Partial Purification of Phytase and Mannanase from *Lactobacillus plantarum* and Kinetic Determination of the Features of the *L. plantarum* immobilized onto the Magnetite Florisil Nanoparticle

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Abstract

In this study, production of the phytase and mannanase was carried out in a natural medium which includes corn (C) and wheat (W) using free and immobilized L. plantarum bacterial strains. Accordingly, test bacteria were inoculated into media containing C (10, 20, 30, 40 gr) and W (10, 15, 20 gr) at different ratios and left to incubate at the growth conditions pH 6.0-6.5 and 35^0C. First, $-NH_2$ group was attached to the support material using APTES. Then, the sub-branch was formed using glutaraldehyde and the resulting Schiff was reduced with sodium borohydride to make the basic compound stable. Activated support material nano florisil was made magnetic with Fe_3O_4 compound and used for the immobilization of the bacteria. It was determined that approximately 50% of the prepared bacteria immobilized to the nanoparticles of magnetite florisil.

The production of phytase and mannanase was performed with both free microorganisms and microorganisms attached to the matrix phytase and mannanase production by L. plantarum using corn and wheat natural media increased by 10-15% compared to those of the free isolates. In light of these findings, it was concluded that magnetite florisil nanoparticles can be used in many areas especially food and agriculture.

Keywords: Immobilization, *L. plantarum*, mannanases, phytase, corn, wheat.

Introduction

Enzymes are mostly protein-based biological catalysts that increase the rate of chemical reactions in living organisms by lowering the activation energy[7,8,12]. Enzyme-catalyzed reactions occur 10^{10} times faster than other reactions[30]. Many industrial enzymes used today are of microbial origin. Thus, the use of microorganisms in the production of industrial enzymes have become widespread. The widespread use of microbial enzymes is predominantly attributed to the higher catalytic activity of the enzymes of microbial origin than that of enzymes of vegetable or animal origin, the ability to produce microbial enzymes at higher amounts, not obtaining undesired byproducts and products, and the lower costs of microbial enzymes[29].

The microbial mannanase and phytase enzymes are extracellularly secreted and stable enzymes that do not lose their activity at a wide range of pHs and temperatures[22,24]. Therefore, mannanases have widespread use in paper and detergent production, pharmaceutical applications and medical fields, while phytases are widely used in the food industry[4,21,25].

Lactic acid bacteria (LAB) are gram-positive, facultative anaerobes that produce lactic acid as the end product of carbohydrate fermentation. *Lactobacillus plantarum* is a gram-positive bacterium and has various strains. It is classified as a heterofermentative bacterium that can utilize a large spectrum of carbon sources[13,18]. There are various studies on the promotion of its use in industrial applications because of its inclusion in the Generally Recognized as Safe (GRAS) list and non-toxicity[1,2,9].

Especially in the last three decades, studies have focused on the immobilization of enzymes to add to their appeal for industrial use. Enzymes are immobilized through physical or chemical attachment to an inert support material that does not directly dissolve in water[15,20,28]. The industrial use of immobilized enzymes has many advantages which include the significantly increased stability of the catalytic activity of the immobilized enzymes, their reusability, easily obtaining pure products, their high stability against environmental factors, the sustainability of their production and cost reduction[5].

In the study, *L. plantarum* was isolated from the natural medium prepared in the study (wheat and corn flour were added to the medium as nutrients) and immobilized onto magnetic florisil nanoparticles (NPs). The activities of the mannanase and phytase produced from free and immobilized bacteria were compared. Then, the potential of these industrially important enzymes for biotechnological production was investigated.

Material and Methods

The preparation of the media and inoculation of the bacteria: In the reaction mediums containing 10 g corn, 10, 15 and 20 g of whole wheat flour were used respectively. The carbon sources were partially sterilized in Petri dishes.

The carbon sources that were weighed according to the type of the mineral substance and mineral amounts [2 g $(NH_4)_2SO_4$, 1.5 g (KH_2PO_4), 1 g ($MgSO_4$), 0.3 g ($CaCl_2$) and 0.03 g ($FeSO_4$)] were added to the Erlenmeyer flasks used in the reaction. The pH of the medium was adjusted to 7.0. A loopful of the bacteria grown in the medium (MRS Oxoid) was inoculated into each Erlenmeyer flask. Then, the flasks were incubated 5 days in an incubator at 35°C. The same procedure was repeated using 20 g, 30 g and 40 g corn flour.

