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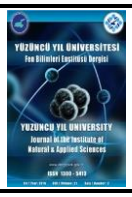

Research Article

Effects of Microwave-Assisted Steam Drying and Freeze-Drying Techniques in the Raw Wheat Germ for the Antioxidant Activity and Microbiological Quality

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Abstract: The purpose of this research was to study the effects of stabilization and storage conditions on the antioxidant activity and microbiological quality of raw wheat germ (R). For this reason, the raw germ samples were stabilized by microwave-assisted steam drying (MWS) and freeze-drying (FD) methods, and they were stored at different storage temperatures (-18, + 4 and 25°C) with polyethylene bags for 90 days. The total phenolics contents (TPC), antioxidant activities (DPPH and ABTS assay methods), and the counts of total mesophilic aerobic bacteria (TMAB), and the total yeast-mold count (YMC) of the germ samples were investigated. Methanol extracts of FD samples showed higher TPC and antioxidant activity than MWS samples significantly ($p<0.05$). Although MWS samples had remarkably lower microbial counts (TMAB and YMC), their antioxidant properties were adversely affected by heat treatment. According to obtained data samples stored at -18 °C showed the most reliable results in terms of tested parameters.

Mikrodalga Destekli Buharlı Kurutma ve Dondurarak Kurutma Tekniklerinin Ham Buğday Ruşeyminin Antioksidan Aktivitesi ve Mikrobiyolojik Kalitesi Üzerine Etkileri

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Anahtar Kelimeler
Dondurarak kurutma,
Mikrodalga destekli buharlı kurutma,
Buğday ruşeymi,
Stabilizasyon

Öz: Bu araştırmanın amacı, farklı stabilizasyon ve depolama koşullarının, ham buğday ruşeyminin antioksidan aktivitesi ve mikrobiyolojik kalitesi üzerindeki etkilerini incelemektir. Bu nedenle ham ruşeym örnekleri mikrodalga destekli buharlı kurutma ve dondurarak kurutma yöntemleriyle stabilize edilmiş ve farklı depolama sıcaklıklarında (-18, + 4 ve 25°C) 90 gün boyunca polietilen torbalarda depolanmıştır. Ruşeym örneklerinin toplam fenolik içerikleri (TFM), antioksidan aktiviteleri (DPPH ve ABTSyöntemleri), toplam mezofilik aerobik bakteri (TMAB) sayıları ve toplam maya-küf sayısı (TMK) araştırılmıştır. Dondurarak kurutulmuş örneklerin metanol ekstraktları, mikrodalga fırın ile stabilize edilmiş örneklere göre istatistiksel olarak daha yüksek antioksidan aktivite göstermiştir ($p<0.05$). Mikrodalga fırın ile stabilize edilmiş örneklerin mikrobiyolojik kalitesi oldukça yüksek olmasına rağmen, antioksidan aktiviteleri ısıl işlemden olumsuz etkilenmiştir. Elde edilen verilere göre -18 °C'de depolanan numuneler, test edilen parametreler açısından en başarılı sonuçları göstermiştir.

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1.Introduction

The purpose of the wheat-milling process is to separate the endosperm from possible by-products such as wheat germ and wheat bran. Wheat germ is one of the main by-products or waste of wheat due to its poor storage stability because of unsaturated fatty acids and the activity of hydrolytic and oxidative enzymes such as lipase and lipoxygenase (Srivastava et al., 2007). Although it is usually evaluated as waste, germ contains valuable nutritional components: 3 times as much protein, 15 times as much sugars, 7 times as much fat, 6 times as much minerals, 6 times as much thiamine, and 15 times as much α-tocopherol when compared to wheat flour (Sudha et al., 2007; Ali et al., 2013). It is also highly valued due to its high content of phenolic acids, flavonoids, and carotenoids and so antioxidant activity (Zhu et al., 2011).

Some researchers have investigated that how this valuable by-product can be used as a commercial product in the food industry by inactivation of the hydrolytic and oxidative enzymes and they have developed several stabilization methods using heat treatments (Xu et al., 2013; Jha et al., 2013; Marti et al., 2014; Li et al., 2016; Zou et al, 2018, Gili et al., 2018; Erim Köse 2021 and 2022).

