

Research Article

Effects of Fermentation on the Quality, Structure, and Nonnutritive Contents of Lentil (*Lens culinaris*) Proteins

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Protein digestibility, secondary protein structure components, sugars, and phenolic compounds were analysed to investigate the effect of fermentation on the quality, structure, digestibility, and nonnutritive contents of lentil (*Lens culinaris*) proteins (LPs). Fermentation was carried out using water kefir seed. The initial pH of the unfermented LPs (6.8) decreased to pH 3.4 at the end of the fermentation on day 5. Protein digestibility increased from 76.4 to 84.1% over the 5 days of fermentation. Total phenolic content increased from 443.4 to 792.6 mg of GAE/100 g after 2 days of fermentation, with the sums of the detected phenolic compounds from HPLC analysis reaching almost 500 mg/100 g. The predominant phenolic compounds detected in fermented LPs include chlorogenic and epicatechin, while traces of rutin, ferulic acid, and sinapic acid were observed. Fermentation played a major role in the changes of the components in the secondary protein structure, especially the percentage of α -helices and random coils. In addition, the reduction in α -helix: β -sheet ratio with the increase in protein digestibility was related to the prolongation of the fermentation time. The model used in this research could be a robust tool for improving protein quality, protein degradation, and nonnutritive nutrients using water kefir seed fermentation.

1. Introduction

Research interest in establishing a clear alternative to the use of plant proteins has increased in recent years. Lentil (*Lens culinaris*) seeds have high protein contents that range from 20.6 to 31.4 g/100 g [1]. Lentil seeds are also high in fibre content and low in fat, making them an attractive alternative source of protein [2]. Interest in lentil proteins (LPs) has grown because of their excellent nutritional benefits, such as their favourable leucine: isoleucine (1.24–1.98) and leucine: lysine (1.08–2.03) ratios [2]. LPs are composed of four protein fractions, i.e., 70% globulins (7S and 11S), 16% albumins, 11% glutelins, and 3% prolamins [3]. Amongst the four main protein fractions in pulses, albumins differ due to their high solubility in water [4].

A key limitation of the use of plant protein in the food industry is its low digestibility resulting from its densely packed rigid system that is mainly due to hydrophobic interactions and disulfide bonds [5]. Digestibility is often used as an indicator to evaluate the proteolysis capability and availability of a protein. Compared with proteins with low digestibility, proteins with high digestibility are considerably more suitable because they have more amino acids for uptake and are thus of higher nutritional benefit. Fermentation can be used to increase the digestibility of plant protein by decreasing the contents of nonnutritive compounds, e.g., phenolics, by promoting cross-linking between proteins, and the synthesis of microbial proteases, which could lead to minute breakdown and release of some of the proteins from the matrix [6–8].

Water kefir seed (WKS) has become a very common microbial consortium due to its excellent content of microorganisms with the accepted probiotic features [9]. The microbial biodiversity and bioactivity of WKS have been intensively investigated and have been shown to originate from several essential anti-inflammatories and antimicrobial bioactive compounds and antioxidants [9, 10]. Some researchers have proven that WKS contains high amounts of acetic acid bacteria and yeasts (*Saccharomyces*, *Candida*, and *Kluyveromyces*), as well as lactic acid bacteria, such as *Streptococcus*, *Lactococcus*, *Leuconostoc*, and *Lactobacillus* [10].

The main purpose of this research is to investigate the effect of WKS on the digestibility, protein quality, and certain nonnutritive components of LPs. LPs are considered to be a strong alternative to soy protein because they are not genetically engineered and do not cause allergic problems. Developing a design for generating fermented LPs with improved nutritional properties is extremely desirable for food technology and industrial food production. However, information on the quality, structure, and nonnutritive contents of LPs is very limited. This study is the first to investigate the effect of WKS on the quality, structure, digestion, and nonnutritive contents of LPs.

2. Materials and Methods

Lentil seeds (*Lens culinaris*) and kefir seeds were purchased from iHerb (California, USA). The samples were transported to the laboratory inside an insulated box and kept at 4°C until use. All chemicals used, unless otherwise stated, were analytical reagent grade. High-performance liquid chromatography (HPLC) grade was used for the HPLC analysis. These chemicals were obtained from Fine Chemical Limited (Boisar, India), Gainland Chemical Co. (Flintshire, UK), Fluka (Buchs, Switzerland), and Sigma-Aldrich (New Jersey, USA).

2.1. Preparation of LPs. LPs with a protein content of $62.98\% \pm 2.18$ (determined using the Kjeldahl method, AOAC method 930.29 [11], and 6.25 as the nitrogen-to-protein conversion factor) were extracted based on the method described by Jarpa-Parra et al. [1] with modifications. Lentil flour was prepared by using an ultra-centrifugal mill with a screen-space size of 0.5 mm. The fine flour of the lentil was placed in a plastic bag and kept at 4°C before extraction. Lentil flour (100 g) was mixed with 1 L of distilled water, and the pH of the slurry was adjusted to 9.5 using 0.1 M NaOH and then stirred (Toanlab, SH-4, Arizona, USA) at 40°C for 2 h at 1,000 rpm. The slurry was centrifuged (Kubota, S700TR, Tokyo, Japan) at $8,500 \times g$ for 15 min. The pH of the supernatant was adjusted to 4.2 using 0.1 M HCl before being left overnight to precipitate. The insoluble solids were then isolated. The precipitate was centrifuged at $1,590 \times g$ for 30 min. The collected supernatant was then freeze-dried (Telstar, LyoQuest plus Eco, Kingston, USA). The lyophilised LPs were stored in clean plastic bags at 4°C before fermentation and analysis.

