MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Microbiology and Biological Sciences (ISSN: 2408-7076) Vol. 6(7) pp. 094-102, August, 2018
Available online http://www.meritresearchjournals.org/mbs/index.htm

Original Research Article

Protein Content and Antibacterial Effect of *Agaricus bisporus* Additive on Chicken Minced Meat

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Abstract

This study focused on the effect of adding *Agaricus bisporus* mushroom to chicken minced meat and its effect on extending shelf life and protein percent within 7 days storage at refrigerator temperature. About 21.75 kg of chicken minced meat was added to dried *Agaricus bisporus* mushroom powder by different concentrations (5%, 10%, 20%, 30%), level of total bacterial count was evaluated, total anaerobic count, *Staphylococcus aureus* and *Escherichia coli* on the different samples on (0 day, 3rd day, 5th day and 7th day) days after refrigeration storage at ±4°C. Also about 50 gm dried *Agaricus bisporus* powder with different concentrations tested against one gram-positive bacteria (*Staphylococcus aureus*) and one gram-negative bacteria (*Escherichia coli*) to measuring the zone of inhibition. Determination of total nitrogen content on meat using Kjeldahl apparatus was also performed. The best reduction effect of different concentrations of *Agaricus bisporus* mushroom observed in case of 20% & 30% conc. Of mushroom powder addition especially against *E. coli,* followed by total aerobic counts while the lowest inhibition effect recorded against *Staphylococcus aureus* followed by total anaerobic count. Protein % decreased by prolong storage period, while, the protein % were increased by adding higher *Agaricus bisporus* concentrations as follow; (19.0%, 19.12%, 19.26, 19.50%, 19.74%) on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations respectively. The study reported the positive reduction effect of *Agaricus bisporus* against *E. coli* (food pathogen), total aerobic count (food spoilage microorganisms, which lead to extending the shelf life of the chicken minced meat product) respectively with less inhibition effect against total anaerobic counts and *Staphylococcus aureus* as gram positive foodborne microorganisms. Further studies is recommended to establish more applications using *Agaricus bisporus* to enriching more types of food to extend shelf life and improve nutritive values of food naturally and safely which helps in improving consumers health.

Keywords: Natural preservatives, white cotton mushroom, food poisoning, Sensitivity Test, fungi

INTRODUCTION

These years, awareness arising to both consumers and food producers about the hazardous of chemical preservatives which is use to improve some physical properties of different food product and/or extending the shelf life of them, this enhances the scientists and food hygienists to begin a lot of experiments which aim to try

some natural products as a natural preservatives to further using them instead of chemicals additives (Sharma *et. Al.,* 2015).

Edible mushroom (*Agaricus bisporus*) white button mushroom is white creamy firm texture with delicate flavor, tasty and inexpensive. It belongs to the macro fungi, having a big size and good enough to be seen grossly by naked eyes. *Agaricus bisporus* mushrooms grow from spores and not seeds. They are essentially Saprophytes organisms (plants without chlorophyll) which extracts nutrients from dead and decaying plant and animal matter and become edible for consumers due to its high nutritive value through its high contents of protein, minerals, vitamins and fiber with very low amount of carbohydrates (Kalaras *et al.,* 2012).

In addition to its broad spectrum antibacterial activity against Gram-negative and Gram-positive bacteria which protect and promote consumers health by lower cholesterol due to its content of some enzymes can lower bad cholesterol level which is known as (LDL). *Agaricus bisporus* mushroom is also rich in iron which enhances synthesis of RBCs and its copper content helps in proper absorption of iron in the body thereby helps in treatment of anemic patients. Mushroom have also phosphorous, calcium, vitamin D, vitamins A, B-complex and C which protect bones and play a role in some metabolic activities. Mushroom is also known as “master antioxidant” due to the presence of sulfur containing amino acids and Ergothioneine which promotes immune system. *Agaricus bisporus* mushroom is rich in conjugated linoleic acid and beta-glucans and aromatase enzyme which have anti-carcinogenic effect especially to prevent against prostate and breast cancer. Mushrooms also have some enzymes which helps to break down starch and decrease blood sugar level by its natural insulin content. In addition to all of the above benefits, mushroom is considered natural antibiotic by its broad bacteriostatic effect not only against gram positive and gram negative bacteria but it also have growth inhibition effect against wide range of other fungal infection which is improving consumers’ immune systems. Mushroom have triterpenes that suppers histamine and have anti-inflammatory properties (Mau *et al.,* 2004; Yamaç and Bilgili, 2006; Ferreira *et al.,* 2007 and Süfer *et. A.,* 2016).

