Nutritional and anti-nutritional evaluation of cookies prepared from okara, red teff and wheat flours

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Abstract

Okara is a nutritious byproduct of soy milk industry. The present study is carried out to effective utilization of okara in cookies preparations. Cookies are prepared with Okara, Red teff and Wheat flours. A 16-run constrained D-optimal mixture experiment was generated using Design-Expert® (Version 8.0, Stat-Ease) software. The three flours were mixed with other ingredients to prepare cookies. Prepared cookies are analysed for proximate, mineral and anti-nutritional compositions. Moisture content, Ash, crude protein, fiber, crude fat, carbohydrate, energy of cookies had ranged between 6.25-8.00%, 1.25-2.4%, 15-18.56%, 7-8.9%, 13-18.2%, 44.52-57.45%, 406-416 K. Cal/100gms respectively. Iron, Zinc and calcium contents of cookies are ranged between 6.20-7.30 mg/100gms, 2.66-20.88 mg/100gms and 16.60-18.00 mg/100gms respectively. Anti-nutritional components (tannin and phytate) rise by the okara ratio increase in cookies composition. Red teff 33-38%, Wheat 18-20% and 45-47 Okara% are the optimum compositions for best nutritional quality cookies.

Introduction

Cookies are popular ready-to-eat snack foods and widely consumed by all age groups especially by children around the world due to their simple manufacturing process, long shelf life and potential for containing high nutrient components (Aziah et al., 2012). Cookies are made from different flours that are characterized by a formula with high in sugar, shortenings and relatively low in water. Commercially available cookies are prepared from white flour that is nutritionally inferior to whole wheat flour (Chavan et al., 1993).

Okara is an insoluble major by-product of the soy milk process, composed of 50% dietary fiber, 25% protein, 10% lipid, and other nutrients (Li et al., 2012). In Ethiopia, cultivation of soybean and utilization of the soy products are drastically increasing (Hailu and Kelemu, 2014), for instance, Faffa foods Share Company reported the business of more than 15 million birr (local currency) in 2011 by soy milk powder and milk production. The huge quantities of okara produced annually have a significant disposal problem because of its high putrefaction capacity (Li et al., 2013). This leads to environmental problems and wastage of the nutrients contained byproduct. Researchers are reported that okara is a good raw material to be used as a dietary supplement in different products since its high nutrient content and low production costs, (O’Toole, 1999; Sengupta et al., 2012).

Teff (Eragrostis tef) is an ancient tropical cereal has its center of origin and diversity in the northern Ethiopian highlands from where it is believed to have been domesticated (Tadesse, 1993). Teff is a minor cereal crop worldwide, whereas in Ethiopia, it is a major food grain, mainly used to make injera, a traditional fermented Ethiopian pancake (Tadesse, 1993; Gebremariam et al., 2012). Red teff has a higher iron and calcium content than other varieties (Abebe et al., 2007). Inadequate iron intake is common in low and middle income countries like Ethiopia, particularly among infants and young children (Gibson et al., 2010) and pregnant women (Coban et al., 2003). Food fortification with iron rich crops like red teff and nutritional supplements may constitute effective strategies to prevent iron deficiency (Stoltzfus, 2011).

Wheat (Triticum aestivum L.) is the most important staple food crop for more than one third of the world’s population and one of the most important cereals cultivated in Ethiopia for the purpose of baked products because of its special protein known as gluten. It contributes more calories and proteins to the world diet than any other cereal crops (Adams et al., 2002; Shewry, 2009). Gluten,
the protein component of wheat is attributed to the dough elasticity and strength, can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water soluble constituents (Wieser, 2007; Kaushik et al., 2013). Some wheat varieties (e.g. *Triticum aestivum*) are suitable for bread making while others (e.g. *Triticum durum*) are suitable for biscuits and cookies making (Sapirstein et al., 2007).

Cookies prepared from the wheat flour or white flour alone may have the nutritional inferiority so the major objective of the present research is to enrich the nutritional component by the utilization of locally available cereal (red teff) flours and soybean (soy milk) by-product (okara).

