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Comparison Of Physicochemical Properties of Biscuits Supplemented With Soy, Kinema, And Buckwheat Flour

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Abstract
Consumer demand for bakery products prepared using composite flour, such as biscuits, is growing. The incorporation of buckwheat flour, soy flour, and kinema flour can be considered in composite flour-based biscuits since they have good nutraceutical properties and their gluten-free nature can help avoid celiac disease. The purpose of this research was to study the physicochemical and functional characteristics of buckwheat flour, soy flour, and kinema flour as well as their potential as functional foods. The biscuits were prepared with a mixture of buckwheat flour, kinema flour, and soy flour in 10%, 30%, and 50% concentrations with whole wheat flour to evaluate the quality and acceptability of the biscuit. The composite flour biscuits were examined for their proximate compositions. The DPPH radical scavenging assay was used to assess the antioxidant properties of the biscuit extract, while the Folin-Ciocalteu method and the Aluminum Chloride Colorimetric Assay were both used to assess the total phenolic and flavonoid contents, respectively. The overall acceptability of biscuits by sensory analysis was highest at a 50% level of Black kinema flour (BLKF). Sample M (BLKF 50 %) had 0.66, 21.06, 1.61, 11.88, and 3.23 % moisture, fat, crude fiber, protein, and ash, respectively. The highest total phenolic and total flavonoid contents were found in black kinema flour (748.22 mg GAE/g and 8.92 mg GAE/g, respectively), while the highest level of antioxidant activity was found in brown kinema flour (313.56 µg/ml). BRKF 50:50 had the highest antioxidant activity (440.43 µg/ml), while BLKF 50:50 had the highest total phenolic and flavonoid contents (692.67 mg GAE/g and 8.60 mg GAE/g, respectively). The qualitative evaluation of the phytochemical content and amino acid in various flour varieties as well as in composite biscuits gave positive results. Thus, the composite flour biscuits could serve as a low-cost functional food.

Keywords: Buckwheat flour, Soy flour, Kinema flour, Supplemented biscuits
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Introduction
In recent years, consumers have become more conscious of the benefits of consuming whole-grain cereals that are gluten-free and high in fiber and protein. As a result, several research has assessed the use of composite flours derived from cereals, pseudocereals, and root crops as a wheat flour substitute in the development of cookies and other useful items. Along with the benefits of composite flours, the difficulty is to make designed goods with acceptable physical and sensory attributes. Millet, rice, green gram, Bengal gram, chickpea, peanut, soybean, buckwheat, and other grain blends have been used to make cookies with high sensory scores[1].

Novel functional foods are intended to supplement daily diets with additional nutrients and health-promoting compounds. Incorporating healthy elements necessitates a change in the original product formula, resulting in a new product with more nutritional value. Such fortification of baked formulations results in a broader diversity of taste and textures that are distinct from their traditional equivalents, as well as increased protein, vitamin, and mineral content and quality benefits. To assure sustainable growth and development, a combined strategy of traditional food fortification and food-to-food fortification using locally accessible, micronutrient-dense foods has been promoted [2].

Wheat flour is the most crucial component in biscuit making since it contains gluten proteins that are not found in other cereal flours. Wheat protein, on the other hand, is deficient in some important amino acids and has a lower protein content than oilseeds and pulses. The use of composite flour technology to supplement wheat with protein-rich components such as oilseeds and pulses could be a way to combat malnutrition [3]. Buckwheat *(Fagopyrum esculentum)* is a pseudo-cereal that grows in simple conditions and could be a viable nutritional supply for the "Starving World" in the near future. Wheat flour contains less lysine, iron, copper, and magnesium than buckwheat flour. Rutin, catechins, and other polyphenols' considerable contents, as well as their potential antioxidant action, are also important. Buckwheat's functional components have health benefits such as lowering blood pressure, lowering cholesterol, managing blood sugar, and reducing the risk of cancer [4].

The leguminous soya plant is a nutrient-dense natural vegetable that produces seeds known as soya beans. It is most commonly utilized as a vegetable oil source and protein substitute because of the presence of proteins and lipids. Soybean is a far less expensive protein source than meat. It contains a high biological value and a well-balanced necessary amino acid pattern, as well as calcium, vitamins, iron, and phosphorus. It is the finest protein source for vegetarians, as it can help to balance our diet's protein shortfall. Because of the nutritious value and acceptability of soy cereals, the nutritional status of vulnerable groups such as pregnant women, school-aged children, and others has greatly improved. Soybean has been discovered to be an excellent source of protein, and by adding an additional soy meal to any food product, the product's protein level may be increased [5]. However, due to its high satiety value brought on by its high oil content, low digestion, green beany flavor, lengthy cooking time, and enduring bitterness, it has limited direct uses. One of the most effective ways to enhance the flavor, texture, and nutritional value of soybeans is fermentation.

