ORIGINAL ARTICLE



An investigation of functional quality characteristics and water interactions of navy bean, chickpea, pea, and lentil flours

Revised: 11 December 2021

Ozan Tas¹ | Ulku Ertugrul¹ | Leonid Grunin^{2,3} | Mecit Halil Oztop¹

¹Department of Food Engineering, Middle East Technical University, Ankara, Turkey

²Department of Physics, Volga State University of Technology, Yoshkar-Ola, Mari El, Russian Federation

³Resonance Systems GmbH, Kirchheim unter Teck, Germany

Correspondence

Mecit Halil Oztop, Department of Food Engineering, Middle East Technical University, Ankara, Turkey. Email: mecit@metu.edu.tr

Funding information Middle East Technical University, Grant/Award Number: TEZ-D-314-2020-10304

Abstract

Legume flours are great sources of protein, dietary fiber, starch, minerals, and vitamins. In recent years, the utilization of different legume flours in food systems has gained attention due to their sustainable and functional properties. This study aimed to characterize and examine the water interactions of different legume flours: navy bean, chickpea, pea, and lentil. For this purpose, in addition to the standard techniques (proximal analysis, Fourier transform infrared, protein solubility, and water solubility/absorption index), time-domain nuclear magnetic resonance (TD-NMR) relaxometry was also performed to explain the molecular interactions in the flours. Based on the results, carbohydrate and protein content of legume flours varied from 67.44 to 72.23 (g/100 g dw) and 23.19 to 27.03 (g/100 g dw) with low fat (0.86-5.44 [g/100 g dw]) and moisture content (6.01-8.14 [g/100 g dw]). Despite the slight differences in their compositions being small, moisture, protein, and carbohydrate contents influenced flour-water interactions. Thus, flour-water mixtures were assessed, and findings showed that water solubility index (WSI) followed the order: chickpea > lentil > navy bean > pea, whereas water absorption index (WAI) followed the order: pea > navy bean > lentil > chickpea. T₂ relaxation times measured by NMR and protein solubility results were also in accordance with these results. The results of this study demonstrated that legume flours that were investigated offered potential for commercial applications. Because various food applications require different flour-water interactions, a suitable flour can be selected by considering these results.

KEYWORDS

flour-water interaction, FTIR, legume flours, proximal analysis, TD-NMR relaxometry

INTRODUCTION 1

Legumes are dicotyledonous seeds of the plants that belong to the family of Fabaceae (or Leguminosae). They are worthwhile crops that can adapt in many lands. Many different edible dry legumes are planted and consumed all over the world (Du et al., 2014). Legumes also have beneficial effects on the environment because they improve soil quality through nitrogen fixation and they are relatively

inexpensive (Semba et al., 2021). The most well-known dry legumes comprise bean, soybean, pea, chickpea, and lentil (Kamboj & Nanda, 2018). In addition, legumes are excellent sources of proteins, rich in essential amino acids, dietary fiber, complex carbohydrates, vitamins, and minerals, as well as include biologically active components such as folic acid and polyphenols (Teterycz & Sobota, 2020). Especially, they are high in carbohydrate (60%-70%) and protein (10%-30%) content (Kumar & Pandey, 2020; Ratnawati et al., 2019).

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. Legume Science published by Wiley Periodicals LLC.

└WILEY_<mark>Legume Science</mark>

Moreover, legumes have been shown to play a significant role in reducing cancer risk and heart diseases, lowering type 2 diabetes, increasing satiety, and thereby reducing obesity (Polak et al., 2015).

Legume flours are known to fortify the nutritional value of foods, and they can be utilized in a food formulation if the functional properties are well-known. They have excellent functional properties such as solubility, gelling, foaming, and emulsifying activity, as well as flavor, water, and oil binding capacity (Mani-López et al., 2021). These properties of legume flours are influenced by the components, especially the proteins, fat, moisture, carbohydrate, and ash (Awuchi & Ogueke, 2019).

Hydration has a critical impact on the functional properties because higher interaction of the solid material in the aqueous phase may lead to synergistic effects on those properties at certain conditions. However, besides being very significant in many food applications, it is quite complicated to explain the mechanism underlying water interactions with the grains or their flours (Miano & Augusto, 2018).

Legume flours have constituents that interact very well with water. This type of interaction is called as water binding, water holding capacity, or water hydration, which are highly important in many food applications (Gharsallaoui et al., 2008). For instance, the quality of a dough is affected by the mechanical, rheological, textural, and sensorial properties, and the flour-water interactions have a significant contribution in controlling these properties (Rehman & Sharif, 2018; Sanjeewa et al., 2010). Studies showed that freshness maintenance, high elasticity, and firm consistency of a baked product can easily be obtained by flour having high water holding capacity (Fu et al., 2016; Kohajdová et al., 2013). In addition, other functional properties such as emulsification activity, foaming, and gelation are strongly affected by flour-water interactions in the processes that nonenzymatic browning reactions occur (frying and roasting) (Ertugrul et al., 2021; Tas et al., 2021; Uysal et al., 2009). The flour-water interaction may also give an idea about the design of a food package and the shelf-life of the products (Aristilde et al., 2017).

Time-domain nuclear magnetic resonance (TD-NMR) relaxometry is a rapid analytical method that can be applied to the materials in the solid or liquid state by considering the population of the mobile protons in the materials (Rodríguez-Alonso et al., 2019). With the help of TD-NMR relaxometry, the physical and chemical properties of the samples can be obtained in a wide range. TD-NMR relaxometry can be used to determine the crystallinity of the samples in solid state through different approaches such as spin-lattice (T₁) relaxation times and magic sandwich echo (MSE) sequence (Berk et al., 2021; Grunin et al., 2019). Furthermore, the qualitative or quantitative analysis of food components such as water, proteins, and fats could be performed (Kirtil et al., 2017). Besides, the TD-NMR relaxometry approach could be a valuable alternative to understand the various legume flour-water interactions. The analysis of spin-spin (T₂) relaxation time can give practical information regarding the hydration behavior of material because T₂ time changes with respect to the population of free water in the system (Ozel et al., 2017). Thus, TD-NMR relaxometry could be performed to analyze the hydration behavior of

different legume flours by considering the changes in the T₂ relaxation times

In this study, selected legume flours, navy bean, pea, chickpea, and lentil, have been studied. The main objectives of this study were to characterize these flours, examine their interactions with water, and explain some of these properties through TD-NMR relaxometry.

