



FORMULATION OF JACKFRUIT SEED PROTEIN ENRICHED CAKE

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Abstract

The study was conducted to establish a formulation for development of jackfruit seed protein (JSP) enriched cake. After extracting from jackfruit seeds, the JSP flour was added as substitutions of wheat flour at a level of 0%, 3%, 6%, 8% and 10% to prepare protein enriched cakes. The cakes were then analyzed to find out the acceptable formulation by assessing their physicochemical properties and sensory attributes. The cakes prepared with 6% JSP isolate secured the maximum sensory scores in terms of color, flavor, taste, texture, and revealed the highest overall acceptability (7.58 ± 1.01) suggesting 'like very much'. The proximate analysis of 6% JSP enriched cake showed 15.26%, 11.59%, 25.91%, 1.06%, 2.03%, and 44.15% moisture, crude protein, crude fat, ash, crude fiber, and carbohydrate respectively. An increase in JSP level in cake formulation decreased the volume, weight, specific volume and water activity while the baking loss showed a reverse trend. The JSP enriched cake also exhibited a reduction in vitamin A content and peroxide value, and an increased calorific value. Microbiological analysis of the JSP enriched cake showed an acceptable quality by 10 days of storage (25 ± 2 °C). Therefore, maximum 6% JSP flour could be added as a substitute of wheat flour to formulate a protein enriched cake having good preferences and overall acceptability.

Keywords: Jackfruit seed, protein cake, malnutrition remedy, sensory score, jackfruit seed utilization.

Introduction

The Jackfruit (*Artocarpus heterophyllus* L.) belonged to family Moraceae, is one of the largest fruits and distributed widely in tropical and sub-tropical countries including Bangladesh, Brazil, India, Indonesia, Malaysia, Sri Lanka, and Thailand (Feili, 2014). It is the national fruit of Bangladesh and locally termed as 'Kanthal'. It grows abundantly in particular areas of Bangladesh especially in hilly areas of Chittagong and Sylhet, and some highlands of Tangail, Mymensingh, Gazipur,

Cumilla and Jessore during May-July (Saha *et al.*, 2016). According to Rahman *et al.* (1995) it is commonly considered as the 'fruit of poor men' due to its availability and lower price in Bangladesh.

In general, the jackfruit seeds constitute 8-15% of total weight of the entire jackfruits (Swami *et al.*, 2012). A jackfruit seed consists of various nutrients including carbohydrates, protein, fibre, minerals, vitamins, and provides about 146.06 kcal/100 g energy (Kumar *et al.*, 1988), and has many natural antioxidant

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properties (Bhushan *et al.*, 2008 and Zzaman, 2012). According to Chowdhury *et al.*, (2012) the jackfruit seeds contain a group of glycoprotein called 'lectin' which shows some anticarcinogenic, antibacterial and antifungal properties (Singh *et al.*, 1991). The two lectins found in jackfruit seeds have been proved to alleviate the immune status of HIV infected patients and possess 13–14 immunological properties (Gupta *et al.*, 2011). Although most of the jackfruit seeds are eaten as snacks, cooked or roasted dishes, a significant amount is discarded and just thrown away as social tradition. Every year a huge amount of jackfruit seeds is damaged due to the lack of proper storage resulting in a great national loss in Bangladesh. Besides, the jackfruit seed is often underutilized in Bangladesh for both human and animal consumption due to the lack of knowledge about its nutrition, potential of the nutrients to be used in food formulations, and appropriate technology for isolation of the nutrients (Gunaseena *et al.*, 1996).

Protein is one of the fundamental nutrients. It is also a structural and functional compound in each cell of human body, and widely involved in various interactions of metabolism. It provides about 10-15% of total dietary energy being the second most chemical compound after water in human body (Gunnars, 2018). Lack of protein in the body generally disrupts the normal growth of children. Therefore, it is necessary to take a rational diet of protein. The major sources of animal proteins include fish, meat, eggs, milk and dairy products which contain all essential amino acids required by human body. However, the vegans and vegetarians may get the essential amino acids by combining various plant protein sources like cereals, pulses and other seeds (Leonard, 2018). The sources of plant proteins generally play a significant role to supply the human nutrition in developing countries particularly

where an average protein intake level is significantly less than that of required amount. With increasing health consciousness, the protein foods from animal origins have been substituted by that of plant origins as new and alternative food sources (Nunes *et al.*, 2003). The jackfruit seeds contain 13.50–17.37% protein depending on the variety and can be utilized as an inexpensive plant protein source (Ocloo *et al.*, 2010). Over the world, many researches are going on various sources of plant proteins and in this case jackfruit seed protein (JSP) is indispensable to fulfil this requirement (Gorinstein *et al.*, 2002) and can be beneficial to increase nutritional values of food products at lower costs.

Furthermore, the JSP contains both essential and non-essential amino acids which impart their specific functions (Miah *et al.*, 2017). The JSP naturally complements the proteins in cereal-based diets as the chemical and nutritional elements (Altschull, 1994). Therefore, it is the time to perform researches regarding the isolation of JSP as an alternative source to reduce the high costs of commercial sources followed by utilizing it in manufacturing protein enriched cakes or other food products to mitigate the national protein deficiency in Bangladesh (Bernardino-Nicanor *et al.*, 2014). Considering these circumstances outlined above the study was conducted to find out an acceptable formulation for preparation of JSP enriched cakes followed by analyzing their physicochemical properties and shelf life.

Materials and Methods

Sample collection

Khaja variety of ripe jackfruit was collected from local markets of Bhaluka Upazila, Mymensingh, Bangladesh. All experiments were carried out at the laboratories of Agro-

processing department of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

The ripe jackfruit was cut manually with a knife and the seeds were separated from bulbs. The seeds separated from bulbs were washed out thoroughly with the tap water to discard unnecessary parts. The arils were peeled out manually and then the seeds were soaked in 5% NaOH (for 2 minutes) and then re-soaked in 5% citric acid (for 2 minutes) at room temperature (25 ± 2 °C) followed by removing the brown layers (Spermoderm coverings) to collect the seed cotyledons only. The cotyledons were again washed thoroughly with tap water.

Preparation of jackfruit seed flour

The washed seeds were chopped into smaller pieces with a sharp knife and dried in the sun. The dried small pieces of seeds were reduced in size through grinding by a grinder machine. The ground seeds were transformed into flour in a blender and then sieved through 75 micro size mesh sieves. The jack fruit seed flour (JSF) was packed in a glass bottle and stored at room temperature (25 ± 2 °C) for further applications.

