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Prolonging the Shelf-life of Probiotic Wheat Germ Containing *Bacillus badius* with Xanthan Gum by Freeze-Dried Encapsulation Method

Kadkhodaei, A. [1], Goli, M. [1, 2*], Ramezani, M. [3]

1. Department of Food Science and Technology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.
2. Laser and Biophotonic in Biotechnologies Research Center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.
3. Microorganisms Bank, Iranian Biological Resource Centre (IBRC), ACECR, Tehran, Iran.

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*Corresponding Author E-Mail:
mgolifood@yahoo.com

ABSTRACT

Wheat germ is a high-nutrient byproduct of wheat milling, however it has a short shelf life and extremely restricted ideal intake due to the intensive activities of lipase and lipoxygenase. In order to increase the storage life of probiotic wheat germ containing Bacillus badius, the freeze drying method was used in this study, and the effect of using xanthan gum: maltoxtrin in different ratios of 0.3:1, 0.1:1, and 0.03:1 as the capsule wall on antioxidant properties and physicochemical characteristics of probiotic wheat germ during 360 days of storage was investigated. Three control treatments were also produced without probiotic bacteria and had the same quantities of gums as the treatments. A pure wheat germ sample was also analyzed on the first day, 180 and 360, along with the encapsulated samples, for better comparisons and the influence of applied treatments. The experiments used a completely random design, and the treatments were analyzed using SPSS software and comparing the averages using Duncan's multi-range test at the 99% confidence level. The use of xanthan gum and maltodextrin as wall materials increased the antioxidant qualities in encapsulated wheat germ, according to the findings of this study. The introduction of Bacillus badius bacteria as a probiotic resulted in a substantial decrease in oxidation indices in wheat germ ($P<0.01$). In general, it can be stated that Bacillus badius bacteria, as a powerful probiotic, can extend the storage time of encapsulated wheat germ. Furthermore, xanthan gum is proposed as a good material for wheat germ encapsulation to enhance shelf life.

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1. Introduction

One of the limitations of using wheat germ is its very short shelf life (a few days to a few weeks) [1]. Wheat germ contains high amounts of saturated fat and hydrolytic and oxidative lipase enzymes, which requires a method to inactivate its enzymes, such as lipases, to prevent lipid oxidation [2]. Contrary to the high nutritional value of wheat germ, it has a limited use for human consumption and its most use is to provide animal feed. For this reason, many efforts have been made to increase its durability in order to provide acceptable products for human consumption [3]. Some of them include inactivating enzymes under thermal cooking, microwave and extrusion treatments, packaging in three-layer composite packages with polyethylene coating, using sourdough biotechnology, using antioxidants, and separating the oil part from wheat germ. Despite the proven effects of the previous methods to stabilize wheat germ, these treatments are in some cases very expensive, not completely soluble, reduce the nutritional value of wheat germ, and the use of antioxidants also poses a risk to the health of the consumer with suspicion and There is doubt [4]. Encapsulation is an effective approach that can increase the chemical/biological stability of sensitive compounds and also protect them from inevitable reactions in food systems [5-7]. Kind*Bacillus badius* One of the oldest members of the genus Bacillus, first isolated in 1919. The optimal temperature for this bacterium is around 37 degrees Celsius and it grows weakly at 28 and 50 degrees Celsius. Bacillus species are gram-positive bacteria that are spindle-shaped and sposa. They often have a chain arrangement and are optional aerobic or anaerobic. This genus is widely distributed in nature and can be found in soil, water, plant surface and in animal body]8[. Bacillus species are used in many medical, pharmaceutical, agricultural and industrial processes due to their wide range of physiological characteristics and ability to produce enzymes, antibiotics and metabolites. Various species produce nutrients such as vitamins (eg, riboflavin, cobalamin, and inositol) and carotenoids and are used to synthesize several health supplements for human consumption.[9 and 10]. In addition, Bacillus spores have a long history of consumption and safe use as probiotics]11[.
Based on this, the purpose of this research was to increase the shelf life of probiotic wheat germ due to the effect of antioxidant properties of probiotics and by using the encapsulation technique by freeze-drying method by xanthan gum-maltodgestrin.

2- Materials and methods

2-1- Materials

Consumable raw materials in the production of wheat germ probiotic capsules include wheat germ (Kalil Javane Company, Iran), xanthan gum and maltodextrin (Sigma Company, USA), Badius probiotic bacteria (Genetic Reserve Center, Iran) and YPG agar and TSA (Merck, Germany) and chemical materials were prepared for chemical analysis of oxidation tests (Merck, Germany).

2-2-plan implementation method

In this research, the microcapsule of probiotic wheat germ using bacteria*Bacillus badius* It was done by freeze drying method. Various proportions of xanthan gum and maltodextrin were used as the walls of the capsules for microcoating according to Table 1. Three control treatments, containing the same proportions of gums used for the treatments, without probiotic bacteria were also prepared. For better comparisons and the effect of applied treatments, a pure wheat germ sample was also examined and tested along with the encapsulated samples. Tests for peroxide, anisidine, totox, acidity, thiobarbituric acid and total count of probiotic bacteria were performed on the samples in rounds on the 0th, 180th and 360th days.

Table 1 Mixing ratio of the Gums used for the walls of the capsules

Treatments	Xanthan	Maltodexterin
0.3 : 1	0.3	1
0.1 :1	0.1	1
0.030 : 1	0.03	1

2-3-microcoating of probiotic wheat germ containing*Bacillus badius*

The amount of 500 grams of each treatment was consumed for each period of time on day 0, 180 and 360 of the maintenance period of the treatment. First, for each treatment, 125 grams of wheat germ was mixed with 115 grams of water, and then 35 grams of bacteria *Bacillus badius* (cfu/gr $1/4 \times 10^8$1.4 × 10^8) was added to it next to the flame. Then, in another part, using a mixer, 300 grams of distilled water was mixed with 125 grams of xanthan gum:

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maltodextrin based on the ratios mentioned in Table 1, and the container containing wheat germ,*Bacillus badius* Distilled water was added to this mixture and divided into 6 plates. In order to reach the appropriate temperature for entering the samples into the freeze dryer, the prepared emulsion was kept in the freezer at -40 degrees Celsius for 24 hours and then transferred to the freeze dryer. In order to prepare the control sample, all the mentioned steps were done except for the addition of bacteria.

2-4-oil extraction

Before conducting the oxidation tests, at first the produced samples were powdered and homogenized using an electric mill. Then, 450 grams of the powdered sample was weighed in a 1000 ml beaker and almost twice the weight of the sample in the amount of 850 ml of petroleum ether was added to it as a solvent. The lids of the beakers were covered with aluminum foil and 48 hours were given for the oil to leave the sample. After the given time, it was passed through the filter paper and the filtered mixed petroleum ether was separated using a rotary machine with a temperature of 40 degrees Celsius and a revolution of 76, and the remaining oil was obtained to perform the tests.

