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Comparison of Nutritional Values of Cookies Produced from Enset Bulla Fortied with Soybean Flour

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Abstract

Cookies are baked products liked by all age groups; containing flour, sugar, and fat and the one which blended with SBF. This study aimed to prepare cookies from bulla that is mixed with wheat flour fortified with SBF at a proportion of flours for making cookies 0%, 10% 15%, and 30. The proximate, functional, microbial, and sensory attributes of bulla-soybean cookies' baking potential were studied using standard analytical methods. Soybean flour (SBF) was produced and used to substitute bulla flour at different substitutional levels viz 70:20:10, 60:20:20, 50:20:30 with 80:20:0 %, bulla: wheat: soybean flour as control. Nutritional analysis of cookies revealed that there are significant differences in their physicochemical properties such as moisture, fat, protein ash, carbohydrate, and fiber contents, which were higher by 30%. However, small differences were observed between cookies produced from all bulla in terms of minerals. Whereas the organoleptic attributes of cookies were deferred significantly ($p \leq 0.05$). However, microbial analysis was evaluated every 30 days up to 210 days and checked the suitability of the best cookies and the total plate count of cookies samples was under acceptable limits. Studied results showed that produced cookies had high amounts of protein in 30 and 20 % fortified cookies. This study might use to promote bulla for their food and nutrition security, the use of fortified cookies, and develop nutritious-based new products and income-generating potential in Ethiopia more emphasis should be given.

Keywords: Bakery product, Cookies, Enset, Fortification, Organoleptic Properties

1. INTRODUCTION

Enset (*Enset ventricosum*) is an herbaceous monocot, a large, banana-like plant that grows up to 11 m high to the tip of the leaves. Enset has been grown in Ethiopia for more than 10,000 years. It is also a crop with many uses as human food, livestock feed, industrial fiber, as rob material in fences and house-building, for mattresses and seats making, as local packaging material, and as a substitute for table plates or umbrellas (Tiruha, H.K., Kebede A.A., and Edessa N.G., (2014)). The recurrent droughts and environmental factors have led to the expansion of Enset cultivation to other parts of the country to secure the food and health of the people. A wide range of adaptations within the species to altitude, soil, and climate have prevalent cultivation of the crop in Western Bale, Southern part, South-Western Oromia including South and East Shewa, Jima, Illubabor, and Wollega. Enset has remarkable significance in the day-today-life of peasant households cultivating the crop as a staple food (Tiruha, H.K., Kebede A.A., and Edessa N.G., 2014) & (Tesfaye, A., and Girma, A, 2017).

Ethiopia is identified as one of the six gene centers of crops such as Tef (*Eragrostis tef*), Noug (*Guizotia abyssinica*), Gesho (*Rhamus prinoides*), Enset (*Enset ventricosum*), Coffee (*Coffea arabica*) and Khat (*Chata edulis*) are originated in Ethiopia. Enset is an orphan or little-researched food crop that's cultivated only in Ethiopia (Asres, A., and Omprakash, S., (2014))

Enset is considered an economic crop in Ethiopia due to its high-yielding potential wide adaptability, resistance to drought, and multifunctional usage. It is a rich source of starch and uses as food locally known in Ethiopia as kocho, bulla, amicho, and workay. Enset provides staple food for 15 million people in Ethiopia after fermentation into kocho and bulla (Mohammed, B., Martin, G., and Laila, M.K., 2013).

When enset pseudo-stem is used to prepare food for humans, the most common way is to make "bulla" that in turn baked into different kinds of bread. Kocho is a decorticating and fermented product of Enset has contains starch-rich products. Bulla is energy-rich: (6.46 and 8.46 MJ/kg fresh weight) respectively food products (Asres, A., and Omprakash, S. , (2014)) & (Mohammed, B., Martin, G., and Laila, M.K. , 2013).

Enset bulla is a good source of essential mineral nutrients that contribute to growth as well as health maintenance and general well-being. The major mineral nutrient in bulla is Mg, Fe, Zn, Na, K, and Ca (Mohammed, B., Martin, G., and Laila, M.K. , 2013).

The use of soybean products in the feed and food industry has increased because it has a high amount of nutritional quality, especially for protein and amino acids. Tofu and soymilk-bred production largely depend on seeded soybeans (Emmanuel, O.A., Gondwe T., Phumzile, M., and Maziya, D.B., 2017). It also has linoleic (omega-6) and alpha-linolenic acid (omega-3), both polyunsaturated fatty acids that are essential nutrients but are also increasingly recognized to reduce the risk of chronic age-related diseases such as cancer and cardiovascular disease. These standards will be useful for all the people concerned with the soybean industry globally (Svejstil, R., Musilova, S., and Rada, V., 2015).

Cookies are highly popular snack baker products consumed by most people, especially in the United States and Europe (Mehmet, B.M., and Fatih E., 2011). They are produced by mixing flour and/or starch with other ingredients through a kneading process, fermented; that contains different toppings, fillings, shapes, and textures as well as a fortification (Rebellato, A.P., Pacheco, B.C., Prado J.P., and Pallone A.L., 2015).