Isolation of jackfruit seed protein

The JSF was poured in distilled water (1:10 w/v) and suspended thoroughly followed by adjusting the pH of the suspension to 12.0 using 4 M NaOH solution. This suspension was stirred up continuously for 1 h and then centrifuged at 12,600 g for 15 min at room temperature (25 ± 2 °C). The pH of the collected supernatant was adjusted to 4.5 using 4 M HCl followed by stirring up for 30 min. The solution was then allowed for precipitation overnight at 4 °C. The resulting precipitate

was collected through centrifugation at 2217 g for 15 min followed by washing 3 times with distilled water to eliminate all other dissolved components. The washed protein was re-suspended thoroughly in distilled water at a rate of 10% total solid (w/v) in the suspension (pH = 7.0) followed by collecting the protein through centrifugation (2217 g, 15 min). The collected protein was dried through a Spray dryer (Yamato ADL-311S Spray Dryer, Inlet Temp. = 40~220 °C, Outlet Temp. = 0~60 °C) by adjusting the inlet and out temperature at 155 °C and 60 °C respectively, and the resulting product was termed as jackfruit seed protein (JSP) flour.

Preparation of cake by incorporating JSP

The cakes were prepared according to traditional method of cake preparation where all major and minor ingredients, except wheat flour, were used according to the standard of commercial plain cake (CPC) formulation (Salehi *et al.*, 2016). To prepare the JSP enriched cakes, JSP flour was incorporated in cake mixes as the substitutes of wheat flour only. In this study, five different cakes were prepared by incorporating JSP flour at five different doses such as $S_1 = 0\%$ JSP flour or control, $S_2 = 3\%$ JSP flour, $S_3 = 6\%$ JSP flour, $S_4 = 8\%$ JSP flour, and $S_5 =$ JSP flour (Table 1). The prepared cakes were packed in transparent polyethylene bags to prevent from drying followed by storing at room temperature (25 ± 2 °C) and refrigeration temperature (4 ± 2 °C) for further analyses. To enhance the study, all experiments were performed triplicate for each treatment.

Sensory assessment of prepared cakes

The sensory assessment of prepared cakes was performed by a '12-members test panel' comprising of postgraduate students,

Table 1. Proportions of JSP and wheat flour used in individual cake preparations

Major ingredients	Ingredients used				
	S ₁	S ₂	S ₃	S ₄	S ₅
JSP flour (g)	0	3	6	8	10
Wheat flour (g)	100	97	94	92	90
Whole egg (pcs)	2	2	2	2	2
Sugar (g)	95	95	95	95	95
Baking powder (g)	2	2	2	2	2
Baking soda (g)	1	1	1	1	1
Vanilla (g)	1	1	1	1	1
Milk powder (g)	5	5	5	5	5
Whole milk (mL)	200	200	200	200	200
Butter (g)	10	10	10	10	10
Salt (g)	2	2	2	2	2

S₁ = cake with 0% JSP flour or control

S₂ = cake with 3% JSP flour

S₃ = cake with 6% JSP flour

S₄ = cake with 8% JSP flour

S₅ = cake with 10% JSP flour

academic staffs and teachers of faculty who were previously experienced in sensory evaluation of bakery products. The prepared cakes were served to the panelists and asked to assign appropriate scores against the sensory attributes such as color, flavor, taste, texture and overall acceptability. The 9-point Hedonic Scales were designed as 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely (Ranganna 1991).

Proximate composition analysis

In terms of proximate composition, the prepared cakes were analyzed to determine the moisture, ash, protein, fat, crude fibre and carbohydrate contents.

Determination of moisture and ash contents

The moisture and ash contents of the cakes were determined according to the procedures as stated in the Standard Official Methods of Analysis (AOAC, 2002). To obtain more accurate results triplicate experiments were performed for the individual analyses of each sample.

Determination of fat content

The fat content of the prepared cakes was determined according to solvent extraction method using Soxhlet apparatus (Ranganna, 2000). For all individual samples triplicate extractions were performed for 16 h where hexane was used as extracting solvent.

Determination of protein content

The protein content of the cake sample was determined by Kjeldahl method. At first,

nitrogen content in the experimental sample was determined through Micro-Kjeldahl apparatus and finally the protein content was calculated by multiplying the nitrogen value with protein factor 6.38 (AOAC, 2002). For more accuracy triplicate analyses were performed for each sample.

Determination of crude fibre content

Crude fiber content in the prepared cake was determined by following the procedure as described by Ranganna (2000) where triplicate analyses were performed for each sample.

Determination of carbohydrate content

Total carbohydrate content in the individual cake was calculated by the subtraction of obtained moisture, ash, protein, fat and crude fibre contents from 100 (AOAC, 2002) as likely as follows:

$$\% \text{ carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ fat} + \% \text{ crude fibre}).$$

Analysis of physical properties

Determination of baking loss of the cake

Before baking, the cake mix was weighed and immediately after baking the baked cake was again weighed on a digital balance at room temperature (25 ± 2 °C). The percent of baking loss of the prepared cakes was calculated according to the following equation-

$$\% \text{ BL} = \frac{(B - C)}{B} \times 100$$

Where

BL = baking loss

B = weight of cake mix before baking (g)

C = weight of the prepared cake immediately after baking (g)

Determination of volume and specific volume

Volumes of the prepared cakes were measured by the rapeseed replacement method (AACC, 1996). At first, rapeseed was poured into a container, having a diameter and height higher than that of the measuring cakes, until it was overflowed. The seed was leveled by passing a ruler across the top of container and then the volume (cm^3) of used rapeseed was measured by a measuring cylinder and kept therein. The container was emptied and a piece of experimental cake was placed into it followed by pouring the rapeseed into the container from the measuring cylinder until it was overflowed. The seed in the container was again leveled by the ruler and volume of the unused rapeseed was measured. The volume (cm^3) of unused rapeseed remaining in the measuring cylinder corresponded to the volume (cm^3) of the experimental cakes (Fasina *et al.*, 1997). Finally, the specific volume (cm^3/g) of each cake was calculated by dividing individual volume (cm^3) by its own weight (g). For each treatment six individual samples (cake) were used for this test.

Color measurement

Color determination of the cakes was performed with a Chromameter (model CR-400b-8209889 Konica Minolta Sensing, Inc. Japan) set called CIELAB color space. Crumb and crust colors of a fresh cake were estimated by a Hunter lab DP 9000 D 52L optical sensor by using L^* , a^* and b^* color scale. For each performance the instrument was standardized with a black and white ceramic plate. The

cakes were scanned at three individual locations and then mean values of L, a, b were recorded. The L^* = lightness/darkness (0 to 100), a^* = redness/greenness (-120 to 120) and b^* = yellowness/blueness (-120 to 120) were estimated (Salehi and Kashaninezad, 2015) and total color changes (ΔE) in individual cakes were calculated (Pathare *et al.*, 2013) as follows-

$$\Delta L = (L - L_0); \Delta a = (a - a_0); \Delta b = (b - b_0)$$

$$\Delta E = \sqrt{(\Delta L)^2 + \Delta a^2 + \Delta b^2}$$

Where

L, a and b are the measured values of JSP enriched cakes

L_0 , a_0 , and b_0 are the respective values of the control samples.

