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Innovation functional food from oyster mushrooms and fermented milk

ابتكـــار غـــذاء وظيفـــي مـــن الفطـــر المحـــاري والحليـــب المتخمـــر

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Abstract

This study was conducted to determine the effect of two levels of oyster mushroom (*Pleurotus ostreatus*) powder, which were (0.5 & 1.00)% under different storage conditions to increase the number of lactic acid bacteria *Lactobacillus plantarum* (LBP) the best treatment was C1(0.5 gm dried oyster mushrooms powder **before storage** with12%recovered skim milk)which amounted to 475.5×10^7 cfu/ml followed by C4 (1.0 gm dried oyster mushrooms powder **after three weeks of storage at a temperature of 30 C°** with 12%recovered skim milk)which amounted to 287.5×10^7cfu/ml compared to control which was 41×10^7 cfu/ml . After a week of storage products manufactured the best treatment in dilution 10^8 was C3 (1.0 gm dried oyster mushrooms powder before storage with 12%recovered skim milk)which amounted to 29.5×10^8 cfu/ml compared to control was 4.0×10^8cfu/ml . Evaluated the models for five treatments sensory and the treatment C1 was closest to the treatment control of the sensory and accept consumer, amounting to 38.42 a degree of flavor, 32.83 degrees of body and texture either acidity amounted to 8.417 degrees compared to control which amounted to (38.63, 34.00, 6.250) degree for each of the attributes of flavor ,strength and textures and acidity respectively. Treatments C3 , C4 got on the lower grades the attributes of flavor ,body and textures amounting to (34.00 , 33.92) degree for the recipes flavor. (29.50 , 28.17) degree for the recipe strength and textures respectively. The recipe acidity got the highest degree , amounting to 10 for each of the treatments C4, C3 While we noted that the numbers of bacteria were fixed for each treatments.

المستخلص

أجريت الدراسة لتحديد تأثير مستويين من مسحوق الفطر المحاري(*Pleurotus ostreatus*) والذي كان بنسبة (0.5 ، 1.0)% وتحت ظروف خزن مختلفة على زيادة اعداد بكتيريا حامض اللاكتيك *L. plantarum* **(LBP)** كانت افضل معاملة هي C1 (0.5 غم فطر المحاري مجفف **قبل خزنه**مع 12٪ من الحليب الفرز المسترجع) و التي بلغت 475.5×10^7cfu/ml تليها C4 (1.0 غم فطر محاري مجفف **بعد ثلاث اسابيع من خزنه على درجة حرارة 30م°** مع 12٪ من الحليب الفرز المسترجع) والتي بلغت 287.5×10^7 cfu/ml مقارنة مع السيطرة التي كانت41×10^7 cfu/ml . بعد أسبوع من خزن المنتوجات المصنعه كانت افضل معاملة للتخفيف 10^8 هي C3 (1.0 غم فطر محاري مجفف قبل الخزن مع 12٪ من الحليب الفرز المسترجع) والتي بلغت29.5×10^8 cfu/ml مقارنة مع السيطرة كانت 4.0×10^8 cfu/ml ، قيمت النماذج للمعاملات الخمس حسيا وكانت معاملة C1 هي الاقرب الى معاملة الكنترول من الناحية الحسية وتقبل المستهلك اذ بلغت 38.42درجة للنكهة ، 32.83 درجة للقوام و النسجة اما الحموضة بلغت 8.417 درجة مقارنة بمعاملة السيطرة التي بلغت 38.63، 34.00 ، 6.250 درجة لكل من صفات النكهة والقوام والنسجة والحموضة على التوالي. بينما حصلت معاملات C4,C3 على اقل الدرجات لصفات النكهة والقوام والنسجة اذ بلغت (34.00 و 33.92) درجة لصفات النكهة و (29.50، 28.17) درجة لصفة القوام لمعاملات C4,C3 على التوالي مقارنة بمعاملة السيطرة اما صفة الحموضة حصلت على اعلى درجة اذ بلغت ١٠ لكل من المعاملات C4,C3 في حين نلاحظ ان أعداد البكتريا كانت ثابتة لكل المعاملات .

Introduction

In the last few years , nutrition science , traditionally ,concentrated on identifying a balanced diet , had emphasized optimized nutrition , this is maximizing life expectancy and quality by identifying food ingredients that ,when added to a balanced diet , improved the capacity to resist disease and enhance health .The development of functional foods reflects this shift in attitudes between diet and health because of the complexity of the term "functionality " . The functional

foods defined by the European scientists that " A food could be regarded as functional if it satisfactorily demonstrated to affect beneficially one or more target functions in the body , beyond adequate nutritional effects , in a way that was relevant to either improved stage of health and well-being and/ or reduction of risk disease(1) .