2.2. Preparation of WKS. In brief, 50 g of kefir seeds mixed with 100 g of brown sugar were preserved in 1 L distilled water for 3 days at 25°C in a refrigerated incubator (Sanyo, MDF-U500VXC, Chicago, USA) before fermentation to maintain seed viability. The supernatant from the kefir seeds was isolated via filtration by using a sterile sieve.

2.3. Design of LP-WKS Preparation and Fermentation. Fermentation was performed for 5 days at 25°C in the MDF-U500VXC refrigerated incubator. Briefly, 5 mL of WKS solution was mixed with 1 g of LPs in a 250 mL conical flask containing 95 mL of distilled water (Figure 1). Samples were collected in triplicate every 24 h during the 5 days of fermentation for analysis.

2.4. Assessment of pH and Total Soluble Solids (TSS). A pH meter (Mettler Toledo, S20, Greifensee, Switzerland) was used to calculate the pH of the unfermented and fermented LPs samples. A digital refractometer (Hanna Instruments, HI 96801, Rhode Island, USA) was used to determine the concentrations of TSS expressed as °Brix, and samples were analysed every 24 h in triplicate.

2.5. Protein Digestibility. The digestibility of LPs and LP-WKS was measured based on the procedure as described by Almeida et al. [12]. Briefly, 250 mg of protein sample was suspended in 15 mL of 0.1 M HCl containing 1.5 mg/mL pepsin and then incubated in a waterbath for 3 h at 37°C. After that, 7.5 mL of M NaOH was added. Subsequently, 10 mL of 0.2 M phosphate buffer (pH 8.0) containing 10 mg of pancreatin, along with 1 mL of 0.005 M sodium azide, was added to prevent microbial growth and development. Then, the protein solutions were incubated at 37°C for 24 h in a waterbath (Mettler, WB22, Schwabach, Germany). After incubation, the protein solutions were centrifuged at $10,000 \times g$ for 20 min. The protein content in the supernatant and the sample was measured using the Kjeldahl method and AOAC method 930.29 [11]. Protein digestibility (expressed in %) was calculated using the following equation:

$$\text{Protein digestibility (\%)} = [(N_S - N_B)/T] \times 100\%, \quad (1)$$

where, N_S and N_B are the nitrogen contents in the supernatant and blank sample, respectively, and N_T is the total nitrogen content of the sample.

2.6. Determination of Total Phenolic Content (TPC). TPC of the unfermented and fermented LPs samples was determined following the procedure described by Alu'datt et al. [13]. A standard curve was prepared for the calculation of TPC by using gallic acid (10 mg/100 mL). A total of 100 µL of the sample was added to 8.4 mL of distilled water, and 500 µL of Folin-Ciocalteu reagent was added and mixed well in the test tube. After 4 min, 1 mL of 5% sodium carbonate was added to the mixture. The contents of the test tube were then mixed by using a vortex. After 1 h, a UV-Vis spectrophotometer (Shimadzu, UV-3600, Kyoto, Japan) was used to

