



Wheat-lentil fortified flours: health benefits, physicochemical, nutritional and technological properties

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Abstract

Lentil (*Lens culinaris*) is among the most consumed legume worldwide, especially in developing country. Lentil seeds provide an excellent source of iron and zinc content, and a high level of protein. Six treatments of composites flours were prepared by blending bread wheat flour (WF) with lentil flour (LF) in different proportion to evaluate the effect of blending on chemical, nutritional and technological characteristics. Our results revealed a significant increase ($p < 0.05$) of nutritional quality parameters such as ash, proteins, fat and energy value. Moreover, total polyphenols, flavonoids and antiradical activity were significantly improved. While, total carbohydrates content was significantly decreased in all fortified flours ratios. In addition, a significant variation of iron and zinc content was observed between composites flour. Regarding physical and technological characteristics, a significant difference was observed between the six treatments. Clarity, whiteness index and gluten strength values decreased with the increase of lentil flour ratios. Furthermore, rheological proprieties of wheat-lentil composites flours dough show that supplementation of lentil flour has significantly decreased water absorption, development time and dough stability, and increased the degree of softening. Overall, wheat-lentil fortified flours until 20% of LF incorporation can provide an ideal ingredient to improve human nutritional quality and health status.

1. Introduction

Moroccan diet is a Mediterranean type based on a large consumption of cereals and fruits and vegetables. The main cereals (wheat, maize and barley) account for 60% of the dietary energy supply [1]. Like several developing countries, Morocco is undergoing a nutritional transition characterized by the coexistence of nutritional deficiencies and diseases linked to overweight and obesity within the same household [2].

Malnutrition is a public health problem in Morocco. Micronutrient deficiency continues to be an underlying cause contributing to maternal and infant mortality [3]. The World Health Organization (WHO) estimate that iron deficiency is one of the most prevalent micronutrients deficiency, affecting around two billion people globally. Children and women in the developing countries are particularly vulnerable with 300 million children and more than 500 million women suffering from iron deficiency anemia worldwide [4]. In Morocco, 37.2% of pregnant women, 31.5% of children aged 6 months to 5 years, 32.6% of women of childbearing age and 18% of men are anemic [3]. To alleviate this problem, kingdom of Morocco developed a National Micronutrient Program based on fortification of flour with electrolytic elemental iron.

Food fortification, which consists of the incorporation of high-protein and micronutrient foods into a widely consumed and available staple food, is one of the main strategies used to improve nutritional quality of third world populations [5]. Legumes may be helpful in solving this problem. Pulse crops are among the most important sources of protein, starch and dietary fiber [6]. In addition, they have high content of essential amino acids, particularly lysine. Thus, the amino acid composition of legumes is complementary to that of cereals [7]. The

combination of cereal and legume proteins would thus provide a better overall balance of essential amino acids [8]. Along with macronutrients, leguminous seeds contain appreciable amounts of vitamins, minerals and a number of health-promoting bioactive substances [9]. Legumes also constitute an important source of polyphenols and have a high antioxidant capacity [10].

Adding legumes to wheat flour could be a good means to develop such foodstuffs, which could be a natural source of bioactive substances that would have a positive impact on health of consumers. The large and the high consumption of wheat flour by the Moroccan population (366 g/person/day) [3] makes wheat flour a good choice as a fortification vehicle.

Numerous studies have been performed to assess the effect of the incorporation of legume flour on the functional properties of dough and its potential use as an ingredient in various food applications [11]–[18].

The aim of the present study was to analyze the effects of partial wheat-flour substitution by lentil flour on physicochemical properties and technological performance of wheat-lentil fortified flour.

2. Material and Methods

2.1. Biological material

Experiments were conducted at INRA-Morocco (Institut National de Recherche Agronomique), during 2016–2017 cropping season. Bread wheat grains (*Triticum aestivum* L.), variety "KHADIJA", and lentil seeds (*Lens culinaris* L.) variety "BAKRIA" registered in Moroccan official catalog were obtained from INRA.

