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STUDYING THE CHEMICAL PROPERTIES OF LACTIC FERMENTER WHICH IS FORTIFIED BY ENCAPSULATED FOLIC ACID BY SODIUM ALGINATE AND IT'S ANTIOXIDANT EFFECTIVENESS

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ABSTRACT

The effect of adding encapsulated Folic Acid(FA) to at a rate of 7.5 mg / 120 mL of Lactic Fermenter (LF) was studied as one of the fortification methods that should be included in the diet to reduce the pathological conditions resulting from a deficiency of FA in the body, as it is one of the vitamins that are not synthesized inside the body, and the results showed that The coefficients that gave the highest percentage of encapsulation were FA encapsulated with sodium alginate. The results of chemical tests of LF stored at refrigerator temperature 5° C for a period from 0- 21d from the addition of standard FA T1 and encapsulated FA T2 showed that the addition of FA did not affect the chemical properties of yogurt, especially T2 represented by LF fortified with encapsulated FA, which makes it qualified to be one of the methods Reinforcing the absence of negative effects on the properties of the obtained product.

Key words: FolicAcid, encapsulation, lactic fermenter, sodium alginate.

دراسة بعض الخصائص الكيميائية للمخمر اللبني المدعم بحامض الفوليك المغلف بألجينات الصوديوم وفعاليته المضادة للأكسدة

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الخلاصة

دُرس تأثير إضافة حامض الفوليك المغلف بمعدل 7.5 ملغم / 120 مل من المُخمر اللبني كإحدى طرق التدعيم التي يجب تضمينها في النظام الغذائي لتقليل الحالات المرضية الناتجة عن نقص حامض الفوليك في الجسم. إذ يعد من الفيتامينات التي لا يتم تخليقها داخل الجسم ، وأظهرت النتائج أن المعامِلات التي أعطت أعلى نسبة للتغليف هي حامض الفوليك المغلف بألجينات الصوديوم، كما أظهرت نتائج الاختبارات الكيميائية للمخمر اللبني المخزن في درجة حرارة الثلاجة 5 م° لمدة تتراوح من 0-21 يومًا من إضافة حامض الفوليك القياسي وحامض الفوليك المغلف T2 أن إضافة حامض الفوليك لم يؤثر على الخصائص الكيميائية للزبادي وخاصة T2 ممثلة بالمُخمر اللبني المدعم بحامض الفوليك المغلف ، مما يجعلها مؤهلة لتكون إحدى طرق لتدعيم لعدم وجود آثار سلبية على خواص المنتج .

الكلمات المفتاحية: حامض الفوليك، التغليف، المُخمر اللبني، ألجينات الصوديوم.

INTRODUCTION

Milk is considered one of the basic sources of human food, which prompted countries to take intense interest in providing milk and its products and strive to develop them in order to achieve self-sufficiency (**Mohammed, 2015**).One of the oldest methods by human practiced is fermentation beings for the transformation of milk into products with an extended storage

