J. Food and Dairy Sci., Mansoura Univ., Vol. 10 (4): 85 - 91, 2019

The Effects of Crude Phenolic-Rich Extract from Mushroom (*Agaricus bisporus*) on Shelf-Life Extension and Quality Attributes of the Feta Like Cheese

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ABSTRACT

The objective of the present study was to prepare the phenolic-rich extract (PRE) from the mushroom (*Agaricus bisporus*) and investigate there *in vitro* antioxidant activity as well as estimation the contents of total phenolic and total flavonoids. The antibacterial activities against gram-positive and gram-negative bacteria were estimated. On the other hand, PRE from *Agaricus bisporus* was estimated as a bio-preservative article in Ultra filtered Feta-like cheese. *In vitro* study with DPPH-assay showed that the antioxidant activity of PRE increased gradually as concentration increases. The antibacterial activity of PRE increased with increasing the concentration of total phenolic compounds. This treatment was found to reduce the total bacterial, coliform, mold and yeast counts in feta like cheese compared with control. The score given for flavor increased gradually with advanced storage to be evaluated as extremely and like very much. It can be concluded that Mushroom (*Agaricus bisporus*) could be added as an additional nutrient to dairy products (UF-Feta like cheese) as it constitutes a new potential source of natural antioxidant and antibacterial agents. The potentiality of this PRE at the level of 100 µg/ml as a useful additive to improve the bacteriological quality and shelf life of the product could be recommended.

Keywords: *Agaricus bisporus*, Phenolic-rich extract, Antimicrobial, Antioxidants, Feta like cheese

INTRODUCTION

Food security and safety of consumer food globally are a growing concern and a serious challenge (Organization, 1992). Concurrently, the demand for shelf life stabilized with optimal nutrition and organoleptic quality has driven the evolution of novel food processing applications (Zink, 1997). Additionally, Microbial contamination of food is one of the major problems that may influence shelf life and may also cause humans sickness. Thus, many synthetic agents are used as preservatives to raise the shelf life for food products. The use of synthetic agents is prevented owing to the probable negative influence of such chemicals on human health and the environment. So, novel antibacterial and disinfectant articles from natural products are strongly wanted. Using natural antibacterial in food protection is progressively becoming more prevalence (Zohri *et al.*, 2013). Natural alternates of preservatives can be established from several sources such as 7S globulin, 11S globulin and its basic subunit from soybean (Mahgoub, Osman, & Sitohy, 2016; Osman, Daidamony, Sitohy, Khalifa, & Enan, 2016; Osman, Mahgoub, & Sitohy, 2013), legumin from chickpea (Osman, Goda, & Sitohy, 2018), lupin protein hydrolysate produced by alcalase (Osman, El-Araby, & Taha, 2016), antibacterial peptide from whey protein (Abdel-Hamid, Goda, De Gobba, Jenssen, & Osman, 2016; Osman, Goda, Abdel-Hamid, Badran, & Otte, 2016), esterified egg proteins (Abdel-Shafi, Osman, Enan, El-Nemer, & Sitohy, 2016), esterified legume proteins (Mahgoub, Osman, & Sitohy, 2011; Mahgoub, Sitohy, & Osman, 2013; Osman, Mahgoub, El-Masry, Al-Gaby, & Sitohy, 2014; AO Osman, Mahgoub, & Sitohy, 2014), essential oils (Mahgoub, Osman, & Ramadan, 2017), and nisin (De Arauz, Jozala, Mazzola, & Penna, 2009). On the other hand, the oxidation in handling and storage of food is important in the food manufacture as participating factor to proteins and lipids oxidation as well as to the forming of not pleasant flavor, texture and color (Ali, 2010).

