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Functional Properties and Injera Making Qualities of Tef (*Eragrostis tef (Zucc.)*) Supplemented with Lupine (*Lupinus spp.*)

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Abstract: This study was conducted to investigate the effect of lupine flour on functional properties and injera making qualities in respect to injera eyes and color of tef-lupine blended injera. There are limited studies on formulating of injera from composite flour with legumes (lupine). The effect of two factors two lupine varieties (Australian sweet lupine and dibettered lupine seed) and blending ratios (0, 2.5, 5, 7.5, 10, 15, 17.5 and 20). Maximum and minimum levels of independent variables were first investigated by doing a preliminary analysis and founded that tef (80-100%) and lupines (0-20%). Response surface methodology was applied to find the formulations and predictive model. Oil absorption capacity and swelling power properties of composite flour decrease as blending ratio of lupines increased and water absorption and foaming capacity increased as blending ratio of lupines increases for both varieties. The L* value and number of eye by injera eye software were 72.77 to 79.84 and 14220.43 to 18929.33, respectively. The L values of blended injera increased as lupine proportion were increased, but the number of injeras eyes decreased. Therefore, the findings of this study were found to be very significant and it is believed that this study could give insights for use of lupine flour mixed with tef flour in home and industry level for making injera.

Keywords: Dibettered Lupine Seed, Injera, Response Surface Methodology

1. Introduction

Injera is fermented, sour leavened, pancake-like, moist, chewy and elastic bread made principally from *tef* (*Eragrostis tef (Zucc.) Trotter*). But it can also make from other cereals like wheat, barley, sorghum or maize or a combination of some of these cereals. It is served in restaurants in Europe, North America, and Israel and is receiving an enthusiastic acceptance [2]. Injera from tef is most preferred due to its softer texture, preferred taste, its colour, and can be rolled without cracking. However, it is more widely consumed by the economically better off urban peoples than by rural households [6, 8]. So for rural households and the urban poor, *tef* is more of a luxury while maize, wheat and rice are necessity food grains. As tef prices go up, even middle income households tend to mix *tef* flour with cheaper cereals such as sorghum maize or rice in preparing injera [10].

Tef *(Eragrostis tef (Zucc) Trotter)* is an important staple cereal crop in Ethiopia. It is cultivated as a major cereal in Ethiopia and represents 19% of the total cereal production, with the largest share area (23.42%, about 2.6 million hectares) under cereal cultivation [26]. It has similar protein content to other more common cereals like wheat, but contains no gluten. Tef amino acid composition is well-balanced and contains relatively higher concentrations of lysine than what is commonly found in other cereals.

Lupines can be divided into sweet lupines, which contain low levels of alkaloids, and bitter lupines, which contain higher levels of alkaloids. Lupine generally contains about twice the amount of proteins found in those legumes that are commonly consumed by humans. Lupine is a good source of nutrients, not only proteins but also lipids, dietary fibre, minerals, and vitamins [17]. Lupine flour has high nutritional value containing about (33-47%) protein, (20-30%) dietary fibre and (6-13%) fat contents and has low glycemic index

(GI) due to little or no starch content.

The functional properties are significantly influenced by the processing of grain flour [18]. Currently, these flours have been used as a food ingredient in the development of health food due to its functional properties for its high protein content suggested that the functionality of proteins is closely related to their physical and chemical properties, such as molecular weight, amino acid composition, processing temperature and ionic strength of the food system [7]. The functional properties such as water absorption, oil absorption and protein solubility affect the processing, texture, and appearance of the product. These are critical to the production of associated foods [20].

It is common in Ethiopia injera were prepared from tef mixed with different cereals like sorghum, barley, wheat, millet, maize, rice or wheat which has protein content of ranges from 8-15% [5], but blending of tef with lupine are not yet practiced in our country even if it have higher amount of proteins contents and minerals. Therefore, effort is needed to improve the nutrient density of tef injera by mixing with locally available and protein rich ingredient like lupine which may be one of the ways of combating protein-malnutrition problem of the country. Initiation is taken to investigate the effects of lupine complemented with tef flour on the functional properties and possibilities of improving the final product quality that is injera in accordance with number of injera eye and colour of produced injera.

2. Materials and Methods

The experimental materials included tef grain and lupine. Tef variety DZ-01-196 (magna) was collected from Deber Zeit Agricultural Research Centre and two varieties of lupine; debittered lupine seed and Australian sweet lupine were brought from Holetta Agricultural Research Centre.

