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Process Optimisation of *Dolichos lablab* Peel Powder and Buckwheat Flour incorporated Biscuits using Response Surface Methodology

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Abstract
The present study was carried out to partially replace refined wheat flour with buckwheat flour and *Dolichos lablab* peel powder to get benefit of their nutraceutical properties and also to utilize *Dolichos lablab* peel, which would otherwise be a waste. The composition of the various ingredients like refined wheat flour (RWF), buckwheat flour (BWF) and *Dolichos lablab* peel powder (DLPP) was optimised using response surface methodology (RSM) using Minitab 17 taking into consideration 20 set of experiments and observing the responses on various parameters like hardness, fracturability, colour and appearance, crispiness, flavor and overall acceptability. The optimized biscuit had 45 % refined wheat flour, 10.65 % *Dolichos lablab* peel powder and 30 % buckwheat flour apart from other ingredients like salt, sugar etc. and the overall acceptability of the biscuit came out to be 8.17. The optimized biscuits were analysed for their nutritional and physicochemical properties. The moisture, protein, fat, ash, crude fibre and carbohydrate content were calculated to be 2.73 %, 8.052%, 15.90 %, 1.48 %, 5.25 % and 66.61 % respectively. The thickness of the optimized biscuits came out to be 0.70 cm, the diameter of the biscuit came out to be 5.80 cm and the spread ratio was 8.32. The shelf life of biscuit was studied at a storage temperature of 37 ℃ for a period of 45 days at an interval of 15 days based on various parameters like moisture, free fatty acid, peroxide value and hydroxy methyl furfural (HMF) content. There was no significant changes in moisture content of biscuits stored at ambient temperature conditions. The change in lipid peroxidation as measured by peroxide value and free fatty acid value were found to increase during storage period. Peroxide value found to increase from 6.967 to 17.905 meq O_2/Kg fat, while free fatty acid increased from 1.22 to 1.6532 % oleic acid respectively. It was observed that with increase in storage time the formation of HMF increased.

Keywords: *Dolichos lablab* peel powder, buckwheat, biscuits, RSM

1. Introduction

One of the most popular ready to eat bakery product biscuits possess several attractive features including palatability, relatively long shelf life and easy storage, more convenient and good eating quality. Due to their increasing demand among consumers the interest in bakery industry is growing fast [19, 21]. For making biscuits soft wheat is more preferable. Now-a-days, composite wheat flours are being widely used because of low protein content (6-14%) and lacking in some of the essential amino acids (lysine) in refined flour [34]. Use of composite flour gives increased quality protein and dietary fibre [19, 41]. Biscuits comprises largest category of snacks foods in world [26, 32]. Baljeet, et al., (2014) studied on quality characteristics of biscuits prepared from incorporation of carrot pomace powder (CPP) and germinated chickpea flour (GCF) which resulted in an increase in protein, ash and crude fibre contents. Wani et al., (2013) studied on the nutritive value of cookies which could be improved by the addition of inexpensive watermelon seed protein concentrate resulting in substantial increase in protein content of cookies. Similarly, Kohajdova et al., (2013) studied on suitability of pea flour for cracker biscuits production which modified the physical properties of final products by different ways (decreasing of volume index, width and spread ratio, increasing of thickness). Neha and Ramesh, (2012) developed biscuits by using soya flour and rice bran and concluded that supplementation of soy flour and rice bran at 15% levels each, would improve the nutritional quality without adversely affecting the sensory parameters. High sensory rating biscuits are produced from blends of wheat and fonio (*Digiteria exilis*) [28], cocoyam and pigeon pea [33], soy and sweet potato [1], cowpea/plantain/wheat flour blends [5] etc. In comparison of other cereals Buckwheat has been reported to possess higher antioxidants due to rutin content [25]. Due to its functional and therapeutic benefits Buckwheat are incorporated in products.

Buckwheat (F*agopyrum esculantum Moench*) is pseudo cereal, highly nutritious and rich in dietary source protein with favourable amino acids and vitamins [10], trace elements [38], essential minerals [40] and starch and dietary fibre [10]. Buckwheat is rich in phenolic compounds like orientin, rutin, vitexin, quercetin, isovitexin, kampferol-3-rutinoside and catechins [14]. Similarly, Phytochemical analysis of *Dolichos lablab* by Al-Snafi A.E., (2017) showed that it contained sugar, alcohol, phenols, essential oils, alkaloids, tannins, flavonoids, saponins, coumarins, terpenoids, pigments, glycosides, anthnanoids, wide range of minerals and many other metabolites. *Dolichos lablab* showed wide range of medicinal activities including antioxidant, anti-microbial, anti-cancer, hypolipidemic, cardiovascular, central nervous, respiratory, immunological, anti-inflammatory, analgesic, antipyretic and other pharmacological effects [3, 12, 27, 31, 35, 36, 39, 43]

Yadav et al., (2012) reported that the addition of DPMF (De-Oiled Peanut Meal Flour) resulted in significant improvement in protein content in wheat flour-DPMF blends. Also, Alam et al., (2014) formulated a fibre enriched herbal biscuits using Tulsi (*Ocimum sanctum*) leaves, drumstick (*Moringa oleifera*) leaves, whole wheat flour, egg white, vegetable oil, margarine (Dalda) and other necessary ingredients. Mišan et al., (2014) worked on improving nutritional profile and antioxidant capacity of gluten-free cookies by drying and grinding Blueberry pomace and by-product of juice production and using it for a gluten-free cookie formulation. Ganorkar and Jain, (2014) reported that incorporating RWF with refined flaxseed flour, increased the fibre content by nine times more than the control. Similarly, Bandyopadhyay et al., (2014) developed mango peel and kernel powder fortified cookies which are rich sources of natural bioactive compounds and play an important role in prevention of diseases. Their results revealed that Mango peel powder (MPP) has higher ash, crude fibre, water holding and oil holding capacity than that of mango kernel powder (MKP). Similarly, Dolichos lablab peel is usually not consumed and thrown as waste. Using it in the powder form in products like biscuits will not only improve its nutritional and functional attributes but would also help in value addition of the waste. The Present work has been done to make fibre rich biscuits by using refined wheat flour, *Dolichos lablab* peel powder and buckwheat flour (RWF-DLPP-BWF) blends.

2. Materials and Method

2.1 Dolichos Lablab Peel Powder Preparation

The *Dolichos lablab* peels were collected as a by-product washed continuously with water to remove dust and other insoluble impurities present in it. *Dolichos lablab* peels were cut into smaller size and then it was dried for 30 minutes in sunshade to remove the surface water and then in hot-air oven (Perfit, India) at 333^{o} K for 2 hours till it becomes crisp. Then the dried *Dolichos lablab* peels were crushed by using a mixer-grinder to obtain it in the powder form. The powder was collected in accurate size by using a 200 mesh sieve and it was preserved using clean polyethylene pouches (Fig. 1).

