bread as shown in Table 1 and the cross section of the different formulations of breads was shown in figure 1.

Figure 1. Cross sectional view of breads made from different formulations

Table 1. Treatment combination and response for the bread formulation

Treatments	PTF (%)	Pregelatinized PTF (%)	Amount of water added (%)	Specific volume (cm^3/g)	Moisture content (%)	pH	Hardness (N/m^2)
T1	40	3.75	35	2.23± 0.01^{g}	48.17±0.01^{b}	4.61±0.03^{def}	46000.0±150.0^{k}
T2	25	5	25	2.46± 0.00^{f}	43.36±0.00^{f}	4.86±0.06^{ab}	16230.0±15.0^{q}
T3	10	3.75	25	2.23±0.01^{g}	38.81±0.01^{k}	4.63±0.01^{de}	41380.0±30.0^{n}
T4	10	2.5	30	2.00± 0.10^{h}	38.79±0.00^{k}	4.55±0.03^{fgh}	51240.0±10.0^{h}
T5	25	3.75	30	3.55±0.06^{b}	42.94±0.00^{g}	4.49±0.01^{h}	80845.0±50.0^{b}
T6	25	5	35	3.66±0.12^{ab}	40.00±0.04^{i}	4.58±0.03^{efg}	32460.0±35.0^{p}
T7	40	3.75	25	3.13±0.03^{d}	44.89±0.01^{c}	4.91±0.00^{a}	97200.0±100.0^{a}
T8	25	3.75	30	3.04±0.05^{de}	41.87±0.10^{h}	4.68±0.03^{cd}	44715.0±5.0^{m}
T9	25	3.75	30	3.32±0.00^{c}	44.19±0.01^{d}	4.52±0.01^{gh}	62380.0±15.0^{f}
T10	10	3.75	35	3.11±0.07^{d}	41.89±0.04^{h}	4.82±0.016^{b}	44980.0±35.0^{l}
T11	40	5	30	2.90±0.05^{e}	41.89±0.03^{h}	4.63±0.02^{de}	77873.0±5.0^{c}
T12	25	2.5	35	3.39±0.03^{c}	49.37±0.01^{a}	4.73±0.02^{c}	49970.0±65.0^{i}
T13	25	2.5	25	3.82±0.03^{a}	43.68±0.00^{e}	4.66±0.02^{cd}	55060.0±35.0^{g}
T14	40	2.5	30	3.12±0.09^{d}	48.11±0.01^{b}	4.23±0.01^{j}	47530.0±35.0^{j}
T15	10	5	30	3.82±0.03^{a}	39.50±0.04^{j}	4.85±0.02^{ab}	34850.0±26.0^{o}
T16	25	3.75	30	3.38±0.02^{c}	41.90±0.01^{h}	4.32±0.00^{i}	74160.0±15.0^{d}
T17	25	3.75	30	3.33±0.03^{c}	43.65±0.05^{e}	4.54±0.03^{fgh}	64610.0±12.0^{e}

The values are presented as Mean ± SD. Means with different superscript letters within a column are significantly different ($P<0.05$)

Effects of independent variable on the specific volume of the bread

Specific volume is one of the important economic parameter and quantitative parameters in the baking process. Loaf volume and loaf weight ratio produce the specific volume. Higher volume and loaf weight have a positive economic value and consumer acceptance (Shittu *et al*, 2007).

Table 1 shows the specific volume of the formulated breads ranged from 2.23 – 3.82 cm^3/g. Contour plots of the surface response of specific volume are shown in figure 2(a), indicating that the specific volume increases with increasing % of pre-gelatinized PTF and water content. However, it was decreased with the substitution of the PTF. Amandikwa *et al*. (2015) stated that the higher water absorption capacity (WAC) of flour had an impact on the specific volume. The specific volume of the loaf decreases with the increasing WAC of the flour. PTF (189.53%) has a higher WAC than wheat flour (81.16%). It may have affected the specific volume of bread (Ravindiran, 2022).