The immobilization of the bacteria: The *L. plantarum* bacteria were inoculated onto the MRS Agar and incubated at 35°C for 24 hours. A loopful of bacteria was collected from the petri dishes and transferred to a sterile tube containing 1 µL pure water. The total volume of the tube was brought to 3 mL by adding 2 µL pure water. After vortexing for 30-40 seconds, the tube was centrifuged at 3200 rpm for 10 minutes. Then, serial dilutions were made and bacteria were inoculated into Petri dishes. The Petri dishes were incubated at 35°C for 24 hours and, then, the bacteria counts were determined.

The determination of the free and immobilized bacteria counts: The *L. plantarum* bacteria were inoculated onto the MRS Agar and incubated at 35°C for 24 hours. After serial dilutions, the bacteria were inoculated into Petri dishes. The Petri dishes were incubated at 35°C for 24 hours and the bacteria counts were determined. For the determination of the immobilized bacteria counts: First, bacteria were immobilized onto the magnetite florisil nanoparticle. For this purpose, 0.1 g of the magnetite florisil nanoparticle was weighed and add 1000 **µl** preculture and 2000 **µl** distilled water. Then, the Eppendorf tube containing the nanoparticle and bacteria was incubated in a shaker at 35°C for 60 minutes and thus, the attachment of the bacteria to the magnetite florisil nanoparticles was achieved.

For the determination of the number of the attached bacteria, the supernatant of the nanoparticle-containing tubes was removed and the pellet was rinsed by pipetting several times with water and re-homogenized with 1000 **µL** sterile pure water. The immobilized bacteria count per mL was determined using dilution counting by taking 1000**µl** samples from the tubes (Table 1).

Table 1
Dilution rates and bacteria counts of the free and immobilized *L. plantarum*

L. plantarum		
Dilution Rates	**Immobilized Cell Count**	**Free Cell Count**
10^{-3}	424	860
10^{-4}	153	180
10^{-5}	21	50
10^{-6}	3	11
10^{-7}	≤ 0	1

The activation of the acrylamide derivate of florisil with glutaraldehyde and the reduction of the Schiff base: To obtain the support material, 10 gr of florisil was weighed and rinsed with 50 µL 5% HNO_3. Then, the support material was neutralized by rinsing with pure water and dried at 120°C. After 1 gr of the dried support material was weighed and add to the 4% (v/v) 3-APTES (3-Aminopropyltriethoxysilane) solution prepared with acetone, the mixture was incubated at 45°C for 25 hours. Then, the activated support material was rinsed with pure water and left to dry for one night in a drying oven at 105°C (Figure 1). One gr of the acrylamide derivative was taken, add 25 mL of the glutaraldehyde solution that was prepared in 50 mM pH 7.0 phosphate buffer to obtain a 2.5% (w/v) solution and then, shaken at room temperature for 2 hours.

Then, the support material was rinsed with pure water and glutaraldehyde was removed. The reduction of the Schiff base in the dried support material was achieved by adding 10 µL of the 0.25 M $NaCNBH_3$ solution in the 50 mM pH 7.0 phosphate buffer medium and stirring for 12 hours at room temperature. The thereby obtained support material was rinsed with pure water and dried.

The preparation of the nanomagnetic florisil nanoparticles: One gr of the activated florisil support material was dissolved in 100 mL 1M acetic acid solution. Then, it was stirred with 3 gr Fe_3O_4 nanomaterial for one night. Following this procedure, the activated florisil compounds were homogenously coated with nano Fe_3O_4 (Figure 2).

The determination of the activity of mannanase: The dinitro-salicylic acid (DNSA) method was followed to measure the activity of mannanase[3,17]. The change in the absorbance of mannanase was spectrophotometrically determined at 540 nm. The activity of mannanase was measured at 6-hour intervals for 5 days and pure water was used as the blank sample.

The determination of the activity of phytase: The Sarıbuğa et al[22] method was followed in the measurements. The activity of phytase was measured at 6-hour intervals for 5 days and pure water was used as the blank sample.

Results and Discussion

In the study, florisil was used as the support material and NH_2 groups were formed on its surface through activation with 3-APTES. Then, the amine groups on the matrix surface were modified with glutaraldehyde to form the Schiff base. The Schiff base was reduced using sodium cyanoborohydride because of its unstable structure. Then, to magnetize the modified florisil support material and increase its surface area, it was treated with the nano Fe_3O_4 compounds weighing three times as much as florisil.