Fig 1: Packaged *Dolichos lablab* peel powder

2.2 Preparation of Biscuits

Weighing of all the ingredients, cleaned and sieved Maida (RWF), BWF, and cleaned and sieved DLPP, sugar, biscuit fat, and all other minor ingredients (like sodium bicarbonate, salt, skim milk powder, dextrose, vanilla and water) was done using weighing balance (Metller Toledo, JB I 603- CIF act, Switzerland). An electric beater was used to beat the fat for about a minute, after which the sugar powder was gradually added and beating was done for about 5 minutes till it was light and fluffy. The vanilla essence was then added and beating was done for 30 seconds until a smooth wet mixture was formed. Salt was added to enhance the flavour with other ingredients. The BWF, DLPP and RWF are weighed, sieved and mixed well. Gradually the wet mixture was added to the dry flour mixture in a bowl and kneaded gently to make soft non sticky dough. The dough was then cut into equal square shapes with uniform thickness using a biscuit cutter and placed in a preheated oven to bake at 180^{0}C for 20 minutes. After baking the biscuits were taken out of the oven and allowed to cool (Fig. 2). The process flowchart has been summarized in Fig. 3.

Fig 2: Biscuits prepared after process optimization

Flow Sheet

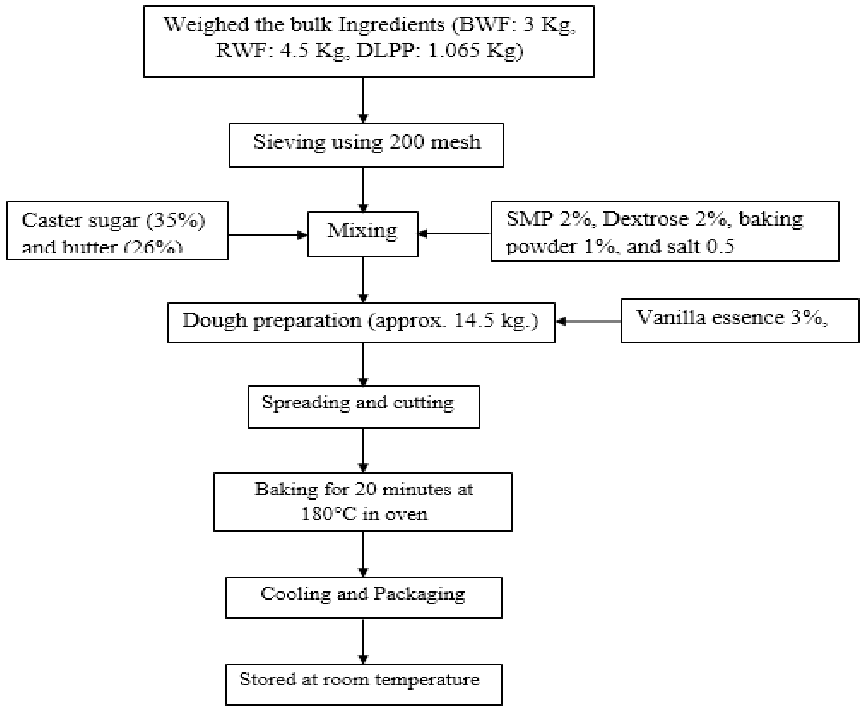


Fig 3: Manufacturing Process of *Dolichos lablab* Peel Powder Biscuits

2.3 Textural Analysis

The textural properties of biscuits hardness and fracturability was measured by texture analyser (TA.XT plus texture profile analyzer, Stable Micro Systems, UK) using 3- Point Bending Rig (HDP/3PB) using 5kg load cell and Heavy Duty Platform (HDP/90), Pre-Test speed: 1.0 mm/s; Test Speed: 3.0 mm/s; Post Test Speed: 10.0 mm/s; Distance: 4 mm; Trigger Force: Auto- 50g; Tare Mode: Auto; and Data Acquisition Rate: 500pps. The peak force (g) and the mean distance at break (mm) were recorded.

2.4 Determination of Physical Characteristics

The total diameter and the thickness were measured in centimetre with the help of a vernier calliper. Diameter (D) of biscuits was determined by placing four biscuits edge to edge. The biscuits were rotated at an angle of 90^o for duplicate readings. The process was repeated thrice to get an average value. The thickness (T) of the biscuits was determined by placing four biscuits stacked on one another. This was repeated thrice to get an average. Spread ratio was calculated as diameter (Length) to thickness ratio [37].

2.5 Sensory Evaluation

The sample of each trial was evaluated for sensory attributes viz, colour and appearance, crispiness and overall acceptability on a 9-point hedonic scale by an experienced panel comprising of five judges selected from the Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

2.6 Statistical Analysis

The experimental data obtained was analyzed with the help of Minitab17 software. After that each individual experiment, responses were analyzed to assess the effect of independent parameters or factors on them. The second order polynomial equation of the following form was fitted to the responses:

$$Y_k = \beta o + \Sigma\beta i\, Xi + \Sigma\beta ii + X^2 + \Sigma\beta ij\, XiXj$$

Where

Y_k= Responses

βo, βi, βii, & βij = constant, linear, quadratic, and cross product regression coefficient

X= are the actual value of the independent variables.

2.6.1 Statistical Optimization

Response surface methodology which involves designing of experiments, selection of levels of variables in experiments runs, fitting mathematical models and finally selecting levels of variables by optimizing the response was used in the study. A central composite rotatable design (CCRD) was used to design the experiments comprising three independent processing parameters or factors or constraints viz. RWF, DLPP and BWF. Twenty experiments were conducted for the present research work. There were six experiments at centre point to calculate the repeatability of the method. The experiments were conducted in randomized order to minimize the effect of unexpected variability in the observed responses because of extraneous factors. Response surface analysis required coding of the values of the processing parameters of factors are BWF, RWF and DLPP. Their lower and upper limit has been shown in Table 1. The rest of the ingredients used in manufacturing of biscuits are kept constant and their quantities are given as: sugar- 35%, vanilla- 3%, salt- 0.5%, butter- 26%, skim milk powder- 2%, dextrose- 2%, baking powder- 1%**.** The processing parameters or factors were optimized with respect to the responses viz. colour and appearance, flavour, overall acceptability, hardness and fracturability of the biscuits. Numerical optimization technique of the Minitab17 software was used for simultaneous optimization of the multiple responses among 20 sets of experiments.

2.7 Physicochemical Analysis

The nutritional parameters such as moisture content, carbohydrate, ash, crude fiber, fat and protein content were determined using method described by AOAC (2000). The physiological changes in the sample viz. Peroxide value, Free Fatty Acid (FFA) content and Hydroxy Methyl Furfural (HMF) content, under storage at 37^o C were determined at an interval of 15 days for a period of 45 days. Peroxide value and Free fatty acid content was determined as per AOCS (2009) methods. HMF was determined by the method suggested by White (1979) with slight modifications in sample preparation.