Mushrooms are a source of bioactive compounds and functional food (Farzaneh, Khanahamadi, Ehsani, & Sharifan, 2018). One of the most used and important mushrooms is *Agaricus bisporus*, which has been applied in classical medicine (Liu *et al.*, 2012). One of the major components of mushrooms is phenolic compounds (Reis, Martins, Barros, & Ferreira, 2012). Phenolic compounds have properties as antioxidant and antimicrobial, among others (Puttaraju, Venkateshaiah, Dharmesh, Urs, & Somasundaram, 2006). Ultrafiltered (UF) Feta cheese is a cheese with a soft and spreadable texture that is produced from milk which has been concentrated by ultrafiltration, to achieve total solids of 35 %, and then enzymatically coagulation of retentate. This type of cheese contains 45-60% fat (on a dry basis), 28% protein (on dry basis) and max. 3% salt and its final pH after 72 hours is 4.8. UF-Feta cheese is a fresh cheese that can be consumed 3 days after production. The shelf life of UF-Feta cheese is max. 2 months (El-Salam, Alichanidis, & Zerfiridis, 1993). In this work, the phenolic-rich extract (PRE) was prepared from *Agaricus bisporus*. Total phenolic, total flavonoid compounds, the antioxidants activity and the antimicrobial activities for this extract were investigated. On the other hand, the application of using the resultant mushroom extract to an enhancement of keeping quality and prolong the shelf life of low salt Feta cheese type was investigated.

MATERIALS AND METHODS

1-Mushroom

Champignons (*Agaricus bisporus*) was purchased from local market, Zagazig City, Egypt.

2-Rennet

Microbial rennet powder (Marzyme – protease Rhizomucor miehei) has obtained from Danisco France- 2, Avenue Brun- Fauiuier- 38470 VINAY (Franc) and used in cheese making after dilution (0.5 g / 20 ml water/10 kg retentate- MPC).

3-Retentate

Retentate of buffalo's milk (40% solids), was obtained from Obour Land Company for food industries (Obour City, Cairo, Egypt).

4-Chemicals

All reagents used in chemicals analysis of cheese and in preparing the microbiological media, were of analytical quality.

5-Phenolic compounds extraction

The mushroom sample (*Agaricus bisporus*) was cleaned manually, cut and lyophilized (Thermo-electron Corporation – Heto power dry LL 300 Freeze dryer). Then, the lyophilized powder was defatted using n-hexane (5 % w/v) in a Soxhlet apparatus as described in (Kavishree, Hemavathy, Lokesh, Shashirekha, & Rajarathnam, 2008) and hexane was separated from sample under reduced pressure at 68 °C in a vacuum evaporator. Twenty grams ground mushrooms were extracted with methanol (200 ml) using magnetic stirrer at 25 °C ±3 °C for 2 h, followed by filtration by filter paper Whatman No.1. The remains were re-extracted twice under the same conditions. Methanol was separated in a vacuum rotary evaporator (BüCHI-water bath-B-480) under reduced pressure at 65 °C. To removing methanol wholly, samples were re-dissolved in distilled water and filtered through a 0.20 µm filter followed by lyophilization (Thermo-electron Corporation–Heto power dry LL 300 Freeze dryer). The lyophilized PRE was preserved in a refrigerator to further analysis.

Total phenolic compounds (TPCs) determination

The TPCs for the PRE from mushroom (1000 µg in 1ml distilled water) were estimated by Foline-Ciocalteu reagent as described by (Singleton, Orthofer, & Lamuela-Raventós, 1999). Absorbance was determined at 765 nm. Gallic acid was applied to gain the standard curve (20 – 200 µg/ ml), and the lowering of Folin-Ciocalteu reagent by the samples was expressed as mg of gallic acid equivalents (GAE) per g of extract. The calibration equation for gallic acid was $y = 0.001x + 0.0563$ ($R2 = 0.9792$), where y is absorbance and x is concentration of gallic acid in µg/ml..

Total flavonoids (TFs) determination

Total TFs for the PRE from mushroom (1000 µg in 1ml distilled water) were estimated according to the protocol of (Ordonez, Gomez, & Vattuone, 2006). The absorbance at 420 nm was estimated. Quercetin was applied to gain the standard curve (20–200 µg/ml), Total flavonoids contents expressed as quercetin equivalent (QE), which was calculated based on the calibration curve. The calibration equation for quercetin was $y = 0.0012x + 0.008$ ($R2 = 0.944$), where y is absorbance and x is concentration of quercetin in µg/ml.

Antioxidant activity evaluation (DPPH-assay)

The antioxidant activity of mushroom PRE at different concentrations (100, 250, 500, 1000, 1500 and 2000 µg extract/1ml solvent) was estimated by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay according to (Hatano, Kagawa, Yasuhara, & Okuda, 1988). 500 µl of each concentration was added to 2500 µl of 0.1 mM DPPH dissolved in methanol. After the incubation period of 30 min at 27 °C ± 3 °C, the absorbance was recorded with the control at 517 nm (Gülçin, Küfrevioğlu, Oktay, & Büyükokuroğlu, 2004). The antioxidant potential of DPPH radicals (%) was studied as follow:PRE

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

Where Abs. control is the absorbance of the control and Abs. sample is the absorbance in the presence of mushroom extract.