2.2. Preparation of composites flours

Whole wheat and lentil seeds were cleaned from dirt by sorting out contaminants such as sands, sticks and leaves. Wheat seeds were milled using automatic laboratory mill (BUHLER) set at 64.28% extraction rate and sieved into fine flour with 250µm particle size. Lentil seeds were milled using an attrition mill and sieved into fine flour of uniform particle size, by passing them through a 1 mm mesh sieve. Wheat flour was mixed with 0, 10, 20, 30, 40 and 50% of lentil seeds flour.

2.3. Chemical analysis

Flours samples were analyzed on dry weight basis in triplicate. Moisture and ash were obtained using the method detailed in (AOAC, 2000), crude protein was determined by multiplying the nitrogen content by a factor of 5.75 (fine wheat flour) and 6.25 (composites flours). Crude fat was defatted by refluxing with 250ml petroleum ether using Soxhelt apparatus (AOAC, 2000). Total carbohydrate was determined by difference, by subtracting the measured protein, fat, ash, and moisture from 100 %. Total energy was calculated by multiplying the percentage of crude protein and carbohydrates by 4 and crude fat by 9.

2.4. Determination of iron (Fe) and zinc (Zn) content

Mineral (Fe and Zn) concentrations in flours were determined using a previously described modified $\text{HNO}_3\text{-H}_2\text{O}_2$ method [19], [20]. Samples flour (0.5g) were placed in individual digestion tubes. Six mL of concentrated (70%) nitric acid (HNO_3) was added to each digestion tube. The digestion tubes were placed in a 90 °C digestion block for one hour, and they were shaken at 15 and 45 minutes. Three mL of 30% hydrogen peroxide (H_2O_2) was then added to each tube. The tubes were kept for 15 min at 90 °C. Finally, 3 mL of 6 M hydrochloric acid (HCl) was added to each digestion tube, and the tubes were kept in the digestion block for 5 minutes. Upon complete digestion the volume was adjusted to 10 mL, and then filtered. Mineral concentrations of the filtrates were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES); (ICP-7000 Duo, Thermo Fisher Scientific). Calibration curves for Fe and Zn concentration were made using serial dilutions of ICP multi-elements standard solution.

2.5. Determination of phenolic content (PC), flavonoids content (FC) and antiradical activity (ARA)

Methanol extracts from wheat, lentil and composites flours were used to determine the total phenols content and the antioxidant activity. The residues of methanol extract were treated with hot sulphuric acid in methanol to free the hydrolysable polyphenols. The phenol content was determined by means of the Folin-Ciocalteu reagent according to the Folin–Ciocalteu procedure [21] modified by [22]. An aliquot of 10 µL of the sample solution was mixed with 100 µL of commercial Folin-Ciocalteu reagent and 1580 µL of distilled water. After a brief incubation at room temperature (5 min), 300 µL of saturated sodium carbonate was added. Absorbance was measured after 2 h at room temperature at 765 nm. All samples were analyzed in triplicate. The correlation between the absorbance and gallic acid concentrations were used to create a calibration standard curve. The phenolic concentration (PC) of the samples was expressed as gallic acid equivalent (mg EAG g⁻¹ DM).

Total flavonoids content (TFC) was determined using a colorimetric method in [23] in 50 μL extraction samples as described by [24]. TFC was expressed as quercetin equivalents (mg of quercetin equivalent per gram sample) through the calibration curve of catechin acid. Linearity ranges of the calibration curves were 2 to 0.0625 $\text{mg}\cdot\text{mL}^{-1}$ ($r = 0.98$).