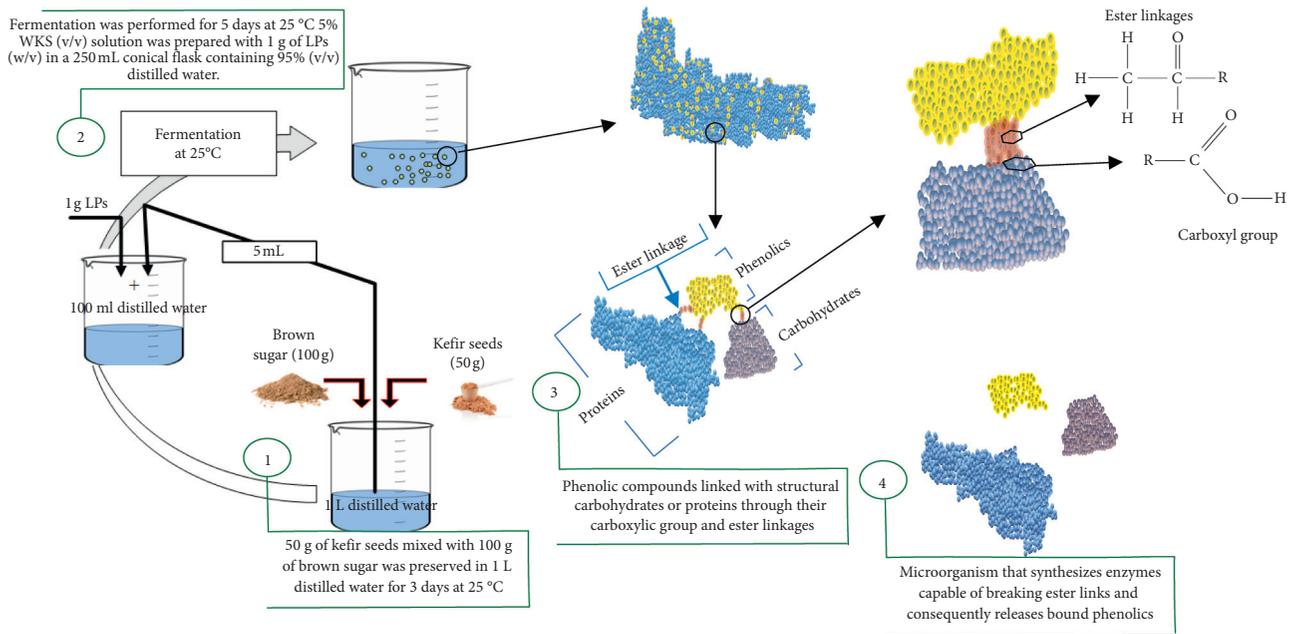


FIGURE 1: Schematic diagram of the fermentation process steps. (1) Preparation of water kefir seeds (WKS). (2) Preparation of protein solution (1%, w/v) and 5% (v/v) WKS in distilled water at 25°C for 5 days of fermentation. (3) Protein complexes with phenolic compounds and carbohydrates connected via ester linkages. (4) Breakdown of the ester linkages by microorganisms and their enzymes, resulting in the release of protein complexes, phenolic compounds, and carbohydrates.

read the absorbance of the samples at 725 nm. TPC of the samples was expressed in milligram of gallic acid equivalents per 100 g (mg GAE/100 g). The analysis was conducted in triplicate for each sample.

2.7. Determination of Sugar and Phenolic Compounds. High-performance liquid chromatography (HPLC) was used to analyse sugars and phenolic components. Each sample was centrifuged for 10 min at 15,000 × g, and the supernatant was filtered with a membrane filter (0.45 μm) before injection into the HPLC system (Agilent, 1200 series, New Jersey, USA) with a refractive index detector. Sugars were separated using a Cosmosil Sugar-D column (4.6 mm × 250.0 mm) at 40°C (column temperature). Acetonitrile and water (75:25, v/v) were used as the mobile phase. The flow rate was set at 1.2 mL/min, and the injection volume was 20 μL. Working standards (consisting of fructose, glucose, and sucrose) were prepared from the stock solution and were used to construct standard curves for fructose, glucose, and sucrose to quantitate the concentration of sugars present in the unfermented and fermented LPs samples.

Phenolic compounds were separated using a Plus C18 column (4.6 mm × 250 mm). The mobile phases A and B were acetonitrile and 1% (v/v) acetic acid solution, respectively. The flow rate was set at 0.7 mL/min, and the injection volume was 40 μL. The samples were read at 272 and 254 nm. Working standards (consisting of catechin, chlorogenic, epicatechin, quercetin, rutin, caffeic acid, ferulic acid, gallic acid, sinapic acid, and syringic acid) were prepared from the stock solution and were used to construct standard curves for these phenolic compounds to quantitate

the concentration of phenolic compounds in the unfermented and fermented LPs samples.

2.8. Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra of the unfermented and fermented samples were determined using an FTIR spectrometer (Shimadzu, IRAffinity-1S, Kyoto, Japan) following the method described by Yasar et al. [14]. The FTIR spectra were measured between 400 and 4,000 1/cm at an interval of 4 1/cm. Each spectrum was created by 18 scans to obtain the best possible signal to noise ratio. The analysed spectra were amide I band, between 1,600 and 1,700 1/cm. The percentages of secondary protein components (% of amide I band) were calculated: β-sheet (1,600–1,639 1/cm), random coil, RC (1,640–1,649 1/cm), α-helix (1,650–1,660 1/cm), and β-turn (1,661–1,699 1/cm) for the samples. A total of 18 spectral scans (triplicate for each protein samples) were carried out in this study.

2.9. Statistical Analysis. Statistical analysis was performed using SPSS software version 23.0 (Chicago, USA). One-way ANOVA was carried out, and Duncan's multiple range test was applied to determine significant differences ($P < 0.05$) between the mean values.

3. Results and Discussion

3.1. Effects of WKS Fermentation on pH and TSS of LPs. The pH value and TSS dropped drastically during the WKS fermentation (Table 1). On day 2 of the fermentation, the pH value and TSS decreased from 6.8 to 3.4 and from 0.70 to

TABLE 1: Changes in the pH, total soluble solids (TSS), protein digestibility, and sugars profile of unfermented lentil proteins (day 0) and water kefir seed fermented lentil proteins (days 1–5).