Any dish rich in *Agaricus bisporus* mushroom can lead to relaxation and decrease its consumer blood pressure due to its high amount of potassium which act as vasodilator to blood vessels and increase attention and memories. *Agaricus bisporus is* also rich in lean protein of about 20-30% of dry mushroom weight which helps in muscle building with very low calories gain and without any side effect (Geo *et al.,* 2005; Barros *et al.,* 2007; Oyetayo *et al.,* 2009and Kosanic *et. Al.,* 2013).

Chicken meat is one of the most popular animal protein to old and young consumers due to its high palatability with low cooking shrinkage and high nutritive values such as amino acids, vitamins and minerals in addition to its inexpensive price when compared with red meat (Hassanin *et. al*., 2014; Thanissery *et. al.,* 2014; Chelebi *et. al.,* 2015 and Oulkeir *et. al.,* 2017).

Nowadays, the world complains of shortage of animal protein in comparison to the increase of popular demand to it which lead to replace some percentage of animal protein in many meat products by plant protein such as soybean which sometime yield to side effects to consumers. Although there isn’t any trial to replace some animal percent by edible mushroom which will increase the shelf life and encourage the nutritive value of the products without any side effect. This study focused on the effect of adding *Agaricus bisporus* mushroom to chicken minced meat and study its effect on final protein percent of the product and extending shelf life of the products when stored at refrigerator temperature.

MATERIAL AND METHODS

Samples Preparation (Süfer *et al.,* 2016)

Fruiting bodies of *Agaricus bisporus* mushrooms were bought about 5kg from the same market then sun-dried for 1 day at 60°C. Then powdered by commercial blender (National, Japan) then stored in glass jars at +4°C. and about 21.75 kilograms of chicken minced meat was added to *Agaricus bisporus* mushroom powder by different concentrations, (5% mushroom powder, 95% chicken minced meat, 10%, 90%, 20%, 80%, 30% and 70%) as following; (5 Kg chicken minced meat, 0.25 Kg *Agaricus bisporus* powder: 4.75 Kg chicken minced meat, 0.5 Kg mushroom powder: 4.5 Kg chicken minced meat, 1kg mushroom powder: 4 kg chicken minced meat and 1.5 kg *Agaricus bisporus* powder, 3.5 kg chicken minced meat) then estimate the level of total bacterial count, total anaerobic count, *Staphylococcus aureus* and *Escherichia coli* on the different samples on (zero day, 3rd day, 5th day, 7th days after refrigeration storage at ±4°C.

Experimental Antibacterial Evaluation of Different Concentrations of *Agaricus bisporus*

About 50 gm dried *Agaricus bisporus* coarse powder was soaked in 300 ml of acetone and methanol then the flasks were covered with aluminum foil and stand for 7days. Then filtered by Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and stock solution of concentration of 10 mg/ml in (acetone and methanol). It was tested against one gram positive bacteria (*Staphylococcus aureus*) (ATCC 25923) and one gram negative bacteria (*Escherichia coli*) (ATCC 25922) were cultured on Muller Hinton then impress *Agaricus bisporus* mushroom discs with different concentrations and incubated at 37°C/24 hours. Then measuring the zone of inhibition (Jonathan

and Fasidi, 2003; Hemashenpagam and Selvaraj, 2010 and Balakumar *et al.,* 2011).

Bacteriological Evaluation of Different Concentrations of *Agaricus bisporus* in Chicken Minced Meat (APHA, 1992)

Chicken minced meat samples with/without different concentration of *Agaricus bisporus* homogenized in a Seward stomacher (400R/UK) and serial dilutions (10^{-1}) then appropriate diluents of each tube were placed on the following media in duplicate as follows:

Estimation of Total Aerobic and anaerobic Counts (APHA, 2002)

Total aerobic count cultured in nutrient agar (Merck, Darmstadt, Germany) by surface plating of 0.1 ml of the serial dilutions from each sample then incubated at 37°C/24 hr. Plates with distinct colonies counted 30-300 were enumerated as Colony Forming Units (CFU). Total anaerobic count by placing 1 ml of samples spread onto duplicate plates of double layers of Reinforced Clostridial medium agar (Oxoid, CM151) incubated anaerobically on (Gaspak plus anaerobic system) at 37°C/48 hr.

