

## Study of some characteristics of Mannoprotein bioemulsifier produced from a local isolate of *Saccharomyces cerevisiae* strain jzt351 and its use in the manufacture of ice cream

Jaffar Z. Thraeib 1 , Alaa Jabbar Abd Al-Manhel1, Ammar B. Altemimi 1

1Department of Food Science, College of Agriculture, University of Basrah, Basrah 61004, Iraq

### Abstract

In this study, the local isolate of *Saccharomyces cerevisiae* strain jzt351, isolated from vinegar starter in the Al-Khiam factory in the city of Basra/Iraq to produce the mannoprotein bioemulsifier. Some characteristics of the bioemulsifier were studied, and after conducting wetting and dilution tests, it was found that it is a type of oil in water bioemulsifier (O/W), in addition to studying the stability of the bioemulsifier towards different conditions of pH, temperature, and salts by used three methods to test the effectiveness of the bioemulsifier, which are oil displacement, emulsifier effectiveness, and emulsification index (E24), as mannoprotein is characterized by its high stability in light of these. the components of the bioemulsifier were estimated and it was found that it contains 91% of mannose sugar and 3.5% proteins. To know the effect of this bioemulsifier on food systems, it was added to ice cream at different concentrations and compared with industrial emulsifiers (monoglycerides), and then the chemical and physical properties characteristics of the manufactured ice cream were studied. It was found that the best effect was for the ice cream to which the bioemulsifier was added at a concentration of 0.2%, and this was confirmed by sensory evaluation of the ice cream samples.

**Keywords:** Mannoprotein, oil displacement, emulsifier effectiveness, bioemulsifier, ice cream.

### Introduction

Bioemulsifiers are surface-active biomolecules produced by microorganisms such as bacteria, yeasts, and fungi[1]. These molecules contain hydrophilic and hydrophobic sides, and thus they work to disperse oil droplets in water, making them molecules with wide applications in the food industry,

pharmaceuticals, cosmetics, and other applications [2]. The danger of industrial emulsifiers to consumer health and their high costs have led orientation researchers to produce emulsifiers from natural sources [3], bioemulsifiers are characterized by having characteristics that are similar to or superior to

industrial emulsifiers, which are non-toxicity and ability to Biodegradation, efficiency at low concentrations, and high stability over a wide range of pH, salinity, and temperatures [4]. Accordingly, these emulsifiers have been introduced into many foods, such as bread, salad dressing, biscuits, cakes, ice cream, and others[5]. Bioemulsifiers have high molecular weights because they consist of a complex mixture of heterogeneous polysaccharides, lipopolysaccharides, lipoproteins or proteins [6]. *Saccharomyces cerevisiae* is one of the industrial microorganisms with wide applications [7, 8], It has a cell wall, the outer layer of which constitutes a high percentage of mannoprotein, which has a major role to set the shape of cell as well as other physiological functions [9]. Mannoprotein is a bioemulsifier glycoprotein with a molecular weight ranging from 14,000 to 15,800 daltons, found in the outer wall of *Saccharomyces* spp. and *Kluyveromyces marxianus*, and can be extracted by thermal or enzymatic treatments, and this bioemulsifier has the ability to stabilize oil-in-water (O/W) emulsions [4]. The structural composition of mannoprotein consists of mannose sugar polymers covalently bonded to proteins and constitutes 30-40% of the dry weight of the cell wall[10]. [11] showed that mannoprotein has several applications in the food industry. It has been used as an emulsifier, stabilizer, and

biopreservative. In addition, mannoprotein derived from yeast has antioxidant properties due to its high content of proteins and carbohydrates [12]. While [13] showed that the difference in the structural properties of mannoprotein is due to the use of different extraction methods and various sources of yeast, which makes it have various functions in the food industries. In addition, [14] researched The possibility of producing this bioemulsifier from agricultural waste such as cassava husk and rice bran, which makes it an environmentally friendly bioemulsifier. Based on the above, the current study aimed to use the Mannoprotein emulsifier produced from a local isolate of the yeast *Saccharomyces cerevisiae* strain jzt351 in the manufacture of ice cream and to study its physical, chemical, and sensory properties.