2.1. Experimental Design

Mixture design was used in this study to determine the ratio of blends of tef and lupine. Maximum and minimum levels of independent variables were first investigated by doing a preliminary analysis at different proportion of lupines and it was found that a maximum of only 20% lupine will be substituted with tef. The proportion of tef from 80-100% and lupine from 0-20% were used. Each formulation had nine runs and was done in triplicate.

In building the model, a regression equation was established to describe the relationship between the response Y and variable X. A predictive model was generated for the two mixture components as follows:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2$$

Where: Y is the predicted response, β_1 and β_2 are linear coefficients, β_{12} is the interaction coefficient and X_1 and X_2 are independent variables.

2.2. Lupine Flour Preparation

The debittering process for the lupine seeds consisted of cleaning, boiling and debittering. Extraneous material and immature and damaged seeds were removed first. The cleaned seeds were boiled in water (1:3 seeds: water (w/w)) for 50 min to destroy thermo labile anti-nutritional factors and to soften the seeds hull. The boiled lupine seeds were debittered with water at room temperature (~25°C). The lupine seeds, during the debittering process, were soaked fully with debittering water and these steps were renewed subsequently in 12hrs intervals for 144 hrs. Afterwards, the whole seed was de-hulled manually and the kernel was dried at 105°C for 3hrs in oven [19]. Prior to the chemical analyses, the seeds were dried and milled into a fine powder by using disk attrition mill. Then sieved with sieve size of 750 μm and packed in polyethylene bags and store at 4°C until required for analysis [21].

The Australian sweet lupine flour were prepared by soaking in boiled water for only 5 minutes and dried in oven 105°C then the dried sample were undergo de-hulling process simply by using local mill and then milled by disk attrition mill.

2.3. Preparation of Tef Flours

Tef grain were manually cleaned and milled by disk attrition mill to fineness (750 μm) level. The flour was kept in air tight sealed plastic bag at room temperature [1] for the duration of the analysis.

2.4. Preparation of Composite Flour

The flour composite blends contained tef and lupine were prepared using a formulation which were generated by mixture design. The dry material individually were blended uniformly to homogenize and then packed in tightly closed clean plastic container that kept at room temperature (25 ± 2°C) until used.
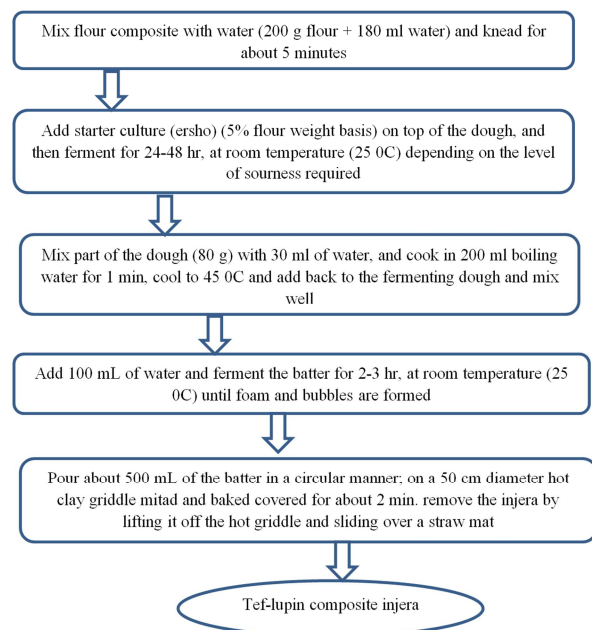
Mix flour composite with water (200 g flour + 180 ml water) and knead for about 5 minutes

Add starter culture (ersho) (5% flour weight basis) on top of the dough, and then ferment for 24-48 hr, at room temperature (25 0C) depending on the level of sourness required

Mix part of the dough (80 g) with 30 ml of water, and cook in 200 ml boiling water for 1 min, cool to 45 0C and add back to the fermenting dough and mix well

Add 100 mL of water and ferment the batter for 2-3 hr, at room temperature (25 0C) until foam and bubbles are formed

Pour about 500 mL of the batter in a circular manner; on a 50 cm diameter hot clay griddle mitad and baked covered for about 2 min. remove the injera by lifting it off the hot griddle and sliding over a straw mat

Tef-lupin composite injera

Figure 1. Injera making flowchart [25].