Textural properties of cakes

The analysis of textural profile of the cakes ($2 \times 2 \times 2$ cm) was carried out by using a texture analyzer (TA-XT Plus, Stable Micro Systems, UK) with a cylindrical probe (diameter = 36 mm), 50% compression and 1.0 mm/s test speed after a removal of crusts of the cakes. A doubled cycle was programmed out and the textural profile was estimated by using a software called Texture Expert 1.05 (Stable Microsystems). Other essential parameters were fixed up as: pre-test speed = 2.0 mm/s, post-test speed = 2.0 mm/s and trigger force = 5 g. The data were analyzed by the texture expert program (version 4.1.2). As the texture parameters only the hardness in terms of firmness (kg/cm) of the triplicate samples were measured and results were determined

from the force-distance curve. According to the literature, hardness was the peak force obtained in the first compression (Bourne, 1990).

Determination of water activity

The water activity (a_w) along with temperature of the protein enriched cakes was determined using the Hygropalm Water Activity meter with 9 volt (LAB Touch- Water Activity CH8853, Lachen, Switzerland).

Determination of energy content

The gross energy content, in terms of heat energy (cal/ g), of the prepared cakes was determined by using an oxygen Bomb Calorimeter (Model: 1341, Parr Instrument Company, USA). At first, the cake sample was pressed into a tablet weighing 1 g and then a 10 cm platinum wire of stainless steel was connected to the two electrodes of the bomb. The tablet was placed in a crucible and the thread was tied up to the platinum wire in order to carry the flame to the tablet. The entire assemble was put in the bomb, the cap was tightened and then the bomb was filled with oxygen up to 25 psi. 2000 ml distilled water was poured into the pail and it was kept in proper position in the outer jacket. The bomb was placed in the pail and connected to the mains of switch box followed by positioning the stirrer, thermometer and lid of the calorimeter accordingly. Immediately after switching on the stirrer initial temperature of water was recorded for about 5 min. The bomb was ignited by pressing the push button for 3-4 sec and the final temperature of the water was recorded while it was constant for 2-3 min.

Determination of water equivalent

The water equivalent of the calorimeter was computed using the following equation where benzoic acid, having heat of combustion 6318 cal/g, was used as the standard material.

$$W = \frac{h \times m + c}{T}$$

Where

W = water equivalence of calorimeter (cal/°C)

h = combustion heat of benzoic acid (6318 cal/g)

m = weight of benzoic acid (g)

T = rise of water temperature in the pail (°C)

C = correction in calories for heat formation in paper, thread and fuse wire.

Calculation of gross heat

The gross heat of combustion i.e. energy production (cal/g) of the bomb was calculated using the following equation

$$\text{Energy (Cal/g)} = \frac{T \times w - c}{m}$$

Where

T = rise of water temperature in pail (°C)

W = water equivalent (cal/°C)

m = weight of sample (g)

C = correction in calories for heat formation in paper, thread and fuse wire

Determination of vitamin A

Vitamin A content of the prepared cake was determined in terms of β -carotene ($\mu\text{g}/100\text{ g}$). At first, 1 g sample was crushed and mixed up with 10 ml of acetone-hexane solution

(acetone: hexane = 4:6) thoroughly and then the suspension was centrifuged at 4000 rpm for 5 min. The dilution factor was fixed up at $DF = 1500/20 = 75$ and optical densities (OD) of the supernatant was measured by UV-vis spectrophotometer (UH5300, HITACHI, Japan) at absorbance 453 nm, 505 nm, 645 nm and 663 nm. The β -carotene ($\mu\text{g}/100\text{ g}$) content was estimated using the following formula (Nagata *et al.*, 1999; Aremu and Nweze, 2017).

$$\beta\text{-carotene } (\mu\text{g}/100\text{g}) = \frac{\text{OD} \times V \times \text{DF} \times (100 \times Y)}{W \times 100}$$

where

V = volume of supernatant taken for spectrophotometry (μg)

Y = % dry matter in the sample

W = weight of sample taken for making the mixture (g)

Finally, the vitamin A content in the experimental cake was determined by the following equation

$$\text{Vitamin A } (\mu\text{g}/100\text{ g}) = 0.167 \mu\text{g } \beta\text{-carotene (Aremu and Nweze, 2017).}$$

Shelf-life study of JSP enriched cake

Determination of peroxide value

At first lipid extract was prepared according to the modified method as stated by Baiano *et al.* (2009). Fifty grams cake sample was thoroughly mixed with 100 ml of hexane in a flask with a stopper and then kept at room temperature ($25 \pm 2\text{ }^\circ\text{C}$) overnight. The whole mix was filtered through No. 1 Whatman filter paper. The extracted lipid was stored for further analyses.

Three grams of the lipid extract was mixed thoroughly with a mixture solution (glacial acetic acid:chloroform = 3:2) and then 1 mL saturated potassium iodide (KI) was added to it. The entire solution was allowed to stand in the dark for 3 minutes. After then 100 mL distilled water and 1 ml starch solution were added to the solution and mixed uniformly by shaking, and titrated against $\text{Na}_2\text{S}_2\text{O}_3$. The peroxide value (PV) of the cake sample was calculated in terms of milliequivalent peroxide per 1000 g fat according to the following equation–

$$\text{PV (meq/kg)} = \frac{S \times M \times 100}{w}$$

Where

S = volume (mL) of sodium thiosulphate solution used in titration

M = molarity of sodium thiosulphate solution

W = weight of cake sample (g)

Microbiological assessment

To assess the microbial quality, the developed cakes were stored at room temperature (25 ± 2 °C) in commercial polyethylene packages and analyzed after 2 days' interval till 14 days. At first, 10 g cake sample was suspended in 90 ml distilled water and blended in a sterile blender (8000 rpm, 25 ± 2 °C) for 2 min. To account for the reasonable numbers of microorganisms a decimal series dilutions of cake suspension were prepared in normal saline solution (1% NaCl). The agar plates were prepared using Poly Dextrose Agar (pH 3.5, 0% tartaric acid). 100 μL decimal solution was spread over the agar and incubated aerobically at 37 °C for 24 to 36 h. To calculate the probable numbers of

microorganisms only 30 –300 colony forming units (CFU) were taken into consideration and accounted for total viable load following the equation, $\text{CFU/mL} = \text{Number of colonies} \times 10 \times (\text{dilution level})^{-1}$ (Ranganna, 1991).