Antimicrobial activity estimation

Agar well-diffusion assay

Agaricus bisporus mushroom PRE were tested for antibacterial activity at different concentrations (0, 50, 100, 200, 500 and 1000 µg/ml) against two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were kindly gained from the Laboratory of Microbiology, Department of Microbiology, Faculty of Science, Zagazig University, Egypt by conventional well-diffusion assay (Nanda & Saravanan, 2009). The clear cultures of bacterial strains were sub-cultured on nutrient broth at 37 ˚C on a rotary shaker at 200 rpm. Every strain was dispersal uniformly onto the single plates using sterile cotton swabs. Wells of 6-mm diameter were made on Müller Hinton Agar (MHA) plates using a gel puncturing tool. Forty µL of each sample (0, 50, 100, 200, 500 and 1000 µg/mL) were carried into each well. After incubation at 37 ◦C for 24 h, the diameter of the inhibition zone was recorded by using a ruler. The lowest concentration of the examined articles that presented visible clear zone on Mueller-Hinton agar plates was regarded as the minimal inhibitory concentration (Abdel-Hamid *et al.,* 2016; Sitohy, Mahgoub, Osman, El-Masry, & Al-Gaby, 2013).

Transmission electron microscopy (TEM)

Structural changes of bacterial cells (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) onto the treatment with PRE (1 MIC) were studied using TEM (JEOL-TME-2100F, Japan) as demonstrated by (Sitohy *et al*., 2013).

Cheese manufacturing

The UF-Feta like cheese was manufactured according to the method described by (Renner & Abd-El-Salam, 1991). Calcium chloride (0.02%) and sodium chloride (3%) were added (Moawad, Galal, & Metry, 2001). PRE from mushroom was added at the rate of (0, 50, 100, 200 µg/mL of retentate); Microbial rennet powder was used in cheese making after dilution (0.5 g / 20 ml water/10 kg retentate-MPC). The resultant cheese of all treatments was packaged into a plastic container (1 Kg), sealed and incubated at 45°C for one hour and then transferred to the refrigerator at 5±2 °C and stored for 28 days. Samples were taken for organoleptic properties, chemical, and microbiological analysis at zero time (just after manufacture), 7, 14, 21, and 28 days.

Examination of cheese

Organoleptic evaluation

Feta like cheese was examined for organoleptic properties as described by (O'sullivan, 2016), with maximum score points of 50 for flavor, 40 for body & texture and 10 for appearance.

Microbiological examination

One gram of cheese was accurately weighted and transferred to a sterile mortar. Cheese was then thoroughly ground in 10 ml of a sterile aqueous sodium citrate solution (2%) to a homogenous mass. The mortar contents were transferred quantitatively to a sterile volumetric flask 100 ml using a sterile saline solution and the volume was made up to the mark to get the final 1/100 dilution of the cheese which was used in final making further dilutions (Association, 1992) for the determination of the different microbial groups as follow:

The total bacterial count (T.C) was determined according to (Digestive Ferments Company. Difco Laboratories, 1953) using Tryptone Glucose Extract Agar (T.G.E.A) medium. Plates were incubated at 37 °C for 2 to 3 days. The total coliform count was estimated by plating suitable dilutions on MacConkey agar medium as described by (Association, 1992). The plates were incubated for 24 hr., at 35±1°C, and the small non-mucoid red colonies were

counted. The total mold and yeast counts were determined according to APHA, 1992, by plating suitable dilutions in duplicates on Sabouraud Dextrose Agar medium (Manual, 1998). Plates were incubated at 28°C for 3 days then the counts were recorded.

Chemical analysis

Total solids (T.S%), Fat/DM content, total nitrogen (TN/DM), soluble nitrogen (SN/TN), non-casein nitrogen (NCN/TN) and non-protein nitrogen (NPN/TN) fractions were determined as described in (Fox, 1997). Titratable acidity (T.A) was determined by the method developed by (Lau, Barbano, & Rasmussen, 1991). Total volatile fatty acid (TVFA) contents were determined by the method described by (Kosikowski, 1982).