In this context, considering the problem of the country's economic losses and environmental impact and to verify whether the importance of this new ingredient is safe for sweet cookies production, this study intends to test/determine the changes of sweet cookies made from both bulla and kocho enset with 20 % of wheat flour by fortifying with varying concentration of soybean flour, obtained after baking (Ogunsina, B.S., Radha, C., and Rndrani D., 2011). Therefore, the objectives of this present research work are to produce quality cookies from bulla-wheat-soybean composite flour as well as compare their nutritional composition, microbial analysis, and sensorial properties and used to avoid losses and environmental pollution and to generate or to harness its potential both in economic and nutritional terms by increasing the contents in cookies.

1.2. Statement of the Problem

Ethiopia has a higher Enset farm. Even though, the consumption of the Enset plant is significant and used to make many known food products like kocho, bulla, and amicho. All of these have low protein and mineral contents, which results in malnutrition (protein case) and lack of knowledge about the nutritional value of enset in consumer regions. Even though there avails enough food, people do not fulfill their daily nutritional requirements. This may be unaware of information about the balanced diet. The low nutritional value can be avoided by fortification and to have new product development, when compared to the choice of other cellulosic plants (Melese, 2013).

In this concern, cookies play an important role in the human diet. It is considered rich in nutrient contents that have protein, minerals, fibers, carbohydrates, and antioxidants. Even though cookies are considered a baker product that contains the most important nutrients for human health, availability in the market yet remains a big problem in Ethiopia. In addition, food composition data are needed to assess or to promote health purposes, evaluate the diet, carry out research on the relationship between diet, health, and disease, and stimulate markets for new ethnic foods (Hawa, A., Satheesh, N., and Kumela, D., 2018). So, produced cookies are used for weight management, alternative food for breakfast, as a source of antioxidants, as increases digestion, and provide quick energy for people.

The motivation that drives to conduct this study was to find the alternative use of enset product by fortification to increase the nutritional values of the end product and also it is taken as the economically feasible method. Hence, the main goal of this study was to compare the nutritional and microbial composition of cookies produced from fermented bulla fortified by soybean flour in West Guji, Oromia, Ethiopia.

2. MATERIALS AND METHODS

2.1. Source of Materials

Wheat flour, margarine, white sugar, iodine salt, powdered milk, sunflower oil, 20 egg album, baking powder, and flour, were purchased from a reputable store in Bule Hora town, South Local Government Area, Ethiopia.

2.2. Processing of Bulla and Soybean into Flour

The fresh bulla enset of Genna varieties that were fermented for 60 days were collected from Abaya Woreda in West Guji zone, Oromia of Ethiopia was prepared according to the method described by (Abrehet, G.F., Kebede, A.A., and Meksud, A., 2018) & (Yemataw, Z., 2018). Then washed three times with distilled water until the impurities were separated, and sundried. The samples were packed in low polyethylene bags, kept in the refrigerator until analysis to prevent moisture loss and fermentation, and transported to the center for laboratory analysis. The collected matured soybean was wet cleaned and steeped by employing distilled water, then drained & dehulled by rubbing with palms and removed the hulls by rinsing with clean water and wet milled

using a grinder. Finally, dried at 60 °C using the oven. The pictorial presentation of raw materials (bulla & soybean) preparation was shown in **Fig** 1. & **2**. as indicated below.

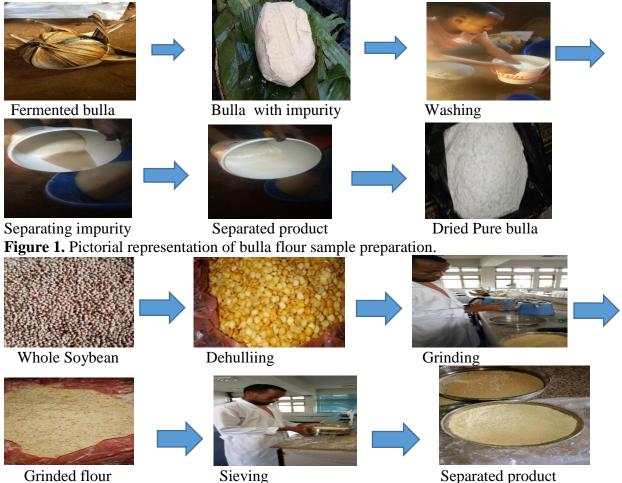


Figure 2. Flow diagram for preparation of soybean flour.

2.3. Production of Cookies and Design

Seven steps were used for the preparation of control and experimental cookies based on different proportions of bulla & other ingredients. Sugar and margarine were accurately weighed, mixed in a stainless bowl, and creamed until the mixture became light and fluffy. Milk powder was added to the cream while still mixing, allowed for 20 min after composite flour, baking powder, and salt was gently poured into the mixture to obtain the dough. The dough was kneaded using a wooden rolling pin, cut into shapes using a cutter, and then placed on oiled flat baking trays. The dough was baked at 120 °C for 25 minutes in an oven until they are light brown, then cool for 30 minutes, then stored for further analysis (**Fig 3.**). The nutritional values, microbiological analysis, and

sensory analysis of the prepared cookies were conducted (Ikuomola D.S., Otutu O.L. and Oluniran D.D., , 2017).