2.5. Preparation of Fermented Dough and Baking of Injera

All ingredients (composite flour + water + ersho (starter culture- from previous batch)) were added accurately and the fermentations of the dough were conducted by following the traditional tef dough preparation procedure as presented by Yoseph Legesse [25]. Injera of the 23 (three control samples (i.e. 100%) for both varieties) formulations were baked at Debre Zeit food science and nutrition laboratory.

2.6. Functional Properties

The water absorption capacity of flour sample was measured according to the centrifugation method of Yu *et al.* (2007). The swelling power of flour was determined according to (AACC, 2000) method. The foaming capacity of the samples was determined using the method described by (Yusuf *et al.*, 2007). Oil absorption capacity of the flour was determined by the method of Adeleke *et al.* (2010).

2.7. Instrumental Measurements of Number of Eyes and Colours of Blended Injeras

Two parallel fluorescent lamps were used to illuminate the sample. The lamps were situated at 10 cm above the sample at the angle of 45° of the sample plane to give a uniform light intensity. Finally the images of injera were captured using camera with resolution of 720 x 1280 pixel was located vertically at a distance of 45 cm from the injera sample. Samples were carried out on the basis of CIE L* ab values [25].

2.8. Statistical Analysis

The statistical analyses of the data were conducted using SAS statistical software package. Comparisons between the varieties were done using one ways analysis of variance (ANOVA) with a probability $P< 0.05$. Design- Expert ®, version 7.0, Stat-Ease, (SaMeep104 Inc., Minneapolis, MN USA) was used to generate experimental test trials and to perform regression equations (Okpala and Okoli, 2013).

3. Result and Discussion

3.1. Alkaloid Content of Lupines

The alkaloid content of two raw lupine varieties was 1.36 mg/100g and 0.75 mg/100g for DLSF (Debittered lupine seed flour) and ASLF (Australian sweet lupine flour), respectively. The alkaloid contents ranged from 6 mg/100g to 7 mg/100g reported by Cerletti and Venturin [9] which was higher than this finding. Both lupine varieties had alkaloid content below the maximum level permitted for lupines for human food use of 20 mg/100g as defined by the Australian [12] and Great Britain national food standards [16].

3.2. Functional Properties of Tef and Lupine Flours

There were significant (P<0.05) differences among the raw materials (tef, ASLF and DLSF). Moreover, even lupine protein derivate with the same protein content may show different functional properties because, for instance, the ratio of the different globulin fractions differs among lupine varieties [9]. The result of WAC for tef was found in harmony with previous studies conducted by Alabi *et al.*, [4] reports 0.99 g/g. The WAC result of lupine flour was found to be within range 2.05 and 2.65 g/g reported by Tizazu and Shemilse [23] for DLSF.

The higher water absorption capacity of the lupine flour could be attributed to the presence of greater amounts of hydrophilic constituents such as proteins. There are economic benefits in adding water to a product which is priced according to its weight, and a positive impact on the shelf life, hence food manufacturers prefer to incorporate food ingredients with high water absorption capacities in their formulations.

The result swelling power of tef was found to be within range between 7.70% and 12.50% which were reported by Kaushal and Sharma [14]. They indicated swelling power is a measure of hydration capacity of starch, because the determination is a weight measure of swollen starch granules and their occluded water. While the swelling power was practically the same for the two lupines varieties with no significant difference, they were significantly lower than that of tef.

Some food proteins are capable of forming good foams, and their capacity to form and stabilize foams depends on the type of protein, degree of denaturation, pH, temperature and whipping methods. The result of DLSF in this study related with that of Alabi et al. [4] which ranged between 65.00 and 68.00%. Better foaming capacity implies greater incorporation of air bubbles.

The results of oil absorption content of tef were found to be within range between 1.00 and 1.55 g/g reported by Ajatta *et al.*, 2016 [3]. Whereas the result obtained for DLSF were similar with [23] reports which ranges between 1.24 and 1.37 g/g. This makes the powder to have potential functional uses in foods such as bakery products. The oil absorption capacity also makes the flour suitable in facilitating enhancement in flavour and mouth feel when used in food preparation. In addition, fat increases the leavening power of the baking powder in the batter and improves the texture of the baked product.

Table 1. Functional properties of raw material flours.