2 MATERIALS AND METHODS

2.1 Materials

Legume flours were purchased from Smart Kimya Tic. Dan. Ltd. Sti. (Izmir, Turkey). All chemicals were purchased from Sigma Aldrich Co. (St Louis, MO, USA).

2.2 Chemical composition

Proximal analysis of the flours was carried out for macronutrients (ash, proteins, fat, and carbohydrates) and moisture by following AACC Methods (AACC, I., 2000). The moisture content of the flours was measured by Karl-Fisher Titration (Hach Company, Loveland, Colorado). The modified Kjeldahl method was evaluated to determine the total protein content of the flours by $N \times 6.25$ (ASTM Standard & E258., 2007). The ash content of the samples was found by incineration at 550 ± 15°C (Thiex et al., 2012). The fat content of the flours was measured with Soxhlet apparatus (EFLAB) by the extraction of the powdered sample with a known weight using hexane as the solvent (Zhao & Zhang, 2013). Total carbohydrates value was calculated by the following formula:

Total carbohydrates $(g/100 g dw) = 100 - (m_{ash} + m_{protein} + m_{fat})$ (1)

2.3 Water solubility index and water absorption index

A modified method (Yousf et al., 2017) was conducted to determine the water solubility index (WSI) and water absorption index (WAI) of flours. Flours were dissolved in distilled water with a ratio of 1:4 and then mixed by an orbital shaker (Daihan Scientific Co., Ltd., Korea) at 200 rpm for 24 h to achieve ideal hydration. The solutions were centrifugated at 4000 rpm for 15 min. The supernatant and the sediment were carefully separated and weighted. WSI and WAI were calculated by the following equations:

$$WAI = \frac{\text{weight of sediment}}{\text{weight of flour}}$$
(2)

$$WSI = \frac{\text{weight of dry solid in supernatant}}{\text{weight of flour}}$$
(3)

2.4 | Protein solubility by Lowry method

The soluble protein content of legume flours was determined by the Lowry method (Lowry et al., 1951) with some modifications. The experiment was conducted on supernatants collected after centrifugation of 5% (w/v) flour solutions at 4000 rpm for 5 min. The supernatant and Lowry reagent were mixed at a ratio of 1:5 and kept at room temperature for 20 min. Later, 250- μ l Folin reagent was added to the mixture. After stirring gently, the final mixture was kept in the dark for 30 min, and absorbance was measured at 750 nm using a spectrophotometer (Optizen Pop Nano Bio, Mecasys Co., Ltd., Korea). Bovine serum albumin (BSA) stock solution at different concentrations (0.03125–1 g/L) was used to prepare the calibration curve.

2.5 | Crystallinity by spin-lattice (T_1) relaxation time and MSE

The flours in powder form were analyzed by both the MSE sequence and saturation recovery sequence of T_1 relaxation time. T_1 relaxation times and crystallinity (%) were analyzed via a 0.5 T (20.34 MHz) benchtop TD-NMR system using the special modules in RELAX 8 software (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany). For measurements, the relaxation period, time of observation, and the number of scans were set as 10^3 ms, 10^6 ms, and 1, respectively.

2.6 | Fat content by TD-NMR relaxometry

The fat content of legume flours was measured using a 0.5-T (20.34 MHz) benchtop TD-NMR system (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany). Measurements were conducted on flours in powder form and Hahn-echo sequence with a repetition time of 300 ms, and 128 scans. MATLAB (R2019b, The MathWorks Inc., USA) was used to analyze the acquired signal.

2.7 | Structural analysis by FTIR spectroscopy

Fourier transform infrared (FTIR) analysis was conducted on legume flours using an IR Affinity-1 spectrometer (Shimadzu Corporation, Kyoto, Japan). An attenuated total reflectance (ATR) accessory was attached to the sample compartment. The spectrum within a 600– 4000 cm⁻¹ range was acquired at a resolution of 4 cm⁻¹ at room temperature. LabSolutions IR software (Shimadzu Corporation, Kyoto, Japan) was used to analyze the baseline-normalized spectrum.

2.8 | Hydration behavior by TD-NMR relaxometry

The same flour-distilled water solution (1:4 ratio) prepared for WSI and WAI analysis was used to investigate hydration behavior. T_2 relaxation times were measured via a 0.5-T (20.34 MHz) benchtop TD-NMR system (Spin Track, Resonance Systems GmbH, Kirchheim/ Teck, Germany). Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was utilized, and acquisition parameters, echo time, number of echoes, and number of scans were selected as 1000 ms, 300–500 ms, and 1, respectively. MATLAB (R2019b, The MathWorks Inc., USA) was used to calculate the relaxation times by considering a mono-exponential behavior.

$$M_{xy}(t) = M_0 \left(e^{-\frac{t}{T_2}} \right) \tag{4}$$

2.9 | Statistical analysis

Multifactor analysis of variance (ANOVA) was performed through MINITAB (Version 19, Minitab Inc., Coventry, UK) to analyze the data having three replicates. To determine the significance level, Tukey's comparison test at p < 0.05 was used.

3 | RESULTS AND DISCUSSION

3.1 | Proximal analysis

The proximate composition analysis of the flours is crucial in many food applications because it may give information regarding the nutritional quality and technological developments of a food product (Cardoso et al., 2019). The proximal analysis of the navy bean, chickpea, pea, and lentil flours is given in Table 1.