#### Materials & Methods

Analytical chemicals were used from the Swiss company Fluka, the German Merck, and the English BDH. The media were prepared by the Indian company Himedia. In a previous study, the yeast *Saccharomyces cerevisiae* strain jzt351 was isolated from vinegar starter in the city of Basra/Iraq, which was registered in the bank. Gene number (OR115510) for the production of bioemulsifier Mannoprotein. The liquid (YEPG) medium was used to grow yeast to produce the bioemulsifier by using volumetric flasks with a capacity of 250 ml

containing 100 ml of the medium. It was inoculated by adding 2 ml of activated yeast and placed in a refrigerated shaking incubator at a speed of 150 rpm at a temperature of 28°C for 48 hrs, then the Mannoprotein was extracted according to what was mentioned by [15]. The crude Mannoprotein precipitate was collected using centrifugation at a speed of 10,000 rpm, then dried and stored at room temperature.

Emulsifier type test

Wetting test

The wetting test was conducted according to what was shown by [16], by placing a drop of the emulsifier on the filter paper, as the droplet remaining on the paper indicates that the emulsifier is water in oil (W/O) type, while the dispersion A drop on a paper indicates that its type is oil in water (O/W).

Dilution test

To conduct the dilution test, follow the method of [16], by adding a drop of the emulsifier to a test tube containing water and oil. The drop being dispersed in the oil and remaining in the water indicates that the emulsifier is the water-in-oil (W/O) type, while the emulsifier is of the oil-in-water (O/W) type, if the drop is dispersed in the water and turns cloudy (foggy) while remaining in the form of the drop in the oil.

Emulsification effectiveness test

The emulsification effectiveness of the Mannoprotein was tested by following three tests:

.1Oil Displacement Test

This test was conducted according to the method described by [17], by adding 50 ml of distilled water to a Petri dish with a diameter of 150 mm, then adding 50 microliters of crude oil to it, after which 100 microliters of the cell-free filtrate was added to the center of the membrane formed in the dish, the diameter of the corona formed was then measured after 30 seconds.

.2Estimation of emulsifier effectiveness

3 ml of the bioemulsifier solution prepared by dissolving 0.08 g of the bioemulsifier in 3 ml of distilled water, according to [18], was mixed with (0.5) ml of olive oil in a test tube and mixed using a mixer (Vortex) for a period 2 minutes and left for 1 hour in an incubator at a temperature of 37°C, then the plank was prepared with the same previous steps without adding the bioemulsifier. After that, the absorbance reading of the tubes was recorded in the spectrophotometer at a wavelength of (400) nanometers, the increase in absorbance is evidence of increased emulsification effectiveness [19]. [

.3Emulsification index test (E24)

The emulsification index was estimated according to the method described by [17], by mixing 2 ml of cell-free filtrate with 2 ml of

sunflower oil in a test tube and shaking with a mixer (Vortex) for 2 minutes and leaving at room temperature for 24 hours. The emulsification index E<sub>24</sub> was calculated as follows:

$E_{24} = \frac{\text{height of the emulsifying layer formed}}{\text{total height of the mixture}} \times 100$ .

Stability test of Mannoprotein bioemulsifier

.1 Testing the stability of the bioemulsifier within various pH ranges

Prepared 10 volumetric flasks with a capacity of 100 ml containing a solution consisting of dissolving 2 g of the dried bioemulsifier in an amount of distilled water and the volume was completed to 100 ml, then the pH was adjusted to (3,4,5,6,7,8, 9,10,11,12) each separately by using a 1 M solution of HCl acid or NaOH base. After that, emulsification effectiveness tests were conducted to determine the stability of the Mannoprotein bioemulsifier within this range of pH [20]