3. Result and Discussion

3.1 Optimization of Biscuits Using RSM

RSM was applied to the experimental data with the help of Minitab 17 software. All the models exhibited statistical significance, as indicated by the F value, R^2 value for all the models was more than 80%, c.v. was less than 10% and lack of fit was also found to be not significant. All told, it can be concluded that all the models were statistically valid for predicting the response. Table 2 shows the central composite rotatable design (CCRD) for the optimization of fibre rich biscuits.

3.1.1 Effects of Ingredients on Hardness of Biscuits

The average hardness of biscuits varied from 6.1 kg/cm3 to 9 kg/cm^3 (Table 2). The minimum and maximum hardness was obtained for the experiment number 12 and 3 respectively. In experiment number 12, level of RWF, DLPP, and BWF was 45g, 5g and 30g respectively. While in experiment number 3 the level of RWF, DLPP, and BWF was 35g, 15g and 20g respectively. The coefficient of determination (R^2) was 0.9219. The "Pred R - Squared" of 0.00% is reasonable agreement with the "Adj R - Squared" of 0 .8517. The quadratic model was significant (P< 0.03). This model can be used to navigate the design space. The data fitted the following quadratic model;

Hardness = - 20.4 + 0.939 x RWF - 0.111 x DLPP + 0.975 x BWF - 0.01119 x RWF x RWF + 0.02103 x DLPP x DLPP - 0.02437 x BWF x BWF - 0.00543 x RWF x DLPP + 0.00093 x RWF x BWF - 0.00157 x DLPP x BWF

Where, RWF = Refined wheat flour
DLPP = *Dolichos labalab* peel powder
BWF = Buckwheat flour

It was observed that with increase in DLPP there is an increase in the hardness and there is little or less effect on hardness due to RWF (Fig. 4a), whereas with increase in BWF there is decrease in hardness and there is very little effect on hardness due to RWF (Fig. 4b). Also, it can be observed that increase in buckwheat is a slight decrease in hardness with increase in DLPP there is a slight increase in hardness (Fig. 4c). By studying all three graphs together we can see that DLPP is the major ingredients which is responsible for increasing the biscuits hardness. Similar studies were carried out by Garg (2015) who reported that more strength was needed to break biscuits incorporated with pea peel powder. This might have resulted from incorporation of protein and fibre rich powder which needs more water to obtain good biscuit dough, and the biscuits prepared from high absorption dough tend to be comparatively harder.

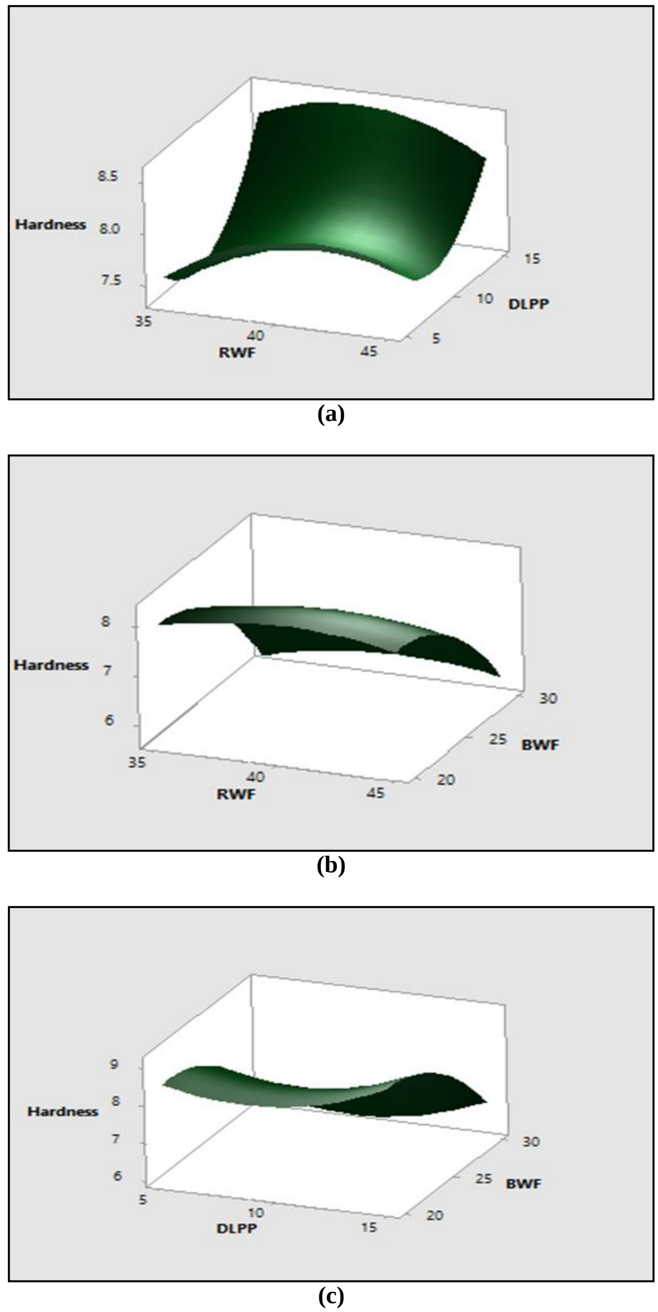


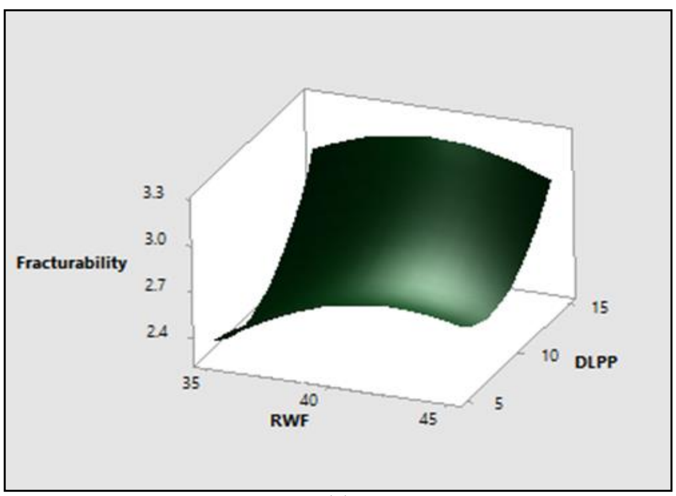
Fig 4: Effect on Hardness due to **(a)** Refined wheat flour and *Dolichos Lablab* peel powder; **(b)** Refined wheat flour and Buckwheat flour; and **(c)** *Dolichos lablab* peel powder and Buckwheat flour.