According to the results, the moisture content values ranged from 6.01 to 8.14 (g/100 g dw) for the flours. The moisture content in chickpea and navy bean flours was significantly higher and followed by lentil and pea flour, respectively (p < 0.05). In the literature, similar

TABLE 1 Proximal analysis of different types of legume flours

Flours	Moisture (g/100 g dw)	Ash (g/100 g dw)	Protein (g/100 g dw)	Fat (g/100 g dw)	Carbohydrate (g/100 g dw)
Navy bean	8.11 ± 0.01 ^a	2.54 ± 0.01^{d}	23.19 ± 0.29 ^c	2.01 ± 0.17^{b}	72.23 ± 0.2^{a}
Chickpea	8.14 ± 0.01^{a}	3.1 ± 0.02^{c}	23.67 ± 0.17 ^c	5.44 ± 0.05^{a}	67.69 ± 0.16 ^b
Pea	$6.01 \pm 0.02^{\circ}$	4.87 ± 0.02^{a}	25.03 ± 0.26 ^b	$1.54 \pm 0.05^{\circ}$	67.83 ± 0.24^{b}
Lentil	6.45 ± 0.02^{b}	4.59 ± 0.01^{b}	27.03 ± 0.12^{a}	0.86 ± 0.05^{d}	67.44 ± 0.14^{b}

Note: Values are expressed in dry weight (dw) as mean \pm SE (n = 3). In each column, different letters represent significant differences (p < 0.05).

results are reported for these flours, as well (Ladjal Ettoumi & Chibane, 2015; Sumargo et al., 2016). It is important to have information about the moisture content of a food material because it is a critical parameter that determines storage conditions: Lower values will lead a longer shelf-life in the product (Hădărugă et al., 2016). Besides, the moisture content of flour may play an important role in hydration behavior.

The ash content of the flours ranged from 2.54 to 4.87 (g/100 g dw). Pea flour had the highest value, followed by lentil, chickpea, and navy bean flours, respectively (p < 0.05). These results also matched the reported values for legume flours in the literature (Khattab et al., 2009).

The protein content of the flours ranged between 23.19 and 27.03 (g/100 g dw). Lentil flour had the highest value, followed by pea, chickpea, and navy bean flours, respectively (p < 0.05). In most of the studies, legume flours are reported to contain high amounts of protein, and these proteins are generally classified as excellent high-quality plant protein (Kavitha & Parimalavalli, 2014; Khattab et al., 2009; Ladjal Ettoumi & Chibane, 2015). As can be seen from the results, these four different legume flours also contain high amounts of protein, and they can be interpreted in food formulations to fortify the food product. Furthermore, the proteins are unique molecules and are interacting with water molecules differently. Thus, they play an essential role in both the hydration and solubility of the flours in the solutions.

Fats are also important because they are a source of essential fatty acids and energy (Di Pasquale, 2009). In Table 1, the fat content of the flours was shown to range from 0.86 and 5.44 (g/100 g dw). The fat content in chickpea flour was much higher among the others, and it was followed by navy bean, pea, and lentil flours, respectively (p < 0.05). It was also stated in the literature that chickpea flour had higher fat content and fatty acids such as oleic and linoleic acid than other legume flours such as navy bean, pea, and lentil, so our results also confirmed these findings (Jukanti et al., 2012).

The results of the proximal analysis of these four legume flours showed that they are rich in carbohydrates. The carbohydrate content of the flours ranged from 67.44 to 72.23 (g/100 g dw). According to statistical analysis, navy bean flour had the highest carbohydrate content, and the other three flours had almost the same amount (p < 0.05). Carbohydrate content of the legume flours was stated to be around 60% or higher in which the main component is starch (Cardoso et al., 2019; Jahreis et al., 2016; Ladjal Ettoumi & Chibane, 2015). In this research, findings for these flours were also similar to reported studies. Because carbohydrates contain compounds like starch, which is interacting with water molecules, they have a great impact on the water and flour interaction in the solutions, as well.

In general, the proximal analysis showed that these legume flours have different chemical compositions, and the results also matched with the reported studies. Moreover, these components and their differences play a key role in the flour and water interactions, and they needed to be evaluated.

3.2 | WSI, WAI, protein solubility, and hydration behavior

Solubility of different legume flours is particularly important for exploring the flour-water interactions and gathering the necessary information for further utilization (Jogihalli et al., 2017). Therefore, the WSI of four different legume flours was studied and reported in Table 2. Statistical analysis proved that the WSI of these flours was significantly different (p < 0.05). The results indicated that pea flour had the lowest WSI, followed by navy bean, lentil, and chickpea flours.

Another way to observe the water-flour interaction deeper is by investigating the WAI of these legume flours. WAI can be defined as the ability of a product to absorb and retain water within its matrix under an external force, and like WSI, it is another approach to observe the flour-water interactions thoroughly (Yousf et al., 2017). Thus, the WAI of flours was also investigated and given in Table 2. According to the results, the highest WAI was observed in pea flour, followed by navy bean, lentil, and chickpea flours (p < 0.05). The opposite relationship between WSI and WAI can be explained by the soluble protein content and insoluble starch content of the flours. In literature, legume flours generally contain 40%-50% starch and 20%-25% protein, and the soluble protein content of the legume flours is the main contributors of WSI because remaining components are mostly insoluble starches, fibers, and fats (Laleg et al., 2016; Morad et al., 1980; Teterycz et al., 2020). When protein solubility and WSI results were compared, the lowest soluble protein content and WSI was observed in pea flour, indicating that both phenomena are interrelated. Furthermore, this claim can be supported by the positive Pearson correlation between WSI and soluble protein content of the flours with a correlation coefficient of 0.946 (p < 0.05). On the other hand, WAI of the flours is mainly affected by the starch content

TABLE 2 T₂ relaxation times, water solubility index (WSI), water absorption index (WAI), and soluble protein content of legume flours

Flours	T ₂ (ms)	Water solubility index (WSI) (w/w)	Water absorption index (WAI) (w/w)	Soluble protein content (%(w/w))
Navy bean	125.29 ± 3.32^{b}	$2.90 \pm 0.01^{\circ}$	2.09 ± 0.01^{b}	11.60 ± 0.02^{b}
Chickpea	100.36 ± 0.14^{c}	3.35 ± 0.002^{a}	1.65 ± 0.001^{d}	12.80 ± 0.01 ^a
Pea	166.72 ± 1.01^{a}	1.9 ± 0.01^{d}	3.20 ± 0.001^{a}	4.49 ± 0.01^{d}
Lentil	119.43 ± 4.82 ^b	3.28 ± 0.01^{b}	1.73 ± 0.001 ^c	10.78 ± 0.02 ^c

Note: Values are expressed as mean \pm SE (n = 3). In each column, different letters represent significant differences (p < 0.05).

because starch is the most considerable portion in insoluble content, and there is no other major component to compete with starch in water absorption (Eliasson, 1983; Rampersad et al., 2003). The competition between protein and starch can be diminished only after achieving optimal water content because proteins can be distributed evenly and oriented broadly in water with covalent, hydrophobic, ionic, and hydrogen bonds, whereas starch granules can absorb water freely and easily only if there is enough water in such a system (Olu-Owolabi et al., 2011; Schopf & Scherf, 2021). In this study, it can be stated that optimal water content was achieved due to the clear distinction between WSI and WAI results.