The number of the studies investigating the immobilization of *Lactobacillus plantarum* onto magnetite nano florisil is

limited. The studies found in the literature have reported that the most suitable pH and temperature values for the attachment of the microorganisms to the activated magnetite florisil via an intermediate glutaraldehyde arm were 5.0-6.0 and 50-65°C respectively. In the study, the trials were carried out at 35°C considering the optimum growth temperature of *L. plantarum*. Using the spread plate method, the attachment rate of the microorganism to the magnetite florisil nanoparticle support material was determined to be around 50%[26].

To determine the optimum pH values for the chitinase and mannanase produced by *L. Plantarum*, tests were performed at pH values between 2.0 and 11.0 and optimum pH values were determined to be between 5.0 and 6.5[23,31]. Duruksu et al[10] have cloned the extracellular endo-1,4-β-mannanase gene of *Aspergillus fumigatus* and transferred it to *Aspergillus sojae* (AsT1) and *Pichia pastoris* (PpT1) GS115. High levels of mannanase production were achieved in both expression systems and the optimum pH values were determined to be around 4.5 and 5.2-5.6, respectively. Chandra et al[6] reported that the mannanase of *Paenibacillus sp*. had optimum activity at pH 5.0. Zamudio et al[31] isolated microbial phytases suitable for use in food fermentations from natural lactic acid bacteria of vegetable origin and reported that the optimum pH values for the phytases were between 5-6.5.

Table 2
Changes in phytase enzyme produced by free *L. plantarum* depending on time and organic feed content

Wheat Amount	Corn Amount	0 (h)	6 (h)	12 (h)	24 (h)	30 (h)	36 (h)	48 (h)	54 (h)	60 (h)	72 (h)	78 (h)	84 (h)	96 (h)	102 (h)	108 (h)	Average
10	**10**	21,65	21,40	29,00	19,65	18,90	20,00	23,32	20,52	21,60	21,85	22,46	27,55	23,43	24,30	23,75	22,63
	20	24,50	20,00	25,00	21,60	20,52	20,90	22,90	17,50	23,75	23,90	23,75	25,38	25,70	24,85	25,38	23,04
	30	25,95	25,90	30,50	35,65	26,50	20,50	23,80	21,50	21,08	21,40	22,70	27,23	23,56	23,32	22,70	24,82
	40	26,50	24,20	28,62	26,50	20,53	20,55	22,70	20,00	23,75	24,20	23,80	23,77	25,72	24,86	25,40	24,07
	Average	24,65	22,88	28,28	25,85	21,61	20,49	23,18	19,88	22,55	22,84	23,18	25,98	24,60	24,33	24,31	23,64
15	**10**	20,25	21,60	26,00	19,50	25,90	19,00	24,30	23,55	22,70	22,68	27,00	24,84	25,95	25,90	27,00	23,74
	20	27,00	23,50	22,75	20,55	22,25	20,55	27,00	23,75	22,00	24,85	24,20	24,30	26,00	23,75	25,25	23,85
	30	29,00	25,92	29,70	30,25	29,20	19,00	23,20	23,70	22,70	22,70	26,00	24,32	26,00	26,20	27,00	25,66
	40	27,55	25,50	28,00	28,32	22,16	20,50	27,25	23,55	22,00	24,75	24,21	24,32	25,94	23,78	25,90	24,92
	Average	25,95	24,13	26,61	24,66	24,88	19,76	25,44	23,64	22,35	23,75	25,35	24,45	25,97	24,91	26,29	24,54
20	**10**	22,50	20,00	25,50	21,25	24,30	20,00	26,50	23,32	22,68	23,75	22,70	30,78	20,15	24,85	25,67	23,60
	20	24,85	22,75	22,75	20,50	21,80	21,60	28,80	21,60	22,15	24,88	25,15	28,10	24,85	27,25	26,00	24,20
	30	29,20	23,76	28,00	27,65	35,70	20,00	26,50	23,25	23,00	24,32	22,70	30,80	29,20	24,86	27,00	26,40
	40	22,70	23,80	27,00	26,00	21,83	22,70	28,65	22,50	22,15	25,00	24,85	27,25	24,86	28,10	25,92	24,89
	Average	24,81	22,58	25,81	23,85	25,91	21,08	27,61	22,67	22,50	24,49	23,85	29,23	24,77	26,27	26,15	24,77
Wheat average	**10**	21,47	21,00	26,83	20,13	23,03	19,67	24,71	22,46	22,33	22,76	24,05	27,72	23,18	25,02	25,47	23,32
	20	25,45	22,08	23,50	20,88	21,52	21,02	26,23	20,95	22,63	24,54	24,37	25,93	25,52	25,28	25,54	23,70
	30	28,05	25,19	29,40	31,18	30,47	19,83	24,50	22,82	22,26	22,81	23,80	27,45	26,25	24,79	25,57	25,62
	40	25,58	24,50	27,87	26,94	21,51	21,25	26,20	22,02	22,63	24,65	24,29	25,11	25,51	25,58	25,74	24,63
AVERAGE		25,14	23,19	26,90	24,79	24,13	20,44	25,41	22,06	22,46	23,69	24,13	26,55	25,11	25,17	25,58	24,32

