

Improving of Nutritional Value of Wheat Bran Subjected to Solid State Fermentation with Pomegranate Peel and Whey

Royalıa TİRYAKİ¹, Ş.Canan BÖLÜKBAŞI²

^{1,2}Atatürk Üniversty, Agriculture Faculty, Animal Science Department Erzurum Türkiye

¹<https://orcid.org/0000-0002-6547-2872>, ²<https://orcid.org/0000-0003-1153-34891>

✉: canan@atauni.edu.tr

ABSTRACT

In this study, it is aimed to improve the nutrient value of the wheat bran (WB) subjected to solid state fermentation with pomegranate peel (PP) and whey (W). In the study, 0, 0.5, 1, 1.5 and 2 g pomegranate peels were added to the wheat bran, respectively, to make it up to 100 grams, and whey was used to ensure fermentation. Experiment was conducted on six treatment groups such as WB + TW (tap water) “the first group”, WB + 0% PP + W “the second group”, WB + 0.5% PP+ W “the third group”, WB + 1% PP + W “the fourth group”, WB + 1.5% PP + W “the fifth group” and WB + 2% PP + W “the sixth group”. Prepared feed samples were placed in Erlenmeyer flasks, 120 mL of tap water was added to the first group and the same amount of whey was added to the other groups and mixed. Eight replications were prepared for each sample. Four of the erlenmeyers prepared for each group were drained without being fermented and dried at room temperature. The remaining erlenmeyers were fermented in 32 °C ± 2 for 48 hours. Weende analyses, phytic acid ratios, phytase activities, antioxidant activities and yeast numbers of fermented and non-fermented feeds were determined in present study. As a result, it was found that fermented wheat bran had a significant increase in yeast content, antioxidant activity, crude protein and crude ash ratios, phytase activity, and a decrease in ether extract and phytic acid ratios.

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Nar Kabuğu ve Peynir Altı Suyu ile Katı Faz Fermantasyonuna Tabi Tutulan Buğday Kepeğinin Besin Değerinin İyileştirilmesi

ÖZET

Bu çalışmada, nar kabuğu (NK) ve peynir altı suyu (PAS) ile katı hal fermantasyonuna tabi tutulan buğday kepeğinin (BK) besin değerinin artırılması amaçlanmıştır. Çalışmada, buğday kepeğine sırasıyla 0, 0,5, 1, 1,5 ve 2 gr nar kabuğu ilave edilerek 100 grama tamamlanmış ve fermantasyonu sağlamak için peynir altı suyu kullanılmıştır. Denemede BK + MS (musluk suyu), BK + %0 NK+PAS, BK + %0,5 NK+PAS, BK + %1 NK + PAS, BK + %1,5 NK + PAS ve BK + %2 NK + PAS olmak üzere altı grup oluşturulmuştur. Hazırlanan yem örnekleri Erlenmayerlere konularak birinci gruba 120 ml musluk suyu, diğer gruplara aynı miktarda peynir altı suyu ilave edilerek karıştırılmıştır. Her yem örneği için sekiz tekerrür olarak hazırlanmıştır. Her grup için hazırlanan erlenmayerlerden dördü fermente edilmeden boşaltılarak oda sıcaklığında kurutulmuştur. Kalan erlenmayerler 32 °C±2'de 48 saat fermente edilmiştir. Bu çalışmada fermente ve fermente olmayan yemlerin weende analizleri, fitik asit oranları, fitaz aktiviteleri, antioksidan aktiviteleri ve maya sayıları belirlenmiştir. Çalışma sonucunda fermente buğday kepeğinin maya içeriği, antioksidan aktivite, ham protein ve ham kül oranları, fitaz aktivitesinde önemli artış, eter ekstraktı ve fitik asit oranlarında ise azalma olduğu belirlenmiştir.

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INTRODUCTION

The poultry sector in the world has been growing continuously over the years and the number of both laying hens and broiler chickens is increasing. The increase in the number of animals brought with it the need for feed.

The widespread use of many plant products such as corn and soy in both human and animal nutrition poses a significant problem in terms of raw material shortage. Therefore, the search for alternative feed materials in poultry nutrition continues. In recent years, it has become very important to make some feedstuffs more functional with different applications (Zhao et al., 2017).

Wheat bran, which contains an average of 12-16% crude protein in its composition, is a very rich feed in terms of B vitamins and minerals such as Fe, Cu, Zn and Mn. However, the high crude fiber content, low crude protein content and the phosphorus it contains in the form of phytin phosphorus limit the use of wheat bran in poultry feed. It is a recent practice to subject wheat bran to solid state fermentation (SSF) in order to increase its use in poultry rations (Zhang et al., 2022).

