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BLENDING STUDIES USING WHEAT AND LENTIL COTYLEDON FLOUR – EFFECTS ON RHEOLOGY AND BREAD QUALITY

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ABSTRACT

Background and objectives: Lentil (*Lens Culinaris*. Medik) is a highly nutritious food staple widely consumed within India subcontinent and the Mediterranean region. Although gaining popularity in western diets, wheat will continue to be a major crop as it can be used to manufacture a wide range of products. The nutritional benefits of lentils are acknowledged, particularly as a source of high protein so the incorporation of lentil flour into wheat-based foods has the potential to improve the nutritive value of a range food products. Twelve blended flours were made using different concentrations of red lentil cotyledon, wheat and additional gluten. A blending study was undertaken to access yeast vitality, rheological properties of dough and baking characteristic of resulting bread.

Findings: High-ratio blends of lentil flour had no negative effect on yeast vitality even at the highest concentration of 40%. Increasing substitution of lentil flour was highly correlated to increased protein (r = 0.98) and ash (r = 0.95) and a concomitant decrease in dough strength but not extensibility. Loaf volume and baking quality were also compromised at higher concentrations. At a concentration of 5% lentil flour, there were no deleterious effects on dough quality traits or on baking quality. The addition of bakers gluten 0.1g /gram flour, had a restorative effect on the rheological and baking characteristics of wheat-lentil composites at higher concentrations of up to 20%.

Conclusion: Our results show that optimal baking quality of wheat-lentil flour can be achieved using either low concentrations of up to 5% lentil flour, or up to 20% lentil flour with the addition of gluten which maintained a superior loaf and crumb quality.

Significance and novelty: The protein and ash content of baked breads significantly increased when wheat was partially substituted with lentil flour. Concentration of lentil flour decreased dough strength and dough development time and decreased loaf volume whilst increasing crumb firmness in resulting bread. The addition of gluten improved the rheological and product quality of bread which allowed higher concentrations of lentil flour to be used in bread making. Balancing the ratio of lentil flour and gluten to optimise the rheological properties will result in a composite wheat-lentil bread with acceptable baking performance and enhanced nutritional benefits for consumers.

KEY WORDS

protein, ash, extensibility, loaf volume, crumb structure, crumb colour, crumb firmness.

INTRODUCTION

Bread made from wheat flour is a major source of nutrients in both western and non-western diets. In many cultures, bread is an important source of protein which also provides dietary vitamins and minerals (Aider, Sirois-Gosselin, & Boye, 2012). Historically, whole and multi-grain bread were commonplace, but white bread became the preference in modern times. Consumers are now more aware of the benefits of whole grain bread from both health and taste perspective. Nutritionally wheat has a relatively low level of protein, 7-15% (Sozer, Holopainen-Mantila, & Poutanen, 2017) when compared to protein content of non-cereal grains such as pulses (Davies-Hoes, Scanlon, Girgih, & Aluko, 2017). The protein content in lentils is reported to range from 23-27%. The amino acids that form lentil proteins are predominantly lysine, leucine, aspartic acid, glutamic acid, and arginine (Boye, Zare, & Pletch, 2010). Lentil flour has a high concentration of lysine (Boye et al., 2010), which is the

limiting amino acid in wheat flour protein (Aider et al., 2012). Lysine is also an essential amino acid which the body cannot produce and has to be gained through nutritional intake (Nosworthy, Tulbek, & House, 2017). Additionally, lentil is an enriched source of fiber, carbohydrates, and vitamins (Sozer et al., 2017). The mineral composition of lentil includes Mg, Ca, Fe, Zn, Mn, Cu and Se. These trace elements are important factors in human metabolic systems (Ray et al., 2014). Other health benefits can be gained through the bioactive components found in lentils (Jarpa-Parra, 2018; Joshi et al., 2013; Takruri & Issa, 2013), such as phenolic compounds, which occur in higher concentrations in the seed-coat (Amarowicz et al., 2009). Lentil cotyledon contains low concentrations of compounds derived from hydroxybenzoic and hydroxycinnamic acids, which are predominantly phenolic acids (Davies-Hoes et al., 2017). These compounds do not have the negative anti-nutritional properties found in some flavonoid and tannin seed coat compounds (Akhtar, Anjum, & Anjum, 2011). Plant phenolic acids are commonly known for their antioxidant activity which can quench the negative effect of oxidative stress in human cells (Mohammed, Ahmed, & Senge, 2012). Research has also shown that these compounds can have anti-cancer, anti-obesity, anti-inflammatory, anti-microbial as well as anti-hypertensive properties (Davies-Hoes et al., 2017; Rochfort & Panozzo, 2007; Takruri & Issa, 2013).

The inclusion of lentil proteins in bread and other baked products is not new, with the most successful examples to date achieved using protein concentrate and protein isolate (Aider et al., 2012). Concentrate or isolate may increase protein levels, but do not include other equally important benefits obtained through the phenolic, macronutrient and increased mineral bioavailability contained in lentil flour (Aider et al., 2012; Jarpa-Parra, 2018）. By utilizing, for example, lentil-cotyledon flour, there is potential to exploit its beneficial functional properties through incorporation of lentil flours into wheat-based bread. The purpose of this study was to investigate the rheological and baking performance of wheat-lentil composite flours; establishing the optimal concentration of lentil flour before negative effects were observed in baking properties, and if such effects could be corrected by fortification with additional gluten.

MATERIALS AND METHODS

Materials

Lentil *cv*. Northfield (*Lens culinaris.* Medik) is a commercially grown variety characterized by a gray seed coat and orange-red cotyledon. The wheat variety *cv.* Elmore (*Triticum aestivum.* L) is a hard-grain, white wheat used for bread making. In preparation for this study, wheat was conditioned to 16% moisture for 24 hours prior to milling on a Buhler laboratory

mill (MLU 202, Buhler, Switzerland) in accordance with AACC Method 26-10.02 (AACC, 2000). Whole lentil seeds were de-hulled, and the fractions of cotyledon and seed coat collected separately using an air aspirator (KimSeed, WA, Australia). The cotyledon was milled to flour using a cyclone mill fitted with a 0.5 mm screen (Laboratory Mill 120, Perten Instruments, Huddinge, Sweden). Wheat and lentil flour composites were prepared in the ratios shown in Table. 1. A second set of composite wheat-lentil flour blends was prepared with the addition of Vital Wheat gluten powder (Melbourne Food Ingredient Depot, Vic. Australia), at a concentration of 0.1g per gram of flour. (Insert Table. 1)

Yeast Activity and CO_2 Production

Yeast activity in each wheat-lentil flour matrix was assessed based on respirational CO_2 generation using a traditional gas water displacement method (Brubaker, 2017). Erlenmeyer flasks (250 mL) containing 150 mL of water and bakers yeast (4 g), (Invicta Group, QLD, Australia), along with 7.5 g of 100% wheat, 60-40% wheat-lentil and 100% lentil flour were added to each flask. Samples underwent rapid stirring using a stir plate for one minute. The flasks were then placed in a 31°C oscillating water bath set at 100 RPM (Paton Scientific, SA, Australia). Each flask was fitted with a rubber stopper and equal lengths of rubber tube which vented CO_2 into inverted measuring cylinders filled with water. Measurements of CO_2 production by water displacement were taken in triplicate during fermentation at 60, 120 and 180 minutes.