5-2-Experiments performed on extracted oil

In order to extract oil, the produced samples were powdered using an electric mill. Each sample was weighed to a certain amount and 600 milliliters of petroleum ether was added to it. The lid of the besars was covered with aluminum foil. After 24 hours, the extracted oil from the sample was passed through a strainer, and the refined mixed petroleum ether was separated from the oil using a rotary machine, and the oil was obtained for testing.

2-5-1-peroxide test

The peroxide value was obtained by iodometric method. For this purpose, 2 grams of extracted oil along with 30 milliliters of acetic acid-chloroform (1:1) and 0.5 milliliters of saturated potassium iodide were poured into an Erlenmeyer flask and placed in the dark for 2 minutes. Then 30 ml of distilled water and 0.5 ml of starch glue were added. The resulting mixture was titrated using 0.1 normal sodium thiosulfate until colorless and the amount of thiosulfate used was recorded. For example, all steps were performed without primary oil [12]. In this relation, S is the amount of sodium thiosulfate used for oil sample titration, B is the amount of sodium thiosulfate used for control titration, N is the normality of sodium sulfate, and W is the oil weight in grams.

$$\text{Peroxide number} = \frac{(S-B)\times N\times 1000}{W}$$

2-5-2-anisidine number

3 grams of the extracted oil was weighed in a flask and it was brought to a volume of 25 milliliters by hexane, and its absorbance was read at a wavelength of 350 nm.$A_bA_bA_b$). 5 ml of solutionA_bA_b was removed and 1 milliliter of anisidine reagent (0.25 grams of para-anisidine, which has been adjusted to 100 with acetic acid) was added to it. After 10 minutes in the dark, the absorbance was read at 350 nm ($A_sA_bA_s$)]13[. $A_S\ A_bA_s$ The amount of fat absorption after reaction with anisidine,A_bA_b A_bAbsorption of fat solution, W is the weight of the sample, V is the volume in which the sample was dissolved in milliliters, and 1.2 is the correction factor for diluting the sample solution with one milliliter of anisidine reagent.

$$\textbf{Anisidine} = \frac{v\times(1.2\times A_S-A_b)}{w}\ \frac{v\times(1.2\times A_S-A_b)}{w}$$

2-5-3-Number of Tutox

Totox number was calculated from the sum of twice the number of peroxide plus the number of anisidine [12].

2-5-4-acidity number

1 gram of sample oil was poured into an Erlenmeyer along with 30 milliliters of ethanol and 1 milliliter of 0.01 phenolphthalein, and the titration was done using 0.1 normal sodium until the purple color was reached [14].

Acidity =2.82×0.1 milliliters of normal consumption/Sample weight

2-5-5-number of thiobarbituric acid

0.2 g of the extracted oil was poured into a 25 ml flask and made up to volume with butanol. 5 ml of it was removed and transferred to a dry laboratory tube. Then 5 ml of thiobarbituric acid solution was added to it and the test tube was kept in a water bath at 95°C. After 2 hours, the tube was removed from the bath and cooled. Finally, its absorbance was read at 530 nm. All steps were also done for the control sample [15].

thiobarbituric acid= (Sample acquisition - witness acquisition) ×50/Sample weight

6-2- General counting

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2-6-1-Preparation of cultivation environments

In order to isolate and count bacteria, yeast and fungi from the samples, it was necessary to prepare the solid isolation media in the first stage. For this purpose, YPG agar and TSA culture mediums were used, the first culture medium was used for the isolation of fungi and yeast and the second culture medium was used for the isolation of bacterioprobiotics in this research. After preparation, the media were autoclaved at a temperature of 121 degrees Celsius for 15 minutes and at a pressure of two atmospheres and distributed in 10 cm plates [16].

2-6-2-Preparation of the delivered biological sample

1 gram of each sample was added to a capped tube containing 9 milliliters of physiological serum. This tube, which contained the original sample, was placed at room temperature for one hour so that the bacteria entered the serum from the surface of the sample. During this period, the vortex was performed several times. Then 1 ml of the tube containing the original sample was transferred to another tube containing 9 ml of physiological serum and vortexed. This process until the preparation of the dilution^{5-}10 was done. In the next step, 100 µl of dilutions^{1-}10f $^{2-}$10. In order to evaluate and count fungi and yeasts, they were spread on YPG agar medium plates in the form of porplates and heated at 25 degrees Celsius for one week. Also, 100 µl of dilutions^{4-}10 and^{5-}10. In order to evaluate and count the probiotic bacteria, it was spread on the plates of TSA culture medium in the form of porplates and heated at 35 degrees Celsius for one week. Three replicates of each of the above samples were prepared in three consecutive weeks and evaluated to confirm the results. To count the microorganism in each plate, the following relationship was used:

= count (CFU/ml)
10x photo dilution x number of microorganisms read on the plate

2-7-Statistical analysis

The data obtained from this research were analyzed in the form of a completely random design. Statistical analysis of samples was done by SPSS. Means were compared using Duncan's test at 95% confidence level and graphs were drawn using Excel software.

3-Results

3-1- Investigation of the effect of xanthan gum treatment, probiotic bacteria and storage time on changes in total acidity number and comparison with the control sample

According to the analysis of variance in Table 2, the independent effect of xanthan gum treatment, probiotic bacteria, storage time and the interaction effect of treatment-probiotic-time on changes in total acidity number (mg/kg) were significant ($P<0.01$). Figure 1 shows the independent effect of xanthan gum treatment on acidity changes. According to the form, the acidity values after adding xanthan gum in amounts of 0.03, 0.1 and 0.3% have been significantly reduced compared to the control sample. Figure 2 shows the independent effect of using probiotic bacteria on acidity changes. According to the shape, the acidity values have been significantly reduced after the addition of probiotic bacteria. Figure 3 shows the independent effect of storage time of 180 and 360 days compared to zero day on changes in acidity values. According to the data, the values of this index have decreased significantly in 180 and 360 days compared to zero day. Figure 4 shows the interaction effect of treatment-probiotic-time on changes in total acidity number (mg/kg). According to the figure, the highest and lowest values of acidity number were observed in the treatments of 0% xanthan gum, without probiotics and on day 360 and 0.1% xanthan gum, without probiotics and on day 360. Acidity values in zero percent xanthan treatments without probiotics increased significantly in 180 and 360 days compared to the control treatment (zero percent xanthan, no probiotics, day zero). Also, the acidity values in the treatments containing xanthan gum, with or without probiotic bacteria and at the time of 180 and 360 days compared to the treatments without xanthan gum and probiotic at both time of 180 and 360 days were significantly reduced. Also, the acidity values in the treatment of 0.03% xanthan, with and without probiotics and at the time of 180 days are significantly higher compared to the treatments with the same conditions and at the times of 0 and 360 days. Although there is no significant difference between two treatments containing 0.03% xanthan with and without probiotics in 180

days. The total acidity values in the treatment of 0.1% xanthan, with and without probiotics and time of 0 and 360 days, compared to the treatment of 0.1% xanthan, with probiotic and time of 180 days, have significantly decreased. Total acidity values in 0.3% xanthan treatment, with and without probiotics and 180 days time compared to 0.3% xanthan treatments with and without probiotics and 360 days time have increased significantly.