Fairouz *et al*. (2018) in their study stated that the addition of pre gelatinized flour increases the specific volume of the bread. In conventional bread, CO_2 was entrapped in the gluten network increasing the specific volume of the bread. Starch granules in the pre gelatinized flour were already swollen before they were combined with other ingredients. As a result, the specific volume of that bread was increased.

Specific volume increased with the degree of hydration and, it may be due to the plasticizer effect of water, which contributes to the extensional properties of the dough during mixing. A higher amount of water is required for hydrating this raw material and for the swelling of starch granules (De La Hera *et al*, 2014).

Effect of the independent variable on the moisture content of bread

Final moisture content is one of the key parameters affecting the final quality of bread. The recommended moisture content in any part of the loaf is 40% (SLS 141: 1992). Figure 2(b) shows that the moisture content of the bread has increased with the PTF substitution. A similar trend was reported by Amandikwa *et al*. (2015) in bread made from wheat-yam flour composite. A higher WAC of PTF increases the moisture content of the bread (Lian *et al*., 2020).

Effect of independent variables on pH of the bread

According to Table 1, the pH of the breads ranged from 4.23 to 4.91. Figure 2(c) shows that pH of the bread decreased with the increased substitution of PTF. High pH loaf could be due to its high protein content with amphoteric properties (Raya *et al*, 2014). PTF has a lower protein content (3.1g/100g) than wheat flour (11.5g/100g) (Mason and Henry, 1989). As a result, the pH of the prepared bread decreased with increasing PTF substitution.

Effect of independent variables on hardness of the bread

Texture is one of the most important quality attributes which affecting the consumer preference, thus soft crumb is related to freshness of bread and felt by the consumers. According to data shown in figure2 (d), maximum hardness (97200.0±100.0 (N/m^2)) is observed in the T7 treatment with a high content of PTF% (40%) and the lowest hardness (16230.0±15.0 (N/m^2) was observed in the T2 with the addition of maximum pregelatinized PTF%. The hardness of the bread was reduced with the increase of pre-gelatinized PTF. A similar effect is observed in gluten-free made with unripe banana flour (Hernández-Aguirre *et al*., 2019). More gas retention during fermentation can result in rise in volume of dough. The hardness of bread gets increased with the incorporation of PTF%. The same tendency was reported by Sadowska *et al*. (2003) in germinated pea flour incorporated bread.