On the other hand, heat treatment increased the shelf life of the germ, decreased the valuable bioactive compounds, antioxidant activity, and increased the rate of Maillard reaction.

For this reason, we investigated the effects of different heat treatments using microwave-oven (MWS/ radiation heat transfer) and lyophilizer (FD/ freezing heat transfer) on the total phenolic contents, antioxidant activity, and microbiological quality of the raw germ, in the present study. The MWS and FD samples were packaged with polyethylene bags and stored under different storage temperatures (- 18, 4, 25 °C) for 90 days. To our best knowledge, there is no previous study related to the application of MWS and FD methods for the stabilization of the wheat germ. This work aims to develop alternative effective stabilization techniques with the least effect on the nutritional value and investigate the best storage conditions for stabilized germ.

2. Material and Methods

2.1. Germ stabilization

The raw and fresh wheat germ flakes were obtained from the commercial wheat mill Konya-Turkey. The germ samples were homogeneously spread on the tray of the unheated microwave oven (Arçelik, MD554, Turkey). MWS stabilization was carried out for 5 minutes at 600 W power. As the source of steam 50 mL of water in a glass cup was put on top of the chamber, consequently, steam was supplied in the oven during the process. The inner temperature of the sample was 110 °C after heating. The stabilized samples were allowed to cool to room temperature (25 °C).

The frozen R samples (-36 °C) were freeze-dried in a lyophilizer (Labconco, Model:117, Czech Republic,) at a condenser temperature of -50 °C for 48 hours. After the MWS and FD treatments, both stabilized samples and control groups were packed with polyethylene bags and stored at -18, +4, and 25 °C for 90 days. The analyses were performed on days 0, 15, 30, 60 and, 90.

2.2. Preparation of extracts

Before TPC and antioxidant activity analysis, the germ extracts were prepared with methanol (100%, by volume). 10 mL of methanol was added to 2.5 g defatted germ and the mixture was shaken in an incubator (Heidolph Unimax 1010, Germany) for 2 h at 250 rpm. The samples were centrifuged at 10000 rpm for 10 min at 20 °C (Hettich Zentrifugen Universal, 32 R, Germany) to obtain the supernatant. This procedure was repeated twice to a final volume of 25 mL (Bakkalbaşı et al., 2012).

2.3. Determination of TPC

The amount of TPC of methanol-germ extracts was determined by the Folin–Ciocalteu's colorimetric method as described by Bae & Suh, 2007. Briefly, 150 µL Folin-Ciocalteu's reagent (1:1, v/v in water) was mixed with 150 µL methanol-germ extracts and 3.0 mL sodium carbonate (w/v in water). The reaction mixture was shaken and allowed to rest for 45 min in dark at room temperature. Finally, absorbance was measured at 765 nm in a spectrophotometer (UV Mini-1240, Shimadzu, Japan). Gallic acid was used as the standard to calculate the calibration curve (R2=0.99). The results were expressed as mg gallic acid equivalents (GAE) in per 100 g of wheat germ.

2.4. Determination of antioxidant activity

2.4.1.DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay

The DPPH radical scavenging activity analysis was conducted according to the method of Brand-Williams et al., (1995). Firstly, DPPH solution (2.4 mL) was mixed with 100 µL germ extract and this mixture was vortexed and then allowed to react for 30 min. in the dark at room temperature. Absorbance was measured at 520 nm in a spectrophotometer (UV Mini-1240, Shimadzu, Japan). %DPPH scavenging activity or % inhibition were expressed as Equality 1 (Dudonne et al., 2009).

$$\text{Inhibition \%} = (\text{Abs control} - \text{Abs sample})/\text{Abs control} \times 100 \quad (1)$$

where Abs is the absorbance of control and sample at 520 nm.

2.4.2. ABTS (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) assay

The ABTS assay was done according to the method of Kırca & Özkan (2007). Firstly, the ABTS radical cation solution was prepared with 7 mM ABTS • + containing 2.45 mM potassium persulfate and then incubated in the dark at room temperature for 16 h. 30 µL of germ extract was mixed with 2970 µL prepared- ABTS+ solution and shaken vigorously and left to stand for 6 min at room temperature. The absorbance of the mixture was determined at 734 nm. The results were expressed as mmol of ABTS in per 1 g of wheat germ.