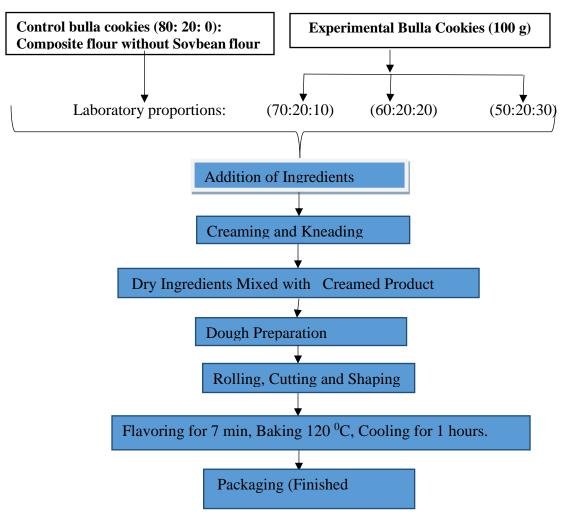


Figure 3. Flow diagram adapted for control and experimental bulla cookies (Ikuomola D.S., Otutu O.L. and Oluniran D.D., , 2017).

2.4. Analysis of Proximate Composition

Proximate compositional analysis such as ash, crude protein, crude fat, crude fiber, and mineral contents determined according to the standard methods described by AOAC, 2003 (Yemataw, Z. , 2018) & (Elisa J., Herla R., Ridwansyah, E. Y., , 2015).

2.4.1. Determination of proximate compositions of the cookies

Cookies were analyzed for ash, moisture, protein, fat, crude, fiber contents & pH according to the methods described in the Association of OAC (*AOAC*, 2000) & ([AOAC]., 2005). The total carbohydrate (CHO) was calculated by the difference method as CHO = (% moisture + % protein + % fat + % ash).

2.5. Total Microbiological Analysis

2.5.1. Nutrient Agar Base (for Bacteria)

Accordingly, the media yeast extract (5 g), peptone (5 g), glucose (10 g), and agar (20 g) were suspended in 1L of distilled water and maintained at a pH of 6.8. The suspension was heated until the sold ingredients were completely dissolved. Then completely sterilized by autoclave at 121 °C for 15 min and cooled to 50 °C. 10 g of the cookies were mixed with 90 mL of 0.8 % NaCl sterile saline water (adjusted to pH of 7) and allowed to maintain the culture for 4 hrs in an incubator at $30 \,^{\circ}$ C. This provided 10^{-1} dilution, further dilutions were made skeptically; similarly, 1 mL of each dilution was then transferred to a petri-dish and poured the above-prepared medium into every individual plate. The plates were then rotated for uniform distribution of inoculums. Then, the plates were inverted and incubated at 30 °C for 48 hrs. Finally, counted the colonies and expressed as **equation 2.1** (Mehmet, B.M., and Fatih E., 2011):

2.5.2. Agar (for yeast, mold, and other fungus) Extraction

The media was prepared by dissolving peptone (5 g), beef extract (3 g), NaCl (5 g), and agar (20 g) in 1L of water and maintaining the pH of the media as 7.0-7.2. Then, 10 g of cookies were powdered & dissolved in 10 mL of sterile saline solution and made homogenate for 1 min. Serial dilutions were made by dissolving 1 mL of homogenates in 9 mL sterile saline, dispensed into test tubes, and 0.1 mL of the dilution $(10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6})$ were spread on sterile petri plates containing nutrient agar media. The plates were incubated at 30 °C for 48 hrs (Appendix 1). The colonies were counted by using a digital colony counter. Counts of visible colonies made were calculated using **equation 2.1** and expressed in cfu/g according to (Safa H. W., Amir G., Farhana A. & Tariq A. S., 2015):

2.6. Evaluation of Organoleptic Properties

The organoleptic properties are evaluated through color, fracture, hardness, taste, aroma, flavor, and overall acceptability measurements using an evaluation form. The panelists were provided with 8 samples of cookies that coded with SBF 0 %, 10 %, 20 %, and 30 % for bulla cookies. The panelists were assigned to score their preference for the various attributes using nine (9) hedonic scales (hedonic evaluation form given in Table 3.3). A semi-trained 15 panelists selected 6 members drawn from Ethiopian Standard Agency (ESA) and Ethiopian Conformity Assessment Enterprise (ECAE) and 6 selected from instructors and staff members to evaluate the total cookies and select the preferable cookies (randomly) product (Thivani M, Mahendran T., and Kanimoly M., 2016) & (Vasantharuba S., Banumathi P., Premalatha M. R., Sundaram S. P. and Arumugam T., 2012)

2.7. Determination of the ShelfLife of Cookies

The shelf-life of cookies was assessed based on the nutritional, sensory qualities, and microbial activities as well as on peroxide values, and acid values. Shelf-life of cookies is carried out by packing them in polyethylene pouches. Then it's stored in airtight containers at ambient temperature and analyzed at an interval of two weeks and noticed the stability of cookies (Thivani M, Mahendran T., and Kanimoly M., 2016).

2.7.1. Determination of Peroxide Value

The titrimetric method of AOAC (2003) as cited in (AOAC, 2003) was used for this study. 30 mL of acetic acid: chloroform solution (2:1) mixture added in a flask and swirled for 30 min to dissolve them. This was slowly titrated with 0.1 N Na₂S₂O₃ with vigorous shaking until the yellow color disappeared. Then added 0.5 mL of 1.0 % starch solution and titrated continuously with vigorous shaking doe's release all iodine from the chloroform layer until the pink color just disappeared. The blank sample was carried out side by side. The peroxide value is determined by using the following relation:

 $\begin{aligned} \text{PeroxideValue (\%)} &= \frac{(\text{Sample reading-blank reading}) - \text{N of Na2S2O3} \times 1000}{\text{Weight of Sample (g)} \times 100} \text{ x 100} \\ & \text{N Na_2S_2O_3 (blank) M= Normality of Na_2S_2O_3 solution} \end{aligned}$

