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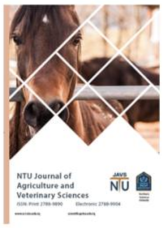

Synbiotic Effects of *Agaricus bisporus* Fungus on *Lactobacillus Acidophilus* Growth and the Properties of Yielded Yogurt

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ABSTRACT

The production of functional foods has increased in recent decades due to their health benefits. The yogurt represents most popular functional food from ancient times until the present. This study was conducted to evaluate the synbiotic effects of *Agraicus bisporus* fungi dried powder on *Lactobacillus acidophilus* growth rate and viability and physiochemical yogurt properties after storage periods (0-7-14-21-28 days) at 6 Cº. The study showed an increase in total proteins, ash, fat, and titratable acidity of fortified yogurt the highest values were obtained in treatment Lb4 after 28 storage days (5.35%,0.71%,3.66%,1.72g respectively) The titratable acidity increased as a result of the addition of dried powder of fungi, while the moisture, carbohydrate, and pH were reduced with progress of storage time, the lowest value of moisture and pH obtained after 28 days in Lb4 (84.29%,3.51 respectively). The study also showed Lb. acidophilus count is dependent on the additive amount and total acidity, the highest count number obtained in treatment Lb4 after 21 days of storage was (177x 10^9 cfu/gr). while the highest viable count obtained after 28 days of storage in treatment Lb3 was (165x 10^9 cfu/gr). The rheological properties of yogurt were also affected by the additives, with a notable development in the hardness and retreat in whey separation, the highest value of hardness obtained in treatment Lb4 after 28 storage days was (111.1 g).

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Introduction

Food and Drug Administration defined yogurt as the final product obtained from fermented milk essentially by *Lactobacillus delbrueckii bulgaricus*, and *Streptococcus salivarius thermophilus*, and contains at least 8.25% non-fat solids, 3.25% milk fat, pH 4.6 or lower, viable bacteria counts at least 107 cfu/gr [1]. Traditional yogurt is an ancient product investigated to solve the problems of fast milk degradation, lactose intolerance, and milk rancidity. The word "yogurt" is Turkish in origin meaning thickened [2]. Recently, developing dairy products and the persistent need to enhance human health appears to be a new trend of products that can promote health named functional food, which uses a combination of specific microorganisms terminologically called probiotics and indigestible polysaccharides called prebiotics [2]. Functional food provides a preventive, curative, and or protective effect against one or more diseases, further its nutritional benefits" [3]. In contrast, probiotics are defined as "a live microbial food ingredient that, when administered in adequate amounts confers a health benefit on the host" like *Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium bifidum* [4]. While prebiotics are defined as "selectively fermented ingredients that result in specific changes, in the composition and/or activity of the GI microbiota, thus conferring a benefit upon host health" like Lactulose, galacto-oligosaccharides, Lactosucrose, and Malto-oligosaccharides [5]. Dairy products are fortified for several purposes, such as increasing nutritional value or improving physicochemical, rheological, and sensory properties [6][7]. Thus we see many fortified yogurt products such as yogurt-fruits, yogurt-vegetables, yogurt-fungi, etc. [8]–[10].

Agaricus bisporus fungus has a high nutritional value, with protein contents 34-44%, carbohydrate 38-48%, fiber 17-23%, ash 8-11%, and fat 3-4% [11]. It also has prebiotic properties due to its content of complex polysaccharides with a variety of polymerizations, such as fucogalactan and glucan, which are used by certain bacteria as nutrients [12]. The fermentation of polysaccharides by probiotic bacteria enhances bacteria viability yogurt's physiochemical and rheological properties by interacting with milk proteins, thus trapping water within the texture and reducing whey separation [13], [14].

Material and Methods

A- Starters and Fungi

1- Traditional yogurt starter *Streptococcus thermophiles* and *Lactobacillus bulgaricus* from Danisco Company.

2- Lactobacillus acidophilus isolation from Al-Amien scientific research centers.

3- Fungus powder: *Agraicus bisporus* fungus was purchased from the local market, washed with deionized water, then cut into slim slices and dried in an oven at 60Cº until reached constant weight, then crushed in a blender to fine powder, stored in a dry clean tightly closed container.