F VALUE			LSD (0.05)		
	Wheat amount (W)	62.70**		**Wheat amount (W)**	0,2
	Corn amount (C)	139.54**		**Corn amount (C)**	0,24
	Time(T)	105.29**		**Time(T)**	0,47
	W X C	2.13*		**W X C**	0,42
	W x T	19.45**		**W x T**	0,81
	C X T	34.08**		**C X T**	0,94
	WX C X T	6.54**		**WX C X T**	1,63.

Table 3
Changes in phytase enzyme produced by immobilized *L. plantarum* depending on time and organic feed content

Wheat Amount	Corn Amount	0 (h)	6 (h)	12 (h)	24 (h)	30 (h)	36 (h)	48 (h)	54 (h)	60 (h)	72 (h)	78 (h)	84 (h)	96 (h)	102 (h)	108 (h)	Average
10	**10**	23,90	26,26	27,35	22,70	29,60	24,00	26,25	27,35	22,20	29,60	31,90	29,00	27,45	28,20	27,60	26,89
	20	27,45	26,00	26,20	25,00	31,70	27,50	26,00	26,20	25,40	31,50	28,00	29,75	25,40	24,35	27,00	27,16
	30	32,20	25,30	26,80	27,35	30,60	32,20	24,50	26,80	27,35	30,55	29,00	29,32	27,50	28,30	31,25	28,60
	40	26,00	25,00	28,40	32,10	30,00	26,00	25,00	28,45	32,10	30,00	28,75	29,00	26,80	28,75	28,10	28,30
	Average	27,39	25,64	27,19	26,79	30,48	27,43	25,44	27,20	26,76	30,41	29,41	29,27	26,79	27,40	28,49	27,74
15	**10**	26,16	26,90	27,70	24,25	28,50	26,20	27,00	27,60	24,30	28,50	28,45	29,80	28,00	29,00	28,10	27,36
	20	26,91	28,00	26,20	27,11	29,75	27,00	28,00	26,20	26,60	29,70	28,75	30,00	27,60	27,40	29,20	27,89
	30	27,25	24,64	28,50	27,50	32,50	27,25	25,00	28,22	27,45	32,45	29,25	30,50	29,50	30,80	28,65	28,63
	40	31,35	24,86	28,30	28,00	26,60	31,45	25,00	27,63	28,00	26,60	28,10	27,00	26,60	28,10	27,00	27,64
	Average	27,92	26,10	27,68	26,72	29,34	27,98	26,25	27,41	26,59	29,31	28,64	29,33	27,93	28,83	28,24	27,88
20	**10**	29,94	25,00	27,00	27,45	29,85	30,00	25,00	27,10	27,45	29,80	27,00	29,75	26,60	30,00	27,00	27,93
	20	28,53	25,00	28,23	27,35	27,00	28,50	26,20	28,25	27,80	27,00	29,00	31,00	25,50	29,40	30,25	27,93
	30	28,75	24,86	27,75	28,25	29,50	28,75	25,00	27,80	28,35	29,50	30,00	30,25	30,00	26,50	27,35	28,17
	40	25,50	25,75	26,48	29,25	27,35	25,25	26,00	26,50	29,00	27,35	27,90	25,70	25,75	28,50	27,70	26,93
	Average	28,18	25,15	27,37	28,08	28,43	28,13	25,55	27,41	28,15	28,41	28,48	29,18	26,96	28,60	28,08	27,74
Wheat Average	**10**	26,67	26,05	27,35	24,80	29,32	26,73	26,08	27,35	24,65	29,30	29,12	29,52	27,35	29,07	27,57	27,39
	20	27,63	26,33	26,88	26,49	29,48	27,67	26,73	26,88	26,60	29,40	28,58	30,25	26,17	27,05	28,82	27,66
	30	29,40	24,93	27,68	27,70	30,87	29,40	24,83	27,61	27,72	30,83	29,42	30,02	29,00	28,53	29,08	28,47
	40	27,62	25,20	27,73	29,78	27,98	27,57	25,33	27,53	29,70	27,98	28,25	27,23	26,38	28,45	27,60	27,62
AVERAGE		27,83	25,63	27,41	27,19	29,41	27,84	25,75	27,34	27,17	29,38	28,84	29,26	27,23	28,28	28,27	27,79

