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Improving Nutritional Qualities of Tomato Pomace by Pleurotus ostreatus and Phanerochaete chrysosporium Fermentation

Sulhattin YASAR[1], Ramazan TOSUN[2]✉
[1]Karamanoglu Mehmetbey University, Faculty of Engineering, Department of Food Engineering, Karaman, [2]Igdır University, Faculty of Agriculture Department of Animal Science, 76000, Iğdır-Türkiye
[1]https://orcid.org/0000-0001-9334-1303, [2]https://orcid.org/0000-0002-8209-6362
✉: ramazantosun68@gmail.com

ABSTRACT

In this study, it was aimed to improve nutrient quality by fermenting tomato pomace with *Pleurotus ostreatus* (P. *ostreatus*) and *Phanerochaete chrysosporium* (*P. chrysosporium*). Tomato pomace was incubated for 21 days at optimized conditions of pH (3.50-5.50), temperature (24-28 oC), moisture content (68% w w-1), aeration (0,25 L min-1) and stirring rates (10 rpm). Three samples taken at each incubation time were chemically analyzed. The results indicated that fermentation with *P. ostreatus* and *P. chrysosporium* significanly increased ash content by 25 and 21%, crude protein content by 16 and 30%, respectively (P<0.05). Fermentation with P. ostreatus decreased ether extract content from 7.22% to 0.29% at 21th day (P<0.05). However, there was an increase of ether extract content with *P. chrysosporium* fermentation (from 7.22 to 11.62% at 21 day) (P<0.05). Crude fiber of tomato pomace with *P. chrysosporium* were reduced by 64% (P<0.05). Both fungal fermentations reduced total reducing sugar content by about 30% (P<0.05). Fermentation with *P. ostreatus* and *P. chrysosporium* significantly changed tannin and pectin levels (P<0.05). As a result, fungal fermentation caused to nutritionally enriched tomato pomace with added active compounds, and could be used as functional feed in animal nutrition.

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Domates Posasının Pleurotus ostreatus ve Phanerochaete chrysosporium Fermentasyonu ile Besleyici Değerinin Artırılması

ÖZET

Bu çalışmada, domates posasını *Pleurotus ostreatus* (*P. ostreatus)* ve *Phanerochaete chrysosporium (P. chrysosporium)* ile fermente ederek besin madde kalitesini artırmak amaçlanmıştır. Domates posası, optimize edilmiş pH (3.50-5.50), sıcaklık (24-28 °C), nem içeriği (%68), havalandırma (0.25 L dk^{-1}) ve karıştırma hızı (10 rpm) koşullarında 21 gün inkubasyona bırakılmıştır. Fungal fermentasyon sonuçlarına göre ham kül içeriği %21-25 oranında ve ham protein içeriği ise %16-30 oranında artmıştır (P<0.05). *P. ostreatus* ile fermentasyonda, 21. günde ham yağ içeriği %7.22'den %0.29'a önemli derecede azalmıştır (P<0.05). Ancak ham yağ içeriği *P. chrysosporium* ile yürütülen fermentasyonda artmıştır (%7.22'den %11.62'e kadar artış) (P<0.05). *P. chrysosporium* ile yürütülen fermentasyonda ham selüloz içeriği yaklaşık %64 oranında azalmıştır (P<0.05). Domates posasının her iki fungal fermentasyonunda da toplam redükte şeker içeriği yaklaşık %30'a kadar düşmüştür (P<0.05). Tanin ve pektin içeriği her iki fungal fermentasyonunda da önemli derecede değişmiştir (P<0.05). Sonuç olarak, fungal fermentasyonu domates posasının besleyici değerinin artmasına, biyolojik olarak fonksiyonel bileşiklerin oluşmasına sebep olmuştur.