Table 2 Analysis of variance of changes in total acidity number (mg of linoleic acid/kg of oil extracted from wheat germ), peroxide value (meq of oxygen/kg of oil extracted from wheat germ), anisidine value, thiobarbituric acid value (mg of malondialdehyde/kg of oil extracted from wheat germ), and totox value (meq of oxygen/kg of oil extracted from wheat germ)

	Mean Square				
Source	Total acidity number	Peroxide value	Anisidine value	TBA value	Totox value
Corrected Model	38418^{***}	4.27^{***}	4.25^{***}	19.73^{***}	35.08^{***}
Intercept	2241884^{***}	188.1^{***}	132.16^{***}	19823.46^{***}	1517.21^{***}
Treatment	96369^{***}	12.33^{***}	11.55^{***}	25.82^{***}	108^{***}
Probiotic	593^{ns}	0.33^{*}	0.000^{ns}	0.06^{ns}	1.40^{ns}
Time	57319^{***}	0.44^{**}	7.51^{***}	106.11^{***}	4.94^{***}
Treatment * Probiotic	381^{ns}	0.05^{ns}	0.003^{***}	0.13^{ns}	0.51^{ns}
Treatment * Time	33063^{***}	5.13^{***}	2.85^{ns}	7.63^{***}	35.06^{***}
Probiotic * Time	383^{ns}	0.47^{**}	0.003^{***}	0.40^{**}	0.94^{*}
Treatment * Probiotic * Time	38418^{ns}	0.06^{ns}	0.011^{ns}	0.03^{ns}	0.31^{ns}
Error	212	0.07	0.007^{ns}	0.06	0.22
R Squared	0.986	0.963	0.996	0.992	0.984
Adjusted R Squared	0.981	0.948	0.994	0.989	0.978

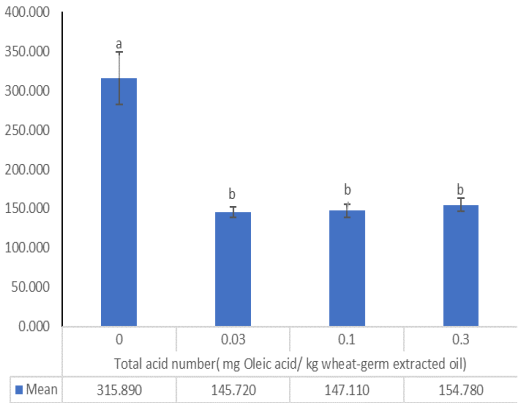

Fig 1 Effect of xanthan gum treatment on total acidity number of probiotic wheat germ oil

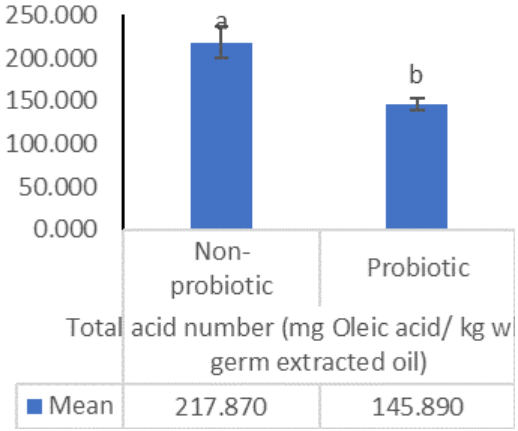

Fig 2 The effect of probiotics on total acidity number of probiotic wheat germ oil

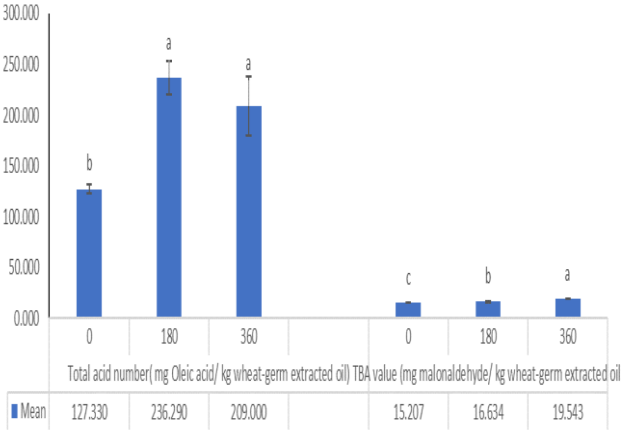

Fig 3 Effect of storage time on total acidity number and thiobarbituric acid value of probiotic wheat germ oil

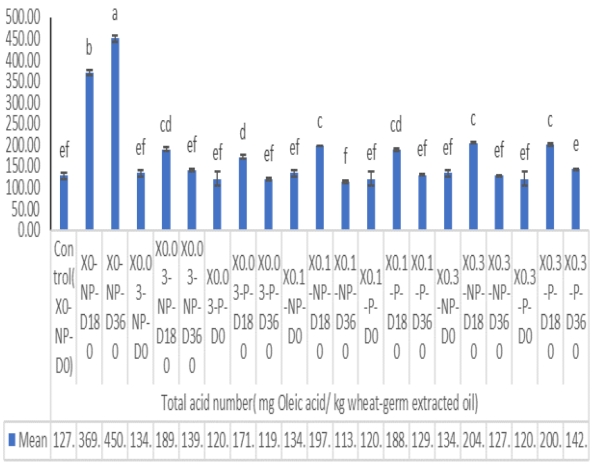

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Fig 4 The interaction effect of treatment-probiotic-time on changes in total acidity number of probiotic wheat germ oil. (X: Xanthan gum; P: Probiotic; NP: Non probiotic; D: day)

3-2- Investigating the effect of xanthan gum treatment, probiotic bacteria and storage time on changes in peroxide number and comparing with the control sample

According to analysis of variance in Table 2, the independent effect of xanthan gum treatment, probiotic and the interaction effect of treatment-probiotic-time on changes in peroxide number (mE/kg) were significant ($P<0.01$). Figure 5 shows the independent effect of xanthan gum treatment on peroxide changes. According to the figure, the amount of peroxide after adding xanthan gum in amounts of 0.03, 0.1 and 0.3% has been significantly reduced compared to the control sample. Figure 6 shows the independent effect of using probiotic bacteria on peroxide changes. According to the figure, the amount of peroxide has been significantly reduced after the addition of probiotic bacteria. Figure 7 shows the interaction effect of treatment-probiotic-time on changes in peroxide value (mE/kg). According to the figure, the highest and lowest peroxide values were observed for the treatment of zero percent xanthan gum, without probiotics, for 360 days and for the treatment of 0.03% xanthan gum, with or without probiotics, for 360 days. The values of peroxide number in zero percent xanthan treatments without probiotics increased significantly in 180 and 360 days compared to the control treatment (zero percent xanthan, no probiotics, day zero). Peroxide number in 0.03% xanthan treatments containing probiotics at 180 and 360 days compared to 0.03% xanthan treatment without probiotics at 180 days and the control treatment was significantly reduced.