The water-flour interactions can be further explained by TD-NMR relaxometry because T₂ relaxation times can give precise, detailed, and valuable information about the dynamic properties of water in a food system (Goetz & Koehler, 2005; Kirtil & Oztop, 2016; Narin et al., 2020). As shown in Table 2, T₂ relaxation times of flours are significantly different (p < 0.05). Also, there is a strong correlation between WSI and T₂ relaxation times of flours with a correlation coefficient of -0.957 (p < 0.05). A negative correlation between these two results is anticipated because as WSI increases, the free water content in the system decreases, and thus, the relaxation time decreases (Kirtil & Oztop, 2016). Therefore, flours with higher WSI were expected to have lower T₂ relaxation times. According to the results, pea flour, which had the lowest WSI, had the longest relaxation time and is followed by navy bean, lentil, and chickpea flours (p < 0.05). Thus, these results showed that legume flour-water interactions could be easily studied via TD-NMR relaxometry.

3.3 | Characterization of legume flours

3.3.1 | Crystallinity

As stated in the proximal analysis, the legume flours contain a high amount of carbohydrates, and the main component in the carbohydrates is starch. Starch is a semi-crystalline carbohydrate polymer, and it has diverse properties like the degree of crystallinity (Kaptso et al., 2016).

In this study, as an alternative to classical methods like X-ray diffraction, TD-NMR relaxometry was performed on the navy bean, chickpea, pea, and lentil flours in powder form, and the results are shown in Table 3. Besides providing useful information regarding free water molecules in the system, spin–lattice (T₁) relaxation times were shown to be also used to characterize the crystal structure of the samples in the solid state. The reported studies showed that longer T₁ values would be associated with a more crystalline structure in the sample (Ilhan et al., 2020; Le Botlan et al., 1998). Moreover, to make a more accurate comparison for crystallinity through T₁ relaxation times, the moisture content of the flours should also be taken into consideration. The moisture content of the flours was shown in proximal analysis that navy bean and chickpea flours had higher values and were followed by lentil, and pea flour, respectively (p < 0.05). According to the T₁ relaxation time results, the values ranged from

TABLE 3 T₁ relaxation times (ms) and crystallinity (%) by magic sandwich echo (MSE) of the flours in powder forms

Flours	T ₁ relaxation time (ms)	Crystallinity (%) by MSE sequence
Navy bean	68.5 ± 0.42^{b}	80.9 ± 0.01^{b}
Chickpea	69.63 ± 0.41 ^b	$71.28 \pm 0.08^{\circ}$
Pea	77.46 ± 0.26^{a}	88.01 ± 0.06 ^a
Lentil	77.12 ± 1.03 ^a	88.07 ± 0.03 ^a

Note: Values are expressed as mean \pm SE (n = 3). In each column, different letters represent significant differences (p < 0.05).

68.5 to 77.46 (ms) for the flours. Pea and lentil flour had the same and higher T₁ values than chickpea and navy bean flours, respectively (p < 0.05). By considering the results, it can be concluded that although lentil flour had higher moisture content than pea flour, their T₁ relaxation times were statistically the same, indicating that lentil flour is more crystalline than pea flour and the higher crystallinity had more effect on the T₁ relaxation time compared with moisture content. On the other hand, the chickpea and navy bean flours had the same moisture content, and their T_1 times were also found to be the same and lower than the pea and lentil flours, which also confirmed that as the moisture content in the system increased, T₁ relaxation times decreased. In addition to the effect of moisture content, the reason for having different crystallinity values may also be explained by the fact that legume starches have a different proportion of amylopectin chains, which is the main factor that is responsible for the crystallinity (Singh et al., 2008). Literature studies have shown that the differences in the amylopectin chains of lentil, navy bean, pea, and chickpea starches obtained from their flours may also affect the crystallinity of these flours. Our results also confirm the literature findings (Hoover & Ratnayake, 2002; Huang et al., 2007; Siva et al., 2019).

In this study, MSE, a nonconventional TD-NMR sequence, was also performed to analyze the crystallinity (%) of these flours. In TD-NMR, the solid and liquid fractions can be detected with the free induction decay (FID) sequence in a sample. FID is based on a single 90° radiofrequency (RF) pulse (Musse et al., 2010). However, FID may not be able to detect all the signals coming from the solid fraction accurately because of the dead time that is the time required for the first data to be obtained (Papon et al., 2011). On the other hand, the MSE sequence performs refocusing the signal in the initial part of the FID and does not require any ringing time (Grunin et al., 2019). Hence, all the signals coming from the solid part can be obtained more accurately. That is why in this study, an MSE sequence was performed to determine the crystallinity (%) of these flours.

According to the MSE results, crystallinity (%) was found to be the highest in lentil and pea flours, followed by navy bean and chickpea flours (p < 0.05). Also, the Pearson correlation analysis was performed between MSE and T₁ values, and a positive correlation with the coefficient of 0.799 (p < 0.05) was obtained. Hence, the TD-NMR approach can be performed to characterize the crystallinity because it provided a much easier and shorter experiment.