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Anahtar Kelimeler
İşlevsel yem
Phanerochaete chrysosporium
Pleurotus ostreatus
Katı faz fermentasyon
Domates posası

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INTRODUCTION

Recently, there has been an increasing demand on the utilisation of agro-industrial waste products causing enviromental pollution to a greater extend. Tomato pomace is a by-product of tomato processing industry, and composed of tomato skin, seed and pulp. The world's annual tomato waste production reaches up to 11 million ton per year including 4 million tons of tomato pomace (FAO, 2016). Tomato pomace contains appreciable amount of proteins, lipids, carbohydrates, amino acids, carotenoids and minerals (Frexio et al., 2012; Liu et al., 2013; Ergun and Urek, 2017; Waldbauer et al., 2017; Ulker et al., 2018). It has mainly been used as feed material or soil fertilizer (Knoblich et al., 2015; Bennamoun et al., 2016). Tomato pomace can directly be fed to ruminant animals as fresh or dried forms and as a part of silage at appreciable amounts. The ruminant animals can easily utilize from the nutrients of tomato pomace as a result of microbial digestion of the rumen (Weiss et al., 1997; Mirzaei-Aghsaghali and Maheri-Sis, 2008; Ziaei and Molaei, 2010; Abdollahzadeh et al., 2010). In contrary, poultry species can not completely utilize from tomato pomace so as to ruminant animals since the digestive tract of most of the poultry species, especially young growing birds, do not sufficiently secret specific enzymes degrading the nutrients such as crude fibre (CF), tannin and pectin which are mostly considered as antinutritional factors (ANFs) (King and Zeidler, 2004; Al-Betawi, 2005; Wadhwa and Bakshi 2016; Yasar and Tosun, 2019). In addition, tomato pomace is a seasonal product and not available throughout the entire year and difficult for conversation due to its high moisture content of 75%. Moreover, drying tomato pomace at commercial scale to produce animal feed has been found not economically feasible (Weiss et al., 1997), but there are novel processing treatments including drying to produce bioactive compounds such as antioxidants, lycopene, oils and protein as food and feed ingredients (Lu et al., 2019).

Tomato pomace has been successfully used as fermentation substrate by the industry for the production of functional bioactive molecules (enzymes, organic acids, aromatic compounds and antimicrobial agents), for improvement of its nutritional qualities and for reduction of its contents of ANFs (Raimbault, 1998; Singhaniaa et al., 2009; Özşölen, 2010; Afşin, 2010; Kurt and Buyukalaca, 2010; Ravichandran and Vimala, 2012; Mukherjee et al., 2016). Solid state fermentation (SSF) is defined as fermentation of insoluble solid substrates immersed in free water by microorganism cultures similar to their natural environment (Afşin, 2010). For instance, several biologically active enzymes inlcuding cellulases and oxidases (Verma and Madamwar, 2002; Rashad et al., 2009; Iandolo et al., 2011; Yoon et al., 2014; Bennamoun et al., 2016; Ergun and Urek, 2017) are produced by the *P. ostreatus* and *Phanerochaete chrysosporium* (*P. chrysosporium*) fermentations. *P. chrysosporium* was considered an ideal fungal microorganism in order to increase the levels of phenolic compounds of apple fruit pomace (Ajila et al., 2011; Yasar and Tosun, 2018).

The fungals of *Rhizopus stolonifer LAU 07*, *Candida utilis*, *Trichoderma viride, Aspergillus niger, Fusarium, P. ostreatus* and *P. chrysosporium* (Villas-Boas et al., 2003; Lateef et al., 2008; Yasar and Tosun, 2018), the yeast of *Saccharomyces cervisiae* and bacteria of *Bacillus subtilis* (Azza et al., 2013) have been used in SSF processes of agricultural by-products for crude protein enrichment. In addition, fungal microorganisms was reported to break down cellulose, hemicellulose and other complex polysaccharides in industrial by-products (Rashad et al., 2009; Díaz-Godínez et al., 2012). Previously studies reported that fermenting tomato pomace with several microorganisms increased the amount of ash and crude protein (CP) and decreased the levels of CF and hemicellulose (Assi and King, 2008; Azza et al., 2013; Roja et al., 2017; Yasar and Tosun, 2019).