Toxicity and Yeast Cell Growth

A liquid yeast starter culture was prepared in three 250 mL Erlenmeyer flasks by combining 10 g of 100% wheat, 60-40% wheat-lentil or 100% lentil flour into 200 mL of RO water. Each sample was pitched with 6 g bakers yeast (Invicta Group, QLD, Australia). Each yeast suspension was placed in an orbital incubator (Ratek Instruments, VIC, Australia) and gently rocked at 67 RPM at 32°C. At time 0, 60, 120 and 180 minutes a dilution series was performed in triplicate for each sample and then plated at 10^{-8} CFU/mL on malt extract agar (Oxoid Limited, VIC, Australia). All plates were then incubated at 18°C for 48 hours. Images of the cell growth on each plate were then taken using a digital camera and analyzed with Matlab R2016b software (MathWorks, Massachusetts, USA) for the number of cell forming colonies on each plate.

Water Soluble Carbohydrate Extraction

Samples were prepared using the method described by Maharjan et al., (2018) with modification. For individual sugar analysis 0.2 g of flour was weighed into Teflon tubes and suspended in 5 mL of reverse osmosis (RO) water. Subsequently each sample was vortexed

and sonicated for 10 minutes and repeated three times. Mixtures were then centrifuged at 10,000 g for 10 minutes (Eppendorf Centrifuge 5430R, Hamburg, Germany). An aliquot of 0.75 mL was then transferred to 2 mL Eppendorf tubes and mixed with 0.75 mL 100% acetonitrile and centrifuged at 3000 g for 10 minutes. The supernatant was filtered through a 0.2 µm PTFE syringe filter (Grace Davidson Discovery Sciences, IL, USA), for UPLC injection to determine concentration of sugars in each sample.

Water Soluble Carbohydrate UPLC Analysis

Water soluble carbohydrate analysis was performed using a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) equipped with UPLC Binary Solvent Manager, UPLC Sample Manager and an Evaporative Light Scattering Detector, (ELSD). Separation was performed using a Waters ACQUITY BEH Amide column (2.1×100 mm, 1.7 µm) at 25°C. The mobile phase consisted of 80% acetonitrile with 0.05% ammonia (Solvent A) and 30% acetonitrile with 0.05% ammonia (Solvent B). The injection volume was 2.0 µL for all samples and the flow rate was kept constant at 0.13 mL/min over a 20-30-minute run time. UPLC ELSD data was analyzed using Empower 3 software to identify water soluble carbohydrate compounds and calculate peak area. Concentration of sugars was determined via calibration curves and individual peak area compared to the retention times and quantification of external standards (Maharjan, Jacobs, Deighton, & Panozzo, 2018).

Total Protein Analysis

Protein percentage of each sample was determined by the Dumas combustion method AACC 46-30.01 (AACC, 2000) using a Leco TruMac analyzer (Leco Corp, St Joseph, MI, USA). Moisture and ash content was predetermined with a thermogravimetric analyzer (Leco Corp, St Joseph, MI, USA). All sample evaluations were completed in triplicate.

Solvent Retention Capacity (SRC)

Solvent Retention Capacity (SRC) of wheat and lentil flour blends was measured to characterize the swelling capacity of the different polymer networks present in flour by the AACC method 56-11.02.(AACC, 2000). SRC values were corrected to 14% moisture using the formula established by Kweon, Slade, and Levine (2011).

Dough Rheology

The dough rheological properties of each wheat and lentil-wheat flour composite were measured with a Brabender Farinograph AT, fitted with a 50 g bowl (Brabender, Duisburg, Germany). Tests were performed in accordance with AACC method 54-21.02 (AACC, 2000). Dough extensibility was measured with an Extensograph E (Brabender, Duisburg,

Germany), by modifying AACC method 54-10.01 (AACC, 2000) for small-scale 50 g physical dough testing. In brief, 50 ± 0.1g of flour was sieved and mixed on a Farinograph AT (Brabender, Duisburg, Germany), fitted with a 50 g bowl. Samples were mixed for 5 minutes and appropriate % water addition containing 1 g of salt was administered through an electronic burette (Brabender, Duisburg, Germany) to obtain a consistency of 500 BU. After mixing, 75 g ± 0.1 g of the developed dough was excised, moulded and proved for 45 minutes then assessed for extensibility using an Extensograph E (Brabender, Duisburg, Germany).

Bread Baking

All bread samples were baked in triplicate using the straight dough method, method 10-09 (AACC, 2000). Loaf volume was determined by the rape-seed displacement method AACC method 10-05 (AACC, 2000). Volume values were expressed as specific volume by dividing with the sample weight.

Crumb Firmness

Crumb firmness was measured using a TA-XT2 Texture Analyzer fitted with a 25mm diameter probe and a 5g load cell, (Stable Micro Systems, Surrey, UK). Tests were conducted in accordance with the AACC method 74-09.01(AACC, 2000). One day after baking, a 25 mm slice was cut using a custom-made slicing box for consistency. Three compressions were administered in each of the top, middle and bottom sections of each slice along the medial line of each sample. The average of force in g to compress each slice to 25% was expressed as a measure of crumb firmness (N).

Crumb Color

Color of bread crumb for each sample was measured in triplicate using the Commission International del'eclairage tristimulus color parameters (CIE) $L^*a^*b^*$ with a Chroma Meter CR-410 colorimeter (Minolta Co. Osaka, Japan)

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) with GenStat statistical analysis software 17th edition (VSN, International, Hemel Hempstead, UK). Means were analyzed for the least significant difference at a probability level of $P < 0.05$. Results are expressed as mean values ± standard deviation. All analyses were conducted in triplicate.