3.1.2 Effects of Ingredients on Fracturability of Biscuits

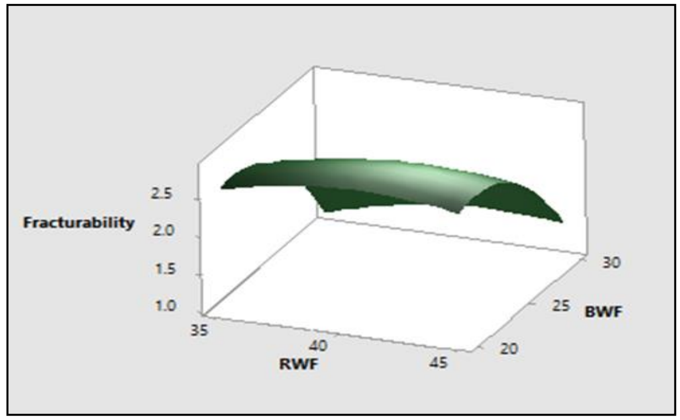
The average fracturability of biscuits varied from 1.23mm to 3.33mm (Table 2). The minimum and maximum hardness was obtained for the experiment number 12 and 3 respectively. In experiment number 12, level of RWF, DLPP, and BWF was 45g, 5g and 30g respectively. While in experiment number 3 the level of RWF, DLPP, and BWF was 35g, 15g and 20g respectively. The coefficient of determination (R^2) was 0.8532. The "Pred R - Squared" of 0.00% is reasonable agreement with the "Adj R - Squared" of 0 .7210. The quadratic model was significant (P< 0.004). This model can be used to navigate the design space. The data fitted the following quadratic model:

Fracturability = - 15.99 + 0.579 x RWF - 0.179 x DLPP + 0.721 x BWF - 0.00718 x RWF x RWF + 0.01305 x DLPP x DLPP - 0.01935 x BWF x BWF - 0.00314 x RWF x DLPP + 0.00184 x RWF x BWF + 0.00354 x DLPP x BWF

It can be observed that with increase in DLPP there is an increase in the hardness and there is little or less effect on fracturability due to RWF (Fig. 5a) while with increase in buckwheat there is decrease in fracturability and there is very little effect on hardness due to RWF (Fig. 5b). Also, increase in buckwheat is a slight decrease in fracturability with increase in DLPP there is a slight increase in fracturability (Fig. 5c). By studying the results we observe that with increase in DLPP the fracturability increased to a great extent, this could be as a result of high fat in the DLPP. Similar studies were carried out by Agiriga and Iwe (2009) who found that fragility of cookies bars increased with increase in groundnut flour. However fragility reduced when RWF and BWF were added. This increase in rigidity is due to increase in carbohydrate starch granules, which is responsible for gel and structure formation in baked goods [20].



(a)



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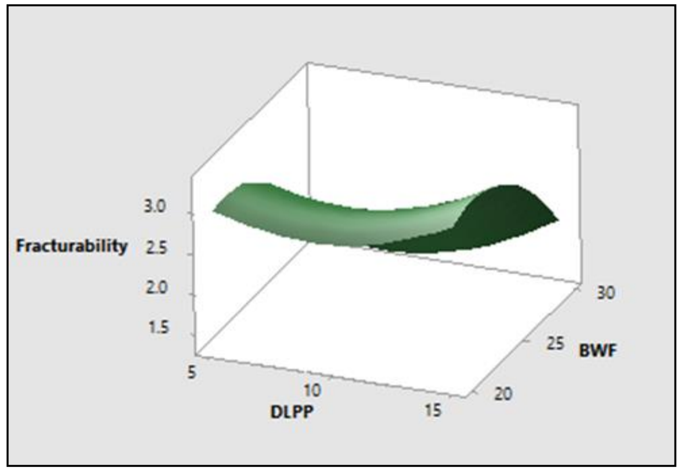


(c)

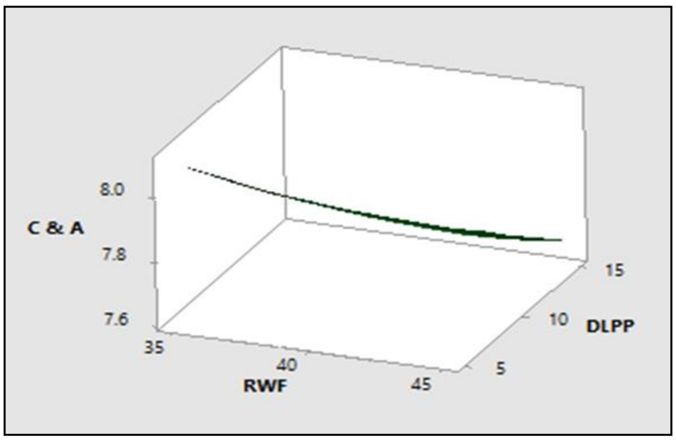
Fig 5: Effect on Fracturability due to **(a)** Refined wheat flour and *Dolichos Lablab* peel powder; **(b)** Refined wheat flour and Buckwheat flour; and **(c)** *Dolichos lablab* peel powder and Buckwheat flour.

3.1.3 Effects of Ingredients on Colour and Appearance of Biscuits

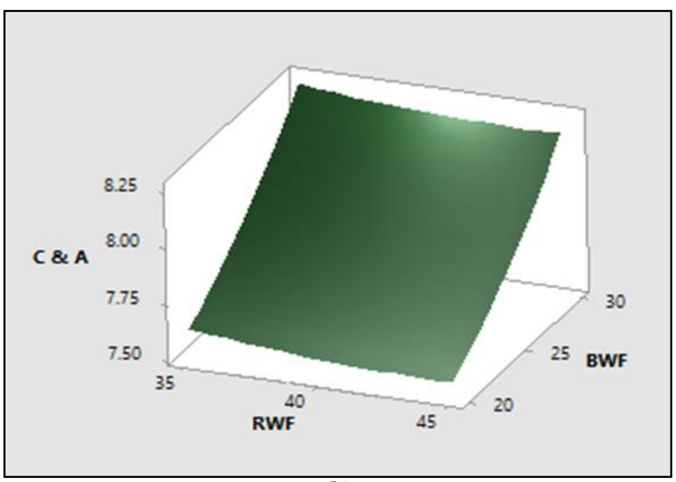
The average score on 9-point Hedonic scale of colour and appearance of biscuits varied from 7.3 to 8.4 (Table 2). The minimum and maximum scores of colour and appearance were obtained for the experiment number 11 and 5 respectively. In experiment number 11, level of RWF, DLPP, and BWF was 40g, 10g and 30g respectively. The coefficient of determination (R^2) was 0.9115. The "Pred R - Squared" of 0.0862 is reasonable agreement with the "Adj R - Squared" of 0 .8319. The quadratic model was significant (P< 0.003). This model can be used to navigate the design space.