Kinema is a sticky, moderately alkaline indigenous fermented soybean food with a mild ammoniacal flavor created by natural fermentation plant food in the local diet. Kinema is a functional meal with various health-promoting properties such as antioxidants, digestible protein, vital amino acids, vitamin B complex, reduced cholesterol level, and so on. It is high in linoleic acid, an important fatty acid, and contains all essential amino acids. During kinema fermentation, total amino acids, free amino acids, and mineral content all rise. Phytosterols (which decrease cholesterol) are elevated during kinema fermentation. Riboflavin and niacin increase in kinema during fermentation [6].

Biscuits are a mainstream bakery item around the world. They are rich in carbohydrates, fat, and calories however low in fiber, vitamins, and minerals which make them unhealthy for day-to-day use. As a result of its agreeableness in all age groups, for longer self-life and for better taste, it is considered as a decent product for protein fortification and other nutritional enhancements [7]. The bakery business is growing at a rate of nearly 10% per year, and its commodities are becoming increasingly popular among all demographics [8]. Biscuits have various appealing characteristics, including a broad consumer base, a long shelf life, greater convenience, and good eating quality. Biscuits are appealing for protein fortification and other nutritional enhancements because of their good eating quality. Biscuits are typically made with refined wheat flour (RWF), however, combining RWF with other components like soybean, buckwheat or kinema flour can improve nutritional quality These features make protein-rich, high-dietary fiber biscuits appealing to a variety of target markets, including School Feeding Programs [9, 10].

Despite the fact that biscuits have long been a staple of many individuals' diets in both developed and emerging nations, there is currently a scarcity of knowledge on the nutraceutical properties of composite flour mix biscuits. As buckwheat flour, kinema flour, and soy flour are gluten-free, substituting these flours with wheat flour will reduce the concentration of the wheat gluten proteins, aiding in the prevention of celiac disease, and so serve as an excellent substitute for wheat flour in biscuit preparation. However, only a few investigations on the biscuit-making behavior of composite flour mixes have been published. This study was carried out to assess the antioxidant properties, phenolic content, and proximate formulation of the combined flour and biscuit compositions.

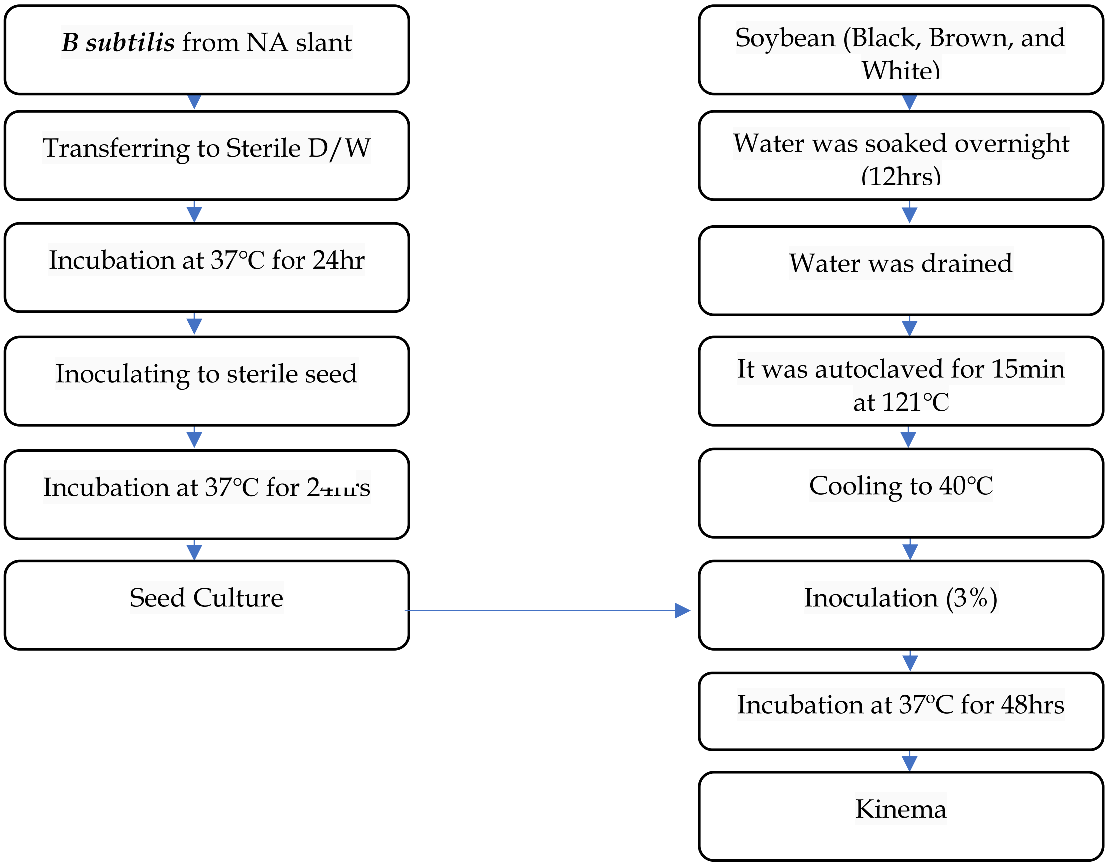
Methods and materials

Collection of sample and Preparation

Soybeans of a different variety (Black, Brown, and White), buckwheat flour, wheat flour, and freshly prepared kinema (for the isolation of *Bacillus subtilis*) were purchased from local vendors in Kathmandu. *Bacillus subtilis* was isolated from the previously prepared Kinema sample. Kinema sample was prepared from a white variety of soybean.