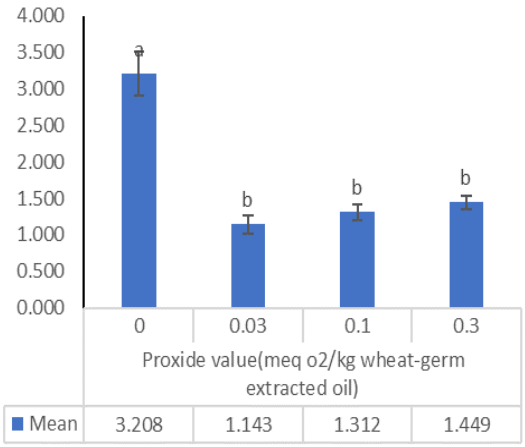

Fig 5 The effect of xanthan gum treatment on the peroxide values of probiotic wheat germ oil

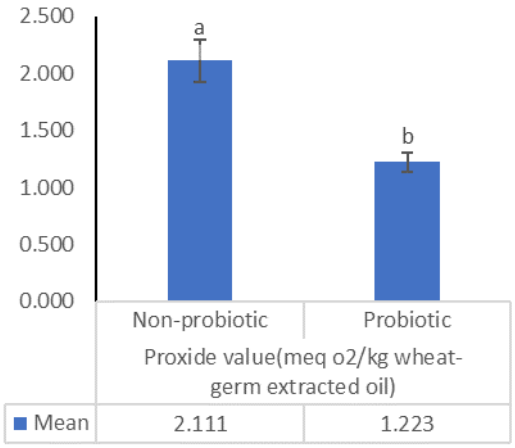

Fig 6 Effect of probiotic bacteria on peroxide values of probiotic wheat germ oil

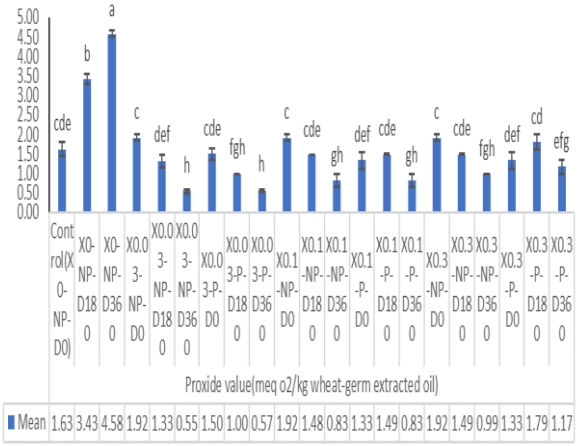

Fig 7 Treatment-probiotic-time interaction effect on the peroxide values of probiotic wheat germ oil. (X: Xanthan gum; P: Probiotic; NP: Non probiotic; D: day)

The values of the above index in the treatment of 0.1 xanthan, with and without probiotics and time of 360 days compared to the same treatments at time of 0 and 180 days, have

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decreased significantly. Also, no significant changes are observed between the 0.1 xanthan treatments, with and without probiotics and 360 days in terms of peroxide value. Peroxide number in 0.3 xanthan treatment, without probiotics and 360 days time compared to 0.3 xanthan treatments, with and without probiotics and 0 and 180 days time has decreased significantly.

3-3- Investigating the effect of xanthan gum treatment, probiotic bacteria and storage time on anisidine number and comparing with the control sample

According to analysis of variance in Table 2, the independent effect of xanthan gum treatment, probiotic, storage time and the interaction effect of treatment-probiotic-time on anisidine changes were significant (P<0.01). Figure 8 shows the independent effect of treatment on anisidine number. According to the shape of anisidine values after adding xanthan gum in amounts of 0.03, 0.1 and 0.3 compared to the control sample without xanthan gum, it decreased significantly. Figure 9 shows the independent effect of probiotic bacteria on anisidine number. According to the shape, the amount of anisidine has decreased significantly after adding probiotic bacteria. Figure 10 shows the independent effect of storage time on anisidine number. This index increased significantly in 180 and 360 days compared to zero time. Figure 11 shows the interaction effect of treatment-probiotic-time on anisidine number. According to the evaluation of the information provided, the zero xanthan treatments without probiotics in 360 days and the 0.3 xanthan treatments without probiotics in 180 days have the highest and lowest anisidine values, respectively. The values of anisidine number in treatments without xanthan and probiotics increased significantly in 180 and 360 days compared to the control treatment. The values of anisidine number in the treatments containing 0.03% xanthan, with and without probiotics increase significantly with increasing storage time. The values of anisidine number in treatments containing 0.1 and 0.3% xanthan, with and without probiotics at 0, 180 and 360 days do not show significant changes in terms of anisidine number values.

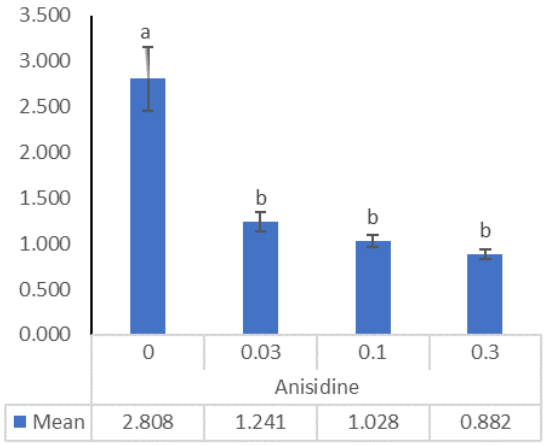

Fig 8 The effect of xanthan gum treatment on the anisidine value of probiotic wheat germ oil

