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ORIGINAL ARTICLE

Formulation and Development of Nutritious Bread from a Mixture of Wheat, Teff and Lupine

Badasa Rata Jalata [1*], Mesfin Wogayehu[1]

[1]Department of Applied Human Nutrition, Faculty of Chemical and Food Engineering, Bahir Dar University, P.O.Box 26, Bahir dar, Ethiopia

Highlights

- Composite flour have higher development time and dough stability compared to white wheat flour
- Lupine and teff substituted bread have higher nutrient profile than white bread
- Red teff substituted bread have higher Fe, Mg, Ca and Zn than both white teff substituted and white bread
- Thirty percent substitution of teff into wheat flour made poor sensorial acceptance

ABSTRACT

Consumption of plant-based foods limited in certain essential nutrients is common in Ethiopia. Therefore, the main approach of this study is to develop nutritious and fibrous bread through supplementation of whole teff and lupine. Wheat flour was replaced with 10-30% of teff (red and white) flour and 10% lupine (sweet and bitter). The straight dough technique was used for baking and ANOVA was used for the analyses. The farinograph properties of the flour used for bread indicated that the water absorption capacity of white teff incorporated flour was higher than the control flour and other composite flour. The development time and dough stability of red teff flour incorporated at 10% was higher than the white flour. In addition, the concentrations of teff have an impact on the mixing tolerance index of the flour. However, increasing amounts of both white and red teff flour have no effect on water absorption capacity and dough development time. The type and concentration of teff and lupine incorporation significantly affected the ash, protein, total carbohydrate contents and texture properties of teff-lupine-wheat bread. The proximate composition of the composite flour reflected a significant increase in protein, fat and fiber contents, whereas the total carbohydrate decreased. Mg and Ca were mainly found in red teff substituted bread while Na, K, and Mg were mainly found in white teff substituted bread. Mn, Fe and Cu are mainly identified in red substituted teff whereas Pb is the only mineral mainly found in white teff. The teff concentration had an impact on Co whereas there was no effect on Cr. The tannin contents of the composite bread were similar to the white bread whereas, the phytate contents were higher. The sensorial attributes indicated that the white bread had superior overall acceptability compared to other composite breads. The findings implied that lupine and teff can be incorporated into wheat flour at 10% for bread making with desirable nutritional composition, and sensory acceptability.

Keywords: Wheat flour, teff, lupine, White bread, Composite bread

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Corresponding Author:
Badasa Rata Jalata
Department of Applied Human Nutrition, Faculty of Chemical and Food Engineering, Bahir Dar University, P.O.Box 26, Bahir dar, Ethiopia
Email: badasa43@gmail.com

1. Introduction

Wheat is widely produced in western countries and imported into many African countries for daily caloric consumption (Chisenga et al., 2020). On the other hand, continuous increases in the price of wheat in global markets, due to inflation, war and hard currency shortage is raising issues on the economic sustainability of wheat importation for Sub Saharan countries, including Ethiopia. Thus, there is a growing interest to encourage the use of locally produced staples for partial substitution of wheat flour in baking (Abass et al., 2018). Teff flour has been identified for partial replacement of wheat flour (Abebe & Ronda, 2014a; Baye, 2014; Geremew Bultosa, 2007; Collar et al., 2015). In Ethiopia, consumption of plant-based food complemented with almost no animal-based food prevails due to the poor economic background and religious–inspired dietary habits (Seleshe et al., 2014). The majority of the community in the northern part of the country are orthodox religion followers, strongly prohibits consumption of animal-based food products for a number of months in a year. This forces individuals to be exclusively dependant on plant-based foods (Shumoy & Raes, 2017). This causes low dietary diversity and reliance on frequently starchy staples, which do not provide sufficient calories and vital micronutrients, which contributes to the burden of malnutrition and micronutrient deficiencies (Carson & Edwards, 2009; UNICEF, 2009). Therefore, alternative means should be researched to increase the diet diversity of the population and thereby minimize the prevalent problem of malnutrition in the country, specifically in the North-Western part of Ethiopia(Yeneabat et al., 2019; Zeray et al., 2019). One of the many and highly recommended approaches is the use of composite flour technology, mainly in bread making processes (Olaoye & Ade-omowaye, 2011). This approach employs the concept of complementing cereals with legumes which are to improve nutritional compositions of some food products such as bread. *Lupinus albus ssp. Graecus* is one of the main legume crop produced and consumed by small holder farmers in the North -Western part of Ethiopia (Nigusse, 2012). They are rich in protein (35-40%)*, as well as dietary fiber and poor in digestible carbohydrates (Arnoldi et al., 2015). The nutritional content of breads can be improved by incorporation of lupine (Dervas et al., 1999). The higher lysine contents of lupine complements with that of wheat flour. The replacement of wheat flour with up to 10% lupine flour can enhance dietary and functional value of bread (Guillamón et al., 2010a). Teff [*Eragrostis tef* (Zucc.) Trotter] is a common cereal produced in Ethiopia. The nutritional profile of teff is good and rich in carbohydrates, fiber and all essential amino acids. Teff is also particularly high in iron and has more calcium, copper and zinc than other cereal grains (Abebe et al., 2007; Campo et al., 2016). However, its tendency to be less elastic, sticky, lower loaf volume and rough texture hinders teff using in baking industry. Teff has low gluten content, inferior gluten viscoelasticity, which results in poorer bread-making quality (Coleman et al., 2013). The potential use of teff flour in bread-making could be more promising if it were used in blends with wheat flour. Bread produced from replacing some portion of the wheat with locally available crops such as teff and lupine would be a best approach to enrich nutrients like iron, protein, and dietary fiber. The lupine has high protein content (38.3 g/100 g) (Guillamón et al., 2010b), while teff has a high content of fiber, Fe and Zn (Baye, 2014; Geremew Bultosa, 2007) which makes them an important complement for wheat flour and the production of high proteinaceous and fibrous bread. Many research works have been done on formulation of composite bread using tubers (Bakare et al., 2015), cassava (Barati, Latif, Freihardt, 2015), teff (Callejo et al., 2016a; Legesse et al., 2022). However, there is a gap on formulation and development of bread from lupine (local varieties) and teff. This work will address utilization of lupine and teff for bread making. Therefore, it is hypothesized composite mixture of wheat; teff and lupine flour have superior nutritional value such as protein content and essential minerals than the white bread.