F VALUE			LSD (0.05)		
	Wheat amount (W)	1,18ns		**Wheat amount (W)**	-
	Corn amount (C)	29,04**		**Corn amount (C)**	0,24
	Time(T)	48,06**		**Time(T)**	0,47
	W X C	48,06**		**W X C**	0,42
	W x T	14,11**		**W x T**	0,81
	C X T	10,98**		**C X T**	0,94
	WX C X T	7,92**		**WX C X T**	1,62

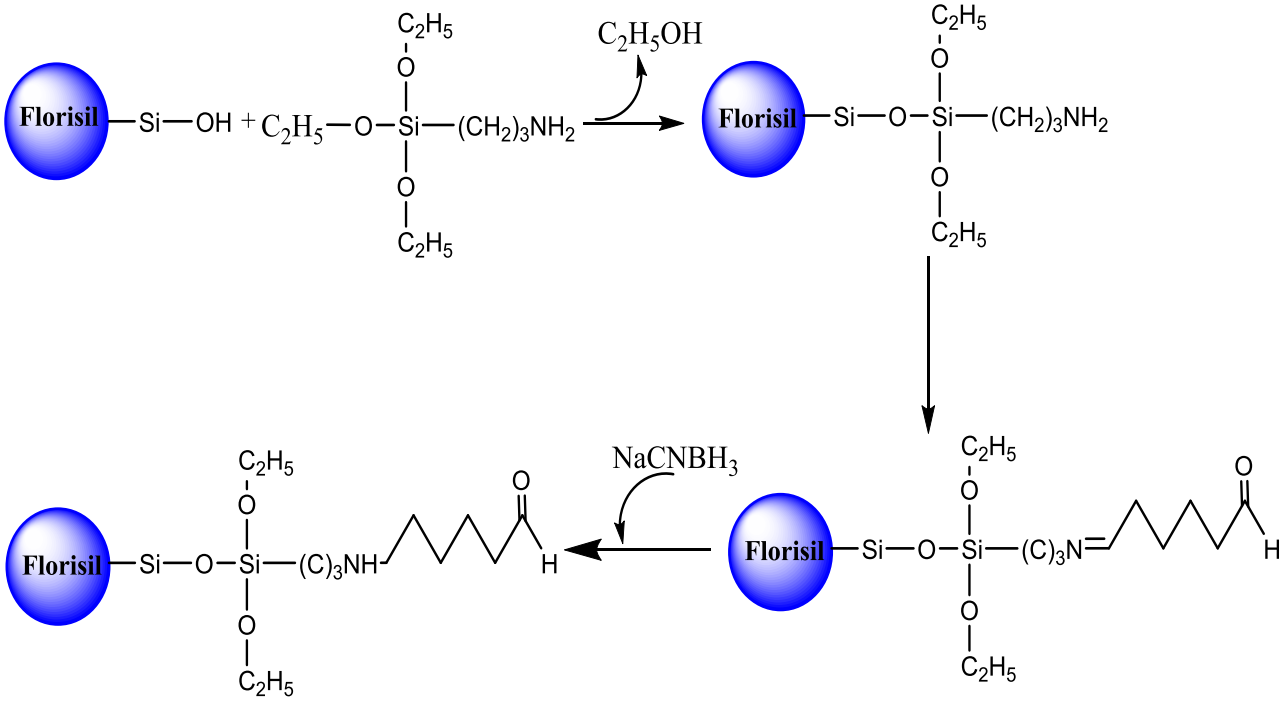

Figure 1: The activation of the florisil support material with 3-APTES

Table 4
Changes in mannanase enzyme produced by free *L. plantarum* depending on time and organic feed content