Solid state fermentation is defined as the growth of microorganisms in a moist environment (Pandey et al., 2008). It has been reported that the rate of yeasts (*Saccharomyces cerevisiae*) increased considerably as a result of solid phase fermentation (Pamir, 1985). Various studies have shown that the nutrient usefulness of feeds increases with the increase in the number of yeast and other microorganisms in the environment (Joseph et al., 2008; Machida & Gomi, 2010; Kumar & Duhan, 2011; Zhang et al., 2014).

Whey, which is a by-product of cheese production, is rich in proteins with high biological value as well as chemical, physical and functional properties. While some of the whey is used in some areas, a significant part of it remains largely as agricultural waste. These wastes cause significant environmental pollution (Ersoy & Uysal, 2002; Özen & Kılıç, 2007; Demir, 2011; Mete, 2012). In our study, it is planned to prevent environmental pollution by fermenting with whey, which is a valuable nutrient, instead of water. At the same time, pomegranate peels, which are another waste material and have a strong antioxidant effect, were added to the wheat bran.

Pomegranate (*Punica granatum* L.), known as tropical and subtropical climate fruit, is a fruit with high antioxidant and antitumor activity (Akbulut et al.,

2010). Approximately 48% of the pomegranate's weight consists of the peel (Zarei et al., 2011). It has been reported that the peel or pulp of the pomegranate, which becomes waste after processing, may be used as an alternative feed source (Sarica, 2011).

In this study, it was aimed to make wheat bran more useful for poultry by mixing it with pomegranate peel in different proportions and subjecting it to solid phase fermentation with whey.

MATERIALS AND METHODS

Feed Material

The wheat bran used in the study was obtained from a commercial feed factory (87.98% dry matter, 3.98% crude oil, 15.65% crude protein, 3.33% crude ash, 13.40% crude fiber, 49.85% 1,1-Difenil 2-pikril hidrazil (% DPPH) inhibition, 0.318 IU/g phytase activity, 4.46% phytic acid ratio and yeast count 5.98×10^3). Pomegranate peels were obtained by drying the peels at room temperature after cleaning the inside of the pomegranates purchased from the market (87% dry matter, 0.95% crude oil, 4.27% crude protein, 2.91% crude ash, 17.04% crude fiber, 80% DPPH. inhibition and 1,151 IU/g phytase activity).

The whey used in solid state fermentation to provide moisture in the environment was obtained daily from a dairy factory (contained: 6.5% dry matter, 0.8% crude fat, 0.85% crude protein, 0.55% crude ash and 6.6 pH).

Before the fermentation process, wheat bran (WB) and pomegranate peels (PP) were ground and passed through a 3 mm sieve. Experimental groups, first group WB+ MS (tap water), second group WB+ W (whey), third group WB + 0.5% PP + W, fourth group WB + 1% PP+W, fifth group WB + % 1.5 PP+W and the sixth group were formed as WB + 2% PP +W. In the study, the feed samples were adjusted to 100 g by adding 0, 0.5, 1, 1.5 and 2 g pomegranate peel to the wheat bran, respectively.

Whey was used to adjust the humidity (optimum 66%) required for solid phase fermentation. The feed samples were prepared in eight replications and placed in 500 ml erlenmeyer flasks. 120 ml of tap water was added to the mixtures in the first group and 120 ml of whey was added to the other groups and mixed homogeneously. The pH values of the feed samples were measured and stabilized between 4-4.5 values ideal for yeast growth was achieved with citric acid (Pandey et al., 2000).

Four of the feed samples prepared for each group were poured on a nylon cover without fermentation and

dried at room temperature. The mouths of the remaining erlenmeyers were covered with cotton and subjected to fermentation for 48 hours in an incubator at 32 ± 2 °C to ensure the growth of yeast (*Saccharomyces cerevisiae*). In order to reduce the temperature increase during the fermentation and to remove the carbon dioxide from the environment, the Erlenmeyer flasks were mixed with the help of sterile glass drumsticks every eight hours. After fermentation, the feed samples were emptied and dried in the shade at room temperature. After all the feeds were dried, crude protein, crude oil, crude fiber, crude ash, dry matter (Kutlu, 2008), phytic acid analysis (Raheja et al., 1973), antioxidant activity (Blois, 1958), phytase activity, and yeast count (Halkman, 2007) was made.

Statistical Analysis

The analyzes of the data on the nutrient content of the feed samples were made using the General Linear Model procedure and the SPSS 17.0 package program. Significance checks of means found significant between groups were determined by Duncan Multiple Comparison Test (Düzgüneş et al., 1983). The differences between the nutritional values of the feeds before and after fermentation were determined by t test.