Evaluation of organoleptic defects

After the sensory evaluation the accepted cake samples such as S_1 , S_2 and S_3 were stored at room temperature (25 ± 2 °C) and continuously observed at 2 days' interval for any organoleptic changes like color, texture and flavor. The color was observed by naked eyes and changes in texture and flavor were perceived manually till 14 days.

Statistical Analysis

The triplicate data obtained from different analyses were statistically analyzed by using R-software and significance differences were observed at 5% level. The Duncan's Multiple Range Test (DMRT) was performed to determine the mean differences between the samples.

Results and Discussion

As considering a good source of protein, JSP has several potentials to value addition. The results obtained in this study were presented and discussed below in terms of physicochemical properties, overall consumer's acceptability and microbial quality of the JSP enriched cakes.

Organoleptic assessment

To assess the consumer's preferences on any food products the hedonic scale is a unique one to provide a valid and reliable results (Stone *et al.*, 2012). In this study, the cake samples showed varying degrees of acceptability in terms of color, flavor, taste, texture, and overall acceptability.

The cake samples without JSP flour (S_1) and containing JSP flour up to 6% (S_3) secured the satisfactory scores in all sensory attributes (Table 2). However, increase in JSP flour in the cake formulations resulted in harder crust and crumb which gradually secured lower scores by the panelists. Besides, the cakes containing JSP flour above 6% (S_4 and S_5) produced a slightly vegetative smell and consequently rejected by all panelists. Therefore, the sensory evaluation result expressed that a partial substitution of wheat flour by upto 6% JSP flour (Table 2) in cake formulations would have a reasonable consumer's preferences.

According to Table 2, in terms of all sensory attributes like color, flavor, taste, texture and overall acceptability, only the samples S_1 , S_2 and S_3 were equally acceptable. Therefore, after the organoleptic assessment all of the further analyses were performed for only samples S_1 , S_2 and S_3 .

Proximate composition of JSP enriched cake

The proximate analysis of the JSP enriched cakes indicated that all composition such as moisture, ash, fat, carbohydrate and vitamin contents were in favorable proportion except the protein content which was found significantly rich. The entire results obtained in proximate composition analysis of JSP enriched cakes were tabulated below (Table 3).

Moisture content

Table 3 shows that moisture content was the highest (17.91%) in control sample (S_1) which was gradually decreased from 17.91% (S_1) to 15.26% (S_3) with the increase of JSP flour in the cake formulation from 0 (S_1) to 6% (S_3), respectively. This variation might be due to the presence of a greater amount of total dry solid (TDS) in the JSP flour which exhibits high emulsifying properties as compared to wheat flour (Juarez-Barrientos *et al.*, 2017). The results obtained in this study corresponds

Table 2. Sensory evaluation of JSP enriched cakes

Type of Sample	Hedonic scores from individual sensory attributes (0-9)				
	Color (mean \pm SD)	Flavor (mean \pm SD)	Taste (mean \pm SD)	Texture (mean \pm SD)	Overall acceptability (mean \pm SD)
S_1	7.50 \pm 0.80 ^a	7.42 \pm 0.90 ^a	7.08 \pm 0.79 ^a	7.67 \pm 1.09 ^a	7.75 \pm 0.67 ^a
S_2	7.42 \pm 0.79 ^a	7.33 \pm 0.78 ^a	7.50 \pm 0.80 ^a	7.67 \pm 0.98 ^a	7.67 \pm 0.78 ^a
S_3	7.33 \pm 0.65 ^a	7.27 \pm 0.03 ^{ab}	7.50 \pm 0.67 ^a	7.63 \pm 1.06 ^a	7.58 \pm 1.01 ^a
S_4	7.25 \pm 0.83 ^a	6.92 \pm 0.08 ^b	6.92 \pm 0.90 ^b	7.25 \pm 0.75 ^b	7.00 \pm 0.85 ^b
S_5	6.83 \pm 0.85 ^b	6.33 \pm 0.98 ^c	6.41 \pm 0.89 ^c	6.58 \pm 0.90 ^c	6.67 \pm 0.89 ^c

Means with same superscripts in a column indicate no significant difference at $p < 0.05$

S_1 : cake with 0% JSP flour (control)

S_2 : cake with 3% JSP flour

S_3 : cake with 6% JSP flour

S_4 : cake with 8% JSP flour

S_5 : cake with 10% JSP flour

to the findings of Sutharshan *et al.* (2001) who reported that an increase in soy flour proportion in cake formulation significantly reduces the moisture content in soybean flour supplemented cakes. Besides, the moisture content in sponge cake significantly decreases with the increase of pumpkin powder (Ghaboos *et al.*, 2018) and marjoram powder (Hafez, 2012) levels in cake formulations when used as substitutions of wheat flour. It is known that the lower in moisture content of cake the more its shelf-life and enhanced the quality.

Ash content

According to Table 3, the ash contents in JSP enriched cakes such as S₂ and S₃ were respectively 1.38% and 1.06% which were significantly lower than S₁ (1.53%). This result is consistent with the findings as obtained by Udoidem and Enwere (2012) who reported that the ash content in soybean powder enriched cake decreases with the increase in soybean powder in the formulation as a substitute of wheat flour. Besides, the decrease in ash content might be due to the increase in JSP flour in cake formulation as well as screening out of a portion of minerals during JSP isolation.

Fat content

In this study, the substitution of JSP flour to cake formulation resulted no significant changes in total fat content. The fat content in the control sample (S₁) was found 25.02% whereas in JSP enriched cakes such as S₂ and S₃ that were respectively 25.35% and 25.91% (Table 3). According to the literature (Eke-Ejiofor, 2013) fat content might be ranged

from 18.93% to 21.79% in the consumers' acceptable cakes after a substitution of flour by African breadfruit flour. Besides, the fat content in sponge cakes is usually ranged from 22.62% to 24.11% when substituted by pumpkin flour (Ghaboos *et al.*, 2018).

Crude fibre content

In this study, the fibre content in formulated cakes decreases gradually with an increase of JSP flour (Table 3) in the formulation from 2.79% (S₁) to 2.03% (S₃) which might be due to the presence of more fibre in wheat flour than the isolated JSP flour. Previous studies reported that the crude fiber of cakes was found to be 1.49 - 2% when substituted by wheat-soy composite flour, and decreases in a formulated cake with an increase of soybean flour in the formulation as a substitute of wheat flour (Udoidem and Enwere, 2012).