2.5. Determination of microbiological analysis

Microbiological analyses of all germ samples were determined according to Harrigan (1998) and Speck (1984). TMAB was enumerated in Plate Count Agar and Potato Dextrose Agar was used for YMC. Results were calculated as cfu/g wheat germ.

2.6. Statistical Analysis

All data were analyzed using software SPSS 18 (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using a one-way ANOVA and Duncan's multiple-range test. ($p < 0.05$).

3. Results

3.1. TPC of wheat germ samples

The TPC of R, MWS, and FD samples were 335, 295.80 and, 343 mg GAE $100g^{-1}$ db, respectively, on the first day of storage (Table 1). The higher TPC of FD samples is due to the effect of lyophilization. The ice crystals formed during lyophilization disrupt the matrix structure of the cell wall and migration of cellular components from the material, consequently, allowing the access of solvent thoroughly in the cell-matrix (Orphanides et al., 2013). Besides, the higher TPC of FD samples might be due to the absence of heat-related damage.

TPC of all samples showed a decreasing trend until the 90th day of storage due to their destruction while an increase was observed on the 90th day. This increase in total phenolic content could be attributed to the degradation of high molecular weight phenolics into the release of simple phenolic compounds with increasing storage days (Amol et al., 2011) In addition others authors have previously observed and reported that formation of new compounds of antioxidant character during storage (Kallithraka et al., 2009; Martinez-Flores et al., 2015). It is possible to say that there is no difference between R and FD samples after the 30th-day of all samples, but MWS samples have always lower TPC values during 90th days of storage, statistically (Table 1). At the end of the storage period, the highest value for TPC (342.10 mg GAE $100g^{-1}$) was determined in the FD sample stored at -18 °C, while the lowest value (304.24 mg GAE $100g^{-1}$) was determined in MWS samples, kept at 25°C. These results show that the FD method is very successful in protecting the valuable phenolic compounds of germ.

Table 1. Total phenolic contents (mg GAE $100g^{-1}$ db) of wheat germ (n=3 ±SD)

		Storage Days				
		Initial	15th day	30th day	60th day	90th day
R	-18 °C	335.00±7.07aA	333.17±1.40aB	325.00±.0.00bB	314.52±0.72cbB	337.76±2.49aB
	+4 °C	335.00±7.07aA	333.62±4.96aB	329.06±.0.74bB	310.91±3.54cB	333.50±4.48abB
	+25°C	335.00±7.07aA	330.17±4.01abB	324.50±.4.24cB	304.90±4.24dB	326.69±0.13bB
MWS	-18 °C	295.80±0.00aA	305.60±0.85bA	303.55±.0.64bcA	300.91±1.00CaA	309.07±7.08bA
	+4 °C	295.80±0.00aA	306.39±2.71bA	300.20±.0.28bcA	300.50±0.39bcA	308.10±3.47bA
	+25°C	295.80±0.00aA	304.30±1.41abA	292.60±.3.39cA	286.91±1.92cdA	304.24±5.30bA
FD	-18 °C	343.00±2.83aB	341.45±2.05abC	336.15±3.04bB	326.10±3.04cB	342.10±4.10aB
	+4 °C	343.00±2.83aB	343.14±0.51aC	338.20±2.26bB	327.15±2.26cB	341.50±2.12aB
	+25°C	343.00±2.83aB	342.53±1.45aC	335.80±1.13bB	320.75±1.13cC	340.60±0.85aC

[a-e] Within each row, different superscript uppercase letters show differences between the storage days within the same analysis group ($p<0.05$). [A-E γ] Within each column, different superscript lowercase letters show differences between treatments within the same analysis group ($p<0.05$). R: Raw wheat germ, MWS: microwave-assisted steam drying wheat germ, FD: freeze-drying germ.