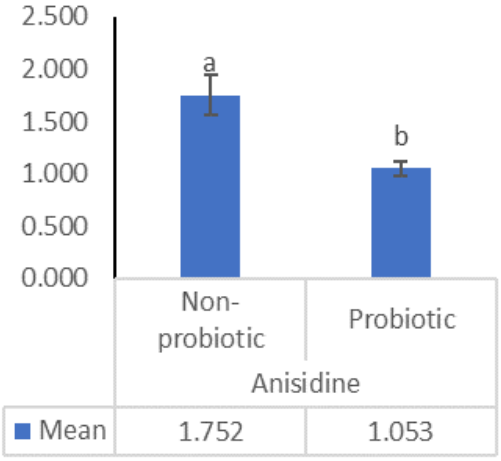

Fig 9 The effect of probiotic bacteria on the anisidine value of probiotic wheat germ oil

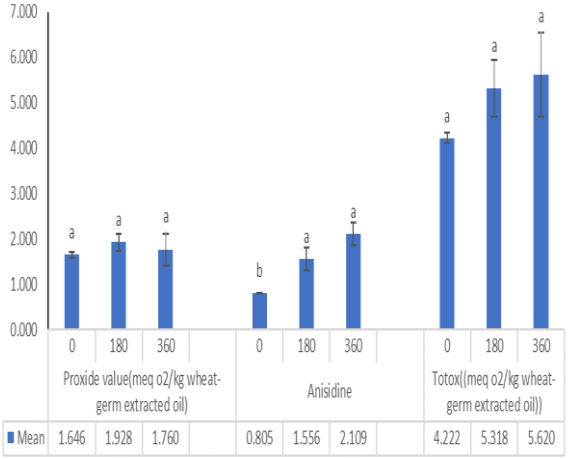

Fig 10 Effect of storage time on the anisidine value of probiotic wheat germ oil

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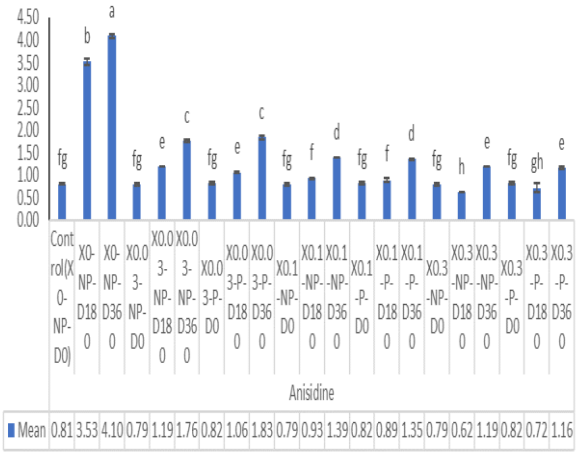

Fig 11 Treatment-probiotic-time interaction effect on the anisidine value of probiotic wheat germ oil. (X: Xanthan gum; P: Probiotic; NP: Non probiotic; D: day)

3-4- Investigating the effect of xanthan gum treatment, probiotic bacteria and storage time on thiobarbituric acid number and comparing with the control sample

According to the analysis of variance in Table 2, the independent effect of xanthan gum treatment, storage time and the interaction effect of treatment-probiotic-time on the changes of thiobarbituric acid number (mg/kg) were significant (P<0.01). Figure 12 shows the independent effect of xanthan gum treatment on changes in thiobarbituric acid number (mg/kg). According to the shape of the values of this index after adding xanthan gum in values of 0.03, 0.1 and 0.3 compared to the control sample without xanthan gum, it decreased significantly. Figure 3 shows the independent effect of storage time on changes in thiobarbituric acid number (mg/kg). The results show that the values of this index increase significantly with the increase of storage days. So that its highest values correspond to the time of 360 days and then 180 days respectively. Figure 13 shows the interaction effect of treatment-probiotic-time on the changes of thiobarbituric acid number (mg/kg). According to the chart, zero xanthan treatments without probiotics in 360 days and 0.3 xanthan treatments with and without probiotics in 180 days have the highest and lowest values of this index, respectively. The values of thiobarbituric acid number (mg/kg) in the treatments containing zero, 0.03 and 0.1 xanthan with and without probiotics increase significantly with increasing storage time. Although for the treatment containing 0.3 xanthan, with and without probiotics, a different trend is observed with increasing storage time. So that the values of thiobarbituric acid (mg/kg) decrease significantly in 180 days compared to 0 and 360 days.

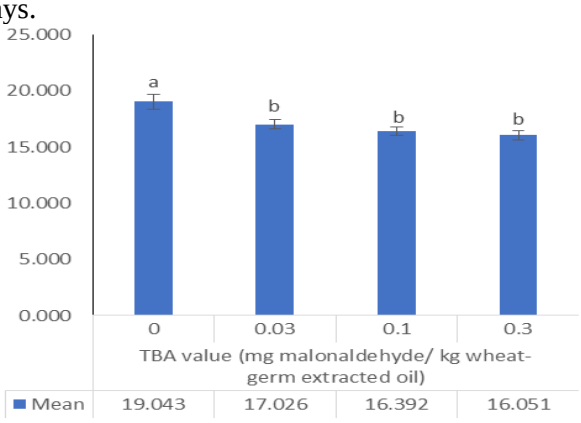

Fig 12 The effect of xanthan gum treatment on the thiobarbituric acid value of probiotic wheat germ oil

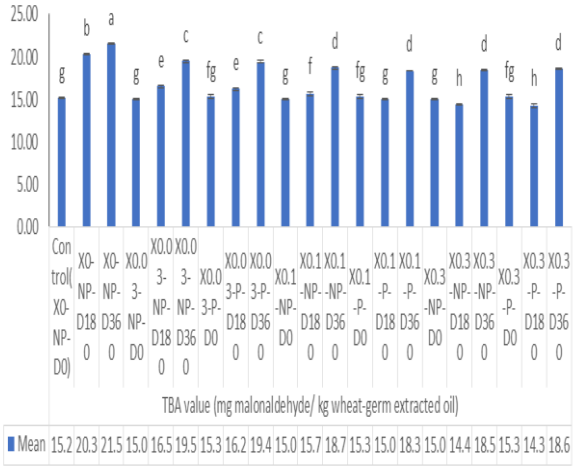

Fig 13 Treatment-probiotic-time interaction effect on thiobarbituric acid value of probiotic wheat germ oil. (X: Xanthan gum; P: Probiotic; NP: Non probiotic; D: day)

5-3- Investigating the effect of xanthan gum treatment, probiotic bacteria and storage time on the Totox number and comparing with the control sample

According to the analysis of variance in Table 2, the independent effect of xanthan gum treatment, probiotic and the interaction effect of treatment-probiotic-time on the changes in Totox number (mE/kg) It was significant (P<0.01). Figure 14 shows the independent effect of xanthan gum treatment and Figure 15 shows the independent effect of probiotics on changes in Totox number (mE/kg). The results show that the values of this index are significantly reduced by adding 0.03, 0.1 and 0.3 xanthan and probiotic bacteria compared to the control sample.