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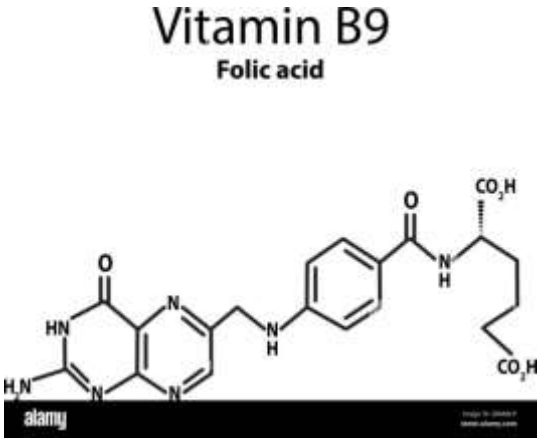
period or shelf-life (**American Oil Chemists Society, 1998**). LF is a fermented milk product and has been noted to be the most widely consumed fermented dessert worldwide. Traditionally, the product is prepared by fermenting cow milk with lactic acid bacteria Streptococcus thermophilus and Lactobacillus bulgaricus. (**Fernandez & McGregor, 1994**). The advantage of lactic acid bacteria is that they are important in food and therapeutic processing and have multiple characteristics that they grow in aerobic and anaerobic conditions, their rapid growth, non-producing toxins, most of them are non-pathogenic, and they resist low pH, and their production of flavoring materials in food as well as their ability to preserve food because of their products The metabolites resulting from fermentation, such as bacteriocins, have been used as probiotics, which are single or combined cultures of microorganisms that have beneficial effects on health, and are important in treating many diseases that affect humans and animals (**Abdulkarem & Hasan, 2022**). The discovery of folic acid (FA) is the result of hard work of Lucy Wills, a medical researcher graduating from Cambridge University with the degree of botanist and geologist. In 1930, she worked in India, having been especially interested in the problem of severe anemia in pregnant poor textile workers. Wills demonstrated amazing accuracy and insistence in her investigation excluding the infectious and parasitic nature of anemia and having come to the conclusion that it was linked to bad monotonous nutrition (**Shulpekova *et al.*, 2021**). FA was an essential vitamin that has functions in the biosynthesis of nucleic acid, and the deficiency of this vitamin leads to neural tube defects (**Grosse *et al.*, 2016**). In an attempt to reduce the risks arising from FA deficiency, many countries have tried to fortify foods with FA. For example, cereal grain products that fortification by FA in USA, white wheat flour fortification by FA in Canada (**Chan *et al.*, 2015**). FA is yellow to yellowish-orange crystalline compound, it is insoluble in water (**Dhanikula *et al.*, 2016**) ,and it cannot by synthesized by humans and mammals and must be provided through the diet such as , asparagus, broccoli, spinach, Brussels sprouts and organ meats like liver and kidney (**Chan *et al.*, 2015**). The chemical formula of folic acid is $C_{19}H_{19}N_7O_6$. The core of the molecule consists of heterocyclic pterin structure, with a methyl group in the sixth position bound to para-aminobenzoic and glutamic acids so that folic acid presents pteroylglutamic acid (Figure 1).



Figure(1): The chemical composition of FA **(Chan *et al.*, 2015)**

Pterin is composed from pyrimidine and pyrazine rings (pteridine) with substituting keto and amino groups in the second and fourth positions. (**Shulpekova *et al*., 2021**). FA is able to degradation under acidic condition(**Ihekoronye & Ngoddy, 1985**). therefore to improve the acceptability of FA it need to be protected from the degradation by the acidic condition in the stomach and encapsulation could be a way to protect FA from release inside the stomach and achieve the release before arriving to jejunum where FA is absorbed (**Pamunuwa *et al*., 2020**). There are many food grade matrices can be used to encapsulated FA such as, alginate, alginate-pectin, whey protein, pectin-whey protein concentrate, maltodextrin-whey protein using one of many techniques for example, spray-drying, nanospray drying and double emulsions (**Pamunuwa *et al*., 2021**).Encapsulation can improve thermal stability for example , the thermal protection of lactoferrin molecules is due to the alginate encapsulation, and the high stability of ß-catotene at 55° C is also due to the alginate encapsulation(**Pamunuwa *et al*., 2021**).

MATERIALS AND METHODS

Sodium alginate was purchased from (Sigma alderch) Germany. FA, Pectin, whey protein, bovine serum albumin (BSA) span 80, calcium chloride were purchased from Qualikems Fine chem. India. UV-VIS Spectrophotometer (Philip harris, England).

Preparation of FA loaded Sodium alginate particles

The particles were prepared by the ionic gelation by **Pamunuwa *et al*., (2020**) . An aqueous solution of Sodium alginate (1% w/ v), Sodium alginate (SA) and bovine serum albumin (BSA) (1% w/ v),Sodium alginate and whey protein (1% w/ v) and Sodium alginate(SA) and Pectin (1% w/ v) were prepared , homogenized by stirring (model- Philip harris). After adjusting the pH to 5 and adding a small amount of span 80, 10mg of FA was added to the solution and stirred for 30 min. Then the solution was gelatinized by adding 50mL of $CaCl_2$ solution as drops. The particles were kept in refrigeration overnight, and then lyophilized.

The encapsulation efficiency

The encapsulation efficiency in this step represents the amount of FA encapsulated in the particles compared to the amount of FA used in the encapsulation process and was determined by preparing a calibration curve for FA **(Sandip *et al*., 2017)**

Table (1): The treatments that used in this study.