Isolation and Presumptive Identification

One gram of powdered Kinema sample (purchased from the local vendor) was added to Nine milliliters of sterile distilled water and was mixed well-using vortex. The tube was heated at 80°C for 10 minutes. The bacterial sample was spread over Nutrient Agar plates as well as HiCrome Bacillus Agar (HBA) and was incubated at 37°C for 24hrs.



Flowchart 1: Flowchart for Kinema Production

Isolated colonies of *Bacillus* spores were selected and sub-cultured on Nutrient Agar (NA) medium for presumptive identification and further work. The organisms were identified based on their morphological and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology. The identification method included Gram's staining and microscopy, Spore staining, Catalase test, Oxidase test, IMVIC test, Starch hydrolysis, Gelatin hydrolysis, Salt tolerance test, Nitrate reduction test, and carbohydrate fermentation tests [11]. After the confirmation of *Bacillus subtilis* colonies from all the above tests, the pure culture of *Bacillus subtilis* was preserved by streaking and incubation at 37°C for 24hrs on nutrient agar slant, then *Bacillus subtilis* from NA slant was transferred using aseptic techniques to nutrient broth, and incubated for 24hrs at 37°C. Then it was preserved under refrigerated conditions. Successive sub culturing was performed every 15 days. The different varieties of kinema were prepared by inoculating the seed culture. The seeds from soybeans were macerated and inoculated with culture broth and incubation was done at 37°C for 24hrs and was employed as culture. For the optimization process, three soybean cultivars (Black, Brown, and White) were used. The beans were optimized based on fermentation time (24hrs, 48hrs, and 72hrs) and fermentation temperature (28°C, 37°C, and 45°C). Moisture content and pH content were determined using the method of a hot air oven and digital pH meter respectively [12]. Titratable acidity and Free fatty acids were determined as described by KC and Rai [13]. Sensory evaluation was done by observing and tasting the samples. Appearance, Smell, and Texture of raw kinema samples were evaluated [14].

Preparation of Kinema

Soybeans (Black, White, and Brown) were purchased at Kathmandu's local market. The soybeans were then physically sifted to eliminate any apparent foreign matter such as straw, big soil, and stones, wires, metals, and other foreign materials. The filth, dust, and sludge that had stuck to the surface were then washed away with water. After the soaking was finished, the excess water was drained. Soaked soybeans were autoclaved for 15 minutes at 121°C. The seeds were then cooled to a temperature of roughly 40°C. Seeds were then macerated with hands to remove the seed coat and expose the cotyledons. Inoculation was done with a previously prepared starter culture that was well mixed by hand. The inoculated seeds were then incubated for 48 hours at 37°C. Kinema was ready after 48 hours of fermentation, having a nutty and musty aroma [15].

Preparation of Kinema flour and Soy flour

Each variety of fresh kinema (Black, Brown, and White) was dried in a hot air oven for 4 hours at 100°C, then processed into smooth flour using a grinder and labeled as Black kinema flour (BLKF), Brown kinema flour

Table 1 Recipe for the preparation of biscuits from buckwheat flour

Ingredients	WC(A)	BW 10:90 (B)	BW 70:30 (C)	BW 50:50 (D)
Wheat flour	100	90	70	50
Buckwheat flour	0	10	30	50
Sugar	40	40	40	40
Fat	35	35	35	35
Baking soda	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3

Table 2 Recipe for the preparation of biscuits from Soy flour

Ingredients	SF 10:90 (E)	SF 70:30 (F)	SF 50:50 (G)	BLSF 10:90 (Q)	BLSF 70:30 (R)	BLSF 50:50 (S)	BRSF 10:90 (T)	BRSF 70:30 (U)	BRSF 50:50 (V)
Wheat flour	90	70	50	90	70	50	90	70	50
Soy flour	10	30	50	10	30	50	10	30	50
Sugar	40	40	40	40	40	40	40	40	40
Fat	35	35	35	35	35	35	35	35	35
Baking soda	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Table 3 Recipe for the preparation of biscuits from Kinema flour

Ingredients	KF 10:90 (H)	KF 70:30 (I)	KF 50:50 (J)	BLKF 10:90 (K)	BLKF 70:30 (L)	BLKF 50:50 (M)	BRKF 10:90 (N)	BRKF 70:30 (O)	BRKF 50:50 (P)
Wheat flour	90	70	50	90	70	50	90	70	50
Kinema flour	10	30	50	10	30	50	10	30	50
Sugar	40	40	40	40	40	40	40	40	40
Fat	35	35	35	35	35	35	35	35	35
Baking soda	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

(BRKF), and White kinema flour (KF). Each variety of soybean (Black, Brown, and White) was washed and oven-dried after being cleaned of dirt and pollutants such as sand, twigs, and leaves. The soybeans were roasted and winnowed before being used. The full-fat soybeans were then ground and sieved into fine, uniform-particle-size flour [16]

Formulation of recipe

Biscuits were made from wheat flour in the form of Aata. Each variation of kinema flour, soy flour, and buckwheat flour was used. Sugar that has been pulverized was employed. As a shortening agent, butter was employed. Other items from the usual supermarket were used, such as salt and sodium bicarbonate. The recipe formulation for composite flour biscuits is as shown in **Table** 1, **Table 2**, and **Table 3** [17].