RESULTS AND DISCUSSION

Yeast Vitality

Yeast is the primary fermentation agent in dough, affecting bread quality by influencing the flavor profile through the production of a range of volatiles (Heitmann, Zannini, Axel, &

Arendt, 2017), and the expansion of gas-cells, which contribute to the architecture of bread crumb (Reinhart, 2011). However, it has been shown that pulse flour may have an inhibitory effect on yeast vitality due to the possible antimicrobial activity of phenolic compounds originating from either the seed coat or cotyledon (Davies-Hoes et al., 2017; Sabel, Bredefeld, Schlander, & Claus, 2017). An additional factor impacting on yeast vitality may occur when the fermentable sugar profile is altered through the introduction of complexed and non-reducing sugars. Complexed sugars, such as the disaccharide-maltose from wheat and lentil or the digalactoside ciceritol from lentil are linked via α 1-6 glyosidic bonds. Catabolism of these sugars requires synthesis of carbohydrate specific hydrolytic enzymes, which only occurs when the monosaccharide and disaccharide glucose and sucrose utilization reaches 50% (Zheng, D'Amore, Russell, & Stewart, 1994). We undertook two experiments in a wheat-lentil flour matrix, to study the effect of yeast activity and yeast-cell growth during fermentation. Measurements of CO_2 produced during fermentation were determined by water displacement (Figure. 1), and yeast activity by cell counts on malt extract agar plates (Figure. 2), were conducted at termination times of 0, 60, 120 and 180 minutes. At the 60, 120 and 180-minute fermentation time point the 40% composite blend showed significantly higher water displacement, ($P < 0.05$) than both the 100% lentil and 100% wheat flour blends. (Insert Figure 1)

At each time point during fermentation, cell growth continued to increase significantly ($P < 0.05$). At the completion of the fermentation period the number of cell counts for each flour sample were as follows; 100% wheat: 1052 ± 14.63 CFM, 60-40%: 1458 ± 37.22 CFM, and 100% lentil: 1278 ± 12.49 CFM. (Insert Figure 2) A positive correlation of lentil flour inclusion on CO_2 production ($r = 0.99$), and cell growth ($r = 0.98$), was observed. Increases in both CO_2 production and cell growth are likely results of increases in bio-nutrient availability, such as the additional sugar matrix derived from the addition of lentil flour which is demonstrated in Figure. 3. Initially, yeast-cell growth is less vigorous in the suspensions containing wheat-lentil composites which may be due to lower concentration of fructose, (5.6 mg/g) and glucose (4.4 mg/g) compared with 14.1 mg/g of fructose and 16.9 mg/g glucose in the suspension containing 100% wheat. The addition of lentil flour has the effect of diluting fructose and glucose loadings (Figure. 3), however the lentil flour increases the concentration of sucrose (19.0 mg/g) which is not detected in the wheat sample and this contributes to an increase in yeast activity in the exponential growth phase and increased CO_2 production. The 40% lentil blend has both a higher cell-growth rate and CO_2 production than 100% lentil flour which can be explained by higher concentrations of maltose in the wheat

(20.8 mg/g) compared with 9.2 mg/g in the lentil flour. Overall, these studies conclude that the addition of lentil flour did not negatively affect yeast cell growth and vitality during 180 minutes of fermentation. (Insert Figure 3)

Protein and Ash Content

The proximal analyses for protein and ash are presented in Table. 2. As expected the addition of lentil flour resulted in a significant increase in both protein and ash levels for each composite sample ($P < 0.05$). Concentration of lentil flour was positively correlated to increase in ash, ($r = 0.95$) and protein ($r = 0.98$) (Table. 3). There were no significant differences for ash values observed between non-gluten and gluten blends. As would be expected the additional gluten in blended flours resulted in a significantly higher protein content (Table. 2) compared to each corresponding non-gluten blend ($P < 0.05$).

Dough Rheology

Dough rheology can be defined as a combination of performance parameters comprising % water addition (WA), dough development time (DDT), dough stability (DS), extensibility (EXT) and maximum resistance, (R max), providing an analytic characterisation of dough which is analogous to baking quality. Primarily, glutenin and gliadin proteins are the major determinants of quality, influencing dough rheology and baking quality. To a lesser extent soluble and insoluble dietary fibre, smaller molecular-weight proteins as well as thiol-containing polypeptides, also affect the end-product quality which is manifested through loaf volume and crumb texture (Dalgetty & Baik, 2006; Issarny, Cao, Falk, Seetharaman, & Bock, 2017). Baking quality is dependent on an optimum balance of rheologically important gluten-forming proteins (Panozzo et al., 2014; Uthayakumaran, Stoddard, Gras, & Bekes, 2000) and the addition of lentil flour into the wheat-flour matrix is expected to disrupt this balance (Bojnanská, Francáková, Lísková, & Tokár, 2012). Rheology measurements quantifying these effects are summarised in Table. 2. In this study, the addition of lentil flour did not cause a significant change in WA for wheat and lentil blends of up to 10% lentil flour. Similar findings were reported by Turfani et al. (2017), who found that WA for wheat and lentil flours of up to 10% was not significantly different from wheat. (Turfani, Narducci, Durazzo, Galli, & Carcea, 2017). In this study blends above 10% showed a significant reduction in WA ($P < 0.05$), which may be attributed to a higher concentration of lentil fiber. Dalgetty et al., (2006), reported that increasing soluble fibers by 6% in doughs made from pea, lentil and chickpea, caused a significant drop in WA. In this study DDT and DS were significantly reduced as lentil flour concentration increased ($P < 0.05$). Turfani et al., (2017) reported similar findings for DS, but not DDT where an increase in DDT for lentil and wheat

flour doughs was reported. The differences in observation for DDT is most likely due to the dehulled lentil cotyledon flour used in this study, which lacks the structural fiber found in hull material that would positively influence WA (Dalgetty & Baik, 2006) . Extensograph results (Table. 2), show that the increasing concentration of lentil flour caused a significant decrease in dough strength determined by R max ($P < 0.05$); however, dough extensibility was not significantly affected. These findings are in agreement with previous research where lentil proteins, albumins and globulins, were found to dilute glutenin and gliadin containing wheat proteins (Bojnanská et al., 2012; Turfani et al., 2017). In a subsequent experiment, baker's gluten was added to each composite flour at the rate of 0.1g /gram flour, to determine if gluten could compensate for the gluten-dilution effect due to the addition of lentil flour (Table. 2). The addition of gluten resulted in an increase in WA, DDT and DS, ($P < 0.05$) as well as an increase in extensibility and R max, ($P < 0.05$). However, the weakening of the gluten matrix, through high concentrations of lentil proteins (i.e. for 40% lentil-flour), could not be restored by the addition of baker's gluten. Overall these results show that there is a low threshold for including lentil flour into wheat flour before rheological properties are noticeably affected. Similar findings have been reported for the rheological properties when blending green lentil flour with wheat flour (Turfani et al., 2017). The fortification of the glutenin-gliadin network, with the addition of vital wheat gluten shows that overall, these blends have a much-improved rheology profile compared to the control blend.