Treatments
Control
T1 (standard FA)
T2 (FA encapsulated with SA 1%(w / v)
T3 (FA encapsulated with SA+BSA)
T4(FA encapsulated with SA+ whey protein)
T5 (FA encapsulated with SA+ Pectin)

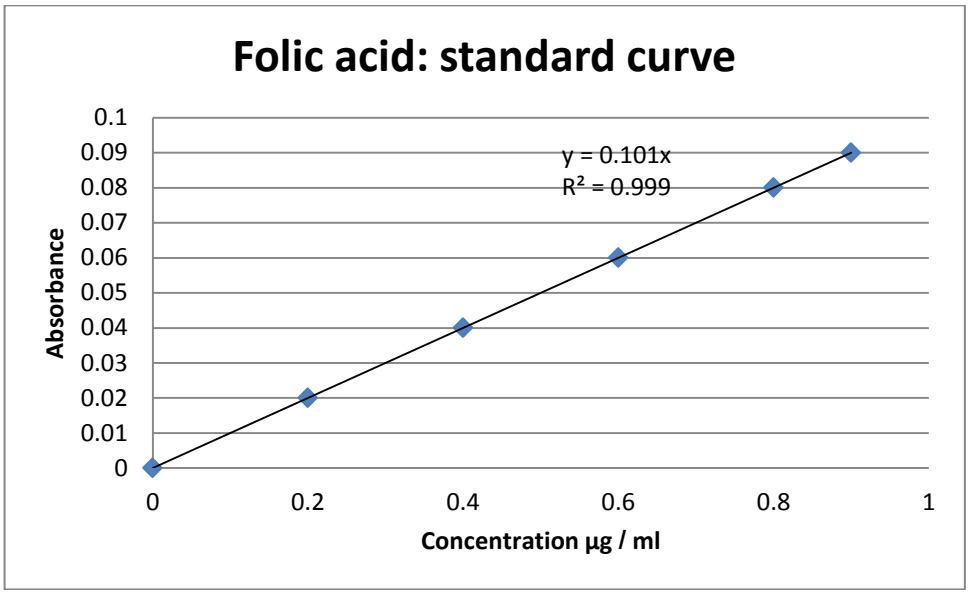
Preparation of stock solution

An 1%(w/v) FA solution was prepared. To obtain a solution with concentration 1000 µg/ mL. And the Preparation of solution, 0.1 of the previous solution was diluted in 10 mL of distilled water to obtain a concentration 100 µg/mL

Determination of ʎ max

From the previous solution an appropriate amount was pipette out and then was scanned in UV-VIS Spectrophotometer in the range of 200-400nm using distilled water as blank . The maximum absorbance was found to be 280nm. And then the Preparation of calibration curve of FA From the standard solution (0.1→ 0.9) mL were pipette out, then diluted by 10mL of distilled water to obtain (10 → 90) µg/mL solution.

Then the absorbance of the solutions was measured at a wavelength of 280nm using distilled water as the blank. Then by taking concentration (µg /ml) on X-axis and absorbance on Y-axis (figure 2). The correlation coefficient R^2 was 0.9999.



Figure(2): FA calibration carve

Encapsulation efficiency (EE)

The percentage of encapsulation efficiency was calculated using the following formula

$$EE = \frac{\text{Amount T} - \text{Amount S}}{\text{Amount T}} \times 100$$

Where:

Amount T is the total amount of FA

Amount S is the amount of FA in supernatant.

LF manufacturing

The LF manufactured as the method in **Jayalalitha *et al.*, (2012**) by receiving a quantity of row milk, full-fat mixture, from the dairy factory, Department of food science, college of Agriculture Engineering Sciences, University of Baghdad, and the the percentage of solids was adjusted to 12.98% using dry milk. Conducting a heat treatment of milk at 90°C for 10 min then cooled to temperature suitable for the starter (45°C). Then the starter culture (*Lactobacillus delbrueckii ssp.bulgaricus* and *Streptococcus salvarius ssp.thermophilus*) was added, and milk was stored in plastic containers with capacity of 120mL. After that 7.5mg of

encapsulated and unencapsulated FA were added then incubated at 42°C For 5 h, then stored in refrigerator at 5°C .

The chemical properties of LF fortified by encapsulated FA

Moisture percentage

The percentage of moisture in the LF was estimated by adding 5g of the sample in a pre-weighted container, and it was put in an oven at a temperature of 105°C until the weight stabilized **(A.O.A.C., 2005**).