3.3.2 | Fat content by TD-NMR relaxometry

In this study, the fat content of the flours was determined by Soxhlet extraction, as well as TD-NMR relaxometry. Although for the determination of fat content, Soxhlet extraction, which is a well-known method in the food industry, would be sufficient, TD-NMR relaxometry was also preferred to indicate that a nondestructive, chemical-free method (TD-NMR) with short operating time (Yildiz et al., 2018) can be used rather than an expensive, hazardous, and time-consuming method (Soxhlet extraction) which results in a large amount of solvent use and waste (Danlami et al., 2014).

Hahn-echo (HE) sequence used in this study is composed of 90° and 180° RF pulses with a waiting time in between (Lee et al., 2021). The signal obtained after 180° RF pulse represents the signal coming from fat only since the signal coming from the free water will decay before acquiring the fat signal (within a few microseconds) (Todt et al., 2006, 2008). Therefore, the intensity of the signal could be a way to measure the fat content of the flours after preparing a calibration curve.

TABLE 4 Fat contents of legume flours determined by timedomain nuclear magnetic resonance (TD-NMR) relaxometry

Flours	Fat content (%)
Navy bean	$1.38 \pm 0.01^{\circ}$
Chickpea	5.54 ± 0.04^{a}
Pea	1.97 ± 0.02^{b}
Lentil	0.96 ± 0.01^{d}

Note: Values are expressed as mean \pm SE (n = 3). Different letters represent significant differences (p < 0.05).



FIGURE 1 Fourier transform infrared (FTIR) spectra of the studies flours

Based on the results represented in Table 4, the fat contents of the flours were found to be significantly different (p < 0.05). The fat contents of the flours ranged from 1.38 to 5.54 (%), and among these flours, chickpea flour had the highest fat content, followed by pea, navy bean and lentil flours (p < 0.05). When fat contents of these flours obtained by TD-NMR relaxometry were compared with fat contents obtained by Soxhlet extraction, there is found to be a strong correlation between these results with a correlation coefficient of 0.977 (p < 0.05). Furthermore, a calibration curve (y = -5.2205x + 1.9454, $R^2 = 0.954$) was obtained by Soxhlet extraction and TD-NMR relaxometry results. The linear relationship between these fat contents showed that TD-NMR relaxometry could be considered a powerful and highly accurate technique for fat content analysis.

3.3.3 | Structural analysis by FTIR spectroscopy

FTIR spectroscopy is a widely used technique to identify the functional groups and structural changes in several food products (Ahmad & Benjakul, 2011), and in this study, it was used to observe the structural differences among different legume flours. Figure 1 shows several important peaks in the FTIR spectrum, which can help to identify the protein, fat, and carbohydrate present in the flours. The first peak that can be detected is at the range of 1000– 1100 cm⁻¹, which is a typical peak for polysaccharides, and this peak indicates the coupling of the C—O or the C—C stretching modes (Guerrero et al., 2013). Furthermore, the intensity of this peak suggests a relative estimation of the polysaccharide content in a system, and the highest intensity belonged to this peak, indicating that the flours consist of mostly polysaccharides.

The peak observed around 1700 cm^{-1} provides information about the C=O stretching modes of fats (Silva et al., 2014), and as can be seen from the figure, chickpea flour had the highest and lentil flour had the lowest intensity. These results were in accordance with the fat contents found in the proximate analysis of the flours. Moreover, Amide I (~1600 cm⁻¹), Amide II (~1500 cm⁻¹), and Amide A (~3300 cm⁻¹) bands, where C=O, C–N, and N–H stretching modes of proteins are observed, can be easily detected from the FTIR spectrum (Demir et al., 2015; Diblan et al., 2018; Guerrero et al., 2013). Overall, these peaks provide information regarding the polysaccharides, fat, and protein in the flours.

4 | CONCLUSION

Legume flours are a great macro and micronutrient source, beneficial to human health, and have a great potential in several food applications due to their functional properties. Although flour-water interaction is a key parameter to understand the functional properties indepth, this interaction has not been thoroughly explored. In this study, navy bean, chickpea, pea, and lentil flours were characterized, and their interactions with water were studied. Furthermore, TD-NMR relaxometry was utilized as an alternative technique. Proximal analysis showed that these flours are rich in carbohydrates and proteins but low in fat and moisture content. Results obtained from TD-NMR relaxometry regarding crystallinity and fat content supported these results. Besides, the proximal analysis results were taken into further consideration to evaluate flour-water interactions.

Flour-water interactions were investigated in many aspects, and different behaviors were observed. It was noticed that the WSI was highest in chickpea flour but lowest in pea flour, whereas navy bean and lentil had a moderate WSI. On the other hand, the highest WAI belonged to pea flour, followed by navy bean, lentil, and chickpea. Thus, the findings of this study may provide a practical means to fortify legume flours in different food formulations depending on the intended use. Also, TD-NMR relaxometry confirmed the results, so this approach might be considered a chemical-free and short operating method to investigate the flourwater interactions.

ACKNOWLEDGMENT

This study was funded by the Middle East Technical University Scientific Research Projects Coordination Unit with project number TEZ-D-314-2020-10304.

CONFLICT OF INTEREST

All the authors have approved the manuscript and agree with the submission. There are no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

ORCID

Ozan Tas ^D https://orcid.org/0000-0001-6421-5092 Ulku Ertugrul ^D https://orcid.org/0000-0003-0142-5054 Leonid Grunin ^D https://orcid.org/0000-0001-6195-042X Mecit Halil Oztop ^D https://orcid.org/0000-0001-6414-8942

REFERENCES

- AACC, I. (2000). Approved methods of the AACC. Association of Cereal Chemists.
- Ahmad, M., & Benjakul, S. (2011). Impact of legume seed extracts on degradation and functional properties of gelatin from unicorn leatherjacket skin. *Process Biochemistry*, 46(10), 2021–2029. https:// doi.org/10.1016/j.procbio.2011.07.018
- Aristilde, L., Galdi, S. M., Kelch, S. E., & Aoki, T. G. (2017). Sugar-influenced water diffusion, interaction, and retention in clay interlayer nanopores probed by theoretical simulations and experimental spectroscopies. *Advances in Water Resources*, 106, 24–38. https://doi.org/10.1016/j. advwatres.2017.03.014
- ASTM Standard E258. (2007). Standard test method for total nitrogen in organic materials by modified Kjeldahl. ASTM International, 1–4. https://doi.org/10.1520/E0258-07.2
- Awuchi, C. G., & Ogueke, C. (2019). Evaluation of patulin levels and impacts on the physical characteristics of grains. *International Journal* of Advanced Academic Research (IJAAR), 5(4), 10–25.
- Berk, B., Grunin, L., & Oztop, M. H. (2021). A non-conventional TD-NMR approach to monitor honey crystallization and melting. *Journal of Food Engineering*, 292(May 2020), 110292. https://doi.org/10.1016/j. jfoodeng.2020.110292