Statistical analysis

Results were statistically analyzed using a computer program "SAS system for windows version 9.00 TS M0" (Guide, 2008) for analysis of variance by one way (ANOVA) and comparison of means by Duncan's multiple comparison test where $P< 0.05$ was considered for significant difference.

RESULTS AND DISCUSSION

1-Total phenolic compounds (TPCs), total flavonoid contents (TFCs) and antioxidants activity

The TPC value is shown in Figure 1. *Agaricus bisporus* mushroom listed in Folin Ciocalteu assay was 164 mg GAE/g. The result obtained in this investigation was higher than results obtained by (Oke & Aslim, 2011; Reis, Barros, Martins, & Ferreira, 2012; Reis, Martins, *et al.*, 2012; Yang, Lin, & Mau, 2002). The TFC value is listed in Figure 1. The result gained for *Agaricus bisporus* mushroom was 100 mg QE/g.

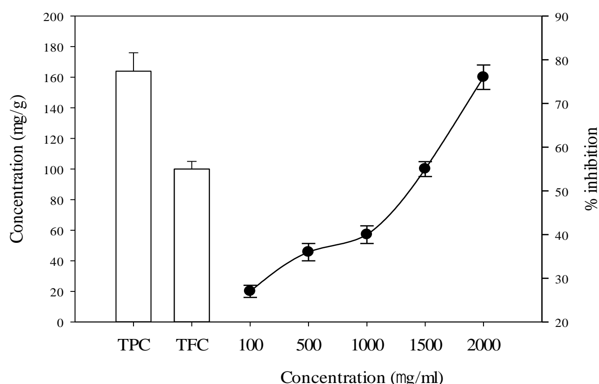

Figure 1. Total phenolic contents (mg GAE/g extract) (□), total flavonoid contents (□) (mg QE/g extract) for *Agaricus bisporus* mushroom and antioxidants activity (-●-) (inhibition %) for phenolic-rich extract from *Agaricus bisporus* at different concentration (100-2000 µg/ml) using DPPH assay.

The antioxidant action of PRE is generally correlated with the total phenolic content (Palacios *et al.*, 2011). Antioxidant activity (% inhibition) for PRE from *Agaricus bisporus* using DPPH assay is presented in Figure 1. The antioxidant activity of PRE from *Agaricus bisporus* increased gradually with increasing concentration of TPCs and TFCs. These results compatible with our results recorded in total phenolic compounds. These results are in agreement with results obtained by (Chirinang & Intarapichet, 2009; Oke & Aslim, 2011; Tsai *et al.*, 2009).

2-Antimicrobial activity estimation

The PRE from *Agaricus bisporus* was subjected at different concentrations (0, 50, 100, 200, 500 and 1000 µg / ml) to Petri dishes containing Müller Hinton Agar (MHA) infected with two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), incubated at 37 °C for 24 h and the inhibition zones diameter (mm) of the resulting are listed in Table 1. The diameter of the inhibition zones increased with the increment of the extract concentration. The minimum inhibitory concentration (MIC) of PRE from *Agaricus bisporus* was 100 µg/ml against *Staphylococcus aureus* and *Bacillus subtilis* and recorded 200 µg/ml against *Pseudomonas aeruginosa* and *Escherichia coli.* It can be noted that, the antibacterial activity of PRE in *Agaricus bisporus* increased gradually with increasing the concentration of TPCs and TFCs. Crude extracts wealthy in mycochemicals of *Pleurotus* species have also certain antimicrobial activities (Carrasco-González, Serna-Saldívar, & Gutiérrez-Uribe, 2017). Organic solvent extracts of *P. ostreatus, P. sajor-caju, P. eryngii* and *P. citrinopileatus* presented clear antibacterial (Gogavekar *et al.*, 2014; Meng *et al.*, 2011). The methanolic extracts containing b-sitosterol, cholestanol, 1,5 dibenzoylnaphthalene and 1,2-benzenedi-carboxylic acid from *P. sajor-caju* presented antibacterial action (Gogavekar *et al.*, 2014).