Parameters	Fermentation period (day)						P value
	0	1	2	3	4	5	
pH	6.87 ^a ± 0.05	4.90 ^b ± 0.01	3.43 ^c ± 0.01	3.38 ^d ± 0.03	3.31 ^e ± 0.01	3.28 ^e ± 0.01	<0.05
TSS (°Brix)	0.70 ^a ± 0.00	0.60 ^b ± 0.00	0.50 ^c ± 0.00	0.47 ^c ± 0.05	0.40 ^d ± 0.00	0.40 ^d ± 0.00	<0.05
Protein digestibility (%)	76.42 ^c ± 3.00	78.64 ^{bc} ± 0.22	81.16 ^{ab} ± 1.47	82.69 ^a ± 0.74	83.70 ^a ± 1.00	84.17 ^a ± 2.10	<0.05
Sugars (g/L)							
Fructose	0.47 ^b ± 0.01	2.41 ^a ± 0.04	0.51 ^b ± 0.02	0.22 ^c ± 0.01	0.11 ^d ± 0.01	0.08 ^d ± 0.01	<0.05
Glucose	ND	1.24 ^b ± 0.06	2.52 ^a ± 0.05	1.10 ^c ± 0.03	0.80 ^d ± 0.04	0.33 ^e ± 0.01	<0.05
Sucrose	5.90 ^a ± 0.32	1.91 ^b ± 0.06	0.11 ^c ± 0.01	0.09 ^c ± 0.04	ND	ND	<0.05

The data presented in the table represent the mean ± standard deviation ($n = 3$). Values with different superscripts within the same row are statistically significant from each other ($P < 0.05$). ND represents not detected.

0.50, respectively. Finally, after 5 days of fermentation, TSS and pH decreased to 0.40 and 3.2, respectively. The release of amino acid and NH_4^+ due to protein degradation can impact the decrease of pH during fermentation [15]. The decrease in the pH during the fermentation process could also be a result of the deposition of lactic acid produced by microbial activity [16–18]. Similar trends were reported in related studies suggesting that *Lactobacillus* spp. led to the reduction in the pH of protein after fermentation by water kefir seed [18, 19]. For the first 3 days, there was a drop in TSS, and a slow decrease was immediately followed until levelling off within 4 days. Similarly, Tu et al. [19] reported that the TSS of the soy-whey decreased sharply, from 9.20 to 4.43 in the first 2 days of fermentation °Brix by water kefir seed, and it reached 3.97 °Brix on day 5.

3.2. Effect of WKS Fermentation on the Protein Quality of LPs.

A major variation was observed in the digestibility of LPs, with digestibility values ranging approximately 76.4–84.2% (Table 1). The digestibility of LPs increased from 76.4 to 82.6% on day 3 of fermentation and reached 84.2% on day 5. A study by Chandra-Hioe et al. [6] on desi chickpea flour showed that the lyophilised yoghurt cultures containing *Streptococcus bulgaricus* and *Lactobacillus* managed to improve the protein digestibility from 70.5 to over 77.2%. Hence, the increase in the protein digestibility of LPs was expected since WKS has been reported to possess a high concentration of yeasts (*Saccharomyces*, *Candida*, and *Kluyveromyces*) and lactic acid bacteria (*Streptococcus*, *Lactococcus*, *Leuconostoc*, and *Lactobacillus*), as well as some acetic acid bacteria [10].

No significant changes ($P > 0.05$) on the digestibility of fermented LPs were observed from day 3 up to day 5 (Table 1). This result could be attributed to the decrease in protein cross-linking stimulated by the existence of antioxidants and the phenolic contents of nonnutritive compounds that can cause proteolytic attacks. The increase of digestibility of fermented LPs is associated with the hydrolysis of protein and nonnutritive compounds in LPs. Studies conducted by Mugula et al. [20] and Pranoto et al. [21] had proved that *Lactobacillus plantarum* has proteolytic activity. Proteolysis of protein leads to the generation of amino acids and peptides during fermentation, leading to an increase in protein digestibility.

3.3. Effect of WKS Fermentation on the Phenolic Compounds of LPs. Phenolic compounds are known nonnutritive compounds that negatively affect protein quality [22–24]. The phenolic compounds cross-linked protein, reducing their responsiveness to the action of enzymes during digestion and fermentation. The TPC and phenolic compound contents of LPs were measured during 5 days of fermentation with WKS. The results (Table 2) revealed that after fermentation, the TPC of fermented LPs increased. The increase might be attributed to the release of bound phenols [25] through microbial fermentation [26]. This finding is in agreement with the findings of several other studies, which showed that TPC and phenolic compounds increased during fermentation with WKS [19, 27, 28]. The use of kefir seeds is considered to be an effective approach for increasing TPC by fermentation [29].

The phenolic compounds detected in LPs included rutin, caffeic acid, quercetin, catechin, chlorogenic, epicatechin, ferulic acid, gallic acid, sinapic acid, and syringic acid (Table 2). The levels of all the detected phenolic compounds increased after fermentation. Caffeic acid and sinapic acid were detected on days 4 and 1, respectively. Syringic acid content remained constant. Moreover, rutin content decreased from 2.76–1.88 mg GAE/100 g on day 2 and then increased to 5.90 mg GAE/100 g on day 4. In the natural environment, phenolics are connected to sugars in the form of bound or phenolic complexes (Figure 1). These phenolic compounds are connected through the hydroxyl groups of protein and carboxylic group of carbohydrate by ester linkages [25, 30]. It was reported that microorganisms and their enzymes could break down these bounds or phenolic complexes into free phenolics and simple sugars during fermentation [31–35]. The sinapic content of fermented LPs was not significantly different ($P > 0.05$) throughout the 5 days of fermentation. Catechin content increased by more than two-fold from 38.67 mg GAE/100 g in unfermented LPs to 70.88 mg GAE/100 g in fermented LPs. Our findings demonstrated that the levels of phenolic compounds, except quercetin, in fermented LPs were significantly higher ($P < 0.05$) than those in unfermented LPs (day 0).

