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Life Science Journal

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

The effect of Oyster mushroom (*Pleurotus ostreatus*) as functional food on yoghurt quality

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Abstract: Oyster mushrooms as health promoter and environmental restorer are gaining more importance during the past two decades. Owing to the high consumption rate of dairy products such as yoghurt, fortification of these products will effectively reduce or prevent diseases associated with nutritional deficiencies. Mushrooms are also considered as functional foods as they elicit their positive effect on human being in several ways. In the current study, different concentrations of dehydrated mushroom powder (DMP; 0.05, 0.1 and 0.2%) were adjunct during yoghurt production. Its property is affected like its physiochemical, nutritional, rheological and sensorial values during 0, 7 and 15 days of storage at 4°C. It was noticed that, the presence of fibre was markedly noted as increased with the mushroom ratios increased. The yoghurt with 0.2% DMP appeared to have significant impact on yoghurt viscosity (7805, 7896 and 7965 mPs during the storage time). In terms of important minerals in tested yoghurt; Calcium was observed in moderate concentrations at zero time with such DMP ratios (0.05, 0.1 and 0.2%, 1254.0877, 1348.9505, and 1438.6377 mg/kg, respectively). Zn and Fe are considered as micronutrients, they found as the highest concentrations in sample 6 (30.3651 and 31.471 mg/kg, respectively). The present investigation revealed that Valine (3.882 g/100gm in sample 3) was the major essential amino acid in casein. While, for non-essential amino acids: Alanine content was found to be highest in sample 2 (5.223g/100gm) followed by Glutamic and Proline in samples 8 and 6 (3.899 and 2.98 g/100gm, respectively). The antioxidants vitamins (C and E) were abundantly found in samples 3 and 4 as 537.0350 18.134 mg/kg, respectively. Rheological and sensorial results indicated that the incorporated yoghurt with 0.1% DMP showed a values of hardness, cohesiveness and gumminess higher than control yoghurt caused a more compact texture and so helped to improve the acceptability properties of yoghurt. As conclusion, the addition of DMP to yoghurt affect significantly the functional yoghurt properties.
[Aisha El Attar, Nour El Hoda Ahmed and Morsi El Soda The effect of Oyster mushroom (*Pleurotus ostreatus*) as functional food on yoghurt quality**.** Life Sci J 2021;18 (6):7-19]. ISSN 1097-8135 (print); ISSN 2372-613X (online). http://www.lifesciencesite.com 2.doi:10.7537/marslsj180621.02.

Key words: Yoghurt Dehydrated mushroom powder, physicochemical properties, Texture analysis, Sensorial evaluation.

1. Introduction:

Mushrooms are consumed over years as a neighbourhood of human diet. Due to the therapeutic value of mushroom, it's categorized as a functional food with the property of disease prevention in humans. The presence of biologically active compounds having different medicinal properties provides a chance to develop edible mushrooms into functional foods with enhanced nutritional value and various health benefits. Genus *Pleurotus* belongs to Tricholomataceae and has about 40 well-recognized species, out of which 12 species are cultivated in several parts of country. *Pleurotus* is an efficient lignin degrading mushroom and may grow well on differing types of lignocellulolosic materials. As, in previous work, Elattar *et al*. (2019) showed that the yield and overall acceptability of *P. ostreatus* were higher when grown on rice straw (RS) or mix of rice and wheat straws (RS+WS) 1:1 (w/w) compared to those grown on other substrates such as saw dust and water hyacinth alone or mixed with wheat straw 1:1 (w/w). In recent years, increasing prosperity in developing countries has led to a considerable growth in interest in mushroom cultivation, which is now seen as a potentially important economic activity for small farmers (Cheung, 2008). Cultivation of this Mushroom is extremely simple and low cost production technology, which provides consistent growth with high biological efficiency. Different species of *Pleurotus* can grow well in variable temperature conditions; hence they are ideally suited for cultivation throughout the year in various regions of tropical country like Egypt. Mushrooms are rich in proteins, vitamins, and minerals and popularly called as the vegetarian meat. Mushroom proteins are considered to be intermediate between that of animals and vegetables (Kurtzman, 1976) because it contains all the nine essential amino acids required for physical body (Hayes and Haddad, 1976). Mushrooms are valuable health foods which are low in calories and supply with essential minerals (Enas *et al.*, 2016). They are an excellent source of vitamin B-complex, including riboflavin and pentothenique acid that provide energy by breaking down proteins, fats and carbohydrates. Mushrooms are also considered a source of important minerals like Copper which plays a role in making red blood cells that carry oxygen through the body. It also maintains bones and nerves healthy. Potassium is a significant mineral that keeps normal fluid and mineral balance that controls blood pressure (Duyff 2006). Recently, many agencies and ministries in Egypt offered small projects to the youth to produce mushrooms in order to provide job opportunities, but the Egyptian customers still are not accustomed to taste, flavour and texture of fresh mushrooms. Moreover, fresh mushroom has low shelf life even if it is stored at refrigerated temperature. Thus, the present work was undertaken to prepare dehydrated mushroom powder (DMF) and utilize it in the preparation of yoghurt. Yoghurt is a functional food product containing probiotic cultures and amino acids (Antontceva *et al.*, 2018). Many manufacturers enrich yoghurts with various vitamins, minerals and natural flavour additives (Vital *et al.*, 2015). Oyster mushroom (*Pleurotus* spp) extract (OME) contains many oligosaccharides and polysaccharides and is therefore considered to be a prebiotic which supports the viability of probiotics. The roles of prebiotic in improving and maintaining human health have been studied extensively (Elsayed *et al.*, 2014, Valverde *et al.*, 2015 and Gupta and Chaturvedi 2020). Otherwise, developments in prebiotics have heightened the need to search for another potential source of prebiotics. Mushrooms seem to be a potential candidate for prebiotics as it contains carbohydrates like chitin, hemicellulose, band β-glucans, mannans, xylans and galactans simplified findings on the composition of carbohydrate in mushrooms. Due to the high consumption rate of dairy products like yoghurt, fortification of those products will effectively reduce or prevent diseases related to nutritional deficiencies. Efforts to combat nutrient deficiencies have centred on supplemental nutrient administration and addition of selected nutrients to the organic phenomenon within the sort of food fortification. Over the past several decades, and because the association between diet and chronic diseases became apparent, supplementation and fortification were also targeted at healthy individuals, with the aim of reducing their risk of future diseases like cardiovascular diseases, diabetes, and cancer (Caballero. 2003). Nutrition scientists have mentioned that fortification of food products using natural resources (fruits,cereal, etc.) is one among the simplest ways to enhance the general nutrient intake of food with minimal side effects (Nestlé, 2013). Therefore, consumers will not only benefit with the prebiotic effect of mushrooms extract, but also enjoying the medicinal benefit of it (Abd El-Razek *et al.*, 2020). This addition is typically around 15% of the total volume of the product and can provide increased acceptance, since not all consumers appreciate plain yoghurts. Furthermore, fruits cause the attenuation of the characteristic sour taste of fermented products. Therefore, the aim of this study was to gauge the nutritional value, the rheological proprieties and sensory evaluation during refrigerated storage (4°C for 15 days) of probiotic yoghurts fortified with mushroom.