Protein percentage

The micro-Kjeldahl method was used to determine total protein content of LF **A.O.A.C(2010**).

Fat percentage

The percentage of fat in the LF samples was determined using the method in **Ling(2008**).

Ash percentage

The percentage of ash in the LF was determined using the method in **A.O.A.C., (2010**).

Carbohydrates percentage

The percentage of carbohydrates in LF was determined using the method that used by **Sadiq & Doosh (2019**).

The antioxidant activity of the lactic fermenter

Peroxide value

Acetic Acid-Chloroform Method To determine the peroxide number of fats, in terms of milli equivalent per Killogram of sample, The Official Method Cd 8-53 of the **American Oil Chemists' Society,** (**1998**) was used.

Acid degree value

The acidic degree value was determined according the Bareau of dairy industry (BDI) used by **Sadiq & Doosh, (2019**).

Statistical Analysis

The Statistical Analysis System- **(SAS, 2018)** program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

RESULTS AND DISCUSSION

The encapsulation efficiency

The results in (Table, 2) obtained by using spectrophotometer showed that FA encapsulated by Sodium alginate was given the higher encapsulation efficiency and that agree with **Pamunuwa *et al*., (2021).**

Table (2): shows the percentage of the encapsulation efficiency .

Treatment	Encapsulation efficiency(%)
T2	95.55
T3	83.54
T4	90.74
T5	93.69

The chemical properties of LF fortified by encapsulated FA

Results in (Table 3) showed The percentage of fat for the control treatment, T1(LF which were fortified by standard FA) and T2 (LF which were fortified by encapsulated FA) immediately after manufacturing was 2.80, 2.81 and 2.81% respectively, Therefore that the percentage of fat increased during the storage period due to the increase in the percentage of solids compounds in exchange for the decrease in the percentage of moisture. The reason for that due to the evaporation of water during the cooled storage period. This agrees with the results **Bibiana *et al.*, (2014**) found, where the percentage was 2.11% .There was not a significant difference at least between the samples (P≤0.05)

Table(3): The percentage of Fat in lactic fermenter during the storage period.

Treatment	Storage period (d)				LSD value
	1	7	14	21	
Control	2.80	2.90	3.03	3.17	0.391 NS
T1	2.81	2.91	3.00	3.15	0.355 NS
T2	2.81	2.92	3.02	3.16	0.361 NS
LSD value	0.318 NS	0.305 NS	0.297 NS	0.326 NS	---
NS: Non-Significant.					

While the results in (Table 4)shows the percentages of carbohydrates, they were after manufacturing for the control, T1, T2, 4.68,4.62 and 4.59% respectively. And it agree with what **Sadiq & Doosh (2019**) found, who used encapsulated Iron to fortified the LF, as their percentage reached 4.85%, it was noted from the results that the percentage of carbohydrates decreased during the storage period, due to the activity of the starter culture, which consumed the lactose sugar present in the LF to convert it into lactic acid. There was not a significant difference at least between the samples(P≤0.05), but there was a significant difference between the samples during the storage period(P> 0.05).

Table (4): The percentage of Carbohydrates in lactic fermenter during the storage period.

Treatment	Storage period (d)				LSD value
	1	7	14	21	
Control	4.68	4.40	4.22	4.00	0.526 *
T1	4.62	4.15	4.10	3.90	0.544 *
T2	4.59	4.19	4.18	4.05	0.425 *
LSD value	0.331 NS	0.382 NS	0.277 NS	0.310 NS	---
* (P≤0.05). NS: Non-Significant					

In addition (Table 5) shows the ash percentages in the various treatments that used, after manufacturing for the control, T1 and T2 was, 0.93 0.92 and 0.94%, as it is close to what was found by **YILDIS & OZCAN(2018**)who concluded that the ash percentage was 0.80%. There was not a significant difference at least between the samples (P≤0.05).

Table (5): The percentage of Ash in lactic fermenter during the storage period

Treatment	storage Period (d)				LSD value
	1	7	14	21	
Control	0.93	0.95	0.98	1.01	0.152 NS
T1	0.92	0.94	0.97	1.02	0.164 NS
T2	0.94	0.95	0.95	1.02	0.149 NS
LSD value	0.116 NS	0.154 NS	0.142 NS	0.088 NS	---
NS: Non-Significant.					