The free radical scavenging capacity of flours extracts was determined using the staple 2,2-diphenyl-1-picrylhydrazyl radical (DPPH $^\circ$) method [22] with some modifications. 200 μL of flours extracts was mixed with 1.8 mL of DPPH $^\circ$ methanol solution (0.039 $\text{g}\cdot\text{L}^{-1}$). Negative control was prepared in parallel by mixing 800 μL of methanol with 1.8 mL of the DPPH $^\circ$ methanol solution. The mixture was incubated for 30 min in dark at room temperature and then the absorbance was determined at 517 nm. The inhibition percentage of the DPPH free radical is calculated as follows:

$$\text{Inhibition Percentage} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.6. Color measurement

Color measurements were carried out using a calibrated colorimeter CR 400 (Konica Minolta). Calibration was performed before each analysis with white and black standard tiles. Determined parameters were L^* (0 black and 100 white), a^* (greenness and redness) and b^* (blueness and yellowness) as defined by CIE (International Commission on Illumination). Additionally, the whiteness index (WI) was calculated as indicated by [25].

2.7. Evaluation of gluten strength and gluten (dry and wet) yield

Gluten strength was estimated using Zeleny sedimentation index according to the AFNOR NF V03-704 standards. This method relies on hydration and swelling properties of gluten proteins in acid medium, and involves measuring sedimentation volume formed by a suspension of flour in an aqueous lactic acid-isopropanol solution. The wet and dry gluten yield of wheat and wheat-lentil composites flours were determined using hand washing method. The dough was washed and gluten retained was collected and weighed for the determination of wet gluten yield. The wet gluten yield was calculated by the formula given below:

$$\text{Wet gluten yield} = (\text{weight of wet gluten} \times 100) \div \text{Weight of flour}$$

The dry gluten yield was determined by drying wet gluten in Perten Glutork 2020 instrument for 4 min and dry yield was calculated:

$$\text{Dry gluten yield} = (\text{weight of Det gluten} \times 100) \div \text{Weight of flour}$$

2.8. Evaluation of dough properties

Dough mixing and stretching properties of samples were studied using farinograph instrument (Brabender® GmbH & Co. KG, Germany). Measurements were conducted according to the constant flour weight procedure of ICC standards no 115/1. Water absorption, dough development time (min), dough stability (min), degree of softening (BU) and farinograph quality number (F.Q.N) were determined. All measurements were performed at room temperature (25°C).

2.9. Statistical analysis

Analysis of variance (ANOVA) was carried out using SAS program (Statistical Analysis System version. 9.1). Differences between respective means were determined using least significant difference (LSD) and considered significant when $p < 0.05$. Mean \pm standard deviation of three replicates were used. Figures were generated using GPP program (GraphPad Prism version 7).

3. Results and discussion

3.1. Nutritional value

Compared with wheat flour, lentil flour contained 98.6% more of proteins, 288% more of ash, 204% more of fat and 2.5% more of total energy as listed in Table 1. Wheat flour showed a higher carbohydrate content (71.56 $\text{g}/100\text{g}$), our findings match with those reported by [20] and [26]. In addition, a significant difference ($P < 0.05$) was obtained between wheat and wheat-lentil composites flour for nutritive parameters (table 2). The supplementation of lentil flour led to an increase of ash (up to 1.11%), proteins (up to 18.63%), lipids (up to 1.27%) and total energy content (up to 347.67 $\text{kcal}/100\text{g}$) compared to sole wheat flour (0.43%, 13.08%, 0.44%

and 342.45kcal/100g respectively). However, the carbohydrate content has decreased as lentil substitution rate increased (71.6% to 65.7%) which is an interesting result for diabetics. These results confirm the previous results of [27]. Indeed, lentils provide slowly absorption of carbohydrate which gives a flatter blood glucose profile even in non-insulin dependent diabetes mellitus [28].