Isolation of *Staphylococcus aureus* (Valls *et al*., 2000)

The enriched broth was plated on Baird-Parker Agar (Oxoid, CM 275) supplemented with Egg Yolk-Tellurite Emulsion (Oxoid, SR 54) for total Staphylococcal count incubated at 37^{o}C/24 hr & 48 hr.

Isolation of *E. coli*

25gm of meat sample was weighed aseptically and placed in 225ml on modified vancomycin-trypticase soy broth (m-VTSB) as it contained vancomycin (40mg/litre) to suppress Gram-positive flora, blend or stomach at low speed for 2min. The meat sample was incubated aerobically in m-VTSB overnight at 41.5±0.5°C then cultured on Eosin Methylene Blue Agar Media (EMB) and incubated at 37 C/24 h; *E. coli* had been grown producing green metallic shine colonies, morphological characters after staining, cultural and biochemical characters were carried out according to Quinn *et al.* (1994).

Estimation of protein percent of Different Concentrations of *Agaricus bisporus* in Chicken Minced Meat (Kjeldahl, 1883)

Collected meat samples transferred in sanitary food bags on ice box to the central laboratory in faculty of veterinary medicine, New valley, Assiut university to determine the total nitrogen content on meat using Kjeldahl apparatus as following; digest 2g of meat sample with 20 ml concentrated H_2SO_4 and catalyst tablet using Kjeldahl digestion flask. Steam distillation by 50 ml of 45% sodium hydroxide solution and 150ml of methyl red indicator then titrated against 100ml 0.1N HCl until the color solution the changed.

% Nitrogen was calculated as follows =

$$\frac{[(\text{ml standard acid} \times \text{N of acid}) - (\text{ml blank} \times \text{N of base})] - (\text{ml std base} \times \text{N of base}) \times 1.4007}{\text{Weight of sample in grams}}$$

Where N=normality

Statistical Analysis (GraphPad Instant, 2009)

The statistical program, GraphPad Instant version 3 for window was used for determination of means, the analysis of variance between the different data and treatment in this study were determined using standard error and analysis of variance ($P<0.05$).

RESULTS

Effect of Different *Agaricus bisporus* Concentration against *Staphylococcus aureus* and *E. coli*:

Figure (1,2) showed the different inhibition zones on sensitivity test against *Staphylococcus aureus* as one of gram positive microorganism using different concentrations of *Agaricus bisporus* mushroom as follows: there were completely negative effect of (5% and 10%) mushroom powder concentrations and about 5.0mm and 10mm inhibition zone of (20% and 30%) mushroom powder concentrations. While Photo 2 showed the different inhibition zones of different *Agaricus bisporus* mushroom concentrations against *E. coli* as one of gram negative microorganism using different concentrations of mushroom powder as follows; there is completely negative effect in case of 5% *Agaricus bisporus* concentration, while the inhibition zone were about (13, 24 and 32) mm in 10%, 20% & 30% *Agaricus bisporus* concentrations.

Evaluation of addition Different Concentrations of *Agaricus bisporus* to chicken minced meat within 7 days of refrigeration storage

Figure (3) illustrated the different reduction effect of different concentrations of *Agaricus bisporus* mushroom powder on total aerobic count as follows; although the total count were increased by time but in all cases the

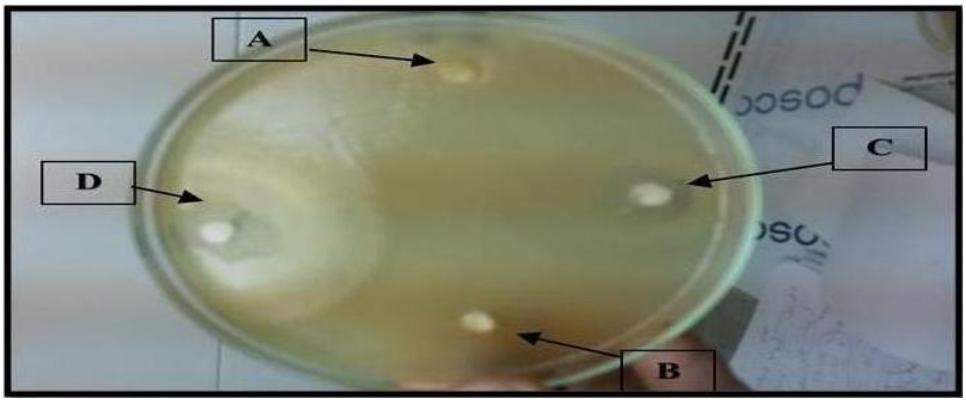

Figure 1. Effect of Different *Agaricus bisporus* Concentrations against *S. aureus*
A) No inhibition zone around the disc containing 5% *Agaricus bisporus* extract
B) No inhibition zone around the disc containing 10% *Agaricus bisporus* extract
C) 0.5 cm inhibition zone around the disc containing 20% *Agaricus bisporus* extract
D) 1.0 cm inhibition zone around the disc containing 30% *Agaricus bisporus* extract.