Materials and Methods

Sample collection

Soybean (Avgat) and both Red teff (H-0199), Wheat (Digalu) were obtained from Jimma Agricultural Research Center and Holeta Agricultural Research Center, Ethiopia, respectively. Eggs were collected from Jimma University College of Agriculture and Veterinary Medicine College poultry farm, milk powder, baking powder, table salt; table sugar and shortening were collected from local markets. The experiment was conducted in laboratories of Post harvest Management (Jimma University) and Ethiopian Public Health Institute, Ethiopia.

Sample preparation

The red teff and wheat were cleaned manually to remove contaminants and immature seeds and inferior materials. They were ground into flour in mill and sieved through a 0.5 mm sieve and packed in airtight polythene plastic bags for further processing (AACC, 2000).

The left over residue (Okara) of the soy milk processing was collected from our co-researcher (working on soy milk with Avgat variety of soybean) and dried in hot air oven at 60°C temperature for 24 hrs and ground into flour using attrition mill and passed through 0.5 mm sieve and packed in polyethylene plastic bags for further usage (Wickramarathna and Arampath, 2003).

Experimental design

A 16-run constrained D-optimal mixture experiment was generated using Design-Expert® (Version 8.0, Stat-Ease) software. The constraints used were 20 to 40% for red teff, 10 to 20% for wheat and 40 to 50% for okara and one considered as control with 100% wheat flour, the total runs in this experiment was 17. The range of constrains were determined based on different literatures (Chen et al., 2003; Gernah, 2007; Aziah et al., 2012; Chinma and Coleman et al., 2013; Nwankezi, 2013; Hrušková and Švec, 2015) and preliminary studies.

Preparation of cookies

Total of 17 Cookies samples were prepared from the Composite flour with minor modification according to the method reported by Aziah et al. (2012). For 1000 g composite flour, Whole egg (60 g), powdered milk (20 g), baking powder (0.1 g), Salt (1 g), sugar (200 g) and 250 ml of distilled water were mixed in a bowl by a dough mixer until a stiff paste was obtained and kept aside. In other mixer creaming of shortening (60 g) was done until foaming occurred. The previous blend was added to the creamy mass of shortening and mixed for 10 minutes at medium speed in a laboratory dough mixer. The dough was allowed to rest for 30 minutes at room temperature and rolled on a floured board using rolling pin to thickness of 0.3 cm. The rolled dough was cut by pre-mould cookies shape, arranged on a grease tray and baked at 120°C for 15 minutes by convection oven (temperature and time was determined in preliminary work). Cookies were cooled to ambient temperature, packed in low density polyethylene bags and kept in airtight containers to subsequent laboratory analysis.

Data collection

Proximate, mineral and anti-nutritional compositions of the cookies samples were determined.

Proximate analysis

Moisture content of the prepared cookies samples were determined by hot air oven according to AOAC method 925.10, (AOAC 2005). Ash content, crude fat and crude fiber were determined by dry basis according to AOAC method 923.03, 920.39 and 962.09, respectively (AOAC 2005). Kjeldahl method was used to determine total nitrogen and was multiplied by the 6.25 to obtain total protein in the sample (AOAC 2005, method 979.09). Total carbohydrates (CHO%) was estimated by difference method (Monro and Burlingame, 1996). Gross energy was calculated according to the method developed by Osborne and Voogt (1978).

Mineral analyses (Ca, Zn and Fe)

The mineral contents were determined by Atomic Absorption Spectrophotometer (Perkin–Elmer, Model 3100, USA, Auto sampler AA 6800, Japan) as per the AOAC (2005) method 985.35. One gram of
cookies sample was converted to ash; known weight of ash was treated with 5 mL of 6 N HCl and dried on the hot plate and 15 mL of 3 N HCl was added and heated on the hot plate until the solution boiled. The solution was cooled and filtered through what men No 1 filter paper in to 50 mL graduated flask then make up with de-ionized water and used to determine Ca, Zn and Fe. The sample and standard was atomized by reduced air-acetylene for Calcium and oxidizing air-acetylene for Zinc and Iron as a source of atomization energy (AACC, 2000). The absorbencies of both the samples and standards were measured at 248.4 nm, 213.9 nm and 422.7 for Iron, Zinc, Calcium. The samples mineral concentrations were determined from standard graph and expressed as mg/100 g.

