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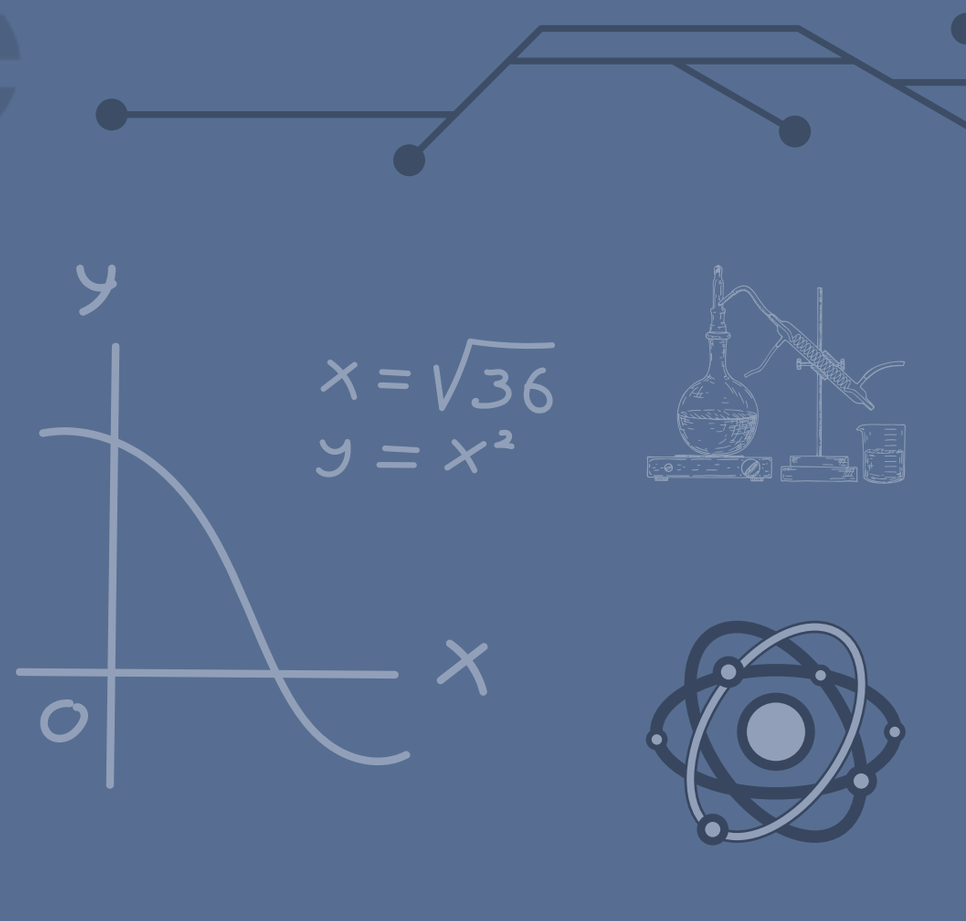

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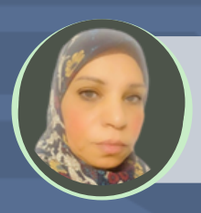

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PREFACE

The I. International Rimar Congress of Pure, Applied Sciences, was organized by Iğdır University in collaboration with Rimar Academy. The primary objective of this event was to compile and disseminate valuable scientific knowledge and make a meaningful contribution to the future.

Remarkably, a substantial number of researchers, both from local and international backgrounds, demonstrated their interest in this conference. The scientific committee meticulously reviewed the submissions and ultimately accepted a select group of individuals, totaling 67 applicants, 41 of them were accepted by the scientific committee.

The core of this conference was the presentation of 38 complete research papers in the Journal, while the remaining researches are publishing in this proceedings book.

I would like to extend my sincere appreciation to all the contributors and scholars who played an essential role in making this conference a resounding success. Your dedication and valuable contributions are deeply respected and acknowledged.

Editor-in-Chief
Prof. Dr. Ghuson H. MOHAMMED

Table of Contents

Utilization of Eucalyptus Honey as Antioxidant to Increase the Shelf Life of Local Cream

Ebtisam F. Mousa [1], Ajeena S. J. [2], Shaymaa Saady Lafta [3]

Abstract

The effect of eucalyptus honey addition on the safety and extend the shelf life of local cream was evaluated. Cream was manufactured by the corporation various concentrations of bee honey (0, 5, 10,15 and 20) % and kept under cool temperature (6 ± 1) °C for 15 days. Several variables were determined to assess the bee's honey influence samples with time of the storage on cream quality which those involved the total viable count, coliform, psychrotrophic bacteria, yeasts and molds as well as peroxide value, acid degree value (ADV), these tests were carried out at the age of 0, 5, 10 and 15 days. The cream sample of bee's honey contain (0%) was the highest sample in total viable count (TVC), psychrotrophic bacteria, coliform, yeasts &molds, PV, ADV while cream samples with 20% eucalyptus honey had the lowest total viable count, coliform, psychrotrophic bacteria, yeast and molds in addition to the PV, ADV. General and regardless of storage period, the microbial preparation and each PV, ADV decreased (P<0.05) when the high concentrations of bee honey was incorporated with the Cream product. Thus, the results showed, the bee honey could utilize as normal preservative to prolong the cream storage period for 15 days in cold storage.

Key Words

Characterization Of Honey; Coliform Bacteria; Antioxidant; Peroxide Value; Acid Degree Value; Dairy Product.

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Introduction

Natural and chemical food additives have been used since ancient times in the field of food preservation in order to improve sensory properties such as color, flavor, texture, and effectively contribute to the prevention of spoilage in its microbial, chemical and physical forms such as oxidation, rancidity and microbial spoilage etc. (JEGFA, 1994 ,Al-Aubadim, 2014; Khalil, A. H., and Sh. S. Lafta2023), honey can be one of them. Honey was one of the best natural nutrients for thousands of years and as a natural sweetener until the advent of sugar production in the fifteenth century (Álvarez-Suárez *et al*., 2010) as well as utilized as a natural antimicrobial and antioxidant food (Lambert *et al.,* 2012). Many studies reported the antimicrobial activity of honey against Gram positive and Gram negative bacteria, molds and yeasts, although, the natural preservative properties of honey are known but only several studies reported the use of honey in foods and beverages preservation (Makarewicz *et al.,* 2012; Mohammed *et al*., 2013, Ali *et al.,* 2016).

Honey has an important role as a natural antioxidant with reducing of food preservatives and sweet healthy product (Popova *et al*., 2007). Honey samples has total phenolic content including antioxidant levels such as catechin, caffeic acid, quercetin, gallic acid and ferulic acid was detected according toAl-Barazinji,Y.M.S. and Zainal,F.K.(2023). Honey includes different types of phytochemicals, in addition organic acids, vitamins, and enzymes which can as an antioxidant sources (Gheldo and Engesth, 2002; Gheldo et al., 2002).

Phenolic compounds found in honey are phenolic acids and flavonoids, which are potential signs of the botanical origin of honey (Küçük *et al*., 2007; Estevinho *et al.*, 2008). Phenolic antioxidant activities are associated with several mechanisms, including: free radical-scavenging, hydrogen-donation, metal ion chelating (Awada,J.M et al,2019) acting as a substrate for radicals such as superoxide and hydroxyl, and singlet oxygen, there is a remarkable relationship between antioxidant activity and phenolic content of honey and inhibition of lipoprotein oxidation in the human serum.It was found that intake honey causes a higher antioxidant effect in the blood than black tea, although its effect in vitro measured that the oxygen radical absorbing capacity (ORAC) activity was five times smaller than black tea (GHELDOF *et al*., 2003).

In general, the darker the honey, the higher its content of phenolic and its antioxidant capacity (GHELDOF *et al*., 2002; ESCUREDO *et al*., 2013; SANT'ANA *et al*., 2014). Researchers *Kačániová et al.* (2011) found that the phenolic compounds extracts of dark honey samples show greater antioxidant effectiveness than clear samples. This is due to variations in

the phenolic compounds profile that depend on the geographical origin honey (*flora predominance*), and according to other work it is stated that dark honey samples have phenolic compounds with higher microbiological inhibitors properties. In addition, to the strongest antiradical activities colour and translucence of honey have been related with pigment content, antioxidant characteristics and pendent particles such as pollen (Beretta *et al*., 2005).

The honey is widely used as an antimicrobial, gingivitis, gastrointestinal tract, skin infections and other diseases (Lebenia, 2004, Ebtisam et al., 2019, Rasheed and Alnuaimmi,2022) and used for wound healing, gastrointestinal and respiratory diseases, eye etc. (Fernandez, 2011). And is useful in reducing the risk of cardiovascular disease, cancer, cataracts and many inflammatory processes. Many researchers associate these properties with honey content of phenolic acids and flavonoids (Blasa *et al*., 2006; Beretta *et al*., 2005).

Objectives: Depending on health characteristics of honey.

In addition, health risks caused by chemical additives moreover, the high amount of money paid to purchase of chemicals for dairy products preservation that encouraged for utilizing natural honey as biological preservative in half and half cream Therefore, the goal of this article, the impact study of bee's honey at the shelf life of cream product based on its properties (antioxidant and antibacterial) like a food preservative, to enhance its shelf life and nutritional qualities.

