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Production of a cupcake with probiotics added by Encapsulation to treat intestinal disorders in children

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Abstract:*The study was conducted in the laboratories of the College of Agriculture, Tikrit University, which included the production of cupcakes fortified with probiotics, which carried out the process of encapsulation before adding it to the cupcake mixture to ensure that the probiotics are preserved for as long as possible and reach the intestines and benefit from it in the treatment of diarrhea cases in pre-school children who are more than disease-prone and the most neglected category of the community, The chemical composition analysis of the samples, sensory evaluation, icing, and bacterial counting were conducted to find out the best addition among the treatments. The results of the chemical composition of the cupcake showed high percentages, including the percentage of moisture in T4, which amounted to 4.89%, and the percentage of fat and ash percentage in T5, which amounted to (4.75, 2.68)%, respectively, and the percentage of protein in the standard treatment and T5, which was 7.97% for both, The percentage of carbohydrates in the standard treatment was 81.94%, while the lowest percentages of moisture, fat, protein, ash and carbohydrates were in treatments T1, standard treatment, T4, T3, and T5, which were (4.56, 4.23, 7.77, 2.16, 79.81)%, respectively. The sensory evaluation of the produced cupcakes had the highest value for evaluation in terms of color in T2, T3, smell in T4, T5, taste in T5 and texture T3, which amounted to (19, 29) respectively, while the lowest value for sensory evaluation was in the standard treatment, which amounted to (16,25,26) respectively, When observing the results of icing for different storage periods, the highest value was in the standard treatment for the length of the storage period, which amounted to (19, 17, 15, 13), respectively, while the lowest value was in the T4 treatment, which amounted to (12, 9, 7, 6) on the Respectively, and that the bacterial count of probiotics during the storage period gave the highest value in treatment T5 for the first day 15 x 10 $^{-6}$, while the rest of the storage period was the highest bacterial count in treatments T4 and T5, which amounted to (13, 11, 10) x 10 $^{-6}$ respectively, while the lowest bacterial number during the storage period appeared in the standard treatment.*

Keywords: cupcakes, probiotics, functional foods.

1. INTRODUCTION

The demand for processed functional foods containing probiotics is increasing due to the increasing awareness of consumers about their positive impact on health. The development of foods that contain appropriate doses of probiotics at the time of consumption

is a challenge, because many factors during processing and storage affect the quality of micro-organisms. The presence of probiotics in food products may also negatively affect their quality and organoleptic properties. Great emphasis has been placed on protecting microorganisms with the help of encapsulation technology, by adding different protective materials (Kamal – Eldin & Chugh, 2020).

these foods contain viable microorganisms in capsules that are used to enhance and strengthen immunity because they contain biologically active compounds. Among the strains used, which are considered safe and effective, are Lactobacillus, Bifidobacterium and Saccharomyces (Skocinska et al., 2018).

Food is the most convenient way to consume probiotics, so many studies have made a lot of choices in the development of functional foods and methods for preparing capsules for probiotics, It is worth noting that most of the foods that supplement probiotics in the market are made from dairy products. The demand for non-dairy products has become more to increase health awareness and as a diversification of choices for probiotic supplement products, although the use of pastries is still limited because these products are baked at high temperatures that lead to the loss of life of microorganisms, so the micro-encapsulation technology has been developed to preserve the greatest possible It is possible from them, thus ensuring that they reach the intestines (Dong et al., 2020).

Much research has dealt with the use of functional foods containing probiotics in the treatment of diarrhea, digestive disorders and allergies in children and the elderly, as Mitchill & Davies (2021) states that children suffering from chronic diarrhea can be treated with foods containing probiotics, which contribute to improving health of the child in a short period of time, Thus, it reduces the risk of children suffering from mental disorders, dehydration and other side effects as a result of diarrhea.

The study conducted by (Manna et al., 2010) provides evidence of an important protective effect of probiotics on acute diarrhea in children aged 1-5 years in a community, and in another community study on the protective effect of probiotics on diarrhea in poor developing countries, it was found Lactobacillus GG is useful as a preventive measure to control diarrhea in undernourished children at increased risk, especially non-breastfed children in the young child age group.

Inflammatory bowel disease, which affects millions of people around the world and colitis, has become clear that a combination of factors, especially genetic background, causes these diseases in addition to the diet followed, so recent studies in the treatment of inflammatory bowel disease have tended to modify the gut by probiotics, Over the past 20 years, many publications have focused on the role of probiotics in the treatment of Inflammatory bowel disease IBD (Jakubczyk et al., 2020).