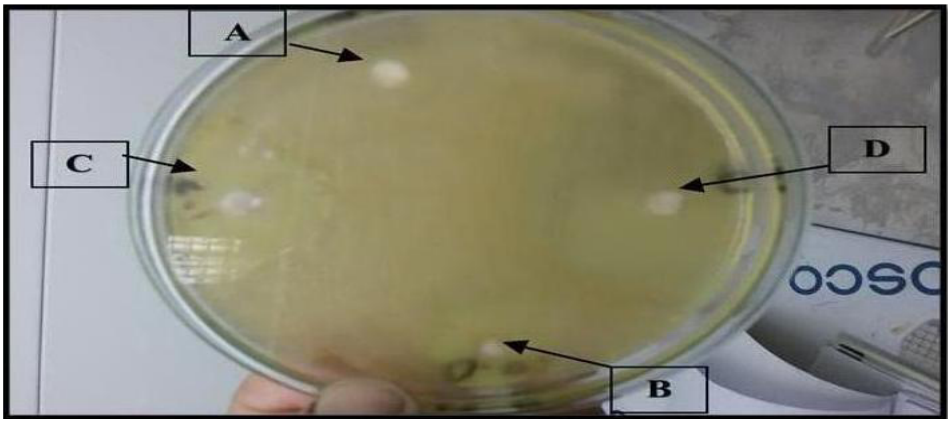

Figure 2. Effect of Different *Agaricus bisporus* Concentrations against *E. coli*
A) No inhibition zone around the disc containing 5% Agaricus bisporus mushroom extract
B) About 1.3 cm inhibition zone around the disc containing 10% *Agaricus bisporus* extract
C) About 2.4 cm inhibition zone around the disc containing 20% *Agaricus bisporus* extract
D) About 3.2 cm inhibition zone around the disc containing 30% *Agaricus bisporus* extract.

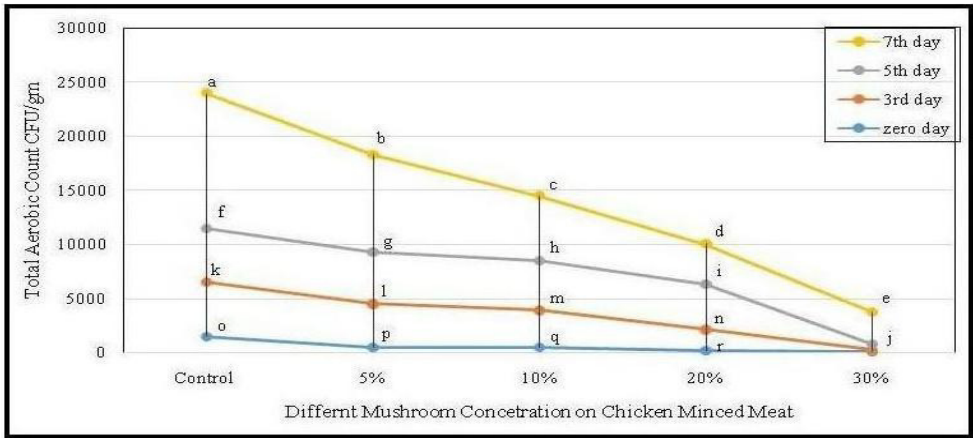

Figure 3. Effect of Different Concentrations of *Agaricus bisporus* Addition to Chicken Minced Meat within 7days of Refrigeration storage on Total Aerobic Count

Means followed by a different letter in the line are significantly different (p>0.05)