Table 1: Effect of lentil flour incorporation on chemical composition (g.100g⁻¹) of composites flours

Flour ratios		Parameters				
Lentil	Wheat	Ash	Protein	Fat	Carbohydrates	Energy value *
0%	100%	0.43 ^g ±0.02	13.08 ^d ±0.1	0.44 ^d ±0.02	71.56 ^a ±0.11	342.15 ^e ±0.16
10%	90%	0.59 ^f ±0.02	12.92 ^d ±0.02	1.03 ^c ±0	71.12 ^{ab} ±0.02	345.40 ^d ±0.14
20%	80%	0.70 ^e ±0	14.59 ^{cd} ±0.4	1.07 ^c ±0.05	69.55 ^{abc} ±0.4	346.13 ^{cd} ±0.22
30%	70%	0.86 ^d ±0.01	15.80 ^c ±0.4	1.15 ^{bc} ±0.05	68.55 ^{bc} ±0.5	347.67 ^b ±0.47
40%	60%	0.97 ^c ±0.01	16.87 ^b ±0.22	1.18 ^{abc} ±0.06	67.17 ^{cd} ±0.16	346.84 ^{bc} ±0.33
50%	50%	1.11 ^b ±0.01	18.63 ^b ±0.37	1.27 ^{ab} ±0	65.10 ^d ±0.38	346.34 ^c ±0.02
100%	0%	1.67 ^a ±0.01	25.98 ^a ±1.78	1.34 ^a ±0.12	58.77 ^e ±1.95	351.01 ^a ±0.35

Data expressed as means±standards deviation (n=3). Values in the same line with different letters are significantly different at p<0.005 using the LSD test; *: kcal.100g⁻¹ in dry weight.

3.2. Effect of lentil flours incorporation on total phenols content (TPC), total flavonoids content (TFC) and antiradical activity (ARA)

The major polyphenolic compounds of pulses consist mainly of tannins, phenolic acids and flavonoids. According to [29] and [30] studies, total phenolic content is directly associated with antioxidant activity [29], [30]. Among food legumes, lentil has the highest phenolic, flavonoid and condensed tannin content [24].

Our results indicated a significant difference (p<0.05) between all ratios for TPC, TFC and ARA (Table 2). In addition, lentil flour yielded a higher content of polyphenols (1.39 mg EAG g⁻¹), flavonoids (0,59 mg EQ.g⁻¹) and showed a higher DPPH scavenging capacity (76.98%) in comparison with wheat flour (0.46 mg EAG g⁻¹, 0 mg EQ.g⁻¹ and 0.85%). According to ANOVA test results, lentil flour addition significantly improved (p>0.05) phenolic compounds, flavonoids content and DPPH scavenging capacity. Based on obtained results, bread made with wheat-lentil mixtures will have a higher antioxidant activity than bread made from wheat flour only; TPC value of WF was 0.46 and 1.01 mg GAE g⁻¹ of LF50%, ARA value of WF was 0.58% and 51.20% for LF50%. Our results confirm those reported by [31] and are consistent with [32]. These investigations indicate that lentil flour is a useful food ingredient for improving the anti-oxidative potential of wheat flour.

Table 2: Antiradical activity (DPPH° scavenging percentage), total flavonoids content ((µg EQ.mg⁻¹) and total phenols content (mg GAeq.g⁻¹) of wheat flour and lentil-wheat composite flours

Flour ratios		Parameters		
Lentil	Wheat	Antiradical activity ¹	Total flavonoids content ³	Total phenol content ²
0%	100%	0,85 ^e ±0,62	0,00 ^c ±0.00	0,46 ^c ±0,09
10%	90%	3,10 ^e ±0,38	0,01 ^c ±0.08	0,49 ^c ±0,09
20%	80%	36,76 ^d ±0,04	0,06 ^c ±0.01	0,61 ^c ±0,05
30%	70%	37,38 ^d ±0,43	0,12 ^c ±0.01	0,90 ^b ±0,04
40%	60%	40,59 ^c ±0,14	0,18 ^{bc} ±0.04	0,92 ^b ±0,03
50%	50%	51,20 ^b ±1,14	0,36 ^{ab} ±0.14	1,01 ^b ±0,02
100%	0%	76,98 ^a ±1,42	0,59 ^a ±0.10	1,39 ^a ±0,09

Data are expressed as means±standards deviation (n=3). The values in the same line with different letters are significantly different at p<0.05 using the LSD test.