2.7.2. Determination of Titratable Acidity

Titratable acidity (TA) is determined by titrating the sample according to the method described in (Tiruha, H.K., Kebede A.A., and Edessa N.G., (2014)). 10 g of prepared cookies were used and homogenized in 90 mL of distilled water using a stomacher (model 400, Seward). Then, determined the TA by titrating an aliquot of cookies filtrate (10 mL) with 0.1M NaOH, using 1 % phenolphthalein as an indicator. The volume of aliquot used was recorded to determine the amount of acid in the sample and expressed the result as in percent of lactic acid as:

 $\% Acid \frac{wt}{wt} = \frac{N \times V \times Eq.wt}{W \times 10}$

Where, N - Normality of NaOH (m Eq/mL), V - Volume of titrant (mL), Eq.Wt. - Equivalent weight of predominant acid (mg /mEq), and W - the weight of the sample

2.8. Statistical Analysis

All statistical analyses were carried out in triplicate times and their mean values were calculated. The results are expressed as mean \pm standard deviation. The mean comparison that performed using Microsoft Excel Origin 6.0 version for raw materials and significant differences were compared at p<0.05 using Microsoft Excel one-way ANOVA. For all data for each blend proportions (i.e. for fortified cookies).

3. RESULTS AND DISCUSSIONS

3.1. Proximate Composition of Fortified Cookies

Proximate compositional analysis of control cookies and soybean flour (SBF) fortified cookies are presented in Table 1. All the measurements for each parameter are measured by employing triplicate measurement and evaluated statistically by one-way ANOVA.

The statistical data mostly obtained in this current study had shown better consistent results with permissible limits. Similarly, most of the proximate composition of the fortified cookies had significant differences, evident through p < 0.05 and with a higher absolute mean difference than LSD (Table 1).

	C	I							
Sample	Conc. of SBF	Ash	Moisture	Fat	Protein	Fiber	СНО	Energy	
	0 %	2.19±0.10	3.75±0.40	3.25±0.44	12.10±2.11	3.02±0.10	75.67±2.00	386.41±0.37	
Bulla	10 %	2.36±0.33	2.54±0.5	4.07±0.22	20.09±0.20	2.32±0.21	68.59±1.50	392.45±0.90	
Cookies	20 %	2.23±0.50	1.90±0.77	6.50±0.30	24.35±0.50	1.53±0.11	65.00±0.80	404.34±2.15	
	30 %	2.51±0.65	1.43±0.80	8.86±0.90	27.54±0.43	2.64±0.12	57.44±0.64	410.81±2.00	

Table 1. Results for textural and organoleptic properties of fortified cookies of the bulla.

<u>Note</u>: ^b - statistically significantly different compared with control (p < 0.05)

^c - no statistically significant difference compared with control (p > 0.05)

Ash Content

From Table 1, it can be seen that as SBF increases the ash contents rises slightly and linearly in all fortified bulla-based cookies (except 20 % bulla blend), whereas 30 % SBF fortified bulla cookies have slightly increased the ash contents as 2.51 ± 0.16 g. In this study bulla were used as base materials; 30% is more nutritious than others. This trend gets followed even after the fortification of base materials with different proportions of SBF. Compared with the ash content of control, the fortified cookies have very slightly increased values, this might be due to the influence of SBF fortification as well as the processing method. But no statistically much significant difference was evidenced with greater alpha value (p > 0.05) in all cases of fortification. Similarly, an earlier study (Singh, M., and Mohamed, A., 2007) also reported increased ash content of cookies (range 0.96-2.02), which is inferior to the currently studied SBF-fortified cookies.

Comparatively, fortified bulla cookie shows the lowest ash content $(2.23\pm0.36 \text{ g})$ as compared to others. These results are in line with the earlier study in (EHNRI). (1998) (Singh, M., and Mohamed, A., 2007) & (Ethiopian Health and Nutrition Research Unit (EHNRI)., 1998)) that reported increased ash content with the corresponding increases in whey protein in cookies production. The results show that an increase in ash content with increasing SBF substitution level might be associated with the ash content of the soybean.

Moisture Content

The moisture content of control cookies is high in all bulla cookies $(3.75\pm0.49 \text{ g}/100 \text{ g})$. In all cases bulla control and fortified cookies have greater moisture content; this is supported by the high amount of moisture and fiber found (Table 4.5). As increasing the SBF from 10-30 %, the moisture content was reduced from 3.75 ± 0.49 to 1.43 ± 0.21 in bulla cookies.

Statistical analysis disclosed that the moisture content of the supplemented cookies had significantly (p < 0.05) decreased compared to that of their control cookies. The moisture content of cookies has been found to increase from 3.14 % to 7.28 % with the supplementation of rice bran (Sally F. S., Buzzard I. M., and Susan, E. G., 2009); but in this current study as SBF supplementation level increases the moisture content of cookies get decreased. Furthermore, the result obtained in this study was found under the limits of Ethiopian Standard Agency (ESA), 2010 (Ethiopian Standard Agency, , 2010) for wafer cookies is recommended as 5.0 g/100 g.