Preparation of composite flour-incorporated biscuits

First, the fat and sugar powder were mixed. Salt was dissolved in water and added to the cream mixture that had been produced. While the creaming process was still going on, composite flour and baking powder were added and thoroughly mixed together for 10 minutes.

Now, the dough was laid out for 15 minutes to reach dough maturity, allowing for easy machinability and a high gloss finish. The dough was matured and rolled out to a thickness of 5-6mm before being cut into a suitable round form. The created biscuit was baked for 40 minutes at 180°C. The biscuits were placed in polypropylene bags and stored in a cold, dry area after cooling to about 35°C [17].

Determination of physical properties of Soybean

Visual inspection was used to determine color, shape, and surface. The soybean seeds were spread out on a wooden tray and checked for color, shape, and surface. Length/breadth ratio, 100 seeds weight, 100 seeds volume, and Bulk density were determined [18].

Proximate analysis

The moisture content and the total ash content of the samples were determined [19]. The crude fat content, crude fiber content, and crude protein contents of the samples were determined as described by Upadhyaya et al [20]. Total carbohydrate was calculated by the difference method.

Total carbohydrate = 100 – (moisture + protein + fat + crude fiber + ash) %

Aqueous Extract Preparation

One gram of each sample was dissolved for 5 minutes in 100 mL of distilled water to create the aqueous extract of the composite biscuit samples. After that, the mixture underwent a 10-minute, 2,000 rpm centrifugation. The collected supernatants were put in a 50 mL plastic bottle with a tight lid and kept in the fridge. For the aim of carrying out various quantitative and qualitative analyses, the supernatant was dried and reconstituted in water [21].

Antioxidant, Total Phenol and Total Flavonoid content of Products

The antioxidant activity was determined spectrophoto-metrically and absorbance was measured at 517nm [12]. The total phenol content was determined by the Folin-Cioacalteu's method (gallic acid as the standard) [21]. The total flavonoid content was determined by the Aluminium chloride colorimetric method [22].

Phytochemical Properties and Amino acids analysis of Products

The qualitative analysis of different flour varieties was done for the phytochemicals: Saponin, Tannin, Terpenoid, and Glycoside. Different qualitative tests such as Ninhydrin, Biuret, Sakaguchi, Xanthoproteic, and Millon's were performed to determine the presence of amino acids [23, 24].

Sensory Analysis

The laboratory-prepared samples were sensory evaluated using a 9-point hedonic scoring system (9 = like extremely, 1 = dislike extremely) for appearance, flavor, color, texture, taste, and overall acceptance. The review was conducted by a panel of ten people, including faculty and students from St. Xavier's College in Maitighar, Kathmandu, Nepal. In a classroom with natural light, each panelist was given coded samples on a plastic plate and a sheet of sensory evaluation cards. Each participant was asked to assess the sample on quality factors such as appearance, flavor, texture, and overall acceptability. Warm water also was offered to rinse their mouths between testing.

Statistical Evaluation

The experiment was carried out in triplicate. Microsoft Excel 2016 was used to do the statistical analysis of mean and standard deviation (SD).

Results

Determination of Physical Properties of Soybeans

The weight and volume of the white soybean were higher than the black and brown soybean. However, the brown soybean had the highest L/B and bulk density (**Table 4)**.

Table 4: Physical Properties of three varieties of soybeans

Parameters	White Soybean	Black Soybean	Brown Soybean
Color	Yellowish White	Black	Brown
Shape	Oblong and elliptical	Oblong	Oblong
Surface	Smooth	Smooth, Glossy	Smooth
Length(mm)	4.54	4.2	4.44
Breadth(mm)	2.8	2.9	2.1
L/B ratio	1.5	1.4	2
Weight (g/100 seeds)	13.1	12.3	12.2
Volume (ml/ 100 seeds)	10.5	9.8	9.6
Bulk density (ml)	1.24	1.25	1.27

Proximate Analysis of Biscuits

The moisture content and crude fat of BW 3 were highest among all the biscuits samples whereas ash content was higher in BRKF 3 sample. BLSF 3 had a higher crude fiber content. The protein content was high in KF 3 whereas the carbohydrate of WC was highest (**Table 5**).

Sensory Evaluation of Biscuits

For composite flour biscuit formulations, sensory scores were collected from 10 semi-trained panelists using a 9-point hedonic rating scale (9=like extremely, 1=dislike greatly). The semi-trained panelists gave their opinions on the color, flavor (taste and aroma), texture, crispiness, and overall acceptability of the biscuits. It was advised to the panelists that they offer scores in the score sheets based on their perceptions. The biscuit made with Black Kinema and Wheat Flour (50:50) was the most popular. The M sample received the greatest score (Color, Flavor, Body and Texture, Crispiness) out of nine, whereas the C and U samples were made from Buckwheat: Wheat flour (70:30) and Brown Soy: Wheat flour (70:30) received the lowest for overall acceptability(**Table 6)**.