RESULTS and DISCUSSION

The mean and variance analysis results of dry matter, crude ash, crude protein, ether extract and crude fiber values of wheat bran subjected to solid phase fermentation with whey and pomegranate peel before and after fermentation are given in Table 1. When the results were examined, it was found that there was no difference between the groups in terms of dry matter, crude ash and crude protein before fermentation (0.hour) and after fermentation (48.hour). It was observed that fermentation had no effect on dry matter.

When the effect of fermentation on the crude ash ratios was examined, it was found that there was an increase in the crude ash ratio of all groups ranging from 0.97% to 20.62%, but this increase was significant ($P<0.05$) only in the WB+ 1.5% PP + W group. When the effect of fermentation on the protein value was examined, it was determined that the crude protein values of the mixtures subjected to fermentation increased significantly ($P<0.05$), except for the WB+ 1.5% PP+ W group, and this increase varied between 9.65% and 23.02%.

It was determined that there were very significant ($P<0.01$) differences in ether extract between both pre-fermentation and post-fermentation groups. The lowest ether extract value before fermentation was observed in WB+TW group, and after fermentation in WB+TW and WB+ W groups. When the effect of

fermentation on ether extract was examined, it was seen that there was a very significant ($P<0.01$) difference in all groups and the ether extract ratio of the feeds decreased between 39.57% and 55.31%. The highest rate of change was observed in the WB+W group with a decrease of 55.311%.

When Table 1 is examined, no significant difference was found between the groups in terms of crude fiber values before fermentation. After fermentation, the difference between the groups was very significant ($P<0.01$) and the lowest crude fiber value was determined in WB+TW, WB+W and WB+ 0.5% PP+ W groups. In addition, it was determined that fermentation had no effect on crude fiber values.

In this study, the increase in the crude ash ratio of the feeds after fermentation was associated with the increase in the yeast count. Because approximately 6-9% of the dry matter of yeast contains crude ash. Phosphorus constitutes most of the crude ash contained in yeast, followed by K, Mg, Ca, Na, respectively. Yeast ash contains small amounts of iron, silicon and sulfur, and trace amounts of copper and zinc (Pamir,1985).

Baran (2017) reported that there were significant changes in the dry matter ratios of barley, wheat and oats subjected to solid phase fermentation with *L. salivarius*. As a result of 48 hours of fermentation, it was determined that the dry matter content of wheat and oats decreased significantly, while the dry matter content of barley increased significantly. The same researcher reported that the rate of crude ash also increased significantly.

Yaşar (2014) reported that there was no change in the dry matter ratio of barley, wheat and oats subjected to fermentation with whey and citrus pulp, but the crude ash ratios increased. Similar results were obtained from studies with different feeds and microorganisms (Rashad et al., 2011; Shi et al., 2017; Hassaan et al., 2015).

In this study, it was found that crude protein levels of feed samples increased due to the effect of fermentation. Similarly, Yaşar (2014) determined that there was a slight increase in the crude protein ratio of barley, oats and wheat fermented with whey. Rashad et al. (2011) fermented the soy by-product "Okora" with six different yeast strains and examined the crude protein ratios. They determined that the crude protein ratio of the fermented product increased by 54% compared to the control feed. Researchers have shown that the reason for this increase in crude protein ratio is the rapid increase in the number of yeast during fermentation. Moore et al. (2007) reported that the crude protein ratio increased by 11-12% after fermentation in wheat bran fermented with different yeast strains and this increase was due to the increase in the number of yeast in the medium. Mathot et al.

(1992) suggested that the increase in crude protein ratio of barley as a result of solid phase fermentation with *Aspergillus niger* is due to the increase in protease activity. Rajesh et al. (2010) reported that crude protein ratio increased by 38% as a result of fermenting various vegetable wastes in solid phase using *Aspergillus niger*. It has been reported that at least 19 types of enzymes are produced due to fermentation. In their research, they attributed the reason for this increase in crude protein rate to the rapid conversion of carbohydrates to fungal protein by *A. niger* 616 used for fermentation. It has been reported that the crude protein ratio of sunflower seed meal fermented with different *Bacillus* strains increased between 3-104%, and the free amino acid content increased from 99.7 mmol/g to 529.1 mmol/g with the effect of the proliferation of microorganisms and the enzyme system (Zhang et al., 2014). Similarly, it was reported by some researchers that the total amino acid content increased as a result of fermentation of soybean meal with *L. plantarum* (Frias et al., 2008).

In the studies summarized above, either the increase in the number of microorganisms or the increase in the enzyme level was shown as the reason for the increase in the amount of crude protein. In this study, in line with the expectations, increases were recorded in the crude protein ratio and yeast count of the feeds after fermentation.

In general, it has been reported that fresh yeast contains 20-30% dry matter and approximately 45-60% of this dry matter consists of crude protein (Pamir, 1985). The significant protein content of yeast body components may be shown as the reason for the increase in crude protein ratio in feed samples.