2. Materials and Methods

2.1. Collection and Preparation of Raw Materials

Samples of lupine (*Lupinus albus*) were collected from Bahir Dar open market (Amado Gebeya). The area was selected because of high market accessibility both for the producers and users. The sampling technique used for buying was purposive sampling, in which the best quality lupine was selected. After the sample was bought from the Bahir Dar open market, it was packed in nylon bags and transported to Food Process Laboratory of Bahir Dar Institute of Technology. Contrary to this the sweet lupine was packed in polyethylene bag and delivered from the Andasa Agriculture Research Institute to Bahir Dar Institute of Technology. Wheat flour, teff grains (red and white) type were purchased from Bahir Dar local market. The lupine grains were sorted for stones, rot and other physical defects, while teff grains were cleaned by sorting, and winnowing. The teff grains were milled using a local attrition mill. This was followed by sieving using standard sieves. The lupine seeds were soaked in a volume of water three times to the weight of the grains in a plastic bucket at 27± 2ºC (room temperature) for 8 h, drained for 10 min, transferred to aluminum trays, and dried in the sun to about 12% moisture. After cooling, the lupine samples were decorticated, winnowed and grounded using an attrition mill at the local shop.

2.2. Composite flour formulation

Composite flours were prepared by replacing wheat flour with teff flour (Red and White) at (0%, and 30%) (Alaunyte et al., 2012) and two types of lupine (Sweet and Bitter) with (10%) (Doxastakis et al., 2002; Guillamón et al., 2010b) at Food Process Laboratory of Bahir Dar Institute of Technology. Eight wheat/teff/lupine flour blends were prepared: wheat flour (100%) which was a control and other composite bread were prepared based on their ratio. Three replicate blends for flour testing and baking were prepared and analyzed independently.

2.2.1 Farinograph testing

The control flour and composite flour samples were tested using a Farinograph (Brabender GmbH & Co. KG, Duisburg, Germany) according to AACC Approved Method No. 54-21 (AACC, 2011). As described in Abang et.al., (2010), Inframatic analyzer was used to obtain an estimate of the moisture content of flour sample and to decide the actual weight of flour samples to be used at the approved 14% moisture basis for the farinogram analysis. This was obtained by the expression;

$$\textit{Required weight of sample} = \frac{(100-14)x300}{(100xM)}$$

where M = Percent moisture content of the sample. Appropriate weight of flour sample was placed in the mixer of the farinogram, which was thermostatically controlled by means of water jacket at a temperature of 30°C. Cold water at 30°C was added to the sample through the attached burette until optimum water absorption content was absorbed by the dough when the farinogram curve was on the 500 line. A new sample was taken and the process was repeated using the suitable water absorption for the mixing and development process. The development of the dough and the resistance offered to mixing were recorded. Farinograph values: water absorption capacity (WAC %), dough development time (DT min.), dough stability time (ST min.), and mixing tolerance index (MTI FU) and time to break down (TBD min.) were evaluated by AACC Method using the Farinogram software (Brabander® Farinograph version: 2.3.6, 1996–2005, Microsoft corporation).

2.3. Baking procedure

A straight dough process for all bread types were performed using the following formula on a 250 g flour (wheat, teff and lupine) basis: 1.8% salt, 0.5% bread improver, 2% dry yeast and 85% water. The composite flours were mixed manually by hand in buckets. Dough (250 g) was placed into aluminum trays and proofed at 48 ^{O}C and (75 ± 5%) relative humidity for 30 min. Subsequently, baking was carried out in a conventional baking oven at 210 ^{O}C for 30 min, and resulting breads were left for one hour at room temperature before analysis. Sensory evaluation was made for all composite flour compared to the control wheat bread.

2.4. Sensory evaluation

The sensory evaluation was performed by forty seven judges from staff and graduate students of Faculty of Chemical and Food Engineering of Bahir Dar University who have previous experience in conducting sensory parameters at food process laboratory. The five-point hedonic scales with score ranging from ‘Like extremely (5)’ to ‘dislike extremely (1)’ were used. The evaluated parameters were taste, flavor (taste and aroma), texture, color, appearance and overall acceptability.

2.5. Chemical composition of bread samples

2.5.1. Proximate composition

2.5.1.1. Moisture content

The moisture content of all bread was determined using drying oven (AOAC, 1990).

2.5.1.2. Total Ash content

The ash content of the bread were determined by muffle furnace (AOAC, 2000).

2.5.1.3. Crude protein

The protein content of bread was estimated from the crude nitrogen content of the sample determined by the Kjeldahl method (Nx6.25) (AOAC, 2000).

2.5.1.4. Fat

Crude fat content of bread was determined by soxhlet technique (AOAC, 2000).

2.5.1.5. Dietary fibre

The total dietary fiber content of white bread and composite bread were determined using Total Dietary Fiber Assay Kits TDF-100A and TDF- C10 (Sigmae Aldrich Inc, St Louis, MO, USA) using a combination of enzymatic and gravimetric methods (AOAC, 1997).

2.5.1.6. Total carbohydrate

Total carbohydrates of both white and composite bread were determined using difference technique (FAO, 1998).

2.5.2. Mineral composition

The minerals content of the bread samples were determined by atomic absorption spectrophotometer (AAS) (Agilent FS240 AA, USA) following (AOAC, 2000). Three grams of composite flour samples were carbonized on a heating plate and ashed in a muffle furnace (Sx2-4-10, Zhejiang, China) at 550℃ until ashing was completed. The white ash was dissolved using 5 mL of 6N HCl, dried on a hot plate, followed by the addition of 7 mL of 3N HCl heating on a hot plate and then finally, the solution was diluted to the 50 mL de-ionized water. The Fe, Zn and Ca contents were determined by AAS using air acetylene as a source of flame energy for atomization. The absorbance for Fe was measured at 248.3 nm, and the Fe content was estimated from a standard calibration curve (0, 0.5, 1.0, 2.0, 3.0 and 4.0 μg/mL) prepared from analytical grade iron wire. The absorbance for Zn was measured at 213.9 nm, and the Zn content was estimated from a standard calibration curve (0.000, 0.125, 0.250, 0.500, 0.750 and 1.000 μg/mL) prepared from ZnO. The absorbance of Ca was measured at 422.7 nm after adding 2.5 mL of LaCl3 to sample solutions. The Ca content was then estimated from the standard solution (0.0, 0.5, 1, 1.5, 2.0 and 2.5 μg/mL) prepared from CaCO3. The Na and K contents were determined using a flame photometer (ELICO CL 378, India) by measuring their emission at 589 and 767 nm, respectively. The Na content was estimated from standard solution (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 μg/mL) prepared from NaCl. The K content was estimated from a standard solution (0, 2, 4, 6, 8, 10 and 12 μg/mL) prepared from KCl.