Different TPC values have been previously reported for wheat germ in the literature. The TPC of aqueous and ethanol extracts of wheat germ were found to be 14.63 and 14.93 mg GAE g^{-1}, respectively (Zhu et al., 2011). Velioğlu et al. (1998) noted 349 mg GAE $100g^{-1}$ in methanol extract of wheat germ, which is consistent with the finding (335 mg GAE $100g^{-1}$) of this study. The TPC reported by Bilgiçli et al. (2006) and De Vasconcelos et al. (2013) in 50 and 70% methanol extracts were 3.01 Mm GAE g^{-1} and 4.07 mg GAE g^{-1}, respectively.

3.2. Antioxidant activity of wheat germ samples

3.2.1. DPPH assay

DPPH values followed the same trend as those observed in TPC values, significantly. FD samples showed higher levels of DPPH activity (81.63%) than R and MWS samples (74.49% and 78.92%) (Table 2), since antioxidant substances are not damaged by high heat treatment.The difference between DPPH values of R and MWS samples was not significant at the initial stage ($p>0.05$). Because the low temperature and short time application in the microwave oven (600 W, 5 min) minimized the loss of antioxidant substances. In addition, in the MWS method, steam was

simultaneously introduced into the microwave oven, thus preventing the germ from burning and serious losses in antioxidant activity (Table 1, 2, 3). A linear decrease in DPPH value was observed during the storage period in all samples. For example, the activity of the R sample reduced from 78.92 to the lowest 70.01% while the activity of the MWS sample 74.49 to 66.26% and the FD sample from 81.63 to 75.16 % at the end of the storage period. The reasons to reduce the activity of DPPH are the destruction of compounds with antioxidative activity such as phenolics, tocopherols, or carotenoids by enzyme, pH, temperature, oxygen, light, etc. (Brandolini & Hidalgo, 2012). At the end of the storage period, the MWS samples had the lowest (66.26%) and the FD samples showed the highest DPPH values (75.84%). The result proved once again that the FD method preserved a very high rate of natural bioactive compounds in germs.

Table 2. DPPH values (% inhibition) of wheat germ (n=3 ±SD)

		Storage Days				
		Initial	15th day	30th day	60th day	90th day
R	-18 °C	78.92±0.11aA	78.45±0.01bB	77.01±0.01cB	71.70±0.16dB	70.01±0.01eB
	4 °C	78.92±0.11aA	78.17±0.23bB	77.62±0.88cB	71.45±0.74dB	70.02±0.03eB
	25°C	78.92±0.11aA	78.85±0.06bB	76.78±0.39cB	71.53±0.04dB	70.31±0.44eB
MWS	-18 °C	74.49±0.69aA	73.05±0.04bA	70.44±0.00cA	67.44±0.52dA	66.26±0.51eA
	4 °C	74.49±0.69aA	73.63±0.04bA	70.16±0.22cA	67.35±0.49dA	66.26±0.08eA
	25°C	74.49±0.69aA	73.22±0.31bA	68.59±0.59cA	67.91±0.13dA	66.59±0.09eA
FD	-18 °C	81.63±1.68aB	81.51±0.01bC	80.91±0.14cC	78.30±0.11dC	75.16±2.91eC
	4 °C	81.63±1.68aB	81.09±0.07bC	80.42±0.09cC	78.18±0.88dC	75.80±0.40eC
	25°C	81.63±1.68aB	81.44±0.62bC	80.56±0.63cC	77.71±0.24dC	75.84±0.66eC

[a-e] Within each row, different superscript uppercase letters show differences between the storage days within the same analysis group (p<0.05). [A-E] Within each column, different superscript lowercase letters show differences between treatments within the same analysis group (p <0.05). R: Raw wheat germ, MWS: microwave-assisted steam drying wheat germ, FD: freeze-drying germ.

3.2.2ABTS assay

The ABTS value of the R, MWS, and FD samples were 17.22, 15.54, and 16.75 mmol troloks g^{-1}, respectively. MWS treatment significantly reduced the ABTS values of all samples. (Table 3). While TPC and DPPH values of the FD sample were higher than R samples, its ABTS value was found to be lower. The lower value of ABTS in FD samples may be due to reducing the water content during freezing, consequently, reducing the activity of ABTS used in the method which is more reactive in aqueous media (Şanlıdere Aloğlu, 2010).