- Cardoso, R. V. C., Fernandes, Â., Heleno, S. A., Rodrigues, P., Gonzaléz-Paramás, A. M., Barros, L., & Ferreira, I. C. F. R. (2019). Physicochemical characterization and microbiology of wheat and rye flours. *Food Chemistry*, 280(September 2018), 123–129. https://doi.org/10.1016/j. foodchem.2018.12.063
- Danlami, J. M., Arsad, A., Zaini, M. A. A., & Sulaiman, H. (2014). A comparative study of various oil extraction techniques from plants. *Reviews in Chemical Engineering*, 30(6), 605–626. https://doi.org/10.1515/revce-2013-0038
- Demir, P., Onde, S., & Severcan, F. (2015). Phylogeny of cultivated and wild wheat species using ATR-FTIR spectroscopy. Spectrochimica Acta
 Part a: Molecular and Biomolecular Spectroscopy, 135, 757–763. https://doi.org/10.1016/j.saa.2014.07.025
- Di Pasquale, M. G. (2009). The essentials of essential fatty acids. Journal of Dietary Supplements, 6(2), 143–161. https://doi.org/10.1080/ 19390210902861841
- Dıblan, S., Kadiroğlu, P., & Aydemir, L. Y. (2018). Ft-Ir spectroscopy characterization and chemometric evaluation of legumes extracted with different solvents. *Food and Health*, 4(2), 80–88. https://doi.org/10. 3153/fh18008
- Du, S., Jiang, H., Yu, X., & Jane, J. L. (2014). Physicochemical and functional properties of whole legume flour. LWT - Food Science and Technology, 55(1), 308–313. https://doi.org/10.1016/j.lwt.2013.06.001
- Eliasson, A.-C. (1983). Differential scanning calorimetry studies on wheat starch—Gluten mixtures: I. Effect of gluten on the gelatinization of wheat starch. *Journal of Cereal Science*, 1(3), 199–205. https://doi.org/ 10.1016/S0733-5210(83)80021-6
- Ertugrul, U., Namli, S., Tas, O., Kocadagli, T., Gokmen, V., Sumnu, S. G., & Oztop, M. H. (2021). Pea protein properties are altered following glycation by microwave heating. *Lebensmittel-Wissenschaft & Technologie*, 150, 111939. https://doi.org/10.1016/j.lwt.2021.111939
- Fu, Z., Che, L., Li, D., Wang, L., & Adhikari, B. (2016). LWT–Food science and technology effect of partially gelatinized corn starch on the rheological properties of wheat dough. LWT - Food Science and Technology, 66, 324–331. https://doi.org/10.1016/j.lwt.2015.10.052
- Gharsallaoui, A., Rogé, B., Génotelle, J., & Mathlouthi, M. (2008). Relationships between hydration number, water activity and density of aqueous sugar solutions. *Food Chemistry*, 106(4 SPEC. ISS), 1443–1453. https://doi.org/10.1016/j.foodchem.2007.02.047
- Goetz, J., & Koehler, P. (2005). Study of the thermal denaturation of selected proteins of whey and egg by low resolution NMR. *LWT - Food Science and Technology*, 38(5), 501–512. https://doi.org/10.1016/j.lwt. 2004.07.009
- Grunin, L., Oztop, M. H., Guner, S., & Baltaci, S. F. (2019). Exploring the crystallinity of different powder sugars through solid echo and magic sandwich echo sequences. *Magnetic Resonance in Chemistry*, 57(9), 607–615. https://doi.org/10.1002/mrc.4866
- Guerrero, P., Garrido, T., Leceta, I., & De La Caba, K. (2013). Films based on proteins and polysaccharides: Preparation and physical-chemical characterization. *European Polymer Journal*, 49(11), 3713–3721. https://doi.org/10.1016/j.eurpolymj.2013.08.014
- Hădărugă, D. I., Costescu, C. I., Corpaş, L., Hădărugă, N. G., & Isengard, H.-D. (2016). Differentiation of rye and wheat flour as well as mixtures by using the kinetics of Karl Fischer water titration. *Food Chemistry*, 195, 49–55. https://doi.org/10.1016/j.foodchem.2015. 08.124
- Hoover, R., & Ratnayake, W. S. (2002). Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chemistry*, 78(4), 489–498. https://doi.org/10.1016/S0308-8146(02)00163-2
- Huang, J., Schols, H. A., van Soest, J. J. G., Jin, Z., Sulmann, E., & Voragen, A. G. J. (2007). Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches. *Food Chemistry*, 101(4), 1338–1345. https://doi.org/10.1016/j.foodchem. 2006.03.039