The mineral element content was calculated using Eq. (1):

$$Element(mg/100gm) = \frac{[(\mu g/mL * df]}{[sample\ mass(db) * 10]}$$

Where; df is a dilution factor (50 mL), and db is sample mass on a dry matter basis.

2.5.3. Anti-nutrient contents Determination

2.5.3.1. Determination of tannin content

The tannin content was determined according to Maxson, E. D. & Rooney (1972). About 1.000 g of the sample mixed with 10 mL of 1% HCl solution in methanol in a screw cap test tube was shaken for 24 h at room temperature on a mechanical shaker (Hy-2(C), Shanghai, China). The solution was centrifuged (sigma 2-16KC, UK) at 1000 rpm for 5 min. One mL of the supernatant was transferred to another test tube and mixed with 5 mL of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol). A 40 mg of D-catechin standard was dissolved in 1000 mL of 1% HCl solution in methanol from which series of standard solutions (0, 12, 24, 36, 48 and 60 μg/mL) were prepared from stock solution by mixing with 5 mL 1% HCl in methanol. The absorbance of samples and the standard solutions were measured at 500 nm using UV-VIS Spectrophotometer [V-630, Jasco (USA)] after 20 min, and the condensed tannins content was estimated from the D-catechin calibration line ($Y = 0.006X + 0.0772, R^2 = 0.993$) The result was expressed as mg/100g.

2.5.2. Determination of phytate content

The phytate content was determined as described in Vaintraub & Lapteva (1988). About 1.00 g samples were extracted with 10 mL of 2.4% HCl in a mechanical shaker (Hy-2(C), Shanghai, China) for 1 h at room temperature and centrifuged (Sigma 2-16KC, UK) at 3000 rpm for 30 min. Clear sample solution (supernatant) or series of standard solution (3 mL) was mixed with 1 mL of wade reagent (solution of 0.03% $FeCl_3.6H_2O$ and 0.3% of sulfosalicylic acid in water), vortex mixed for 5s. Then absorbance was measured at 500nm UV- VIS spectrophotometer [V-630, Jasco (USA)] using 3mL de-ionized water as blank. The absorbance of blank was subtracted from the sample, the phytate content was determined from standard solutions (0, 4.5, 9, 18, 27 and 36 μg/mL) calibration line ($Y = -0.0145X + 0.7128, R^2 = 0.992$) prepared from sodium phytate (analytical grade) in 0.2 N HCl. The result was expressed as mg/100g.

3. Data analysis

The statistical processing of the data was performed using R 4.1.1 software. Proximate composition and sensory analysis data were analyzed with ANOVA. Means were subject to multiple comparisons using Tukey's HSD test at 5% level of significance. Where data normality and equal variance assumptions were not met, the non-parametric Kruskal-Wallis tests were used. The analyses were done using transformed data, where necessary, but raw data were used for ease of explanation.

4. Results and Discussion

4.1. The effect of teff and lupine addition on dough rheological properties

As indicated in Table 1 and Figure 1 the addition of lupine and teff into white flour affected the dough rheological characteristics. The rheological properties obtained from Farinograph of teff and blends are presented in Table 1.

Viscosity (Fu): The white bread had lower viscosity (481 Fu) compared to $WTSL_3$ bread (504 FU). As the concentration of teff increased the viscosity of the dough was increased except for the $WTBL_3$.

Water absorption: It is the point chosen by the baking business as a mark of water to flour ratio in bread dough. The WAC plays a major role in the functionality of dough especially with related to dough consistency. It is important to determine taste, texture, and dough performance during proofing and baking (Mubanga et al., 2020a). The water absorption of the white dough (51.9 ml/100g) was lower than composite dough ranged from 60.50 ml/100g to 100.4 ml/100g. The lower incorporation of teff with bitter lupine had lower water absorption whereas, it revealed vice versa for the sweet lupine. The water absorption of flours blended with white teff increased, which indicated higher water absorption capacity of white teff flour compared to red teff. This is in line with (Doxastakis et al., 2002; Mohamed et al., 2006) which indicated that dough made from composite flour absorbed more water than that made from wheat flour alone. The absorption of more water during mixing is a typical characteristic of composite starches (Doxastakis et al., 2002) and fibers. The water absorption capacity of the composite bread samples increased with the concentration of teff. This is ascribed due to the small starch granule size nature with increased surface area (Geremew Bultosa, 2007). The effect of added lupine and teff matrices on the flour water absorption (WA) is shown in Fig. 1 where the added ingredients increased the WA. The highest WA value was due to the presence of more concentration of fiber and protein in teff (Abebe & Ronda, 2014a).

Dough development time (DDT) is the time from the first addition of water to the time the dough reaches the point of greatest torque. During this phase of mixing, the water hydrates the flour components and the dough is developed well. The development time of the control dough ($P<0.05$) was shorter than red teff substituted bread (1.2 vs 4.6 min). The development time of white bread have lower development time compared to the composite bread. This showed that the composite flours with different water absorption had different dough development time as recorded on a kymograph chart. Therefore, the level of substitution had affected the arrival and development time of the composite flours. The development time for the composite bread prepared from white teff is the lowest compared to others, which was 0.8 min. This might be due to the low gluten protein contents of the composite flours and higher amount of bran particles in which may interfere in the immediate development of gluten and hydration of endosperm (Mubanga et al., 2020b). The composite bread prepared with sweet lupine (0.8 and 1 min) had lower dough development time compared to other composite breads (4.6 and 3.4 min). It is noticeable from the kymograph profile that the white flour peaked to 500 BU in less than two min, while the composite breads needed around 3-4 min. The presence of higher protein (Mohamed et al., 2006) and fibrous food matrices in the blend delayed gluten formation, thus increasing the mixing time.

Dough stability time (DST)

Stability time is the spot between arrival time and departure time, which indicates the strength of flour (Mubanga et al., 2020b). Similarly, as cited by Mohammed et al., (2009) from (Schiller, 1984) DST indicated how much tolerance the flour has to over or under mixing. The composite bread dough stability during mixing was twice compared to the control bread. However, this is against Hallén et al (2004) in which cow pea flour protein was substituted with wheat flour. Red teff substituted bread had higher dough stability time ($P < 0.05$) compared to the white teff substituted bread. The stability time of the composite dough was longer (1.4 to 3.8 min) than that of the control except for $RTSL_1$ which was less than the control (1.1 min). The dough stability time values of the blends were ranged from 1.1 to 3.8 min. These trends didn't agree with the reports of Bakare et al., (2015).