Table 1. The Inhibition zones diameter (mm) induced in two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) using agar well diffusion assay under the influence of different concentrations (0, 50, 100, 200, 500 and 1000 µg/ml) of phenolic-rich extract from *Agaricus bisporus*.

Microorganisms	Inhibition zone diameter (mm)					
	0	50	100	200	500	1000
Gram +ve						
Staph. aureus	0±0	0±0	11±0.05	14±0.06	27±0.03	33±0.02
B. subtilis	0±0	0±0	10±0.03	15±0.02	25±0.01	36±0.05
Gram -ve						
Pse. aeruginosa	0±0	0±0	0 ±0	14±0.04	19±0.07	29±0.05
E. coli	0±0	0±0	0 ±0	11±0.05	18±0.08	27±0.07

The treated and untreated (control) bacterial cells (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were studied using TEM to estimate the mode of action of PRE from *Agaricus bisporus* as antibacterial agent against gram positive and gram negative bacteria. The effect of PRE from *Agaricus bisporus* on the *Staphylococcus aureus* (gram-positive bacteria) and *Pseudomonas aeruginosa* (gram-negative bacteria) are shown in Figure 2. The control (untreated cells) of *Staphylococcus aureus* and *Pseudomonas aeruginosa* looked normal with right components, while the processed cells exposed different points of the antibacterial action of the extracts. Adsorption of extracts to the cell surface occurred, followed by whole lysis of the cell wall and demolition of the cell membrane. Based on TEM results, the antibacterial activity may be due to the interaction between the components in PRE from mushroom such as b-sitosterol, cholestanol, 1,5 dibenzoylnaphthalene and 1,2-benzenedi-carboxylic acid (Gogavekar *et al.,* 2014) and cell wall and membranes.

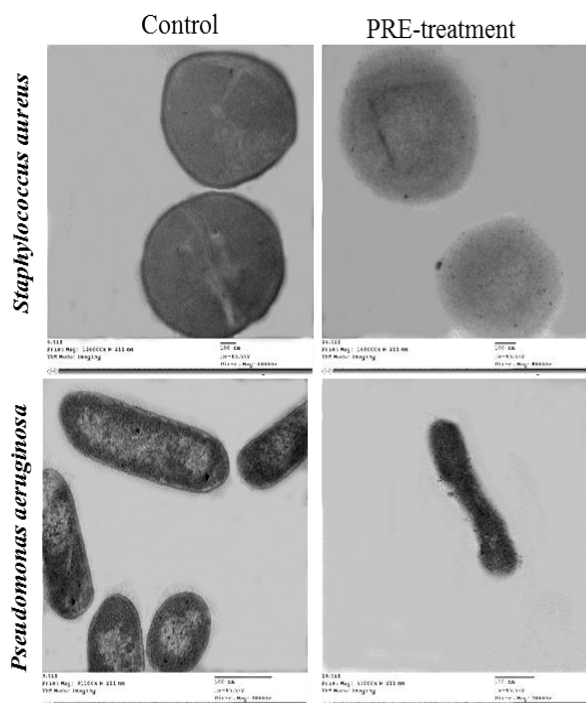

Figure 2. Transmission electron microscopy (TEM) of *Staphylococcus aureus* and *Pseudomonas aeruginosa* treated with phenolic-rich extract (PRE) from *Agaricus bisporus* at 1 MIC concentrations (200 µg/ml) at 37°C as compared to control.

3-Chemical composition of UF Feta like cheese as affected by mushroom PRE

The composition of all treatments has been presented in Table 2. The addition of PRE to UF retentate at levels if 50 and 100 µg/ml did not show significant ($p < 0.05$) effect on moisture content of Feta like cheese. However, at concentration of 200 µg/ml moisture content of Feta like cheese was significantly ($p < 0.05$) higher than control one. With the advance of storage period moisture content of feta like cheese of all treatments significantly ($p < 0.05$) decreased up to the end of storage period. Data in the same Table indicated that mushroom PRE treated feta like cheese showed significant ($p < 0.05$) lower fat content than control one. Also, it could be noticed that fat content of cheese from all treatments showed significant ($p < 0.05$) increase with the progress of storage period. However, the total nitrogen content of treated cheese showed opposite trend, whereas the treated cheese had significant ($p < 0.05$) higher total nitrogen content than control cheese. It could also noticed that there were significant ($p < 0.05$) differences in salt content of control and treated cheese up to the 100 µg/ml, but increasing the concentration of mushroom extract at 200 µg/ml resulted significant ($p < 0.05$) decrease in salt content of resultant cheese. The compositions of all treatments were in agreement with the composition recommended by Codex Alimentarius Commission, "Standard for Cheese in Brine" (Jalili, 2016).