3.3. Influence of lentil flours incorporation on Mineral content (Zn and Fe)

A significant difference (p<0.05) was obtained between wheat flour and fortified wheat- flours ratios for both zinc and iron contents (table 3). The lowest value of zinc and iron content was found in wheat flour 28.83 mg kg⁻¹ for zinc and 35.69 mg kg⁻¹ for iron, while the maximum value of zinc and iron content in lentil flour was 51.84 mg kg⁻¹ and 59.42 mg kg⁻¹. Iron concentration was different from that obtained by [33] who found 45.4 mg kg⁻¹ for wheat flour and 53.2 mg kg⁻¹for lentil flour. Likewise, Iron content in wheat flour was similar to that reported by [34]. Zinc content in lentil flour was in accordance with [20] results, where zinc concentration of 19 lentil genotypes ranged from 44 mg kg⁻¹ to 54 mg kg⁻¹.

Wheat flour fortification resulted in a significant ($p < 0.05$) improvement of zinc and iron content. Zinc content increased from 28.83 mg kg^{-1} (0% LF) to 48.66 mg kg^{-1} (50% LF) and iron content from 35.7 mg kg^{-1} (0%) to 58.03 mg kg^{-1} (30%). Iron content in fortified wheat flour has proportionally increased from 10% to 30% and stabilized at 40% and 50% ratios. Obtained results indicate that lentil has the potential to provide an excellent natural source of Fe and Zn when added at 30% ratio. Our results confirm those obtained by [33], [35].

Table 3: Fe and Zn content (mg kg^{-1}) of wheat, lentil and lentil-wheat composite flours.

Flour ratios		Parameters	
Lentil	Wheat	Zinc	Iron
0%	100%	$28,83^b \pm 7,66$	$35,69^c \pm 2,33$
10%	90%	$42,20^a \pm 0,68$	$46,45^b \pm 7,71$
20%	80%	$44,24^a \pm 3,27$	$54,36^{ab} \pm 2,01$
30%	70%	$44,00^a \pm 2,88$	$58,03^a \pm 0,39$
40%	60%	$46,05^a \pm 3,14$	$56,12^{ab} \pm 3,56$
50%	50%	$48,66^a \pm 0,34$	$56,17^{ab} \pm 0,19$
100%	0%	$51,84^a \pm 1,75$	$59,42^a \pm 1,20$

Data are expressed as means \pm standards deviation ($n=3$). The values in the same line with different letters are significantly different at $p < 0.05$ using the LSD test

3.4. Influence of lentil flour incorporation on color propriety

Appearance, mainly color, is an important attribute of flour quality. Wheat flour had higher luminance (L^*) and whiteness index (WI) value than lentil flour. While, lentil flour had higher (b^*) value as listed in table 4. Analysis of variance showed that lentil flour addition significantly ($p < 0.05$) affected the color of composite flours. This change is probably due to bran contents of lentil flour resulting in greater amounts of pigment. Clarity or luminance value of wheat-lentil mixture decreased significantly as well as the incorporation rate increase (84.92 ± 1.25 to 92.21 ± 0.11). The same trend was obtained for whiteness index (85.96 ± 0.18 to 76.82 ± 1.19). Yellow-blue chromatic component (b^*) of wheat flour (8.66 ± 0.21) increased by the increase of lentil flour incorporation ratios (11.65 ± 0.14 to 17.57 ± 0.51).

Table 4: Color measurements of wheat, lentil and lentil-wheat composite flours.