Protein Functionality Studies Based on the Solvent Retention Capacity

The test for solvent retention capacity (SRC) (Bettge, Morris, DeMacon, & Kidwell, 2002) is an alternative method for investigating protein functionality. Results are summarized in (Table. 2). The result for each SRC test for straight blends (i.e. without additional gluten) specified that an increase in lentil flour concentration significantly decreased gluten levels, lactic acid (LA-SRC) in all blends, ($P < 0.05$). Starch damage, sodium carbonate (SC- SRC) showed a significant decrease when lentil concentration reached 10%, ($P < 0.05$). Pentosan, sucrose (SU-SRC) decrease was only significant at the 15% level, ($P < 0.05$), (Insert Table 2 & 3). Water absorption, (WA-SRC) showed a significant increase at the 15% level, ($P < 0.05$), which is contrary to the decrease in WA confirmed by the Farinograph at the 15% level. A possible reason for this difference is that WA-SRC acts as a reference within the SRC method, and is able to hydrate the three functional components simultaneously (Kweon et al., 2011). Farinograph water addition is based on kinetic interactions of polymer network development (Kweon et al., 2011), it is possible that once gluten-gliadin formation is complete less hydrophilic regions are available for water-binding (Tuhumury, 2014; Turfani

et al., 2017). However, in the SRC model full gluten development does not occur under the same shear force as it would on the farinograph, and this may be due to the thermodynamic nature of the SRC model. This could leave exposed hydroxyl groups of both lentil and wheat proteins available for water binding which would be reflected as a rising WA-SRC. Blended wheat-lentil flours containing additional gluten, 0.1g/gram flour, maintained higher gluten levels as reflected in LA-SRC values, but a significant decrease in LA-SRC between all blends substituted with gluten was observed as lentil concentration increased ($P < 0.05$). As was expected there was a significantly lower level of starch damage, (SC-SRC) in gluten substituted samples compared with straight wheat and lentil blends ($P < 0.05$). Pentosan and gliadin SRC showed significant decrease when lentil flour exceeded 15% for blends with additional gluten ($P < 0.05$). All SRC values were significantly higher in wheat-lentil and gluten blends compared to straight wheat-lentil blends ($P < 0.05$). WA-SRC followed a similar trend to the non-gluten variants; a significant increase was observed only when lentil flour concentration reached 15%, ($P < 0.05$). The WA-SRC values in all gluten substituted samples were significantly higher than straight wheat-lentil blends ($P < 0.05$). Overall results from SRC were positively correlated with the rheology data except for WA-SRC which was negatively correlated (Table. 3). Strong relationships were observed, such as the high positive correlation between lentil concentration and protein level. However, LA-SRC and SU-SRC values, are indicative of a weakened gluten structure. SRC characteristics also show a strong relationship with Extensograph and Farinograph results, where the increase in lentil concentration correlated to a lower R max, and DS. This was also reflected physically in reduced loaf volume (Table. 3).

Bread Baking Characteristics

Loaf volume, crumb texture and crumb color, were used as a measure of bread-making quality. With increasing concentrations of lentil flour, loaf volume progressively reduced, and the crumb colour darkened (Table. 4). Visually there was greater browning of crust as lentil concentration was increased, which could be influenced by both the colour of lentil flour and increased Maillard reaction during baking driven by a high lysine content (Turfani et al., 2017). Loaf volume (Figure. 4) was significantly reduced in all blends with and without gluten addition as lentil flour concentration increased, ($P < 0.05$). However, the loaf volume for all breads with added gluten retained significantly higher loaf volumes than all blends without gluten until lentil concentration reached 40%. Similar findings for reduced loaf volume have been reported in composite breads of wheat and chickpea (Mohammed et al., 2012) as well as lentil and bean flours (Kohajdová, Karovičová, & Magala, 2013). A

reduction in loaf volume is most-likely due to low hydration of wheat gluten as characterized by the Farinograph results for WA (Table. 2 & 3). Not only is gluten diluted by the presence of lentil protein, but the inclusion of lentil flour also initiates competition between gluten-gliadin and lentil proteins, albumin and globulin. Because lentil proteins have a greater number of hydroxyl groups, they have a greater affinity for water binding (Turfani et al., 2017). The deleterious effect that lentil flour has on the dilution of wheat gluten concentration corresponds with lower LA-SRC and SC-SRC values (Table 2). Hydration is also partially effected by lower levels of damaged starch which was also reflected through lower SC-SRC values (Bojnanská et al., 2012). Lentil fibre has also been reported to compromise gluten-gliadin strand formation (Dalgetty & Baik, 2006; Wang, Rosell, & de Barber, 2002). However, given that the addition of gluten to each wheat-lentil composite significantly increased loaf volume for blends with less than 40% lentil concentration, it is likely that the dilution of gluten when blending with dehulled cotyledon flour is the dominant cause of reduced loaf volume. It was determined that the concentration of gluten used in this experiment could compensate for any loss in loaf volume resulting from the addition of 5 to 15 % lentil flour, ($P < 0.05$). (Insert Figure 4 & Figure 5)

Crumb Texture

Crumb texture and firmness are important attributes contributing to the overall appeal of bread including visual appearance of bread, mouth feel and overall end-product. These attributes can be objectively determined using a texture analyser which measures the force-deformation profile of multiple compressions (Figure. 6). The addition of lentil flour had a significant effect on increasing crumb firmness from 64 N at the 100% wheat level to 350 N at 20% lentil addition, ($P < 0.05$). There was no significant difference observed for firmness between the wheat control and wheat-lentil blends with additional gluten until lentil concentration reached 20% ($P < 0.05$). Thus indicating that the addition of gluten had a restorative effect on the dough prior to baking which aligns with the observations of loaf volume. The crumb firmness of both variants of the 40% lentil blend exceeded analyser limits and therefore is not shown. Increase in firmness is likely associated with lower water absorption and reduced loaf volumes through the dilution of rheologically important proteins which are essential during dough fermentation (Lu, Brennan, Serventi, & Brennan, 2018). Reduced R max values and a strong correlation between extensograph results, lentil concentration, protein level, as well as LA, SU and SC-SRC values (Table. 3), support this finding. (Insert Figure 6)

Bread Color

Color is an important trait which consumers readily identify with quality and taste perception. The addition of lentil flour decreased the lightness of crumb color as measured by a decrease in CIE L* (Table 4). No significant difference was observed between the CIE L* values of the control and the 5% lentil loaves without and with the additional gluten. All blends above 5% lentil had significantly lower CIE L* value, ($P < 0.05$). The a* value of the crumb is an indicator of redness which is determined by positive a* value (Aider et al., 2012). The control breads mean CIE a* value of (– 0.88 ± 0.08) is indicative of a crumb with a slightly green hue. In contrast, the CIE a* values for the supplemented samples were positive indicating that as lentil flour concentration increased the crumb became significantly more red, ($P < 0.05$). Breads that were supplemented with more than 5% lentil flour had significantly higher CIE b* values, ($P < 0.05$) indicating an increase in yellow hue in the crumb. The ΔE vales provide a measure of noticeable difference of perception between two colour values, in this case between each blend and the control (100% wheat flour). For ΔE there was no significant difference between the control and 5% wheat-lentil blends. For breads containing additional gluten the difference in ΔE did not become significant until the concentration of lentil reached 20%, ($P < 0.05$). Overall, as lentil supplementation was increased breadcrumb developed a red and yellow hue; in straight wheat-lentil blends significant change for ΔE was observed at the 5% lentil level, ($P < 0.05$), however subsequent blends up to 20% lentil did not have significantly different ΔE values to each other. (Insert Table 4)