Degree of dough softening (DS)

The degree of softening for white bread (81 Fu) was lower compared to the $RTSL_3$ bread (251 FU). Degree of dough softening (DS) has increased as the concentrations of teff is increased ($P < 0.05$). However, the teff with bitter lupine had an inverse relationship. The DS of composite bread prepared from red teff is lower compared to the white one.

Table 1. The farinograph properties of the white bread and composite breads.

Farinograph properties	Bread blending ratio								
	B	$RTSL_1$	$RTSL_2$	$WTSL_1$	$WTSL_2$	$RTBL_1$	$RTBL_2$	$WTBL_1$	$WTBL_2$
Arrival time	0.7a	0.8a	2.5c	0.5a	0.7a	2.0b	1.8b	1.2a	0.8a
Water volume (ml) (Corrected to 500 FU)	150.8a	140.7a	173.1b	272.7b	276.8c	273.4c	176.2b	277.2c	272.2c
Departure time	1.8a	4.3b	4.7b	3.9a	5.3c	2.9a	4.4b	4.0a	4.2a
Breakdown time	1.9a	4.7c	4.9c	4.1b	5.5c	2.9a	4.6c	4.1b	4.2a
Mixing Tolerance Index (MTI)	420b	430b	405b	450c	430b	460c	380a	410b	400a
Viscosity (Fu)	481a	497b	489a	494b	504b	466a	502b	499b	484a
Water absorption (ml/100g) (Corrected to 14%)	51.9a	60.5a	61.2a	97.4b	100.4b	99.5b	63.6a	101b	61.6a
Development time (min)	1.2a	4.6c	1.2a	3.4b	0.8a	5c	3b	2.6a	3.4b
Dough stability (min)	1.3a	1.1a	---	2.6b	1.3a	3.8b	3.6c	1.4a	3.2b
Degree of softening (FU)	81a	113b	251c	133b	180b	66a	165b	235c	109a

**B= Control bread,* $RTSL_1$=*Red teff-sweet lupin,* $RTSL_2$= *Red teff-sweet lupin,* $WTSL_1$= *White teff-sweet lupin,* $WTSL_2$= *White teff-sweet lupin,* $RTBL_1$= *Red teff bitter lupin,* $RTBL_2$= *Red teff bitter lupin,* $WTBL_1$= *White teff bitter lupin,* $WTBL_2$= *White teff bitter lupin*

*** The subscripts 1 and 2 shows the concentration of teff which is 10 and 30% respectively*

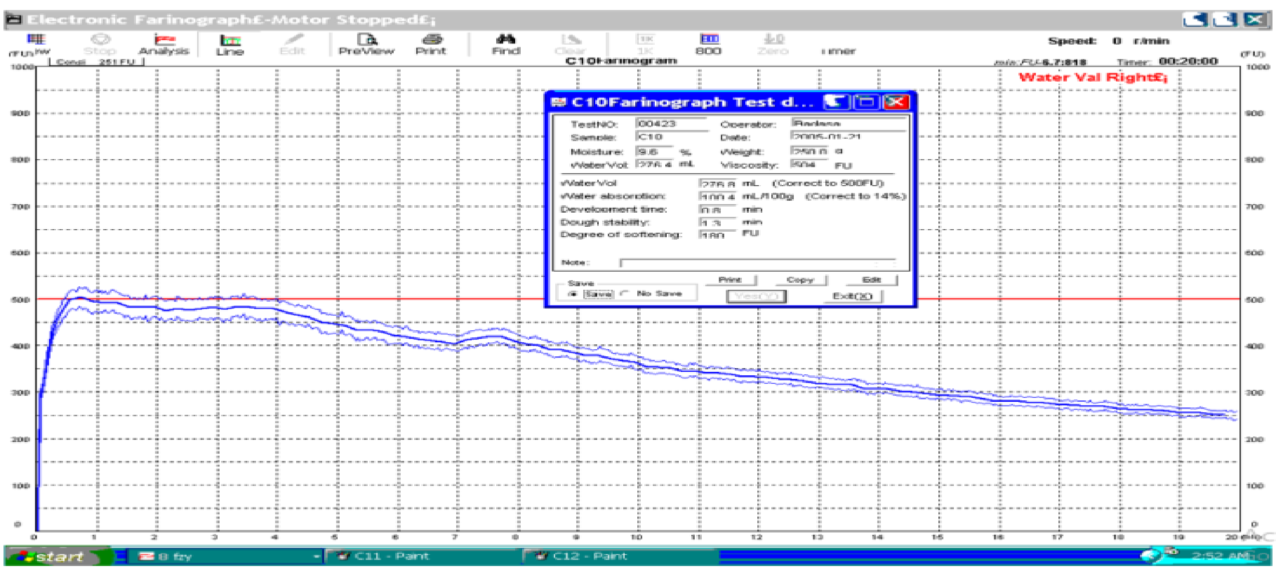
Values with different letters across the rows are not statistically significant (p>0.05)

Water volume (wv)