Materials and Methods

Honey samples

The eucalyptus honey was taken from beekeeping places in Baghdad capital. They were utilized as found in concentration 100%.

Cream preparation

The raw cream was manufactured as 28 -35 % of fat in College of Agriculture (Dairy manufactory) at University of Baghdad. After applied the pasteurization, honey was mixed with cream treatments in the concentration of (0, 5, 10, 15, 20) % using homogeneous manner. These concentrations have been determined after the first sensory evaluation. The industrial antioxidant was added as 200 ppm to other cream treatment as a comparison sample.

Those samples contain:

- T_1: Control sample (0 %) honey
- T_2: Cream with honey (5 %)
- T_3: cream with honey (10 %)
- T_4: Cream with honey (15 %)
- T_5: Cream with honey (20 %)
- T_6: Cream with BHA (200 ppm)

Finally, the processed cream was tinned as well as kept at (1 ± 6) °C for fifteen days until microbiological analysis, peroxide value (PV), acid degree value (ADV) were applied in (0, 5, 10 and 15) day in cold temperature using refrigerator.

Microbial examination for honey cream

The media of nutrient agar was used for counting of total bacterial count and psychrotrophic bacterial count, counting of the coliform bacteria using MacConkey agar media. These tests applied by the pour-plate methods according to Harrigan (1998). The plates of total bacterial count, coliform and psychrotrophic bacteria were incubated at 37˚ C for 48 hours and 7 ˚±1 ˚C for 10 days respectively. And the enumeration of yeasts and molds was carried out using the Potato Dextrose Agar and incubated at 22 - 25 °C for 5 days. The viable bacterial colonies were counted by colony counter as a cfu unit / gram.

Peroxide Value (PV)

Peroxide value of the cream sample was determined according to the method described in A.O.A.C. (2000). 5gram of extracted was accurately weighed into 250 ml conical flask and dissolved in 30 ml of a mixture of solvent (60% glacial acetic acid and 40% chloroform). 0.5 ml of saturated potassium iodide solution was added with strong shake into 2 minutes and stored in a dark place for 5 minutes, after that 30 ml of the distilled water and 0.5 ml of the starch solution at 1% concentration was added, titrated with accurately standardized solution of 0.01N sodium thiosulphate. The titration was carried out with uninterrupted shaking until reached to end point. PV was calculated on the basis of the number of mille equivalents of sodium thiosulphate per 1000 g fat as in the following equation:

Peroxide value (meq/ kg fat) = milliliters of sodium thiosulphate x normality of sodium thiosulphate/ weight of fat sample (g)

Acid Degree Value (ADV)

The level of free fatty acids released as a result of hydrolysis of fat was expressed in the manner described by FRANKEL and TARASSUK (1955) by adding 25 g of the cream form to a test tube containing 5 ml of 95% ethyl alcohol .After sealing the tubing nozzle with a Teflon-lined stopper, the mixture was thoroughly shaken for 1 minute, then 7.5 ml of the mixture (40% ethyl ether and 60% petroleum ether) was added to the tube with re-shaken for 1 minute, then centrifuged was carried out using the SBV centrifuge and filled at 1500 rpm for 3 minutes of flow. 5 ml of the upper ether layer in the tube was pulled and transferred to a flask containing 15 ml ethyl alcohol (95%) with five drops of 1% alcoholic Phenolphthalein equivalent to pink and titration with standardized solution of 0.025 N Potassium hydroxide. Acid degree value of the fat was calculated as follows:

ADV = (The volume of base consumed in the titration with the sample - the size of the base consumed in the titration with the blank) / weight of sample (g)) x 100

Statistical analyses

Those analyses were carried out to investigate the influence of sample and shelf life on microbial preparation as well as each acid degree value (ADV), peroxide value (PV). Least significant difference (LSD) between the mean values of treatments (SAS, 2012).

Results and Discussion

Total bacterial count

The results in Table (1) showed a significantly decrease ($P<0.05$) in the (TVC) cream samples with an increase in the concentration of eucalyptus honey added compared to un-treated cream T1 (0% honey) during the refrigerated storage period, where the log. number of bacteria reached 6. 27 cfu/g in the un-treated cream after 15 days of storage period while the numbers were 4.51, 4.46 ,4.43 ,4.28 cfu/g in the samples of cream containing 5,10 ,15 and 20 % honey respectively and reached 4.49 cfu/g in the treatment of cream containing BHA compound. The 20% treatment gave the lowest TVC due to the level of bee's honey was increase.

These results are in agreement with other studies, when the honey was mixed with samples (cheese) as 15% honey that couldn't be beneficial as antimicrobial in cheese or other dairy products which are non-stabilized or long time for storage (Belewu and Morakinyo, 2009). In flavored ice cream made from camel milk with a mixture of vanilla and coconut using natural

ingredient (honey), the microbial count showed significantly (P<0.05) lower in total bacterial count (Ahmed and El Zubeir, 2015 Mohammed, M. F,2021).

The results of Mohammed et al. (2013) showed that beef sausage mixed with 2.5, 5 and 7.5% of bee honey levels decreased in TVC as 6.82, 6.64 and 4.98 log, and honey might be beneficial as antimicrobial in meat products which was unstable. Al-Jabri (2005) was also mentioned that blending of camel milk with honey might be good source for nutrition and protection of microbial infection. The results reported by Ali *et al. (*2016) showed that bee honey followed by bacterial supernatant at 30% concentration were the best in preservation all three kinds of fruit juices (apple, orange and pomegranate juices) for 21 days of storage at 4°C. Some of the antimicrobial activity created from the bees (the enzymes producing peroxide, the acids of honey, carbohydrates, defensin-1, compouns similar to antibiotic-) and some of the other created from the plants (methyl gly- oxal, polyphenols) while the third (maillard products) might be created through storage of honey (Bogdanov, 2011).

Table 1 - The microbial counts of cream samples in cold storage on 6 ± 1°C.

Treatments	Storage Periods	Total Viable Count	Coliform	Yeast & Molds	Psychrotrophic
T1	0	4.36	0.14	0	3.68
	5	4.62	0.90	1.84	3.99
	10	5.40	2.27	2.27	4.85
	15	6.27	2.55	2.55	5.20
T2	0	4.32	0.079	0	3.57
	5	4.41	0.30	2.07	3.74
	10	4.48	0	2.51	3.83
	15	4.51	0	2.75	3.90
T3	0	4.25	0	0	3.43
	5	4.38	0	0	3.49
	10	4.43	0	0	3.56
	15	4.46	0	0	3.59
T4	0	4.07	0	0	3.32
	5	4.21	0	0	3.43
	10	4.34	0	0	3.47
	15	4.43	0	0	3.51
T5	0	3.93	0	0	3.29
	5	4.08	0	0	3.30
	10	4.27	0	0	3.39
	15	4.28	0	0	3.44
T6	0	4.27	0.11	0	3.50
	5	4.39	0.84	0	3.65
	10	4.45	1.97	0	3.66
	15	4.49	2.23	0	3.68
LSD		* 1.059	* 0.683	* 0.588	* 0.942

* rate of triplicate. T1= 0 % honey (honey cream free), T2= cream contain honey (5 %), T_3 = Cream contain honey (10 %), T_4= Cream + honey (15 %) and T_5= Cream + honey 20 %).

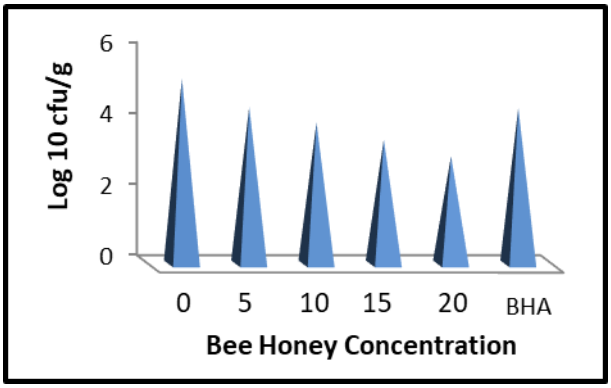

Figure 1- Influence of bee honey concentration in TVC period in TVC

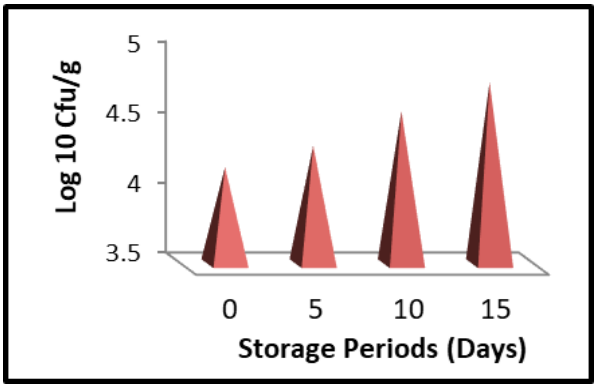

Figure 2- Influence of storage (log 10 cfu/g) of cream

Coliform Bacteria

The highest log. numbers of coliform bacteria was reported for the cream without eucalyptus honey (T1) followed by BHA treatment (T6), the results revealed the honey treated cream samples with 10, 15 and 20% have no coliform bacteria (table 1), these results supported the findings of Ahmed and El Zubeir (2015) they reported, there was not coliform bacteria growth in all ice cream treatments using natural ingredient (honey) during storage. Those study was agreement with Belewu and Morakinyo (2009), they found that the bacterial growth were prevented during the experimental periods when the 15% honey was added to cheese sample. In terms of coliform, total count, mold and yeast of ginger-honey cream (breakfast cream with 30 % fat) with ginger powder about 5% and honey about 30% was within standard range according to the microbial terms (Tajik and Jalali, 2014, Abdulkarem, K., A., Hasan, S., K.(2022).