In the current study, it was observed that the ether extract ratios of the feeds decreased with the effect of fermentation. It is known that approximately 1-7% of yeast consists of fat and similar substances (Pamir, 1985). It has been reported that this rate is between 2% and 23.67% in baker's yeast. The fat ratio in yeast varies according to the nutritional status of the yeast. While well-fed yeasts have high fat content, starved yeasts have little or no fat. High temperature, excessive ventilation and abundant nutrients increase the amount of fat in the yeast cell (Pamir, 1985). Similar to this study, Rashad et al. (2011) determined that the ether extract ratio of Okora fermented with *S. cerevisia* decreased by 6.67%. In many previous studies, it was reported that the ether extract level of feed fermented with different microorganisms decreased (Ilyemi et al., 2006; Lateef et al., 2008). Baran (2017) determined that the ether extract ratio of wheat and oat fermented with *Lactobacillus salivarius* increased, while the ether extract ratio of barley decreased. Shi et al. (2017) reported that a numerical reduction in ether extract ratio of corn-soybean

blended feed fermented with *Bacillus subtilis* and *Enterococcus faecium*

In this study, it was observed that fermentation had no effect on crude fiber values. However, Rashad et al. (2011) reported a 7.38% reduction in crude fiber as a result of fermentation of Okora with *S. cerevisiae*. Researchers attributed the decrease in crude fiber ratio to enzymes that break down cellulose/hemicellulose secreted by yeasts (Lateef et al., 2008). Moore et al. (2007) reported a decrease in the crude fiber ratio of wheat bran fermented with different commercial yeasts. Researchers explained this decrease by the fact that yeasts may have individual enzyme activities and interact differently with soluble and insoluble fibers. As a result of the solid phase fermentation of canola pulp with 10^7 spore/g *Aspergillus niger*, it was determined that the ratio of acid detergent fiber, neutral detergent fiber and crude fiber decreased by 66%, 78% and 25%, respectively (Safari et al., 2012).

The mean and variance analysis results of yeast (*Saccharomyces cerevisiae*) numbers, % DPPH, phytase activity values and phytic acid ratios of wheat bran subjected to solidstate fermentation with whey and pomegranate peel before and after fermentation are given in Table 2.

It was not determined that there was no significant ($P>0.05$) difference between the groups in terms of phytase activity before fermentation. However, it was observed that there was a very significant ($P<0.01$) difference between the groups in terms of phytase activity after fermentation and the lowest value occurred in the WB+TW group. In the study, it was determined that fermentation increased phytase activity significantly ($P<0.01$). Depending on the fermentation, an increase in phytase activity was observed ranging from 9.39% to 47.42% (Table 2).

When the phytic acid values were examined, it was found that there was no significant difference between the groups before fermentation. After fermentation, there were significant ($P<0.01$) differences between the groups in terms of phytic acid and the highest phytic acid ratio was determined in the WB+TW group. It was observed that there was a decrease in the phytic acid ratio between 63.16% and 68.65% depending on the effect of fermentation, and this decrease was very significant in all groups.

When Table 2 was examined, it was seen that there was a very significant ($P<0.01$) difference between the groups in terms of DPPH both before and after fermentation. The lowest DPPH rate before fermentation was observed in the WB+TW group, and the highest value was observed in the WB+2% PP+W group. After fermentation, the lowest DPPH inhibition rate was found in the WB+TW group, while the highest value was found in the WB+2% PP+W group. When the

effect of fermentation on antioxidant values was examined, it was observed that DPPH value increased

significantly with the effect of fermentation, except for WB+TW and WB+0.5% PP+W groups.

Table 1. Dry matter, crude protein, ether extract, crude ash and crude fiber rate of fermented and non-fermented feeds.

Çizelge 1. Fermente ve fermente olmayan yemlerin kuru madde, ham protein, eter ekstraktı, ham kül ve ham selüloz oranı.