There was an increase in ABTS values of R samples stored under different temperatures whereas the MWS and FD samples did not show significant differences during storage (p>0.05). The lowest antioxidant activity was determined in the MWS sample at 4°C (16.17 mmol trolox g^{-1}).

Table 3. ABTS values (mmol trolox g^{-1}) of wheat germ (n=3 ±SD)

		Storage Temperatures				
		Initial	15th day	30th day	60th day	90th day
R	-18	17.22±0.03aB	17.50±0.06aC	19.28±0.39bB	20.61±0.06cB	20.17±0.18cB
	4 °C	17.22±0.03aB	17.45±0.01aB	19.05±0.06bC	20.11±0.02cB	19.66±0.38cC
	25°C	17.22±0.03aB	17.53±0.02bB	19.72±0.19cB	20.04±0.04dB	19.94±0.06cdB
MWS	-18	15.54±0.47aA	15.43±0.18aA	16.09±1.13aA	16.71±0.75aA	16.41±0.04aA
	4 °C	15.54±0.47aA	15.30±0.35aA	16.11±0.16abA	16.57±0.46bA	16.17±0.05abA
	25°C	15.54±0.47aA	15.71±0.08aA	15.76±2.04aA	16.20±0.17aA	16.45±0.00aA
FD	-18	16.75±0.01aB	16.99±0.04aB	17.99±0.09abAB	18.70±1.05bAB	17.15±0.67aA
	4 °C	16.75±0.01aB	17.02±0.75aB	18.02±0.28abB	18.84±1.00bB	17.52±0.42abB
	25°C	16.75±0.01aB	17.48±0.64aB	18.75±0.03aAB	18.03±1.00aA	16.67±1.58aA

[a-e] Within each row, different superscript uppercase letters show differences between the storage days within the same analysis group (p<0.05). [A-E] Within each column, different superscript lowercase letters show differences between treatments within the same analysis group (p <0.05). R: Raw wheat germ, MWS: microwave-assisted steam drying wheat germ, FD: freeze-drying germ.

3.3. Microbiological analyses of wheat germ samples

The TMAB value of the R sample was determined as 3.81 log cfu g^{-1} at the start of the storage, and a linear increase was detected during 90 days (Table 4). At the end of the storage, R samples which were stored at 25˚C had the highest value (5.39 log-cfu g^{-1}) among all germ samples and it can be stated that samples stored at 25˚C are not suitable storage temperature for wheat germ according to microbiological criteria. As a result of the heat treatment applied during the MWS method, the amount of TMAB had a decreasing tendency. During microwave applications, high-frequency waves pass quickly from the inner of food and thus heat transfer from the center to the surface takes place (Meda et al., 2017). So, the amount of energy absorbed by food and microorganisms increases in the microwave oven. Therefore, the power level of 600 W applied for 5 minutes was sufficient to reduce the amount of TMAB in this study. On the other hand, FD samples were found to be statistically different from R samples at the beginning of storage (3.46 log-cfu g^{-1}), this difference gradually closed from the 30th day. In all germ samples, the lowest TMAB value was determined in samples stored at -18°C. YMC value (Table 5) was limited completely in MWS samples because the internal temperature of the sample reached 110 °C in the oven. Although the samples of FD are found at very low temperatures and humidity levels at the beginning of storage, it has been reported that yeast spores maintain their vitality at -15 ° C for 160 weeks (Çakmakçı et al., 2011). In this case, it is seen that yeast spores cannot be inhibited with the FD method. A linear increase was observed in R and FD samples during storage but the rate of increase in FD samples was less than in the R samples. It was determined that yeast-mold growth was quite rapid in samples stored at room temperature (25°C) compared to other temperatures.At the end of the storage, the highest YMC value was observed in the R sample stored at room temperature with a value of 3.13 log-cfu g^{-1} while the YMC value in the FD sample under the same conditions was determined as 2.49 log-cfu g-.