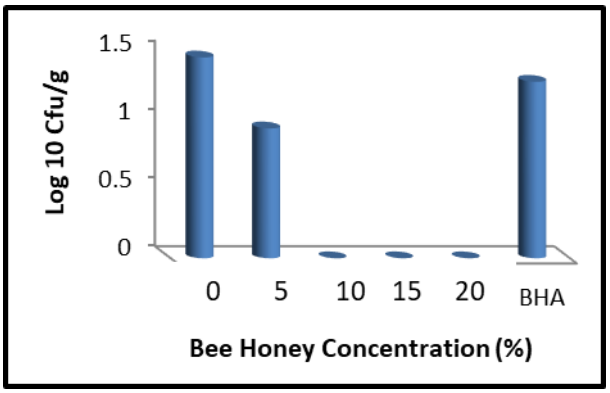

Figure 3- Influence of bee honey concentration of coliform count (log 10 cfu/g) cream

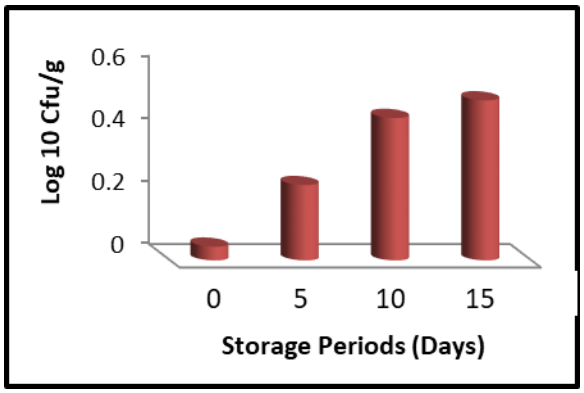

Figure 4- Influence of bee honey on coliform count (log 10cfu/g) cream

Furthermore, Mohammed et al, (2013) mentioned there aren't growth of coliform bacteria was noted in honey sausage samples with increased storage periods , whereas, the sausage sample which has 0% bee honey had the highest growth in PV, TVC, total coliform bacteria, E. coli, S. aureus and psychrotrophic during storage periods. However, sausage treatment has bee's honey about 7.5% was the lowest in all previous bacteria ($P<0.05$).

The major antibacterial effect of honey are related to the level of hydrogen peroxide, and non-peroxide antibacterial substances with different chemical origin, On the other hand, phenolics and flavonoids had prevented the growth a large number of bacteria included Gram-positive and Gram-negative (ESTEVINHO et al; 2008), the hight osmotic possibility of honey, due to its high concentration of sugar lead to collapse of the membranes of bacterial and so th microbial growth were inhibited (MUNDO et al., 2004). The low honey pH and also, aromatic acids, Millard products have an antibacterial activity. An antibacterial honey protein as defensin-1 .The honey bacteria make antibiotic-such as antifungal peptide compounds, like bacillomycin F (LEE *et al.*, 2008), and Lysozyme (LEON-RUIZ et al., 2013). The hard antibacterial effectiveness of Manuka honey was due to the existence of the methylglyoxal (Bogdanov, 2011).

Yeasts and Molds

Results of this study showed that no growth of yeast and mold was noticed in the honey cream treatments except for T2 treatment, which showed fungal growth after 5 days of refrigerant storage. Also there was growth in the un-treated creamT1 (Table 2), this may be due to possible contamination during labor or preparation of cream or refrigerated storage. It was also noted that cream containing honey(10,15, 20) % and industrial antioxidant BHA had a significant inhibitory effect of growth and production of toxins for different types of molds(Ahmed & Elmar,2004) Pure Honey has been noted to have inhibitory effects on growth of fungi and diluted honey appears able for inhibiting toxin manufacturing (Al-Waili and Haq,2004,Doosh,K. *et al*., 2016).

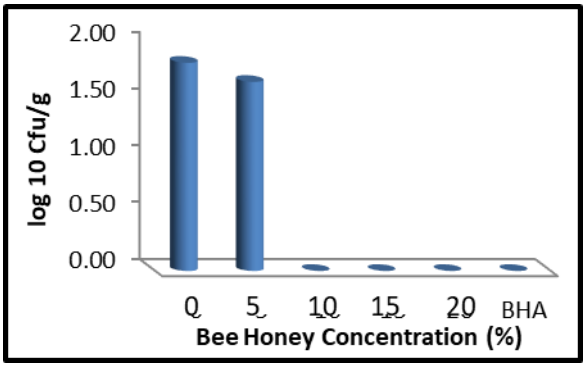

Figure 5- Influence the concentration of bee honey on duration on Yeasts and Molds in cream products

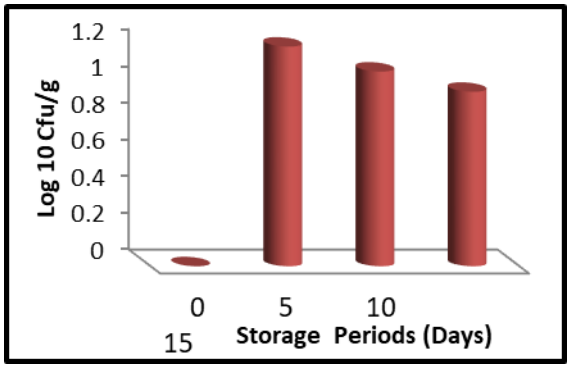

Figure 6- Influence the storage Yeasts and Molds of cream products.

Yeasts & Molds

The KOC *et al*., (2009) showed that honey models from various floral sources were evaluated for their capacity to prevent the growth of fourty strain of yeast (Candida albicans, C. krusei,Cglab- rata.and Trichosoporon spp.) and approved with KACANIOVA et al, (2011) who mentioned that various unifloral honey from Slovakia was also exhibited antifungal effectiveness against Penicillium crustosum, P.expansum, P. griseo -fulvum, P. raistrickii and P. verrucosum ,most of them had high concentration more than 10%. The fungicide effect of honey against *Candida albicans* is due to the effect of honey flavonoids (CANDIRACCI *et al*., 2011).

Psychrotrophic bacteria

As shown in Table (1), obviously the treatment T1 (0% honey) noted the highest count of Psychrotrophic bacteria, their log. numbers increased and reached 5. 20 cfu /g after 15 days of refrigerated storage, while there was decrease significantly ($P \leq 0.05$) in the log. number of bacteria with an increase in the concentration of honey added to the cream after 10, 15 days of storage period, where it was 20% honey (T5) reported the lowest count .The result herein was consistent with the data observed by Krushan *et al.* (2007) these authors observed that milk containing 50 mg\ ml honey stored at 4^0c had a significant inhibitory effect about 50% of the growth of bacteria compared to the samples of milk free of honey preserved in the refrigerator for 3-6 days. It was noted that the percentage of inhibition increased with the increased concentration of honey added.

The honey fortified with meat and poultry products was lead to decrease the growth of bacteria (MOLAN, 1992). The effect of honey flavour, antimicrobial and nutrition on processed turkey meat and also reduce lightly the account of bacteria thus promoting the shelf life stability

(EL-Sukhon *et al*., 1994). The finding by Lee et al. (1998) corresponded with those results that mentioned here, the honey perhaps beneficial for inhibiting the growth of bacteria in less stable of meat products that need long time of storage.