Groups	Dry Matter %		Crude Ash %		Crude Protein %		Ether Extract %		Crude Fiber %	
	B.F	A.F	B.F	A.F	B.F	A.F	B.F	A.F	B.F	A.F
WB+TW	92.36±0.36	91.76±0.19	3.80±0.15	4.52±0.16	15.44±0.42B	18.41±0.66A*	4.29±0.01bA	2.37±0.25bB**	13.09±0.31	12.22±0.14b
WB+W	89.15±1.22	89.74±2.18	4.07±0.04	4.11±0.10	15.29±0.92B	18.10±0.50A*	5.17±0.09aA	2.31±0.41bB**	13.08±0.70	12.28±0.23b
WB+0.5% PP+W	88.97±0.97	91.05±0.39	3.62±0.08	4.16±0.03	14.94±0.34B	17.90±0.18A*	5.49±0.01aA	3.11±0.08aB**	13.59±0.58	12.56±0.13b
WB+1%PP+W	89.91±1.20	89.47±1.34	3.96±0.05	4.06±0.09	15.20±0.72B	18.70±0.60A*	5.17±0.09aA	3.09±0.26aB**	13.10±0.09	13.29±0.09a
WB+1.5% PP+W	88.85±0.15	90.48±0.62	3.54±0.01B	4.27±0.05A*	16.37±0.95	17.95±0.13	5.13±0.02aA	3.10±0.14aB**	13.03±0.40	13.20±0.22a
WB+2% PP+W	89.13±0.79	88.89±0.04	3.80±0.15	4.22±0.02	15.31±0.32B	17.65±0.44A*	5.46±0.04aA	3.18±0.06aB**	13.54±0.17	13.24±0.13a
P	ns	ns	ns	ns	ns	ns	**	**	ns	**

a-c: The averages shown with different letters in the same column are different from each other. A-B: Different letters indicate that the mean of each parameter is different from each other before and after fermentation. B.F: before fermentation, After fermentation, WB: Wheat bran, TW: Tap water, Pomegranate peel, W: Whey, *: P<0.05, **: P<0.01 ns: Not significant.

Table 2. Phytase activities, phytic acid ratio, antioxidant activities and yeast numbers of fermented and non-fermented feeds.

Çizelge 2. Fermente ve fermente olmayan yemlerin fitaz aktiviteleri, fitik asit oranı, antioksidan aktiviteleri ve maya sayıları

GROUPS	Phytase Activities (IU/g KM)		Phytic Acid Ratios (%)		Antioxidant Activities (%)		Yeast (<i>Saccharomyces cerevisiae</i>) Numbers %	
	B.F	A.F	B.F	A.F	B.F	A.F	B.F	A.F
WB+TW	4.15±0.06	4.54±0.36b	4.36±0.07A	1.60±0.3aB**	50.45±0.82e	52.56±0.82e	4.67 X 10±0.01 ³ B	6.09 X 10±0.03 ⁶ A **
WB+W	3.83±0.09	5.56±0.26aA**	4.61±0.02A	1.44±0.0cB**	53.35±0.22dB	59.09±0.45dA**	6.00 X 10±0.04 ³ B	6.30 X 10±0.04 ⁷ A **
WB+0.5% PP+W	4.52±0.34B	5.21±0.09aA*	4.43±0.02A	1.46±0.03cB**	54.50±2.24d	60.32±0.25cd	6.55 X 10±0.02 ³ B	6.10 X 10±0.05 ⁷ A **
WB+1% PP+W	4.05±0.20B	5.978aA**	4.48±0.01A	1.42±0.04cB**	56.95±2.77cB	64.02±0.10cA**	4.00 X 10±0.03 ³ B	6.19 X 10±0.03 ⁷ A **
WB+1.5% PP+W	4.58±0.17B	5.67±0.14aA**	4.59±0.01A	1.45±0.02cB**	62.70±1.17bB	69.67±1.25bA*	5.90 X 10±0.02 ³ B	6.23 X 10±0.02 ⁷ A **
WB+2% PP+W	4.09±0.21B	5.50±0.08aA**	4.60±0.06A	1.56±0.02bB**	65.40±1.03aB	76.22±1.26aA**	5.00 X 10±0.04 ³ B	6.12 X 10±0.01 ⁷ A **
P	0.000**	0.000**	ns	0.000**	**	**	ns	ns

a-c: The averages shown with different letters in the same column are different from each other. A-B: Different letters indicate that the mean of each parameter is different from each other before and after fermentation. B.F: before fermentation, After fermentation, WB: Wheat bran, TW: Tap water, Pomegranate peel, W: Whey, *: P<0.05, **: P<0.01 ns: Not significant.

There was no significant difference between the pre-fermentation and post-fermentation groups in terms of yeast count. However, it was determined that yeast counts of the feeds increased significantly (P<0.01) with the effect of fermentation in all groups.

Tran and Sauvart (2004) reported that most of the phosphorus in cereals and oilseeds (2/3) is in the form of phytate, of which 28.2% is in the form of phytin

phosphorus. In a previous study, it was reported that phytase activity increased as a result of soaking wheat bran (Morris & Ellis, 1980). In the present study, it was observed that phytase activity increased as a result of fermentation, while the phytic acid ratio decreased significantly in the groups to which both tap water and whey were added. In a study by Safari et al. (2012), it was determined that the amount of

phosphorus in phytin decreased by 74% as a result of solid phase fermentation of canola meal with 10^7 spore/g *Aspergillus niger*. Carlson and Poulsen (2003) stated that phytate levels in barley and wheat fermented by soaking at different temperatures decreased significantly due to fermentation. In another study, it was stated that a diet soaked with whey for 3 hours at 40 °C significantly increased P absorption in pigs (Näsi et al., 1995). Baran (2017) determined that the phytic acid ratio in barley, wheat and oats subjected to solid phase fermentation with *Lactobacillus salivarius* decreased significantly due to fermentation, while phytase activity increased significantly in barley and wheat.