Protein content

The protein content of the developed cakes was increased from 6.39% (S₁) to 11.59% (S₂) with an increase of JSP flour in the cake formulation (Table 3) which might be due to the increase of JSP in the blend. The results found in this study is consistent with the finding as reported by Fukushima (2011) who stated that in rock cakes the protein content of wheat-soy composites significantly increases with an increase in parts of soybean flour in the formulation. Besides, addition of JSP flour to cake formulations also increases the essential amino acids content in the end product and thereby has a greater potential to overcome the protein-malnutrition-problem over the world (Akubor and Ukwuru, 2003).

Carbohydrate content

The carbohydrate content determined by difference method (Table 3) was higher in S_1 (46.36%) than in S_2 (45.92%) and S_3 (44.15%). According to Eke-Ejiofor (2013) the carbohydrate in the cakes usually ranges from 47.76% to 48.72% when the wheat flour is substituted by sweet potato and African breadfruit flour respectively. In addition, the JSP flour possesses relatively low carbohydrate as compared to wheat flour (Salunkhe *et al.*, 1992) which might cause the gradual decrease of total carbohydrate in the JSP enriched cakes (Table 3).

Physical properties of JSP enriched cake

The JSP enriched cakes were analyzed for different physical properties including weight, volume, specific volume, firmness, baking loss and water activity, and obtained results were tabulated (Table 4) below.

Weight, volume and specific volume

Table 4 shows that weights of formulated cakes were decreased with the increase of JSP flour in formulation. The control sample (S_1) had an average weight of 189.28 ± 0.03 g which was decreased to 188.12 ± 0.03 g and 187.32 ± 0.03 g for S_2 and S_3 , respectively.

Baking of the control sample (S_1) resulted in a crumb structure with numbers of air-bubbles as well as a significantly higher loaf volume (448.33 ± 2.89 cm³) as compared to that of JSP supplemented cakes such as S_2 (440.00 ± 5.00 cm³) and S_3 (420.00 ± 5.00 cm³). Increased proportions of JSP flour in the cake formulation led to a reduction of loaf volume and flattered shape. The results obtained in this study is approximately similar to previous studies where pine leaf powder (Lee and Lee, 2013) and marjoram powder (Hafez, 2012) were incorporated in the cake formulation. The gluten content of wheat flour contributes to the improved gas retention capacity resulting in an acceptable uniform crumb structure and better loaf volume of cakes (Paraskevopoulou *et al.*, 2015). In this study, the wheat flour was substituted partially by JSP flour that might cause the decreased volume and flattered shape of JSP enriched cakes (Ashwini *et al.*, 2009). Reduced expansion of JSP supplemented cakes might be attributed to decreased cake stability during baking due to the structural changes. A similar phenomenon was found in sponge cakes prepared with apple pomace (Masood *et al.*, 2002). In previous studies (Ngo and Taranto, 1986; Paton *et al.*, 1981) it has been reported that good cake mixtures

Table 3. Proximate composition of JSP enriched cakes

Samples	Proximate composition					
	% Moisture (mean \pm SD)	% Ash (mean \pm SD)	% Fat (mean \pm SD)	% Fibre (mean \pm SD)	% Protein (mean \pm SD)	% Carbohydrate (mean \pm SD)
S_1	17.91 \pm 0.04	1.53 \pm 0.03	25.02 \pm 0.08	2.79 \pm 0.02	6.39 \pm 0.02	46.36 \pm 0.38
S_2	15.73 \pm 0.05	1.38 \pm 0.03	25.35 \pm 0.05	2.23 \pm 0.03	9.37 \pm 0.02	45.92 \pm 0.04
S_3	15.26 \pm 0.04	1.06 \pm 0.04	25.91 \pm 0.31	2.03 \pm 0.04	11.59 \pm 0.03	11.59 \pm 0.03

S_1 : cake with 0% JSP flour (control)

S_2 : cake with 3% JSP flour

S_3 : cake with 6% JSP flour

Table 4. Physical properties of JSP enriched cakes

Samples	Physical parameters					
	Weight (g) (mean \pm SD)	Volume (cm ³) (mean \pm SD)	Sp. Volume (cm ³ /g) (mean \pm SD)	Firmness (kg/cm) (mean \pm SD)	Baking Loss (%) (mean \pm SD)	Water activity (mean \pm SD)
S ₁	189.28 \pm 0.03	448.33 \pm 2.89	2.37 \pm 0.02	1.40 \pm 0.16	7.82 \pm 0.03	0.80 \pm 0.01
S ₂	188.12 \pm 0.03	440.00 \pm 5.00	2.34 \pm 0.03	1.61 \pm 0.14	8.28 \pm 0.01	0.77 \pm 0.00
S ₃	187.32 \pm 0.03	420.00 \pm 5.00	2.17 \pm 0.06	1.95 \pm 0.22	10.68 \pm 0.02	0.74 \pm 0.00

S₁: cake with 0% JSP flour (control)

S₂: cake with 3% JSP flour

S₃: cake with 6% JSP flour

must retain sufficient viscosity to prevent the incorporated air bubbles from the rising to surfaces and being lost during initial heating. The baking time also should be pre-defined so that the inherent air bubbles are properly and uniformly distributed by water vapor and carbon dioxide gas during the cake maturation. In the study, the specific volumes of the JSP enriched cakes showed a descending trends, like 2.37 ± 0.02 cm³/g (S₁), 2.34 ± 0.03 cm³/g (S₂) and 2.17 ± 0.06 cm³/g (S₃), to JSP flour concentration in cake formulation (Table 4). This physical variation, as observed in this study, is consistent with literatures (Baldi *et al.*, 1965; Donelson and Wilson, 1960) described to weaken gluten matrix which is responsible to retain the generated gases in baked foods.

Texture

Texture of the JSP enriched cakes was measured in terms of hardness or firmness. According to Table 4, the hardness of different cake samples was differed from 1.40 ± 0.16 kg/cm (S₁) to 1.95 ± 0.22 kg/cm (S₃). Therefore, the cakes supplemented with JSP show more hardness than the control one (S₁). Reduction of aeration in the cake mixture and excessive heat resulted in protein coagulation and consequently led to a dense structure causing a

decrease in volume and an increase in hardness as well as requiring a maximum force for compression of the JSP enriched cakes. The result obtained in this study is consistent with previous study where Ghaboos *et al.* (2018) reported that the cakes become harder with increasing the concentration levels of pumpkin powder and β -glucan concentrates (Kalinga and Mishra, 2009) in the cake formulation. Similar trends haven been stated by Majzoobi *et al.* (2014) while studied the effect of oat on characteristics of sponge cake. In addition, the frequently occurred case hardening can accelerate the crumb dehydration (Lebesi and Tzia, 2011) resulting in an increased hardness of the cakes.