The aim of the study is to produce a food product (cupcake) fortified with probiotics because the segment of children aged (1-5) years is the most neglected group in terms of nutrition and the most vulnerable to diseases, and the lack of studies in Iraq on functional foods for children of these ages and its provision in food Various other than dairy products and the use of Encapsulation technology in preserving and benefiting from probiotics, and studying the extent of the effect of probiotics on the chemical properties and sensory characteristics of the product, and the effect of different storage periods on the activity of probiotics

2. MATERIALS AND WORKING METHODS:

Probiotic strains

Activated probiotic strains were obtained from the mixture of Lactobacillus acidophilus, L.rhamnosus, and Bafidobacterium actireglaris, in equal proportions, and microencapsulation was carried out before adding them to the cupcake mixture.

Minute Encapsulation

Perform the encapsulation method as mentioned by (Dong et al., 2020) with some modification

As the packaging material, starch was used instead of alginate and pectin, and sesame oil was used instead of canola oil due to its availability in the market. The method was carried out by adding (1, 2, 3, 4, 5) ml of biomass mixed with (10, 20, 30, 40, 50) ml of starch suspension, 10 ml of sesame oil with 0.1% Tween 80 and stirred for a period of time for 15 minutes to allow emulsification and encapsulation to occur. Then, 20 mL of 0.1Molar of CaCl2 was rapidly added down the side of the beaker to break up the emulsion. Capsules were harvested from the solution by centrifugation at 5000 rpm for 10 min.

How to prepare Cupcake

The method mentioned by Al-Nasiri (2021) was followed, where both sugar and fat were mixed until they became homogeneous and the color of the mixture turned white, the baking powder and the dried fruit pieces that had been previously cut into small pieces were added to the flour and mixed well, the eggs were added to the sugar and fat mixture and vanilla was added before Mix the eggs with the rest of the ingredients, then add the milk and mix well, add the flour gradually and alternate with the milk until you get a homogeneous dough, avoiding excessive mixing.

After completing the mixing process, the probiotics are added according to the treatments (with different proportions) and the dough (Buttermilk) is poured into the molds, greased and sprinkled with a layer of light flour (prepared) and a quantity of the buttermilk is placed so that it does not exceed half of the mold while avoiding the dough spilling on the sides of the mold.

The baking process (grilling) is at a temperature of 177 °C for 45-40 minutes, and the oven is heated before starting the mixing process to ensure that the heat is distributed evenly inside the oven. After the baking process is finished, we take out the molds from the oven, then leave them for a while until they are at room temperature, and then store them until the analysis is done.

Table (1) Materials and proportions used to prepare cupcakes

Country of Origin	trade mark	Weight	the ingredients	No.
Kuwait	Al-Fakhir	100 g	flour	1
Available in the Iraqi market	Al-Maedah	80 g	Sugar	2
Egypt	Janah	50 g	fat	3
Available in the Iraqi market	Al-Maedah	one egg	eggs	4
Iraq	Danon	50 ml	milk	5
Iraq	Family	2 g	baking powder	6
Iraq	Family	2 g	vanilla	7

Chemical tests conducted on cupcakes:

Moisture estimation:

The moisture content was estimated according to the mentioned method (2019) A.O.A.C by Rapid moisture test device
at a temperature of 105°C until the weight is fixed.

Fat estimation:

The percentage of fat was estimated according to the method mentioned in (2019) A.O.A.C, using the extraction device (Soxhelet) using petroleum ether.

Protein Estimation:

The percentage of protein was estimated by the Microchaldal method according to what was mentioned in (2019) A.O.A.C. Then the amount of nitrogen produced was multiplied by the factor 6.38 to extract the protein percentage.

Ash Estimation:

The standard method mentioned in (2019) A.O.A.C was followed at a temperature of 550 °C and the samples were left until obtaining a whitish gray color.

Carbohydrate rating:

It was mathematically estimated by the difference between the components (moisture, fat, protein and ash) subtracted from the 100 mentioned in (2019) AOAC and as follows:
Total Carbohydrates - 100 = (Moisture % + Fat % + Protein % + Ash %).

Sensory evaluation of the cupcake:

The sensory evaluation of fresh laboratory cake was carried out according to the evaluation form shown in Figure (1) and the scores were distributed according to what was mentioned by Ayoubi & Porabolghasem (2019) and the evaluation was conducted by fifteen assessors of two teachers and students of the Department of Food Sciences.

Figure (1) Sensory evaluation form for cupcake samples

30 texture	30 Taste	20 odor	20 color	Treatment	No.
				Control	1
				T1	2
				T2	3
				T3	4
				T4	5
				T5	6

Refrigerating storage:

The cupcake samples were stored in sterile plastic bags at 4 ℃ for two weeks, and every 3 days the glaciation of the samples was measured.