CONCLUSION

Blending lentil cotyledon and wheat flour together in baking can enhance the nutritional value of breads, most noticeably through gains in total protein. The difference in composition between lentil and wheat proteins does change the gluten network matrix, which impacts on bread quality but in particular loaf volume and crumb firmness. The quality of the bread can be restored with the addition of baker's gluten; however, this may contribute to increased costs. Blends of wheat-lentil with 20% lentil inclusion have high protein content but very low water absorption which leads to loaves with extremely poor volume and dense crumb structures. Thus, high ratio lentil blends may show better performance in different baked products like biscuit or extruded products such as noodles. Overall this study showed that in blends of up to 20% lentil flour and supplemented with baker's gluten, results can be obtained that have good loaf volume, firmness and crumb structure. Gluten is often targeted as pernicious, but as demonstrated in this study, can be used to create enriched composite lentil and wheat breads with many additional benefits for the consumer. More research is

403 required to understand the physiochemical properties of lentil and other legume proteins
404 which will provide a greater scope for incorporation in different types of baked products.
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(Table 1)

Table. 1. Composite blended wheat, lentil flour and gluten ratios as %

Sample*	Wheat Flour %	Lentil Flour %	Gluten %
Control	100.0	0	0
5% Lentil	95	5	0
10% Lentil	90	10	0
15% Lentil	85	15	0
20% Lentil	80	20	0
40% Lentil	60	40	0
Control	91	0	9
5% Lentil	86	5	9
10% Lentil	82	9	9
15% Lentil	77	14	9
20% Lentil	73	18	9
40% Lentil	55	36	9

* Composite blended wheat & lentil flour ratio.

Blend Ratio	**100 % W**	**5% L**	**10% L**	**15% L**	**20% L**	**40% L**
Protein (%)	13.80 ± 0.13**a**	14.30 ± 0.12**b**	15.30 ± 0.07**c**	15.68 ± 0.07**d**	16.35 ± 0.03**e**	19.02 ± 0.08**f**
Ash (%)	0.538 ± 0.02**a**	0.614 ± 0.01**ab**	0.712 ± 0.05**bc**	0.800 ± 0.01**cd**	0.890 ± 0.0**d**	1.210 ± 0.02**e**
Lactic acid SRC (%)	140.4 ± 2.03**a**	127.1 ± 1.64**b**	111.8 ± 0.78**cd**	107.0 ± 0.55**d**	95.9 ± 0.43**e**	74.3 ± 1.08**f**
Sucrose SRC (%)	98.7 ± 1.83**a**	99.0 ± 0.30**a**	98.4 ± 1.25**a**	95.2 ± 1.38**b**	89.3 ± 1.31**c**	76.8 ± 0.81**d**
$N2CO_3$ Na_2CO_3 SRC (%)	89.9 ± 3.55**a**	84.7 ± 0.79**b**	80.9 ± 0.95**c**	79.6 ± 2.45**c**	77.5 ± 0.62**cd**	73.0 ± 0.81**e**
Water SRC (%)	70.2 ± 0.04**a**	70.4 ± 0.61**a**	70.2 ± 0.44**a**	73.2 ± 0.19**b**	73.6 ± 0.62**b**	80.7 ± 0.60**c**
Farinograph						
Water absorption (%)	68.4 ± 0.10**a**	70.8 ± 0.06**a**	70.1 ± 0.34**b**	67.9 ± 0.29**b**	66.3 ± 0.06**c**	61.0 ± 0.21**d**
Development time (min)	9.31 ± 0.01**a**	8.17 ± 0.01**b**	5.55 ± 0.05**c**	5.28 ± 0.05**d**	5.05 ± 0.05**d**	4.91 ± 0.05**d**
Stability (MIN)	15.11 ± 0.07**a**	10.33 ± 1.73**b**	4.98 ± 0.33**c**	4.80 ± 0.33**c**	2.73 ± 0.24**d**	1.71 ± 0.25**e**
Extensograph						
Energy (cm^2)	106.00 ± 7.02**a**	87.33 ± 2.88**a**	62.33 ± 2.88**b**	52.00 ± 9.81**c**	42.67 ± 0.58**c**	21.00 ± 1.73**d**
Extensibility (cm)	16.87 ± 0.06**a**	17.80 ± 1.37**a**	18.43. ± 0.20**a**	18.53 ± 0.38**a**	17.83 ± 0.15**a**	18.7 ± 0.40**a**
Resistance (BU)	244.0 ± 17.3**a**	223.7 ± 17.3**a**	179.3 ± 5.1**b**	143.0 ± 19.1**c**	134.3 ± 3.1**d**	69.33 ± 5.69**e**
Max. resistance (BU)	404.0 ± 21.63**a**	328.3 ± 25.65**b**	233.0 ± 18.77**c**	189.0 ± 10.82**d**	165.7 ± 10.97**e**	78.33 ± 3.06**f**
Blend Ratio	**100% L ^{Glu}**	**5% L ^{Glu}**	**10% L ^{Glu}**	**15% L ^{Glu}**	**20% L ^{Glu}**	**40% L ^{Glu}**
Protein (%)	18.35 ± 0.12**a**	19.10 ± 0.10**b**	19.63 ± 0.06**c**	20.23 ± 0.06**d**	20.53 ± 0.06**e**	23.10 ± 0.10**f**
Ash (%)	0.621 ± 0.10**a**	0.632 ± 0.02**ab**	0.718 ± 0.01**ab**	0.777 ± 0.01**cd**	0.846 ± 0.01**d**	1.563 ± 0.01**f**
Lactic acid SRC (%)	166.4 ± 1.27**a**	149.2 ± 0.60**b**	131.9 ± 0.55**cd**	126.1 ± 0.64**d**	117.8 ± 0.29**e**	70.8 ± 0.80**f**
Sucrose SRC (%)	107.6 ± 1.83**a**	106.0 ± 0.30**a**	106.2 ± 1.22**a**	103.8 ± 0.40**ab**	98.6 ± 0.96**b**	81.2 ± 0.41**c**
Na_2CO_3 SRC (%)	91.2 ± 0.49**a**	87.6 ± 0.72**bc**	85.1 ± 0.5**cd**	84.4 ± 0.34**d**	83.4 ± 0.82**d**	67.4 ± 0.75**e**
Water SRC (%)	73.6 ± 0.27**a**	74.3 ± 0.24**a**	74.5 ± 0.54**a**	75.9 ± 0.58**b**	77.0 ± 0.73**b**	83.8 ± 0.27**c**
Farinograph						
Water absorption (%)	72.08 ± 0.12**a**	74.86 ± 0.59**ab**	77.26 ± 0.02**b**	76.96 ± 0.05**b**	75.03 ± 0.17**c**	64.3 ± 0.06**d**
Development time (min)	16.50 ± 0.02**a**	9.51 ± 0.01**b**	9.47 ± 0.03**c**	9.32 ± 0.27**c**	9.05 ± 0.03**c**	7.22 ± 0.10**d**

Table.2. Rheology Characteristics of Blended Flours[z]