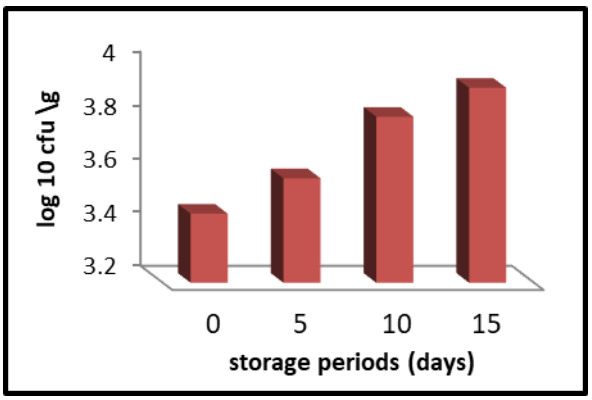

Figure 7- Impact the honey of bee proportion at duration on Psycho Psychotrophic bacteria (log 10 cfu/g) cream

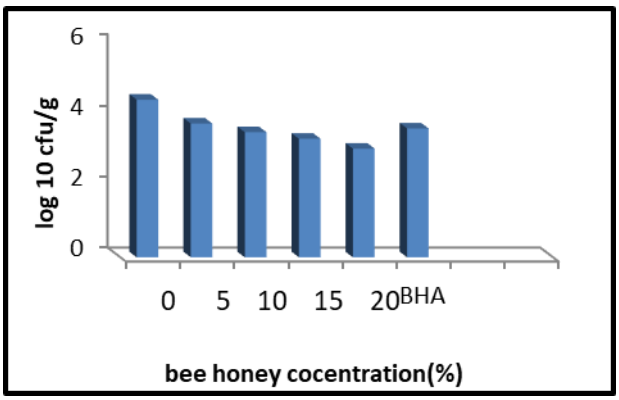

Figure 8- Psychotrophic bacteria (log 10 cfu/g) cream trophic bacteria (log 10 cfu/g) cream

Peroxide Value (PV)

Peroxide value of the un-treated cream T1 (0 % honey) was higher than all the treatments tested. However it's noted that, added eucalyptus honey to locally cream due to the decreased clearly (significantly P≤0.05) in PV value for the honey treatments (T2, T3,T4,T5) for all ages compared to un-treated cream, the PV values of honey treatments were less than 10 meq /Kg (fig. 8) ,these results were in accordance with The specification of Iraq device central standardization and control quality (1990) which text on that the peroxide value does not exceed 10 meq /Kg ,While the results showed that T2 and T6 treatments has slightly increased (no significantly P≤0.05) PV values at 15 days of storage (fig. 9), and the lowest PV was observed in the cream treated with 20% (T5) bee honey for all age (Table 2)

When the increase in honey level, the peroxide value was decline due to possability of presence the antioxidant compounds in honey. The Antioxidants properties of (Gharbia citrus and Minia cotton) honey samples as a result of the presence of phenolic and flavonids (EL-Kalyoubi, 2013). in addition, the Total phenolic componds such as quercetin, catechin, gallic acid, caffeic acid and ferulic acid was appreciated as an antioxidant in honey by Al Lawati et al, (2014). Those results were coresponded with the results of Antony et al. (2002), they referred that added of more than 15% of dry honey inhibited the evolution of oxidation compounds in processed turkey meat. Moreover, those findings were agreed with Mohammed (2006) who

found that the lowest PV was noted in meat sausage fortified with honey and who concluded that the decline in peroxide value with augmentation in bee's honey level.

Table 2- The Peroxide Value of cream samples in 6 ± 1°C.

Treatments	Store Duration (days)				LSD value
	0	5	10	15	
T1	4.85	12.87	34.07	42.89	23.67
T2	4.85	5.91	9.34	10.53	7.65
T3	4.85	5.59	9.96	9.02	7.35
T4	4.85	5.36	6.77	8.85	6.45
T5	4.85	5.23	6.63	8.56	6.32
T6	4.85	5.71	8.75	10.39	7.42
LSD value	4.85	6.78	12.58	15.04	---
LSD: Treatments: 11.224 *, Days: 10.575 NS, Treatments x Day: 18.63 * * (P ≤0.05)					

* The numbers = rate for 3 repeaters. (T_1= free honey cream, T_2= cream + (5 %) honey, T_3= cream + (10 %) honey, T_4= cream +(15 %) honey, T_5= cream +(20 %) honey.

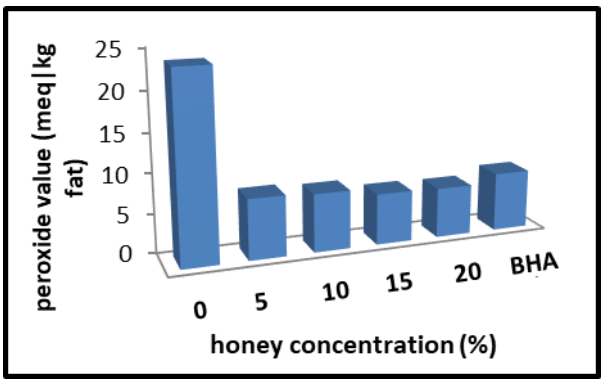

Figure 9- Impact of bee's honey concentration on PV duration on PV (meq. /Kg) cream

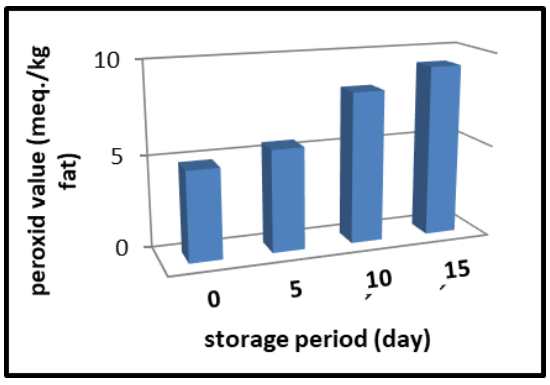

Figure 10- Impact of storage (meq. /Kg) cream

Acid Degree Value (ADV)

The impact of adding the honey to the local cream was studied with different concentrations 5, 10, 15 and 20% on the development of ACID DEGREE VALUE (ADV) in order to follow up the lipid degradation in the cream by the enzymes of milk-based lipases or enzymes for psychrophilic bacteria and the estimation of volatile short-chain free fatty acids responsible for the rancid flavor in the cream. Table (3) shows that the ADV value for all treatments were the same after the manufacturing process directly as at 0.4946 meq /100 g fat they are within the acceptable limits of the ADV value (The specification of Iraq device central standardization and control quality/cream, 1990).

But after five days of cold storage there was a significant increase (P≤0.05) in ADV values for T1 (un-treated) and T6 (BHA) cream treatments while the T2, T3, T4, T5 had the lowest values of ADV for the previous time period.This is due, of course, to the fact that honey added

to the cream, which contributed to prevent the development of the ADV caused by the action of lipases enzymes whether lipases originally found in milk before the manufacture of cream, including Lipoprotein lipases or the lipases enzymes of psychrophilic bacteria, the first enzymes inhibited by the cream pasteurized temperature,while the bacterium enzymes resist even the sterilization temperatures (AL-Ameri,2002, Salih,F.A. et al, 2017; Sadiq, I. H., and K. S. Doosh, 2019).) so they are mainly responsible for increasing of ADV values in the cream treatments during cold storage.

The addition of honey to the cream treatments has led to a significant decrease ($P \leq 0.05$) in the preparation of these bacteria, The presence of the antimicrobial factors in honey may be responsible for the low psychrotrophic bacteria counts in the honey cream treatments and thus control of ADV values, especially in the 20% honey cream treatment (T5) ,which obtained the lowest ADV values compared to the un-treated treatment (T1) in which there was significant evolution in ADV values for the same previous storage ages which became rejected according to the gradient adopted the values of the ADV ,where the number exceeded 2.0 meq / 100 g fat (The specification of Iraq device central standardization and control quality/ cream ,1990) at 15 days of storage for the un-treated cream treatment (Fig.10) , in such case cream samples be un accepted by consumers.

This rise in the ADV values due to the liberation volatile stumpy chain free fatty acids like butyric acid and caproeic in high concentrations those results are corresponded with Hakeem (2008), Doosh and AL-Mosawi (2010) Hussein, A. R *et al.* (2018). The impact of the storage duration on ADV of honey cream sample was displayed in fig (11), the highest value was at zero day during stored for 0, 5, 10 and 15 days and the ADV value was insignificant increased ($P>0.05$).

Table 3- The acid degree Value of the cream samples throughout storage in refrigerator at 6°C ± 1.

Treatments	(days)				LSD value
	0	5	10	15	
T1	0.4946	1.282	2.1489	2.94	1.716
T2	0.4946	0.7113	0.9342	1.4221	0.891
T3	0.4946	0.6943	0.8632	1.268	0.830
T4	0.4946	0.6509	0.8147	1.2079	0.792
T5	0.4946	0.6011	0.7721	1.0651	0.733
T6	0.4946	0.9725	1.1342	1.5891	1.047
LSD value	0.4946	0.8187	1.1112	1.5820	---
LSD: Treatments: 0.802 * , Days: 0.544 * , Treat. x Day: 1.176 * * ($P<0.05$)					

* The numbers = rate for 3 repeaters. (T_1= free honey cream, T_2= cream + (5 %) honey, T_3= cream +(10 %) honey, T_4= cream +(15 %) honey, T_5= cream +(20 %) honey.