2. Materials and Methods:

1. Mushroom cultivation

The *P. ostreatus* was cultivated on a 1:1 (w/w) mixture of rice and wheat straws (RS+WS), (Elattar *et al.*, 2019). Straws were cut into small pieces (2-3 inches long) and soaked in water at room temperature for 1-2 hr, then rinsed with clean water 2-3 times. Their moisture content was also adjusted to 65%. Then, CaCO3 was added at a final concentration of 0.2% (w/w) of the total substrate. All substrates were filled into polyethylene bags and autoclaved at 121 °C for 80 min, then cooled down to room temperature and inoculated with 5 g spawn of *P. ostreatus* per 100 g of the substrate wet weight. The inoculated bags were incubated in darkness at 20–25 °C and 80-95% relative humidity for 3 weeks until the substrates were completely colonized with mycelium. Bags were punctured from the 4 sides to facilitate primordial initiation.

2. Preparation of dehydrated mushroom powder (DMP)

After cutting fresh mushrooms into 2 mm thick slices, they spread over aluminum trays in a single layer and kept overnight at -80^{o}C, then subjected to freeze- dryer Zirbus (Vaco-5-ll Germany). The freeze dryer was programmed to operate primary drying cycle for 20 h at 0^{o}C shelf temperature and 0.6 mbar chamber pressure, followed by secondary drying cycle at 15^{o}C shelf temperature for 2 h and 0.4-0.5 mbar chamber pressure. The freeze-dried mushroom was electrically grounded to obtain dehydrated mushroom powder (DMP). The dehydrated mushroom powder was packed, sealed in plastic bags under vacuum and stored in a refrigerator (4°C) until used.

3. Preparation of yoghurt with different percentages of dehydrated mushroom powder (DMP):

Yoghurt was made in the Cheese Research Laboratory, Department of Dairy Science and Technology, Alexandria University. Raw buffalo milk was standardized to 7% fat in milk. Different percentages (control, 0.05, 0.1, 0.2 of DMP) were added to whole buffalo milk as different treatments. The milk was then pasteurized at 95˚C for 10 min and cooled to 42˚C using an Actini model Acti-Joule pasteurizer. One percent of probiotic culture (*Lactobacillus acidophilus* LA-5 probiotic culture nu-trish LA-5 (CHR-HANSEN) was immersed to the milk and left to one hour at 42˚C. Then the probiotic milk was mixed with 3% (v/v) of active commercial starter culture YoFlex- L903 (The thermophilic lactic culture type yoghurt -YoFlex- L903(CHR-HANSEN). All samples were poured into 50-mL bottles, incubated at 42 °C. The fermentation was terminated at pH 4.7±0.1 and the samples stored at 4^{o}C for 0, 7 and 15 days for later tests (Physicochemical, texture and sensorial analysis). Dried DMP yoghurt samples using freeze- dryer Zirbus (Vaco-5-ll Germany) as mentioned previously, were used to determine their contents of minerals, amino acids and water soluble vitamins.

4. Yoghurt analysis

4.1. Physicochemical analysis of DMP yoghurt samples

Yoghurt samples were analysed for moisture using the moisture analyser (Mettler Toledo model HR73), total protein by Kjeldahl method, (AOAC, 2003). The pH of yoghurt samples was measured by macerating 20 g of yoghurt in 20 ml of deionized water using a glass electrode (Criston, Basic 20). The percent fat was determined by Gerber method, (AOAC, 2003). Crude fibre was determined according to the AOAC 2003 using sulphuric acid. Colour analyses for samples were conducted via a high performance colour measurement spectrophotometer (UltraScan®VIS Spectrophotometer, Hunter Associates Laboratory Inc., Va, USA). The instrument was first standardized using a white tile (top of the scale) and a black tile (bottom of the scale). Yoghurt sample was placed at the specimen port; the tri-stimulus values of the colour namely; L*, a* and b* were measured where: L: value represents darkness from black (0) to white (100), a: value represents colour ranging from red (+) to green (-) and b: value represents yellow (+) to blue (-) as described by Seçkin and Baladura (2012). The apparent viscosity of yoghurt was determined by using a digital Rotary Viscometers MYR (VR 3000-Model L, Viscotech Hispania, S.L, El Venrell, Spain). Yoghurt sample was placed in a clean dried glass beaker of 120 ml capacity. The spindle was inserted and centred in the tested sample until the fluid's level become at the immersion groove on the spindle shaft. The measurement was always carried out at 20˚C by using spindle L3 after 30 seconds at speed of 12rpm. The viscosity reading was recorded as mPas (Nilsson *et al.*, 2006).

4.2. Minerals determination of DMP yoghurt samples

Metals (Magnesium, Calcium, Sodium and potassium) and heavy metals (Zinc, Copper, Iron and Manganese) were determined using Atomic Absorption Spectrophotometers Shimadzu model (AA- 6650) according to Paul *et al.*, 2014. Phosphorus presence was determined using the colorimetric method (Ch. Zinzadze. 1935).