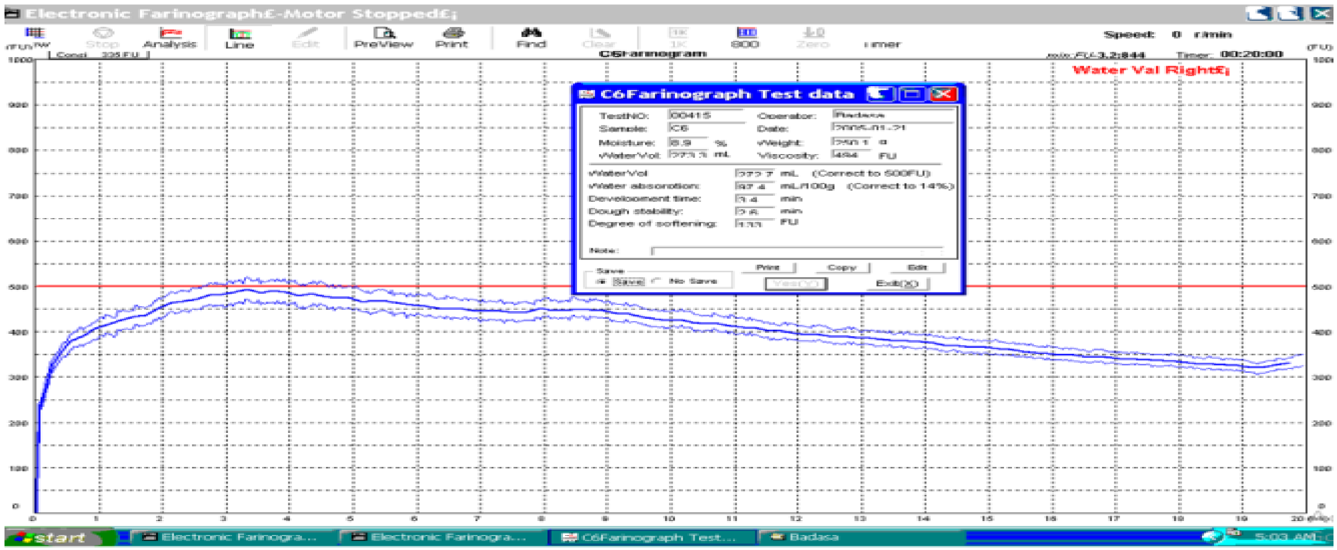
The water volume required for mixing of white bread was lower compared to the composite bread. White teff substituted flour had higher water volume consumption compared to the red teff at the same level of concentration.

Departure time (DT) was the time required for the curve to drop below the 500 BU consistency line. All the blends of lupine and teff had longer departure times compared to the white flour. Breakdown Time also showed an index of the relative strength of flours. The breakdown time values ranged from 2.9 to 4.9 min. The white flour (1.9 min) showed less resilience than the blends of composite flour.

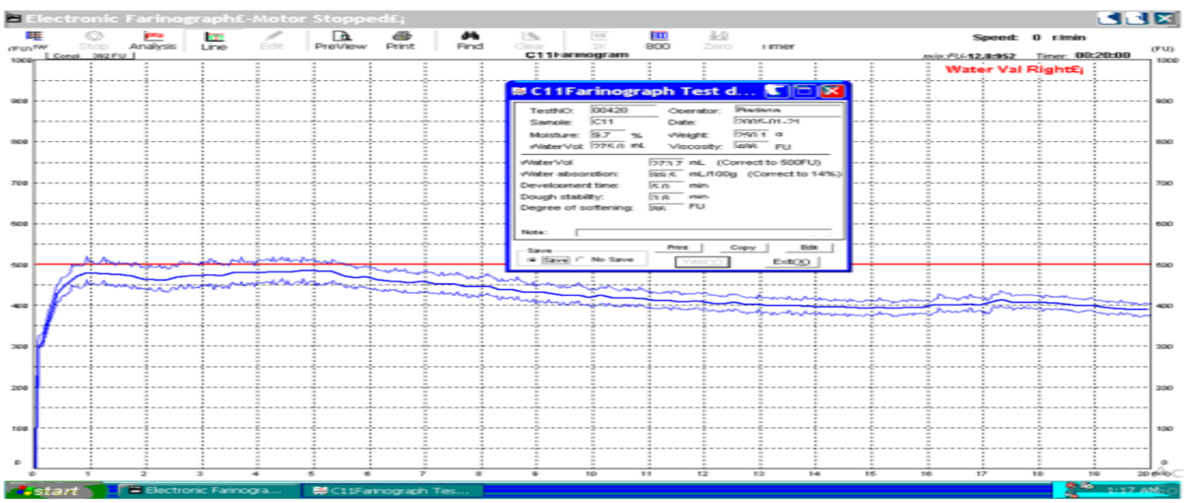
The mixing tolerance index (MTI) is the difference in Brabender Units between the top of the curve and the top of the curve measured 5 min after the peak is reached. Higher MTI values indicate weaker flour, i.e. flour with inferior bread-baking quality (Mohamed et al., 2006). Flours having higher MTI values are the ones with inferior bread-baking quality (Hallén et al., 2004). The mixing tolerance index (Fig. 1 and Table 1) of white teff composite dough was higher to red teff irrespective of its blending ratio. Samples incorporated with more both red and white teff have lower MTI values (Table. 1). The RTSL$_1$ sample revealed the lowest stability among all samples and the modest MTI value. Therefore, it would be the sample with the lowest baking quality. From the profiles described, the RTBL$_3$ sample would have the best baking quality of all because of its lowest MTI value. As the level of flour blends in composite dough increased from 10 to 30%, water absorption and mixing tolerance index were not revealed any significant change, yet dough stability differs.



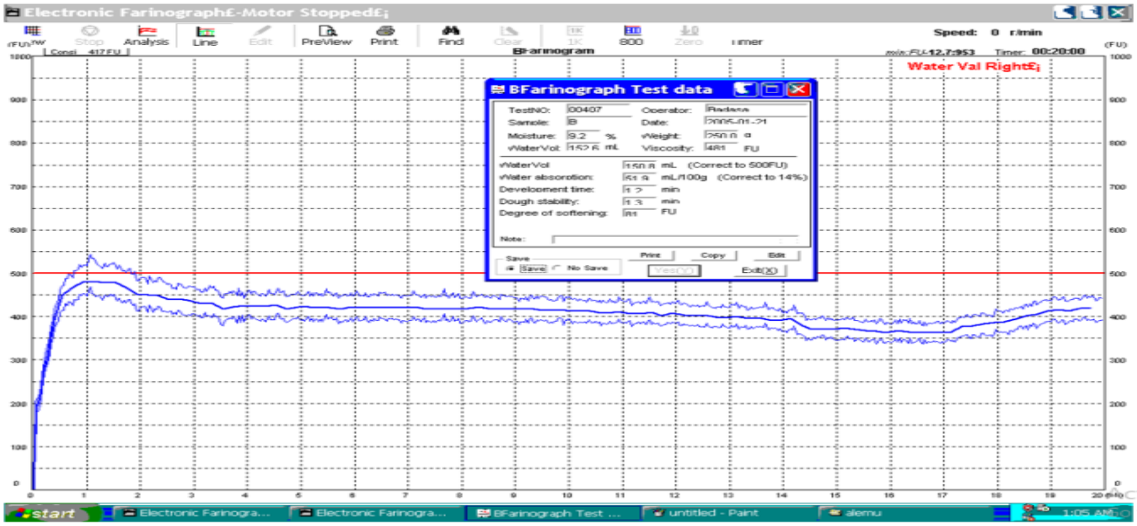
a. Control (wheat flour) kymograph



b. Wheat flour, red teff and sweet lupine blend ($RTSL_1$) kymograph



c. Wheat flour, White teff and bitter lupine blend ($WTBL_3$) kymograph



d. Wheat flour, White teff and sweet lupine blend ($WTSL_3$) kymograph

Fig. 1: Selected figures of the kymograph of the B, $RTSL_1$, $WTBL_3$, and $WTSL_3$ respectively.