Quantitative Analysis of Bioactive Compounds in Flour Samples

Among 8 different flours, brown kinema flour showed an excellent antioxidant activity whereas black kinema flour had the highest total phenolic and total flavonoid content (**Table 7**).

Table 5: Proximate Analysis of Biscuits

S. N	Sample	Moisture	Ash	Crude Fat	Crude Fiber	Protein	Carbohydrate
1.	WC	2.5 (0.18)	1.47 (0.21)	16.75 (0.47)	0.37 (0.17)	7.26 (0.58)	71.65 (1.07)
2.	BW 1	1.93 (0.06)	1.57 (0.31)	16.89 (0.12)	0.37 (0.05)	9.85 (0.04)	69.39 (0.38)
3.	BW 2	2.53 (0.07)	1.87 (0.15)	18.36 (0.58)	0.86 (0.05)	10.18 (0.17)	66.21 (0.59)
4.	BW 3	2.73 (0.25)	2.13(0.15)	21.71 (0.4)	0.96 (0.04)	10.97 (0.02)	61.51 (0.2)
5.	KF 1	1.95 (0.04)	2.33 (0.1)	16.97 (0.17)	1.31 (0.08)	11.1 (0.09)	66.34 (0.27)
6.	KF 2	0.96 (0.03)	2.57 (0.1)	18.69 (0.22)	1.48 (0.03)	12.26 (0.18)	64.04 (0.35)
7.	KF 3	0.68 (0.08)	3.04 (0.08)	20.9 (0.26)	1.53 (0.06)	13.43 (0.36)	60.42 (0.68)
8.	SF 1	2.51 (0.02)	2.37 (0.08)	17.56 (0.31)	1.22 (0.66)	10.43 (0.15)	65.9 (0.85)
9.	SF 2	1.9 (0.04)	2.19 (0.12)	18.63 (0.36)	1.67 (0.12)	10.55 (0.38)	65.07 (0.67)
10.	SF 3	0.89 (0.06)	1.77 (0.13)	21.16 (0.61)	1.9 (0.07)	11.43 (0.1)	62.84 (0.48)
11.	BLSF 1	2.52 (0.02)	2.39 (0.02)	17.36 (0.62)	1.24 (0.13)	9.56 (0.31)	66.93 (0.62)
12.	BLSF 2	2.09 (0.16)	2.17 (0.14)	19.1 (0.45)	1.7 (0.28)	10.3 (0.12)	64.65 (0.27)
13.	BLSF 3	0.97 (0.03)	1.87 (0.1)	21.38 (1.37)	2.14 (0.17)	10.83 (0.11)	62.8 (1.52)
14.	BLKF 1	2.12 (0.18)	2.34 (0.13)	18.14 (0.25)	1.31 (0.06)	10.95 (0.04)	65.14 (0.1)
15.	BLKF 2	1.79 (0.11)	2.78 0.11)	19.83 (0.3)	1.43](0.07)	11.64 (0.08)	62.53 (0.23)
16.	BLKF 3	0.66 (0.05)	3.23 (0.23)	21.06 (0.9)	1.61 (0.15)	11.88 (0.08)	61.56 (1.24)
17.	BRSF 1	2.47 (0.02)	2.34 (0.13)	17.45 (0.79)	1.19 (0.16)	10.15 (0.09)	66.41 (0.99)
18.	BRSF 2	1.79 (0.03)	2.09 (0.1)	17.76 (0.25)	1.49 (0.07)	10.48 (0.02)	66.38 (0.28)
19.	BRSF 3	0.71 (0.09)	1.78 (0.12)	20.53 (0.47)	1.82 (0.11)	11.45 (0.39)	63.72 (0.64)
20.	BRKF 1	1.99 (0.02)	2.42 (0.09)	18.61 (0.39)	1.18 (0.06)	10.71 (0.04)	65.09 (0.44)
21.	BRKF 2	1.6 (0.18)	2.82 (0.06)	19.43 (0.18)	1.29 (0.02)	11.35 (0.14)	63.51 (0.42)
22.	BRKF 3	0.67 (0.09)	3.37 (0.24)	20.53 (0.36)	1.42 (0.07)	11.8 (0.03)	62.21 (0.58)

Table 6. Sensory Evaluation of the biscuits supplemented with Soy, Kinema, and Buckwheat flour