3.4. Effect of WKS Fermentation on the Sugars Profile of LPs.

The functional activity of WKS was attributed to brown sugars [19], which consist mainly of sucrose (over 85%) and

TABLE 2: Changes in the total phenolic content (TPC, expressed in mg GAE/100 g) and phenolic compounds (expressed in mg/100 g) of unfermented lentil proteins (day 0) and water kefir seed fermented lentil proteins (days 1–5).

	Fermentation period (day)						P value
	0	1	2	3	4	5	
TPC	443.40 ^e ± 8.20	621.89 ^{cd} ± 9.01	792.62 ^a ± 6.45	678.60 ^b ± 12.18	631.44 ^c ± 1.79	614.13 ^d ± 5.47	<0.05
Phenolic compounds							
Catechin	38.67 ^e ± 0.54	71.97 ^c ± 0.62	74.21 ^b ± 0.90	77.31 ^a ± 0.26	76.33 ^a ± 0.18	70.88 ^d ± 0.53	<0.05
Chlorogenic	96.51 ^f ± 1.11	104.19 ^c ± 0.88	123.57 ^a ± 0.49	117.69 ^b ± 0.37	112.91 ^c ± 0.70	111.03 ^d ± 0.18	<0.05
Epicatechin	121.42 ^f ± 0.61	148.69 ^c ± 0.66	185.35 ^a ± 1.02	158.51 ^b ± 0.87	157.18 ^c ± 0.33	153.88 ^d ± 0.38	<0.05
Quercetin	15.96 ^d ± 0.77	16.47 ^d ± 0.51	17.73 ^c ± 0.20	18.90 ^b ± 0.72	21.68 ^a ± 0.45	4.17 ^e ± 0.13	<0.05
Rutin	2.76 ^c ± 0.06	2.21 ^d ± 0.09	1.88 ^d ± 0.04	5.84 ^a ± 0.30	5.90 ^a ± 0.09	5.10 ^b ± 0.66	<0.05
Caffeic acid	ND	ND	ND	ND	5.54 ^b ± 0.07	7.25 ^a ± 0.05	<0.05
Ferulic acid	4.28 ^e ± 0.05	4.49 ^d ± 0.07	4.49 ^c ± 0.04	5.25 ^a ± 0.05	5.06 ^b ± 0.05	5.04 ^b ± 0.03	<0.05
Gallic acid	13.37 ^e ± 1.02	22.61 ^c ± 0.50	24.13 ^b ± 0.46	35.93 ^a ± 0.76	21.41 ^c ± 1.13	19.21 ^d ± 0.60	<0.05
Sinapic acid	ND	1.87 ^a ± 0.04	3.32 ^a ± 2.75	1.49 ^a ± 0.03	1.35 ^a ± 0.03	1.23 ^a ± 0.02	>0.05
Syringic acid	53.29 ^d ± 1.10	54.60 ^d ± 0.59	59.14 ^b ± 0.55	61.82 ^a ± 1.13	63.27 ^a ± 0.13	56.49 ^c ± 1.36	<0.05
Phenolic compounds (Σ)	346.26	427.10	494.21	482.74	470.63	434.28	

The data presented in the table represent the mean ± standard deviation ($n=3$). Values with different superscripts within the same row are statistically significant from each other ($P<0.05$). ND represents not detected.

TABLE 3: Changes in the percentage of secondary protein components (β -sheet, random coil (RC), α -helix, and β -turn) of unfermented lentil proteins (day 0) and fermented lentil proteins (days 1–5) based on FTIR measurements.

Secondary protein components	Peak (1/cm)	Fermentation period (day)						P value
		0	1	2	3	4	5	
β -sheet								
	1,614.42	9.19	9.89	11.09	11.10	10.42	9.47	
	1,622.13	6.89	7.58	7.85	8.03	7.65	6.83	
	1,633.71	11.47	12.83	13.41	14.17	12.94	11.69	
β -sheet (Σ)		30.55 ^a	30.30 ^a	32.35 ^a	33.30 ^a	31.01 ^a	27.99 ^a	$P > 0.05$
RC (Σ)	1,645.28	17.99 ^f	19.60 ^e	21.59 ^d	25.47 ^c	30.12 ^b	35.12 ^a	$P < 0.05$
α -helix (Σ)	1,654.07	18.26 ^a	15.91 ^b	9.52 ^c	8.67 ^d	8.45 ^d	7.53 ^e	$P < 0.05$
β -turn								
	1,668.43	9.52	9.58	9.68	8.13	7.64	7.61	
	1,681.93	11.61	10.39	10.50	9.72	8.22	8.09	
	1,693.50	15.05	14.22	16.36	14.69	14.04	13.74	
β -turn (Σ)		36.18 ^a	34.19 ^a	36.54 ^a	32.54 ^a	29.90 ^a	29.44 ^a	$P > 0.05$
Ratio ¹		59.77	52.51	29.42	26.00	27.24	27.90	