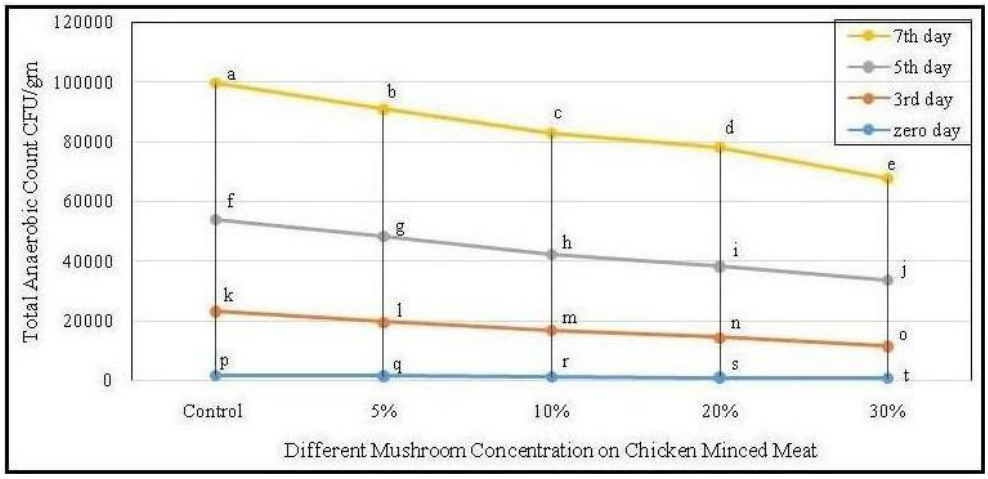

Figure 4. Effect of Different Concentrations of *Agaricus bisporus* Addition to Chicken Minced Meat within 7days of Refrigeration storage on Total Anaerobic Count

Means followed by a different letter in the line are significantly different (p>0.05)

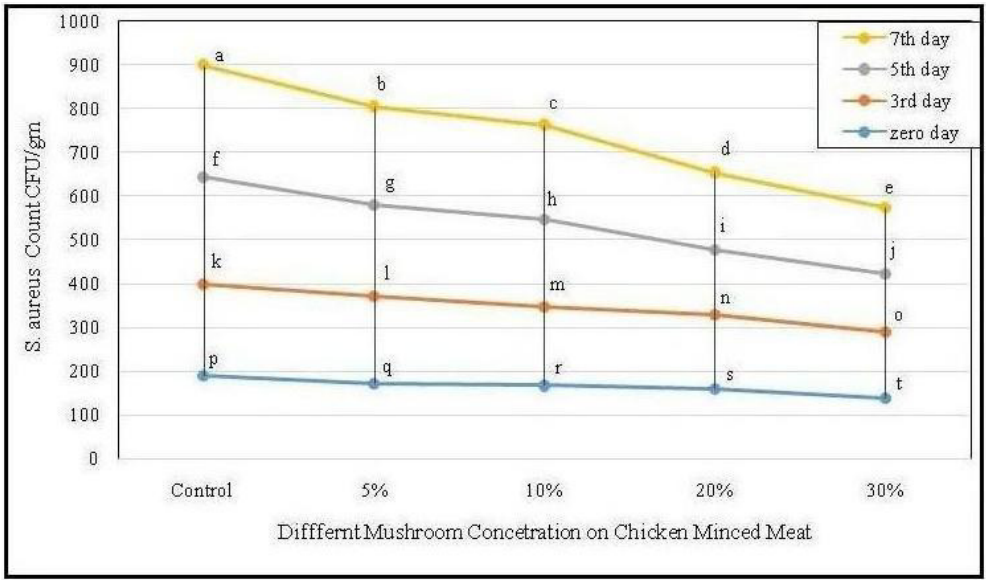

Figure 5. Effect of Different Concentrations of *Agaricus bisporus* Addition to Chicken Minced Meat within 7days of Refrigeration storage on *S. aureus*

Means followed by a different letter in the line are significantly different (p>0.05)

higher concentrations of *Agaricus bisporus* resulted in lower total aerobic counts. The mean total aerobic counts on the zero day were 1.5×10^3, 5.0×10^2, 5.0×10^2, 2.0×10^2, 1.0×10^2 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. While on 3rd day, the mean total aerobic counts were 5.0×10^3, 4.0×10^3, 3.4×10^3, 2.0×10^3, 1.5×10^2 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. The mean total aerobic count on the 5th day of storage were 5.0×10^3, 4.8×10^3, 4.6×10^3, 4.2×103, 5.0×10^2 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. The mean total aerobic counts recorded on 7th storage day were; 1.3×10^4, 9.0×10^3, 6.0×10^3, 3.7×10^3, 3.0×10^3 on control, 5%, 10%, 20%, 30% mushroom concentrations samples respectively. These counts were significantly increased (*P > 0.05*) with extended storage time. Figure (4) demonstrated the different reduction effect of different concentrations of *Agaricus bisporus* on total anaerobic count as following; although the total count were increased by time but in all cases the higher concentrations of *Agaricus bisporus* mushroom powder resulted in lower total anaerobic counts. The mean total anaerobic counts on the zero day were; 1.5×10^3, 1.4×10^3, 1.1×10^3, 1.0×10^3, 8.5×10^2 on control, 5%, 10%, 20%, 30% mushroom powder concentrations samples