Sample	Color	Taste	Texture	Flavor	Crispiness	Overall acceptability
A (WC)	7.5	7.5	7.3	7.3	7.3	6.8
B (BW 10:90)	6	6	6.7	5.9	7.1	6.1
C (BW 70:30)	4.7	4.7	4.7	4.7	5.2	5.3
D (BW 50:50)	5.9	5.9	6.5	6	6.7	6.4
E (SF 10:90)	5.7	5.7	6.7	5.6	6.9	6.8
F (SF 70:30)	6.3	6.3	6.2	6.2	6.2	6.7
G (SF 50:50)	6.2	6.2	6.8	5.9	7.3	6.4
H (KF 10:90)	6.5	6.5	6.5	6.2	7.8	6.6
I (KF 70:30)	6.9	6.9	6.9	6.5	7.6	6.6
J (KF 50:50)	6.3	6.3	6.2	5.9	7	6.2
K (BLKF 10:90)	6.5	6.5	7.1	6.6	7.4	6.5
L (BLKF 70:30)	6.6	6.6	6.7	6.7	6.9	6.7
M (BLKF 50:50)	6.9	6.9	7	6.8	7.5	7.2
N (BRKF 10:90)	6.2	6.2	6.8	6.2	7.3	6.1
O (BRKF 70:90)	6.9	6.9	7.3	6.6	7.4	7
P (BRKF 50:50)	6.5	6.5	6.9	6.5	7.7	6.7
Q (BLSF 10:90)	6.1	6.1	6.6	6.3	6.5	6
R (BLSF 70:30)	5.7	5.7	6.2	6.4	5.4	5.4
S (BLSF 50:50)	5.8	5.8	6.4	6.1	6.7	6.1
T (BRSF 10:90)	5.8	5.8	6.6	5.8	5.9	6.2
U (BRSF 70:30)	5.8	5.8	6	5.5	4.8	5.3
V (BRSF 50:50)	5.3	5.3	5.8	6.1	6.1	5.9

Quantitative Analysis of Bioactive Compounds in Biscuits

BRKF 3 had the highest antioxidant activity whereas the total phenolic content and the total flavonoid content of BLKF 3 were found to be the highest (**Table 8**).

Qualitative Test of Amino acid in Biscuits

All the tests gave positive results, which indicate the presence of amino acids in the biscuits (**Table 9**).

Table 7: Quantitative Analysis of bioactive Compounds in flour samples

S. N	Sample	Antioxidant Activity (µg/ml)	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
1.	Wheat Flour	135.76µg/ml	214.88	5.59
2.	Buckwheat Flour	185.74 µg/ml	397.11	6.88
3.	Soy Flour	163.24 µg/ml	513.78	8.06
4.	Kinema Flour	233.63 µg/ml	706	8.70
5.	Black Soy Flour	173.35 µg/ml	561.56	8.17
6.	Brown Soy Flour	173.24 µg/ml	549.33	7.95
7.	Black Kinema Flour	208.03 µg/ml	748.22	8.92
8.	Brown Kinema Flour	313.56 µg/ml	731.56	8.38

Table 8: Quantitative Analysis of Bioactive Compound in Biscuit sample

S. N	Sample	Antioxidant Activity (µg/ml)	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
1.	WC	259.22	107.11	4.30
2.	BW 1	271.53	277.11	5.16
3.	BW 2	294.52	320.44	6.02
4.	BW 3	377.53	359.33	6.45
5.	KF 1	385.31	474.89	5.37
6.	KF 2	393.62	571.56	6.12
7.	KF 3	402.77	682.67	6.55
8.	SF 1	366.91	243.78	5.26
9.	SF 2	377	397.11	6.88
10.	SF 3	377.6	448.22	8.06
11.	BLSF 1	331.49	289.33	5.59
12.	BLSF 2	340.35	378.22	7.20
13.	BLSF 3	340.79	523.78	8.17
14.	BLKF 1	393.92	483.78	5.80
15.	BLKF 2	404.51	577.11	7.20
16.	BLKF 3	405.32	692.67	8.60
17.	BRSF 1	341.47	277.11	5.16
18.	BRSF 2	367.10	374.89	6.88
19.	BRSF 3	368.26	443.78	7.95
20.	BRKF 1	407.04	480.44	5.69
21.	BRKF 2	408.66	570.44	7.31
22.	BRKF 3	440.43	681.56	7.74

Table 9: Qualitative Test of Amino Acid in Biscuits

Sample	Ninhydrin	Biuret	Sakag-uchi	Xantho-proteic	Millon's
WC	+	+	+	+	+
BW 1	+	+	+	+	+
BW 2	+	+	+	+	+
BW 3	+	+	+	+	+
KF 1	+	+	+	+	+
KF 2	+	+	+	+	+
KF 3	+	+	+	+	+
SF 1	+	+	+	+	+
SF 2	+	+	+	+	+
SF 3	+	+	+	+	+
BLSF 1	+	+	+	+	+
BLSF 2	+	+	+	+	+
BLSF 3	+	+	+	+	+
BLKF 1	+	+	+	+	+
BLKF 2	+	+	+	+	+
BLKF 3	+	+	+	+	+
BRSF 1	+	+	+	+	+
BRSF 2	+	+	+	+	+
BRSF 3	+	+	+	+	+
BRKF 1	+	+	+	+	+
BRKF 2	+	+	+	+	+
BRKF 3	+	+	+	+	+

Qualitative Analysis of Phytochemical Properties in Flour Samples

All of the flour samples gave positive results. This indicates the presence of phytochemicals (Saponin, Tannin, Terpenoid and Glycoside) in all of the flour samples (**Table 10**).