Phytate

Phytate content (mg/100 g) was determined by method described by Vaintraub and Lapteva, (1988). Cookies sample (0.0573 g) was extracted with 10 mL of 0.2 N HCl for 1 hr at an ambient temperature then centrifuged at 3000 rotations per minute for 30 minutes. The clear supernatant was collected and 3 mL of supernatant solution was mixed with 2 mL of Wade reagent (0.03% solution of FeCl₃+6H₂O containing 0.3% sulfosalicylic acid in water). The absorbance was read at 500 nm measured using UV-Vis spectrophotometer (CE1021, England). The phytate concentration was calculated from the difference between the absorbance of the sample and blank. The amount of phytic acid was calculated using phytic acid standard curve.

Condensed tannin

Condensed tannins were determined by Maxson and Rooney, (1972). One gram of sample was extracted with 10 mL of 1% HCl in methanol for 24 hours at room temperature by mechanical shaking (Edmund Buhler, USA) then centrifuged at 3000 rotations per minute for 5 minutes. One mL of the supernatant was mixed with 5 mL of vanillin HCl reagent (equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol) and allowed for 20 minutes at room temperature. Finally, the absorbance was read at 500 nm using UV-Vis Spectrophotometer. A stock catechin solution was used as the standard and value of tannin was expressed in mg of catechin in 100 gram of sample.

Data analysis

The response variables measured from the 17 formulations including control were analyzed using Minitab®, Version 16, software. The statistical analyses included verifying that the model does not have significant lack-of-fit, and the normal distribution and constant variance assumptions on the error terms are valid. Independence assumption was valid through the random order of the runs by testing the significance of each term, constructing contour plots for each response variable to determine the best formulation for each response and, finally, determined the optimum point that optimizes all response variables (Montgomery, 2013).

Results and Discussion

Analysis of variance (ANOVA), p-Values of all the properties were presented in Table 1 with lack of fit values. The cookies proximate composition and anti-nutritional and mineral vales were presented in Table 2. The predicted regression models for all the parameters (proximate, energy, mineral and Anti-nutritional) were given in Table 3.

Moisture contents of cookies were within the range of 6.25 to 8.00% (Table 2). The highest moisture content was determined in the cookies prepared from 40% red teff, 10% wheat and 50% of okara; while the lowest moisture content was observed in the cookies made from 40% red teff, 20% wheat and 40% okara. The moisture content of cookies was increased with the increase in okara flours, this is attributed to the fact that soy flour can absorb and holds higher amount of moisture in baking process (Park et al., 2015; Cheng and Bhat, 2016). Moisture content of cookies in present study are similar with Joel Ndife and Fagbemi, (2014) who prepared cookies from 50% soy flour supplemented with wheat flour. The present result was not significantly difference in both linear and quadratic models, but, significance difference was observed in the blend of wheat with okara. The experimental results of R² values indicated that the models were satisfactory and lack of fit value showed non-significant (Table 1).

The ash content of the cookies showed significance difference in quadratic model. Highly significant difference (p<0.01) was observed between red teff and okara and also significantly different (p<0.05) in red teff with wheat (Table1). The ash content of the cookies sample varied between 1.25 to 2.4% (Table 2). The highest ash content was observed in cookies made from 35% red teff, 15% wheat and 50% okara. Whereas the lowest results were recorded for cookies prepared from 40% red teff, 20% wheat and 40% okara. The result of this study indicated that the ash content of the blends was increased steadily with increasing okara flour. This might be due to the better ash content of soybean than others. Legumes have
been reported to be good sources of ash (Tasnima, 2015). Similar result was reported by Joel Ndife and Fagbemi, (2014) for cookies prepared from 50% soy flour supplemented with wheat flour.