It has been suggested by researchers that wheat bran, which is rich in many nutrients, is a good source of antioxidants due to the phenolic compounds it contains. In this study, the antioxidant efficiency of the bran used was determined as 49.85%. Cingöz et al. (2017), in their analysis of wheat bran samples, reported that the antioxidant capacity increased due to the increase in the amount of phenolic substances in the bran. In the current study, it was observed that the antioxidant activity increased due to the increase in the ratio of pomegranate peel in the mixture, and the highest antioxidant value was determined in the group with 2% pomegranate peel added. The DPPH inhibition of the pomegranate peel used in the experiment was determined as 80%.

When Table 2 was examined, it was determined that fermentation had a significant effect on the DPPH inhibition values of the feeds. In previous studies, it was reported that as a result of fermentation, free phenolic compounds in the structure of the feed increased proportionally and accordingly the antioxidant activity increased (Martins et al., 2011; Tapati & Kuhad 2014). Similarly, Rashad et al. (2011) found that the antioxidant activity of ochora fermented with yeast strains is quite high compared to the unfermented one. Moore et al. (2007) reported that the antioxidant activity of wheat bran fermented with yeast increased significantly. Tosun (2017) determined that the antioxidant level of apple and tomato pulps, which were subjected to fermentation with *A. niger* for 72 hours, increased significantly. Similarly, Baran (2017) reported that the phenolic content of barley and oats subjected to fermentation with some bacteria increased by 70-300%, and accordingly, there was an increase in the antioxidant value of the feeds by 59-92%. In solid phase fermentation studies, it has been reported that depending on the high enzyme activity in fermented feeds, phenolic compounds in bound form become free and therefore their antioxidant capacity increases (Bhanja & Kuhad 2014). Similar to the present study, Bölükbaşı et al. (2019) found that there was a significant increase in the yeast count of barley subjected to solid phase fermentation with whey.

CONCLUSION

It was found that as a result of fermentation of wheat bran, which is insufficient in terms of nutrient content in poultry feed, with pomegranate peel and whey, protein ratio, phytase and antioxidant activity increased, while phytic acid level decreased. In addition, it was determined that with the increase in the number of yeast, it gained probiotic properties.

Author Contributions

Author 1: Designed the study, made laboratory analyzes, collected data. Author 2: Designed the study, performed the statistical analysis and wrote the paper. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of Interest Statement

All authors declare that there are no conflicts of interest in their articles that may affect the results or comments.

REFERENCES

- Akbulut, G., Yıldız, Atilla., & Yalınca, R. (2010). Nar: Bileşimi ve Potansiyel Sağlık Etkileri, *Kafkas Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 3(2), 53-64.
- Baran, B. (2017). *Arpa, Yulaf Ve Buğdayın Lactobacillus Salivarius Kullanılarak Katı Faz Fermantasyon İle Besin Madde Bileşiminin Zenginleştirilmesi*. (Tez No: 472354) [Yüksek Lisans Tezi, Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Zootekni Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Bhanja, D. T., & Kuhad, R.C. (2014). Upgrading the antioxidant potential of cereals by their fungal fermentation under solid-state cultivation conditions, *Letter Applied Microbiology*, 59(5), 493-499. doi:http://dx.doi.org/10.1111/lam.12300.
- Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 26, 1199-1200.
- Bölükbaşı Aktaş, Ş.C., Dumlu, B. ve Ahmadova, R. (2019). Katı faz fermentasyonu ile arpanın besin değerinin iyileştirilmesi. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, 14(3), 315-323., Doi: 10.17094 (Yayın No: 5835038)
- Carlson, D., & Poulsen, H.D.(2003). Phytate degradation in soaked and fermented liquid feed—effect of diet, time of soaking, heat treatment, phytase activity, pH and temperature. *Animal Feed Science and Technology*. 103 (2003), 141-154.
- Cingöz, A., Akpınar, Ö., & Sayaslan, A. (2017). Farklı kepek fraksiyonlarının fonksiyonel özellikleri ve hamur reolojik özelliklerine etkisi. *Journal of Agricultural Faculty of Gaziosmanpaşa University*. 34 (3), 128-138.