B- Experience Design

Six groups of treatments:

[C]-Traditional culture (control)

[Lb]-Traditional culture + *Lactobacillus acidophilus*

[Lb1]-Traditional culture + *Lactobacillus acidophilus+0.5% Agraicus bisporus*

[Lb2]-Traditional culture + *Lactobacillus acidophilus+1% Agraicus bisporus*

[Lb3]-Traditional culture + *Lactobacillus acidophilus+3% Agraicus bisporus*

[Lb4]-Traditional culture + *Lactobacillus acidophilus+5% Agraicus bisporusthen*

C- Chemical analysis of yogurt

1-Moisture determination:

Take 2g of yogurt (or mushroom), and put it in the oven at 105Cº until getting constant weight [15].

2-Total nitrogen determination:

Using Keldahl's method, take 5 g of sample in Keldahl cylinder, 12g of CuSO4, 1 ml of CuSO4.5H2O, Conc. 20 ml H2SO4 98%, digest for 30 min at 200Cº then 90min at 420Cº, distillation, finally titration with 0.1M HCl, the result multiply with milk factor of protein 6.38 [15].

3-Milk fat determination:

Using Gerber method put 10 ml H2SO4 90-91%, in a Gerber tube (butyrometer 1-6 fat%), then 10.75 ml yogurt, and 1 ml Amyl alcohol, close the tube, and shake it until homogeneous, keep it in a water bath for 5 min at 65°C then centrifugation for 5 min at 11,000 rpm, return the tube in water bath 5 min at 65 Cº, then read the result immediately from scale [16].

4-Ash determination:

By direct incineration of the sample (yogurt or mushroom) in a muffle furnace for 6h at 550 Cº, transfer to a desiccator until cool then weighting. Ash content measured by the formula

Ash%= $\frac{IW-FW}{IW} * 100$

IW, initial weight

FW, final weight [15].

5-Total carbohydrate determination:

By applying the formula:

Total carbohydrate%= 100 – (protein%+fat%+moisture%+ash%) [15].

D- Titratable acidity and pH

1-Total acidity determination: take 10g of yogurt mix with 30 ml distilled warm water + 1 ml of phenolphthalein indicator, shake well, then titrate against standard 0.1N NaOH [16].

2-pH value: take 50g of mixed yogurt at 20Cº, and immerse pH meter directly in the sample [15].

E- Microbiological examination

Total count of lactobacillus acidophilus: Use Lactobacillus MRS Agar (MRS Agar) M641I media from Himedia company, take 1 ml from 10^7 dilutions put into a petri dish,, then pour the media above it at 42Cº swirl gently, incubate at 37Cº for 48h [17].

F-Spontaneous Whey separation

Take 50g of yogurt 5Cº in a beaker, place the beaker at a slop angle of 45º, and after 5, Remove the filtered whey with a syringe in a time not exceeding 10s [18].

G- Hardness

Take 50 ml of yogurt analyzed by texture analyzer apparatus CT3 (4500) Brookfield Engineering Lab. Set the initial and final speed of equipment at 1mm/min

H- Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different different treatments on study parameters. The least significant difference-LSD was used to significantly compare the means in this study [19].

K- Chemical analysis of mushroom

1-Total nitrogen determination:

Using Keldahl's method, take 0.2 g of dried powder of mushroom in Keldahl cylinder, 12g of CuSO4, 1 ml of CuSO4.5H2O, Conc. 20 ml H2SO4 98%, digest for 30 min at 200Cº then 90min at 420Cº, distillation, finally titration with 0.1M HCl, the result multiply with the factor of protein 5.70 [15].

2-Mushroom fat determination:

5 g of mushroom was taken and 50 ml of hexane: methanol (30:20) extraction system was added to it and the SAXOLITE device was used at 50°C for 6 h, after which the hexane layer was taken and the solvent was expelled using a rotary evaporator device, and the percentage of fat to the weight of the sample was calculated [20].

Results and Discussion

Table 1 shows the chemical composition of the crude powder of *Agaricus bisporus*, these results were diverse in carbohydrate contents when compared with [21], the result showed the total carbohydrate was (42.20 %) compared to (34.54%), the differences in result of carbohydrate return to the method, in this research used calculation by the formula, this method isn't accurate enough because it adds non-carbohydrate parts to the calculation such as fibers, organic acids, and vitamins., while [21] used chemical method to determination the carbohydrate, other differences in ingredients return to fungi part, maturation, climate changes, and genetic diversity from fungi species [22].