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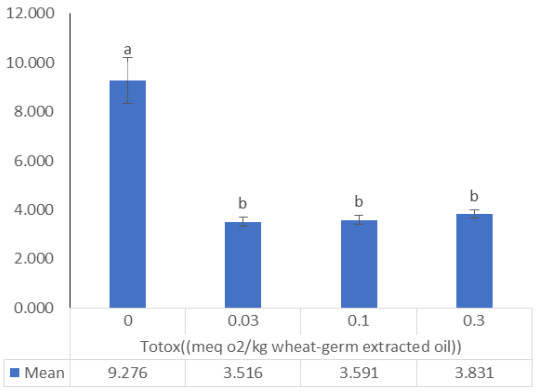

Fig 14 The independent effect of xanthan gum treatment on changes in totox value of probiotic wheat germ oil

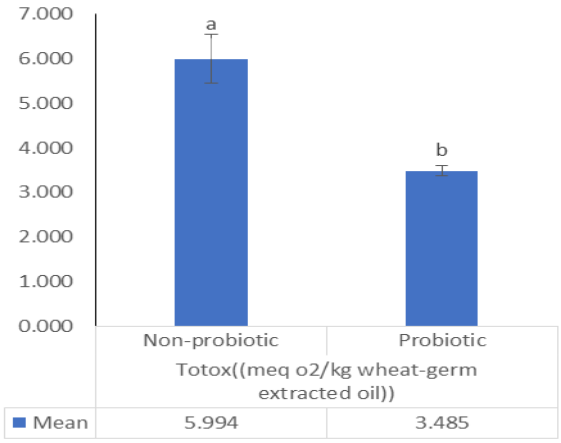

Fig 15 The independent effect of probiotics on changes in totox value of probiotic wheat germ oil

Figure 16 shows the interaction effect of treatment-probiotic-time on changes in Totox number (milliequivalent/kg). According to the chart, zero xanthan treatments without probiotics in 360 days and 0.03 xanthan treatments with and without probiotics in 360 days have the highest and lowest values of this index, respectively. The values of this index in treatments without xanthan and probiotics increase significantly with increasing storage time compared to the control sample. In the case of other treatments containing different amounts of xanthan, with and without probiotics, an inverse trend is observed with increasing storage time.

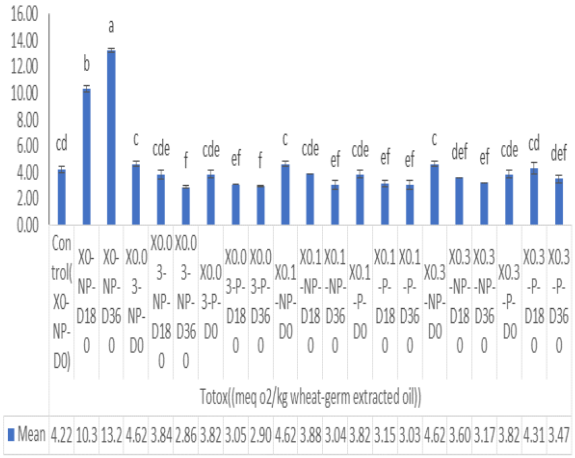

Fig 16 The interaction effect of treatment-probiotic-time on changes in the totox value of probiotic wheat germ oil. (X: Xanthan gum; P: Probiotic; NP: Non probiotic; D: day)

3-6- Investigating the effect of xanthan gum treatment, probiotic bacteria and storage time on probiotic count (CFU/gram)

According to analysis of variance in Table 3, the independent effect of xanthan gum treatment and the interaction effect of treatment-probiotic-time on probiotic count index (CFU/gram) were significant (P<0.01). According to Figure 17, by adding 0.3 xanthan, it decreases significantly compared to 0.03. Although there is no significant difference between 0.1 and 0.3 xanthan treatments in terms of this index. Figure 18 shows the interaction effect of treatment-probiotic-time on probiotic count index (CFU/gram). The highest and lowest values of this index were observed in the treatments containing 0.03, 0.1 and 0.3 xanthan, containing probiotics and 360 days, respectively. Although there was no significant difference between the treatments containing 0.03, 0.1 and 0.3 xanthan, on day zero in terms of counting this index.

Table 7 Analysis of variance of probiotic count (CFU/gram)

Source	Log Sum of Squares	df	Log mean square	F-value
Corrected Model	16.915	5	16.216	10207.78***
Intercept	17.564	1	17.564	227823.68***
Treatment	16.613	2	16.312	12738.59***
Time	14.134	1	14.134	84.55***
Treatment * Time	16.613	2	16.312	12738.59***
Error	13.286	12	12.207	-

R Squared = 1.00 (Adjusted R Squared = 1.00)

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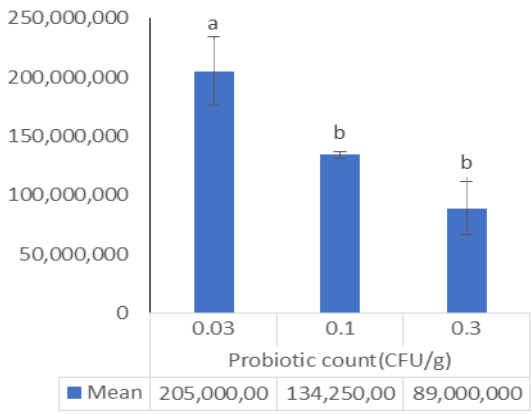

Fig 17 Independent effect of xanthan gum treatment on probiotic bacteria count

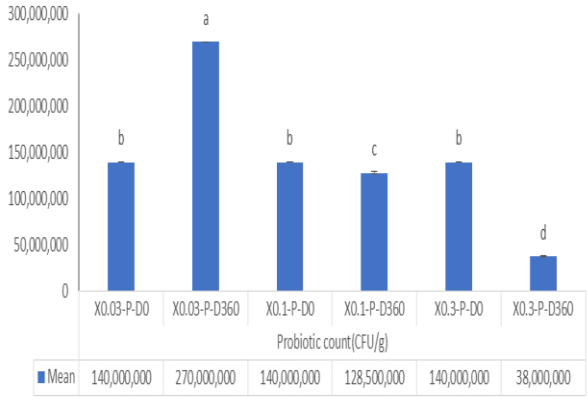

Fig 18 Treatment-probiotic-time interaction effect on probiotic bacteria count. (X: Xanthan gum; P: Probiotic; D: day)

3-7- Investigating the effect of xanthan gum treatment, probiotic bacteria and storage time on mold and yeast counts

According to the analysis of variance in Table 4, the independent effect of xanthan gum treatment, probiotic and the interaction effect of treatment-probiotic-time on changes in mold and yeast count (CFU/g) were significant ($P<0.01$). Figures 19 and 20 show the independent effect of xanthan and probiotic treatment on the values of this index, respectively. According to the graphs, the highest and lowest values of this index were observed in the 0.1 and 0.3 xanthan treatments, respectively. Also, no significant difference was observed between these treatments with the amount of xanthan 0.03 in terms of mold and yeast counts. Also, the addition of probiotic bacteria significantly increases this index compared to the sample without probiotics. Figure 21 shows the interaction effect of treatment-probiotic-time on mold and yeast count index (CFU/gram).