Protein content of prepared cookies was ranged from 15% to 18.56% (Table 2). The maximum protein content was found in cookies prepared from the blending of 30% red teff, 20% wheat and 50% okara; lowest protein content was found in cookies made from blending of 40% red teff, 20% wheat and 40% okara. These trends might be due to the high amount of okara flour in the product and high protein content of soybean (Wickramarathna and Arampath, 2003). The results of the present study is supported by the results of Tasnima, (2015) who reported protein content of 17.8% in biscuits prepared from soybean and wheat flour and protein values of cookies prepared in the present study have high comparability with the Olaoye et al. (2006) study. Result of the blended cookies in present study had highly significant protein contents than the control (cookies made with wheat). Batool et al. (2015) stated that combination of cereals with legumes provide better overall essential amino acid balance, helping to overcome the world protein malnutrition problems. Addition of legumes like soybean have important where many people can hardly afford high protein foods because of their high cost and produced the desired effect of increasing the protein content of the blends. It is a potential way to increase the nutritional value of traditional cookies prepared from wheat flour.

The fiber content of cookies samples prepared from composite flours was varied between 7-8.9% (Table 2). Cookies prepared from blends of 40% red teff, 10% wheat and 50% okara was showed the highest value, in contrast, blends of 40% red teff, 20% wheat and 40% okara were showed the lowest fiber content than other blended samples. Significant difference (p<0.05) was observed for both the linear and quadratic models (Table 1). Significance difference was also observed for red teff with okara and wheat with okara. The result of the present study was in agreement with study reported by Joel Ndife and Fagbemi, (2014) who reported 5.73% of crude fiber in cookies prepared from 50% soy flour supplemented with wheat flour. The increased fiber content of cookies have several health benefits, as it will aid digestion often associated with products from refined grain flours (Slavin, 2005; Brownlee, 2011; Elleuch et al., 2011). Other studies have also showed that combining okara with soft wheat flour resulted an increased dietary fiber contents as compared to the use of soft wheat flour alone (Rinaldi et al., 2007).

Significant difference (p<0.05) was observed in the fat content of the cookies in quadratic models (Table 1). The fat content of all cookies samples was varied between 13-18.2% (Table 2). The results were observed that significantly difference between red teff with okara and also significantly difference in wheat with okara. The highest value of fat content was determined in cookies prepared from 30% red teff, 20% wheat and 50% okara, whereas the lowest value of fat content recorded in cookies prepared from 40% red teff, 20% wheat and 40% okara. This might be because of relatively higher amount of fat found in okara. This result is in agreement with results reported by Akubor and Ukmuru, (2003) who reported the fat content of the biscuits prepared from wheat and soybean increased from 14.6 to 24.0% with increase in soy bean flour. Similarly Iwe and Ngoddy, (1998) also concluded that soy beans have been reported to be a good source of oil. Furthermore, Rita and Adiza, (2010) also authenticated that addition of soybean flour increases the fat content in fortified cookies while the amount of carbohydrate is reduced. Generally the soybean have been reported to contain an appreciable quantity of minerals and fat (Onyeka and Dibia, 2002; Plahar et al., 2003).
Carbohydrate content of cookies made from red teff, wheat, and okara were highly significant difference in quadratic model. Significance difference was observed, highly significant difference between red teff with okara and wheat with okara respectively (Table 1). Total carbohydrate content of cookies samples ranged between 44.52 to 57.45% (Table 2). The highest carbohydrate content was observed in cookies sample prepared from 40% red teff, 20% and okara of 40% whereas least carbohydrate content in cookies from wheat 30% red teff, 20% wheat, and 50% okara. The results of this study showed that okara is not a good source of carbohydrate when compared to wheat and teff. The carbohydrate contents decreased with the increase in the proportion of okara in the composite flour supplemented cookies, the same was supported by Akpapunam et al. (1997). The decreased carbohydrate content of the cookies with addition of red teff and okara flours would be useful to people who need low carbohydrate foods leading to enhanced health for overweight and obese persons. However, cookies which made from control (100% wheat) had reported the highest carbohydrates content; this is due to higher amount of carbohydrate in wheat. Generally, the decreased trend in carbohydrate could be due to the low content of carbohydrate in the added okara compare to wheat flour. This is agreed with the finding of Iwe and Ngoddy, (1998) who reported that soybean is poor sources of carbohydrate. Although, cookies carbohydrate value resulted in this study is still lower than the regular value of wheat-based cookies thus, it could be possible to develop low-carbohydrate cookies using okara.