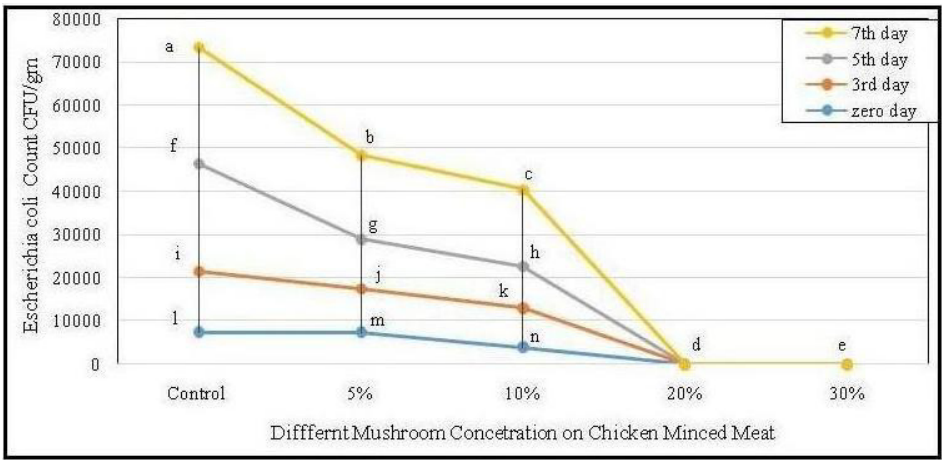

Figure 6. Effect of Different Concentrations of *Agaricus bisporus* Addition to Chicken Minced Meat within 7days of Refrigeration storage on *Escherichia coli*

Means followed by a different letter in the line are significantly different (p>0.05)

Table 1. Effect of Different Concentrations of *Agaricus bisporus* Addition to Chicken Minced Meat within 7 days of Refrigeration Storage on Protein %

Conc./ storage period	Control	5%	10%	20%	30%
Zero day	19.00	19.12	19.26	19.50	19.74
3rd day	18.50	18.63	18.74	19.0	19.24
5th day	18.00	18.12	18.24	18.50	18.75
7th day	17.70	17.92	18.07	18.30	18.54

respectively. While on 3rd day the mean total anaerobic counts were; 2.2×10^4, 1.8×10^4, 1.6×10^4, 1.3×10^4, 2.2×10^4 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. The mean total anaerobic count on the 5th day of storage were; 3.1×10^4, 2.9×10^4, 2.6×10^4, 2.4×10^4, 2.2×10^4 on control, 5%, 10%, 20%, 30% mushroom powder concentrations samples respectively. The mean total anaerobic counts recorded on 7th storage day were; 4.6×10^4, 4.3×10^4, 4.1×10^4, 3.9×10^4, 3.4×10^4 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. These counts were significantly increased (*P > 0.05*) with extended storage time. Figure (5) declared the different reduction effect of different concentrations of *Agaricus bisporus* on *Staphylococcus aureus* count as follows; although the total count were increased by time but in all cases the higher concentrations of *Agaricus bisporus* resulted in lower total aerobic counts. The mean total aerobic counts on the zero day were; 1.9×10^2, 1.7×10^2, 1.7×10^2, 1.6×10^2, 1.4×10^2 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. While on 3rd day the mean *Staphylococcus aureus* were; 2.1×10^2, 1.9×10^2, 1.8×10^2, 1.7×10^2, 1.5×10^2 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations samples respectively. The mean *Staphylococcus aureus* on the 5th day of storage were; 2.4×10^2, 2.1×10^2, 2.0×10^2, 1.5×10^2, 1.4×10^2 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations samples respectively. The mean *Staphylococcus aureus* recorded on 7th storage day were; 2.6×10^2, 2.2×10^2, 2.1×10^2, 1.7×10^2, 1.5×10^2 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. These counts were significantly increased (*P > 0.05*) with extended storage time.