The chemical analysis of yogurt showed an increase in total proteins, fats, carbohydrates, and ash on day one of the storage period dependent on the amount of fungi powder added, this increase in total solids is due to the high percentage of these components in dried fungi powder, the results agree with [23] they found an increase in total solids of yogurt after addition of mushroom powder. This increase means an increase in the nutritional value of the product, as well as synergism action with probiotics by award-specific nutrition compounds like monosaccharides, polysaccharides, and folate, these components stimulate the growth activity of probiotics [17], also *Agraicus bisporus* a wealthy source of polyphenols that reduces the potential oxidative effects (radical scavengers agents) came from the presence of oxygen in the environment, these compounds promote probiotics proliferation [19] [20], all these factors affect on the total count of Lb acidophilus, the results showed the *Lb acidophilus* counts increasing significantly (P≤0.05) by increasing in total solids of yogurt, and the highest count obtained was $177*10^9$ in treatment Lb4 after 21 days of storage as shown in table 4, this result agrees with [24] they observed the increasing in total solids enhanced the growth of starter bacteria. The chemical analysis of yogurt also showed significant elevation (P≤0.05) in titratable acidity as shown in Table 3, the highest value found was 1.72g (as lactic acid) in Lb4 after 28 days of storage, because of fermented sugar by microbial action that converts carbohydrates to organic components like lactic, acetic and formic acids [25]. *Lb acidophilus* has the α-Galactosidases enzyme; this enzyme can degrade the oligosaccharide into a monosaccharide that is fermented into organic acids, leading to an increase in titratable acidity value [26]. On the contrary, there is a significant decrease (P≤0.05) in pH value due to the accumulation of organic acid in yogurt crud; the lowest value in pH was 3.51 in the same treatment this result agrees with [25] their findings show that there is an increase in total acidity and a decrease in pH value over time due to the fermentation of sugars into acids caused by microbial action. Also, there is a noticeable decrease (P≤0.05) in moisture at the late stage of storage, this depends on the number of fungi added and the evaporation ratio from the surface of the yogurt., the lowest value of moisture was 84.29% after 28 days of storage in Lb4 treatment, this agrees with [27] that observed a decline in moisture with increased storage time.

Agaricus bisporus as a prebiotic gives *Lb acidophilus* tolerance to some abnormal environmental conditions such as high acidity, so these microorganisms can grow under elevation acidity as shown in Table 4 [28]. The highest Lb acidophilus counts obtained was 177 x 10^9 cfu\g in Lb4 after 21 days of storage but, we show a significant decrease (P≤0.05) in Lb acidophilus numbers in successive storage time 28 days of storage because the high pH and low availability of sugar and nutrients in the last stage of storage resulted in a decreased growth rate of Lb. acidophilus, consistent with [28], shows a reduction in the number of cultural bacteria dependent on pH value in the last stage of storage. Fortification of yogurt by fungi significantly increased (P≤0.05) the *Lb acidophilus* counter, while didn't have notable effects on the traditional starter counter, as shown in

Table 4, an increase in the count number means more changes in yogurt characters due to microbial action, Table 4 showed a significantly progressive effect (P≤0.05) in yogurt whey separation dependence on fungi addition, that because polysaccharide like fucogalactan and glucan compounds in fungi that interact with milk proteins that lead to form contracting non-retained water curd [29]. The lowest values of whey separation were (1.27 and 1.22 ml\100g) in C, and Lb treatments, and the highest value was 1.99 l/100g in Lb4 treatment, because of the decampment of the liquids from the curd structure by the contraction force generated between polysaccharides and milk proteins, this result agrees with [30] they observed an increase whey separation when increasing fungi powder ratio. On the contrary, the value of hardness significantly increased (P≤0.05) by time exceeded, the highest value equals 111.1g in Lb4 after 28day of storage, which agrees with [24] showed that the hardness depends on the total solids, especially the amounts of protein, thus increasing the binds between proteins and other molecules resulting in a hard curd

Table 1 Chemical analysis of *Agaricus bisporus* powder

Components	Percentage %
Total proteins	36%
Total carbohydrates	42.2%
Ash	1.35%
Fats	0.8%