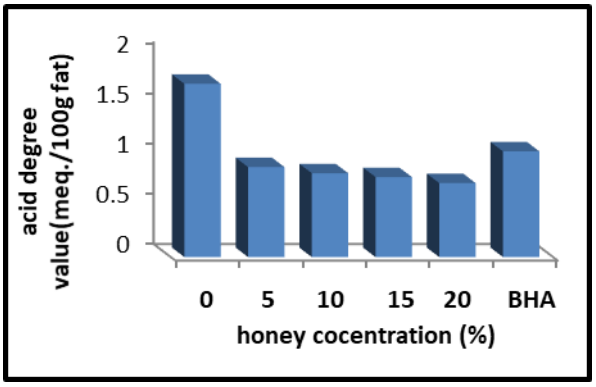

Figure 11- Impact of bee's honey concentration on storage period on ADV (meq/100 g fat) cream

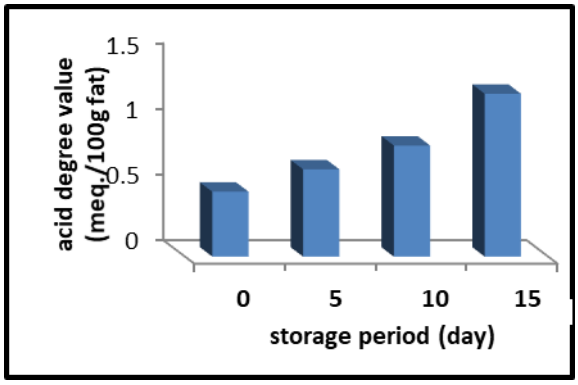

Figure 12- Impact of ADV (meq/100 g fat) cream

CONCULSIONS

Treatment of cream sample with honey resulted in reduce the growth or persistence of the total viable count, total coliform, yeasts & molds and psychrotrophic count, the honey cream was in the standard range according to the microbial terms. The utilization of bee honey in cream processing cause a high decline in peroxide and ADV value during the increasing in bee's honey proportions. The outcome of this project detected that honey could be utilized to improve quality and nutrition value of the products by adding healthy ingredient. Honey of bee could improve the cream properties that included storage the honey of bee cream at cold degree for 15 days, thus, it could utilize as a natural preservation to prolong its shelf life.

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Manufacture of Therapeutic Yogurt Enriched with Iron and Study Its Role in Some Health Indicators of White Mice

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Abstract

The present investigation was carried out with the intention of examining the effects of treating anemia by supplementing milk used for producing yogurt with both encapsulated and non-encapsulated iron. The current study included preparing three different yoghurt treatments: the first was produced as a positive control treatment (C+) with no additions, the second consisted of the addition of non-encapsulated iron to milk that was prepared for the manufacturing process with a concentration of 4 mg/100 ml (T1 treatment), and the third was added to the milk prepared for its manufacture encapsulated iron with sodium alginate at a concentration of 4 mg/100 ml to represent T2 treatment. Three groups of experimental mice in which induced anemia were induced, each group was fed on one of the three types of yogurt mentioned above in addition to a fourth group of mice they were left to feed on the standard diet to represent the negative control C -, The effect of iron fortification yogurt on health indicators related to anemia was studied, in addition to the effect on the rate of weight gain. The results showed that feeding on the standard diet and yogurt fortified with encapsulated iron significantly affected the rate of weight gain of laboratory animals more than yogurt fortified with non-encapsulated iron. Additionally, as compared to the control group C+ fed non-iron-fortified yogurt, C-, and T1, there was an increase in the concentration of transferrin and ferretin compounds, a percentage of iron in the serum, the average volume of red blood cells, the average amount of hemoglobin present in them, an analysis of the mean concentration of hemoglobin per globular, and platelet examination.

Key Words

Yogurt; Encapsulated Iron; White Mice; Anemia.

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Introduction

Due to its better nutritional content and increased human health, the usage of functional foods has expanded in recent years. Dairy products have become recognized as one of the most popular functional foods (Casmalla *et. al.*, 2017). Implementing functional foods primarily aims to boost immunity and lower the risk of long-term health issues such high blood pressure, cholesterol, atherosclerosis, and heart disease (Alzobaay, 2015). Functional foods are defined as those that include products that contain a modified food or any food ingredient that can give a health benefit in addition to its nutritional benefit (Ayat and Shakir, 2021). Fermented dairy and meat products, as well as other fermented meals, are some of the most well-known functional foods (Alzobaay, 2015). Foods that include iron, particularly supplements containing iron, are regarded as functional foods due to their positive impact on health (Al-Hadedee *et. al.*, 2019). Due to its widespread consumption, yogurt is one of the most popular dairy products worldwide (Radi and Doosh, 2023). Even though yogurt is a contemporary food, its history dates back thousands of years, to the time when cows, sheep, and goats first appeared on the scene. Although its exact origins are unknown, it is claimed to have originated in the Middle East (Khalil and Lafta, 2023). It is also believed that it was discovered about 5000 BC in Mesopotamia (Kosikowski *et. al.*, 1997).

Several studies have indicated that yogurt has several physiological and protective functions, including prevention of HIV infection, diabetes, obesity, prevention of urinary tract infections, cancer diseases, and lowering of blood cholesterol levels (Al-Mosowy *et. al.*, 2021). Iron deficiency is one of the most common and widespread nutritional disorders, according to the latest statistics. Conducted by the World Health Organization (World Health Organization, 2011), there are about 1.5 billion people suffering from iron deficiency worldwide. The most stable chemical element known to science, iron, is necessary for both animal and human existence. 4 to 5 g of iron are typically found in the bodies of properly nourished individuals (Abdullah and Alzobaay, 2019). It serves as a fundamental component of many organic molecules and enzymes found in all living things. Hemoglobin, for example, is made up of the heme chain, which has an iron atom in the center of an organic ring (Abbas and Shakir, 2021). Previous studies indicate that 20-45% of women of reproductive age and 70% of children in developing countries suffer from anemia (Al-Hadedee *et. al.*, 2019). Its deficiency causes a decrease in the rate of growth and poor cognitive degree in children, poor pregnancy outcomes and a reduced ability to work in adults and can also reduce enzymes and reactions in which iron participates as a cofactor (Powers and O'Brien, 2019). A non-significant iron deficiency would

also cause some Negative effects including cirrhosis, hepatitis, intestinal irritation, diarrhea, joint pain, hormonal disorder, heart disorder, and osteoporosis (Khairi, *et. al.*, 2020).

In order to find the best ways to compensate for the deficiency in this element in the food consumed and to fill the daily need of it, by fortifying the foods that are eaten in abundance, such as yogurt, which provides many micronutrients such as calcium as well as macronutrients such as carbohydrates, proteins and fats, which is a low-cost besides being a basic or semi-daily food for many people, the objective of the present research was to investigate the impact of consuming yogurt enriched with iron on several nutritional metrics. Red blood percentage, average volume of red blood cells, average amount of hemoglobin present in it, analysis of the mean concentration of hemoglobin per globular and platelet examination and an increase in the concentration of transferrin and ferritin compounds, the percentage of iron in the serum and the total binding capacity of the iron on mice with induced anemia.

Materials and methods:

Source of milk and iron:

Whole cow milk was acquired from the dairy manufacturing plants of the University of Baghdad, Department of Food Science/College of Agricultural Engineering Sciences. The powdered milk, a regale French brand that was utilized to change the amount of total solids in the experimental yogurt was purchased from Baghdad's local markets. Ferrous sulfate was used in both encapsulated and non-encapsulated forms as a form of iron. Sodium alginate is used in cold spraying techniques to encapsulate the iron.

Process of microencapsulation:

The process was carried out using the methodology outlined in (Nai *et. al.*, 2015) as follows:

1. For the preparation of encapsulated iron, a solution of 2 grams' sodium alginate in 100 milliliters of distilled water was used for the cold spraying. The mixture was stirred for 30 minutes at 100 rpm using a magnetic stirrer.
2. After the mixture had been in a shaker incubator for 15 minutes, ascorbic acid and ferrous sulphate were added (5mg/100ml), and the mixture was shaken for another 15 minutes at 100 rpm at 25C˚. The product was microencapsulated and refrigerated for three to four hours to solidify after the mixture was sprayed with $CaCl_2$ (for hardness). After removing CaCL2 (by washing it with water), the microcapsules were freeze-dried and kept in a freezer at -20°C. They were then rinsed with distilled water.

Functional yogurt manufacturing:

The manufacturing process for function yogurt was carried out following the method described by (Martirosyan and Singh, 2015). Three components were taken from a certain quantity of cow milk. The first part was free of any additions which represents the positive control treatment C+. The second part of the milk was prepared by fortifying it with non-encapsulated iron at a concentration of 4 mg/100 ml and adding 5 mg of ascorbic acid which represents (T1) treatment. The third component, (T2) treatment, was supplemented with 5 mg of ascorbic acid and 4 mg of encapsulated iron per 100 milliliters. The milk was heated to 90 °C for 10 minutes in each treatment, and then it was cooled to 42 °C. Starting cultures (3% each of Streptococcus salivarius, Thermophile s, and Lactobacillus delbruecki ssp. bulgaricus) were added and wrapped in 150 ml plastic containers. The samples were then incubated at 42±1°C until full coagulation (3.5 hours) or until the pH reached 4.6. After that, the samples were removed from the incubator and placed in a refrigerator to cool and be stored at (5±1) °C.

Laboratory animals:

Forty male white Albino BALB/C lab mice utilized in this investigation were acquired from Al-Nahrain University's animal house. The mice used were 3-4 months old. They were fed a standard diet for three days and placed in special cages made of plastic, under controlled conditions in terms of temperature and ventilation, which was within 25±2°C, and there were around 12 hours of darkness and 12 hours of light throughout the illumination period, and during the adaptation period of 3 days, the mice were provided with food and sterile water throughout the duration of the experiment. Table (1) demonstrate the proportions and components of the standard diet on which the laboratory animals were fed in the animal house, given that the animals were able to acquire food and water as required. The effects of both encapsulated and non-capsulated iron at a concentration of 4 mg/100 ml yogurt on anemia markers and the pace of weight gain were investigated.