Legume Science

- Ilhan, E., Pocan, P., Ogawa, M., & Oztop, M. H. (2020). Role of 'D-allulose' in a starch based composite gel matrix. *Carbohydrate Polymers*, 228(April 2019), 115373. https://doi.org/10.1016/j.carbpol.2019. 115373
- Jahreis, G., Brese, M., Leiterer, M., Schäfer, U., & Böhm, V. (2016). Legume flours: Nutritionally important sources of protein and dietary fiber. *Ernahrungs-Umschau*, 63(2), 36–42. https://doi.org/10.4455/eu. 2016.007
- Jogihalli, P., Singh, L., Kumar, K., & Sharanagat, V. S. (2017). Physicofunctional and antioxidant properties of sand-roasted chickpea (*Cicer arietinum*). *Food Chemistry*, 237, 1124–1132. https://doi.org/10.1016/ j.foodchem.2017.06.069
- Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L): A review. British Journal of Nutrition, 108(SUPPL. 1), 11–26. https://doi. org/10.1017/S0007114512000797
- Kamboj, R., & Nanda, V. (2018). Proximate composition, nutritional profile and health benefits of legumes—A review. *Legume Research*, 41(3), 325–332. https://doi.org/10.18805/LR-3748
- Kaptso, G. K., Njintang, N. Y., Nguemtchouin, M. G. M., Amungwa, A. F., Scher, J., Hounhouigan, J., & Mbofung, C. M. F. (2016). Characterization of morphology and structural and thermal properties of legume flours: Cowpea (Vigna unguiculata L. Walp) and Bambara groundnut (Vigna subterranea L. Verdc.) varieties. International Journal of Food Engineering, 12(2), 139–152. https://doi.org/10.1515/ijfe-2014-0146
- Kavitha, S., & Parimalavalli, R. (2014). Effect of processing methods on proximate composition of cereal and legume flours. *Journal of Human Nutrition and Food Science*, 2(6), 1051–1055.
- Khattab, R. Y., Arntfield, S. D., & Nyachoti, C. M. (2009). Nutritional quality of legume seeds as affected by some physical treatments, part 1: Protein quality evaluation. LWT - Food Science and Technology, 42(6), 1107–1112. https://doi.org/10.1016/j.lwt.2009.02.008
- Kirtil, E., Cikrikci, S., McCarthy, M. J., & Oztop, M. H. (2017). Recent advances in time domain NMR & MRI sensors and their food applications. *Current Opinion in Food Science*, 17, 9–15. https://doi.org/10. 1016/j.cofs.2017.07.005
- Kirtil, E., & Oztop, M. H. (2016). 1H nuclear magnetic resonance relaxometry and magnetic resonance imaging and applications in food science and processing. *Food Engineering Reviews*, 8(1), 1–22. https:// doi.org/10.1007/s12393-015-9118-y
- Kohajdová, Z., Karovičová, J., & Magala, M. (2013). Effect of lentil and bean flours on rheological and baking properties of wheat dough. *Chemical Papers*, 67(4), 398–407. https://doi.org/10.2478/s11696-012-0295-3
- Kumar, S., & Pandey, G. (2020). Biofortification of pulses and legumes to enhance nutrition. *Heliyon*, 6(3), 4–9. https://doi.org/10.1016/j. heliyon.2020.e03682
- Ladjal Ettoumi, Y., & Chibane, M. (2015). Some physicochemical and functional properties of pea, chickpea and lentil whole flours. *International Food Research Journal*, 22(3), 987–996.
- Laleg, K., Cassan, D., Barron, C., Prabhasankar, P., & Micard, V. (2016). Structural, culinary, nutritional and anti-nutritional properties of high protein, gluten free, 100% legume pasta. *PLoS ONE*, 11(9), 1–19. https://doi.org/10.1371/journal.pone.0160721
- Le Botlan, D., Casseron, F., & Lantier, F. (1998). Polymorphism of sugars studied by time domain NMR. *Analusis*, 26(5), 198–204. https://doi. org/10.1051/analusis:1998135
- Lee, I., Vo, J., Gao, Q., Chang, P., & Swanson, G. (2021). Single-laboratory validation study of a rapid TD-NMR method for quantitation of total fat in sunflower oil powder. *Journal of AOAC INTERNATIONAL*, 104, 1323–1327. https://doi.org/10.1093/jaoacint/qsab022
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1951), 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

- Mani-López, E., Palou, E., & López-Malo, A. (2021). Legume proteins, peptides, water extracts, and crude protein extracts as antifungals for food applications. *Trends in Food Science and Technology*, 112(March), 16– 24. https://doi.org/10.1016/j.tifs.2021.03.035
- Miano, A. C., & Augusto, P. E. D. (2018). The hydration of grains: A critical review from description of phenomena to process improvements. *Comprehensive Reviews in Food Science and Food Safety*, 17(2), 352– 370. https://doi.org/10.1111/1541-4337.12328
- Morad, M. M., Leung, H. K., Hsu, D. L., & Finney, P. L. (1980). Effect of germination on physicochemical and bread-baking properties of yellow pea, lentil, and Faba bean flours and starches. *Cereal Chemistry*, 57(6), 390–396.
- Musse, M., Cambert, M., & Mariette, F. (2010). NMR study of water distribution inside tomato cells: Effects of water stress. *Applied Magnetic Resonance*, 38(4), 455–469. https://doi.org/10.1007/s00723-010-0139-7
- Narin, C., Ertugrul, U., Tas, O., Sahin, S., & Oztop, M. H. (2020). Encapsulation of pea protein in an alginate matrix by cold set gelation method and use of the capsules in fruit juices. *Journal of Food Science*, 85(10), 3423–3431. https://doi.org/10.1111/1750-3841.15433
- Olu-Owolabi, B. I., Afolabi, T. A., & Adebowale, K. O. (2011). Pasting, thermal, hydration, and functional properties of annealed and heatmoisture treated starch of sword bean (*Canavalia gladiata*). International Journal of Food Properties, 14(1), 157–174. https://doi.org/10. 1080/10942910903160331
- Ozel, B., Dag, D., Kilercioglu, M., Sumnu, S. G., & Oztop, M. H. (2017).
 NMR relaxometry as a tool to understand the effect of microwave heating on starch-water interactions and gelatinization behavior. *LWT Food Science and Technology*, *83*, 10–17. https://doi.org/10.1016/j. lwt.2017.04.077
- Papon, A., Saalwächter, K., Schäler, K., Guy, L., Lequeux, F., & Montes, H. (2011). Low-field NMR investigations of nanocomposites: Polymer dynamics and network effects. *Macromolecules*, 44(4), 913–922. https://doi.org/10.1021/ma102486x
- Polak, R., Phillips, E. M., & Campbell, A. (2015). Legumes: Health benefits and culinary approaches to increase intake. *Clinical Diabetes*, 33(4), 198–205. https://doi.org/10.2337/diaclin.33.4.198
- Rampersad, R., Badrie, N., & Commissiong, E. (2003). Physico-chemical and sensory characteristics of flavored snacks from extruded cassava/pigeonpea flour. *Journal of Food Science*, 68(1), 363–367. https://doi.org/10.1111/j.1365-2621.2003.tb14166.x
- Ratnawati, L., Desnilasari, D., Surahman, D. N., & Kumalasari, R. (2019). Evaluation of physicochemical, functional and pasting properties of soybean, mung bean and red kidney bean flour as ingredient in biscuit. *IOP Conference Series: Earth and Environmental Science*, 251(1), 012026. https://doi.org/10.1088/1755-1315/251/1/012026
- Rehman, S., & Sharif, M. K. (2018). Rheological and baking performance of composite flours. (June).
- Rodríguez-Alonso, E., Vergeldt, F. J., & van der Goot, A. J. (2019). TD-NMR to understand water-binding food properties. *Magnetic Resonance in Chemistry*, 57(9), 603–606. https://doi.org/10.1002/mrc.4815
- Sanjeewa, W. G. T., Wanasundara, J. P. D., Pietrasik, Z., & Shand, P. J. (2010). Characterization of chickpea (*Cicer arietinum* L.) flours and application in low-fat pork bologna as a model system. *Food Research International*, 43(2), 617–626. https://doi.org/10.1016/j.foodres.2009. 07.024
- Schopf, M., & Scherf, K. A. (2021). Water absorption capacity determines the functionality of vital gluten related to specific bread volume. *Food*, 10(2), 0–12. https://doi.org/10.3390/foods10020228
- Semba, R. D., Ramsing, R., Rahman, N., Kraemer, K., & Bloem, M. W. (2021). Legumes as a sustainable source of protein in human diets. *Global Food Security*, 28(January), 100520. https://doi.org/10.1016/j. gfs.2021.100520
- Silva, S. D., Feliciano, R. P., Boas, L. V., & Bronze, M. R. (2014). Application of FTIR-ATR to Moscatel dessert wines for prediction of total phenolic