4.2. Composite bread development with teff and lupine

The composite bread with higher nutritional value and lower sensorial acceptability is developed from wheat flour, teff and lupine as shown in Fig.2. It also revealed that the higher incorporation of teff and lupine have an impact on the sensorial quality of breads, and its overall acceptability. The results showed that wheat can be substituted with lupine (bitter and sweet) and teff (red and white) in bread making up to a level of 10%, without negatively affecting bread quality. Higher rate of teff inclusion generally led to lower sensorial acceptance.

a. **b.**

c.

d.

Fig 2. a) The composite flour prepared from all ingredients (before mixing), b) control bread prepared from the wheat flour, c) composite bread from red teff, bitter- lupine and wheat, d) composite bread prepared from white teff, sweet-lupine and wheat flour.

4.2.1. Proximate Composition

Table 2 shows the chemical composition of white bread (WB), Red teff sweet lupine (RTSL), Red teff bitter lupine (RTBL), White teff sweet lupine (WTSL and White teff bitter lupine (WTBL), that were used to prepare a nutritious and fibrous bread. Composite breads have similar moisture content compared to the control bread (P>0.05). $WTSL_1$ had the highest moisture content (4.56 g/100g on wet mass basis) compared to $WTBL_1$ (3.15 g/100g). The moisture contents of the composite breads were found to be in the range of 3.15 – 4.56g/100g which was lower than the report of Guillamón et al., (2010) varying between 11.7 and 12.1 g/100 g. The differences might occur due to the bread samples were dried for further storage before conducting the proximate analysis.

Table 2. The proximate composition of white bread (WB), Red teff sweet lupine (RTSL), Red teff bitter lupine (RTBL), White teff sweet lupine (WTSL and White teff bitter lupine (WTBL) prepared by straight dough technique.

Samples	Protein (g/100g)	Fat (g/100g)	Moisture content (g/100g)	Ash (g/100g)	Crude Fibre (g/100g)	Total Carbohydrate (g/100g)
B	9.70±0.45a	3.55±0.19a	4.01±0.12a	3.88±0.18a	1.01±0.12a	77.90±0.86a
$RTSL_1$	13.49±0.72b	5.63±0.21a	3.92±0.23a	11.60±0.14b	1.92±0.17a	62.41±0.68b
$RTSL_2$	12.85±0.66c	4.89±0.07a	4.12±0.26a	12.81±0.12c	2.52±0.24a	62.17±0.44b
$WTSL_1$	11.83±0.9a	5.74±0.61a	4.21±0.25a	5.91±0.27a	3.21±0.29b	69.07±0.08c
$WTSL_2$	12.27±0.46a	6.24±0.23b	4.56±0.21a	3.52±0.19a	2.96±0.31c	67.83±0.54b
$RTBL_1$	11.82±0.59a	7.52±0.21c	4.12±0.03a	8.70±0.16d	2.12±0.23a	65.69±0.01b
$RTBL_2$	11.82±0.29a	6.77±0.53d	4.41±0.51a	5.04±0.19a	2.40±0.21a	68.53±0.31b
$WTBL_1$	12.51±0.15d	6.52±0.31e	4.27±0.15a	2.22±0.21a	2.27±0.25a	72.18±0.65d
$WTBL_2$	12.88±0.31e	5.80±0.11a	3.15±0.16a	6.02±0.41a	3.15±0.26d	68.98±0.59b

**For all bread types Nitrogen factor of 6.25 were used. **All test parameters were reported on dry basis.*
** Mean values having different letters in each column differ significantly ($p\leq0.05$)*

The ash content of the composite breads were significantly higher ($p<0.05$) compared to refined wheat bread. The ash content of the white bread 3.8808 g/100g had a significance difference to $RTSL_1$ 11.6084 g/100g, $RTSL_1$ 12.8192 g/100g, and $WTBL_1$ 8.7076 g/100g. The ash content of the red teff supplemented bread was increased with its incremental level compared to the white teff bread, implying that red teff mainly impacted by inorganic nutrients (Mohammed et al., 2009). These are influenced by the agronomic practices (threshing on flour) used (Geremew Bultosa, 2007), since the samples are purchased from farmers and have unseen surface contaminants. It might be also from teff grain's high bran composition, which indicates milling performance by indirectly enlightening the amounts of bran removed (G. Bultosa & Taylor, 2004). The ash content of the $RTBL_1$ bread was lowest compared to other composite breads.

White bread had the highest total carbohydrate content, while 30% red teff substituted bread had the lowest content. This indicated that the concentration of teff had an impact on the total carbohydrates contents of the composite bread ($P<0.05$). The total carbohydrate content ranged from 62.17g/100g - 77.902g/100g in which as the amount of whole teff incorporation increased the total carbohydrates decreased. It is supported by the report of Bultosa, (2007) on the amylose content of 13 teff varieties ranged from 20 to 26 %. However, there was no effect on type of teff used for the bread making.

The crude fat content of the composite bread was higher compared to the white bread. However, the concentration and variety of the teff and lupin had no difference between tested samples. The crude fat content of teff is higher than that of wheat (Baye, 2014)

As indicated in Table 2 the crude protein content of the composite breads were higher than the refined white bread ($P<0.05$). However, the composite breads have similar protein content among each other. The concentration and variety of teff used had no difference, which was similar to (Geremew Bultosa, 2007)**.** However, it was contrary to the result of (Abebe & Ronda, 2014b) who found white cultivars had higher (10.5%) protein content and (Callejo et al., 2016a) who found higher protein content in red teff. The protein content found in this work was higher compared to (Abebe & Ronda, 2014b; Callejo et al., 2016b). This might be due to the higher protein contents of lupine (38.3 g/100 g) used (Guillamón et al., 2010b). The differences of results between studies might be due to agronomic practices and ecological situation. As expected, our bread composites contained higher protein than the control, in which the lupine contributed its share in the blends. However, it was lower than the report of Guillamón et al., (2010) 13.6-13.9 g/100 g on wheat flour supplemented with lupine flour. At the highest concentration of both red and white teff the crude fiber had a significant difference ($P<0.05$) on the bread fiber composition. The concentration of teff has a significant impact on the fiber content of the composite bread in which 30% teff substituted bread has the highest crude fiber (3.1516 g/100g) content. This implies that whole teff had higher fiber content than both lupin and wheat. This result was also supported by other findings in which crude fiber, total and soluble dietary fiber content of teff is several folds higher than that found in wheat, sorghum, rice, and maize (Baye, 2014; Geremew Bultosa, 2007).