Nitrogen fractions and TVFA Table 3 shows the effect of addition of mushroom PRE to UF retentate on proteolysis and formation of TVFA during storage of feta like cheese. Data indicated that treated cheese had significant ($p < 0.05$) lower SN, NPN, AAN and TVFA contents that control cheese either fresh or during storage period. These results could be due to the antimicrobial and antioxidants activities of mushroom PRE, which reduced the microbial population in cheese. This in turn reduced the proteolysis rate during cheese storage.

Table 2. The chemical composition of UF Feta like cheese during storage period (4 week) for control and treated samples with different mushroom phenolic-rich extract (PRE) concentrations (0, 50, 100 and 200 µg/ml).

Parameter	Storage period (week)	Concentration of PRE (µg/ml of retentate)			
		0	50	100	200
Moisture %	Fresh	69.78^{bc}	70.14^{ab}	70.19^{ab}	70.41^{a}
	1	69.15^{de}	69.36^{cd}	69.41^{cd}	69.55^{cd}
	2	68.06^{g}	68.51^{fg}	68.83^{ef}	69.08^{de}
	3	66.67^{ij}	67.08^{hi}	67.1^{hi}	67.24^{h}
	4	64.51^{l}	65.98^{k}	65.98^{k}	66.56^{j}
Fat/dry matter %	Fresh	41.86^{h}	41.36^{i}	40.55^{j}	40.25^{j}
	1	43.76^{bcd}	42.49^{fg}	42.42^{fg}	41.05^{i}
	2	43.83^{bc}	43.31^{de}	42.87^{ef}	42.04^{gh}
	3	45.00^{a}	43.55^{cd}	42.52^{f}	42.73^{f}
	4	45.08^{a}	44.09^{b}	44.09^{b}	43.36^{cd}
Acidity (as Lactic acid %)	Fresh	0.13	0.17	0.15	0.14
	1	0.13	0.16	0.15	0.13
	2	0.18	0.15	0.14	0.12
	3	0.22	0.14	0.13	0.12
	4	0.23	0.13	0.12	0.09
Salt /dry matter %	Fresh	10.87^{f}	10.78^{f}	10.75^{f}	10.28^{g}
	1	12.23^{c}	12.14^{cd}	12.00^{cde}	11.65^{e}
	2	12.29^{c}	12.15^{cd}	12.15^{cd}	11.66^{e}
	3	12.39^{c}	12.29^{c}	12.16^{cd}	$11.81d^{e}$
	4	13.92^{a}	13.43^{b}	13.38^{b}	12.38^{c}
T.N /D.M	Fresh	5.64^{bcd}	5.86^{b}	6.31^{a}	6.34^{a}
	1	5.46^{cde}	5.68^{bc}	5.9^{b}	5.67^{bc}
	2	5.21^{efg}	5.36^{def}	5.42^{cde}	5.50^{cde}
	3	5.02^{gh}	5.08^{fg}	5.35^{def}	5.39^{cde}
	4	4.24^{i}	4.32^{i}	4.73^{h}	4.93^{gh}

Values in the same raw having different letters are significantly differed *p>0.05*
Values are presented as means ± SD.

Table 3. The Nitrogen fractions of UF Feta like cheese during storage period (4 week) for control and treated samples with different mushroom phenolic-rich extract (PRE) concentrations (0, 50, 100 and 200 µg/ml).