The gross energy of cookies samples were varied from 406.06 to 416.20 kcal/100g (Table 2). The higher gross energy value content was observed in cookies sample prepared from a formulation of red teff 30%, wheat 10% and Okara 50% while the lower gross energy value was observed in cookies sample prepared from a formulation of red teff 40%, wheat 17% and Okara 43%. The high energy content of cookies sample prepared from a formula consisting of high proportion of soy bean okara flour. The result was not significant difference (p<0.05) was observed in the gross energy content of mixed flours cookies sample between among all interaction composite flour but, no significance between both linear and quadratic models (Table 1). Onabanjo et al. (2009) reported that the high content of legumes and oil crops flour further increased the energy density of the products developed from different formulations. This result was in agreement with the other findings obtained by Aleem Zaker et al. (2012) who reported that blending of soy flour in biscuit preparation with wheat showed increment of total energy up to 462.3 kcal/100g.

Mineral contents of cookies (Iron, Zinc and Calcium)
The iron content of cookies samples was varied between 6.20 to 7.30 mg/100gm (Table 2). The highest Fe content determined in cookies prepared from 40% red teff, 10% wheat and 40% okara, while the lowest result value of Fe was reported for cookies from 34% red teff 20% wheat and 46% okara. This might be due to an increase in proportion of red teff
flour and to some extent with the level of okara. Similarly, Abebe et al. (2007) reported that Teff is a good source of iron content. The compositions of Fe showed no significance different in both linear and quadratic model and all possible interactions of mixed samples of red teff, wheat and okara (Table 1). Similar results had been reported as soy fortified chapattis contained higher iron, than whole wheat flour chapattis (Khetarpaul and Goyal, 2009; Khan et al., 2012).

In the present study the zinc content of cookies samples was varied from 2.66 to 2.88 mg/100 g (Table 2). The highest Zn content was determined in cookies made from 40% red teff, 10% wheat, and 50% okara, while the lowest identified from 34% red teff, 20% wheat and 46% okara cookies. The calcium content of cookies was increase with the increment of some red teff and okara proportion in the mixed flour cookies sample. Calcium content was found significantly (p<0.05) in red teff with okara in quadratic model (Table 1). The results also revealed that an increased Ca contents were observed when there were high concentration of okara and red teff flour in the cookies. Higher mineral content in the present study found in different cookies may be attributed to higher concentration of Calcium in the soybean okara and red teff (Mengesha, 1966; Li et al., 2012). A similar result has also been reported as soy fortified chapattis contained higher Ca than whole wheat flour chapattis reported (Khetarpaul and Goyal, 2009; Khan et al., 2012).

### Anti-nutritional contents

Tannin content of cookies ranged from 13.5 – 18.79 mg/100g (Table 2) and was not significantly affected by the proportion of the mixture components (red teff, Wheat and okara) used for cookies preparation in both linear and quadratic models (Table 1). The highest tannin content was determined in the cookies of 30% red teff, 20% wheat, 50% of okara and the lowest value was obtained from the mixture of 40% red teff, 20% wheat and 40% of okara. This indicates that high amount of tannin in the cookies was increased by the amount of okara increased. Samuel et al. (2012) reported that soybean contains high amount of tannins. The findings of the present study denotes that tannin content of prepared food is increased by the increasing the level of soy flour (El-Shemy et al., 2000). Tannins are known to present in food legumes that decrease the protein quality of foods and interfere with dietary iron absorption (Admassu, 2009; Tadelel, 2015). According to Adeparusi, (2001), tannins affect protein digestibility and adversely influence the bioavailability, obtained from plant sources lead sing to poor iron and calcium absorption. The raise in tannin in cookies is in line with okara proportion could have effect on nutrients bioavailability and absorption so; tannin is one of the factors to be considered during optimizing the proportion of okara supplemented with wheat and red teff. Although tannin has detrimental effect, it has several benefits in processed food and human health. The antimicrobial property of tannic acid can be used in food processing to increase the shelf-life of certain foods (Chung et al., 1998).