- Demir, M. K., (2011). Effects of cheese and yoghurt dairy by-products on some properties of bread, 4th international congress on food and nutrition together with 3rd SAFE Consortium International Congress on Food Safety, İstanbul, Türkiye, 12-14 October.
- Düzgüneş, O., T. Kesici, & F. Gürbüz. (1983). *Statistical Methods I*. Ankara University, Agriculture Faculty No: 861, 229 sy
- Ersoy, M. & Uysal, H., (2002). Süttozu, peyniraltı suyu tozu ve yayıkaltı karışımları ile üretilen kefirlerin özellikleri üzerine bir araştırma I. Bazı Kimyasal Özellikler, *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 39 (3), 64-71.
- Frias, J., Song, Y.S., Cristina, E.G., & De Mejia, E.G. (2008). Conception v.v, immunoreactivity and amino acid content of fermented soybean Products. *Journal of Agricultural and Food Chemistry*, 56, 99-105.
- Halkman, K. (2007). Gıdaların Mikrobiyolojik Analizi. *Orlab On-Line Mikrobiyoloji Dergisi*, 05, 2-6.
- Hassaan, M. S., Soltan, M. A., & Abdel-Moez, A.M. (2015). Nutritive value of soybean meal after solid state fermentation with *Saccharomyces cerevisiae* for Nile tilapia, *Oreochromis niloticus*. *Animal Feed Science and Technology*, 201, 89-98.
- Iluyemi, F.B., Hanafi, M.M., Radziah, O., & Kamarudin, M.S. (2006). Fungal solid state culture of palm kernel cake. *Bioresource Technology Journal*, 97, 477-482.
- Joseph, I., Paul, R.R., & Bhatnagar, D. (2008). Effect of solid state fermentation on nutrient composition of selected feed ingredients. *Indian Journal of Fisheries*, 55(4), 327-332.
- Kumar, A., & Duhan, J.S. (2011). Production and characterization of amylase enzyme isolated from *Aspergillus niger* mtcc-104 employing solid state fermentation. *International Journal of Pharma and BioSciences*, 2(3), 250-258.
- Kutlu, H.R. (2008). *Yem Değerlendirme ve Analiz Yöntemleri*. Çukurova Üniversitesi Ziraat Fakültesi Zootekni Bölümü, Ders Notu, 65 sy, Adana.
- Lateef, A., Oloke, J.K., Gueguim Kana, E.B., Oyeniye, S.O., Onifade, O.R., Oyeleye, A.O., Oladosu, O.C., & Oyelami, A.O. (2008). Improving the quality of agro-wastes by solid-state fermentation: Enhanced antioxidant activities and nutritional qualities. *World journal of microbiology & biotechnology*, 24, 2369-2374.
- Machida, M., & Gomi, K. (2010). *Aspergillus: Molecular Biology and Genomics*, Caister Academic Press, p.238.
- Martins, S., S.I. Mussatto., G. Martínez-Avila, J., Montañez-Saenz, Aguilar, C.N., & Teixeira, J. A. (2011). Bioactive phenolic compounds: production and extraction by solid-state fermentation. A review, *Biotechnology Advances*, 29, 365-373.
- Mathot, P., Debevere, C., Walhain, P., Baudart, E., Théwis, A., & Brakel, J. (1992). Composition and nutritive value for rats of *Aspergillus niger* solid fermented barley. *Animal Feed Science and Technology*, 39(3-4), 227-237.
- Mete, H. (2012). Peyniraltı Suyunun Ekmekçilikte Değerlendirilmesi ve Ekonomik Önemi, *Tekirdağ S.M.M.M. Odası Sosyal Bilimler Dergisi*, 1,06.
- Moore, J., Cheng, Z., Hao, J., Guo, G., Guo-Liu, J., Lin, C., & Yu, L. (2007). Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. *Journal of Agricultural and Food Chemistry*, 55, 10173-10182.
- Morris, E.R. & Ellis, R. (1980). Bioavailability to rats of iron and zinc in wheat bran: response to low-phytate bran and effect of the phytate/zinc molar ratio. *The Journal of Nutrition*, 110, 10, 2000-2010.
- Näsi, M., Helander, E., & Partanen, K. (1995). Availability for growing pigs of minerals and protein of high phytate barley-rapeseed meal diet treated with *Aspergillus niger* phytase or soaked with whey. *Animal Feed Science and Technology*, 56, 83-98.
- Özen, A.E., & Kılıç, M. (2007). Peyniraltı suyundan elde edilen serum proteinlerinin fonksiyonel özellikleri. *Gıda Teknolojileri Elektronik Dergisi*, (3), 45-49.
- Özen, N., Haşimoğlu, S., Çakır, A., & Aksoy, A. (2012). *Yemler Bilgisi ve Yem Teknolojisi*, Atatürk Üniversitesi Ziraat Fakültesi Ders Yayınları no:50, ss 258, Erzurum.
- Pamir, M.H. (1985). *Fermantasyon Mikrobiyolojisi*, Ankara Üniversitesi Ziraat Fakültesi Yayınları no 936, ss 267, Ankara.
- Pandey, A., Bogar, B., Szakacs, G., Linden, J.C., & Tengerdy, R.P. (2008). Optimization of phytase production by solid substrate fermentation. *Current Developments in Solid-state Fermentation*, 33, 183-189.
- Pandey, A., Soccol, C.R., & Mitchell, D. (2000). New developments in solid state fermentation: 1-Bioprocesses and products. *Process Biochemistry*, 35, 1153-1169.
- Raheja, R.K., Kaur, C., Singh, A., & Batia, I. S. (1973). New colorimetric method for the quantitative of phospholipids without aid digestion. *Journal of Lipid Research*, 14, 695.
- Rajesh N., Imelda-Joseph, & Paul Raj, R. (2010). Value addition of vegetable wastes by solid-state fermentation using *Aspergillus niger* for use in aquafeed industry. *Waste Management* 30, 2223-2227.
- Rashad, M.M., Mahmoud, A.E., Abdou, H.M., & Nooman, M.U. (2011). Improvement of nutritional quality and antioxidant activities of yeast