Protein Content

The protein content of cookies increased from 12.10 ± 3.22 to 27.54 ± 2.11 g/100 g with the increase of SBF supplementation from 10 to 30 % for bulla. Protein content in all cases (even triplicate measurements) of 30 % (control and fortified cookies) was found higher than 0% bulla samples. The maximum protein content of the present study was reported in 30 % SBF-fortified cookies in bulla (27.54±2.11 g/100 g). Similar trends of results were observed in many cases of earlier research as by fortifying with wheat protein casein enriched cookies (protein, 4.35 g/100 g) (Ufot E.I., Etini A.D., and Florence, A.B. , 2018) & (Parliament, E., 2006).

Remarkably, all those earlier studied fortified cookies have lower protein than the presently studied cookies prepared from bulla fortified with SBF (range $20.09\pm5.13 - 30.47\pm2.01$ g/100 g). This might be due to the fortification of base materials (bulla) with soybean flour that is having a good amount of protein than acha, kidney bean flour, rice bran, and whole wheat & flour. Similarly, the protein content of the cookies gets increasing order upon fortification of bulla as, 0% 10% < 20% < 30% SBF.

Fat Content

The triplicate measurements of the fat content of bulla cookies were done and the obtained data presented with mean values are shown in Table 4.5. It shows that the increase in the fat content ranged from $3.25\pm0.38-8.86\pm0.75$ g/100 g in bulla cookies upon the fortification of SBF (0-30 %).

The maximum value of fat was measured as 8.86±0.75 g/100 g in 30 % SBF-fortified bulla cookies; This is due to the level of fortification may be exhausted early than 30 % SBF fortification in bulla, because it has greater water absorption capacity and moisture content. The results obtained in this study do not agree with the Ethiopian Standard Agency (ESA), 2010 which permitted a maximum fat content of about 1.2 % (Ethiopian Standard Agency, , 2010) & (Food and Nutrition Board, Institute of Medicine, 2001). This is supporting the results of the current study, though the cookies have higher fat content than recommended by ESA, 2010 (Ethiopian Standard Agency, , 2010). This is due to the replacement of base materials (bulla) by the increment of oil/fat-rich SBF under fortification, which is also the fact that the fortified cookies do have greater fat content than the control.

Fiber Content

The obtained results show that, in bulla-based cookies unfortified control (contains 80 % bulla and 15 % wheat) cookies have maximum fiber content $(3.02\pm0.30 \text{ g}/100 \text{ g})$ than fortified other cookies. This is because bulla and wheat have more fiber than SBF (oil seed flour). Hence, in an enhancement of SBF (0-30 %) into bulla-wheat mixture under fortification, SBF reduces the fiber content of the bulla-wheat mixture. In this context, the 15 % SBF-fortified cookies have significantly reduces the fiber content to 1.53±0.67 from 3.03±0.30 g/100g. The regulation of European Parliament Council (EPC) No: 1924/2006 in nutrition and health claims made on foods stated that the food product does contain at least 3 g of fiber per 100 g or at least 1.5 g of fiber per 100 kcal (WHO., (2003):) & (Andres, B.A., Barreto P.V., Garcia J.P., Mirabel J., and Martinez, M.J., 2013). Henceforth, the currently studied all kinds of bulla-based cookies and control cookies are confirmed as fibrous foodstuff for consumption. According to Food and Nutrition Board, Institute of Medicine, 2001 (Food and Nutrition Board, Institute of Medicine, 2001) & (Shahid, M., Masood S.B., Faqir M.A., and Haq, N., 2008) current recommendations, the average daily requirement of dietary fiber is 25 g/day for women younger than 50; 21 g/ day for women older than 50; 38 g/day for men younger than 50. Thus, the bulla from wild enset and its food products could be a valuable source to accomplish the dietary fiber for human nutrition.

Carbohydrate Content

The results indicate that in all cookies the amount of carbohydrate gets decreased from control $(75.67\pm2.80 \text{ g}/100 \text{ g})$ to SBF 30 % $(57.00\pm2.00 \text{ g}/100 \text{ g})$ in bulla. The results reveal that the bulla-

based (control) cookie has a slightly greater amount of carbohydrates. The increment of carbohydrates in the bulla-wheat mixture greatly improved in bulla (as $57.00\pm2.00 \text{ g} \rightarrow 75.67\pm2.80 \text{ g}$) by only 26.59 %. So, absolute improvement of carbohydrates had taken place in the bulla sample upon preparation of cookies by replacement of 0% with 20 % of wheat.

In the case of fortification of bulla-wheat mixture with SBF with different proportions (as from 0-30 %), significantly (p < 0.05) reduces as in the range of 10-25 % the carbohydrate content. This might be due to the supply of protein and fat by SBF.

The present results agree well with other research, which reported that the incorporation of plant proteins, soy flour, flaxseed, pigeon pea, and sorghum, mustard flour increased the protein, fiber, and ash content of the final products. Also, higher values were reported in cookies than in control (Nwosu A.N., and Akubor, P.I., 2008) & (Banusha S., and Vasantharuba, S., 2014)

4.7. Evaluation of pH, Titratable Acidity, Peroxide Value, and Shelf Life of Cookies

The pH, titratable acidity, and peroxide value were evaluated for bulla cookies. The result of the mean value is presented in Table 3. Generally, the pH of the bulla samples mainly affects by enset varieties, processing methods, and fermentation time (Shahzad H., 2006). The pH of composite bread prepared from wheat and defatted cashew (*Anticardium occidentale L.*) kernel flour ranged from 5.64 ± 1.01 to 6.22 ± 2.11 (Mayara, B., Marta S., and Manuel, G. (, 2019), which was higher than the presently studied results (except 30 % bulla cookies having pH 6.92 ± 0.07). In the presently studied samples, pH increased from 5.54 ± 0.21 to 6.92 ± 0.07 in bulla cookies during fortification by 10-30 % of SBF. In this study, the pH of 30% bulla cookies was found higher than 0% SBF bulla cookies.