Table 2: Effect of Treatment and Age of crude in chemical composition

Treatment	Age of curd	Moisture %	Ash %	Fat %	Protein %	Carbohydrates %
C	1	87.99	0.62	3.32	3.36	4.71
	7	87.96	0.62	3.36	3.38	4.68
	14	87.90	0.63	3.38	3.43	4.66
	21	87.83	0.65	3.4	3.49	4.63
	28	87.78	0.66	3.44	3.53	4.59
Lb	1	87.93	0.63	3.32	3.37	4.75
	7	87.82	0.64	3.46	3.44	4.64
	14	87.79	0.65	3.49	3.48	4.59
	21	87.65	0.76	3.51	3.53	4.55
	28	87.57	0.77	3.54	3.59	4.53
Lb 1	1	87.66	0.63	3.32	3.50	4.89
	7	87.59	0.64	3.44	3.58	4.75
	14	87.52	0.66	3.45	3.66	4.71
	21	87.46	0.67	3.48	3.71	4.68
	28	87.36	0.68	3.54	3.75	4.67
Lb 2	1	86.36	0.63	3.33	4.68	5.00
	7	86.28	0.64	3.48	4.76	4.84
	14	86.22	0.65	3.50	4.81	4.82
	21	86.09	0.67	3.52	4.93	4.79
	28	86.01	0.68	3.53	5.04	4.74
Lb 3	1	85.42	0.64	3.33	4.81	5.80
	7	85.29	0.66	3.51	4.93	5.61
	14	85.15	0.68	3.53	5.06	5.58
	21	85.10	0.67	3.57	5.12	5.54
	28	85.04	0.68	3.58	5.19	5.51
Lb 4	1	84.70	0.65	3.34	4.95	6.36
	7	84.55	0.66	3.55	5.09	6.15
	14	84.44	0.68	3.58	5.20	6.10
	21	84.34	0.70	3.59	5.29	6.08
	28	84.29	0.71	3.66	5.35	5.99
LSD value		2.682 NS	0.275 NS	0.296 NS	0.893 *	1.267 *

* (P≤0.05).

Table 3: Effect of Treatment and Age of crud in pH and Titratable Acidity

Treatment	Age of curd Day	PH	Total acidity as gram lactic acid

C	1	4.50	0.91
	7	4.41	1.00
	14	4.38	1.04
	21	4.28	1.12
	28	4.18	1.17
Lb	1	4.48	0.94
	7	4.38	0.97
	14	4.20	1.15
	21	4.12	1.23
	28	3.98	1.29
Lb1	1	4.46	0.98
	7	4.21	1.14
	14	4.13	1.22
	21	3.96	1.32
	28	3.90	1.37
Lb2	1	4.46	0.94
	7	4.38	1.04
	4	4.09	1.25
	21	3.91	1.36
	28	3.80	1.48
Lb3	1	4.34	1.09
	7	4.10	1.22
	14	3.95	1.33
	21	3.87	1.41
	28	3.75	1.52
Lb4	1	4.20	1.15
	7	3.91	1.36
	14	3.78	1.50
	21	3.63	1.66
	28	3.51	1.72
LSD value		0.498 *	0.377 *

* (P≤0.05).

Table 4: Effect of Treatment and Age of crude in bacteria count, Whale exudation, and Hardness

Treatment	Age of curd\day	*Lb. acidophilus* CFU\g *10^9	Starter bacteria CFU\g *10^9	Whey Separation ml\100g	Hardness g
C	1	0	111	1.65	80.2
	7	0	126	1.80	82.4
	14	0	130	1.10	87.44
	21	0	146	1.15	87.48
	28	0	155	1.27	87.51
Lb	1	98	100	1.58	84.1
	7	99	123	1.49	90.0
	14	118	140	1.36	89.20
	21	125	145	1.22	92.12
	28	129	150	1.10	97.4
Lb1	1	110	81	1.77	90.44
	7	114	97	1.64	80.23
	14	134	99	1.56	85.3
	21	144	114	1.47	99.3
	28	152	120	1.40	90.4
Lb2	1	116	85	1.86	79.8
	7	124	90	1.80	85.1
	14	133	94	1.73	95.7
	21	143	100	1.66	100.0
	28	150	113	1.54	99.23
Lb3	1	124	92	1.91	70.88
	7	128	97	1.81	90.7
	14	133	103	1.75	87.16
	21	147	114	1.68	90.12
	28	165	121	1.60	92.1
Lb4	1	130	128	1.99	85.33
	7	145	133	1.83	88.34
	14	162	139	1.75	92.8
	21	177	143	1.66	99.4
	28	160	147	1.63	111.1
LSD value		22.701 *	24.966 *	0.481 *	10.825 *

* (P≤0.05).

Conclusion

Yogurt fortification by *Agaricus bisporus* powder notably increased total solids and stimulated the growth and viability *of Lb. acidophilus*, also enhancing the hardness property.

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