A functional food must remain food and it must demonstrate its effect in amounts that could normally be expected to be consumed in the diet .It was not a pill or capsule , but part of the normal pattern food (2)

Recently ,there has been an increasing trend of consumer awareness towards the demand for functional foods which have enhanced the health of consumer. Apart from other food ingredients ,prebiotics have been among those which have attracted much attention recently (3) . A prebiotic has been a non-digestible and selectively fermented ingredient that allow specific changes ,both in the composition and /or activity in the gastrointestinal microbiota that conferred benefits upon host well being and health (4).They could not be digested by α –amylase or other hydrolyses in the upper gut section of the intestinal tract (5).

Human intestinal microbiota was composed of more than 400 bacterial species ,which made it a complex bacterial ecosystem .Lactobacilli and Bifidobacteria were predominant members of the gut microbiota , they have been best known for their beneficial and health promoting properties (6).This allows Lactobacillus genera the most important probiotic strains for humans use (7).

In this study has based on fermented milk by one of lactic acid bacteria (*Lactobacillus plantarum*) and mushrooms(oyster mushrooms). Many symbiotic relationships between probiotic and prebiotic have been studied in order to maximize their beneficial effects **(8)**. Lactobacilli were best known to utilize prebiotics in the GI tract based on the fact that they contain relatively high amount polysaccharides as β-glucan (pleuran) and other dietary fiber components as chitin, hemicelluloses, mannans , xylans and galactans, respectively which enable them to break down polymers (the prebiotics)into smaller units and make it available as a substrate during fermentation (9,10).

Oyster mushroom polysaccharides could stimulate the growth of colon microorganisms (probiotic), then beneficial colon microorganism would be fermented polysaccharide (β-glucan) (4) .Organic acids would be produced by lactic acid bacteria as a result of fermentation, thus providing an acidic environment in the colon which indirectly suppresses the growth of pathogens. This mechanism would allow prebiotics to manipulate the composition of colonic microbiota in human gut(11)thus improving the host health in return .These included enhancement immune function ,improved digestion and elimination of faeces as well as reducing the potential of getting irresistible bowl syndrome (11)

many studies have now confirmed that the prebiotics incorporated in the diet were a valid approach to the dietary manipulation of colonic microbiota . this concept has gained global attention and was being manipulated for human health purpose .Because of increasing demand for prebiotics, there was a need to find a new source of prebiotics which was relatively low price as compared to commercially available prebiotics .Extensive studies were now focused on the prebiotic potential of polysaccharides extracted from natural source such as oyster mushrooms (*Pleurotus ostreatus*) and bran barely (*hordeum vulgare*) .Apart from their unique taste and crispy texture(4,9).This has been actually associated with dietary content of oyster mushrooms which served as substrate for fermentation of lactic acid bacteria. Therefore, the objective of this study was : prepared a fermented milk with *Lactobacillus planetarium* and oyster mushrooms *(Pleurotus ostreatus*), then Estimated the number of bacteria in the product lactic fermented under conditions of storage coolant and sensory evaluation**.**

Materials and Methods

1-Pleurotus ostreatus

Fresh samples of oyster mushrooms **Pleurotus ostreatus** were supplied by the laboratories of the department of food science , Faculty of Agriculture University of Baghdad . Took 100 grams of fruiting bodies of fresh pre-stored were cut into small pieces and placed in an electric oven on the

fan air to get rid of the moisture resulting from fruiting bodies and dried at a temperature of 50 C° and while the stability of weight to keep the chemical and medical compounds . Took the same amount of fruiting bodies (100 g) fresh and conducted in the process of storage for 3 weeks were cut into small pieces and put it in plastic containers intended for this purpose, and closed the foil of clear plastic, which has the ability to adhesion and used incubators equipped organizer heat(Thermostat) was installed temperature 30 ° C . The dried fruiting bodies samples of each case were weighed and ground into powder . The samples kept in the refrigerator for a while in use.

2-Lactic Acid Bacteria Lactobacillus plantarum

Strains of Lactobacillus plantarum (LBP) were obtained from the laboratories of the department of food science , Faculty of Agriculture University of Baghdad .

3-Culture media and solutions utilize under study

MRS broth and MRS agar(oxoid) ,was prepared the media as instructed by the company processed.

-Peptone water

dissolved 0.1 mg from peptone in 100 ml of distill water and distributed in test tubes 9 ml of each tube then entered in steam autoclave device at 121 C° , 15 min and 15 Joe .