From the obtained results, a higher TA value (0.41 ± 0.02) was noticed in the control bulla-cookie has lower pH (5.54±0.21); but, the lowest value of TA (0.28±0.02) obtained in 30 % SBF fortified with the greater pH (6.92±0.07). This study is almost showing good agreement with that of bulla prepared from the Messena variety with higher TA (about 0.3 %), and the corresponding lower pH value (0.22) (Shovon, (2018)). Similar trends of TA (0.36 to 0.61) and pH (4.0 to 4.46) were reported in (Afsana K., Tahmina S., and Shahidul, K.M. , 2016) also.

The obtained results of the current study reveal that TA varies from 0.41 ± 0.02 - 0.28 ± 0.02 . Hence, the rise in TA of all kinds of cookies and decreases in their corresponding pH shows that the acid

concentrations in the flour blends are appreciating and this has a benefit in the storage life of the flour blends.

The variations in the peroxide value (Table 3) of the cookies were probably due to the integration of the wheat and soybean flours which have high total phenol contents and antioxidant activity. A similar result was reported (Ogundare, A.O., and Adetuyi, F.C., 2003) on the flaxseeds containing phenols, antioxidants, and lignans which prevent the formation of peroxides. This is also supported by the storage stability of flaxseed reported that peroxides levels were relatively unchanged when stored at room temperature for 10 months (Food and Nutrition Board, Institute of Medicine, 2001), similar results were obtained in this study.

Thus, presently studied results revealed that the fortification had no adverse effects on the physical parameters of cookies. Many causes influence the shelf life of product-like moisture, microbial spoilage, enzymatic changes, and oxidation. The cookies were found to be stable when packed and stored at room temperature. Hence, the shelf life of the studied cookies had no significant impact on physical parameters even after 90 days of storage at ambient temperature.

Sample	Concentration	pН	ТА	Peroxide Value
	0 %	5.54±0.21	0.41±0.02	0.82 ± 0.09
Bulla Cookies	10 %	5.78±0.013	0.37±0.04	0.76 ± 0.09
Dulla Cookles	20 %	6.31±0.18	0.32±0.04	0.66 ± 0.08
	30 %	6.92±0.07	0.28±0.02	0.44 ± 0.05

Table 3. Evaluation of pH, titratable acidity, and peroxide value for shelflife of fortified cookies.

Values are Mean \pm SD, triplicate analysis

<u>Notes</u>: Values in the same row with different samples are statistically significant from each other (p < 0.05). SBF 0 (control): 80% bulla + 20% wheat + no SBF; SBF 15: 65% bulla + 20% wheat + 20% SBF; SBF 30: 50% bulla + 20% wheat + 30% SBF.

4.8. Microbiological Analysis of Fortified Cookies

The results of the microbial counts of cookies produced from fortified bulla are shown in Table 4. Results indicate that the total viable microbial counts in 0% cookies are as high at seven months of storage as in 30% bulla cookies; this is because 0% contains high amounts of fiber than 30%

bulla raw materials. This is an indication that the cookies in all cases were prepared under good hygienic conditions and the integrity of the packaging.

Microbes such as total plate counts, total coliform, Lactic acid bacteria (LAB), E.coli, and yeast and mold were noticed at 7th month of storage in control bulla cookies as $0.43 \times 10^2 \pm 0.03$, $0.85 \times 10^2 \pm 0.01$, $0.01 \times 10^2 \pm 0.005$ and $0.11 \times 10^2 \pm 0.01$ cfu/g, respectively. Most of the microbial counts in the current study were found (Table 4.9) below the minimum and maximum point (between 10¹ and 10⁷ cfu/g) of microbe recommended by standards of the American Association of Cereal Chemists (AACC) (Snezana Z., Ana K., Darko M., Jovanka L.P., Nikola T., Sanja O., and Stanislava, G., 2019). Also, AACC recommends the microbial examination in terms of the total plate counts as ranged 1.00 - 1.75 cfu/g, which stated minimal and within acceptable limits after three months of storage (Ľubomíra, J., Miroslava K., Jana P., Simona K., Adriana P., and Alica, B., 2013). Accordingly, all the prepared cookies of this study are within the acceptable limit as the total plate counts ranged between $0.1 \times 10^2 \pm 0.01 - 1.9 \times 10^2 \pm 0.02$ cfu/g starting from the 0th day to the 210th day. Furthermore, the results revealed that the cookies containing SBF had less total plate count than controlled cookies in both cases. This might be due to the anti-microbial properties of SBF.

The bacterium usually originates from raw ingredients of bulla flour and goes on to break down cookies. Upon the three fortification systems (10,20 and 30 % SBF), the bacterial counts measured in cookies during the first five months were found with no growth in many cases but lower counts in 10 % and 30 % SBF-fortified bulla cookies. At this instant, 30% SBF-fortified bulla cookies showed 0.24×10^2 yeast and molds, respectively. Such growth of microbes developed in 20 % SBF-fortified bulla cookies up to 6 months of storage.