Crude fiber contains substances that are resistant to digestions. Since teff is consumed as a whole grain with bran and cell wall, it might be the major contributor for the dietary fiber. In addition to this starch digestion rate index and lower rapidly digestive starch were achieved from teff substituted breads.

4.2.1 Mineral contents of the white bread and composite bread

From Table 4 both white and composite bread had high concentration of macro elements (Mg, Ca, Na and K) and microelements (Zn and Fe). The other minerals Cu, Co, Pb and Cr are mainly found in trace amounts.

Table 3. Detailed description of the mineral fraction of the different bread types, expressed as mg/100 g, including the microelements Fe, Cu, Zn and Mn, and the macro elements Ca, Mg, Na, K, detected through Induced Plasma Couple spectrophotometer and flame photometry.

Minerals	bread varieties								
	B	RTSL$_1$	RTSL$_2$	WTSL$_1$	WTSL$_2$	RTBL$_1$	RTBL$_2$	WTBL$_1$	WTBL$_2$
Mg	2.860±0.029a	4.543 ±0.132b	3.048 ± 0.065b	2.508 ± 0.071a	4.171 ± 0.105b	4.146 ± 0.042b	5.266 ±0.107c	2.573 ± 0.063a	2.040 ± 0.033a
Ca	7.473±0.053a	7.925 ±0.102a	1.570 ± 0.026b	2.001 ± 0.024b	2.936 ± 0.068b	9.564 ± 0.025c	8.377 ±0.018c	3.372 ± 0.066b	1.459 ± 0.018b
Cr	0.043±0.000a	0.056 ±0.000a	0.043 ± 0.000a	0.047 ± 0.000a	0.052 ± 0.000a	0.059 ± 0.000a	0.039 ±0.000a	0.054 ±0.000a	0.049 ± 0.000a
Mn	0.117±0.000a	0.212 ±0.000b	0.132 ± 0.001a	0.124 ± 0.001a	0.144 ± 0.001a	0.825 ± 0.008c	0.723 ±0.004c	0.386 ± 0.004d	0.297 ± 0.004b
Fe	1.194±0.020a	1.109 ±0.023a	0.782 ± 0.002b	0.620 ± 0.007b	1.022 ± 0.007a	2.993 ± 0.035c	3.404 ±0.040c	0.976 ± 0.003a	0.744 ± 0.002d
Co	0.025±0.000a	0.057 ±0.000b	0.062 ± 0.000b	0.061 ± 0.000b	0.062 ± 0.000b	0.048 ± 0.000c	0.029 ±0.000a	0.054 ± 0.0004b	0.060 ± 0.002b
Cu	0.071±0.001a	0.070 ±0.001a	0.055 ± 0.001b	0.053 ± 0.001b	0.063 ± 0.000b	0.094 ± 0.000c	0.073 ±0.000a	0.058 ± 0.001b	0.060 ± 0.001b
Zn	0.516±0.001a	0.557 ±0.008a	0.934 ± 0.009b	0.451 ± 0.007a	0.473 ± 0.019a	0.566 ± 0.002a	0.487 ±0.005a	0.474 ± 0.006a	0.428 ±0.005a
Pb	0.127±0.002a	0.310 ±0.010b	0.276 ± 0.002b	0.294 ± 0.002b	0.330 ± 0.005b	0.277 ± 0.002b	0.204 ±0.002b	0.279 ± 0.001b	0.272 ± 0.004b
Na	29.528±6.125a	35.108±3.25a	91.93±7.25b	40.83±2.63a	45.52±2.56a	37.68±2.35a	40.736±2.36a	42.79±4.23a	39.450±3.95a
K	300.08±1.254a	355.81±5.32a	924.113±5.26b	4130.82±4.36a	460.16±5.63a	381.59±6.32a	412.12±6.43a	432.49±5.67a	399.283±6.25a

The mean concentration mineral composition of white bread and composite bread prepared from lupine, teff and wheat flour in (mg/100gm) measured using AAS.

**B= Control bread,* RTSL$_1$=*Red teff-sweet lupine,* RTSL$_2$= *Red teff-sweet lupine,* WTSL$_1$= *White teff-sweet lupine,* WTSL$_2$= *White teff-sweet lupine,* RTBL$_1$= *Red teff bitter lupine,* RTBL$_2$= *Red teff bitter lupine,* WTBL$_1$= *White teff bitter lupine,* WTBL$_3$= *White teff bitter lupine*

*** The subscripts 1 and 2 shows the concentration of teff which is 10 and 30% respectively*

** Mean values having different letters in each row differ significantly ($p \leq 0.05$)*

As expected, the most prevalent and abundant minerals were the macro elements like, magnesium, calcium, sodium and potassium. The least quantified minerals were cobalt and chromium. The red teff substituted bread had a higher iron and calcium content compared to the white teff. Red teff substituted bread had a significant effect on retaining the essential minerals like calcium and iron compare to other bread samples (Abebe et al., 2007).

Red teff and bitter lupine substituted bread have higher content of iron compared to the white teff and sweet lupine substituted bread. The Lead content of white bread is lower compared to the composite one. The composite bread with red teff and sweet lupine has higher contents of Zinc compared to other type of bread composition (Callejo et al., 2016b). Red teff with bitter lupin have higher content of Manganese compared to other types of bread contents. Breads that contain 10% red teff flour were found containing 3-5 times Fe, twice Zn as compared to the white wheat bread.