Experiments on animals

At the beginning of the experiment, each animal was weighed individually to know its weight, and the hemoglobin level of all was measured to know the percentage of it in blood using a Haemometer by drawing 20 microliters of blood from the animal and adding it to the tube and then distilling it with a 0.1 M solution of HCL Until the color matched with the device and the percentage was read on the tube, then the animals were starved for three days at varying intervals for the purpose of causing malnutrition and artificial or induced anemia, after which the animals were grouped up into the following four groups:-

1. The first group is referred to as the negative control group (C-), where mice received only standard food throughout the trial.
2. The second group of mice, which represents the positive control C+, received a typical meal along with an oral dosage of 0.1 ml/day of control yogurt throughout the trial.
3. The third group, the (T1) Treatment mice, were given a regular meal along with an oral dosage of 0.1 milliliters of non-encapsulated yogurt fortified with iron each day for the course of the study.
4. The fourth group consisted of (T2) treatment mice, were given a typical meal along with an oral dosage of 0.1 ml of yogurt that had been enriched with iron each day for the duration of the trial.

Nutrient Requirements of Laboratory Animals

The American Institute of Nutrient Requirements of Laboratory Animals' nutritional and physiological requirements were followed in preparing the mice's regular meal, which includes the following ingredients as shown in (Table 1): -

1. Casein free of vitamins.
2. The mixture of metals was prepared in the laboratory according to (Al-Badri, 2021).
3. The mixture of vitamins prepared from Coli-Vita Company.
4. Sucrose from local markets.
5. Corn oil from local markets.
6. Corn starch from local markets.

Table1- Ingredients and proportions of the basic diet used for feeding laboratory mice.

Contents	gm/kg
Casein	200
Corn oil	70
Celluse and fiber	50
Melt vitamins	10
Minerals	35
Colin	2
Corn starch	46
Sucrose	200

Blood sample collection:

After the experiment, the mice were fasted for around 8 hours, after which they received an injection of ketamine (including Xylazine) into a muscle to induce anesthesia, and their hearts' blood was extracted using a proper syringe. One milliliter of blood was transferred into sterile, dry test tubes along with a solution of Ethylene diamine tetra acetic acid (EDTA), an anticoagulant. Standard blood tests were performed on this sample.

Biochemical Analysis

- Complete blood count (CBC): An automated analyzer was used to perform this test (Ayat and Shakir, 2021).
- Estimation of the number of red blood cells by means of a test (RBC): For the detection of the red blood cells number, hemoglobin Hb levels in the blood was carried out according to Ayat and Shakir article (2021).
- Estimation of the percentage of red blood cell volume (PCV): Following the methodology outlined in Ali and Shaker article (2023), the percentage of red blood cell volume (PCV) was estimated.
- Estimation of the volume of red blood cells (mean corpuscular volume MCV): Measuring the mean amount of hemoglobin in one red blood cell, mean corpuscular hemoglobin (MCH). Inside these cells and their small size leads to a decrease in the proportion of hemoglobin (Hillman and Ault, 2002).
- Estimation of the mean corpuscular hemoglobin concentration (MCHC): To estimate the mean corpuscular hemoglobin concentration and plt, as mentioned by Al-Mosowy *et. al.* (2021).
- Estimation of ferritin: Ferritin, which is the main form of iron stored in cells, was estimated according to the method mentioned in Hallberg *et. al.* article (1993).
- The estimation of transferrin: The carrier of iron in the blood, was estimated following the method mentioned by Hallberg *et. al.* article (1993) according to the following equation:

$$\text{Transferrin\%} = \frac{\text{S-iron}}{\text{Tibc}} \times 100$$

- Estimation serum–iron (S-iron) and total iron binging capacity (TIBC): Serum–iron (S-iron) and total iron binging capacity (TIBC) was estimated according to Ayat and Shakir article (2021).

Results and Discussion

Study of the effect of feeding on yogurt fortified with non-encapsulated iron and encapsulated iron on average weights of mice:

After 20 days from the initiation of the trial, the data shown in table (2) demonstrate the impact of yogurt fortified with encapsulated and non-capsulated iron on the degree of final weight increase. Four groups of mice were served standard diets: the first group received only the standard die which represented the negative control (C-) group, the second group received oral doses of yogurt without any additions which represented the positive control (C+) group, the third group received oral doses of yogurt fortified with non-encapsulated iron (T1 treatment), and the fourth group received standard diets plus oral dosing of yogurt enriched with encapsulated iron (T2 treatment).

The data indicates that the (T2) group, which gained 9.349 g of weight after 20 days in mice with induced anemia, and the (T1) group, which gained 8.099 g, had the greatest rates of weight increase. These findings were greater than those of the C+ group, which showed a 6.068 g increase, and C-group, which gained 3.576 g. The diet of C+, which is high in protein and essential minerals like calcium, is the cause of these variations in findings between C- and C+, which helped in gaining more weight, and the differences between (C- and C+) and (T1 and T2) due to the iron in the yoghurt which improved the nutritional importance of yogurt, which made it an integrated source for treating anemia.

Also, from the results, encapsulated iron is more efficient than non-encapsulated iron in helping to regain weight faster.

These results are consistent with Rasheed, Luti and Alaubydi aryicle (2020), which reported that pigs gained much more weight when they are given iron supplements compared to the control treatment that was fed on bread iron supplements, which led to correcting anemia and balancing the physiological mechanisms of the digestive system and weight. According to the statistical analysis, there was a significant difference ($P<0.05$) between the T2 treatment and C- and C+ after 20 days of the trial, however there was no significant difference ($P<0.05$) in the average original weight of the mice.

Table 2- The average weight growth of the mice in each group after 20 days.

Treatment	Initial Weight Rate (G)	Weight Rate After 20 Day (G)	Weight Gain (G) After 20 Days
C-	24.987	28.563	3.576
C+	29.180	35.248	6.068
T1	30.628	38.727	8.099
T2	30.550	39..899	9.349
:LSD value P < 0.05	**6.17NS**	*** 8.04**	*** 3.617**

Investigation of the impact of consuming yogurt supplemented with both encapsulated and non-encapsulated iron on several blood markers:

1. **The concentration of hemoglobin (Hb**).

The Hb test was used in accordance with the procedure outlined by Beutler and Waalen (2006) as a reliable measure of iron bioavailability. The effects on hemoglobin levels in yogurt supplemented with encapsulated iron and non-encapsulated iron are shown in Table (3). Following a three-day period of starvation and 20 days following the experiment's beginning, the findings show a significant difference ($P<0.05$) in hemoglobin concentration, with (T2) treatment showing the highest level of rise at 20 days, and this increase was significant at the level of ($P<0.05$) compared with the C- treatment of 2.34 g/cm^3 As for the T1 treatment, it amounted to 7.61 g/cm^3 of blood and the C+ treatment of 2.46 gm/cm^3 of blood. The high percentage of hemoglobin Hb is due to the increase in the concentration of iron in the blood due to the fortification of yogurt with quantities of encapsulated and non-encapsulated iron, as Fe enters the composition of hemoglobin, which It consists of heme, which is iron, and globin, a protein (one molecule of iron binds 4 globin protein molecules (Ayat and Shakir, 2021).This is consistent with what was found (Haro-Vicente *et.al.*, 2008) of an increase in hemoglobin Hb concentration after feeding mice with fruit juice fortified with different iron salts after 21 days.

According to the statistical analysis, after 20 days of the experiment, outcomes demonstrate a significant difference ($P<0.05$) in the mean of hemoglobin concentration between different treatments, however, there was no significant difference ($P<0.05$) in the mean of the initial hemoglobin concentration.

Table 3- The rate for variation in hemoglobin Hb concentration over a 20-day period in groups of mice receiving various treatments.

Treatment	Primary hemoglobin (g/dl)	Hemoglobin level after 20 days (g/dl)	Decrease in hemoglobin after 3 days (g/dl)	Increase in hemoglobin after 20 days (g/dl)
C-	10.16	12.62	4.25	2.46
C+	10.66	13.00	2.37	2.34
T1	9.63	17.24	4.73	7.61
T2	10.16	18.58	4.31	8.42
LSD value P<0.05	**1.27 NS**	**4.55 ***	**2.09 ***	**2.81 ***

2. The count of Red blood cells (RBC).

Red blood cells are globules in the form of concave discs, with a thin wall and no nucleus. They contain hemoglobin. Food must contain iron because it is part of hemoglobin anemia (Nai *et. al.*, 2015). The effects of yogurt enriched with encapsulated and non-capsulated iron on the total number of red blood cells (RBC) in laboratory animal blood are shown in Table (4).

The number of RBC in T2 was 8.49×10^{6} \ mm^{3} compared with the T1 represented by the yogurt fortified with non-capsulated iron, which was 7.77×10^{6} / mm 3 while the positive control C+ was 7.37×10^{6} / mm 3 and the lowest mean of the number of red blood cells was in the C-، which amounted to 6.89×10^{6} / mm3. As iron is one of the key elements involved in the development of red blood cells, a reduction in the number of red blood cells might be caused by a lack of production in the bone marrow, where iron is released from stores and increased iron absorption from the digestive canal increase the biosynthesis of red blood cells (Al-Mosowy *et. al.*, 2021).