4.3. Amino acids determination of DMP yoghurt samples

The amino acid profile of yoghurt was performed using modified PICO-TAG method (Cohen *et al.*,1989) carried out in a HPLC system. Sample was injected and loaded on Pico-Tag amino acids column (150 x 3.9 mm) stainless steel. Detection of the phenylthiocarbamyl (PTC) derivatives was done by ultraviolet absorption measurements using a fixed wavelength (254nm) Waters detector. Samples were hydrolysed in 6 N HCl for 24 h at 110 °C under vacuum conditions and then, the hydrolysate was derivatized using the PITC method and injected on the HPLC using a sodium acetate/triethylamine/acetonitrile elution method. Each sample analysis was made in triplicate and expressed in g/100 g DM.

4.4. Water soluble vitamins content of DMP yoghurt samples

Chromatographic experiments were conducted using an Agilent 1260 infinity HPLC Series (Agilent, USA) equipped with quaternary pump, a hyper clone TM BDS C18, 130A 100 mm x 4.6mm (Phenomenex®USA) operated at 35˚C. Both isocratic and binary gradients were generated using this system. Separation is achieved using a binary linear elution gradient with 25 mM NaH_2PO_4 pH = 2.5 (v/v) and methanol (HPLC grade). The injected volume was 20uL. Detection variable wavelength detector (VWD) set at 270nm. Vitamin standards were obtained from Sigma Aldrich (Milwaukee, WI).

4.5. Texture Profile Analysis (TPA) of DMP yoghurt samples

Textural properties of yoghurt were evaluated using a texture analyser (TA1000, Lab Pro (FTC TMS-Pro), USA). Yoghurt samples were measured as cups (30X30 cm), samples were allowed to stand at ambient temperature for at least 1h before testing. A two-bite penetration test was performed and operated at a crosshead speed 1 mm/sec, and penetration distance of 10 mm. Hardness, adhesiveness, cohesiveness, springiness and gumminess were evaluated in triplicate (Bourne, M 2002 and Szczesniak1963).

4.6. Sensorial evaluation of DMP yoghurt samples

Final products, which were stored at (4˚C), were allowed to rest at room temperature (25˚C), 10 min before evaluation. The samples were evaluated using a 10-point Hedonic scale (ISO and IDF 2009). The tested yoghurts were evaluated for organoleptic characteristics; colour, taste, smell, texture, appearance, and overall acceptability supplemented by a scale of ten categories as: 1 = dislike extremely; 2 = dislike much; 3 = dislike moderately; 4 = dislike slightly, 5 = neither dislike nor like, 6 = like slightly; 7 = like moderately; 8 = like much; 9 & 10 = like extremely. The sensorial evaluation was determined using scale ranging from excellent (score = 10) to very poor (score = 0) as extremes. Ten panellists (Staff members, under graduated and graduated students) of the faculty of agriculture, Alexandria University., showed sensory evaluation on tested yoghurt samples with different concentration of DMP. Also the panellists were being asked to list any flavour defects.

4.7. Statistical analyses

All data were expressed as mean values ± SD. Statistical analyses were performed via Statistical Package for Social Science (SPSS Version 20). Statistical analyses were performed using one-way analyses of variance (ANOVA) followed by Duncan's test. Sensory properties were analyzed statistically by two-ways analyses of variance using (ANOVA) followed by t-test (Tukey test). Differences were considered significant at $p < 0.05$.

3. Results and Discussions:

1. Physicochemical analysis of DMP yoghurt samples

The gross chemical composition of dried mushroom powder (DMP) was illustrated in Elattar *et al.,* (2019). Buffalo's milks are used for the manufacture of yoghurt and are very widespread in countries around the Mediterranean, Middle Eastern countries, southern Russia, and the Indian subcontinent. Buffalo milk contains about twice as much butterfat as cow milk, making it highly suitable for processing various types of yoghurt and resulting in creamy textures and rich flavour profiles.

Chemical analysis:

Table 1 demonstrates that the moisture contents in yoghurt samples fortified with 0.2% DMP varied from 83.45, 83.19 and 83.13% at zero, 7 and 15 days of storage, respectively. On the other hand, the moisture contents in control yoghurt were the lowest all-over the storage time (83.39, 83.03 and 82. 77%).This might be because the increase of dry substances (DMP) in the content, strengthens the structure of the fermented milk product. There was no effect of storage period (zero, 7 and 15 days) on fat percentage. As, fibre offers a variety of health benefits and is essential in reducing the risk of chronic disease such as diabetes, obesity, cardiovascular disease and diverticulitis. The protein and crude fibre contents showed an increasing trend with the increasing of the percentage of the *P. ostreatus* powder (DMP) added to milk.

Physical analysis:

The yoghurt fortified with DMP concentrations (0.05, 0.1 and 0.2 %) didn't affect the rate of pH decreasing. While, for all the tested samples, the pH values after 15 days of storage showed decreased values (4.48, 4.30, 4.36 and 4.29). It was interesting to notice that, the pH of yoghurt fortified with 0.2% DMP all over the storage time (4.75, 4.33 and 4.29 at zero, 7 and 14 days of storage, respectively) were the lowest values when compared to the other tested yoghurt. These results were in concurrent with Tupamahu and Budiarso 2017, Antontceva *et al.*, 2018 and 2019. As, the addition of *P. ostreatus* powder to milk increased the glycolytic activity of yoghurt cultures. An addition of DMP did not affect significantly ($p<0.05$) changes in instrumental colour of yoghurt samples (Table 1). Compared with control yoghurt, L* values (lightness or whiteness) were slightly decreased in samples with added DMP. Values for lightness were the highest in control yoghurt, this is caused by the creamy colour of DMP. This investigation was noticed previously using Hedonic test on colour by Tupamahu and Budiarso (2017). The values of a* (redness) and b* (yellowness) were not significantly affected by DMP addition, compared with control yoghurt. The a* values of tested yoghurt samples ranged from (-0.44 to -0.28, -0.45 to -0.3 and -0.45 to -0.32 at 0, 7 and 15 days of storage, respectively). On the other hand, the high DMP concentration (0.2%) had a significant effect on b* values during the storage period (8.88, 8.92 and 9.04, respectively). The increasing L*, a* and b* values during the storage period could be related to the moisture decreasing. The demonstrated viscosity in yoghurt samples differed significantly during storage period (Table 1). The yoghurt with 0.2% DMP appeared to have significant impact on yoghurt viscosity (7805, 7896 and 7965 mPs at 0, 7 and 15 days, respectively). The observed high viscosity in yoghurt could be attributed to the inclusion of fibre in fermented milk (Donkor *et al.*, 2007 and Sendra *et al.*, 2010). While, the values of control yoghurt all over the storage period ranged from 7507 to 7523 mPs. The evolution of viscosity during storage showed continuous increase in the viscosity of yoghurt samples and attributed this increment to pH reduction during storage, allowing for gel contraction (Coggins *et al.,* 2010). The addition of DMP in yoghurt formula led to significant increase in the water holding capacity of the final product depending on the level of addition (results not shown).