Raw materials	WAC (g/g)	OAC (g/g)	SP (%)	FC (%)
tef	1.03±0.02b	1.47±0.01b	8.24±0.01a	1.89±0.02c
DLSF	2.42±0.01a	1.84±0.05a	1.30±0.01b	75.77±0.54a
ASLF	2.48±0.01a	1.79±0.04a	1.31±0.01b	61.73±0.27b
CV (%)	1.16	2.51	3.19	2.67

Values are in Mean ± SD on dry weight basis. Means within a column with the different letter are significantly different at P < 0.05. DLSF=debittered lupine seed flour, ASLF=Australian sweet lupine flour, WAC=Water holding capacities, OAC=Oil absorption capacity, SP=Swelling power, FC=foaming capacity, CV=Coefficient of variation, LSD=least Significant difference

3.3. The Effects of Lupine Varieties and Blending Ratios on Functional Properties of Tef-lupine Composite Flour

It was revealed from the results that the water absorption capacity increased slightly as the percentage of lupine flour increased. This is maybe due to the hydrophilic nature of lupine proteins [16].

The higher foaming capacity (15.77%) was observed in composite flour which has 20% DLSF and followed by 20% of ASL (12.71%). The composite flour with 2.5% blending proportion ASLF had the lowest foaming capacity (2.50%). The ability of the flours to form foam depends on the presence of the flexible protein molecules, which may decrease the surface tension of water [22]. Protein in the dispersion may cause a lowering of the surface tension at the water air interface, thus always been due to protein, which forms a continuous cohesive film around the air bubbles in the foam [12].

The swelling power of composite flour was found to be the highest (8.24%) for both lupines at 2.5% blending proportion whereas, the lowest (7.60%) swelling power was observed at 20% of both lupines. Swelling power was high for samples with highest percentage of *tef* flour for both varieties of composite flours. And this is the function of the starch granules, with heat and water starch granules absorbs the water and swells resulting in thicker consistency [14].

Table 2. *Effect of varieties and blending ratios on functional properties of tef-lupine blended flours.*

Tef (%)	DLSF (%)	WAC (g/g)	OAC (g/g)	FC (%)	SP (%)
100	0	1.00±0.01[i]	1.47±0.01[a]	1.82±0.00[n]	8.28±0.01[a]
100	0	1.01±0.01[i]	1.47±0.01[a]	1.89±0.02[n]	8.28±0.01[a]
100	0	1.00±0.01[i]	1.47±0.01[a]	1.90±0.01[n]	8.27±0.01[a]
97.5	2.5	1.08±0.01[h]	1.46±0.01[ab]	3.44±0.03[l]	8.24±0.00[b]
95	5	1.18±0.01[g]	1.46±0.01[ab]	4.98±0.03[i]	8.21±0.01[c]
92.5	7.5	1.22±0.01[f]	1.44±0.02[cd]	6.35±0.28[i]	8.01±0.02[d]
90	10	1.24±0.01[e]	1.44±0.08[cd]	8.28±0.06[g]	7.91±0.01[g]
90	10	1.23±0.07[ef]	1.43±0.01[de]	8.22±0.12[g]	7.93±0.10[fg]
85	15	1.33±0.01[b]	1.42±0.00[ef]	10.71±0.09[e]	7.86±0.00[ij]
82.5	17.5	1.38±0.01[a]	1.41±0.00[fg]	12.12±0.27[c]	7.85±0.01[jk]
80	20	1.39±0.01[a]	1.39±0.01[h]	15.77±0.03[a]	7.61±0.01[l]
80	20	1.39±0.01[a]	1.39±0.01[h]	15.74±0.06[a]	7.60±0.01[l]
80	20	1.38±0.00[a]	1.39±0.03[h]	15.74±0.03[a]	7.61±0.00[l]
Tef (%)	ASLF (%)				
97.5	2.5	1.03±0.01[j]	1.46±0.00[ab]	2.50±0.27[m]	8.24±0.01[b]
95	5	1.09±0.01[h]	1.45±0.01[bc]	4.43±0.28[k]	8.24±0.01[b]
92.5	7.5	1.18±0.01[g]	1.44±0.00[cd]	5.19±0.27[j]	8.03±0.01[d]
90	10	1.22±0.01[f]	1.42±0.01[ef]	7.55±0.20[h]	7.94±0.01[ef]
90	10	1.22±0.08[f]	1.42±0.01[ef]	7.59±0.22[h]	7.96±0.04[e]
85	15	1.26±0.01[d]	1.40±0.01[h]	10.19±0.27[f]	7.89±0.01[h]
82.5	17.5	1.30±0.01[c]	1.39±0.07[h]	11.35±0.28[d]	7.88±0.01[hi]
80	20	1.31±0.01[c]	1.37±0.02[i]	12.71±0.01[b]	7.62±0.01[l]
80	20	1.31±0.00[c]	1.37±0.01[i]	12.70±0.00[b]	7.60±0.02[l]
80	20	1.30±0.01[c]	1.37±0.01[i]	12.70±0.01[b]	7.61±0.01[l]
CV (%)		3.01	2.51	6.25	3.19
LSD		0.01	0.01	0.16	0.02