Colour & Appearance = 11.04 - 0.075 x RWF - 0.1817 x DLPP - 0.103 x BWF + 0.00071 x RWF x RWF + 0.00035 x DLPP x DLPP + 0.00235 x BWF x BWF + 0.00082 x RWF x DLPP + 0.00018 x RWF x BWF + 0.00418 x DLPP x BWF

It was be observed that with increase in DLPP there is a decrease in the colour & appearance and there is little or less effect on colour & appearance due to RWF (Fig. 6a) while with increase in buckwheat there is increase in colour & appearance and there is very little effect on colour & appearance due to RWF (Fig. 6b). Also, increase in buckwheat is a slight decrease in colour & appearance with increase in DLPP there is a slight decrease in colour & appearance (Fig. 6c). By analyzing the results obtained it can be seen that with increase in DLPP, there is a decrease in colour and appearance, a burnt colour was obtained as the DLPP was increased. Maillard browning occurs during baking in the presence of amino acids and reducing sugars, it can be said that more maillard reactions occured in biscuits containing higher amount of *Dolichos labalab* in combination with BWF as it contains higher amount of amino acids. Similar findings were reported by Chakrabarti et al., 2017 where, increase in soyabean flour resulted in a decrease in the colour and appearance



(a)



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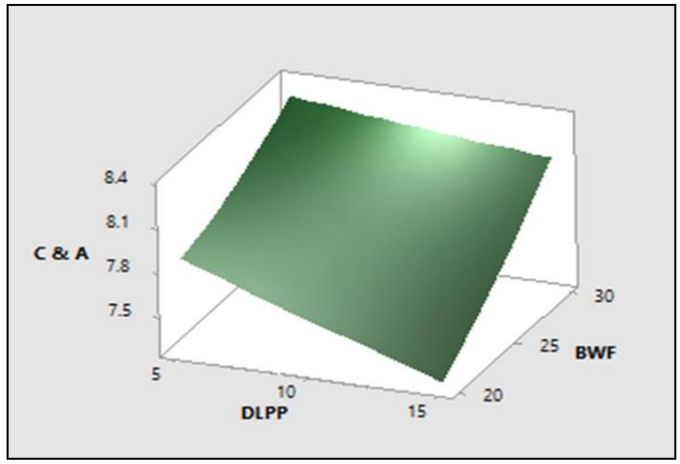


(c)

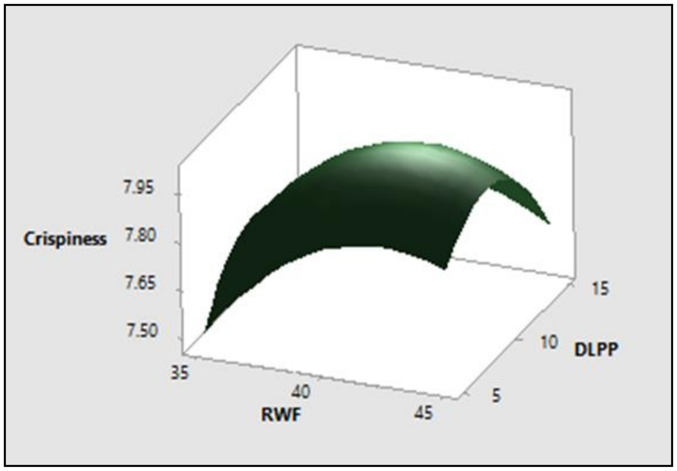
Fig 6: Effect on color and appearance due to **(a)** Refined wheat flour and *Dolichos Lablab* peel powder; **(b)** Refined wheat flour and Buckwheat flour; and **(c)** *Dolichos lablab* peel powder and Buckwheat flour.

3.1.4 Effects of Ingredients on Crispiness of Biscuits

The average score of crispiness of biscuits varied from 7.3 to 8.2 (Table 2). The minimum and maximum scores of crispiness were obtained for the experiment number 11 and 5 respectively. In experiment number 11, level of RWF, DLPP, and BWF was 45g, 15g and 20g respectively. While in experiment number 5 the level of RWF, DLPP, and BWF was 40g, 10g and 30g respectively. The coefficient of determination (R^2) was 0.8115. The "Pred R - Squared" of 0.00% is reasonable agreement with the "Adj R - Squared" of 0.5796. The quadratic model was significant ($P< 0.022$). This model can be used to navigate the design space. The data fitted the following quadratic model:

Crispiness = - 1.95 + 0.444 x RWF + 0.185 x DLPP - 0.055 x BWF - 0.00653 x RWF x RWF - 0.00843 x DLPP x DLPP - 0.00243 x BWF x BWF - 0.00178 x RWF x DLPP + 0.00478 x RWF x BWF + 0.00178 x DLPP x BWF

It was observed that with increase in *Dolichos labalab* peel powder there is an decrease in the crispiness and there is little or less effect on Crispiness due to RWF (Fig. 7a) while, increase in buckwheat there is decrease in Crispiness and there is very little effect on Crispiness due to RWF (Fig. 7b). Also, increase in buckwheat is a slight increase in Crispiness with increase in DLPP there is a slight decrease in Crispiness (Fig. 7c). By observing the results obtained it can be seen that with increase in DLPP there is a decrease in crispiness this may be due to high fat content. Similar study was carried out by Chakrabarti (2017) who found that crispiness of biscuits decreased with increase in soyabean flour. However, crispiness increased when RWF and BWF were added. This increase in crispiness due to high fiber content in in these flours.



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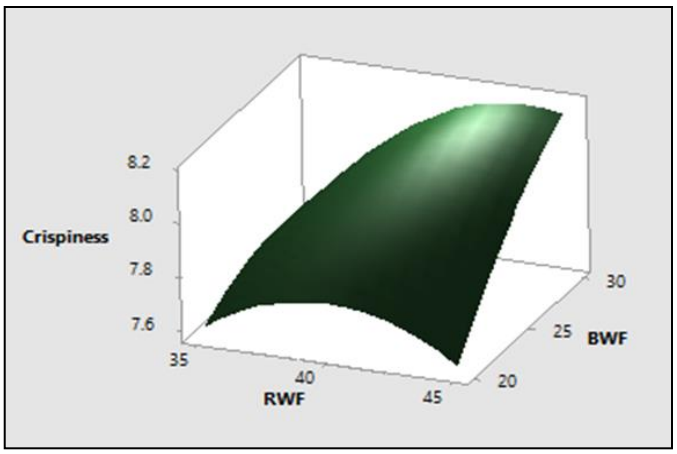