Table 8 Analysis of variance of mold and yeast counts (CFU/gram)

Source	Log sum of squares	df	Log mean square	F-value
Corrected Model	10.67	11	9.63	18799.27***
Intercept	10.53	1	10.53	149744.57***
Treatment	9.93	2	9.63	18956.77***
Probiotic	10.20	1	10.20	70219.31***
Time	6.86	1	6.86	32.24***
Treatment * Probiotic	8.92	2	8.62	1840.70***
Treatment * Time	9.73	2	9.43	11839.99***
Probiotic * Time	9.34	1	9.34	9646.04***
Treatment * Probiotic * Time	10.14	2	9.84	30809.73***
Error	6.73	24	5.35	-

R Squared = 1.000 (Adjusted R Squared = 1.000)

The values of this index were respectively in the treatments of 0.1 xanthan, containing probiotics, day 360, treatment 0.03 xanthan, containing probiotics and day 180, treatment 0.1, without probiotics, day 180, 0.3 xanthan, containing probiotics, day 360, xanthan 0.1 treatment, containing probiotics and day 180 increased and in xanthan 0.3 treatment, containing probiotics, day 180, xanthan 0.03 treatment, without probiotics and day 360 and xanthan 0.3 treatment, without probiotics, day 360 180 decreases significantly. The values of this index in xanthan 0.03 and 0.3 treatment, without probiotics and time of 180 and 360 days are not significantly different from each other.

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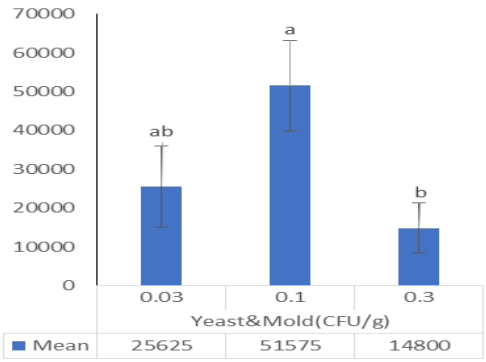

Fig 19 Independent effect of xanthan gum treatment on mold and yeast counts

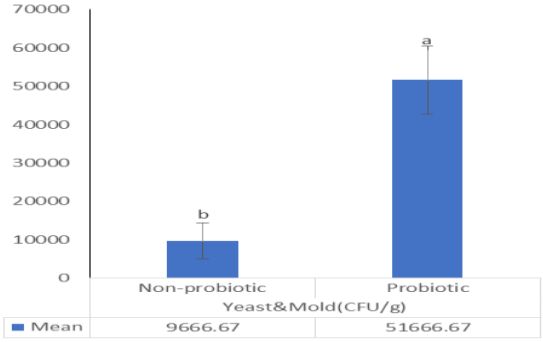

Fig 20 Independent effect of probiotics on mold and yeast counts

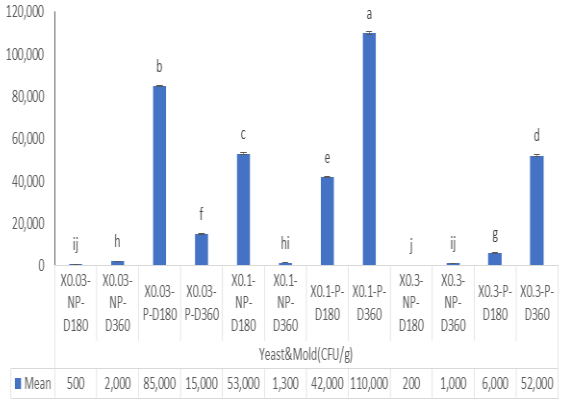

Fig 21 Treatment-probiotic-time interaction on mold and yeast counts. (X: Xanthan gum; P: Probiotic; NP: Non probiotic; D: day)

4-Discussion and conclusion

4-1- Determining the best ratio of xanthan gum and maltodextrin for capsule production by examining different ratios to form capsule walls

Improved delivery of encapsulated materials means that these materials are fully delivered to the target point after controlled release, and this depends on choosing the right coating material for encapsulation. Adding amounts of maltodextrin: xanthan equal to the ratio of 1:0.3, 0.1:1 and 0.03:1 significantly reduces the total acid value (mg/kg), peroxide (mEq/kg). , anisidine, the number of thiobarbituric acid (mg/kg) and totox (mEq/kg). Wheat germ oil is subject to oxidation during storage due to the presence of lipase and lipoxygenase enzymes. Wheat germ oil undergoes oxidation in unfavorable storage conditions due to the high concentration of polyunsaturated fatty acids. Different studies evaluated different methods and parameters to increase the shelf life and persistence of oil during storage. Encapsulation of wheat germ was evaluated by different coating materials such as maltodextrin, whey protein concentrate, sodium caseinate, gum arabic, chitosan. The encapsulation efficiency of maltodextrin: sodium caseinate in a ratio of 1:3 was significantly higher than other coating materials. In addition, the oxidative stability of the encapsulated oil and the integrity of tocopherol during 24 days of storage at 15 and 45 °C were significantly higher compared to wheat germ oil. Also, they reported that the Totox values of the capsules were significantly reduced compared to fresh oil [17]. The reported results are consistent with the results of this research.

2-4- Investigating the effect of time on changes in the oxidative characteristics of encapsulated wheat germ

In the treatments containing 0.03, 0.1 and 0.3 xanthan, the values of total acid number increased significantly in 180 days compared to 0 and 360 days. Also, the values of this index decrease significantly in 360 days in the above treatments compared to 180 days. In general, oils that contain unsaturated or polyunsaturated fatty acids are prone to oxidation. Microparticles prepared from Arabic gum and its ternary mixtures with maltodextrin and whey protein isolate show high protection of sunflower oil against oxidation [18]. To prevent the oxidation of lipids and improve the release parameters of flaxseed oil, the oil was encapsulated in the wall of lentil or pea protein isolate and maltodextrin polymers [19]. Compared to unencapsulated oil, encapsulated flaxseed oil did not undergo oxidation during 25 days of storage at room temperature. The amount of peroxide in the control treatment increases significantly with increasing storage time. In the treatment of 0.03 xanthan, with the increase of storage time in 180 and 360 days, and in the treatments of 0.1 and 0.3 xanthan, in 360 days compared to the time of 0 and 180 days, the values of the above index decrease significantly. Durant et al. [20] reported that

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after encapsulation of wheat bran oil in sodium alginate grains, its stability characteristics improved during storage. The stability of the seeds was tested by evaluating the production of fatty acid hydroperoxide, tocopherol, tocotrienol and carotenoid degradation. The seeds have increased oil stability at 4°C for 30 days with high levels of isoprenoids and low content of hydroxyl peroxide fatty acids.