2. Determination of minerals in dried DMP yoghurt samples

Today, yoghurt has moved from being a "health food" to being an ordinary "healthy food" that peoples of all ages enjoy (Han *et al.*, 2012). DMP is a good source of minerals containing macro elements such as potassium, phosphorus, magnesium and sodium and micro elements such as calcium, zinc, iron, copper and manganese (Elattar *et al.*, 2019). In DMP yoghurt different micro- and macro elements were detected, the average contents, expressed as milligrams per kilograms (dry weight of mushrooms), are given in Table 2. In terms of important microelements; Calcium was 2022.2291 and 1781.9085 mg/kg for yoghurt fortified with 0.1% DMP and control, respectively after 15 days of storage. These values were superior to all the rest of tested yoghurt. While, the moderate concentrations of Calcium were noticed in all the fortified yoghurt at zero time with such DMP ratios (0.05, 0.1 and 0.2%, 1254.0877, 1348.9505, and 1438.6377 mg/kg, respectively). Zn and Fe are also considered as micronutrient which our body needed in small quantities. The body primarily uses iron as part of the red blood cell proteins myoglobin and haemoglobin, which are essential for transporting and storing oxygen, it also plays an important role in the formation of hormones, the health and development of the nervous system, and basic cell functioning (https://ods.od.nih.gov/factsheets/%20Iron-HealthProfessional/). Iron was found as doubled concentration in sample 6 when compared with control yoghurt (31.4707 and 16.8961 mg/kg, respectively). Moreover, zinc supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste and smell (https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/).

The highest concentration of Zinc was observed in samples 2, 6 and 3 (31. 0329, 30.3651 and 29.6786 mg/kg, respectively).

Table 1: Physicochemical analysis of yoghurt samples fortified with dehydrated mushroom powder (0.05, 0.1 and 0.2%) at zero, 7 and 15 days of storage at 4°C.

Storage time	Zero time				7days				15 days			
Treatment / Parameters	**Control**	**(0.0)**	**(0.1)**	**(0.2)**	**Control**	**(0.05)**	**(0.1)**	**(0.2)**	**Control**	**(0.05)**	**(0.1)**	**(0.2)**
Chemical parameters												
Fat (%)	7	7	7	7	7	7	7	7	7.1	7.1	7.1	7.1
Moisture (%)	83.39± 0.39	83.47 ±0.16	83.39 ±0.14	83.45 ±0.07	83.03± 0.04	83.04± 0.2	83.04± 0.25	83.19± 0.13	82.77± 0.35	83.2± 0.28	82.87± 0.38	83.13± 0.14
Protein (%)	4.4± 0.01	4.59± 0.02	4.72± 0.01	4.8± 0.04	4.42± 0.04	4.30± 0.04	4.36± 0.03	4.29± 0.01	4.48± 0.04	4.66± 0.01	4.79± 0.05	4.87± 0.03
Crude fibre (%)	0.0± 0.01	0.34± 0.15	0.43± 0.2	1.85± 0.15	0± 0.01	0.3± 0.1	0.38± 0.11	1.62± 0.23	0± 0.03	0.27± 0.10	0.36± 0.14	1.01± 0.26
Physical parameters												
pH	4.79± 0.05	4.79± 0.3	4.79± 0.07	4.75± 0.06	4.51± 0.01	4.33± 0.069	4.4± 0.0	4.33± 0.05	4.48± 0.03	4.30± 0.04	4.36± 0.03	4.29± 0.01
Colour												
L*[1]	87.83± 0.05	87.60 ±0.03	87.51 ±0.00	87.44 ±0.02	87.86± 0.03	87.66± 0.06	87.58± 0.02	87.50± 0.01	87.92± 0.01	87.72 ±0.02	87.63± 0.01	87.54± 0.00
a*[2]	-0.44± 0.01	- 0.39± 0.00	- 0.28± 0.02	- 0.33± 0.00	-0.45± 0.01	-0.41± 0.00	-0.30± 0.00	-0.35± 0.02	-0.45± 0.00	- 0.43± 0.03	-0.32± 0.01	-0.36± 0.01
b*[3]	8.11± 0.00	8.55± 0.01	8.73± 0.01	8.88± 0.02	8.17± 0.03	8.58± 0.00	8.76± 0.01	8.92± 0.02	8.00± 0.01	8.40± 0.00	8.76± 0.01	9.04± 0.02
Viscosity	7507 ±560	7521 ±325	7652 ±685	7805 ±645	7515 ±570	7532 ±765	7726 ±435	7896 ±540	7523 ±680	7540 ±720	7836 ±685	7965 ±345

[*1] L: values (lightness or whiteness), [*2] a: values (redness), [*3] b: values (yellowness)

Potassium is necessary for the normal functioning of all cells. It controls the heartbeat, safeguards proper function of the muscles and nerves, and is vital for synthesizing protein and metabolizing carbohydrates. Auspiciously, the tested yoghurt noticed a high concentration of Potassium in all fortified yoghurt with DMP especially samples 4 and 6 (235.3763 and 234.2995 mg/kg, respectively). Another macro element is Magnesium which is an essential mineral required by the body for maintaining normal muscle and nerve function, preserving a healthy immune system. The superior concentration of Mg was perceived in samples 3,4 and 7 (68.0348, 68.7621 and 68.0378 mg/Kg, respectively).