Wheat Amount	Corn Amount	0	6	12	24	30	36	48	54	60	72	78	84	96	102	108	Average
10	**10**	75,00	56,25	78,75	76,50	80,00	68,75	77,25	77,25	72,50	80,00	59,50	95,00	87,00	85,00	84,50	76,88
	20	85,00	77,50	60,00	59,83	62,00	60,25	62,50	61,25	58,76	67,50	52,50	77,50	78,00	68,75	65,75	68,21
	30	110,00	105,00	96,25	94,50	95,00	91,25	85,00	82,17	87,50	90,00	80,00	75,25	85,00	90,25	90,00	90,48
	40	90,00	85,00	83,00	75,00	81,25	77,50	68,75	63,50	67,50	78,75	62,50	65,00	65,50	65,00	73,75	73,47
	Average	90,00	80,94	79,50	76,46	79,56	74,44	73,38	71,04	71,57	79,06	63,63	78,19	78,88	77,25	78,50	75,95
15	**10**	73,75	52,50	71,25	70,00	72,50	65,00	71,50	72,25	63,75	77,50	58,25	97,50	87,00	87,50	85,00	73,68
	20	55,00	50,00	59,50	58,75	60,00	58,75	60,25	63,25	60,25	63,75	53,25	78,75	80,50	62,75	61,25	61,73
	30	107,50	102,50	94,50	92,50	91,25	87,50	81,25	77,50	90,25	85,92	75,00	74,75	78,75	80,50	85,00	86,98
	40	88,75	83,75	82,50	73,75	81,25	75,00	67,50	61,25	68,75	72,50	61,25	63,25	64,50	62,25	75,00	72,08
	Average	81,25	72,19	76,94	73,75	76,25	71,56	70,13	68,56	70,75	74,92	61,94	78,56	77,69	73,25	76,56	73,62
20	**10**	62,50	52,25	64,50	60,00	62,50	60,50	66,25	69,50	62,50	77,00	57,00	87,00	87,50	82,50	80,25	68,78
	20	51,25	53,75	56,25	53,75	57,00	52,25	56,00	61,75	55,00	61,25	52,00	76,50	76,25	61,25	60,00	58,95
	30	107,50	92,50	85,00	81,25	87,50	85,00	76,25	74,50	70,00	80,00	67,50	72,50	71,75	75,00	77,50	79,42
	40	82,50	81,00	76,50	70,00	77,50	74,50	66,25	60,00	67,50	66,25	56,25	57,50	62,50	61,00	67,50	68,45
	Average	72,81	69,88	70,56	66,25	71,13	68,06	66,19	66,44	63,75	71,13	58,19	73,38	74,50	69,94	71,31	68,90
Wheat Average	**10**	70,42	53,67	71,50	68,83	71,67	64,75	71,67	73,00	66,25	78,17	58,25	93,17	87,17	85,00	83,25	73,12
	20	69,06	64,69	58,58	57,44	59,67	57,08	59,58	62,08	58,00	64,17	52,58	77,58	78,25	64,25	62,33	63,19
	30	103,75	100,00	91,92	89,42	91,25	87,92	80,83	78,06	82,58	85,31	74,17	74,17	78,50	81,92	84,17	85.60
	40	87,08	83,25	80,67	72,92	80,00	75,67	67,50	61,58	67,92	72,50	60,00	61,92	64,17	62,75	72,08	71,33
AVERAGE		82,58	75,40	75,67	73,26	75,65	71,35	69,90	68,68	68,69	75,03	61,25	76,71	77,02	73,48	75,46	73,34

F VALUE	**Wheat amount (W)**	122.55**	**LSD (0.05)**	**Wheat amount (W)**	1,01.
	Corn amount (C)	497.79**		**Corn amount (C)**	1,17
	Time(T)	33.36**		**Time(T)**	2,27
	W X C	4.59**		**W X C**	2,03
	W x T	2.08**		**W x T**	3,92
	C X T	27.77**		**C X T**	4,53
	WX C X T	1.77**		**WX C X T**	7,85