Stability (MIN)	20.0 ± 0.12**a**	14.99 ± 2.89**b**	13.89 ± 0.49**b**	9.30 ± 0.11**c**	9.07 ± 0.51**c**	2.23 ± 0.16**d**
Extensograph						
Energy (cm^2)	90.33 ± 6.02**a**	87.34 ± 10.41**a**	94.33 ± 1.41**a**	99.33 ± 15.57**a**	92.00 ± 4.58**a**	37.67 ± 0.58**e**
Extensibility (cm)	19.37 ± 1.37**a**	19.37 ± 1.65**b**	20.30 ± 0.15**bc**	21.77 ± 1.37**bc**	21.87 ± 0.99**c**	16.57 ± 1.17**d**
Resistance (BU)	308.3 ± 10.97**a**	255.3 ± 2.6**a**	221.3 ± 8.6**a**	218.3 ± 17.9**a**	204.7 ± 16.6**a**	187.0 ± 67.5**f**
Max. resistance (BU)	416.67 ± 6.03**a**	390.8 ± 24.50**a**	344.7 ± 15.57**a**	241.3 ± 10.57**b**	297.3 ± 20.84**b**	204.0 ± 72.75**b**

[z] Data are mean ± SD. Values in the same column with different alphabetical letters differed significantly as determined by ANOVA following a Tukey's HSD test ($P < 0.05$). W= Wheat flour, L= Lentil Flour, Glu= additional gluten 0.1g per gram (W/W).

Table.3. Rheology Characteristics of Blended Flours[Z]

Non-Gluten														
Variables	**Lentil**	**Protein**	**LA-SRC**	**SU-SRC**	**SO-SRC**	**WA-SRC**	**WA**	**DT**	**S**	**Energy**	**EXT**	**Resistance**	**R Max**	**Volume**
Protein	0.98***	…	…	…	…	…	…	…	…	…	…	…	…	…
LA-SRC	-0.92**	-0.97***	…	…	…	…	…	…	…	…	…	…	…	…
SU-SRC	0.97***	-0.96**	0.90*	…	…	…	…	…	…	…	…	…	…	…
SC-SRC	-0.86*	-0.94**	0.99***	0.83*	…	…	…	…	…	…	…	…	…	…
WA-SRC	0.98***	0.96**	-0.88*	-0.99***	-0.83*	…	…	…	…	…	…	…	…	…
WA	-0.91**	-0.91*	0.82*	0.97***	0.73	-0.96**	…	…	…	…	…	…	…	…
DT	-0.64	-0.77	0.88*	0.60	0.92**	-0.60	0.52	…	…	…	…	…	…	…
S	-0.72	-0.83*	0.93	0.68	0.97**	-0.67	0.57	0.98***	…	…	…	…	…	…
Energy	-0.86*	-0.94**	0.99***	0.83*	0.99***	-0.83*	0.75	0.94**	0.97	…	…	…	…	…
EXT	0.63	0.70	-0.77	-0.49	-0.83*	0.55	-0.37	-0.85*	-0.85	-0.81	…	…	…	…
Resistance	-0.91*	-0.97**	0.99***	0.90*	0.97	-0.91*	0.84*	0.88*	0.91	0.9***	-0.76	…	…	…
R Max	-0.85*	-0.93**	0.99***	0.82*	0.99***	-0.83*	0.74	0.94**	0.97	0.99***	-0.82*	0.98***	…	…
Volume	-0.93**	-0.98***	0.99***	0.91*	0.99**	-0.91*	0.83*	0.85*	0.91	0.99***	-0.76	0.98***	0.98***	…
Gluten														
Variables	**Lentil**	**Protein**	**LA-SRC**	**SU-SRC**	**SO-SRC**	**WA-SRC**	**WA**	**DT**	**S**	**Energy**	**EXT**	**Resistance**	**R Max**	**Volume**
Protein	0.99***	…	…	…	…	…	…	…	…	…	…	…	…	…
Lactic_SRC	-0.99***	-0.99***	…	…	…	…	…	…	…	…	…	…	…	…
Sucrose_SRC	-0.96**	-0.96**	0.94**	…	…	…	…	…	…	…	…	…	…	…
Sodium_SRC	-0.98***	-0.98***	0.98***	0.97**	…	…	…	…	…	…	…	…	…	…
Water_SRC	0.98***	0.977***	-0.95**	-0.99***	-0.98***	…	…	…	…	…	…	…	…	…
WA	-0.68	-0.68	0.62	0.85*	0.76**	-0.82*	…	…	…	…	…	…	…	…
DT	-0.72	-0.74	0.78	0.58	0.69	-0.61	0.13	…	…	…	…	…	…	…
S	-0.97**	-0.97**	0.97**	0.88*	0.92**	-0.91*	0.51	0.83*	…	…	…	…	…	…
Energy	-0.80	-0.82*	0.78	0.92**	0.89*	-0.90*	0.95**	0.40	0.67	…	…	…	…	…
EXT	-0.45	-0.47	0.43	0.64	0.61	-0.61	0.88*	0.09	0.28	0.88*	…	…	…	…
Resistance	-0.84*	-0.84*	0.88*	0.68	0.77	-0.71	0.20	0.93**	0.92**	0.43	0.03	…	…	…
R Max	-0.94**	-0.92**	0.93**	0.84**	0.85*	-0.86*	0.45	0.74	0.96**	0.57	0.13	0.90*	…	…
Volume	-0.97**	-0.97**	0.95**	0.99***	0.98***	-0.99***	0.82*	0.61	0.89*	0.91*	0.63	0.72	0.86*	…

[z] Lentil = addition; SRC = solvent retention capacity; LA-SRC = lactic acid SRC; SU = sucrose SRC; SC-SRC = sodium carbonate SRC; WA-SRC = water SRC; WA = water absorption; DT = dough development time; S = stability; EXT = extensibility. * , ** and *** = significant correlation at the 5, 1 and 0.1% levels, respectively.

(Table 4)

Table. 4. LAB and ΔE values for loaf crumb color[Z]

Treatment	CIE L*	CIE a*	CIE b*	ΔE
100% Wheat	82.94±1.83**a**	-0.88±0.08**a**	14.70±0.23**a**	0.00±1.69**a**
5% Lentil	80.54±1.69**a**	0.31±0.03**b**	12.25±0.22**b**	3.63±1.94**ab**
10% Lentil	78.03±1.82**a**	1.07±0.44**c**	14.96±0.29**bc**	5.29±2.55**b**
15% Lentil	78.01±0.03**a**	1.10±0.20**c**	15.22±0.21**bc**	5.34±0.71**b**
20% Lentil	76.66±0.15**a**	1.41±0.19**c**	16.60±0.32**c**	6.95±0.66**b**
40% lentil	69.49±4.63**c**	2.29±0.27**c**	24.09±1.39**b**	16.7±6.29**c**
5% Lentil + Glu	81.38±2.21**a**	0.15±0.23**a**	13.16±1.70**a**	2.42±4.14**a**
10% Lentil + Glu	79.18±0.97**ab**	0.85±0.20**b**	14.80±0.26**a**	4.14±1.43**b**
15% Lentil + Glu	78.75±1.49**ab**	0.70±0.21**b**	14.15±0.27**a**	4.51±1.97**b**
20% lentil + Glu	76.66±0.15**b**	1.41±0.19**b**	16.60±0.32**a**	6.87±0.73**c**

[Z] Data are mean ± SD. Values in the same column with different alphabetical letters differed significantly as determined by ANOVA following a Tukey's HSD test ($P < 0.05$).