Table 2. Macro and micro elements contents of dried yoghurt samples fortified with dehydrated mushroom powder (0.05, 0.1 and 0.2%) at zero and 15 days of storage at 4◦C.

samples	Cu mg/Kg	Fe mg/Kg	Mn mg/Kg	Zn mg/Kg	Ca mg/Kg	Mg mg/Kg	K mg/Kg	Na mg/Kg	P mg/Kg
1	1.0986	16.8961	0.6771	16.6226	*468.5485*	58.2351	*202.2196*	87.7001	*152.5*
2	1.4359	25.8962	1.8049	31.0329	1254.0877	66.3532	227.5798	96.5772	160
3	1.5053	21.6678	1.3230	29.6786	1348.9505	68.0348	232.7205	95.1195	160
4	1.2552	23.4967	1.0084	29.2737	1438.6377	68.7621	235.3763	107.7307	152.5
5	1.1202	22.7175	2.1560	28.6652	1781.9085	61.9657	204.4057	92.1025	170
6	1.7730	31.4707	1.9523	30.3651	1246.8549	65.2572	234.2995	95.7468	182.5
7	1.2413	23.2367	1.2829	27.8643	2022.2291	68.0378	227.9371	99.8546	*152.5*
8	1.1939	*18.4235*	*0.7398*	26.2500	1356.3571	66.7857	233.4490	98.2908	160

[1] control yoghurt at zero time of storage, [2] yoghurt fortified with 0.05% of mushroom powder at zero time of storage, [3] yoghurt fortified with 0.1% of mushroom powder at zero time of storage, [4] yoghurt fortified with 0.2% of mushroom powder at zero time of storage, [5] control yoghurt at 15 days of storage, [6] yoghurt fortified with 0.05% of mushroom powder at 15 days of storage, [7] yoghurt fortified with 0.1% of mushroom powder at 15 days of storage, [8] yoghurt fortified with 0.2% of mushroom powder at 15 days of storage.

3. Amino acids determination of dried DMP yoghurt samples

The production of yoghurt is one of the most representative examples of bacterial symbiosis, so that one stimulates the growth of the other (Courtin *et al.,* 2004). The action of *L. bulgaricus* is chargeable for the hydrolysis of most the protein with the formation of free amino acids and oligopeptides. The lactobacillus, in fact, possesses an endopeptidasis (PRTB), localized on the cell surface of the microorganism with serine proteinase activity, that hydrolyzes proteins efficiently, working better at pH 5.2/5.8 (Courtain *et al.,* 2002). The release of free amino acids is an essential process in the yoghurt production for two reasons: first, it stimulates streptococcus activity (Beshkova *et al.,* 1998) and second, it allows the formation of compounds liable for its typical aroma. *S. thermophilus* growth, in fact, is stimulated by certain amino acids released by the action of the lactobacillus and actually it uses most of the amino acid amount (50-60%). The biggest amount of free amino acids is produced during the first four hours of fermentation and after words there is a decrease due to the bacterial utilization (Herve-Jimenez *et al.,* 2008). The aromatic compounds production, however, is a result of the activity of both bacteria. The *L. bulgaricus* produces acetaldehyde (most accountable for the aroma), while *S. thermophilus* produces mainly diketones. The principal milk proteins such as casein and whey proteins constitute a favourable balance of amino acids, comprised of essential and non-essential amino acids in varying concentrations. The body needs 20 different amino acids to keep up healthiness and normal functioning. People must obtain nine of these amino acids, called the essential amino acids such as Histidine, isoleucine, leucine, methionine, phenylalanine, Threonine, Tryptophane and Valine. The present investigation revealed that Valine (3.882 mg/100g in sample 3) was the major amino acid in casein while histidine and methionine (3.578 and 2.225 g/100gm in sample 8 and 4, respectively) were second among all essential amino acids as shown in Table 3. Alanine content was found to be highest in sample 2 (5.223g/100gm) followed by glutamic and proline in samples 8 and 6 (3.899 and 2.98 g/100gm, respectively) as non-essential amino acids. However, minor differences were noticed in Leucine, Isoleucine, Cysteine and Methionine contents of all the tested samples (Table 3). Leucine plays a definite role in protein metabolism and therefore the translation initiation pathway of muscle protein synthesis. It is also involved in reversible phosphorylation of proteins that control mRNA binding to the 40S ribosomal subunit (Anthony *et al.*, 2001). The metabolism of branched chain amino acids seems to be connected, above all with the formation of aromatic compounds such as diketones (Radke-Mitchell and Sandine, 1986), especially during the fermentation phase (30/45°C). Furthermore, it was observed that the glutamic acid is used, again by *S. thermophilus*, for the synthesis of arginine (Arioli *et al.*, 2007). This could explain the decrease in Glutamic acid observed in control yoghurt at zero time (sample1, 3.674 g/100g) compared to control yoghurt after 14 days of storage (sample 5, 1.424 g/100gm). On the other hand, all the treatment samples with mushroom powder had a high percentage of glutamic acid after 14 days of storage compared with its presence at zero time **(**Table 3). The decrease observed for the threonine in sample 4 and 8 (1.160 and 0.613 g/100gm), however, could be explained by the fact that this amino acid is used by both bacteria in the production of acetaldehyde, a molecule which contributes to the development of the aroma in the yoghurt (Germani *et al.,* 2014).

4. Water soluble vitamins determination of DMP yoghurt samples.

Vitamins are compounds which play a task as cofactors within the body. Fermented milk products such as yoghurt can be supposed as vitamin sources. However, owing to the capacity of some starters to synthesize B- complex vitamin that's necessary for their growth, there are different vitamin contents in yoghurts. Furthermore, evaluation of vitamins is more difficult since processes like heat treatment, incubation time, temperature and storage conditions change the vitamins content in yoghurt (Rao and Shahani 1987).

Table. 3: The profile of amino Acids g/100gm of dried yoghurt samples fortified with dehydrated mushroom powder (0.05, 0.1 and 0.2%) at zero and 15 days of storage at 4◦C.