Table 10: Phytochemical tests of different flour samples

S. N	Sample	Saponin	Tannin	Terpenoid	Glycoside
1.	Wheat Flour	+	+	+	+
2.	Buckwheat Flour	+	+	+	+
3.	Soy Flour	+	+	+	+
4.	Kinema Flour	+	+	+	+
5.	Black Soy Flour	+	+	+	+
6.	Brown Soy Flour	+	+	+	+
7.	Black Kinema Flour	+	+	+	+
8.	Brown Kinema Flour	+	+	+	+

Photograph 1: Different varieties of Kinema prepared using seed culture

Photograph 2: Biscuits prepared from different composite flours

Discussion

Bakery items such as biscuits are becoming more popular in our diets since they are simple to use, inexpensive, and a good source of protein and nutrients. As, wheat flour is the main ingredient in the biscuit's preparation, it has important carbohydrates and certain proteins however, it is deficient in minerals, particularly calcium, zinc, and iron. Adding food categories including pulses, oilseeds, herbs, and other nutrients to wheat flour-based baked goods improves their nutritional quality. Buckwheat, unlike grains, contains more crude protein, is high in lysine, and is gluten-free, making it essential from a medical and nutritional standpoint. Soy flour is rich in protein that boosts the diet's overall protein quantity and quality. Because of the existence of several health-promoting bioactive chemicals created during microbial fermentation, fermented soybean products are of tremendous interest. Microorganisms obtained from fermented soy products have been involved in the production of polyphenols (isoflavones, phenolic acids, and flavonols), bioactive peptides, γ-aminobutyric acid, antimicrobial compounds, fibrinolytic enzymes, vitamins, and exopolysaccharides, among other bioactive substances [25]. Therefore, adding such nutrients into bakery items such as biscuits would not only improve their nutritional value but will also introduce novel texture features and sensory acceptance.

The moisture content in the supplemented biscuits ranged from 0.66% to 2.73%. The moisture content of wheat control biscuits was found to be 2.52%. Among, the three different buckwheat flour-incorporated biscuits the highest moisture content was observed on BW 3 (2.73%). The increase in moisture content is attributable to an increase in protein content. Sangroula [17] reported a correlation between increased protein content and increased moisture content in bakery products. Similarly, Chopra et al [1] also reported the moisture content of baked goods increased proportionally with the rise in their protein content. When the BWF concentration was increased, the biscuit's protein level increased, which could be due to the incorporation of low-protein soft wheat flour. It was also found that sample BW 3 observed the highest ash content (2.13%), Crude fat (21.71%), crude fiber (0.96%), and protein (10.97%) among the three different concentration levels of buckwheat biscuits. However, the carbohydrate content was found to be the lowest (61.51%) whereas it was higher on BW 1 (69.39%). After the addition of buckwheat flour the ash, moisture, fat, fiber, and protein content of the biscuits was increased whereas the carbohydrate content was decreased [26].

BLSF 1 had the highest moisture content (2.52%) of the three soybean cultivars used in biscuits, followed by BRSF 1 (2.47%), SF 1 (2.51%), BLSF 2 (2.09%), SF 2 (1.9%), BRSF 2 (1.79%), BLSF 3 (0.97%), SF 3 (0.89%), and BRSF 3 (0.71%). Moisture content decreased as the proportion of soy flour increased. A study by Banurekha and Mahendran [27] reported a decrease in moisture content with the increase in soy flour. This may be due to the low moisture content of the beans; soy flour had a higher amount of total dry solid with excellent emulsifying properties than wheat flour. As the proportion of soy flour in the blend increased, the moisture content of the biscuit decreased. BLSF 1 (2.39%) had the highest ash content, while SF 3 had the lowest ash content (1.77%). The moisture and ash contents of the composite flour biscuits decreased with increasing levels of soy flour. The sample SF 3 (21.16%) had the highest crude fat level, while BRSF 1 had the lowest (17.45%). BLSF 3 (2.14%) had the highest crude fiber content, whereas BRSF 1 (1.19%) had the lowest. The crude fat, crude fiber, and protein content of the soy-supplemented biscuits were higher than the control biscuits. The increase in protein content could be attributed to the addition of soybean flour to the flour mix. Because soybean is a high-protein legume, adding soy flour to the biscuits will certainly enhance the protein level. As the protein, fat, and essential amino acid composition of soy flour increases, it has a larger potential for overcoming protein-calorie malnutrition around the world. The increase in fat content could be attributed to the addition of soybean flour to the flour blend. This may be related to the fact that soy flour has a higher fat content than wheat flour [16].