Table 4. TMAB counts (log-kob g^{-1}) of wheat germ (n=3 ±SD)

		Storage Temperatures				
		Initial	15th day	30th day	60th day	90th day
R	-18 °C	3.81±0.14aC	3.97±0.02abC	4.11±0.04bB	4.21±0.01bC	4.72±0.19cC
	4 °C	3.81±0.14aC	4.09±0.01aC	4.39±0.19bAB	4.43±0.02bB	4.94±0.07cB
	25°C	3.81±0.14aC	4.27±0.07bC	4.66±0.13cB	4.95±0.04dC	5.39±0.04eB
MWS	-18 °C	2.75±0.05aA	3.00±0.00bA	3.33±0.04cA	3.47±0.01dA	3.64±0.06eA
	4 °C	2.75±0.05aA	3.17±0.02bA	3.41±0.04bcA	3.71±0.24cdA	3.88±0.13dAA
	25°C	2.75±0.05aA	3.44±0.00bA	3.67±0.02cA	3.87±0.08dA	4.07±0.10eA
FD	-18 °C	3.46±0.06aB	3.72±0.07bB	4.01±0.01cB	4.14±0.01dB	4.26±0.04eB
	4 °C	3.49±0.01aB	3.86±0.04bB	4.23±0.04cB	4.50±0.04dB	4.75±0.04eB
	25°C	3.46±0.06aB	4.10±0.01bB	4.39±0.08cB	4.73±0.06dB	5.20±0.04eB

[a-e] Within each row, different superscript uppercase letters show differences between the storage days within the same analysis group (p<0.05). [A-E]Within each column, different superscript lowercase letters show differences between treatments within the same analysis group (p <0.05). R: Raw wheat germ, MWS: microwave-assisted steam drying wheat germ, FD: freeze-drying germ.

Table 5. Yeast-mould counts (log-kob g^{-1}) of wheat germ (n=3 ±SD)

		Storage Days				
		Initial	15th day	30th day	60th day	90th day
R	-18 °C	2.35±0.06aC	2.37±0.01abC	2.41±0.01abcC	2.43±0.01bcC	2.49±0.01cC
	4 °C	2.35±0.06aC	2.45±0.03bC	2.58±0.04cC	2.65±0.03cC	2.78±0.04dC
	25°C	2.35±0.06aC	2.60±0.14abC	2.70±0.14bC	2.83±0.04bC	3.13±0.07cC
MWS	-18 °C	0.00A	0.00A	0.00A	0.00A	0.00A
	4 °C	0.00A	0.00A	0.00A	0.00A	0.00A
	25°C	0.00A	0.00A	0.00A	0.00A	0.00A
FD	-18 °C	1.91±0.01aB	1.99±0.01bB	2.10±0.02cB	2.12±0.02cB	2.17±0.02dB
	4 °C	1.91±0.01aB	2.04±0.06bAB	2.11±0.01bB	2.20±0.01cB	2.28±0.02dB
	25°C	1.91±0.01aB	2.11±0.01bB	2.25±0.04cB	2.39±0.01dB	2.49±0.01eB

[a-e] Within each row, different superscript uppercase letters show differences between the storage days within the same analysis group (p<0.05). [A-E] Within each column, different superscript lowercase letters show differences between treatments within the same analysis group (p <0.05). R: Raw wheat germ, MWS: microwave-assisted steam drying wheat germ, FD: freeze-drying germ

4. Discussion and Conclusion

In the present study, not commonly used methods MWS and FD were applied for stabilization of raw wheat germ and the effects of these methods on nutritional and microbiological quality of raw germ were discussed. While the TPC and DPPH values of samples followed the order FD>R>MWS,

the ABTS values of samples showed descending order as R>FD>MWS. In terms of microbial counts, MWS samples gave the lowest TMAB, and YMC was not detectable. The overall evaluation of the results showed that FD is an excellent method to minimize losses in bioactive compounds and antioxidant activity in wheat germ. However, it is difficult to use in industrial-scale production as it is not suitable for microbial safety. Thanks to this study, the effects of FD using as a pretreatment can be investigated following the same parameters for different cereal wastes like a germ. Our results also revealed that MWS is a more preferable and safe stabilization method and -18 °C was the most reliable temperature for the storage of stabilized wheat germ according to obtain microbially safe productAs a result, it was observed that the bioactive compounds and antioxidant capacity did not change much at different storage temperatures. Therefore, it seems that it is not necessary to store at -18°C or 4°C as it increases energy consumption in the endustrial scale.

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