Amino acid	1	2	3	4	5	6	7	8
Aspartic	0.758	0.412	*0.380*	0.902	0.413	0.569	0.559	0.804
Glutamic	3.674	1.653	1.238	3.893	1.424	1.927	1.832	3.899
Serine	1.356	1.104	1.145	1.654	*0.928*	1.260	1.419	1.439
Glycine	0.203	0.084	0.079	0.104	0.058	0.080	0.109	0.216
histidine	3.371	2.183	2.333	*1.944*	1.968	2.813	2.541	3.578
Arginine	0.834	0.825	0.826	1.194	*0.637*	0.925	1.045	0.885
Threonine	*0.578*	0.664	0.764	1.160	0.591	0.844	0.748	0.613
Alanine	*2.486*	5.223	5.144	3.775	3.946	*2.399*	3.108	3.816
Proline	1.895	2.871	2.912	*1.166*	2.254	2.980	2.866	2.011
Tyrosine	*0.590*	0.988	0.927	0.767	0.776	1.084	0.839	0.626
Valine	2.689	2.532	3.882	3.249	2.899	2.791	2.548	2.853
Methionine	*1.758*	1.638	2.218	2.225	2.181	2.014	2.314	1.865
Cysteine	0.071	*0.055*	0.073	0.069	0.160	0.090	0.080	0.075
Isoleucine	*0.254*	0.302	0.359	0.388	0.362	0.417	0.366	0.270
Leucine	0.739	0.836	*0.606*	0.941	0.962	0.921	0.736	0.785
Phenylalanine	*0.700*	1.069	0.925	0.975	1.493	1.379	1.582	0.743
Lysine	*1.165*	1.837	1.355	1.382	2.239	1.693	2.067	1.236

[1] control yoghurt at zero time of storage [2] yoghurt fortified with 0.05% of mushroom powder at zero time of storage, [3] yoghurt fortified with 0.1% of mushroom powder at zero time of storage, [4] yoghurt fortified with 0.2% of mushroom powder at zero time of storage, [5] control yoghurt at 15 days of storage, [6] yoghurt fortified with 0.05% of mushroom powder at 15 days of storage, [7] yoghurt fortified with 0.1% of mushroom powder at 15 days of storage, [8] yoghurt fortified with 0.2% of mushroom powder at 15 days of storage.

The approximate vitamins content of yoghurt samples is shown in Table 4. Vitamins C and E which act as antioxidants, cleansing the body of dangerous free radicals and toxins that can cause chronic illness. Vit. C found in significant amounts within yoghurt samples (537.0350 and 331.739 mg/kg in samples 3 and 7, respectively). During a storage of yoghurt samples (2 and 4 at zero time), Vit C decreased with a percentage of 17.31 till 54.4 % in samples 6 and 8 at 14 days, respectively. The initial Alphatochopherol (Vit E) contents in different samples varied from 0 to 18.130 mg/kg, in samples 1 and 4, respectively. After 15 days of storage, the values of Vit E were reduced. A number of factors such as oxygen, light, heat, alkali, trace minerals, and hydroperoxides can cause decomposition of vitamin E (Bramley *et al.*,2000).

Folic acid (Vit B9) is also abundant in yoghurt samples which established in samples 3,4 and 8 as 315.229, 474.528 and 393.484 mg/kg, respectively. It aids the body make healthy new red blood cells. Folate is also important for the synthesis and repair of DNA and other genetic material, and it is necessary for cells division.

Vitamin B6, also known as pyridoxine, that our body needs for several functions. It's significant to protein, fat and carbohydrate metabolism and therefore the creation of red blood cells and neurotransmitters. The highest value of B6 (221.089 mg/kg) found in Sample 4. Table (4) shows B2 (riboflavin) contents as affected by DMP addition on yoghurt. Slightly increasing of Vit B2 was observed after 15 days' storage. Sample 8 showed the highest riboflavin content after the end of storage.

5. Texture profile analysis of DMP yoghurt samples

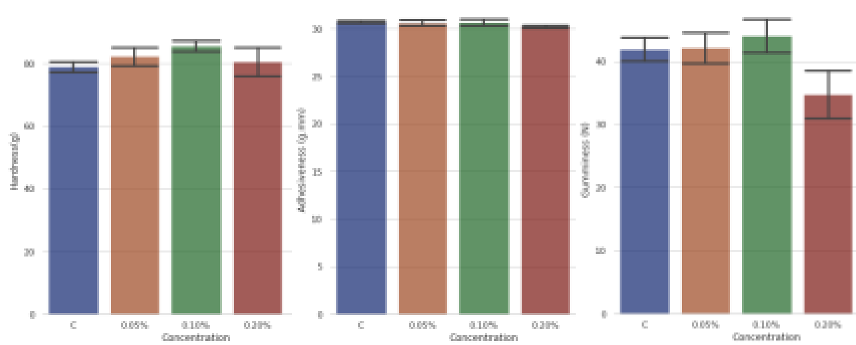
Texture profile analyses of DMP yoghurt with different concentrations during the storage (0, 7 and 14 days) are demonstrated in (Fig 1A, 1B and 1C) representing hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness, respectively. Either fresh or along the storage period, enriched yoghurt with 0.05 and 0.1% DMP (Fig 1A, 1B and 1C) recorded higher values of the hardness and gumminess as compared to control yoghurt. The increased hardness may result in improvement of the yoghurt texture making it less susceptible to rearrangements

Table 4. Water soluble vitamins (mg/Kg) in dried yoghurt samples fortified with dehydrated mushroom powder (0.05, 0.1 and 0.2%) at zero and 15 days of storage at 4◦C.