Coliforms (E.coli) in experimental samples increased in bulla significantly with increased SBF fortification. The coliform count in the unpacked sample $(4.7 \times 10^4 \text{ cfu/g})$ was observed much higher than the safety limit for human consumption as reported in (L'ubomíra, J., Miroslava K., Jana P., Simona K., Adriana P., and Alica, B. , 2013). Hence, the microbial study of the present investigations shows that the microbial counts (in TPC) are within the accepted range, and so the cookies made are safe to consume. Thus, microbiological studies have proved it to be safe, thus making it an excellent and novel product.

Table 4. Effects of microbial activity on fortified cookies produced from fermented bulla during 7
months of study.

Types	Micro	Microbial count ($\times 10^2$ cfu/100g) on bulla cookies measured during the storage (in months)																																
(no/1	In 0 %	6,	SF	BF	(co	nt	rol)			In	1	0 %	%, S	BF				In 20 %, SBF							In 30 %, SBF								
00 g)	0	1	2	2	3	4	5	6		7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
Total plate count	-	-	-		-	1	+	-	0.4	1	-	-		-	-	-	-	0.3 6	I	+	-	_	-	-	+	0.1 6	-	-	-	+	-	-	0.0 8	0.1 4
Total Colifo rm	-	_	-		-	-	_	+	0.0)	-	_	-	-	-	-	0.	0.4	1	_	-	_	_	1		0.5 4	-	-	_	-	_	+	0.3 5	0.6
LAB	-	-	-	+ .	-	١	-	1	0.8		-	+	-	-	-	+	-	0.2	1	-	-	-	+	-	+	1.0 5	-	-	-	-	+	-	0.3 2	0.1 0
E. coli	-	-	-		-	1	+	-	0.0)	-	-	-	-	+	-	+	0.1 4	+	-	-	-	-	-	-	0.3 4	-	-	-	+	-	0.2	+	0.4 4
Yeast and Mold	-	-	-	-	ł	-	-	-	0.		-	-	-	+	-	0. 2	+	0.1	-	-	-	-	-	+	-	0.2 2	+	-	-	-	+	-	0.2 2	0.2 1

Negative sigh (-): No microbial growth, and Positive sign (+): Very small amounts of microbial growth

Value in each cell represents in average ± SD triplicate analysis.

Note: SBF 0 (control): 80% bulla + 20% wheat + no SBF

SBF 20: 65% bulla + 20% wheat + 20% SBF

SBF 30: 50% bulla + 20% wheat + 30% SBF

4.9. Textural and Organoleptic Evaluation of Fortified Cookies

The scores of textural and organoleptic evaluation of color, hardness, tractability, taste, aroma, flavor, and overall acceptability were assessed by expert panelists and the obtained results are given in Table 5.

Sample	Conc. of							
	SBF	Color	Fracture	Hardness	Taste	Aroma	Flavor	Overall acceptability
	0 %	7.16±0.28	7.75±0.25	7.50 ± 0.50	6.25±0.66	7.08±0.38	6.50 ± 0.50	7.03±0.18
Bulla Cookies	10 %	7.66±0.28	9.00±1.00	7.16±0.76	6.75±0.25	6.66±0.57	7.33±0.57	7.67 ± 0.42
	20 %	8.83±1.04	9.33±0.57	7.58 ± 0.14	8.50 ± 1.32	7.58 ± 0.52	7.25 ± 0.66	8.97±0.30
	30 %	9.00±1.00	8.83±1.04	7.00 ± 1.00	7.91±0.80	8.00 ± 0.00	8.33±0.57	7.93±0.02

Table 5. Results for textural and organoleptic properties of fortified cookies of the bulla.

Values are Mean ±SD, triplicate analysis

Color is a very important criterion for the initial acceptability of baked products by the consumer and may be considered a critical index of the baking surface. The mean score value of the color had increased from 7.16 ± 0.28 to 9.00 ± 1.00 in bulla cookies with the supplementation (0 to 30 %). The highest score (9.00 ± 1.00) was shown in 30 % SBF-supplemented cookies of bulla while the lowest mean score (7.16 ± 0.28) was observed in its control. Color depends both on the physicochemical characteristics of the raw dough (i.e., water content, pH, sugars, and protein content) and on the operating conditions applied during baking. Generally, the desirable color of cookies is "golden brown" and it's observed at different time-temperature combinations that significantly affect the color of cookies (Akoja, S.S., and Coker, O.J., 2018).

Cookies with bulla substituted by 20 % SBF were slightly darker than control cookies, and further increases the darkness (brown) by increasing fortification as in 30 % SBF (Figure 4.1). This could be due to the Maillard reactions from reducing sugars in the dough, resulting in more color during baking (Banusha S., and Vasantharuba, S. , 2014). Thus, the present study reveals that the prepared cookies are in the acceptable range of color for consumption even though does have slight brownish color on the increment of fortification level of SBF up to 30 %.

In bulla cookies, the mean score for hardness revealed that the control cookies had the highest score of 7.58 ± 0.14 , while the lowest mean score (7.00 ± 1.00) was noticed in 30 % SBF-supplemented cookies. This may be due to the availability of more carbohydrates in

30% than in 0%. This observation is contradictory to the results for breaking strength of flaxseed cookies (Noorfarahzilah, M., Lee, J.S., Sharifudin, M.S., Mohd Fadzelly, A.B., and Hasmadi, M., 2014). In a similar study (Yemataw, Z., 2018), it was found that the fracture of cookies increases with increased groundnut flour. The amount of absorbed water during dough mixing tends to reduce expansion and increases hardness.