(Figure 1)

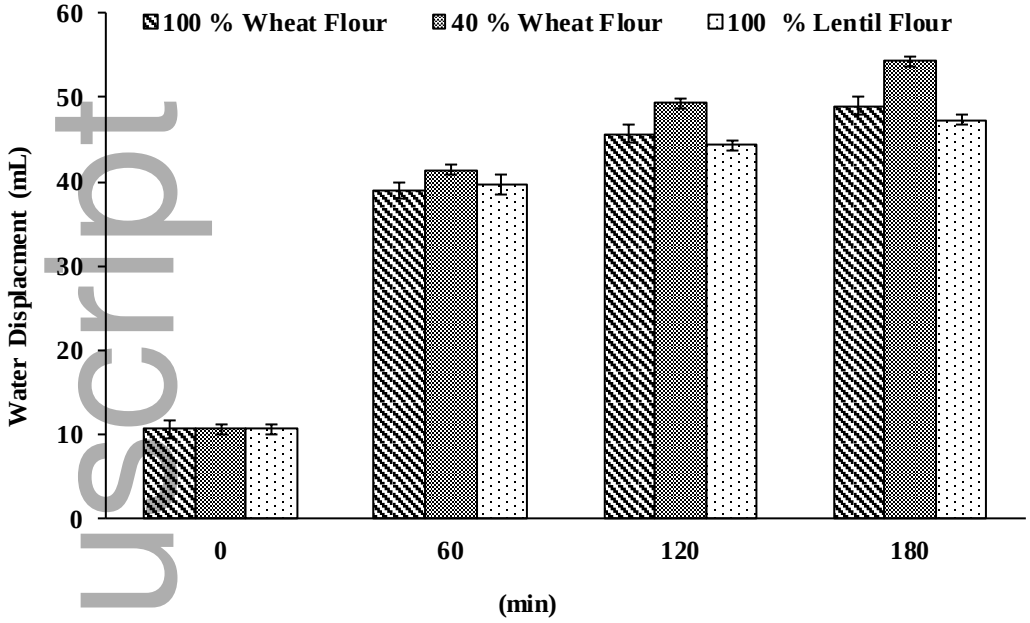

Figure.1. CO_2 production during fermentation of 100% wheat, 60-40% wheat-lentil, and 100% whole lentil flour.

(Figure 2)

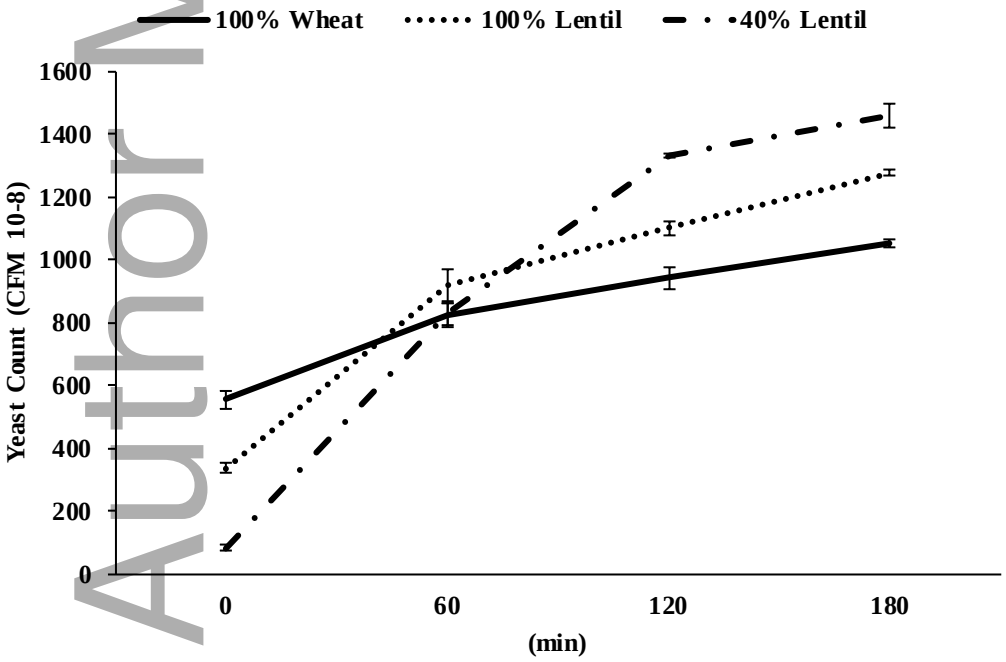

Figure. 2. Cell growth during fermentation of 100% wheat, 60-40% wheat-lentil, and 100% whole lentil flour.

(Figure 3)

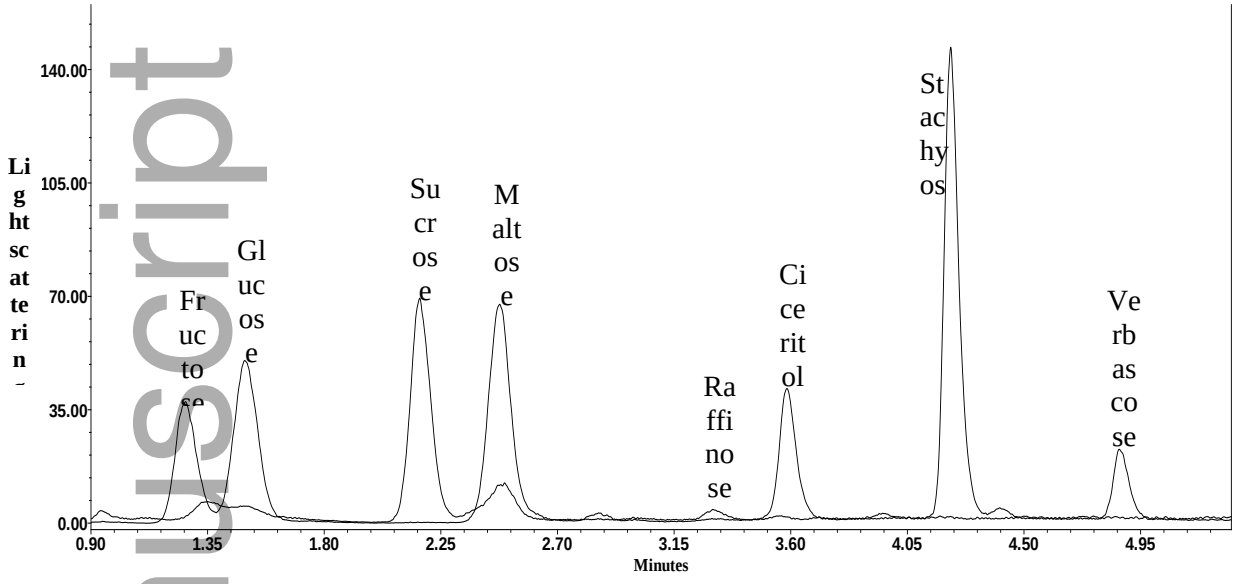

Figure. 3. Chromatogram of the sugar profile of wheat flour; full line and lentil flour; broken line analyzed by UPLC/ELS.