and flavonoid contents and antioxidant capacity. *Food Chemistry*, 150, 489–493. https://doi.org/10.1016/j.foodchem.2013.11.028

- Singh, N., Nakaura, Y., Inouchi, N., & Nishinari, K. (2008). Structure and viscoelastic properties of starches separated from different legumes. *Starch/Staerke*, 60(7), 349–357. https://doi.org/10.1002/star. 200800689
- Siva, N., Thavarajah, P., Kumar, S., & Thavarajah, D. (2019). Variability in prebiotic carbohydrates in different market classes of chickpea, common bean, and lentil collected from the American local market. *Frontiers in Nutrition*, 6(April), 1–11. https://doi.org/10.3389/fnut.2019. 00038
- Sumargo, F., Gulati, P., Weier, S. A., Clarke, J., & Rose, D. J. (2016). Effects of processing moisture on the physical properties and in vitro digestibility of starch and protein in extruded brown rice and pinto bean composite flours. *Food Chemistry*, 211, 726–733. https://doi.org/10. 1016/j.foodchem.2016.05.097
- Tas, O., Ertugrul, U., Oztop, M. H., & Mazi, B. G. (2021). Glycation of soy protein isolate with two ketoses: d-Allulose and fructose. *International Journal of Food Science & Technology*, 56(11), 5461–5470. https://doi. org/10.1111/ijfs.15218
- Teterycz, D., & Sobota, A. (2020). Legume flour as a natural colouring component in pasta production. *Journal of Food Science and Technology*, 57(1), 301–309. https://doi.org/10.1007/s13197-019-04061-5
- Teterycz, D., Sobota, A., Zarzycki, P., & Latoch, A. (2020). Legume flour as a natural colouring component in pasta production. *Journal of Food Sci*ence and Technology, 57(1), 301–309. https://doi.org/10.1007/ s13197-019-04061-5
- Thiex, N., Novotny, L., & Crawford, A. (2012). Determination of ash in animal feed: AOAC official method 942.05 revisited. *Journal of AOAC INTERNATIONAL*, 95(5), 1392–1397. https://doi.org/10.5740/ jaoacint.12-129
- Todt, H., Guthausen, G., Burk, W., Schmalbein, D., & Kamlowski, A. (2006). Water/moisture and fat analysis by time-domain NMR. *Food Chemistry*, 96(3), 436–440. https://doi.org/10.1016/j.foodchem.2005.04.032

- Todt, H., Guthausen, G., Burk, W., Schmalbein, D., & Kamlowski, A. (2008). Time-domain NMR in quality control: Standard applications in food. *Modern Magnetic Resonance*, 1739–1743. https://doi.org/10.1007/1-4020-3910-7_196
- Uysal, N., Sumnu, G., & Sahin, S. (2009). Optimization of microwaveinfrared roasting of hazelnut. *Journal of Food Engineering*, 90(2), 255– 261. https://doi.org/10.1016/j.jfoodeng.2008.06.029
- Yildiz, E., Guner, S., Sumnu, G., Sahin, S., & Oztop, M. H. (2018). Monitoring the effects of ingredients and baking methods on quality of glutenfree cakes by time-domain (TD) NMR relaxometry. *Food and Bioprocess Technology*, 11(10), 1923–1933. https://doi.org/10.1007/s11947-018-2152-z
- Yousf, N., Nazir, F., Salim, R., Ahsan, H., & Sirwal, A. (2017). Water solubility index and water absorption index of extruded product from rice and carrot blend. *Journal of Pharmacognosy and Phytochemistry JPP*, 6(66), 2165–2168. http://www.phytojournal.com/archives/2017/ vol6issue6/PartAD/6-6-326-909.pdf
- Zhao, S., & Zhang, D. (2013). A parametric study of supercritical carbon dioxide extraction of oil from *Moringa oleifera* seeds using a response surface methodology. *Separation and Purification Technology*, 113, 9– 17. https://doi.org/10.1016/j.seppur.2013.03.041

How to cite this article: Tas, O., Ertugrul, U., Grunin, L., & Oztop, M. H. (2022). An investigation of functional quality characteristics and water interactions of navy bean, chickpea, pea, and lentil flours. *Legume Science*, e136. <u>https://doi.org/10.1002/leg3.136</u>