Phytate content of the cookies was ranged from 85–115 mg/100g (Table 2). The high and low amount of phytate was recorded in the cookies prepared from the proportion of 40% red teff, 10% wheat and 50% okara, while the lowest identified from 34% red teff, 20% wheat and 46% okara cookies. The calcium content of cookies was increase with the increment of some red teff and okara proportion in the mixed flour cookies sample. Calcium content was found significantly (p<0.05) in red teff with okara in quadratic model (Table 1). The results also revealed that an increased Ca contents were observed when there were high concentration of okara and red teff flour in the cookies. Higher mineral content in the present study found in different cookies may be attributed to higher concentration of Calcium in the soybean okara and red teff (Mengesha, 1966; Li et al., 2012). A similar result has also been reported as soy fortified chapattis contained higher Ca than whole wheat flour chapattis reported (Khetarpaul and Goyal, 2009; Khan et al., 2012).
and 50% okara and 40% red teff, 17% wheat, 43% okara respectively. This might be due to the high amount of phytate content found in okara (Li et al., 2012) than wheat and red teff. Phytate content was not significantly influenced by the proportion of red teff, wheat and okara in both models. In present study, Phytic aid content increased by the more supplementation of soy okara. Phytate content is high in legumes and decreases the bioavailability of essential minerals and bioavailability of proteins by forming insoluble phytate-mineral and phytate-protein complexes (Admassu, 2009; Tadele, 2015).

**Optimization based on proximate composition**

The white area of Figure 1 indicates the optimum point of formulation to develop composite cookies with best chemical composition which can serve the expected purpose. Thus the optimum point which includes all the optimum points of fat%, fiber%, protein%, carbohydrate%, gross energy Kcal/100gm, Iron mg/100gm, Calcium mg/100gm, and Zinc mg/100gm was indicated in Figure 1. From the numerical optimization determined that the cookies prepared from teff 33-38%, Wheat 18-20% and 45-47 Okara% would show best overall quality. From the optimal value it can be seen that the amount of Okara can be used from the lowest value to the maximum without affecting the nutritional content whereas the optimum nutritional content was found at the maximum amount of okara. In optimization process the superimposition of contour plot regions of interest considered Protein > 15.4%, Fat > 14%, Fiber > 7.4%, Carbohydrate >45.11%, Energy (kcal/100gm), Iron > 6.2 mg/100g, Zinc >2.66 mg/100g, Calcium >16.6 mg/100g. This all optimum result of chemical composition was preferable to 1-11 years old children’s according to the nutritional guidelines.

**Conclusion**

Based on present study the moisture content, Ash, crude protein, fiber, crude fat, carbohydrate and energy of cookies had ranged between 6.25-8.00%, 1.25-2.4%, 15-18.56%, 7-8.9%, 13-18.2%, 44. -52-57.45% and 406-416 KCal/100gms respectively. Furthermore Iron, Zinc and Calcium of cookies are ranged between 6.20-7.30 mg/100gms, 2.66-20.88 mg/100gms and 16.60-18.00 mg/100gms respectively. The optimum nutrient quality of the cookies prepared from wheat, red teff and okara was found to be in the range of teff 33-38%, Wheat 18-20% and 45-47 Okara%. In conclusion, incorporation of Okara and the red teff in to cookies flours the overall nutritional quality of product has improved and successfully utilized the okara in the cookies production. However, the anti-nutritional factors of the okara can not ruled out and further research has to initiate to decrease the anti-nutritional factors in okara.

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**Interest of conflict:**

No conflict showed.

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