¹Ratio of α -helix: β -sheet.

a small amount of glucose and fructose [36]. Sucrose content in the LP-WKS solution decreased with the prolongation of fermentation time. On day 2 of the fermentation, sucrose content reduced to 0.11 ± 0.01 g/L, while glucose and fructose contents increased to 2.52 ± 0.05 and 0.51 ± 0.02 g/L, respectively (Table 1). Pranoto et al. [21] reported that lactic acid bacteria utilised sugars as a source of energy for growth and to increase the number of colonies. After day 2, glucose, fructose, and sucrose concentrations decreased until the end of the fermentation, i.e., day 5. The findings suggested that the microbial consortium of WKS can utilise sucrose, glucose, and fructose as a carbon source in the LP-WKS solution. This outcome is in agreement with the findings reported by Martínez-Torres et al. [37]. Similarly, these changes have also been reported in a recent study by Tu et al. [19], whereby glucose and fructose contents in the soy-whey product increased at the expense of sucrose during the first 2 days of fermentation, after which the content of these sugars decreased until the end of the fermentation process on day 5.

3.5. Influence of WKS Fermentation on the Secondary Protein Structure of LPs. In this research, FTIR spectroscopy was used to characterise secondary protein structure in the amide I region of LPs that had been fermented for 5 days. Table 3 provides the components of the secondary structures of LPs after fermentation with WKS. β -turns (1,660–1,699 1/cm), α -helices (1,653 ± 2 1/cm), RC (1,647 1/cm), and β -sheets (1,600–1,639 1/cm) were the main components of the secondary protein structures. In contrast to the secondary structure of LPs, the protein conformations of unfermented LPs were dominated by α -helices (18.26%), β -turns (36.18%), RC (17.99%), and β -sheets (30.55%). The percentage of secondary protein components in WKS-fermented LPs differed from that of unfermented LPs. In particular, the contents of α -helix and RC changed with the prolongation of fermentation time. These modifications revealed the reorganisation of the degraded LPs during fermentation. Numerically speaking, β -sheets and β -turns are not significantly different from other secondary protein structures (RC or

α -helices). Carbonaro et al. [38] proved that a significant negative relationship exists between the increase in β -sheet percentage and protein digestibility. The percentage of α -helices decreased to 15.91% after the first day of fermentation (day 1), but the magnitude of this decrease was attenuated after day 2 of the fermentation. However, the α -helix content of fermented LPs significantly decreased ($P < 0.05$) on the last 3 days of the fermentation (day 3 until 5) compared with that of unfermented LPs. Besides, clear evidence show that the degradation of fermented LPs resulted in a significant increase ($P < 0.05$) in RC from 17.99 to 19.60% on day 1 and subsequently increased to 35.12% on day 5. This increasing trend indicated that the LPs had aggregated.

The reduction in α -helix percentage is a clear indicator of the increased digestibility of proteins [39]. The results for the changes in secondary protein components are entirely in parallel with the results of the present study, whereby the RC content increased and α -helix concentration decreased. However, the extent of this shift in this study was greater than that in the study by Wang et al. [39] and Yasar et al. [14]. Consequently, the effects of bacterial fermentation on secondary protein components were more beneficial than those of industrial food processes involving extreme heating and pressure treatment conditions, such as cooking, extrusion, and pelleting [40].

Almost all fermentation processes greatly decreased or fully degraded α -helices and increased RC components. In this study, the magnitude of these modifications was substantial. Alterations are significantly associated positively with enhanced protein quality [40]. The increase in the digestibility of fermented LPs was proven in this study. In addition, a decrease in the α -helix: β -sheet ratio is related to enhancing intestinal protein digestibility [40]. The α -helix: β -sheet ratio decreased from 59.77 to 29.42% on day 2 and continuously decreased to 26.00% on day 3.

4. Conclusion

The quality of the overall protein had changed by day 2 of the fermentation. The fermentation resulted in important changes in the protein, secondary structure component, digestibility, and nonnutritive contents of LPs. The proportional increases in TPC and some phenolic compounds after fermentation were attributed to the actions of microorganisms and their enzymes presented naturally in WKS.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

M. Alrosan collected test data and drafted the article. M. Alrosan and T.C. Tan designed the study, interpreted the results, and revised the manuscript. A.M. Easa, S. Gammoh, and M.H. Alu'datt contributed equally to this work.