Flour ratios		Minolta color			
Lentil	Wheat	L^* value	a^* value	b^* value	WI
0%	100%	$94,8^a \pm 0,12$	$-0,54^a \pm 0,01$	$8,66^f \pm 0,21$	$89,89^a \pm 0,25$
10%	90%	$92,21^d \pm 0,11$	$-0,83^a \pm 0,01$	$11,65^e \pm 0,14$	$85,96^b \pm 0,18$
20%	80%	$88,08^a \pm 2,5$	$-0,89^a \pm 0,23$	$14,15^d \pm 0,28$	$81,39^c \pm 1,84$
30%	70%	$88,24^{ba} \pm 0,67$	$-0,95^a \pm 0,2$	$15,27^c \pm 0,24$	$80,70^c \pm 0,59$
40%	60%	$89,97^{ba} \pm 1,7$	$-0,99^a \pm 0,12$	$15,94^c \pm 0,31$	$79,36^{bc} \pm 1,36$
50%	50%	$84,92^{bc} \pm 1,24$	$-1,01^a \pm 0,15$	$17,57^b \pm 0,51$	$64,61^c \pm 1,20$
100%	0%	$72,61^c \pm 1,48$	$-0,6^a \pm 0,24$	$22,39^a \pm 0,08$	$76,82^d \pm 1,19$

Data are expressed as means \pm standards deviation ($n=3$). The values in the same line with different letters are significantly different at $p < 0.005$ using the LSD test

3.5. Effect of lentil flour addition on gluten strength and gluten content

The effect of fortification of wheat flour with lentil flour on gluten strength, wet gluten and dry gluten yield is illustrated in figure (1.a and 1.b). The gluten is an important component of wheat flour that gives texture and strength to baked wheat products, moreover the gluten content affect directly the quality of wheat flour [36]. The Sedimentation test values varied from zero for lentil flour to 22 (ml) for wheat flour. So, the gluten strength of fortified flours decreased gradually as the fortification level increased and is more affected in composites flours (30%, 40%LF and 50%LF). Therefore, the technological quality of these fortified flours will be less than 10%LF and 20%LF. The wet gluten yield ranged between 28.05 and 13.6%, for wheat flour and 30%LF respectively. Also, the dry gluten yield ranged between 10.6 and 5.61% for 0%LF and 30%LF respectively. Fortified flours

with lentil had significantly lower wet and dry gluten yield than wheat flour only. Thus, the sedimentation test provide an indicator for gluten strength and a higher index shows good quality [37]. The decrease of gluten strength is mainly caused by gluten reduction, this reduction resulted by the substitution of wheat gluten proteins by those of lentil flour.