-Skim milk media

dissolved 12g skim milk powder in 100 ml distill water and entered steam autoclave at 121C° , 5min and 15Joe. The purpose of the skim milk was activated (LBP) for three consecutive times by inoculation of 5% and incubated at 37C° / 24 h until the advent of coagulation (11).

3- **addition mushrooms powder to skim milk media**

added Mushrooms(powder) to the skim milk media in different proportions(0.5 , 1.0) % sterilized in autoclave 121C° , 5 min ,15Joe then cooled at 37 C° and inoculated by 5% from LBP (12), then incubated at 37 C° until the advent of coagulation, measured pH of treatments (0.5 , 1.0) % .

4- **Estimate the total number of bacteria (LBP) in vitro**

Prepared a series of decimal dilution by dissolved 0.1mg pepton in 100ml distill water) as mentioned previously in paragraph 2 and inoculated tubes by 1 ml from starter prepared previously in paragraph 3 and followed by (Pour plate) as a mentioned from (13) by using MRS agar , incubated dishes at 37 C° for 48 hours under anaerobic conditions in a container deflated after the end of cuddling period calculated prepare colonies developing device using colony counter .

5- **Sensory evaluation of the product**

Evaluated treatments for the product depending on the form by Trout& Nelson and some modified (12) .

Features	Upper Class in the original form	Upper class in the modified form	Neam of product	Notice
Flavor	45	40		
body and texture	30	35		
*The numbers of bacteria	-	15		
Acidity	10	10		
Appearance	10	10		
Packaging	5	5		

*Evaluated according to the number of bacteria) on the numbers as follows:

*The numbers of bacteria	Score
$0\text{-}10^5$	0
$10^5\text{-}10^6$	3
$10^6\text{-}10^7$	6
$10^7\text{-}10^8$	9
$10^8\text{-}10^9$	12
$10^9\text{-}10^{10}$	15

6- statistical analysis

Used complete random design (CRD) in a analysis of the effect of various treatments in studied traits and differences compared to the significant difference between the means and use the program SAS 2001 based on statistical analysis

Result and Discussion

The results were shown in the table1. impact of oyster mushrooms Added to the development of bacteria farms *L.plantarum*, and noted by the results that there was a significant increase on the level of($p< 0.01$) in bacterial numbers at dilution 10^7 for each treatment compared to control before storage , amounting numbers of live bacteria in the media of skim milk by 12% after 24 hours of cuddling at 37 C^o for each of the control treatment and treatment with added oyster mushrooms were represented C, C1,C2,C3,C4 (41.0, 475.5 , 104.3 , 153 , 287.5) respectively. Noted the results of treatment outweigh the C1 (media of skim milk recovered by 12% and supplemented with 0.5% dried oyster mushrooms before storage , referred to its in advance in materials and methods in paragraph oyster mushrooms) had given the highest numbers for the development of bacteria at 37 C^o for 24 h ,which amounted to 475.5×10^7 cfu/ml this was attributed to oyster mushrooms fortified of dietary fiber especially β-glucan and another chemical compounds(18) . This was agreement with the result by (14) who studied product from 0.5% oyster mushroom with 3% skim milk ,96% goat skim milk which amounted lactic acid bactreia 1.1×10^{10} cfu/ml(14).Another explanation have been presented by(16) that used lactobacilii as a starter cultures for *Pleurotus ostreatus* . The results showed that the numbers of live bacteria in media of the skim milk recovered 12% and rates of 0.5, 1.00% dried oyster mushrooms, fell within the high numbers that were between 10^7-10^8cfu/ml.The microorganism was incorporated at levels 10^7 -10^8 cfu/ml for maintaining the viability, stability and functionality of probiotics not only during processing but during storage is essential to delivering the health benefits of these microorganisms to consumers (12,15) .