Having evaluated all the above stated results, *P. ostreatus* and *P. chrysosporium* could ideally act as best fungal microorganisms to nutritional enrichment of tomato pomace at optimum conditions of 4.0–5.0 of pH, 25-35 °C of temperature and low stirring rates at occational intervals, which were selected from the literature and optimized and controlled throughout the study using a modern bioreactor. Therefore, the objective of this study was to test the effect of two fungal microorganisms used in SSF on the changes in nutritional composition of pomace.

MATERIALS and METHODS

Tomato pomace optained from a local provider dried and ground to pass a sieve with 3 mm were supplemented with nutrients as shown in Table 1 and was further autoclaved at 120 ℃for 15 min. Two fungal microorganisms, *Pleurotus ostreatus* (*P. ostreatus*) and *Phanerochaete chrysosporium* (*P. chrysosporium*) obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany) were cultivated according to the supplier instruction to collect inoculating spores. Optimum fermentation conditions fixed in the study (Table 1) were selected from the literature and optimized by using a laboratory bioreactor of 2.5-3 L working capacity, LabforEtOlH 5 (Infors Ltd., Switzerland). A blank fermentation experiment was conducted with no fungal inoculation. The fixed pH values were well optimized by peristaltic pumps using buffer solutions of 0.1 M sodium acetate (pH=1.5) and 0.1 M sodium bicarbonate (pH=9.75).

At each sampling period (days), three independent

samples were taken from each of fermentation experiments (Table 1) were consequently analysed three times as replicates for the determinations of nutritional and antinutritional factors parameters.

Table 1. Experimental design with optimised fermentation parameters fixed throughout the fermentation period
Çizelge 1. Fermantasyon süresi boyunca optimize edilmiş fermantasyon parametreleriyle deneme deseni

Experiments	pH	Moisture (%)	Temparature (°C)	Aeration (L min^{-1})	Sampling period (day)	Inoculation rate (spor g^{-1})
I	3.5-4.0	68.0	24-28	0.25	0, 7, 14, 21	2.50 x 10^6 *P. ostreatus*
II	5.0-5.5	68.0	24-28	0.25	0, 7, 14, 21	1.00x10^6 *P. chrysosporium*
III**	4.0-4.5	68.0	24-28	0.25	0, 7, 14, 21	None

*Added nutrients in experiment I, II and III were 20 g $(NH_4)_2SO_4$, 10 g NH_4Cl, 10 g CH_4N_2O and 60 g Molasses, and there was a constant rate of stirring rate (10 rpm for 2 min at every 12 h). **Blank fermentation, a non-pH optimised experiment (initial pH of 4.00 did not change throughout the fermentation period) under sterile fermentation conditions, the same as in experiments I and II

Thus, 9 independent replicates per treatment were obtained, and data was analysed according to a general linear model (GLM) of variance analysis, where the differences between the treatments were separated at 0.05 significance level using SPSS software (IBM SPSS Statistics 22.0 for Windows). Fungal growth was determined by the method of TS ISO 21527-2: 2008 and the contents of dry matter (DM, %), ash %, crude protein (CP, %), crude fiber (CF, %), ether extract (EE, %) and reducing sugar (RS, %) by the methods specifically expressed in AOAC (2005). Pectin (Wang and Zhang, 1999) and tannin (Chemesova and Chizhikov, 2004) were spectrophotometrically analysed.

RESULT and DISCUSSION

The results of Table 2 that the growth rate of *P. ostreatus* in experiment I and *P. chrysosporium* in experiment II significantly increased by 3 log at the end of 21 days of fermentation ($P<0.05$). And the pH was well controlled in the pre-fixed ranges in both experiments (Table 1), indicating a successful fermentation of fungal microorganism on tomato pomace.