Vitamin name / mg/Kg	1	2	3	4	5	6	7	8
Vit C	67.683	100.608	537.035	295.562	*45.384*	83.188	331.739	134.451
Alphatochopherol (Vit E)	0.0	8.403	12.088	18.130	*1.645*	4.305	6.342	6.625
Vit B2	3.623	4.325	5.911	18.973	*4.565*	6.328	12.562	21.234
Vit B3	0.0	0.0	0.0	0.0	0.0	8.204	0.0	0.954
Vit B6	41.033	45.064	53.304	221.089	0.0	26.616	*28.0624*	179.230
Vit B9	15.126	19.432	315.229	474.528	8.705	*8.964*	213.868	*393.0484*
Vit B12	0.1158	0.090	0.629	6.62166	*0.862*	0.8230	4.25920	*6.33560*

[1] control ogurt at zero time of storage [2] yoghurt fortified with 0.05% of mushroom powder at zero time of storage, [3] yoghurt fortified with 0.1% of mushroom powder at zero time of storage, [4] yoghurt fortified with 0.2% of mushroom powder at zero time of storage, [5] control yoghurt at 15 days of storage, [6] yoghurt fortified with 0.05% of mushroom powder at 15 days of storage, [7] yoghurt fortified with 0.1% of mushroom powder at 15 days of storage, [8] yoghurt fortified with 0.2% of mushroom powder at 15 days of storage.

within its network and consequently less susceptible to shrinkage and serum expulsion, which were previously reported (Metry and Awayss 2009 and De Souza Oliveira *et al.*, 2012). The most pronounced effects were observed with 0.2% DMP addition, which was significantly softer than the other treatments due to dissolution in the texture of the yoghurt observed after the formation of curd compared to the other treatments. It is dissimilar to what presented by Aritonang (2011). The cohesiveness was higher in control, 0.05% and 0.1% DMP yoghurt all-over the storage period. So, it can be attributed to the compact texture of the yoghurt made with 0.05% and 0.1% DMP than that made with 0.2% DMP. The obtained data from Fig (1A and B) revealed that yoghurt contained 0.2% DMP had the lowest gumminess values when compared to the other yoghurt samples. On the other hand, the highest value was noticed from yoghurt contained 0.1% DMP, this may be due to the increase in the gel strength by the cross- linking of protein chains which led to the stabilization of the three dimensional network of the yoghurt gel (Hassan *et al.*, 1995). The stable springiness and adhesiveness values of yoghurt containing DMP were noticed during the storage period (Fig 1A, 1B and 1C). From our results, we can observe that the addition of 0. 1% of mushroom powder showed a values of hardness, cohesiveness and gumminess higher than control yoghurt caused a more compact texture and so helped to improve the textural properties of yoghurt. Our results were incompatible with Tupamahu and Budiarso 2017 which showed that the most flavoured texture is the 1% DMP. The reason of this conflict can be attributed to the preparation method of DMP used in our study.



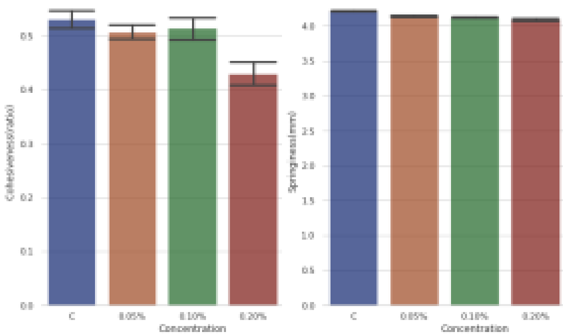


Figure 1A. Evaluation of texture parameters (hardness, Adhesiveness, Gumminess, Cohesiveness and springiness) on tested yoghurt fortified with different percentages of mushroom powder (DMP), over zero time of storage at 4◦C.

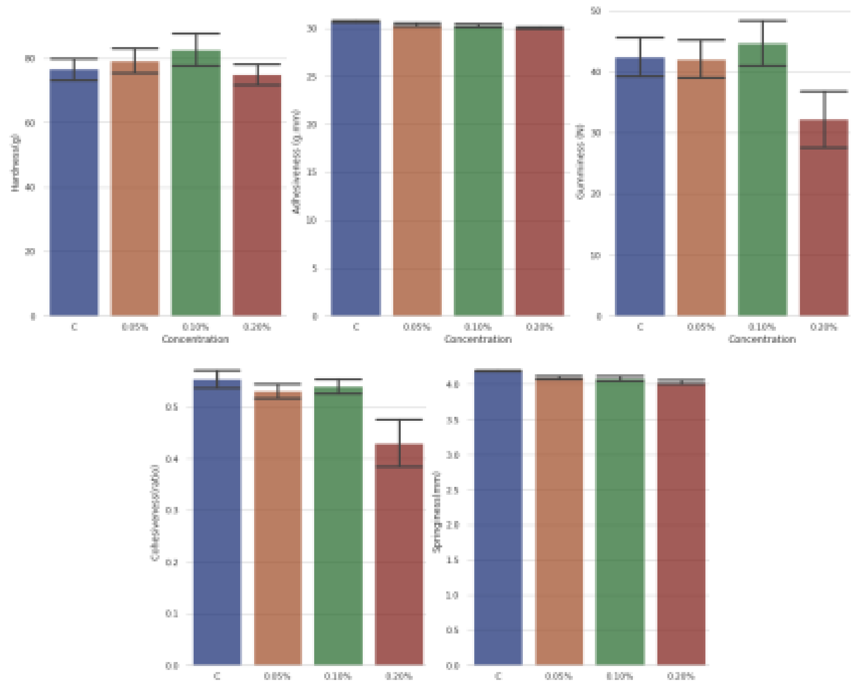
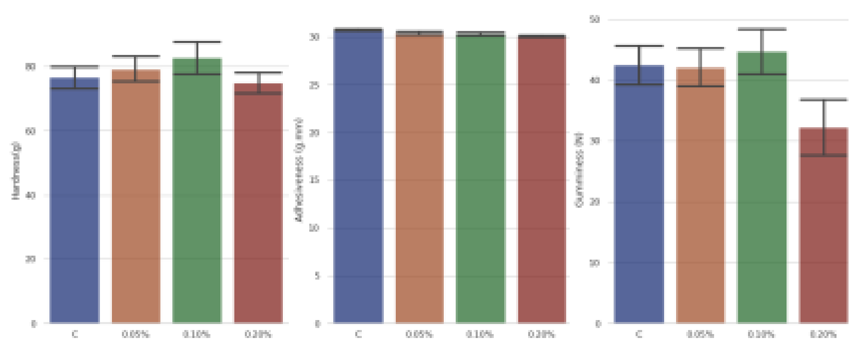


Figure 1B. Evaluation of texture parameters (hardness, Adhesiveness, Gumminess, Cohesiveness and springiness) on tested yoghurt fortified with different percentages of mushroom powder (DMP), over 7 days of storage at 4◦C.