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References

- [1] M. Jarpa-Parra, F. Bamdad, Y. Wang et al., "Optimization of lentil protein extraction and the influence of process pH on protein structure and functionality," *LWT-Food Science and Technology*, vol. 57, no. 2, pp. 461–469, 2014.
- [2] S. S. Yadav, D. McNeil, and P. C. Stevenson, *Lentil: An Ancient Crop for Modern Times*, Springer Science, Berlin, Germany, 2007.
- [3] J. I. Boye, S. Aksay, S. Roufik et al., "Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques," *Food Research International*, vol. 43, no. 2, pp. 537–546, 2010.
- [4] S. R. Bean and G. L. Lookhart, "Recent developments in high-performance capillary electrophoresis of cereal proteins," *Electrophoresis*, vol. 22, no. 8, pp. 1503–1509, 2001.
- [5] S. Utsumi, "Plant food protein engineering," *Advances in Food and Nutrition Research*, vol. 36, pp. 89–208, 1992.
- [6] M. V. Chandra-Hioe, C. H. M. Wong, and J. Arcot, "The potential use of fermented chickpea and faba bean flour as food ingredients," *Plant Foods for Human Nutrition*, vol. 71, no. 1, pp. 90–95, 2016.
- [7] C. Reyes-Moreno, E. Cuevas-Rodríguez, J. Milán-Carrillo, O. Cárdenas-Valenzuela, and J. Barrón-Hoyos, "Solid state fermentation process for producing chickpea (*Cicer arietinum* L) tempeh flour. Physicochemical and nutritional characteristics of the product," *Journal of the Science of Food and Agriculture*, vol. 84, no. 3, pp. 271–278, 2004.
- [8] L. A. Shekib, "Nutritional improvement of lentils, chick pea, rice and wheat by natural fermentation," *Plant Foods for Human Nutrition*, vol. 46, no. 3, pp. 201–205, 1994.
- [9] F. A. Fiorda, G. V. De Melo Pereira, V. Thomaz-Soccol et al., "Microbiological, biochemical, and functional aspects of sugary kefir fermentation—a review," *Food Microbiology*, vol. 66, pp. 86–95, 2017.
- [10] K. L. Rodrigues, T. H. Araújo, J. M. Schneedorf et al., "A novel beer fermented by kefir enhances anti-inflammatory and anti-ulcerogenic activities found isolated in its constituents," *Journal of Functional Foods*, vol. 21, pp. 58–69, 2016.
- [11] Association of Official Analytical Chemists (AOAC), *Official Methods of Analysis of AOAC. Method 930.29*, AOAC International, Rockville, MD, USA, 19th edition, 2012.
- [12] C. C. Almeida, M. L. G. Monteiro, B. R. C. D. Costa-Lima, T. S. Alvares, and C. A. Conte-Junior, "In vitro digestibility of commercial whey protein supplements," *LWT-Food Science and Technology*, vol. 61, no. 1, pp. 7–11, 2015.
- [13] M. H. Alu'datt, I. Alli, K. Ereifej, M. Alhamad, A. R. Al-Tawaha, and T. Rababah, "Optimisation, characterisation and quantification of phenolic compounds in olive cake," *Food Chemistry*, vol. 123, no. 1, pp. 117–122, 2010.
- [14] S. Yasar, R. Tosun, and Z. Sonmez, "Fungal fermentation inducing improved nutritional qualities associated with altered secondary protein structure of soybean meal determined