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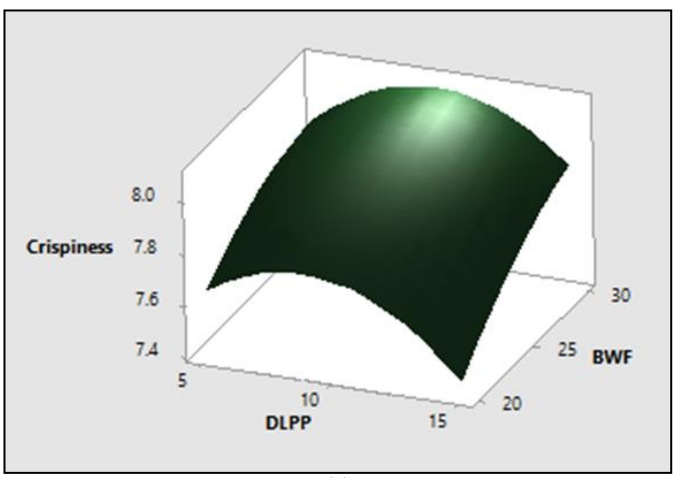


(c)

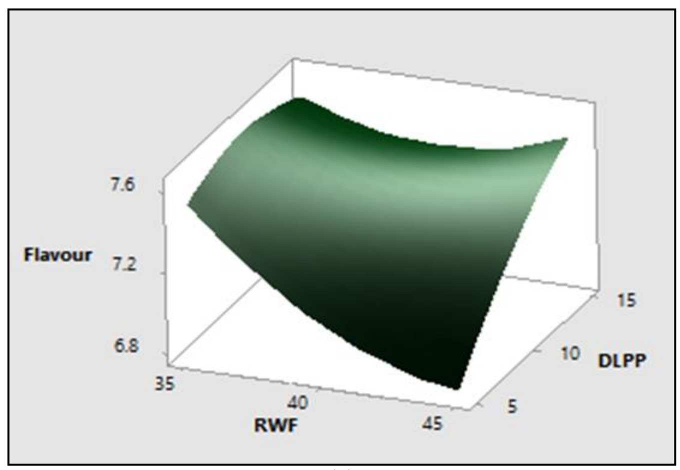
Fig 7: Effect on crispiness due to **(a)** Refined wheat flour and *Dolichos Lablab* peel powder; **(b)** Refined wheat flour and Buckwheat flour; and **(c)** *Dolichos lablab* peel powder and Buckwheat flour.

3.1.5 Effects of Ingredients on Flavor of Biscuits

The average score of flavor of biscuits varied from 6.8 to 8.6 (Table 2). The minimum and maximum scores of flavor were obtained for the experiment number 18 and 5 respectively. In experiment number 18, level of RWF, DLPP, and BWF was 45g, 5g and 20g respectively. While in experiment number 5 the level of RWF, DLPP, and BWF was 40g, 10g and 30g respectively. The coefficient of determination (R^2) was 0.8639. The "Pred R - Squared" of 0.00% is reasonable agreement with the "Adj R - Squared" of 0.7414. The quadratic model was significant (P< 0.003). This model can be used to navigate the design space. The data fitted the following quadratic model:

Flavour = 24.05 - 0.503 x RWF - 0.218 x DLPP - 0.518 x BWF + 0.00562 x RWF x RWF - 0.00393 x DLPP x DLPP + 0.01407 x BWF x BWF + 0.00725 x RWF x DLPP - 0.00225 x RWF x BWF + 0.00175 x DLPP x BWF

It was observed that with increase in DLPP there is an increase in the flavour and there is less effect on flavour due to RWF (Fig. 8a) while with increase in buckwheat there is increase in flavour and there is very little effect on flavour due to RWF (Fig. 8b). Also, it can be observed that increase in buckwheat is a slight increase in flavour and very little effect due to DLPP (Fig. 8c). Results of sensory evaluation of flavour showed that increase in RWF reduced the flavour of biscuits. This is accordance with the studies of Swanson and Akoh (1994) who found that biscuits made with wheat flour had a better flavour compared to biscuits made of composite flour due to trapping of fat by the lipoprotein matrix present in wheat flour. However with increase in DLPP and BWF there is a slight increase in flavour of the biscuits.



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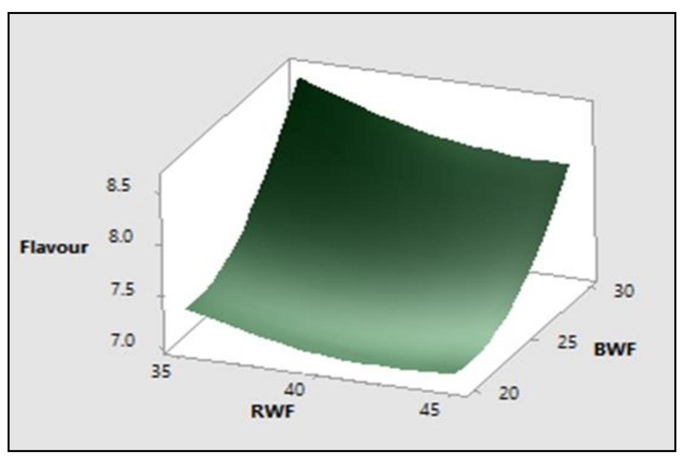

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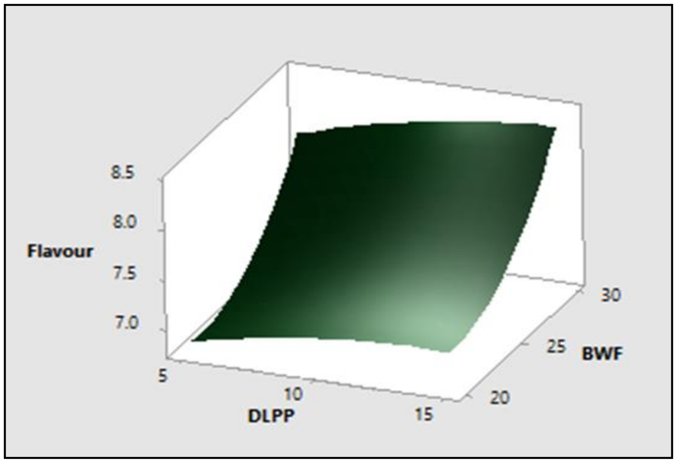


(c)

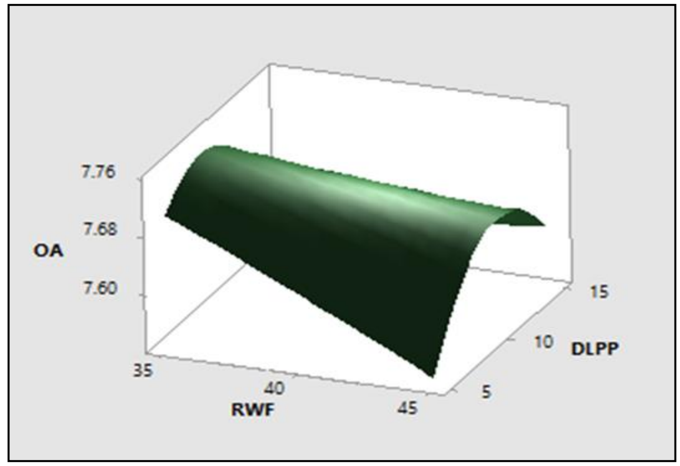
Fig 8: Effect on Flavor due to **(a)** Refined wheat flour and *Dolichos Lablab* peel powder; **(b)** Refined wheat flour and Buckwheat flour; and **(c)** *Dolichos lablab* peel powder and Buckwheat flour.