Baking loss

According to Table 4, the baking losses from JSP enriched cakes were increased with the increase of JSP flour in formulation and that were $(7.82 \pm 0.03)\%$, $(8.28 \pm 0.01)\%$ and $(10.68 \pm 0.02)\%$ for S₁, S₂ and S₃ respectively. The baking loss obtained in this study is significantly lower than that of Lee (2015) who found from 11.20% to 14.54% baking loss during preparation of cakes by incorporating *Rubus coreanus* powder as the substitution of wheat flour. Besides, the baking loss observed

in this study is consistent with the previous studies where sponge cakes have been formulated by incorporating pine leaf powder (Lee and Lee, 2013) and yacon powder (Lee and Son, 2011).

Water activity

It is apparent from the results (Table 4), the a_w varied among different samples from 0.80 ± 0.01 (S_1) to 0.74 ± 0.00 (S_3). Table 4 shows that there was a gradual decrease in a_w against the increase in JSP flour in cake formulations which might be due to the lower water absorption rate and/or lower water binding capacity of the JSP molecules. Qureshi *et al.* (2017) reported that there is a gradual increase in a_w against the increase in concentrations of dried grapefruit albedo powder since the dried grapefruit absorbs more water through osmosis. Since the a_w is directly related to moisture content, its lower value implies a lower probability for microbial contamination and thereby propagation in the food products.

Color measurement of JSP enriched cake

All of the color data obtained for the JSP enriched cake were expressed in terms of CIE $L^*a^*b^*$ (CIELAB) values respectively

corresponded to lightness, redness and yellowness (Fig. 1 and Table 5).

The crust colors of experimental samples were affected by the substitution of wheat flour with JSP flour in the cake formulation. As the level of JSP flour increased in the formulation, the crust colors became darker while determined by the Chroma-meter (Table 5). The crust of the control sample (S_1) was lighter, more reddish and yellow than that of other samples (S_2 and S_3). In case of crumb colors, as the level of JSP flour increased in the formulation, the L^* , a^* and b^* values were decreased indicating a darker, and less reddish and yellowish crumbs. The results found in this study is consistent with the findings obtained by Majzoobi *et al.* (2014) who reported an increase in darkness (lower the L value) of crust and crumb colors while prepared the oat fiber enriched sponge cake. A similar color characteristic has also been reported in previous study (Gomez, 2008) where the wholegrain wheat flour was incorporated into cake formulation.

The color change in baked cakes might be due to the denaturation of JSP, Maillard reaction between the JSP and polyphenols compounds by oxidation reaction, and the caramelization reaction of sucrose present in the cake

Table 5. Color measurement of JSP enriched cakes

Sample	Crust color			Crumb color		
	L^* (mean \pm SD)	a^* (mean \pm SD)	b^* (mean \pm SD)	L^* (mean \pm SD)	a^* (mean \pm SD)	b^* (mean \pm SD)
S_1	60.45 \pm 4.08	14.31 \pm 2.38	36.65 \pm 1.24	90.16 \pm 2.02	5.67 \pm 0.30	26.78 \pm 0.91
S_2	59.15 \pm 5.54	14.31 \pm 2.38	36.02 \pm 3.28	81.69 \pm 3.45	2.73 \pm 0.44	25.44 \pm 0.95
S_3	54.74 \pm 7.22	14.76 \pm 1.22	35.93 \pm 0.42	80.82 \pm 4.83	1.82 \pm 0.24	24.02 \pm 1.41

S_1 : cake with 0% JSP flour (control)

S_2 : cake with 3% JSP flour

S_3 : cake with 6% JSP flour

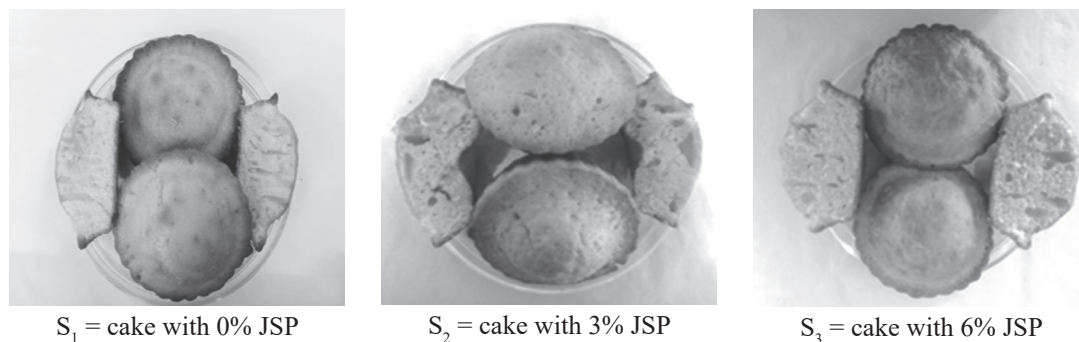


Fig. 1. Changes in color of cakes for differences in JSP flour content to formulation. The images were snapped out immediately after preparation the cakes.

formulation during baking. In addition to the folding process in confectioneries and bakery products the browning reaction is greatly accelerated by the color and type of the added flour which is apart from the wheat flour (Kim *et al.*, 2012).

Energy content of JSP enriched cake

According to Fig. 2, the level of gross energy varies among different samples. The gross energy values were observed as $468.60 \pm$

0.674 kcal/100 g, 466.45 ± 1.012 kcal/100 g and 462.10 ± 1.38 kcal/100 g in cakes with substitution of 0% (S_1), 3% (S_2) and 6% (S_3) JSP flour respectively (Fig. 2). The calorific values obtained in this study for JSP enriched cakes are consistent with previous studies. According to Elsebaie, and Mostafa (2018) energy content in baked cakes decreases from 516.7 (kcal/100g) to 457.57 (kcal/100g) due to the increased amount of stevia leaves powder in cake formulation. In literatures the

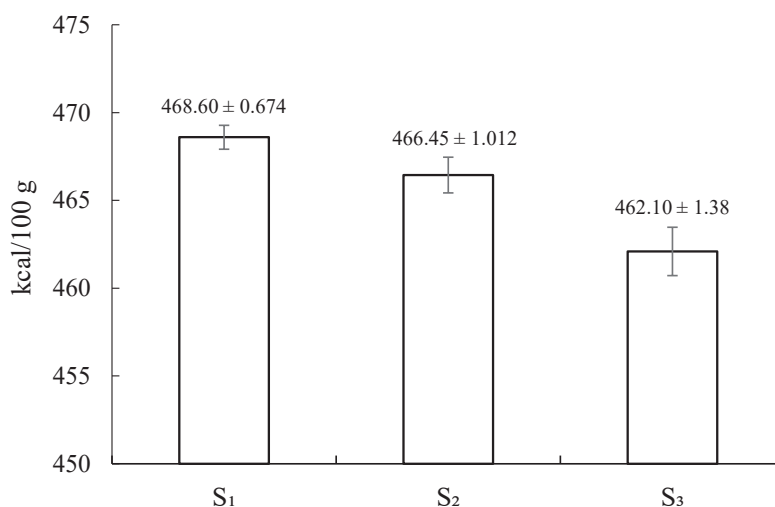


Fig. 2. Effect of JSP on energy content in different cakes. Here S_1 = 0% JSP flour in cake (control), S_2 = cake with 3% JSP flour and S_3 = cake with 6% JSP flour. The data have been expressed as (mean \pm SD).

calorie contents in cakes have been reported to be decreased while the formulation are based on sweet potato (Aziah *et al.*, 2011), plantain peel (Akubor and Ishiwu, 2013) and mango peel powder (Alloush, 2015) as the partial substitution of wheat flour.