Penetration Measurement

It was estimated using the penetration measuring device whose picture is in Appendix (1) and according to the method mentioned by Al-Hadithi (2020), the penetration of the cake during storage was estimated by using a (local) device to estimate the penetration by measuring the depth distance in millimeters caused by the metal cone installed in the device by the measuring ruler is installed on its side.

Bacterial count of cupcake samples during different storage period

Bacterial colonies were counted after dilution of the samples and culturing 1 ml on Petri dishes containing MRS culture medium and incubated at 37° C for 48 hours. Adhikari et al. (2003).

Statistical Analysis

Statistical analysis was carried out using the SPSS program provided by Microsoft and used in data analysis, which was used for the cupcake sensory evaluation tables.

3. RESULTS AND DISCUSSION:

Chemical composition of cupcakes:

Table (2) shows the chemical composition of the produced cupcake, as the results showed that there were significant differences at the level of $p < 0.05$ for all compositions. The highest moisture content was between samples and standard treatments in treatment T4, which amounted to (4.89)%, while the lowest moisture content Between the samples, the standard treatments and T1 were (4.57, 4.56)%. The reason for the difference in moisture percentage is attributed to the increase in the percentage of starch added to the mixture, which was used in the encapsulation, which increases the absorption of a percentage of water. Kosmala et al. (2017). As for the lipid ratio of the treatments, it was superior to the T5 treatment, which amounted to (4.75)%, while the lipid ratio decreased in the standard treatment, which amounted to (4.23)%, The difference between the proportions of the fat is the result of the formation of emulsions in the mixture, which are difficult to separate easily, and the molds used in the manufacture of cupcakes are made of paper, and the paper may absorb part of the fat, causing a loss in the ratio . Majzoobi et al.(2020).

The results for protein showed an increase in the percentage of protein in the two treatments (standard and T5), which amounted to (7.97)% for both, and the percentage of protein decreased in treatment T3, which amounted to (7.82)%, and the percentage of ash increased in treatment T5 to reach (2.68)%, While the lowest level of ash was recorded in the standard and T3 treatments, which amounted to (2.17, 2.16) %, respectively. As for the percentage of

carbohydrates, it increased in the standard treatment, which amounted to (81.94)%, while the percentage of carbohydrates decreased in the T5 treatment, which amounted to (79.81)%.
Table (2) The chemical composition of the cupcake product

%carbohydrate	% Ash	%Protein	% Fat	Moisture %	treatments	ت
81.94	2.17	7.97	4.23	4.57	standard treatment	1
80.90	2.22	7.87	4.45	4.56	T1	2
80.12	2.56	7.88	4.67	4.77	T2	3
80.83	2.16	7.82	4.55	4.64	T3	4
80.59	2.23	7.77	4.52	4.89	T4	5
79.81	2.68	7.97	4.75	4.79	T5	6

T1: 1 ml of probiotics + 10 ml of suspension of starch, gelatin, sesame oil and 80% tween
T2: 2 ml of probiotics + 20 ml of suspension of starch, gelatin, sesame oil and 80% tween
T3: 3 ml of probiotics + 30 ml of suspension of starch, gelatin, sesame oil and 80% tween
T4: 4 ml of probiotics + 40 ml of suspension of starch, gelatin, sesame oil and 80% tween
T5: 5 ml of probiotics + 50 ml of suspension of starch, gelatin, sesame oil and 80% tween

Sensory evaluation of the cupcake product:

Table (3) shows the sensory evaluation of the produced cupcake, as significant differences were observed at the level of $p < 0.05$ for the studied traits and treatments, as it was noted that the sensory evaluation of color increased in the two treatments T2 and T3, reaching (19) for each, while the color decreased to (17). In the T4 treatment, the distribution of cupcakes in the oven and the distribution of heat affects the color of the product, and the treatments did not enter the oven all at once, but rather in the form of successive aggregates, and this affects the temperature and the time it takes to form the color of the product, while the smell was the highest level. In treatment T5, which amounted to (29), While the lowest level was in the standard treatment, which amounted to (16), the additions, although they are few, still give desirable smells to the product, which were the result of the internal reactions taking place in the product from Caramel reactions and Maillard reactions in addition to enzymes and others in the presence of heat, and when observing the taste, it showed Treatment T5 had the highest level of taste among the treatments, as it reached (29), While the lowest level of taste was in the standard treatment, which amounted to (25), that the standard treatment does not contain any additives, which always gives it the lowest levels in the evaluation, and that the sensory evaluation of texture showed its results to exceed the two treatments T3 and T4, which amounted to (29) for both, The lowest level of texture in the standard treatment was (26), the good mixing of the mixture and the distribution of the mixture in the molds enhances the texture of the product and gives a good and acceptable appearance to the consumer.