- by FTIR spectroscopy," *Measurement*, vol. 161, Article ID 107895, 2020.
- [15] E. A. Tepari, G. Nakhla, B. M. Haroun, and H. Hafez, "Co-fermentation of carbohydrates and proteins for biohydrogen production: statistical optimization using response surface methodology," *International Journal of Hydrogen Energy*, vol. 45, no. 4, pp. 2640–2654, 2020.
- [16] B. Çabuk, A. K. Stone, D. R. Korber, T. Tanaka, and M. T. Nickerson, "Effect of *Lactobacillus plantarum* fermentation on the surface and functional properties of pea protein-enriched flour," *Food Technology and Biotechnology*, vol. 56, no. 3, pp. 411–420, 2018.
- [17] R. Doblado, J. Frías, R. Muñoz, and C. Vidal-Valverde, "Fermentation of *Vigna sinensis* var. carilla flours by natural microflora and *Lactobacillus* species," *Journal of Food Protection*, vol. 66, no. 12, pp. 2313–2320, 2003.
- [18] J. Jia, B. Ji, L. Tian et al., "Mechanism study on enhanced foaming properties of individual albumen proteins by *Lactobacillus* fermentation," *Food Hydrocolloids*, vol. 111, Article ID 106218, 2020.
- [19] C. Tu, F. Azi, J. Huang, X. Xu, G. Xing, and M. Dong, "Quality and metagenomic evaluation of a novel functional beverage produced from soy whey using water kefir grains," *LWT-Food Science and Technology*, vol. 113, Article ID 108258, 2019.
- [20] J. K. Mugula, T. Sorhaug, and L. Stepaniak, "Proteolytic activities in Togwa, a Tanzanian fermented food," *International Journal of Food Microbiology*, vol. 84, no. 1, pp. 1–12, 2003.
- [21] Y. Pranoto, S. Anggrahini, and Z. Efendi, "Effect of natural and *Lactobacillus plantarum* fermentation on in-vitro protein and starch digestibilities of sorghum flour," *Food Bioscience*, vol. 2, pp. 46–52, 2013.
- [22] D. Hahn, L. Rooney, and C. Earp, "Tannins and phenols of sorghum," *Cereal Food World*, vol. 29, pp. 776–779, 1984.
- [23] Y. P. Gupta, "Anti-nutritional and toxic factors in food legumes: a review," *Plant Foods for Human Nutrition*, vol. 37, no. 3, pp. 201–228, 1987.
- [24] B. D. Oomah, F. Caspar, L. J. Malcolmson, and A.-S. Bellido, "Phenolics and antioxidant activity of lentil and pea hulls," *Food Research International*, vol. 44, no. 1, pp. 436–441, 2011.
- [25] C. M. Liyana-Pathirana and F. Shahidi, "Importance of insoluble-bound phenolics to antioxidant properties of wheat," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 4, pp. 1256–1264, 2006.
- [26] T. M. Đorđević, S. S. Šiler-Marinković, and S. I. Dimitrijević-Branković, "Effect of fermentation on antioxidant properties of some cereals and pseudo cereals," *Food Chemistry*, vol. 119, no. 3, pp. 957–963, 2010.
- [27] B. A. Acosta-Estrada, J. A. Gutiérrez-Urbe, and S. O. Serna-Saldívar, "Bound phenolics in foods, a review," *Food Chemistry*, vol. 152, pp. 46–55, 2014.
- [28] A. Gunenc, M. H. Yeung, C. Lavergne, J. Bertinato, and F. Hosseinian, "Enhancements of antioxidant activity and mineral solubility of germinated wrinkled lentils during fermentation in kefir," *Journal of Functional Foods*, vol. 32, pp. 72–79, 2017.
- [29] F. Aiello, D. Restuccia, U. G. Spizzirri, G. Carullo, M. Leporini, and M. R. Loizzo, "Improving kefir bioactive properties by functional enrichment with plant and agro-food waste extracts," *Fermentation*, vol. 6, no. 3, p. 83, 2020.
- [30] T. Bhanja, A. Kumari, and R. Banerjee, "Enrichment of phenolics and free radical scavenging property of wheat koji prepared with two filamentous fungi," *Bioresource Technology*, vol. 100, no. 11, pp. 2861–2866, 2009.
- [31] C. M. Ajila, S. K. Brar, M. Verma, R. D. Tyagi, and J. R. Valéro, "Solid-state fermentation of apple pomace using *Phanerochaete chrysosporium*-liberation and extraction of phenolic antioxidants," *Food Chemistry*, vol. 126, no. 3, pp. 1071–1080, 2011.
- [32] P. Jamal, Z. M. Idris, and M. Z. Alam, "Effects of physico-chemical parameters on the production of phenolic acids from palm oil mill effluent under liquid-state fermentation by *Aspergillus niger* IBS-103ZA," *Food Chemistry*, vol. 124, no. 4, pp. 1595–1602, 2011.
- [33] D. Knockaert, J. Van Camp, K. Struijs, C. Wille, and K. Raes, "Potential of lactic acid fermentation to produce health beneficial compounds from vegetable waste," in *Proceedings of the Paper Session Presentation at 23rd International ICFMH Symposium FoodMicro 2012: Global Issues in Food Microbiology*, Istanbul, Turkey, September 2012.
- [34] M. D. S. Oliveira, E. P. Cipolatti, E. B. Furlong, and L. D. S. Soares, "Phenolic compounds and antioxidant activity in fermented rice (*Oryza sativa*) bran," *Food Science and Technology (Campinas)*, vol. 32, no. 3, pp. 531–537, 2012.
- [35] V. A. Q. Santos, C. G. Nascimento, C. A. Schmidt, D. Mantovani, R. F. Dekker, and M. A. A. Da Cunha, "Solid-state fermentation of soybean okara: isoflavones biotransformation, antioxidant activity and enhancement of nutritional quality," *LWT-Food Science and Technology*, vol. 92, pp. 509–515, 2018.
- [36] J. M. Dos Santos, S. P. Quináia, and M. L. Felsner, "Fast and direct analysis of Cr, Cd and Pb in brown sugar by GF AAS," *Food Chemistry*, vol. 260, pp. 19–26, 2018.
- [37] A. Martínez-Torres, S. Gutiérrez-Ambrocio, P. Heredia-Del-Orbe, L. Villa-Tanaca, and C. Hernández-Rodríguez, "Inferring the role of microorganisms in water kefir fermentations," *International Journal of Food Science & Technology*, vol. 52, no. 2, pp. 559–571, 2017.
- [38] M. Carbonaro, P. Maselli, and A. Nucara, "Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: a Fourier transform infrared (FT-IR) spectroscopic study," *Amino Acids*, vol. 43, no. 2, pp. 911–921, 2012.
- [39] Z. Wang, Y. Li, L. Jiang, B. Qi, and L. Zhou, "Relationship between secondary structure and surface hydrophobicity of soybean protein isolate subjected to heat treatment," *Journal of Chemistry*, vol. 201410 pages, 2014.
- [40] S. Salazar-Villanea, W. H. Hendriks, E. M. A. M. Bruininx, H. Gruppen, and A. F. B. Van Der Poel, "Protein structural changes during processing of vegetable feed ingredients used in swine diets: implications for nutritional value," *Nutrition Research Reviews*, vol. 29, no. 1, pp. 126–141, 2016.