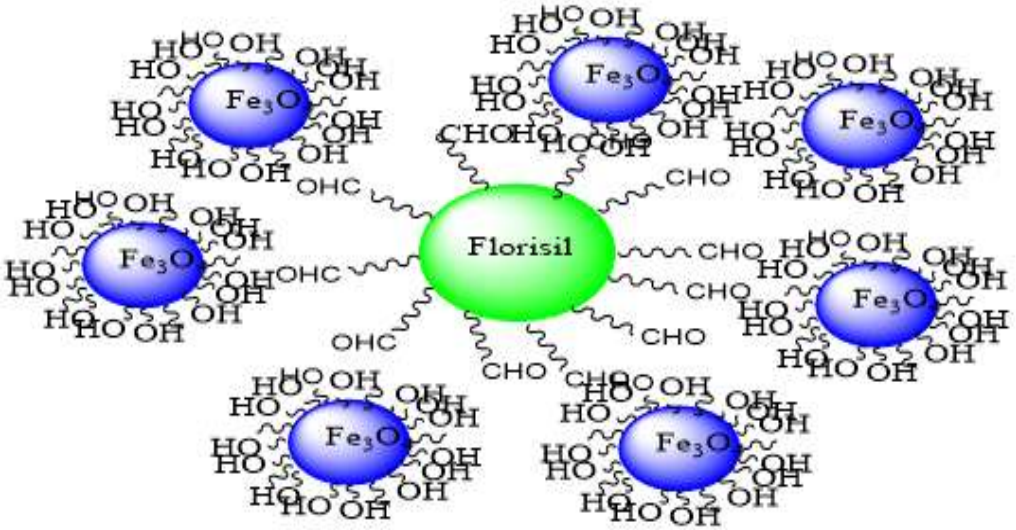

Figure 2: The coating of the activated florisil support with nano Fe_3O_4

Table 5
Changes in mannanase enzyme produced by immobilized *L. plantarum* depending on time and organic feed content

Wheat Amount	Corn Amount	0	6	12	24	30	36	48	54	60	72	78	84	96	102	108	Average
10	10	83,25	60,50	72,50	69,25	76,25	91,50	86,00	83,25	76,00	96,50	94,50	74,25	81,00	78,25	75,50	79,90
	20	75,63	63,00	73,25	68,25	82,25	91,75	89,25	69,50	76,75	95,00	94,25	70,50	83,25	80,25	78,75	78,25
	30	74,00	60,00	67,00	61,25	65,50	81,00	74,00	61,75	68,00	82,50	80,25	78,75	79,00	65,25	75,00	71,55
	40	75,50	61,50	64,75	59,75	61,50	76,25	74,50	64,75	68,00	86,25	72,75	68,75	65,50	64,75	62,75	68,48
	Average	77,09	61,25	69,38	64,63	71,38	85,13	80,94	69,81	72,19	90,06	85,44	73,06	77,19	72,13	73,00	74,92
15	10	100,50	64,75	73,75	74,00	80,75	96,50	90,00	81,25	72,50	99,25	101,25	84,25	85,00	80,75	78,50	84,20
	20	79,50	64,25	70,75	66,50	77,50	84,50	83,75	68,00	69,50	93,75	90,25	68,50	82,75	78,25	75,00	76,85
	30	71,25	61,50	67,50	62,75	66,50	81,00	76,75	64,75	74,00	80,00	82,75	87,00	81,25	82,75	62,75	73,50
	40	77,25	60,75	66,75	61,50	60,25	77,00	75,55	64,50	69,50	77,75	75,50	72,50	68,75	65,00	63,75	69,09
	Average	82,13	62,81	69,69	66,19	71,25	84,75	81,51	69,63	71,38	87,69	87,44	78,06	79,44	76,69	70,00	75,91
20	10	77,00	64,25	75,25	76,25	80,75	97,25	90,25	78,25	76,75	102,25	98,75	75,25	85,00	83,50	80,00	82,72
	20	72,50	65,00	67,25	64,00	68,50	85,00	74,00	66,25	70,50	92,50	90,00	60,00	81,00	78,75	76,25	74,10
	30	78,75	62,00	67,00	59,50	65,75	77,50	77,50	64,50	70,50	81,25	80,00	84,75	76,75	71,25	62,00	71,93
	40	76,75	57,50	62,25	59,50	59,00	71,50	69,50	59,00	67,75	77,50	74,75	70,25	65,75	63,25	61,75	66,40
	Average	76,25	62,19	67,94	64,81	68,50	82,81	77,81	67,00	71,38	88,38	85,88	72,56	77,13	74,19	70,00	73,79
Wheat Average	10	86,92	63,17	73,83	73,17	79,25	95,08	88,75	80,92	75,08	99,33	98,17	77,92	83,67	80,83	78,00	82,27
	20	75,81	63,81	70,42	66,25	76,08	87,08	82,33	67,92	72,25	93,75	91,50	66,33	82,33	79,08	76,67	76,48
	30	75,00	61,75	67,17	61,17	65,92	79,83	76,08	63,67	70,83	81,25	81,00	83,50	79,00	73,08	66,58	72,58
	40	76,50	59,92	64,58	60,25	60,25	74,92	73,18	62,75	68,42	80,50	74,33	70,50	66,67	64,33	62,75	67,99
AVERAGE		78,56	62,16	69,00	65,21	70,38	84,23	80,09	68,81	71,65	88,71	86,25	74,56	77,92	74,33	71,00	74,87