3.1.6 Effects of Ingredients on Overall Acceptability of Biscuits

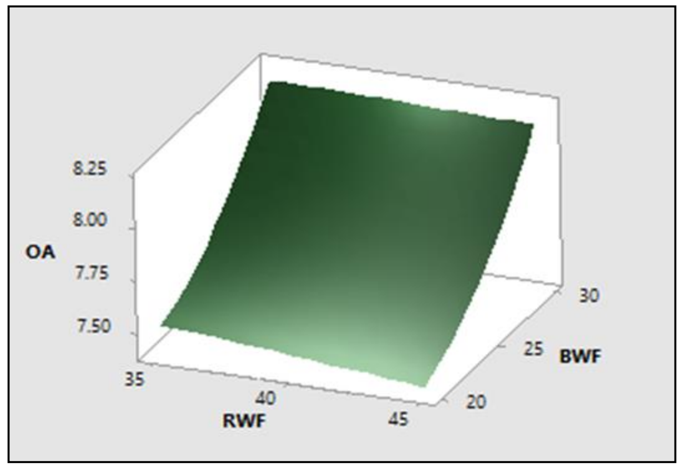
The average score of overall acceptability of biscuits varied from 7.3 to 8.4 (Table 2). The minimum and maximum scores of Flavour were obtained for the experiment number 11 and 5 respectively. In experiment number 11, level of RWF, DLPP, and BWF was 45g, 15g and 20g respectively. While in experiment number 5 the level of RWF, DLPP, and BWF was 40g, 10g and 30g respectively. The coefficient of determination (R^2) was 0.8538. The "Pred R - Squared" of 0.00% is reasonable agreement with the "Adj R - Squared" of 0.7222. The quadratic model was significant (P< 0.004). This model can be used to navigate the design space. The data fitted the following quadratic model:

Overall Acceptability = 11.02 - 0.041 x RWF - 0.073 x DLPP - 0.229 x BWF - 0.00011 x RWF x RWF - 0.00403 x DLPP x DLPP x 0.00477 x BWF x BWF + 0.00214 x RWF x DLPP + 0.00086 x RWF x BWF + 0.00256 x DLPP x BWF

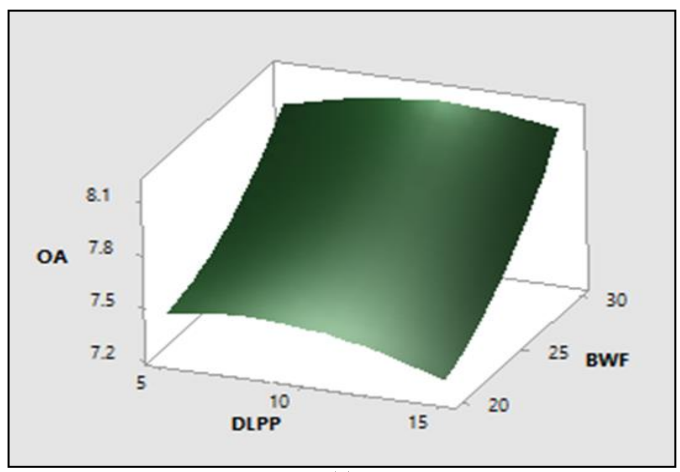
It can be observed that with increase in *Dolichos labalab* peel powder there is an increase in the overall acceptability and there is no much effect on overall acceptability due to RWF (Fig. 9a) while an increase in buckwheat there is increase in overall acceptability and there is very little effect on overall acceptability due to RWF (Fig. 9b). Also, increase in buckwheat there is an increase in overall acceptability and very little effect due to DLPP (Fig. 9c). The observations in the present study were close agreement with the findings of Garg (2015), who studied the effect of pea peel powder on the functional properties and the potential of pea peel powder and RWF in biscuits production. They observed no significant differences in texture, flavor, taste, and overall acceptability of biscuits.



(a)



(b)



(c)

Fig 9: Effect on Overall acceptability due to **(a)** Refined wheat flour and *Dolichos Lablab* peel powder; **(b)** Refined wheat flour and Buckwheat flour; and **(c)** *Dolichos lablab* peel powder and Buckwheat flour.

3.2 Analysis of the Optimized Product

The method adopted for the process optimization was based on numerical method (Table 3). On the basis of constraints suggested, Minitab17 software selected the solution as given in Table 4 with desirability of 0.8670.

3.2.1 Proximate Analysis of Optimized Biscuits

The proximate composition of the optimized product was calculated. The Moisture content was 2.73 %, protein content was 8.052%, fat content was 15.90 %, ash content was 1.48 %, crude fibre was 5.25 % and carbohydrates was 66.61 %. From the proximate composition of the biscuits we can see that the carbohydrate content is very high in the biscuits which can be attributed to the extremely high content of carbohydrate in RWF and BWF. Protein content is acceptable due to nearly equal protein content of all three ingredients. The protein content was found to be 8.052g/100g. This result is in agreement with Divyashree et al., 2016 who reported that buckwheat incorporation increased the nutritional status of biscuits due to high protein content and also by Garg (2015) who reported that pea peel powder incorporation increased protein content. The crude fiber content is high due to high fiber content in DLPP. The crude fiber was found to be 5.25/100g. This result is in agreement with Garg (2015) who reported that pea peel powder incorporation increased nutritional status of biscuits due to high fiber content.

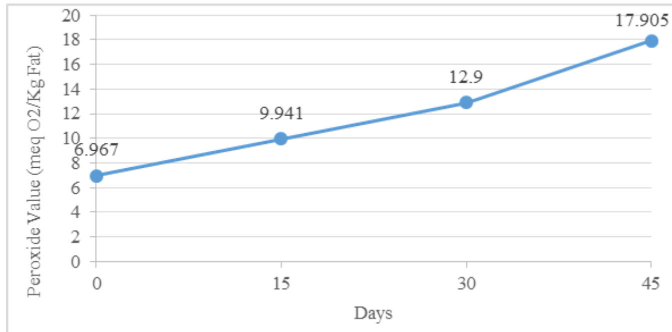
3.2.2 Physical Characteristics

The thickness of the optimized biscuits came out to be 0.70 cm, the diameter of the biscuit came out to be 5.80 cm and the spread ratio was 8.32. Spread ratio or diameter of biscuits has long been used to determine the quality of flour for producing biscuits [16]. Biscuits having higher spread ratios are considered the most desirable [23]. These results agreed with Garg (2015) who found that diameter of biscuits made from pea peel powder and BWF was (5.72 cm), and also showed that spread ratio of biscuits incorporated with pea peel powder and BWF was increased. The results obtained in this study were similar to the findings reported by Divyashree et al., 2016 for wheat and buckwheat composite biscuits. Okaka and Isieh, 1990 also reported a similar trend in biscuits from wheat and cowpea flours.