Figure (6) declared the different reduction effect of different concentrations of *Agaricus bisporus* on *E. coli* count as following; although the total count were increased by time but in all cases the higher concentrations of mushroom powder resulted in lower *E. coli*. The mean *E. coli* on the zero day were; 7.5×10^3, 7.5×10^3, 4.0×10^3, 0.0, 0.0 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. While on 3rd day the mean *E. coli* were; 1.4×10^3, 1.9×10^4, 9.0×10^3, 0.0, 0.0 on control, 5%, 10%, 20%, 30% mushroom powder concentrations samples respectively. The mean *E. coli* on the 5th day of storage were; 2.5×10^4, 1.2×10^4, 9.8×10^3, 0.0, 0.0 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. The mean *E. coli* recorded on 7th storage day were; 2.7×10^4, 1.9×10^4, 1.8×10^4, 1.7×10^2, 1.5×10^2 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations samples respectively. These counts were significantly increased (*P > 0.05*) with extended storage time. The best reduction effect of mushroom recorded in case of *E. coli*, followed by milder effect in the total aerobic count. In the other hand milder

reduction effect reported in case of total anaerobic counts and *Staphylococcus aureus.*

Effect of Different Concentrations of *Agaricus bisporus* Addition to Chicken Minced Meat within 7 days of Refrigeration Storage on Protein % in table (1)

Table 1 declared that the protein % decreased by prolong storage period, while, the protein % were increased by adding higher *Agaricus bisporus* concentrations as follows; about (19.0%, 19.12%, 19.26, 19.50%, 19.74%)on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations samples respectively. While on 3rd day it were; 18.5%, 18.63%, 18.74%, 19.0%, 19.24 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. While on the 5th day of storage protein % were; 18.0%, 18.12%, 18.24%, 18.50%, 18.75% on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations samples respectively. The mean protein% recorded on 7th storage day were; 17.7%, 17.92%, 18.07%, 18.30%, 18.54% on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations samples respectively. These counts were significantly increased (*P > 0.05*) with extended storage time.

DISCUSSION

The consumption of chicken meat products is growing due to its rich source of proteins, minerals and vitamins (FAO, 2013 and Ledesma *et al.,* 2016). In the other hand*, Agaricus bisporus is* one of the highly perishable organism which needs high care during handling and consuming them without prolong storage or after short refrigerated storage. Drying of *Agaricus bisporus* powder can be kept up to one year. *Agaricus bisporus* is one of macrofungus which have antimicrobial activities which can be used as food additive and it can reduce the occurrence of foodborne pathogens (Abah, and Abah, 2010).

Effect of Different *Agaricus bisporus* concentration test against *Staphylococcus aureus* as one of gram positive microorganism using different concentrations of *Agaricus bisporus* powder as following: there were completely negative effect of (5% and10%) *Agaricus bisporus* concentrations and about 5.0mm & 10.0 mm inhibition zone of (20% and 30%) *Agaricus bisporus* concentrations. While the inhibition zones of different *Agaricus bisporus* concentrations against *E. coli*as one of gram negative recorded; there is completely negative effect in case of 5% *Agaricus bisporus* powder concentration, while the inhibition zone were about (13, 24 and 32) mm in 10%, 20% and 30% *Agaricus bisporus* concentrations. Nearly similar results were recorded by Manjunathan and Kaviyarasan (2010) who measured the ~~of~~ inhibition zone of *Agaricus bisporus* powder against *Staphylococcus aureus* about (9-11mm) and about (0-11 mm in diameter) against *E. coli*. Higher results recorded by Akyuz *et. al.,* (2010) who measured the inhibition zone of *Agaricus bisporus* powder against *Staphylococcus aureus* (18-22 mm), and not recorded any inhibition zone against *E. coli* according to Jagadish *et al.,* (2009). In the other hand, Demirhan *et al.* (2007) reported about (9.3 mm) against *E.coli* and *Staphylococcus aureus* (8.0 mm) by adding *Agaricus bisporus* powder. While the inhibition activity against *Staphylococcus sp.* were (7.0-7.6 mm), *E. coli* (7.0-8.2 mm) according to Iwalokun *et al.,* (2007). Akyuz *et al.* (2010) estimated the inhibition zone activity against *Staphylococcus aureus,* about 10.5 mm. Uzun *et al.* (2004) recorded about (17.0-19.0 mm) against *Staphylococcus aureus* and about (8.0-8.5 mm) *E. coli* by adding *Agaricus bisporus* powder extract.