The most commonly found macroelements in white bread are Magnesium, Calcium, Potassium and Sodium (Brier et al., 2015; Carson & Edwards, 2009) as cited in (Bakare et al., 2016). Lupine flours have abundant sources of minerals such as phosphorus, iron, zinc and calcium (Emire, 2015) whereas, Teff flours have a higher Fe, Ca, Zn and Cu content compared to other cereals (Hager et al., 2013). Substitution of 10 to 30% teff and 10% lupine flour into the bread resulted significantly higher amounts of micro-elements compared to the white wheat, these results were in comparable with (Abebe & Ronda, 2014b; Alaunyte et al., 2012). These elements were Mg, Ca, Na, K, Mg, Mn, Fe, Cu and Co regardless of the type of combination. However, there was no significant effect on the concentration of Cr.

4.3. The anti-nutritional composition

Table 4 shows the levels of tannin and phytate composition found in white and composite bread. The data shows that the phytate content was in the ranges of 13.326 ± 1.67 to 48.063 ± 1.73 mg/100g on dry matter basis, whereas the tannin was below detection level for all bread types. $WTSL_2$ and $RTBL_2$ have the highest concentration of phytic acid compared to other breads. However, the levels of the toxic substances were not high enough to cause a public issue as about 80 mg/g diet is detrimental to health. However, the toxicants might have the capacity to reduce the nutrient bioavailability. The $WTSL_2$ has higher concentration of phytate compared to other composite breads. This shows that complimenting white teff at higher concentration have a significant impact on the concentration of phytate. Still, the data shows the phytate composition was much lower compared to injera from white teff (102mg/100gm) and injera from red teff (117 mg/100gm) (Abebe et al., 2007). This finding is in parallel with Abebe et al., (2007) in which absorption of iron from fermented tef injera will not be hindered by phytate, unless the foods are consumed together with others.

Table 4. Tannin and phytate concentrations of white bread and composite bread samples from wheat, teff, and lupin on dry matter basis (mg/100gm).

Antinutritional types	Samples								
	B	$RTSL_1$	$RTSL_2$	$WTSL_1$	$WTSL_2$	$RTBL_1$	$RTBL_2$	$WTBL_1$	$WTBL_2$
Tannin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Phytate	13.326±1.67a	21.021±2.43b	22.746±1.99b	41.878±2.29c	61.209±3.2d	17.844±2.96b	48.063±1.73c	13.537±2.48a	29.704±1.84b

**BDL=below detectable level, The different letter values show the significance difference across the row.*

4.4. Sensorial attributes

Table 5. The hedonic sensory mean scores of the white bread and composite bread samples prepared from lupin (bitter and sweet) and teff (white and red).

Sensory attributes	samples								
	B	$RTSL_1$	$RTSL_2$	$WTSL_1$	$WTSL_2$	$RTBL_1$	$RTBL_2$	$WTBL_1$	$WTBL_2$
Texture	4.222a	3.5a	3.916b	4.230a	3.554c	4.083a	3.083d	4.063a	3.529e
Flavor	4.234a	3.875a	3.583a	4.083a	4.835a	3.916a	3.933a	3.753a	3.658a
Aroma	4.125a	3.7714a	3.966a	4.235a	3.7326a	3.932a	3.833a	3.657a	3.827a
Crumb	4.375a	3.942b	3.757b	3.753b	3.245c	4.333a	3.235c	3.666b	2.562c
Crust	4.625a	3.875b	3.416c	4.254a	3.825b	4.133b	2.833c	3.433b	3.114c
Color	4.375a	4.324a	3.254b	4.333a	4.023a	4.257b	3.254b	2.833c	2.556c
Taste	4.125a	4.375a	3.5833b	4.325a	3.556c	4.333a	3.416d	3.933a	3.265e
Overall Acceptability	4.571a	3.8714a	3.417b	4.253a	4.258a	4.253a	3.189c	3.835a	3.529c

** Mean values having different letters in each row differ significantly ($p \leq 0.05$)*

The sensory evaluation scores of the white bread and composite breads were presented in Fig. 2.and table 5. The sensorial evaluation scores showed that the composite flour breads obtained lower scores compared to the white bread in terms of taste, aroma and overall acceptability. Overall acceptability profiles were evaluated by a semi-trained panels. $RTSL_1$and $RTBL_1$ breads were liked moderately, while $RTSL_2$ and $RTBL_2$ samples had lower liking scores.

According to the descriptive sensory evaluation, the bread made with wheat flour had less flavour than those made from a mixture of wheat flour, teff and lupin. The inclusion of lupin produces a slight flavour deviation (Guillamón et al., 2010b). Incorporation of tef flour from 30% imparted discrete negative effects in terms of taste, and overall acceptability. The darker color in red teff substituted bread was from the original color of the sample which is associated with a healthier product, and necessarily desirable in bread. Similarly, Ben-Fayed & Stojceska (2008) as cited by (Coleman et al., 2013) found that red teff bread had a darker color feature while its crumb were yellowish which might be the contribution of lupine The finding also confirmed that breads containing 0–10% teff flour were better accepted than breads containing 20– 30% teff flour (Coleman et al., 2013). The same investigators found that breads containing 0–10% teff flour were better acknowledged than breads containing 20–30% teff flour. From baking experiments (Table 3) breads prepared with lupin and teff flour substitutes, dark crust colour for the red teff, yellowish crumb colour for the bitter lupin types were observed. In the case of color and overall acceptability, white teff substituted bread were meet the panelist perception.

5. Conclusions

The study attempted to identify bread formulations using cereal-pulse composite bread from gluten free and underutilized crops (lupine). The rheological properties like water absorption, dough stability and development time of 10% red teff and lupin had a positive impact compared to other bread flours. Bread substituted with lupin and teff have diverse nutritional properties that were evidenced by their higher contribution to protein, fiber, Fe and Zn content. Addition of 30% teff flour to wheat flour changed some bread making properties of the blend and sensorial features like taste, color and overall acceptability. Red teff substituted bread contributed to dark crumb color and cracked crust structure. Sensory evaluation of the composite breads with higher teff composition had lower overall acceptability scores. However, the composite bread formulations with higher teff concentration had higher protein content, fat and crude fiber yield. At the last, we can wrap up that the incorporation of 10% lupine and teff to wheat flour is practical, and that the nutritional attributes are enhanced. The sensorial attributes were lower as the concentration of teff was increased to 30%. The experimental results indicated that it is possible to produce healthy and nutritionally rich in proteins, fat and fiber, by substituting wheat flour with teff and lupin at 10% without significantly affecting its rheological, chemical and sensorial attributes. The use of lupin and teff flour in mixing with wheat flours may produce acceptable baked products with the advantages of teff's fibrous, and lupin's proteinaceous flour.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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