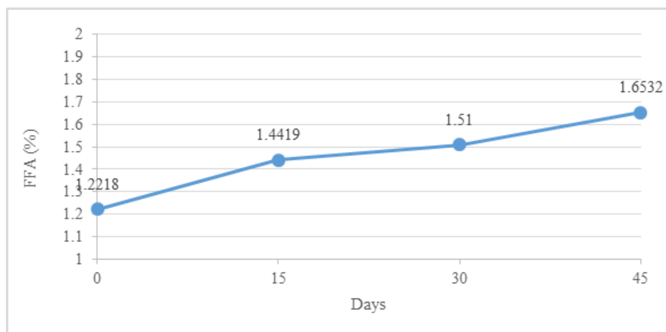
3.3 Determination of Shelf Life of Optimized Products

3.3.1 Change in Hydroxy Methyl Furfural (HMF), Free Fatty Acid and Peroxide Value during Storage

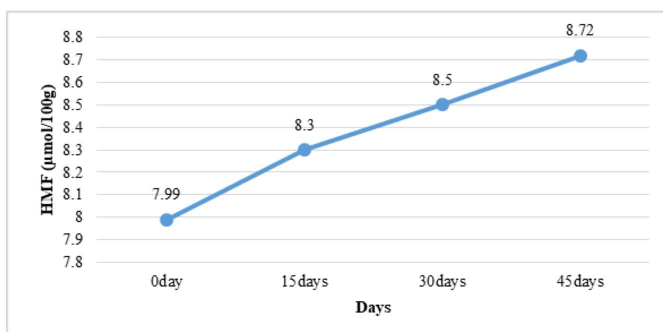
The change in lipid peroxidation as measured by peroxide value and free fatty acid value were found to increase during storage period. Peroxide value found to increase from 6.967 to 17.905 meq O_2/Kg fat (Fig. 10a), while free fatty acid increased from 1.22 to 1.6532 % oleic acid respectively (Fig. 10b). This result is in agreement with Divyashree et al, 2016 who reported that BWF and chia seed flour enriched biscuits shown peroxide value of 7.09 meq O_2 /kg fat and free fatty acid value of 1.4218 % oleic acid. Along with many other flavor and colour related substances, HMF is formed in the Maillard reaction as well as during caramelization. In these foods it is also slowly generated during storage and with increasing time [4]. In fresh biscuits HMF value recorded was 7.99 µmol/100g which increased to 8.3 µmol/100g after storage of 15 days at ambient temperature. After 30 days of storage HMF increased to 8.5 µmol/100g and after 45 days of storage the HMF increased to 8.72 µmol/100g (Fig. 10c). It was observed that formation of HMF was lesser in initial days of storage at ambient temperature. It can therefore be concluded that with increase in storage time the formation of HMF increases. This result is in agreement with Chakrabarti et al., 2017 who reported that cassava flour and soya bean flour enriched biscuits shown HMF value increased from 7.945µmol/100g to 8.5µmol/100g.



(a)



(b)



(c)

Fig 10: Change in **(a)** Peroxide value; **(b)** FFA content; and **(c)** HMF content upon storage.

Table 1: Constraints of RSM

Factor	Name	Low limit	High limit
A	Refined wheat flour	35	45
B	*Dolichos lablab* pod powder	5	15
C	Buckwheat flour	20	30

Table 2: Experimental runs and actual values of factors used in central composite rotatable design

Run	RWF	DLPP	BWF	Hardness	Fracturability	Colour and appearance	Crispiness	Flavor	Overall acceptability
01	40	10	25	7.4	2.55	7.8	8.0	7.2	7.66
02	45	10	25	8.0	2.96	7.9	7.9	7.4	7.73
03	35	15	20	9.0	3.33	7.4	7.4	7.5	7.43
04	40	10	25	7.8	2.33	7.9	8.1	7.2	7.73
05	40	10	30	6.3	1.66	8.4	8.2	8.6	8.40
06	40	10	25	7.5	2.49	7.8	8.1	7.3	7.73
07	40	10	25	7.5	2.50	7.9	8.0	7.2	7.7
08	45	15	30	6.5	2.0	8.1	7.8	8.5	8.13
09	40	10	25	7.8	2.59	7.8	8.0	7.3	7.70
10	40	05	25	8.5	3.2	8.0	7.6	7.3	7.63
11	45	15	20	8.6	3.0	7.3	7.3	7.3	7.30
12	45	05	30	6.1	1.23	8.2	8.0	7.3	7.83
13	35	10	25	7.5	2.23	7.9	7.6	7.6	7.70
14	40	10	20	8.6	2.90	7.4	7.4	7.4	7.4
15	40	10	25	7.6	2.66	7.8	8.1	7.4	7.90
16	35	05	20	7.9	2.60	8.0	7.6	7.2	7.60
17	35	15	30	6.5	1.88	8.1	7.5	8.4	8.0
18	45	05	20	8.3	2.85	7.9	7.6	6.8	7.43
19	40	15	25	8.67	2.98	7.7	7.7	7.2	7.53
20	35	10	25	7.5	2.32	7.8	7.6	7.6	7.66

Where, RWF= Refined wheat flour,
DLPP=*Dolichos labalab* Pod Powder,
BWF=Buckwheat flour

Table 3: Levels of responses fixed for optimization

Response	Goal	Lower	Upper
Overall acceptability	Maximum	7.3	8.4
Flavor	Maximum	6.8	8.6
Colour and appearance	Maximum	7.3	8.4
Crispiness	Maximum	7.3	8.2
Hardness	Minimum	6.1	9.0
Fracturabality	Minimum	1.23	3.33

Table 4: Optimized product chosen by Minitab 17 Software

Factors			Responses						Desirability
RWF	DLPP	BWF	OA Score	Flavor	C & A	Crispiness	Fracturability	Hardness	
45	10.65	30	8.17	8.16	8.19	8.15	1.4479	5.8931	0.8670

Where, OA= overall acceptability, C & A= colour and appearance

4. Conclusion

From the study it can be concluded that BWF and DLPP can be used successfully to be incorporated in biscuits. The longer shelf life of biscuits, based on various physico-chemical parameters, makes large scale production and distribution possible. This studies would support industrial utilization and the consumption of under-utilized peels such as *Dolichos lablab* peel, pea peel etc. However, this study can be carried out to increase the functional ingredients of biscuits making it beneficial for overall health.

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