Vitamin A content of JSP enriched cake

In this study, Vitamin A content in different JSP enriched cakes was estimated in terms of a group of nutritional unsaturated organic compounds such as retinol, retinoic acid, retinal, several pro-vitamins A and carotenoids (mostly the β -carotene).

As shown in Fig. 3, the highest amount (0.0192 ± 0.0003 mg/100 g) of vitamin A was found in the control cake (S_1) whereas 0.0187 ± 0.00012 mg/100 g and 0.0183 ± 0.00023 mg/100 g were found in S_2 and S_3 , respectively, i.e. the vitamin A content in JSP enriched cakes decreased against the increase of JSP flour in the formulation (Fig. 3). However, a reverse trend was found in previous studies

where vitamin A content was increased in the baked cakes with an increase of pumpkin powder (Ghaboos *et al.*, 2018) and carrot powder (Salehi *et al.*, 2016) in formulation as a substitution of wheat flour which might be due to the presence of excessive β -carotene in pumpkin and carrot powder as compared to JSP flour.

Shelf life of JSP enriched cake

The shelf life of JSP enriched cakes were estimated in terms of Peroxide value (PV), microbial analysis (Total plate count) and organoleptic changes throughout the storage (25 ± 2 °C) period of 14 days.

Peroxide value

The effect of JSP on peroxide value (PV) in JSP enriched cakes were observed at room temperature (25 ± 2 °C) by 2 days' interval and obtained results were tabulated below (Table 6).

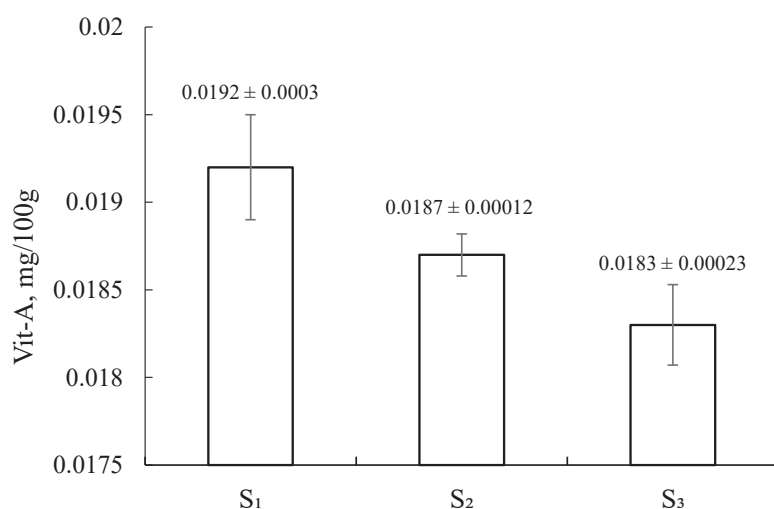


Fig. 3. Effect of JSP on vitamin A content in different cakes. Here S_1 = 0% JSP flour in cake (control), S_2 = cake with 3% JSP flour and S_3 = cake with 6% JSP flour. The data have been expressed as (mean \pm SD).

Table 6. Peroxide value (PV) of JSP enriched cake

Storage period (day)	Level of peroxide value (mEq/kg)		
	S ₁ (mean ± SD)	S ₂ (mean ± SD)	S ₃ (mean ± SD)
0	5.83 ± 0.05	4.90 ± 0.05	4.62 ± 0.02
2	6.33 ± 0.15	5.19 ± 0.08	5.02 ± 0.06
4	7.36 ± 0.03	6.28 ± 0.07	5.67 ± 0.04
6	10.49 ± 0.12	8.91 ± 0.08	6.96 ± 0.02
8	12.43 ± 0.03	10.92 ± 0.03	8.54 ± 0.04
10	15.03 ± 0.07	13.95 ± 0.13	9.94 ± 0.03
12	17.47 ± 0.01	15.81 ± 0.04	10.86 ± 0.08
14	19.01 ± 0.05	16.97 ± 0.02	12.35 ± 0.17

S₁: 0% JSP flour in cake (control)

S₂: cake with 3% JSP flour

S₃: cake with 6% JSP flour

According to Table 6, the PV in the JSP enriched cake was decreased with an increase of JSP flour in the cake formulation and the control sample (S₁) exhibited the highest PV throughout the storage period showing a higher oxidation process than that of S₂ and S₃. The results obtained in this study for PV (Table 6) indicate that all JSP enriched cakes could be preferably acceptable within a storage period of 14 days because the PV ranging from 10 to 20 mEq/kg for most of the food products is generally defined as rancid but still remain acceptable and for a PV \geq 20 mEq/kg the food products would be considered already been rancid and become unacceptable for consumption (Pearson, 1976). Besides, the results (Table 6) suggest that JSP is effective in suppressing the oxidation in JSP enriched cakes. While added into the cake formulations the antioxidants present in JSP flour might prevent the lipid peroxides formation during storage and delayed oxidation (Lu *et al.*, 2010). This result indicated that lipid oxidation in cakes could be inhibited by the addition of antioxidant (Juskiewicz *et al.*, 2008) from JSP flour.

Microbial analysis

Total plate counts, in terms of bacterial and fungal count, of the JSP enriched cakes were determined during the storage period of 14 days after 2 days' interval. The obtained results, as shown in Table 7, varied highly among different samples.