Taste is the greatest important sensory feature for the recognition of products. The maximum score of 8.50 ± 1.32 was shown by SBF 15 % in bulla, while the lowest mean score of 6.25 ± 0.66 and 4.66 ± 0.94 were noticed respectively, in the control bulla. Likewise, Noor Farahzilah, *et al.* (2012) reported that during the baking experiment, crumb acidity increased with the addition of lentils and chickpeas, thus the cookies became tastier and richer nutrients (Noorfarahzilah, M., Lee, J.S., Sharifudin, M.S., Mohd Fadzelly, A.B., and Hasmadi, M., 2014). The flavor score of fortified cookies from retinyl acetate (vitamin A) ranged from 6.86-7.14 (Veronika, K., Zlatica, K., Jolana K., and Michaela, L., (2018)), which were partially higher and lower than those the presently studied sample flavor score as 4.75 ± 0.25 - 8.33 ± 0.57 . In the present study, the flavor score of all bulla SBF-fortified cookies has steadily increased and are significantly different when compared to their control cookies.

Overall acceptability is determined based on quality scores obtained from the evaluation of the color, fractability, hardness, taste, aroma, and flavor of the cookies. The increase in overall acceptability is due to an increase in quality score in response to the practicability, taste, color, hardness, aroma, and flavor of the SBF-supplemented cookies. The results obtained in this study are in closer agreement with the earlier study (Romee, J., Saxena, D.C., and Sukhcharn, S. , 2018), which reported similar results regarding the overall acceptability score of sensory evaluation in soy flour-fortified cookies. Again, this result confirms the report of <u>USDA (2009)</u> that plantains have fat levels range of 0.2-0.5 % (USDA, 2009).



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<u>Note</u>: SBF 0 (control): 85 % bulla + 15% wheat + no SBF; SBF 15: 65% bulla + 15% wheat + 10 % SBF; SBF 15: 65% bulla + 15% wheat + 15% SBF; SBF 30: 50% bulla + 15% wheat + 30% SBF.

CONCLUSIONS

This study was conducted to determine the nutritional composition of cookies such as moisture, ash, fat, protein, carbohydrates, and fiber which were measured as (ranged from control cookies) and minerals (calcium, magnesium, iron, and zinc contents) of cookies showed increased result. It can also be concluded that lactic acid bacteria and yeasts were the dominant microorganisms during kocho fermentation and are indicative of their cometabolic activities and have a longer shelf life. Further, mostly no growth of any kind of microorganisms developed on SBF-fortified cookies up to 5th month of storage of the prepared cookies.

Furthermore, the result of organoleptic analyses concluded that the color score of 30 % SBF bulla cookies in 0% attained more brownish color than other samples. Thus, cookies containing 30 % SBF and 20 % SBF were of much acceptable limit and found the most preferable.

RECOMMENDATIONS

This study investigated the fermented bulla of enset in the form of cookies supplemented with soybean and wheat which is a good source of protein and minerals. The following recommendations are made based on a holistic view of the subject area:

- Encouraging the use of fortified cookies and developing nutritious-based new products and hardness (fracture) in bulla cookies needs improvement.
- Research should be undertaken on the use of some flavoring agents to prolong the shelf life of cookies.
- This study might use to promote bulla for their food and nutrition security, the use of fortified cookies, and develop nutritious-based new products and income-generating potential in Ethiopia more emphasis should be given.

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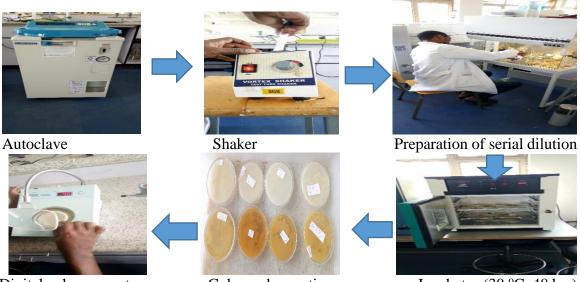
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APPENDICES

APPENDIX - 1: Some of microbial examination flow chart in the laboratory.



Digital colony counter

Colony observation

Incubator (30 °C, 48 hrs)

a 1	Conc. of SBF	I						
Sample		Ash	Moisture	Fat	Protein	Fiber	Carbh.	Energy
	0 %	2.19±0.10	3.75±0.40	3.25±0.44	12.10±2.11	3.02±0.10	75.67±2.00	386.41±0.37
Bulla Cookies	10 %	2.36±0.33	2.54±0.5	4.07±0.22	20.09±0.20	2.32±0.21	68.59±1.50	392.45±0.90
	20 %	2.23±0.50	1.90 ± 0.77	6.50±0.30	24.35±0.50	1.53±0.11	65.00±0.80	404.34±2.15
	30 %	2.51±0.65	1.43±0.80	8.86±0.90	27.54±0.43	2.64±0.12	57.44±0.64	410.81±2.00

<u>Note</u>: Values in the same row with different samples are statistically significant from each other (p < 0.05). SBF 0 (control): 80 % bulla + 20% wheat + no SBF; SBF 10: 70 % bulla + 20% wheat + 10 % SBF; SBF 20: 60% bulla + 20% wheat + 20% SBF; SBF 30: 50% bulla + 20% wheat + 30% SBF.

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