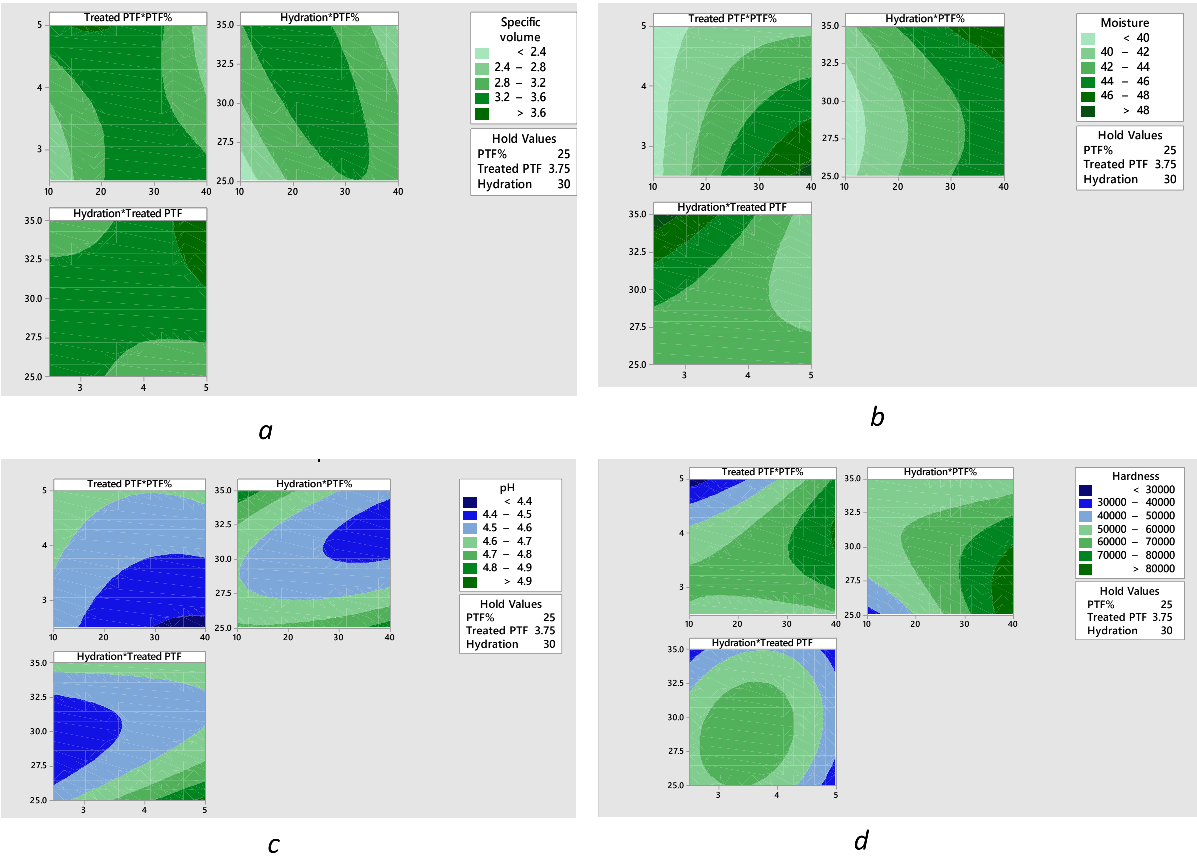

Figure 2. The effect of treatment on the quality parameters of bread; a: The specific volume, b: Moisture content, c: pH, d: Hardness

Optimization of bread flour formulation

The optimized bread flour formulation was selected based on the maximum specific volume, minimum hardness, and minimum moisture content and optimum pH as per the SLS 141: 1992. The best formulation of 10 % PTF, 5 % pregelatinized PTF and 35% water content was identified as the optimized composition for PTF- incorporated bread.

Sensory evaluation

The sensory evaluation was conducted to compare the developed bread against the 100% wheat bread. However, there is no significant difference in the overall acceptability of both breads (Figure 3)

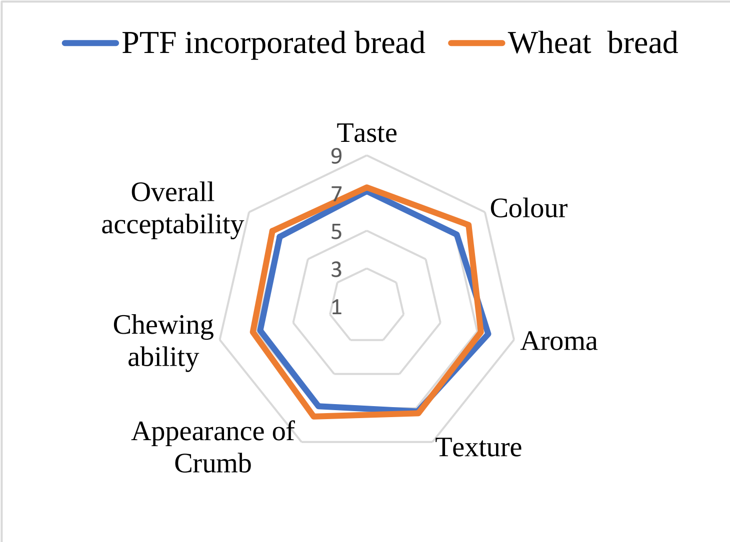

Figure 3. Sensory evaluation of bread

Proximate compositions and physiochemical properties of bread

Proximate analyses of the optimized bread formulation against the control is shown in table 2. The total sugar content of the selected composite flour bread was 35.49±0.00 g/100 g, which is significantly higher than the control (35.35±0.00 g/100 g). However, there was no significant difference in the total fat content between two breads. The energy content of the optimized bread (416.17±1.89) is significantly higher than control (399.78±4.49) and that may be due to the increased fibre content as the PTF bread has a significantly higher crude fiber (4.60±0.01) content than the control.