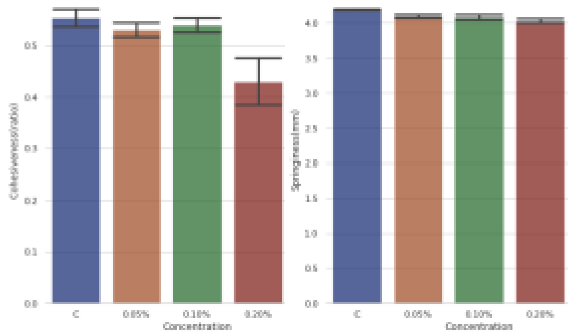


Figure 1C. Evaluation of texture parameters (hardness, Adhesiveness, Gumminess, Cohesiveness and springiness) on tested yoghurt fortified with different percentages of mushroom powder (DMP), over 14 days of storage at 4◦C.

6. Sensorial evaluation of DMP yoghurt samples.

The sensory evaluations of fortified yoghurt with different ratio of DMP at zero, 7 and 15 days of storage is illustrated in (table 5). Generally, there were significant differences ($p<0.05$) among treatments for body and texture, flavour, appearance and overall acceptability of sensory evaluation during storage. Set-style yoghurt should have smooth consistency without cracks or holes, custard like, semi solid consistency without any surface whey (Aswal *et al.*, 2012). These mentioned characters were noticed with yoghurt fortified with DMP (0.05, 0.1 and 0.2%). Table (5) and Fig (2A and 2B) revealed that, yoghurt fortified with 0.1% DMP at zero time and 7 days of storage scored the best scores of overall acceptance (9.65 and 9.1, respectively). This could be related to texture profile analysis results in Fig (1A, 1B and 1C). These results are compatible to what was obtained with Tupamahu and Budiarso (2017). On the other hand, the most overall acceptance value after 15 days of storage (Fig. 2C) was noticed in yoghurt with 0.2% DMP (9.4) when compared with added 0.05 and 0.1% DMP in Yoghurt (8.95 and 8.75, respectively). This observation can be related to the test balance between the sour and mushroom taste in yoghurt after 15 days of storage. The more addition of mushroom powder till 0.2% appeared a highly digestion of protein matrix and whey off as agree with Aritonang 2011 which observed an important role of acid in protein agglomeration, acid causes coagulation over yoghurt protein thus forming a coagulation or lumps that will increase in amount as time passes.

Table 5. Sensory evaluation average of yoghurt samples fortified with different percentages of DMP (0.05, 0.1 and 0.2%) at zero, 7 and 15 days of storage at 4°C

Sample / parameters	*Storage Time*	*Control*	*0.05*	*0.1*	*0.2*
Body and texture (10)	Zero time	8.88±0.73	9±0.46	9.62±0.37	7±0.53
Flavour(10)		8.88±0.79	8.88±0.95	9.5±0.71	8.5±1.2
Appearance (10)		9.12±0.73	9.62±0.37	9.88±0.18	7.5±0.71
Over all acceptance (10)		8.95±2.12	9.1±1.6	9.65±1	7.9±1.67
Body and texture (10)	7 days	8.62±0.7	9±0.76	9.12±0.5	7±0.53
Flavour (10)		9±0	9.19±0.59	9.13±0.83	7.75±1.83
Appearance (10)		8.76±0.74	9±0.53	9±0.53	6.12±1.32
Over all acceptance (10)		8.85±1.22	9.1±1.58	9.1±1.77	7.15±3.2
Body and texture (10)	15 days	8.26±0.83	8.76±0.74	9±0.76	9.5±0.46
Flavour (10)		8.75±1.16	9.19±0.65	8.25±1.46	9.25±0.71
Appearance (10)		8.28±0.89	8.86±0.53	8.86±0.76	9.58±0.53
Over all acceptance (10)		8.45±2.58	8.95±1.49	8.75±2.31	9.4±1.36

A B C

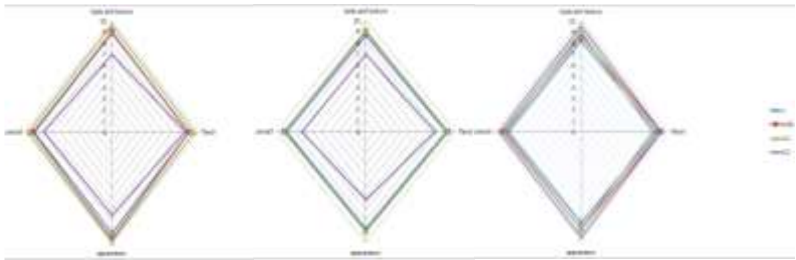


Figure 2. Sensory evaluation average of yoghurt fortified with different percentages of DMP (0.05,0.1, and 0.2) at (A) zero , (B) 7 and (C)15 days of storage at 4◦C

Conclusion

The incorporation of DMP affect significantly the yoghurt quality, which relates to the texture profile analysis, nutritional values. As, the fortification of 0.1 DMP increased each Hardness and Gumminess all over the storage period. The addition of 0.1 and 0.2 provided most preferred yoghurt according to their flavour, body & texture and appearance. Conferring to the nutritional values. The antioxidant vitamins were markedly observed in yoghurt fortified with 0.1 and 0.2% DMP for Vit C. The highest values of vit B-complex was detected in yoghurt fortified with 0.1 and 0.2% MDP. So, the addition of 0.1 or 0.2% DMP on yoghurt is recommended in order to enhance the both texture and nutritional parameters of yoghurt.

Acknowledgments

This study is a part of project entitled: Vigorous mushroom fermented milk summit proceeding. Funded by the Academy of Scientific Research and Technology, Egypt. Project ID 1035.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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6/18/2021