Evaluation of additional different concentrations of *Agaricus bisporus* to chicken minced meat within 7 days of refrigeration storage were increased by time but in all cases the higher concentrations of *Agaricus bisporus*. The best reduction effect observed in case of 20% & 30% conc. of mushroom powder addition especially against *E. coli,* followed by total aerobic counts while the lowest inhibition effect recorded against *Staphylococcus aureus* followed by total anaerobic count. The counts were significantly increased (*P > 0.05*) with extended storage time. Similar results recorded by (Jean van, 2001; Baise, *et. al.,* 2002; Sheena et *al,* 2003; Farkas 2003; Hur *et. al.,* 2004; Shimimura *et. al.,* 2007 and Abah, and Abah, 2010; Dasic, *et. al.,* 2013; Hafel *et al.,* 2014; Thillaimaharan, *et. al.,* 2016) observed the strong inhibition effect of *Agaricus bisporus* against gram negative microorganisms and mild effect on gram positive bacteria. that antimicrobial activities of *Agaricus bisporus.* Špoljarić, *et. al.,* (2015) recorded similar inhibition effect of *E. coli* on broiler digestive tract specially after feeding the broilers on different mushroompowder concentrations, while it didn't show any inhibition effect against *Staphylococcus aureus.* (Barros *et al.* 2007_b; Barros *et al.* 2008_b; Ozen *et al.* 2011) reported that *Agaricus bisporus* is more effective on *E. coli* that any gram negative bacilli bacteria even in higher concentrations. Waithaka*, et. al.,* (2017) recorded antimicrobial activities of mushroom powder. Cristiane*, et. al.,* (2016) observed the lack of significant difference in the size of inhibition between the bacterial pathogens using different *Agaricus bisporus* extract concentrations. Aishah and Wan Rosli, (2013) recorded that there were not any significant difference between control sample and 5%, 10%, 20% and 30% *Agaricus bisporus* at *(P<0.05*) and noted that meatball contained 5% *Agaricus bisporus* mushroom was more inhibition effect other than control samples and 10%. Same results reported in pork patties which contained *Agaricus bisporus* powder by (Süfer *et. al.,* 2016).

Effect of different concentrations of *Agaricus bisporus* addition to chicken minced meat within 7 days of Refrigeration Storage on Protein % declared that the protein % decreased by prolong storage period, while, the protein % were increased by adding higher *Agaricus bisporus* concentrations as following; about (19.0%, 19.12%, 19.26, 19.50%, 19.74%) on control, 5%, 10%, 20%, 30% mushroom powder concentrations samples respectively. While on 3rd day it were; 18.5%, 18.63%, 18.74%, 19.0%, 19.24 on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations samples respectively. While on the 5th day of storage protein % were; 18.0%, 18.12%, 18.24%, 18.50%, 18.75% on control, 5%, 10%, 20%, 30% *Agaricus bisporus* powder concentrations samples respectively. The mean protein % recorded on 7th storage day were; 17.7%, 17.92%, 18.07%, 18.30%, 18.54% on control, 5%, 10%, 20%, 30% *Agaricus bisporus* concentrations samples respectively. These counts were significantly increased (*P > 0.05*) with extended storage time. Naturally mushrooms contain twenty one different types of amino acids ranging between 2.66-2.90g/100g. of protein which covered about 5% of the daily recommended allowance. Protein level can be affected by the storage method such as refrigeration, frozen and by the preparation method while the highest protein amount found in raw *Agaricus bisporus* which was about 3.31 g/100g. Basically mushrooms is consumed mainly for their nutritional value due to its rich content of (proteins, water, fibers, minerals, carbohydrates) with low calories due to its low fat. In addition to it's highly palatability which enhance aroma and flavor specially when mixed with other foods. Mushrooms also are widely used for medicinal benefits such as antimicrobial and a lot of other benefits (Poucheret *et. al,* 2006; Öztürk *et. al.,* 2011 and Xu, et. al., 2011).

CONCLUSION

The results concluded that additional different concentrations of *Agaricus bisporus* to chicken minced meat within 7 days of refrigeration storage showed that the inhibition effects were increased by time. The consumption of minced chicken meat products which was mixed with *Agaricus bisporus* "white button mushroom" have a lot of nutritive and prophylactic antimicrobial enrichments to the consumers especially by children. This study also reported the positive reduction effect of *Agaricus bisporus* against *E. coli* (food pathogen), total aerobic count (food spoilage microorganisms, leading to extend the shelf life of the chicken minced meat product) respectively with less inhibition effect against total anaerobic counts and *Staphylococcus aureus* as gram positive foodborne microorganisms. The study recommended further studies to establish more applications using *Agaricus bisporus* nutritive, antimicrobial and other medical properties to enriching more types of food to extend shelf life and improve nutritive values of food naturally and safely which improves consumers health.

ACKNOWLEDGEMENTS

This work was financed by the author only and wasn't carried out within any framework of project.

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