Values are in Mean of triplicate data ± SD on dry weight basis. BR=blending ratio, DLSF=debittered lupine seed flour. ASLF=Australian sweet lupine flour, WAC=Water absorption capacities, OAC=Oil absorption capacity, SP=Swelling power, FC=foaming capacity.

The oil absorption capacity is a prominent factor in food formulations as it improves flavour and increases the mouth feel of foods. The oil absorption capacity of composite flour up to 5% of both lupines ranged in between 1.46 g/g and 1.45 g/g without significant difference, while the lowest oil absorption was observed in 20% ASLF with 1.37 g/g. Oil absorption capacity of food component is important for various applications because it relies mainly on this capacity to physically entrap oil by a complex capillary attraction process and this property of flour leads to better flavour retention, a consistency trait and an increase in mouth-feel [15]. Low oil absorption capacity indicates the enhanced hydrophilic character of proteins in the flours. Oil absorption capacity is exhibited by the proteins in the flour, which physically bind to fat by capillary attraction. These proteins expose more non-polar amino acids to the fat and enhance hydrophobicity as a result of which flours absorb oil [22].

3.4. Effect of Varieties and Blending Ratio on Number of Eyes and Colour of Tef-lupine Injera

The interaction effect of the varieties and blending proportions on the number of eyes of injeras is represented by the data shown in Table 3. The numbers of holes of injera was significantly ($P<0.05$) affected by interaction effect. From the interactions of lupine varieties and blending ratio, the number of eyes of the blended injera up 10% of both lupines ranges from 18805.33 to 18961.21 with no significance difference among them. While, the minimum number of eyes of injeras were obtained from 20% (14220.33) ASLF variety followed by 20% (14222.67) DLSF variety

with no statistical differences between them. This is due to the protein content difference between the raw materials [13].

The colour of blended injera were significantly (P < 0.05) affected by interactions of lupine varieties and blending proportions Table 3. From the interactions of the two varieties of lupine with blending ratio, the L* values of injera show increasing trends with increasing the blending ratio of lupine for both varieties. From the blending ratio interactions effect a higher L*value was obtained between 79.84 and 76.69 from 20 and 17.5% of both lupines varieties were

blended with tef without statically difference. As the proportion of lupine increased there was also an increasing of yellowness (b) colour of the product. This effect was expected because of the more intense yellow colour of lupine flour. These results agree with those obtained by Dodok et al., 1993 [11], who observed that Lupine seeds contain high levels of carotenoids and zeaxanthin which give the cotyledon (kernel) bright yellow colour and triggered the change in the yellowish colour of bread produced from a composite flour of wheat and lupine.

Table 3. *Effect of variety and blending ratio on number of eyes and colour of tef-lupine injera.*