The results shown pH values for farms(LBP) for the treatment and control of treatments (0.5 , 0.1) % dried Oyster mushroom at 10^7 before a week of storage, amounting to(4.0 , 3.6 , 3.6 , 3.5 , 3.6) Respectively, as there was a decrease in pH values depending on the numerical therapeutic of bacterial and this was confirmed by (12). After a week of storage , decreased number of LBP per treatments compared to treatments before storage clearly. there was not a significant increase on the level of($p< 0.01$) in bacterial numbers at dilution 10^8 for each treatment compared to control after storage ,amounting to (4.0 , 15.0 , 11.5 , 29.3 , 9.6)this was agreement with (`17) Who noted that the bacteria suffer during the storage period of diminishing in numbers, especially when using concentrates of lactic acid bacteria and suggested that lived default for the product ranges between one week to three weeks . Noted the results of treatment outweigh the C3 (media of skim milk recovered by 12% and supplemented with 1.0 % dried oyster mushrooms before storage) had given the highest numbers for the development of bacteria at 37 C^o for 24 h ,which amounted to 29.3×10^8 . In addition to low in pH slightly for each treatments during storage ,amounting to (3.8, 3.5 ,3.5 ,3.5 , 3.5) This is due to the storage conditions, especially temperature refrigeration during storage, especially the oyster mushroom was rich with numerous compounds such as lipids ,protein and minerals .This Has led to a decline in numbers during the storage period if we use the fiber directly as(β-gluacn) extracted from oyster mushrooms there may be a clear numerical increase during storage (4 ,9).

Indicated Table 2. the results of sensory evaluation of the product fermented for treatment C , C1 ,C2 ,C3, C4 there were significant (($p<0.05$)for flavor &($p< 0.01$) the rest of features) decrease of the sensory qualities of the product compared to control treatments .Results of this research was to approach (12 , 14) found in their study . Noted from the result for the treatment C1 got the best results of sensory evaluation in terms of the qualities of flavor, Conception & textures, acidity and the numbers of bacteria which amounted to (38.42 , 32.83, 8.417 ,9.00) score and were closest to the control treatment compared to the rest of treatments , followed by treatment C2, while noting

that the treatments C4 and C5, the corruption of flavor as it got a degree 34 out of 40 score each of them . While the degree of body& textures degrees 29.50, 28.17, respectively, for both treatments however the pH value got to score 10 of the total 10 degrees either total numbers of bacteria remained stable, as was the case with the rest of the treatments.The result showed that consumer accepted treatment C1, C2 got the results similar results to the results of control. Results also showed the lack of acceptance of Iraqi consumers like so flavors especially for treatments C3 and C4 .Failure to accept consumer of treatments C3 and C4 did not mean the failure of the product, especially the pH was among the highest degrees are required in addition to the numbers of the bacteria remained stationary as the rest of the treatments, so we suggest freeze drier product of treatmentsC3 and C4, and entered it into other products as in the manufacture of bread fermented or juice fermented and hold Other studies on them . Noted from the results that the numbers of bacteria were fixed in each treatments because it lied within the limits required in the evaluation form previously referred to in paragraph 5

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Table 1. The effect utilized different concentrations of oyster mushrooms towards the numbers of Lactobacillus plantarum

*oyster mushrooms concentration%	*Numbers of *L.planturum* dilution (10^7)before storage	*Numbers of *L.planturum* dilution (10^8)after storage
C	a41.0	a4.0
C_1	b475.5	a15.0
C_2	c104.3	a11.5
C_3	d153	a29.5
C^4	e287.5	a9.6
LSD p<0.01	51.65	

*The different letters within each table refer to the significant difference between the means ,by (p<0.01).*Data were represented as mean of 2 replicate. *C was represented as control , C_1 was represented as 0.5% oyster mushrooms before storage , C_2 was represented as 0.5% oyster mushrooms after three weeks of storage at a temperature of 30 C°, C_3 was represented as1% oyster mushrooms before storage, C4 was represented as 1% oyster mushrooms after three weeks of storage at a temperature of 30 C°.

Table 2. The sensory evaluation of the product fermented by form Trout Nelson with some modification.

Features * Treatments	* Flavor °40	**Conception & textures °35	**Acidity °10	The numbers of bacteria °15
C	**a**38.63	**a**34.00	**a**6.250	9
C1	**a**38.42	**b**32.83	**b**8.417	9
C2	**a**36.50	**b**31.92	**c**9.500	9
C3	**b**34.00	**c**29.50	**d**10.000	9
C4	**b**33.92	**d**28.17	**d**10.000	9
LSD	LSDp<0.05 2.225	LSD p<0.01 1.017	LSD p<0.01 0.5099	----------

*The different letters within each table refer to the significant difference between the means ,by (p<0.05).** The different letters within each table refer to the significant difference between the means ,by (p<0.01). *Data were represented as mean of 6 evaluator . *C was represented as control , C_1 was represented as 0.5% oyster mushrooms before storage , C_2 was represented as 0.5% oyster mushrooms after three weeks of storage at a temperature of 30 C°, C_3 was represented as1% oyster mushrooms before storage, C4 was represented as 1% oyster mushrooms after three weeks of storage at a temperature of 30 C°.