At 0 day no viable load was found for all of the fresh samples undergone a high baking temperature (Table 7). The total viable loads were increased with the storage periods and the highest one was accounted for S₁ [(5.30 ± 0.17) × 10⁹ cfu/g] after 14 days (Table 7). According to Table 7, the JSP enriched cakes (S₂ and S₃) exhibit a slower rate of microbial growth than that of control one (S₁). The slower growth of microorganisms in JSP enriched cakes might be due to the increased solute contents in cake formulations that would result in a reduced a_w in the baked cakes. After 8 days of storage control sample (S₁) showed the total plate count (8.30 ± 0.10) × 10⁶ cfu/g which became (7.30 ± 0.30) × 10⁷ cfu/g after 10 days. On the other hand, (6.80 ±

$0.17) \times 10^6$ cfu/g and $(6.20 \pm 0.13) \times 10^6$ cfu/g total viable loads were accounted for S_2 and S_3 respectively after 10 days of storage and these found to be proliferated to $(2.15 \pm 0.05) \times 10^7$ cfu/g (S_2) and $(1.78 \pm 0.21) \times 10^7$ cfu/g (S_3) respectively after 12 days (Table 7). The microbial counts obtained in this study (Table 7) is significantly differed from the previous studies where Morkos *et al.* (2013) accounted for the bacterial loads in butter cakes prepared with chick pea and peanut and found an increase in total microbial load after a storage period for 4 weeks. Agu and Okoli (2014) studied on microbial quality of biscuits and reported that no mold was found within 4 days and total plate counts were found to increase in the biscuits after 20 days of storage.

According to the FAO/WHO expert consultation for microbiological specification (CAC/RCP 15, 1976) for egg products, maximum 10^6 mesophilic aerobic bacteria would be safe for human consumption (Frazier and Westhoff, 1995). Therefore, the

JSP enriched cakes prepared in this study are microbiologically safe for human consumption (Ali *et al.*, 2008) before 10 days of storage (25 ± 2 °C) while stored in polyethylene packages without using any externally added preservatives.

Organoleptic stability of JSP enriched cake

The storage stability of food products provides a great importance on ultimate quality of foods. In this study, the cakes were prepared without any preservatives and stored in local polyethylene package at room temperature (25 ± 2 °C). After storage for 6 days the JSP enriched cakes started to change their sensory qualities and all cake spoiled within 14 days (Table 8).

In the context of organoleptic quality attributes, an acceptable smooth texture quality was observed in all of the cakes (S_1 , S_2 and S_3) formulated and prepared in this study by 8 days of storage (Table 8). According to Table 8, all sensory attributes of control (S_1) and JSP

Table 7. Microbial analysis of JSP enriched cakes

Storage period (day)	Total viable load (cfu/g)		
	S_1 (mean \pm SD)	S_2 (mean \pm SD)	S_3 (mean \pm SD)
0	Found no colony	Found no colony	Found no colony
2	$(2.30 \pm 0.10) \times 10^3$	$(2.13 \pm 0.02) \times 10^3$	$(2.07 \pm 0.70) \times 10^3$
4	$(3.50 \pm 0.21) \times 10^5$	$(1.67 \pm 0.11) \times 10^5$	$(1.07 \pm 0.15) \times 10^5$
6	$(5.70 \pm 0.18) \times 10^5$	$(4.90 \pm 0.15) \times 10^5$	$(3.30 \pm 0.10) \times 10^5$
8	$(8.30 \pm 0.10) \times 10^6$	$(3.90 \pm 0.19) \times 10^6$	$(3.20 \pm 0.41) \times 10^6$
10	$(7.30 \pm 0.30) \times 10^7$	$(6.80 \pm 0.17) \times 10^6$	$(6.20 \pm 0.13) \times 10^6$
12	$(8.80 \pm 0.41) \times 10^8$	$(2.15 \pm 0.05) \times 10^7$	$(1.78 \pm 0.21) \times 10^7$
14	$(5.30 \pm 0.17) \times 10^9$	$(9.50 \pm 0.34) \times 10^8$	$(7.90 \pm 0.19) \times 10^8$

S_1 : 0% JSP flour in cake (control)

S_2 : cake with 3% JSP flour

S_3 : cake with 6% JSP flour

Table 8. Storage study of protein enriched cake at room temperature (25 ± 2 °C)

Storage period	Sample	State of organoleptic attributes				Remarks
		Color	Flavor	Taste	Texture	
0 day	S ₁	Ok	Ok	Ok	Ok	Acceptable
	S ₂	Ok	Ok	Ok	Ok	Acceptable
	S ₃	Ok	Ok	Ok	Ok	Acceptable
6 days	S ₁	Ok	Ok	Ok	Ok	Acceptable
	S ₂	Ok	Ok	Ok	Ok	Acceptable
	S ₃	Ok	Ok	Ok	Ok	Acceptable
8 days	S ₁	White spot	Slightly off-flavor	Slightly unpleasant	Ok	Acceptable
	S ₂	Ok	Ok	Ok	Ok	Acceptable
	S ₃	Ok	Ok	Ok	Ok	Acceptable
10 days	S ₁	Spot with mycelium	Off-flavor	Slightly unpleasant	Moderately hard	Moderately acceptable
	S ₂	White spot	Off-flavor	Slightly unpleasant	Moderately hard	Moderately acceptable
	S ₃	White spot	Slightly off-flavor	Slightly unpleasant	Moderately hard	Moderately acceptable
12 days	S ₁	Spot with mycelium	Off-flavor	Unpleasant	Hard	Slightly acceptable
	S ₂	White spot	Off-flavor	Slightly unpleasant	Moderately hard	Moderately acceptable
	S ₃	White spot	Slightly off-flavor	Slightly unpleasant	Moderately hard	Moderately acceptable
14 days	S ₁	Whitish mycelium	Off-flavor	Unpleasant	Hard	Unacceptable
	S ₂	Whitish mycelium	Off-flavor	Unpleasant	Hard	Unacceptable
	S ₃	Whitish mycelium	Off-flavor	Unpleasant	Hard	Unacceptable

S₁: 0% JSP flour in cake (control)

S₂: cake with 3% JSP flour

S₃: cake with 6% JSP flour

enriched cakes (S₂ and S₃) became moderately acceptable from 10 to 12 days of storage and these were turned into unacceptable at the end of 14 days' storage period. The rate of degradation accelerated with formation of mycelial growth on the cake surfaces (Table 8). The extended shelf-life of the JSP enriched cake might be attributed due to the presence and formation of organic acids (Bansal &

Bansal, 2011; Yadav *et al.*, 2011) as well as lower pH and reduced a_w in the baked cakes.

Conclusions

The JSP isolate is a noble source for protein and could be used in various food formulations like cake, biscuit, bread, yoghurt etc. Since cakes are consumed by all ages of people, one of the most convenient ways to utilize JSP isolate is

to prepare supplementary foods like cakes. The JSP enriched cakes could eventually minimize the protein malnutrition among people, especially children, in a developing country like Bangladesh. Besides, the JSP fortified cakes exhibit a superiority in storage quality. Assessing the physicochemical properties and storage stability of the JSP enriched cakes it could be concluded that mixing of 6% JSP isolate in formulation as the substitution of wheat flour yields cakes with attractive and acceptable sensory attributes including color, taste, texture, flavor and overall acceptability.

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