Among the three different kinds of kinema flour-infused biscuits, BLKF 1 had the highest moisture content (2.12%)

and BLKF 3 had the lowest (0.66%). The ash content of BRKF 3 (3.37%) was found to be significantly higher than that of KF 1 (2.33%). BLKF 3 had higher crude fat and fiber levels (2.06%) and (1.61%), respectively, while KF 1 had lower crude fat content (16.97%) and BRKF 1 had lower fiber content (1.18%). KF 3 had the highest protein content (13.43%), while BRKF 1 had the lowest (10.71%). The highest carbohydrate content was found in KF 1 (66.34%), while the lowest was found in KF3 (60.42%). A similar trend was observed in the study by Shrestha and Noomhorm [28] where the composite flour-supplemented biscuits were high in protein and ash content than the conventional biscuits.

The ability of the flours to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals ranged from 159.76µ/ml (wheat flour) to 313.56µ/ml (Brown kinema flour). The highest DPPH activity was observed in BRKF 3 (440.43µ/ml), whereas the lowest activity was found in WC (259.22µ/ml) in the composite cookies. The potential of the biscuits to scavenge DPPH free radicals rises as the quantity of composite flour increases. This was in agreement with the report of Gbenga- Fabusiwa et al [21] where an increase in the proportion of composite flour in the composite biscuits, the ability to scavenge DPPH free radicals increased. It's noteworthy that the DPPH of the composite biscuit is higher than that of composite flour. Another study by Baba et al and Sharma and Gujral [29,30] also reported an increase in the antioxidant activity of cookies after baking and microwave roasting. This may be due to the melanoidins, the brown pigments which are the products of maillard reaction [31].

The total phenolic compound in the composite flour ranged from 214.88 mg GAE/g to 748.22 mg GAE/g, whereas the total phenolic compound in the biscuits was 107.11 mg GAE/g to 692.67 mg GAE/g. The phenolic content of composite biscuits is substantially higher than that of control biscuits. The overall flavonoid concentration of composite flour was expressed as milligram quercetin equivalent (mg QE). The total flavonoid content of black kinema flour had the highest value of 8.92 mg QE/g, while wheat flour had the lowest value of 5.59 mg QE/g. The total flavonoid content of the composite biscuits followed a similar pattern, with BLKF 3 (8.60 mg QE/g) having the greatest value and WC (4.30 mg QE/g) having the lowest. All of the composite flour and biscuit blends generated had higher flavonoid content, according to the findings. The significant difference between the total phenolic and flavonoid contents of the composite flour and the biscuits suggests that baking may hasten the breakdown of the biscuit's total phenolic and flavonoid contents. The results from the **Tables** indicated that the total phenolic contents and flavonoid contents were decreased in the composite biscuits when compared to the equivalent of composite flour. It could be related to the baking process which causes thermal degradation and transformation. A similar trend was observed in Gbenga- Fabusiwa et al [21] where polyphenols were shown to be significantly reduced by fermentation, drying, and alkalization after processing.

Biscuit samples M (BLKF 50:50) had the best overall acceptability ratings of 7.2 while Sample C (BW 70:30) and Sample U (70:30) gained a lower score of 5.3. The general acceptability of the composite cookies ranged from neither liked nor disliked to liked moderately. Color, Taste, Texture, Flavor, and Crispiness are the most important components in determining a product's acceptability, as well as its market success. The color of biscuits prepared from kinema flour and buckwheat flour was darker and more preferred by the panelists. Browning of the biscuit samples could have been caused by Maillard-type reactions caused by the presence of reducing sugars, proteins, and amino acids, as well as caramelization caused by the effect of high processing temperatures. As the level of buckwheat flour in the formulation was increased, the sensory scores for texture, appearance, and flavor of biscuits decreased. Defatted-soy flour substitutes in wheat bread and biscuits resulted in a beany flavor, aroma, and aftertaste [32]. Beany flavors are commonly linked with legumes in food. The baking factors (temperature and time variables); the state of the cookie constituents, such as fiber, starch, and protein (gluten) whether harmed or unharmed, and the quantity of absorbed water during dough mixing, will all contribute to the overall acceptance outcome [33, 34]. All the results of this study indicate that the biscuits prepared from composite flours of wheat, buckwheat flour, kinema flour, and soy flour were generally accepted and had better physical as well as nutrient composition. Therefore, all the formulations could be used in biscuits production according to energy and nutrient needs.

Conclusion

The physical properties, proximate composition, antioxidant properties, phenolic contents, as well as phytochemical properties, and amino acids analysis of the composite flour biscuits, were studied. The crude protein, crude fiber and ash content of the composite flour biscuits were comparatively increased than the control biscuits. The findings of this research revealed that, the biscuits produced with buckwheat flour, soy flour, and kinema flour substitution were nutritionally superior to that of the whole wheat biscuits. This study also concludes that after the inclusion of composite flour, the nutritional quality and bioactive components of the biscuits appeared to improve. This research also comes to the conclusion that the formulation of composite flour reduces the quantity of gluten in the biscuits which would be favorable to celiac disease sufferers. Furthermore, the development of kinema flour-based biscuits could serve as a springboard for the production of high-value functional food products made from kinema, which are gradually getting extinct in Nepal. This research could pave the way for buckwheat flour, soy flour, and kinema flour to be used in other food products.

Competing Interest

This study does not involve any competing interests.

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Ethical Approval and Consent

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