Table (3) Sensory evaluation of the produced cupcake

30 texture	30 Taste	20 odor	20 color	Treatment
26 d	25 d	16 d	16 d	**standard treatment**

28 b	27 c	17 c	18 b	**T1**
28 b	28 b	18 b	19 a	**T2**
29 a	27 c	18 b	19 a	**T3**
29 a	28 b	19 a	17 c	**T4**
27 c	29 a	19 a	18 b	**T5**

Effect of storage on penetration measurement (hardening or glaciation):

The penetration test is an important indicator for following up on the phenomenon of glaciation, and it is one of a group of tests that study changes in textures such as hardness and firmness.

The results in Table (4) show significant differences for the means at the level of $p < 0.05$. The highest averages were for the first day in the standard treatment, as it was noted that they give the highest values, which amounted to (19.0), while the lowest averages were in the T4 treatment, which amounted to (12.0), When the samples were stored for four days at a temperature of 4°C, the results showed significant differences in their averages, as it was observed that glaciation appeared in all samples, and the standard treatment gave the highest of those averages, which amounted to (17.0), While treatment T4 decreased significantly, reaching (9.0), while on the eighth day of storage, the results showed that the standard treatment had the highest averages of glaciation resistance, which amounted to (15.0), and that treatment T4 had the lowest significant mean, reaching (7.0).), and on the twelfth day of storing samples, the standard treatment significantly resisted glaciation, as it gave the highest averages, which reached (13.0), while the T4 treatment showed remarkably glaciation, which gave the lowest values, reaching (6.0), It is noticed from measuring the glaciation of cupcakes and the effect of storage on it, that adding probiotics and starch suspension did not solve the problem of glaciation in the samples, unlike the standard treatment that showed the least effect of glaciation.

Storage period as days				**treatments**
twelfth day	Eighth day	Fourth day	First day	
13.0 a	15.0 a	17.0 a	19.0 a	**standard treatment**
10.0 c	13.0 b	14.0 b	16.0 b	**T1**
11.2 b	12.0 c	12.0 c	15.0 c	**T2**
7.0 e	9.0 e	11.0 d	13.0 e	**T3**
6.0 f	7.0 f	9.0 e	12.0 f	**T4**
9.5 d	10.4 d	12.3 cb	14.5 d	**T5**

Counting the genera of probiotics for different storage periods

Table 5 shows the activity of probiotics in the cupcake produced is important, as differences appeared in the probiotic cells during the different storage periods of the treatments, and the bacterial numbers differed among the same treatments in the same time period, depending on the added percentage of bacterial cells used during the encapsulation, as it was noted that the highest percentage per day The first was in the T5 treatment compared to the standard treatment as it was 15 x 10^{-6} colonies, The increase in the proportion of probiotics in the

treatment is due to the fact that the percentage of addition after the encapsulation procedure was 5 ml, while the lowest colonies were in the standard treatment, which amounted to 2×10^{-6}, and on the fourth day, the highest percentage was in the two treatments T4 and T5, reaching 13×10^{-6} While the lowest percentage of colonies was in the standard treatment, and on the eighth and twelfth storage days for the same storage conditions, the highest percentage of bacterial colonies was in treatments T4 and T5, as they reached (11×10^{-6} and 10×10^{-6}), respectively. As for the lowest percentage on the eighth and twelfth storage days for the same storage conditions, it was in the standard treatment, which amounted to 1×10^{-6}. Adding probiotics to the cupcake mixture in different proportions and using the Encapsulation method helped keep the probiotics active in different periods of time and in different numbers that can be used.

Table (5) Preparation of probiotics during different storage periods at a temperature of 4° C

Number of probiotics for cupcake samples during different storage periods				treatments
twelfth day	Eighth day	Fourth day	First day	
$^{-6}10 \times 1$	$^{-6}10 \times 1$	$^{-6}10 \times 2$	$^{-6}10 \times 2$	**standard treatment**
$^{-6}10 \times 3$	$^{-6}10 \times 5$	$^{-6}10 \times 6$	$^{-6}10 \times 6$	**T1**
$^{-6}10 \times 3$	$^{-6}10 \times 5$	$^{-6}10 \times 6$	$^{-6}10 \times 7$	**T2**
$^{-6}10 \times 6$	$^{-6}10 \times 6$	$^{-6}10 \times 8$	$^{-6}10 \times 9$	**T3**
$^{-6}10 \times 10$	$^{-6}10 \times 11$	$^{-6}10 \times 13$	$^{-6}10 \times 13$	**T4**
$^{-6}10 \times 10$	$^{-6}10 \times 11$	$^{-6}10 \times 13$	$^{-6}10 \times 15$	**T5**

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