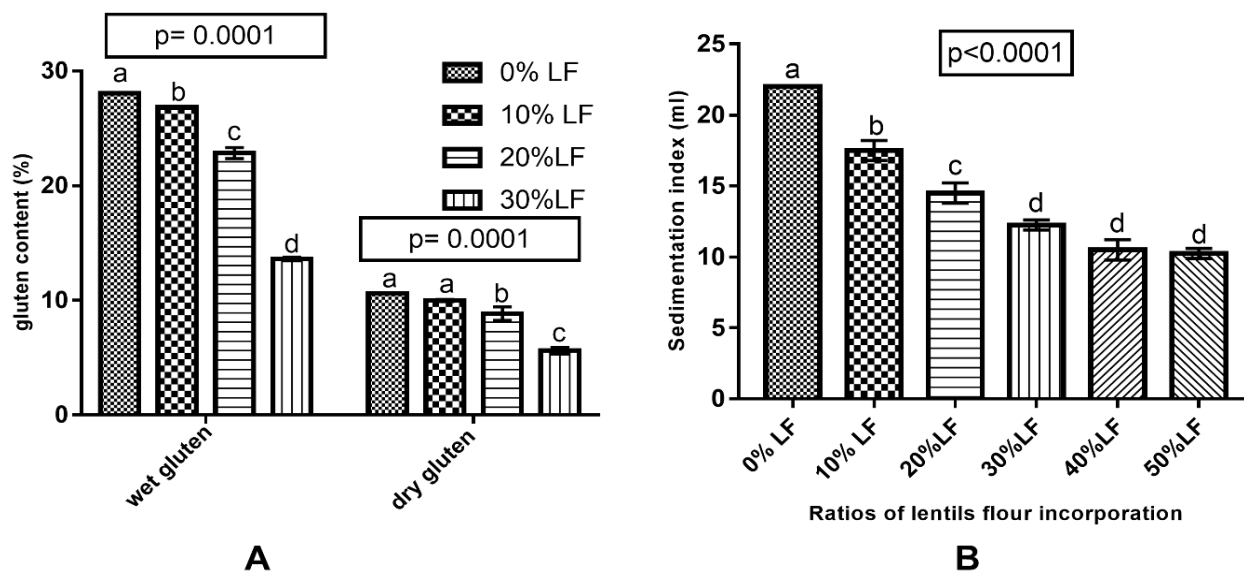


Figure 1: Influence of lentil flour (LF) incorporation on gluten strength (1.A), wet and dry gluten content (%) (1.B).

3.6. Effect of lentil incorporation on dough properties

Information on the rheological properties of dough is useful for predicting the potential of wheat flour and also the quality of the final product [12]. Blending both wheat flour and lentil flour may affect the viscoelastic and mixing properties of dough. The influence of lentil flour incorporation in wheat flour on farinographic characteristics is illustrated in figure 2. Water absorption (WA) is one of the most fundamental quality parameter of wheat flour. The required amount of water to produce dough with optimum consistency is determinate with the farinographic water absorption (WA).

Water absorption increased significantly from 53.8 % (100% WF) to 61.4% (50% LF ratio). Our results are consistent with [27] and [38], who found that WA increased from 58.6% to 61.9% (10% to 50% LF) and from 59.73% to 74.90% (10% to 30% of LF) respectively. In addition [39], [14] and [12] founded a similar effect when various legume flours were added to wheat flour. This increase probably caused by the higher portion of protein and fiber of fortified flours delivering a greater hydration capacity [40], [41]. Dough stability of wheat flour showed the highest value (3.29 min), while the incorporation at 50% of lentil flour showed the lowest one (1:25 min). Our results are in consistence with those reported by [27].

The results of development time (DDT) required to reach maximum consistency of dough, show that fortified flours has the higher DDT compared to wheat flour. In addition, DDT increased by the increase of lentil flour ratios. Dough of fortified flours was very strong with a long developing time thus, increases energy demands to produce dough of optimal consistency. These results accord with those reported by [42] for wheat flours supplemented with lentil and bean, [12], [43] for wheat flours supplemented with chickpea. According to [41] the increase in DDT could be explained by interactions between the non-wheat proteins and gluten leading to a delay in the hydration and development of gluten in the presence of these ingredients [41]. Furthermore, fortified flours had a significant increase in degree of softening (DS) values (136 to 176 BU) compared to wheat flour (136 BU). These results are consistent with [27] (116.2 to 145.4 BU (10 to 50% of lentil flour)). The addition of lentil flours in the blend increased the DS because gluten content lowered and the protein network became weaker and softer [27]. The degree of softening (DS) is an index of dough strength. Higher DS values indicate stronger dough [12], greater tolerance to mixing and greater flexibility in blending operations [38]. Our results are consistent with [27].