- fermented soybean curd residue. *African Journal of Biotechnology*, 10(28), 5504-5513.
- Safari, O.M., Farhangi, C., Carter, B., Yakhchali, S., Bai, M., & Sangatash, M. (2012). Study on the effect of solid state fermentation with *Aspergillus niger* on antinutritional factors of canola protein concentrate with aim of using in the diet of rainbow trout (*Oncorhynchus mykiss*). The proceedings of the 1th International and the 4th National Congress on Recycling of Organic Waste in Agriculture, 26-27 Isfahan, Iran, p:1-12.
- Sarıca, Ş. (2011). Nar suyu yan ürünlerinin hayvan beslemede kullanım olanakları, *Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi*, 28(2), 97-101.
- Shi, C., Zhang, Y., Lu, Z., & Wang, Y. (2017). Solid-state fermentation of corn- soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* for degrading antinutritional factors and enhancing nutritional value. *Journal of Animal Science and Biotechnology*, 8, 50.
- Tapati, B.D., & Kuhad, R.C. (2014). Enhanced production and extraction of phenolic compounds from wheat by solid-state fermentation with *Rhizopus oryzae* RCK2012. *Biotechnology Reports*, 4, 120-127.
- Tosun, R. (2017). *Fungal Mikroorganizmalar İle Katı-Faz Fermentasyonuna Tabi Tutulan Elma Ve Domates Posasının Besin Madde Bileşiminin Kanatlı Hayvanlar İçin Yararlı Hale Getirilmesi*. (Tez No: 475165) [Yüksek Lisans Tezi, Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Zootekni Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Tran, G., & Sauvant, D. (2004). Chemical data and nutritional value. Pages 1724 in D. Sauvant, J. M. Perez, and G. Tran, eds. Tables of composition and nutritional value of feed materials: Pigs, poultry, cattle, sheep, goats, rabbits, horses, fish. 2nd ed. Wageningen Academic Publishers, Wageningen, the Netherlands and INRA Editions, Versailles, France.
- Yaşar, S. (2014). Etlik civciv ve piliçlerde buğday, arpa ve yulaf dane yemlerinin peynir suyu ve narenciye posası ile fermentasyona uğratarak besleme değerlerinin artırılmasına yönelik çalışmalar. TÜBİTAK Proje Raporu. Proje Kodu: 1002, Proje No: 113O201
- Zarei, M., Azizi, M., & Zeinolabedin, B. S. (2011). Evaluation of physicochemical characteristics of pomegranate (*Punica granatum* L.) fruit during ripening. *Fruits*, 66, 121-129.
- Zhang, S.T., Shi, Y., Zhang, S., Shang, W., Gao, X., & Wang, H. (2014). Whole soybean as probiotic lactic acid bacteria carrier food in solid-state fermentation. *Food Control*, 41, 1-6.
- Zhang, A. R., Wei, M., Yan, L., Zhou, G. L., Li, Y., Wang, H.M., Yang, Y.Y., Yin, W., Guo, J.Q., Cai, X.H., Li, J.H., Zhou, H., Liangy, Y.X. (2022). Effects of feeding solid-state fermented wheat bran on growth performance and nutrient digestibility in broiler chickens. *Poult Sci*, 101, 102402.
- Zhao, H.M., Guo, X.N., Zhu, K.X. (2017). Impact of solid state fermentation on nutritional, physical and flavor properties of wheat bran. *Food chem*, 217, 28-36.