F VALUE			LSD (0.05)		
	Wheat amount (W)	170.74**		Wheat amount (W)	0.23
	Corn amount (C)	4254.24**		Corn amount (C)	0,26
	Time(T)	1783.82**		Time(T)	0.51
	W X C	136.37**		W X C	0,45
	W x T	20.96**		W x T	0,87
	C X T	129.07**		C X T	1,01
	WX C X T	26.13**		WX C X T	1,75

Sarıbuğa[23] isolated phytase from *Lactobacillus sp*. strains and performed its characterization. The maximum activity levels of the enzymes obtained from *Lactobacillus plantarum* and *Lactobacillus acidophilus* were determined to be 167.3 EU/mL at pH 6.0 and 163.1 EU/mL at pH 5.0. The results have shown that *Lactobacillus plantarum* and *Lactobacillus acidophilus* were suitable for industrial use because of their characteristic properties. The results obtained in this study agree with the results obtained in other studies found in the literature.

In the samples collected from the reaction media containing corn (10g, 20g, 30g and 40gr) and wheat (10g, 15g and 20gr) and both free *L. plantarum* isolates and isolates immobilized onto the magnetite florisil nanoparticles, the time-dependent changes in the production rates of the phytase and mannanase enzymes were monitored. Table 2,3,4 and 5 show the activity levels of phytase and mannanase determined by the measurements performed for 5 days at 6-day intervals.

The results showed that the activity levels of the enzymes generally increased with increasing bacteria count and reached their highest levels at a certain point, then decreased as a result of the culture conditions. As the duration of the experiment was prolonged, the production of mannanase and phytase decreased. This is attributable to the acidic nature of the *L. plantarum* bacteria, which results in a decrease in the pH of the medium. The comparison of the pH levels at the beginning and end of the experiment revealed that the initial pH levels of 6.0-6.5 reached 4.0-4.5 at the end of the experiment. This acidic change in the medium was associated with the decrease in the enzyme production.

According to the results (Table 2), the highest phytase production from free *L. plantarum* bacteria was 35.65 EU/mL and measured at the 24th hour in the medium containing 30 g corn and 10 g. The highest production obtained from the bacteria immobilized onto the nanoparticle was 32.50 EU/mL and measured at the 30th hour in the medium containing 30 gr corn and 15 gr wheat (Table 3). The results revealed that the activity of the phytase obtained from the free *L. plantarum* bacteria was higher than that of the phytase obtained from the immobilized bacteria.

Phytase was first obtained and immobilized from *A. niger*[27]. Konietzny and Greiner[14] determined the K_m values of the immobilized and free phytase using the same substrate and

reported that the K_M value of the immobilized phytase was higher than that of the free phytase. In their study, Mckelvie et al[16] used immobilized enzymes and determined that immobilized phytase had relatively higher activity and resistance.

The highest mannanase production was obtained in the medium containing 30 g corn and 10 gr wheat and determined to be 110.00 EU/mL at the first measurement (Table 4). In the case of immobilization onto the nanoparticle, the highest production was 102.25 EU/mL and measured at the 72nd hour in the medium containing 10 g corn and 20 g wheat (Table 5). In their study, El-Naggar et al[11] investigated β-mannanase production via the adsorption of the local *Aspergillus niger* strain isolated from coconut fibers onto sponge cubes, luffa pulp, pumice particles, clay particles and ceramic pieces and reported that compared with the free cultures, the optimized medium (adsorption onto pumice particles) showed a relatively higher β-mannanase activity and the activity of β-mannanase was determined to reach 90.87 U / mL, which was approximately 2.3-fold of the yield determined for the basal medium.

As seen in the table, the partial decline of the phytase and mannanase obtained from the immobilized bacteria was mainly due to an immobilization rate of about 50%. Considering the 50% covalent bonding of the bacteria to the support material, the desired enzyme activity was reached using lower amounts of bacteria. Thanks to the attachment, the isolates have become stable and enzyme production have increased. Judging from the high phytase and mannanase activity obtained in the study, it was concluded that *L. plantarum* successfully utilized the natural nutrients (corn and wheat) added into the medium as carbon sources. The investigation of the effect of different carbon sources on the production of bacterial phytase and mannanase showed that compared with the standard medium, bacterial growth was higher and the enzyme activity was increased by 10-15%.

Conclusion

The results revealed that the *L. plantarum* bacteria immobilized onto the magnetite florisil nanoparticle efficiently utilized the carbon sources. In the future, the bacteria can be designed as a preparation and offered to the service of various industrials fields (pharmacology, food, etc.) as a starter culture and used safely. Furthermore, we are of the opinion that maximum yields can be achieved with minimal costs.

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