Parameter	Storage period (week)	Concentration of PRE (µg/ml of retentate)			
		0	50	100	200
S.N / T.N	Fresh	18.62^{n}	17.77^{p}	17.5^{r}	14.80^{t}
	1	20.17^{m}	18.43^{o}	17.63^{q}	16.73^{s}
	2	24.33^{f}	22.95^{g}	22.31^{j}	21.55^{k}
	3	25.00^{e}	22.62^{h}	22.52^{i}	21.01^{l}
	4	33.98^{a}	33.81^{b}	29.58^{c}	26^{d}
N.P.N / T.N%	Fresh	6.88^{p}	5.78^{q}	8.24^{n}	5.76^{q}
	1	9.50^{i}	8.77^{m}	8.77^{m}	7.72^{o}
	2	9.84^{j}	9.73^{k}	11.48^{g}	8.78^{m}
	3	10.90^{h}	10.71^{l}	13.2^{d}	12.45^{f}
	4	17.50^{a}	16.19^{b}	13.50^{c}	13.11^{e}
A.A.N /T.N	Fresh	6.23^{m}	5.42^{q}	4.92^{s}	5.20^{r}
	1	7.03^{n}	5.56^{p}	5.98^{n}	5.84^{o}
	2	7.79^{e}	$7.02h^{i}$	6.27^{l}	6.50^{k}
	3	8.10^{c}	7.62^{f}	7.00^{i}	6.87^{j}
	4	8.99^{a}	8.25^{b}	7.97^{d}	7.50^{g}
TVFA (ml 0.1 N NaOH/100 gm)	Fresh	11.95^{n}	10.36^{o}	10.20^{o}	10.00^{p}
	1	13.90^{j}	13.50^{k}	13.20^{l}	12.92^{m}
	2	14.90^{g}	14.64^{h}	14.55^{h}	14.32^{i}
	3	18.00^{d}	16.60^{e}	16.00^{f}	15.87^{f}
	4	24.20^{a}	20.40^{b}	20.04^{c}	20.00^{c}

Values in the same raw having different letters are significantly differed *p>0.05*
Values are presented as means ± SD.

4-Microbiological profile of feta like cheese as affected by addition of mushroom PRE

Data of microbial profile of mushroom extracts treated feta like cheese are shown in Table 4. Data indicated that addition of mushroom PRE to UF retentate showed significant ($p < 0.05$) reduction in total bacterial, coliform, mold and yeast counts of resultant feta like cheese either fresh or during storage period. Data indicated that total bacterial, mold and yeast counts of control cheese gradually increased during the storage period. However, these microbial counts of treated cheese gradually decreased during the storage period. Moreover, it could be noticed that coliforms were not detected in treated cheese after the first week of storage. But both molds and yeasts were not detected in the treated cheese at the third week of storage period.

5-Effect of mushroom PRE on organoleptic properties of cheese

Table 5 shows the organoleptic scores for control and experimental feta like cheese made with added mushroom PRE at different concentrations. Hedonic scale for flavor control and treated samples were evaluated to be between like moderately and like very much. The score given for flavor increased gradually with advanced storage to be evaluated as like extremely and like very much. Body and texture of fresh control and treated samples were found to be between like moderately and like slightly. This property was improved also with advanced storage to be between like moderately and like very much. The appearance of control sample and treated ones followed the same trend as body and texture.

Table 4. Microbiological profile of UF Feta like cheese during storage period for control and treated samples with different mushroom phenolic-rich extract concentrations (0, 50, 100 and 200 µg/ml).

Treatment	**Total bacterial counts × 10^6 cfu/g**				**Coliform × 10^2 cfu/g**			
Storage period (week)	**0**	**50**	**100**	**200**	**0**	**50**	**100**	**200**
Fresh	83×10^6	55×10^6	34×10^6	12×10^6	4×10^2	2×10^2	1×10^2	N.D
1	110×10^6	38×10^6	21×10^6	2×10^6	3×10^2	1×10^2	N.D	N.D
2	177×10^6	25×10^6	9×10^6	N.D	N.D	N.D	N.D	N.D
3	202×10^6	18×10^6	3×10^6	N.D	N.D	N.D	N.D	N.D
4	235×10^6	7×10^6	N.D	N.D	N.D	N.D	N.D	N.D
Treatment	Moulds × 10^3 cfu/g				Yeasts × 10^3 cfu/g			
Storage period (week)	0	50	100	200	0	50	100	200
Fresh	15×10^3	11×10^3	8×10^3	3×10^3	76×10^3	51×10^3	21×10^3	13×10^3
1	18×10^3	7×10^3	3×10^3	1×10^3	93×10^3	38×10^3	5×10^4	3×10^3
2	20×10^3	5×10^3	2×10^3	N.D	131×10^3	24×10^3	2×10^3	N.D
3	21×10^3	3×10^3	N.D	N.D	183×10^3	11×10^3	N.D	N.D
4	23×10^3	1×10^3	N.D	N.D	211×10^3	7×10^3	N.D	N.D

N.D: Not detected **Values are presented as means ± SD.**

Table 5. Organoleptic properties of UF Feta like cheese during storage period (4 week) for control and treated samples with different mushroom phenolic-rich extract (PRE) concentrations (0, 50, 100 and 200 µg/ml).