Table 2. Fungal growth rate and pH of fermenting substrate at 0, 7, 14 and 21 days of fermentation
Çizelge 2. 0, 7, 14 ve 21 günlük fermantasyonda fungal gelişim oranı ve fermente substratın pH değeri

Days	cfu g^{-1}			pH		
	Experiments-I *(P. ostreatus)*	Experiments-II *(P.chrysosporium)*	Experiments III (Control)	Experiments I *(P.ostreatus)*	Experiments II *(P.chrysosporium)*	Experiments III (Control)
0	$7.9x10^5±0.05^d$	$3.07x10^5±0.05^c$	0.0±0.05	4.05±0.20	5.50±0.10	4.00±0.10
7	$8.0x10^7±0.10^b$	$1.7x10^8±0.20^a$	0.0±0.10	3.70±0.20	5.30±0.15	4.00±0.10
14	$1.3x10^7±0.13^c$	$5.0x10^6±0.05^b$	0.0±0. 10	3.70±0.25	5.30±0.20	4.00±0.15
21	$3.9x10^8±0.15^a$	$1.3x10^8±0.12^a$	0.0±0.10	3.60±0.25	5.40±0.21	4.00±0.15

^{a,b,c,d} Different supercripts showed significant differences between the fermentation periods at each of the coloumn parameters .

No significant changes were seen in nutritional composition of tomato pomace fermented with no fungal inoculations ($P>0.05$) (Table 3). Fermenting tomato pomace with two different fungal inoculants significantly influenced its nutritional composition ($P<0.05$). The contents of ash significantly increased by the fermentations of both fungal species ($P<0.05$). The highest increases in ash content were 25% at 14 days of *P. ostreatus* and 35% at 21 days of *P. chrysosporium* fermentations. There were 16 and 30% increases in the CP content at the 14 days of *P. ostreatus* and at 21 days of *P. chrysosporium* fermentations, respectively were significant ($P<0.05$). The fermentation of tomato pomace with *P. ostreatus* significanly reduced its EE content from 7.22% at 0 day to 0.29% at 21 days. In contrary, there was a gradual increase in the EE content of tomato pomace with the *P. chrysosporium* fermentation (from 7.22% at 0 day to 11.62% at 21 day). As overall there was no significant effect of *P. ostreatus* fermentation on the CF content, whereas an approximate 3-fold decrease in the CF content of tomato pomace was obtained from the *P. chrysosporium* fermentation (15.24% at 0 day to 5.47% at 21 day). The RS contents of tomato pomace were significantly reduced by about 30% in both fermentation cases ($P<0.05$).

Ash content of tomato pomace increased in both fungal fermentations. Dei et al. (2008) reported increased ash content of industrial by-products as a result of fungal fermentation. The reason for the inc rease in ash content was elaborated that during fermentation, microorganisms secrete enzymes which degrade complex minerals such as phosphorus in phytic acid form, and as a result of fermentation, the liberated

minerals are released and thus the ash content increases. Many other studies found increased ash content of the fermenting substrates as a result of fermentation (Dei et al., 2008; Okpako et al., 2008; Aguilar et al., 2008; Altop et al., 2018).

Table 3. Nutritional composition of tomato pomace affected by *P. ostreatus* and *P. chrysosporium* fermentation
Çizelge 3. P. ostreatus ve P. chrysosporium fermantasyonunun domates posasının besin madde bileşimine etkisi

Inoculant	Days	DM, %	Ash, %	CP, %	EE, %	CF, %	RS, %
Experiments I (*P. ostreatus*)	0	31.75±0.57	4.66±0.28^{b}	34.64±0.51^{c}	7.22±0.02^{a}	15.24±2.25	14.04±0.07^{a}
	7	33.60±1.23	4.78±0.41^{b}	40.24±0.07^{a}	0.40±0.01^{b}	14.74±0.94	9.57±0.02^{c}
	14	29.60±0.42	5.81±0.25^{a}	40.07±0.17^{a}	0.45±0.01^{b}	14.75±0.30	10.63±0.12^{b}
	21	31.60±0.19	5.14±0.14^{ab}	37.65±0.08^{b}	0.29±0.01^{c}	16.58±0.49	10.17±0.20^{b}
Experiments II (*P.chrysosporium*)	0	31.75±0.37	4.66±0.28^{b}	34.64±0.51^{d}	7.22±0.02^{d}	15.24±2.25^{a}	14.04±0.07^{a}
	7	34.66±0.52	5.67±0.83^{ab}	42.35±0.28^{c}	10.22±0.03^{c}	14.14±1.18^{a}	9.48±0.03^{b}
	14	32.69±2.24	5.46±0.31^{b}	43.37±0.14^{b}	10.67±0.06^{b}	11.50±0.38^{b}	9.91±0.03^{b}
	21	34.80±1.91	6.31±0.30^{a}	44.96±0.25^{a}	11.62±0.01^{a}	5.47±1.00^{c}	9.53±0.35^{b}
Experiments III (Control)	0	32.00±0.10	4.50±0.21	34.70±0.01	7.00±0.01	15.00±1.00	14.50±0.01
	7	32.10±0.20	4.25±0.05	34.35±0.23	7.20±0.01	15.60±0.05	14.00±0.30
	14	32.00±0.12	4.60±0.23	34.50±0.50	7.40±0.02	15.30±0.20	13.90±0.40
	21	32.10±0.61	4.70±0.11	34.40±0.41	7.10±0.02	15.30±0.50	14.00±0.20