Mahilrajan *et al*. (2017) stated that palmyrah flour-based products reduce the postprandial insulin response and helps to control the type 2 diabetes due to the high fiber content.

The total ash content of the PTF incorporated bread is significantly higher than the control and that may also related to the high fibre content. According to the SLS 141:1992 specification for white bread (second revision), the maximum acid-insoluble ash in the bread is 0.1%.acid-insoluble ash of PTF incorporated bread was 0.072±0.0, and comply with the standard. The incorporation of PTF into bread has significantly ($P<0.05$) increased the total sugar, crude fiber and energy content.

Although sodium, calcium and magnesium contents had slight increase with PTF substitution, there was no significant effect on PTF addition.

Table 2. Proximate compositions of optimized formulation and the control composition of bread

Proximate & nutritional composition	Optimized bread	Control bread
Total sugar (g/100g)	35.49±0.00[b]	35.35±0.00[a]
Total fat (g/100g)	2.06±0.01[a]	2.04±0.01[a]
Crude fiber (g/100g)	4.60±0.01[b]	3.51±0.02[a]
Total ash (%)	1.22±0.08[b]	1.54±0.14[a]
Acid insoluble ash (%)	0.072±0.00[b]	0.058±0.01[a]
Sodium (mg/100g)	423.77±3.72[a]	394.15±9.83[a]
Potassium (mg/100g)	243.9±22.00[a]	322.8±21.5[a]
Calcium (mg/100g)	61.33±6.11[a]	35.00±3.00[a]
Magnesium (mg/100g)	147.00±9.00[a]	167.40±8.25[a]
Energy (Kcal/100g)	416.17±1.89[b]	399.78±4.49[a]

The values are presented as Mean ± SD. Means with different superscript letters within a row are significantly different (P < 0.05).

Crust and crumb colour

In general, a lower L* value implies a darker crust, a positive a* value means a reddish crust and a higher b* value indicates a yellowish crust. According to table 3, the bread crust of the best mixture has a lower L* value (25.31) than the control, which indicates a significantly darker crust. Gelatinization has an impact on the colour of the crust. The Maillard reaction which takes place by reacting proteins and reducing sugars produces coloured components as well as some flavoring compounds. It gives bread its unique colour. Therefore, the color of the bread crust made from PTF demonstrates a slight deviation since the bread was substituted with only 10% of PTF (Jalali *et al.*, 2020).

The crumb colour of the optimized bread and the control is not significantly different. The b* value of optimized bread crumbs indicates a higher yellowness which may attained by the PTF flour, as it is has a yellowish colour than wheat flour.

Table 3. Variation of colour of the 10% PTF bread and the control

Sample	Colour					
	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
Optimize	25.31 ± 1.58^{b}	15.03 ± 0.32^{a}	20.72 ± 0.84^{b}	29.79 ± 0.78^{a}	4.24 ± 1.14^{a}	15.47 ± 1.81^{a}
Control	32.34 ± 2.69^{a}	12.85 ± 1.48^{a}	17.31 ± 0.82^{a}	36.73 ± 4.72^{a}	5.58 ± 4.50^{a}	13.91 ± 1.10^{a}

The values are presented as Mean ± SD. Means with different superscript letters within a column are significantly different ($P < 0.05$).

Functional properties of bread

The addition of bioactive compounds such as phenolic antioxidants to bread has a growing trend to improve the health benefits. PTF is a rich source of phenolic compounds containing various active components such as tannins, saponins and polyphenolics. The total phenolic content (TPC) of the 10% PTF incorporated bread was 1.307±0.11 mg GAE/g and it was more significant than the bread made from wheat flour (1.088±0.03 mg GAE/g). The higher phenolic content provides many health benefits to humans.

Conclusion

The bread formulation with 10% PTF, 5% pre-gelatinized PTF and 35% water is the best formulation as it has the most favorable physiochemical, nutritional, and sensory properties. PTF incorporated bread contains high crude fiber and total polyphenol content providing nutritional and health benefits to humans.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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