Storage period (week)	**properties**	**Concentration of PRE (µg/ml of retentate)**			
		0	**50**	**100**	**200**
Fresh	Flavor	7.33^a	7.17^b	7.09^b	7.04^b
	Body & Texture	6.98^a	6.49^b	6.43^b	6.39^b
	Appearance	7.28^a	6.88^b	6.55^b	6.48^b
1	Flavor	8.25^a	7.61^b	7.50^b	7.45^b
	Body & Texture	7.35^a	6.88^b	6.58^b	6.49^b
	Appearance	7.55^a	6.95^b	6.73^b	6.65^b
2	Flavor	9.09^a	8.30^b	8.15^b	8.06^b
	Body & Texture	8.41^a	7.57^b	7.48^b	7.38^b
	Appearance	8.00^a	7.65^b	7.55^b	7.40^b
3	Flavor	9.25^a	8.16^b	8.15^b	8.1^b
	Body & Texture	8.52^a	7.78^b	7.60^b	7.49^b
	Appearance	8.27^a	7.55^b	7.45^b	7.40^b
4	Flavor	9.45^a	8.70^b	8.64^b	8.50^b
	Body & Texture	8.60^a	7.80^b	7.78^b	7.69^b
	Appearance	8.56^a	7.75^b	7.64^b	7.60^b

Values in the same raw having different letters are significantly differed *p>0.05*
Values are presented as means ± SD.

CONCLUSION

Mushroom (*Agaricus bisporus*) could be added as an additional nutrient to dairy products (UF-Feta like cheese) as it constitutes a new potential source of natural antioxidant and antibacterial agents. This treatment was found to reduce the total bacterial, coliform, mold and yeast counts in feta like cheese compared with control. The potentiality of this phenolic rich extract at level of 100 µg/ml as a useful additive to improve the bacteriological quality and shelf life of the product could be recommended.

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تأثيرات المستخلص الخام الغني بالمواد الفينولية من فطر عيش الغراب (*Agaricus bisporus*) على إطالة فترة الصلاحية وجودة الجبن شبيه الفيتا

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الهدف من هذه الدراسة هو تحضير المستخلص الغني بالفينول (PRE) من فطر عيش الغراب (*Agaricus bisporus*) ودراسة نشاط مضادات الأكسدة في المختبر وكذلك تقدير محتويات مجموع الفينول والفلافونويدات الكلية. تم تقدير الأنشطة المضادة للبكتيريا الموجبة والسالبة لجرام. من ناحية أخرى، تم تقييم المستخلص الغني بالفينول من الفطر كمواد حافظة حيوية في جبن شبيه الفيتا المصنع بالترشيح الفائق. في الدراسة المعملية لاختبار DPPH أظهرت أن نشاط مضادات الأكسدة من المستخلص الغني بالفينول زاد تدريجيا مع زيادة التركيز. ازداد نشاط المستخلص الغني بالفينول المضاد للميكروبات مع زيادة تركيز المركبات الفينولية الكلية. وجد أن هذه المعاملة أدت إلى الحد من العدد البكتيري الكلي، وبكتيريا الكوليفورم ، والفطريات والخمائر في الجبن شبيه الفيتا مقارنة مع الجبن الكنترول. زادت درجات التحكيم الحسي المعطاة للنكهة تدريجياً بتقدم فترة التخزين. يمكن أن نخلص إلى أنه يمكن إضافة مستخلص فطر عيش الغراب (*Agaricus bisporus*) كعامل مغذٍ إضافي لمنتجات الألبان مثل الجبن شبيه الفيتا لأنه يشكل مصدرًا جديدًا محتملًا للعوامل المضادة للأكسدة الطبيعية ومضادات الجراثيم. يمكن التوصية بإمكانية هذا المنتج عند مستوى 100 ميكروجرام/مل كإضافة مفيدة لتحسين الجودة البكتريولوجية وإطالة فترة تخزين للمنتج.