(Figure 4)

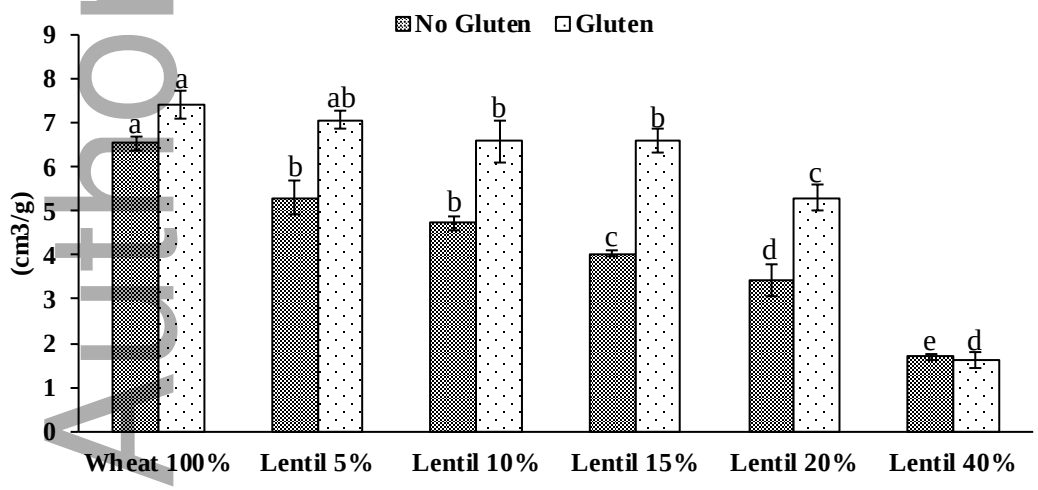

Figure. 4. Specific loaf volume of baked breads with and without gluten. Letters that are the same are not significantly different, at ($P < 0.05$).

(Figure 5)

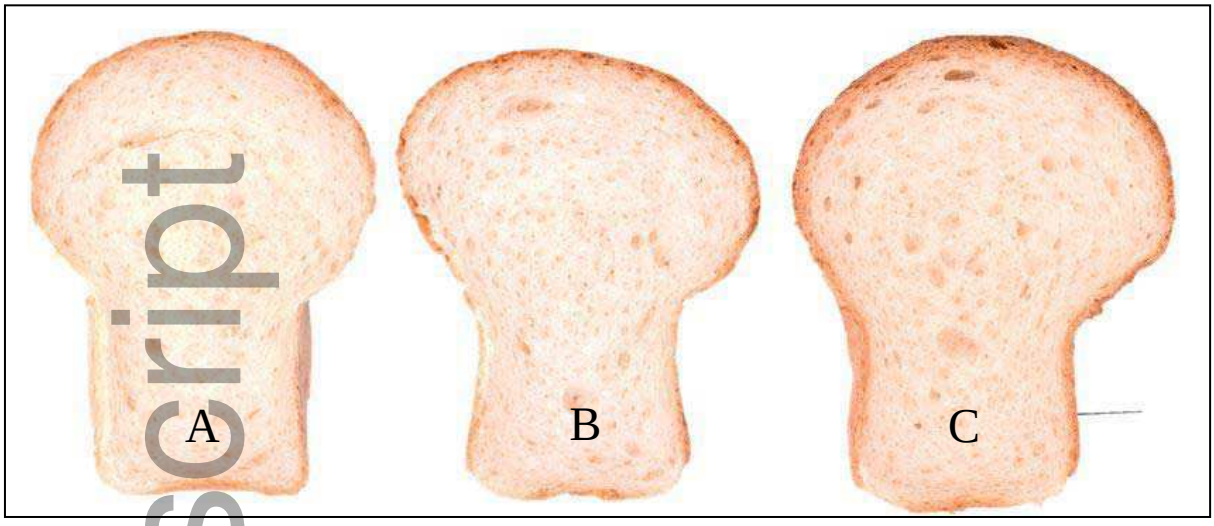

Figure. 5. A; 100% wheat, Volume 6.53 cm^3/g, protein 13.8g. B; 15% lentil, Volume 4.44 cm^3/g, protein 15.7g and C; 15% lentil + Gluten, Volume 6.59 cm^3/g, protein 20.2g.

(Figure 6)

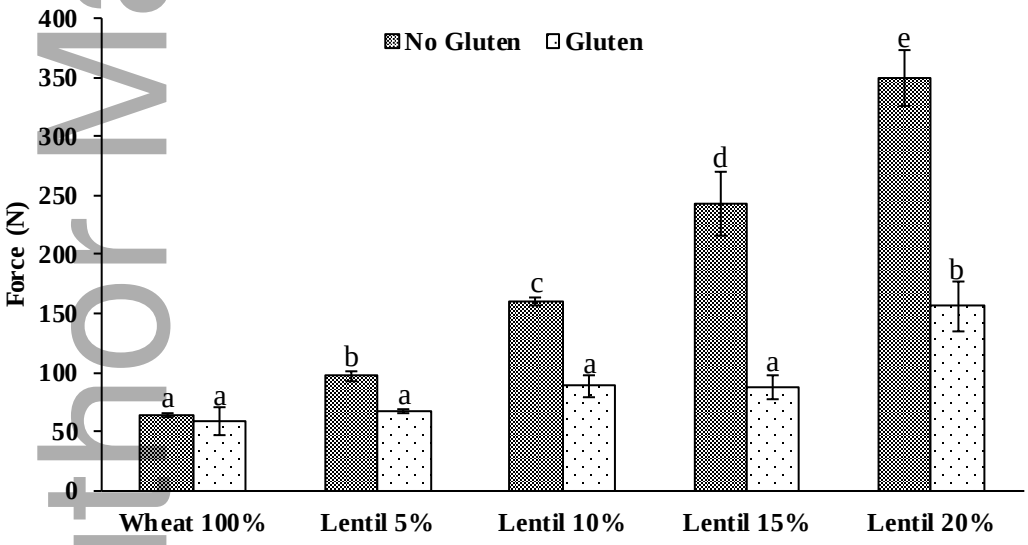

Figure. 6. Crumb texture of baked breads blends with and without gluten. Letters that are the same are not significantly different, at ($P < 0.05$).