According to the results, the values of all three above indicators in the control sample and the values of anisidine, totox and thiobarbituric acid in the xanthan 0.03 treatment increase significantly with increasing storage time. Also, in the treatment of 0.1 and 0.3 xanthan, the amounts of anisidine and thiobarbituric acid increased significantly in 360 days compared to 0 and 180 days. The values of Totox number decrease significantly with the increase of storage time in 360 days compared to zero day. Totox value is often used in the food industry and integrates the results of past and present conditions of certain oils [21]. Good quality oil should have a Totox value less than 4 [22] and oil samples with a Totox value above 10 are considered unacceptable [21]. Changes in the amount of totox provide information about the progress of the formation of primary and secondary oxidation products. Therefore, the determination of Totox value has been widely used to estimate the oxidative deterioration of lipids [23]. Sun and Gunaskaran reported that increasing the concentration of whey protein isolate from 0.2 to 2% by weight significantly reduced the formation of hydroperoxide during 8 days of storage [24].

4-3-Effect of using bacteria*Bacillus badius* As a probiotic in wheat germ

Independent effect of bacteria*Bacillus badius*As a probiotic in wheat germ, it causes a decrease and an increase in total acid number, peroxide value, anisidine number, totox number, ash and powder density and yeast mold count in samples containing probiotics compared to samples without probiotics. It has been reported that various secondary metabolites biosynthesized by bacteria living in marine organisms have good bioactivity. Meanwhile, the secondary metabolite section*Bacillus badius* Effectively inhibited hydrogen peroxide (approximately 73%). Our results show that secondary metabolites*Bacillus badius* They may be useful not only as an antioxidant but also as an anti-inflammatory agent. Reactive oxygen species play a role in inflammation, and as a result, antioxidants play an important role in inflammatory diseases [25]. Showing anti-oxidative and anti-inflammatory activity in*Bacillus badius* It may confirm this relationship. As a result of the presence of strong antioxidant compounds in secondary metabolites*Bacillus badius*approved.

5. Conclusion

The use of xanthan gum and maltodextrin as wall materials improves the antioxidant properties of encapsulated wheat germ. It also produced significant antioxidant properties during 360 days of storage time. According to the role of bacteria*Bacillus badius* As a probiotic in significantly reducing oxidation indices in wheat germ, this bacterium can be mentioned as a strong antioxidant and effective in increasing the storage time of encapsulated wheat germ.

6- Gratitude

The authors of the article are grateful to the colleagues at the Laser and Biophotonics Research Center in Biotechnologies at Isfahan Islamic Azad University (Khorasgan) for their scientific and research cooperation in order to achieve this research.

7- Conflict of benefits

The authors have no conflicts of interest to declare.

8- Resources

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افزایش عمر نگهداری جوانه گندم پروبیوتیک حاوی *باسیلوس بادیوس* به کمک صمغ زانتان به روش انکپسولاسیون از نوع خشک کن انجمادی

عسل کدخدایی[۱]، محمد گلی[۱،۲*]، محدثه رمضانی[۳]

۱- گروه علوم و صنایع غذایی، واحد اصفهان (خوراسگان)، دانشگاه آزاد اسلامی، اصفهان، ایران.

۲- مرکز تحقیقات لیزر و بیوفوتونیک در فناوریهای زیستی، واحد اصفهان (خوراسگان)، دانشگاه آزاد اسلامی، اصفهان، ایران.

۳- بانک میکروارگانیسم ها، مرکز ملی ذخایر ژنتیکی و زیستی ایران، جهاد دانشگاهی، تهران، ایران.

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*مسئول مکاتبات:

mgolifood@yahoo.com

چکیده

جوانه گندم محصول جانبی آسیاب گندم با مواد مغذی بالا است، اما به دلیل فعالیت شدید لیپاز و لیپوکسی‌ژناز، ماندگاری کوتاهی داشته و مصرف بهینه بسیار محدودی دارد. از این‌رو، در پژوهش حاضر، جهت افزایش عمر نگهداری جوانه گندم پروبیوتیک حاوی *باسیلوس بادیوس* از روش انکپسولاسیون از نوع خشک کن انجمادی استفاده شد و تاثیر استفاده از صمغ زانتان : مالتودگسترین در نسبت‌های مختلف ۰/۳ : ۱ ، ۰/۱ : ۱ و ۰/۰۳ : ۱ به عنوان دیواره کپسول‌ها بر خواص آنتی‌اکسیدانی و ویژگی‌های فیزیکوشیمیایی جوانه گندم پروبیوتیک طی ۳٦۰ روز نگهداری بررسی شد. سه تیمار شاهد، حاوی همان نسبت‌های صمغ‌های استفاده شده برای تیمارها، بدون باکتری پروبیوتیک نیز تهیه گردید. جهت مقایسات بهتر و اثر تیمارهای اعمال شده، یک نمونه جوانه گندم خالص نیز در روز اول، صدو هشتاد و سیصدو شصتم همراه با نمونه‌های کپسوله شده مورد بررسی و آزمون قرار گرفت. آزمون‌ها در قالب طرح کاملا تصادفی انجام شد و تیمارها با استفاده از نرم افزار اس پی اس اس و مقایسه میانگین‌ها با آزمون چند دامنه‌ای دانکن در سطح اطمینان ۹۹ درصد ارزیابی شدند. نتایج این تحقیق نشان داد که کاربرد صمغ زانتان و مالتودگسترین بعنوان مواد دیواره، سبب بهبود ویژگی‌های آنتی‌اکسیدانی در جوانه گندم کپسوله شده گردید. استفاده از باکتری *باسیلوس بادیوس* به عنوان پروبیوتیک، باعث کاهش معنی‌دار شاخص‌های اکسایشی در جوانه گندم شد(P<۰/۰۱). باکتری *باسیلوس بادیوس* بعنوان پروبیوتیکی قوی می‌تواند بر افزایش زمان نگهداری جوانه گندم کپسول شده تاثیرگذار باشد. هم‌چنین صمغ زانتان به عنوان ماده مناسب جهت انکپسولاسیون جوانه گندم جهت افزایش ماندگاری پیشنهاد می‌شود.