^{a,b,c,d} Different supercripts showed significant differences between the fermentation periods at each of the coloumn parameters.

Lateef et al. (2008), clearly showed that fungal fermentation using the strain of *Rhizopus stolonifer LAU 07* significantly improved nutritional qualities of some agro-wastes by increasing CP contents by 35-90% and reducing CF by 7.0 to 44% ($P<0.05$). Similar improvement rates were also reported by several other fungal species including *P. ostreatus* and *P. chrysosporium* in the study of Assi and King, (2008); Rashad et al. (2009 and 2010); Díaz-Godínez, (2012) and Yasar and Tosun, (2018). In fungal fermentation fungal growth and reproduction of micelles formed as a result of the substrate used in fermentation has been reported to increase the CP content (Altop et al., 2018). On the other hand, Oboh and Akindahunsi (2003) claimed that the increased CP content was due to the increased enzymes which are in nitrogenous nature. It is thought that the increase of CP content in the fermentation of tomato pomace with *P. ostreatus* and *P. chrysosporium* is due to the above reasons. However, in our study, the amount of increased CP content differed in the case of both microorganisms, showing that the type of fungal microorganisms may have affected the degree of increased CP. As overal the *P. ostreatus* and *P. chrysosporium* fermentations were found useful for increasing CP of tomato waste products.

The content of EE in tomato pomace was nearly consumed by the strain of *P. ostreatus* in our study. *P. ostreatus* was also previously shown to reduce the EE contents of oil-seed meal (Yasar and Tosun, 2018). The reduced lipid content could be due to the accumulation of lipids by some strains of fungal microorganisms which have lipase enzyme activity (Lateef et al., 2008; Agbo and Prah, 2014; Altop et al., 2019) since some fungal microorganisms should assimilate lipids from the fermenting substrates in order to produce other biomasses (Tinoco et al., 2011; Iandolo et al., 2011; Frexio et al., 2012; Jannathulla et al., 2018). In contrast *P. chrysosporium* significantly increased the EE content of tomato pomace. This increase in EE is thought to be due to the release of lipolytic enzymes that break down glycerol and fatty acids in some microorganisms. Because Onweluza and Nwabugwu (2009) reported that the EE increases when millet (*Pennisetum americanum*) and Pigeon pea (*Cajanus cajan*) fermented and as a reason for the secreted lipolytic enzymes during fermentation. However, in the previous studies, EE decreases; and lipolytic enzyme secretion and the increase in EE in fermentation are not sufficient in the literature.