Following 20 days of the trial, the statistical analysis outcomes show a significant difference (P<0.05) in the total number of red blood cells between (T2) treatment compared to other treatments.

Table 4- levels of every red blood cell, the volume of the stacked cells, the average volume of a red blood cell, the average quantity of hemoglobin contained in a single red blood cell, the measurement of the mean corpuscular hemoglobin concentration, and the blood count of the mice treatment groups following 20 days.

Treatment	RBC Cell/mm^3	%PCV	MCV femtoliter	MCH picogram	MCHC gm/dl	PLT Platelet/mcL
C-	6.89	38.86	40.5	14.8	34.7	408
C+	7.37	40.00	42.7	14.2	35.6	453
T1	7.77	52.72	43.2	15.7	36.1	578
T2	8.49	56.74	47.2	15.5	36.1	628
LSD value p<0.05	**1.07 ***	**4.72 ***	**5.61 ***	**2.83 NS**	**3.78 NS**	**74.91 ***

3. The percentage of red blood cell volume (PCV):

The packed cell volume (PCV) was 56.74% for the T1 indicating an elevation in comparison to 52.72% of the T2 treatment, and the 40% of the positive control treatment C+. The lowest PCV was in the C- treatment, which amounted was 38.86%. After 20 days of the trial, the statistical analysis findings show a significant difference (P<0.05) in PCV between (T2) treatment and the other two treatments, C- and C+.

4. Mean corpuscular volume (MCV):

The results in table 4 indicate a significant difference (P<0.05) in MCV after 20 days of the experiment in the groups of the treated mice, being the C- and C+ group, the T1 group (the yogurt is fortified with the non-capsulated iron) and the T2 group (the yogurt is fortified with the encapsulated iron), the results were 40.5, 42.7, 47.2 and 43.2 femtoliter, respectively.

5. Mean corpuscular hemoglobin (MCH):

Table (4) presents the statistical analysis findings, which show that after 20 days of the trial, there was no significant difference (P<0.05) between (C-) negative control, (C+) control, and the (T1) treatment. (the yogurt is fortified with the non-capsulated iron), and the T2 treatment (the yogurt is fortified with the encapsulated iron), as they were 14.8, 14.2, 15.5 and 15.7 pg., respectively.

6. Mean corpuscular hemoglobin concentration (MCHC):

The results of the statistical analysis in Table 4 shown that there was no significant difference (P<0.05) after 20 days of the experiment between the C-, C+, and the T1 treatment

(the yogurt is fortified with the non-capsulated iron), and the T2 treatment (the yogurt is fortified with the encapsulated iron), as they were 34.7, 35.6, 36.1, and 36.1 gm /dl respectively.

7. Platelets (plt):

The results shown in Table 4 illustrate the effect of feeding on yogurt fortified with the iron and with encapsulated iron on the number of platelets. The results indicate an increase in the number of platelets in the group of T1-treated mice (the yogurt is fortified with the non-c

apsulated iron), as it was 628× 10^3 / mm^3 compared with the group of T2-treated mice (the yogurt is fortified with the encapsulated iron), which was 578×ˣ10^3 \mm^3. In turn, these two results were higher when compared with the C- and C+ treatments that had a PLT count of 408 × 10^3 and 453× 10^3 /mm^3, respectively. The increase in PLT after the end of the experiment is thought to be caused by the possibility of counting the small-sized red blood cells that lack some chromosomes as platelets (Al-Badri, 2021). Statistical analysis reveals a significant difference ($p < 0.05$) in the assessed platelet count between (C+) and (C-) control treatment groups and the other mice groups.

8. Ferritin concentration:

Ferritin is mostly a cytosolic protein, but it is also released into the bloodstream in trace levels that correspond to the body's iron reserves (Cook, Flowers and Skikne, 2003). Plasma ferritin can be used to determine the differences that reflect non-heme iron levels in the liver and spleen better than other blood indices (Smith *et. al.*, 1984).

Table (5) presents the results of a study on the impact of yogurt supplemented with both encapsulated and non-capsulated iron on blood ferritin levels. Following 20 days, the findings demonstrated a significant difference ($P<0.05$) in the ferritin concentrations across the various treatments; the group of mice treated with (T2) had the highest ferritin concentration, 15.52 ng/ml. This is a substantial rise ($P<0.05$) in contrast to the (C-) and (C+) concentrations of 11.56 and 12.28 ng/ml, respectively. The value for the T1 treatment was 14.55 ng/ml. The results of the statistical analysis also indicate that there is a significant difference ($P<0.05$) between the iron fortified treatments (T1 and T2) and the control treatments (C+ and C-). It is noticeable that ferritin values increased in treatments (T1 and T2) that were fortified with iron in comparison to the control treatments. This proves the role of iron in increasing the levels of ferritin absorbed by the body as has (Rasheed, Luti and Alaubydi, 2020) indicated that when the mice were fed with iron-fortified milk, the increase in ferritin content was higher compared to the negative control group of mice.

Table 5- The levels of ferritin, transferrin, iron in serum and total iron-binding capacity of groups of different treatments mice after 20 days

Treatment	Ferritin ng/ mL	Transferrin µg/Dl	S-iron µg/mL	Tibc µg/dl
C-	11.56	16.19	68	420
C+	12.28	16.33	66.8	409
T1	14.55	17.97	81.8	455
T2	15.52	17.98	83.8	466
LSD value P<0.05	**2.39 ***	**2.51 NS**	**7.33 ***	**32.75 ***

9. Concentration of transferrin:

Transferrin is a glycoprotein that binds to iron in the plasma and controls the level of iron in the biological fluids. Following 20 days, the results in table (20) demonstrated a significant difference (P<0.05) between the various treatments, with the (T2) group of treated mice exhibiting the highest amount of transferrin concentration (17.98 micrograms/deciliter). This increase is significant (P<0.05) when compared with the C- treatment and the C+ treatment, having 16.19 µg/dL and 16.33 µg/dL, respectively. As for treatment T1, it reached 17.97 µg/dL.

The statistical analysis reveals a significant difference (P<0.05) between the control treatments (C+ and C-) and the iron-fortified treatments (T1 and T2). It is noticeable that the transferrin values increased in treatments (T1 and T2) when compared with the control treatments, this proves the role of iron in increasing transferrin levels. As (Abbas and Shakir, 2021) mentioned, feeding anemic mice diets containing iron supplements Fe^{+3} or Fe^{+2} increased the levels of transferrin in the serum compared to the control group. Also (Hallberg *et. al.*, 1993) mentioned that there was a significant difference (P<0.05) in transferrin levels between the groups of infants and young children who were fed with iron-fortified milk compared with the control group.

10. Serum iron and the total iron-binding capacity (Tibc)

The serum iron test aims to measure the amount of iron that passes into the body, that is, the iron that binds to transferrin in the blood. Table (5) demonstrates the impact of yogurt enriched with encapsulated and non-capsulated iron on blood iron concentration levels.

Notably, there is a statistically significant difference (P<0.05) between the various treatments. At the 20-day mark, the (T2) group of treated mice experienced the highest level of iron concentration in their serum, measuring 83.8 µg/ml. This represents a significant increase (P<0.05) over the levels of the control treatments (C- and C+), which amounting to 68 µg/ml and 66.8 µg/ml, respectively, and 81.8 µg/ml for the treatment T1.

The statistical analysis results show a significant difference (P<0.05) between the (T1 and T2) treatments and the control (C+ and C-) treatments. Notably, the levels of iron increased in the iron-supplemented (T1 and T2) treatments when compared to the control treatments, demonstrating the role of iron in raising serum iron concentrations.

The effects of yogurt enriched with encapsulated and non-capsulated iron on the overall iron binding capacity are shown in Table (5). When comparing the Tibc levels in (T2) treatment to those in the control treatments (C- and C+), which amounted to 420 µg/dL and 409 µg/dL, respectively, there was a substantial rise (P<0.05) to 466 µg/dL. The (T1) treatment's Tibc was 455 µg/dL.

The statistical analysis's findings show that there is a significant difference (P<0.05) between (T1 and T2) treatments and the (C+ and C-) control treatments. It is clear from the results that there is an increase in iron concentration in (T1 and T2) treatments that were supported with iron when compared with the control treatments and this proves the role of iron in increasing the total iron-binding capacity.

Conclusion

From the results that were reached, it can be said that fortification of yogurt with encapsulated iron effectively contributed to restoring the weight of mice that caused induced anemia, in addition to raising the level of hemoglobin in blood, the number of red blood cells, the level of compacted volume of cells, the ratio of both ferritin and transferrin, and contributed to the elimination of Anemia is more effective than non–encapsulated iron, so we recommend manufacturing this inexpensive therapeutic yogurt and giving it to people who suffer from anemia.

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Studying activity silver Nanoparticles of Arirthrospira platensis In Some Biofilm Bacteria

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Abstract

Arirthrospira platensis was conducted at the University of Kufa, Faculity of Science / from November 2021 to February 2022 . Escherichia coli and pseudomonas eroginosa are among the diagnosed bacteria that are obtained from Al-Amin Research Center and advanced biotechnology in the holy city of Najaf. The antibacterial activity of three concentrations (100, 150, and 200 mg/ml) of each extract is assessed in three replicates using the spreading fine agar method.The average diameter and three-dimensional structure of the A. platensis silver nanoparticles were measured using an atomic force microscope (AFM). The average diameter was found to be 2.07. Silver nanoparticles that were biosynthesized shown antibacterial action against the study's microorganisms.