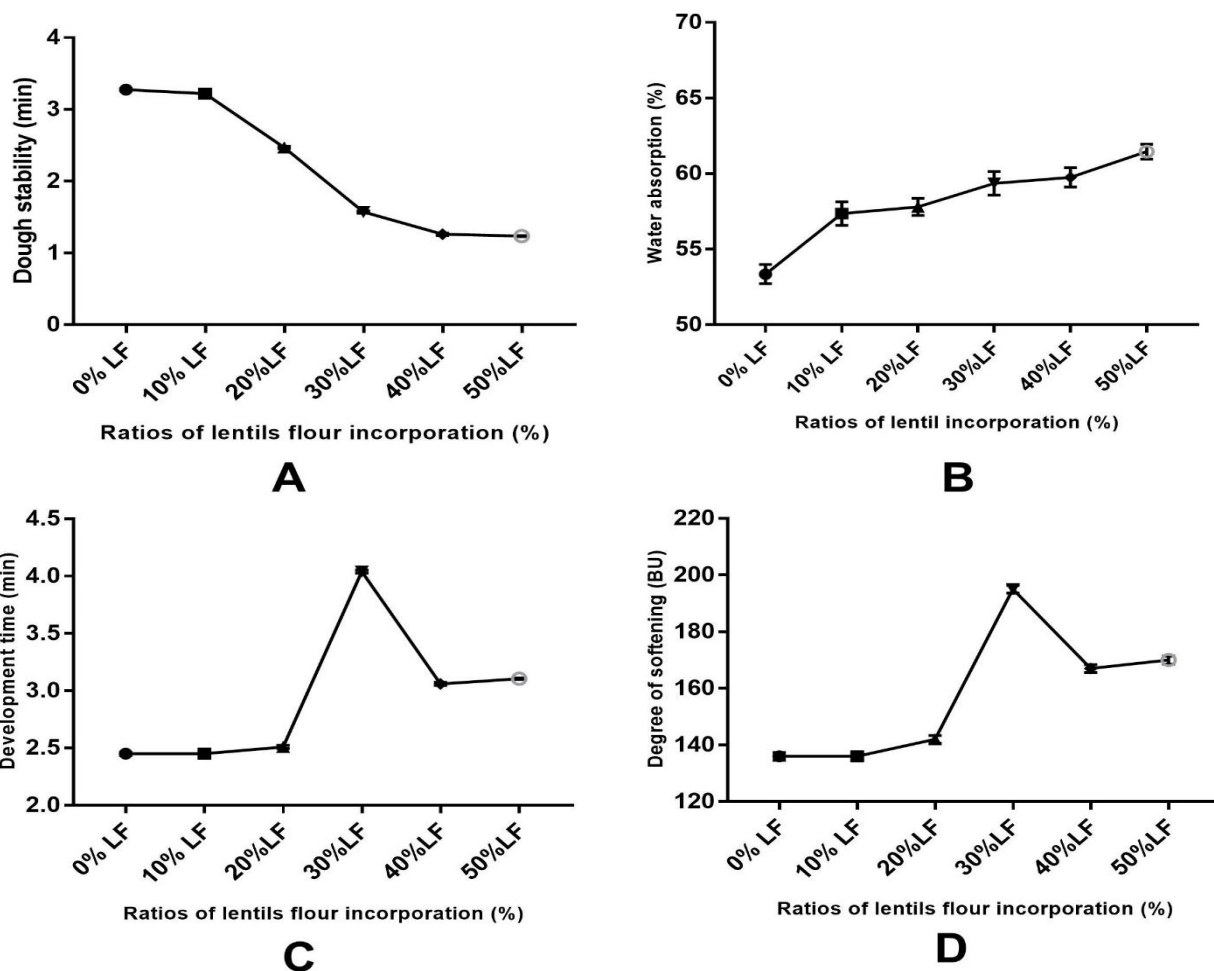


Figure 2: Influence of lentil flour (LF) incorporation on dough stability (2.A), water absorption (2.B), development time (2.C), and degree of softening (2D.).

Conclusion

The current study underlined the nutritional benefits of adding lentil-wheat based flour which promised a beneficial human health effect. Indeed, this investigation revealed that proteins, ash, fat content and energy value has increased especially in blend with higher ratios of fortification; while total carbohydrates content decreased by increasing lentil flour ratio. Furthermore, iron and zinc contents were improved as well as the phenolic compound. Consequently, the antioxidant activity was increased which can help to heal several pathologies which are the main cause of oxidative stress.

From a technological point, lentil flour alters rheological proprieties of dough by increasing its incorporation ratios, decreases gluten strength and dough stability, increases water absorption time, development time and the degree of softening. In addition, the clarity (L^*) and the whiteness index (BI) decreased while the chromatic component (b^*) increased with the increase of lentil flour incorporation ratio. Thus, the wheat flour fortification by lentil flour might be a good option for controlling protein malnutrition, diabetic disease and iron and zinc deficiency.

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