Some microorganisms generally use easily soluble carbohydrates such as starch and sugar to meet carbon requirements during fermentation, and then prefer to use complex carbohydrates or other nutrient as carbon sources (Papagianni, 2007; Altop et al., 2019). And as well Xie et al. (2016) and Altop et al. (2019) reported that during solid state fermentation, fungals release enzymes such as cellulase, hemicellulose, which break down structural carbohydrates, and these enzymes break down structural carbohydrates. It was concluded that the microorganism used as a carbon source as a reason for the decrease in RS and HS content in fermentation of tomato pomace with *P. chrysosporium*. On the other hand, *P. chrysosporium* is a white-rot-fungus, the release of enzymes that break down structural carbohydrates is thought to be high and therefore the HS content of tomato pomace may be reduced. In *P. ostreatus* fermentation, the HS content did not change, but the RS content decreased significantly, suggesting that this microorganism did not use either the carbon requirement of *P. chrysosporium* or the HS as a carbon source or does not release enough enzymes to break down structural carbohydrates

There was no significant effect on the contents of ANFs

(tannin and pectin) of tomato pomace of the fermentation carried out without fungal microorganisms in this study (P>0.05) (Table 4). However, fermenting tomato pomace with *P. ostreatus* and *P. chrysosporium* significantly influenced these compounds (P<0.05). There was an average of 60% decrease in the tannin content at the 14 day of the fermentations carried out both fungal microorganisms (P<0.05). Fermentation of tomato pomace with *P. ostreatus* did not significantly change the pectin content (P>0.05). However, there was a significant sporadic effect of *P. chrysosporium* fermentation on the pectin content, which was increased (P<0.05) by 48% at 14 day and then decrease (P<0.05) by 28% at 21 day of in the pectin contents.

The pectin and tannin are usually available in the tomato peel tissue, and its degradation is very important to free up important biologically active compound such as phenolic compounds and lycopene with antioxidant property (Lavecchia and Zuorro, 2008; Rodríguez-Fernández et al., 2011; Saleh et al., 2018). Biz et al., (2016) reported that they produce pectinase enzyme by fermenting agricultural by-products with *Aspergillus oryzae*. Enzyme production in the presence of pectin in the fermentation of microorganisms in the presence of pectin to break down the enzyme is connected to secrete. In our studythe pectin content of tomato peels was extracted and released into the matrix of fermentation substrate at the 14 day of fermentation with both fungal species, due to a possible increase in the activitiy of pectinase.

Table 4. Influence of fungal fermentation on the contents of tannin and pectin of tomato pomace
Çizelge 4. Fungal fermantasyonunun domates posasının tannin ve pektin içeriğine etkisi

Microorganisms	Days	Experiments I *(P. ostreatus)*	Experiments II *(P. chrysosporium)*	Experiments III (Control)
Tannin, %	0	13.17±0.84[a]	13.17±0.84[a]	13.20±0.02
	7	4.91±0.10[b]	6.55±0.34[b]	13.00±0.50
	14	4.98±0.80[b]	5.52±1.43[b]	13.00±0.20
	21	5.36±0.12[b]	6.99±0.36[b]	12.90±0.01
Pectin, %	0	3.43±0.31	3.43±0.28[b]	3.40±0.02
	7	4.19±0.46	3.77±0.69[b]	3.32±0.06
	14	3.91±0.19	5.09±0.49[a]	3.64±0.09
	21	4.47±0.81	2.47±0.23[c]	3.40±0.05

[a,b,c] Different superscripts showed significant differences between the fermentation periods at each of the column parameters.

Finally, the degradation of tomato peels could be said to be completed by fungal fermentations. This mechanism was more pronounced with the fermentation using *P. chrysosporium*. Thus, it could possible that some important phenolic compounds are released during the fungal fermentations and this lead to a possible increase of antioxidant capacity.

The tannin content of tomato pomace decreased in fermentation with both fungal microorganisms. In previous studies, it has been reported that in fermentation microorganisms break down tannin into smaller molecules such as gallic acid, catechin, glucose and gallicatechin (Rodriguez et al., 2008; Nazarni et al., 2016; Shang et al., 2019). In this study, it is thought that the decrease in tannin content secretes tannase enzyme and the tannin content is converted to gallic acid, catechin, glucose or gallicatechin.

CONCLUSION

As a result, the fermentation of tomato pomace by fungal microorganism yielded an enrichment of nutritional qualities and added some biologically functional compounds. The fermented tomato pomace with improved nutritional qualities is holding a great potential in farm animal nutrition.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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