Results in (Table, 5) showed The percentage of protein in control, T1 and T2 was 4.51, 4.50 and 4.51%, respectively, immediately after processing, and it is consistent with what **Qureshi *et al.*, (2011**) found, as the percentage reached 4.21%. The slight increase in protein levels during the storage period is due to the decrease in moisture levels due to evaporation during the storage period, which in turn led to an increase in the solids percentages. There was not a significant difference at least between the samples (P≤0.05).

Table (5): The percentage of Protein in lactic fermenter during the storage period.

Treatment	storage Period (d)				LSD value
	1	7	14	21	
Control	4.51	4.62	4.67	4.70	0.314 NS
T1	4.50	4.61	4.68	4.71	0.286 NS
T2	4.51	4.63	4.68	4.71	0.291 NS
LSD value	0.267 NS	0.225 NS	0.197 NS	0.202 NS	---
NS: Non-Significant.					

Compared to the humidity levels of the various treatments, (Table 6) shows the decrease in the humidity levels was observed during the storage period. The reason for this due to the evaporation of the water in the LF during the 21(d) storage period. The moisture percentages of the control, T1 and T2 immediately after manufacturing were 88.71, 87.16 and 88.82%, respectively, instead of after 21(d) storage, the percentage reached 83.33, this results consistent with what **Sadiq & Doosh(2019)** mentioned. There was not a significant difference at least between the samples (P≤0.05).

Table (6): The percentage of Moisture in lactic fermenter during the storage period.

Treatment	storage Period (d)				LSD value
	1	7	14	21	
Control	88.71	87.21	87.05	86.33	3.05 NS
T1	87.16	86.96	86.31	86.02	3.11 NS
T2	88.82	87.93	87.63	86.14	3.27 NS
LSD value	3.78 NS	3.22 NS	3.09 NS	2.17 NS	---
NS: Non-Significant.					

Acid Degree Value and Peroxide Value

The results in (Table, 7) showed the Peroxide Value (PV) which is an indicator of fat oxidation and Acid Degree Value (ADV), which is an indicator of fat rancidity, both leads to spoilage of foodstuffs with an unpleasant taste. Where PV immediately after manufacture, for control, T1 and T2 were 0.24, 0.24 and 0.24 meq/kg of fat There was a significant difference at least between the samples (P≥0.05). The cause of rancidity in the product is due to the lipase enzyme While ADV of the control, T1 and T2 were 0.13, 0.23 and 0.12 . That shows the treatment of LF fortified with emcapsulated FA was the lowest in terms of values until the last day of storage period, when it reached 0.37 meq/kg while in T1 it was 1.15 mmeq/kg . There was a significant difference at least between the samples (P≥0.05). Noticed from the obtained results that the LF fortified with standard folic acid increased rancidity in them, as the acidic nature of the vitamin worked to stimulate the lipase enzyme and thus the rancidity increased compared to the samples of LF fortified with encapsulated folic acid. We find that the encapsulation prevented the release of folic acid and thus restricted its Affecting action on the lipase enzyme as much as possible until the end of the storage period, where a rise in the lipid acidity values was observed as a result of the start of folic acid liberation from the sodium alginate capsule surrounding it.

Table (7): Effect of treatment and period in PV and ADV.

Treatment	storage Period (d)	PV(meq)	ADV(meq)
Control	1	0.24	0.13
	7	0.36	0.14
	14	0.55	0.16
	21	0.67	0.17
LSD value	---	0.205 *	0.056 NS
T1	1	0.24	0.13
	7	0.37	0.25
	14	0.75	0.49
	21	0.83	1.15
LSD value	---	0.268 *	0.441 *
T2	1	0.24	0.12
	7	0.36	0.18
	14	0.60	0.37
	21	0.70	0.39
LSD value	---	0.285 *	0.143 *

* (P≤0.05).
NS: Non-Significant

CONCLUSION

The fortification of LF with FA did not lead to a change in the chemical properties of LF, and therefore it is qualified to use FA as one of the nutritional fortification methods.

RECOMMENDATIONS

Manufacturing nanoparticles of folic acid and studying the effect of the size of the particles on the characteristics of the product and the necessary awareness of the importance of folic acid for all ages, especially the elderly, by increasing research that includes fortifying it with consumer food.

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