Tef%	DLSF%	Number of eyes	L*	a	b
100	0	18953.12±64.12[ab]	71.66±0.05[fgh]	0.45±0.21[b]	5.13±0.07[b]
100	0	19017.67±51.51[a]	71.45±0.39[fgh]	0.80±0.10[a]	5.10±0.37[b]
100	0	18956.67±58.96[ab]	71.79±1.17[fgh]	0.28±0.63[c]	5.16±2.82[b]
97.5	2.5	18929.33±63.89[ab]	72.77±1.44[efgh]	0.18±0.20[c]	7.12±0.07[b]
95	5	18832.01±41.35[ab]	74.79±0.36[cdef]	0.21±0.09[c]	7.52±1.60[ab]
92.5	7.5	18796.67±12.04[ab]	75.42±1.01[bcdef]	0.33±0.20[c]	7.70±0.62[ab]
90	10	18791.33±40.93[ab]	75.20±1.12[bcdef]	0.13±0.26[def]	8.80±0.93[ab]
90	10	18828.08±41.05[ab]	75.29±1.07[bcdef]	0.12±0.59[def]	7.78±2.23[ab]
85	85.15	16496.33±52.53[c]	76.55±0.05[bcde]	0.10±0.76[def]	9.09±0.38[ab]
82.5	17.5	15449.67±30.55[d]	76.96±0.21[bcd]	0.06±0.47[ef]	9.38±1.42[a]
80	20	14225.67±39.84[e]	77.55±0.70[abc]	0.08±0.11[def]	9.42±1.88[a]
80	20	14233.07±21.15[e]	77.69±1.58[abc]	0.09±0.68[def]	9.42±1.20[a]
80	20	14222.33±13.65[e]	77.67±2.47[abc]	0.07±0.11[def]	9.45±1.14[a]
Tef%	ASLF%				
97.5	2.5	18961.21±21.26[ab]	72.87±0.36[efgh]	0.21±0.14[c]	3.25±0.28[c]
95	5	18834.33±19.29[ab]	73.94±0.02[defg]	0.17±0.20[d]	4.17±0.08[c]
92.5	7.5	18821.05±18.88[ab]	75.88±2.18[bcdef]	0.16±0.29[de]	5.10±0.46[b]
90	10	18805.33±15.86[ab]	75.93±3.63[bcdef]	0.13±0.30[def]	6.13±0.24[bc]
90	10	18839.52±17.55[ab]	76.21±1.89[bcdef]	0.14±0.22[def]	6.15±0.08[bc]
85	15	16500.33±21.39[c]	77.11±0.24[abc]	0.10±0.09[def]	8.82±0.29[ab]
82.5	17.5	15458.33±11.63[d]	77.93±11.02[abc]	0.07±0.07[def]	8.94±1.65[ab]
80	20	14232.33±13.05[e]	79.84±0.16[a]	0.07±0.15[def]	9.44±1.15[a]
80	20	14244.13±11.79[e]	79.81±5.77[a]	0.05±0.28[f]	9.45±0.47[a]
80	20	14220.43±12.52[e]	79.80±4.57[a]	0.05±0.25[f]	9.47±0.25[a]
CV (%)		7.91	2.94	2.75	2.89
LSD		247.47	4.71	0.10	2.25

Values are Mean ± SD in a column with the same letter are not significantly different (p>0.05). DLSF=debittered lupine seed flour and ASLF=Australian sweet lupine flour.

3.5. Predictive Models for Number of Eyes and Colour Values of Injera

The predictive model of numbers of injeras eyes and colour especially lightness which is the more dominant are shown below in Table 4. Tef shows the greater coefficient value for number of injeras eyes in both lupine varieties. The higher the coefficient value indicates that the higher effect on

the response on the produced injeras.

The colour (lightness) of produced injera was scored higher coefficient values by lupine varieties rather than tef and the blended injera colour determined by the software results indicate there was not agreed with the sensory acceptability test scores these was due to the subjective character of sensory tests panellists but not instruments [24].

Table 4. *Regressions models for eyes and colour of tef-lupine injera by software.*

Tef: DLSF	Predictive model $Y=\beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2$	Model Prob>F	Adj R°	R°	Lack of fit
Number of eyes	Y=18974.19101T- 0.000012L+0.000015T*L	0.0001*	0.9898	0.9998	0.5578 (ns)
Lightness	Y=71.40104T+80.5358L+34.03937T*L	0.0058*	0.9691	0.9743	0.5734 (ns)
Tef: ASLF					
No of Eyes	Y=18954.48895T-0.000014L+0.000017T*L	0.0001*	0.9899	0.9999	0.0881 (ns)
Lightness	Y=71.70969T+81.48756L+37.08492T*L	0.0001*	0.98	0.9833	0.1505 (ns)

βi=coefficients, (T)=*Tef*, (L)=Lupine, Y=response for each parameters, *=Significant at P < 0.05, (ns)=not significant, ASLF=Australian sweet lupine flour and DLSF=debittered lupine seed flour

4. Conclusion

Varieties and blending ratios were found to have effect on the functional properties, number of eye and colour quality of tef-lupine blended injera. Blending ratio was the most significant factor that had an effect on functional properties, number of eye and colour quality of tef-lupine blended injera. Adding lupine proportion had significantly increases water absorption capacity and foaming capacity and decreases the oil absorption capacity and swelling power of composite flour. Instrumental measurements of colour values of composite injera shows that number of eyes decreased as lupine proportion increased and colours (lightness) were increased for both lupine varieties. There should be a further investigation on the effect of lupine on injera eye and colour quality of injera made from different tef varieties in addition with magna (DZ-01-196) variety which was used in this study.

Conflict of Interest

The author declare that they have no competing interests.

Acknowledgements

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