Key Words

Silvernanopracticles; Spirolina; Biofilmbacteria

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Introduction

The blue-green bacterium Arirthrospira platensis grows best in alkaline environments (Mao et al., 2005). It is used as a food agent because of its high carbohydrate content, high nutritional value, and presence of essential fatty acids, carotenoids, quality protein, complex vitamins, vitamin E, copper, manganese, magnesium, iron, selenium, and zinc. It also produces a wide range of secondary activities that carry out the normal activities of cyanotoxins, including hepatotoxins, neurotoxins, cystotoxins, cutaneous toxins, and toxic irritants (Wiegand and Pflugmasher, 2005).

The antibacterial activity of A. platensis was investigated by Barzkarand et al. (2019) in Italy, who discovered that the presence of phycocyanin, lipids, and short peptides could be associated to the activity of the plant (Akbarizare et al., 2020) explore the anti-cancer biological main metabolite predictions from cancer cell. A. nigrum is a naturally occurring source of agrochemicals that are useful for controlling fungus and pests. (Bruneton, 1995)

According to Parthasarathi et al. (2011), nanotechnology is regarded as the fourth industrial revolution in the history of human civic progress. It works with a wide variety of material types, such as metals, ceramics, polymers, and biomaterials.. It has already made significant contributions to the advancement and development of a number of goods, including clothing (Filipowska et al., 2011), medical devices and diagnostics (Yi-binet al., 2015), pharmaceuticals (Vijayakumar and Menakha, 2015), food and agricultural products, and sunblocks, sunscreens, and antibiotics.

Numerous varieties of nanomaterials (NM), such as carbon nanotubes (CNT), bimetallic composites (BMC), and metallic and non-metallic nanoparticles (NPs), were characterized (Sharma et al., 2017).

For the synthesis of nanoparticles (NPs), a range of physical and chemical processes are used, such as vapor deposition, irradiation, laser ablation, sol gel, sono-chemical method, co-precipitation, and other techniques (Goad & Hamad, 2017). These methods are inefficient in terms of money and release harmful substances.

Materials and methods:

Biogenic AgNO3 nanoparticles: characterisation through biology .Antimicrobial activity is one of the techniques used to characterize the biological properties of AgNO3 nanoparticles.

Silver nanoparticles' antibacterial properties: AgNO3 nanoparticles, which are biosynthesized from samples, were tested for their antibacterial efficacy against various

pathogenic bacterial strains that were identified and identified by the Al-Ameen laboratory located in the city of Al Najaf.

Antibiotics and antibiotics susceptibility test: Tests for antibiotic susceptibility and antibiotics. Six antibiotics were employed in the current study: Trimethoprim 5, Cefixime 10, Doxycycline 10, and Meropenem 10 mcg in addition to Cephalexin 30 and Oxacillin 10. A few bacterial colonies were put into test tubes with 5 milliliters of distilled water, according to Hombach et al. (2017). These were then compared to the standard turbid McFarland in paragraph 2.10, which provides an approximate number of cells (1.5 x 108) cell / ml. Muller Hinton Agar was ready and cleaned. The sterile plates were filled with 20 milliliters of the sterilized media, which was then left to solidify at room temperature. Each plate was individually infected with a bacterial specimen using a sterile cotton swab.

The antibiotic disks were placed on the Muller Hinton Agar medium using sterile forceps, and they were then incubated for 24 hours at 37°C. Following incubation, measure the inhibition zones and compare them with (CLSI 2020) to determine the impact of antibiotics on bacterial growth..

Biosynthesis of silver nanoparticles using A.platensis using optimal condition to product: A. platensis is used in the biosynthesis of silver nanoparticles under ideal conditions. Green synthesis and characterisation of silver nanoparticles produced by A. platensis The synthesized materials in the current investigation were distinguished by color change. After being incubated in a dark environment for 24 hours, the mixture's color changed to a reddish brown as seen in photos (1-3). In the current investigation, the proportion of nanoparticles that were produced after AgNO3 a reacted with A. platensis extracts was superior.

Results and Discussion

The antibiotic susceptibility:

The Clinical and Laboratory Standards Institute (CLSI 2020) disc diffusion method was utilized to determine which bacteria were sensitive to which species. Antibiotics in contemporary medicine, antibiotics are overused and misunderstood. Antimicrobial resistance is the most urgent problem, as it poses a serious threat to public health (Ares et al., 2013), which highlights the importance of plants.

We used the Clinical and Laboratory Standards Institute (CLSI 2020) disc diffusion method to identify the species-specific sensitivity of the bacteria antibiotics. Antibiotics are overused and misunderstood in modern medicine.According to Ares et al. (2013), antimicrobial

resistance is the most pressing issue because it seriously jeopardizes public health and emphasizes the significance of plants.

Characterization of biogenic silver nanoparticle:

A- SEM analysis: The findings revealed that the silver nanoparticles in A. platensis were homogenous and well-dispersed, with a diameter of (5) nm. The majority of the particles had spherical forms (1). The ability of each plant to biosynthesize silver nanoparticles varied, as shown by the findings of the SEM characterisation of the particles. produce associated with Abdulhassan (2016) and Aldujaili et al. (2015).

B- Atomic Force Microscopy: The shape, average diameter, and roughness of the silver nanoparticles that were biosynthesized from the two microorganisms were shown by the AFM image.As a result, the final structure's size and shape may be adjusted using the current density and etching time. The average diameter of the silver nanoparticles that were biosynthesized from A. platensis was 10 nm. The silver nanoparticle's granularity accumulation distribution charts and three-dimensional photographs were displayed in Figure (2).

Table 1- Antibiotics used in current study with their manufacturer and origin

No.	Antibiotic name	Icon	Concentration	Manufacturer name
1	Cefixime	CFM	10 mcg	Bioanalyse
2	Cephalexin	CL	30 mcg	Bioanalyse
3	Doxycycline	DO	10 mcg	Bioanalyse
4	Meropenem	MEM	10 mcg	Mast group LTD
5	Oxacillin	OX	10 mcg	Bioanalyse
6	Trimethoprim	TMP	5 mcg	Biolab

Table 2- The antibiotic sensitivity test against studied bacterial isolates

ANTIBIOTIC / **BACTREIA**	**Meropenen**	**OXACILLIN**	**TRIMRTHOP**	**DOXY-CYCLINE**	**Ceftriaxone**
E.faecalis	S	R	S	S	R
s.aereus	S	R	S	S	S

Figure 1- SEM Micrograph of silver nanoparticles synthesized **(by** A-*A.platensis*)

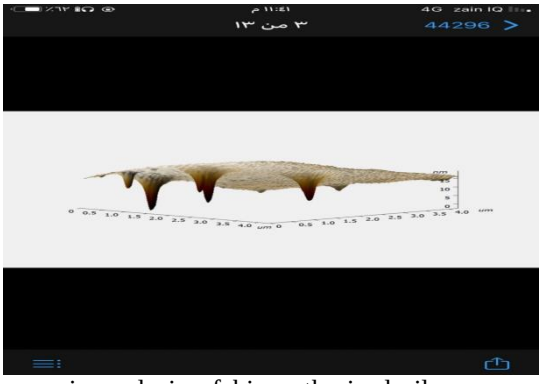
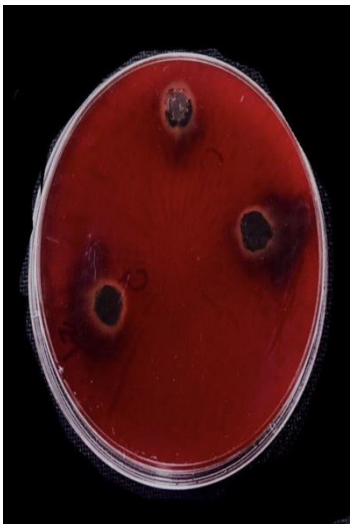


Figure 2- Atomic Force Microscopic analysis of biosynthesized silver nanoparticle from *A.platensis (*three dimension of biogenic silver nanoparticles)

Antimicrobial activity of nanomaterial's

Silver nanoparticles fabricated by.*A.platensis* AgNPs found to be inhibition by *S.aereus* inhibition zone *15.6* , *E.feacalis* inhibition zone was 14.4.

inhibition zone15.4



E.feacalis inhibition zone13

Figure 3-The use of nano silver is placed with the zone of inhibition for each bacteria in *A.platensis*

This outcome is consistent with research done by Usharani et al. (2015), who found that the highest zone of inhibition against S. aureus was demonstrated by an ethanolic extract of A. platensis. Additionally, Parisi et al. (2009) discovered that phenolic compounds isolated from A. platensis using ethanol have strong antibacterial action against S. aureus. More lipids are found in the walls of Gram-negative bacteria than Gram-positive bacteria, and these lipids influence the inhibition of the bacteria by preventing the active chemicals from penetrating the bacteria (El-deen, 2011).

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