.2 Testing the stability of the bioemulsifier at various temperatures

This test was conducted according to what was mentioned by [21], using three volumetric flasks with a capacity of 100 ml containing a solution consisting of dissolving 2 g of dried bioemulsifier with an amount of distilled water, and the volume was completed to 100 ml and incubated at a temperature of (63°C for 30 minutes), (100°C for 15 minutes), and (121°C for 15 minutes), each separately,

then cooled to 30°C. After that, emulsification effectiveness tests were conducted to determine the stability of the bioemulsifier [20]. [

.3 Testing the stability of the emulsion at different salt concentrations

Prepared three volumetric flasks with a capacity of 100 ml containing a solution consisting of 2 g of dried bioemulsifier with an amount of distilled water, and the volume was completed to 100 ml, to which sodium chloride (NaCl) was added at a concentration of (0,1,2,3)% each separately. After that, emulsification effectiveness tests were conducted to determine the stability of the Mannoprotein within this range of salt concentrations [21]. [

Determination of the percentage of proteins and sugars in the (Mannoprotein(

Estimation of sugar content

The phenol-sulfuric acid method mentioned by [22] was adopted to estimate the percentage of total sugars based on the mannose sugar in the Mannoprotein, which included using different volumes of the standard mannose solution (100 µg/ml) and then adding it in test tubes. It included (0, 0.2, 0.4, 0.6, 0.8, 0.9, 1) ml, then the volume of each tube was completed to 1 ml using distilled water, then 1 ml of 5% phenol solution was added to each tube with shaking using a Vortex device, then added Add 5 ml of concentrated sulfuric acid

solution to each tube and shake it again, then leave it to cool to room temperature. The previous process was repeated, replacing the standard mannose sugar with a 120 µg/ml Mannoprotein solution in a volume of 1 ml. The spectrophotometer reading for each tube was recorded at a wavelength of (490) nm. Finally, the relationship between the prepared concentration and spectrophotometer reading was drawn..

#### Estimation of protein content

The percentage of protein in the Mannoprotein was estimated using the method of [23], which included preparing different volumes of a solution of the standard protein albumin (bovine serum albumin) BSA at a concentration of 0.0125% in test tubes that included (0, 0.1, 0.3, 0.5, 0.7, 0.8, 1) ml and complete the volume to 1 ml with distilled water, then add 4 ml of the solution including (a mixture of 2% solution of potassium sodium tartrate with 1% solution of copper sulphate in a ratio of 1:1 (v/v) and mixed with 2% carbonate solution Sodium at a ratio of 50:1 (v/v) with vigorous shaking using a Vortex device and leaving for 10 minutes. After that, 0.4 ml of Folin's reagent was added and also mixed and left for 30 minutes. This process was repeated with the replacement of bovine serum protein with Mannoprotein solution of 125 µg/ml in a volume of 1 ml.

Then, the spectrophotometer reading of the prepared tubes was recorded at a wavelength of 600 nm.

Adding the bioemulsifier Mannoprotein to milk ice cream

The method mentioned in [24] was followed to test the effectiveness of the bioemulsifier (Mannoprotein) in the manufacture of ice cream, with some minor modifications. Four models of ice cream were manufactured (A, B, C, D) with the basic components. treatment(A) representing which consists of 8% fat, 11% non-fat solids, 12% sucrose, 0.2% carboxymethyl cellulose, 0.05% vanilla, to which 0.2% monoglyceride (artificial emulsifier) is added and the volume is completed with water to 100 ml, while The same components of the mixture were used except for replacing the commercial emulsifier with the bioemulsifier at different concentrations, including 0.05% (treatment B), 0.1% (treatment C), and 0.2% (treatment D). After that, the treatments were mixed individually using an electric mixer and then pasteurized at a temperature of 80 °C for 30 seconds and left in the refrigerator overnight at a temperature of 5 °C. After that, the mixtures were added to the ice cream maker, then they were packed in appropriate containers and chemical, physical and sensory tests were conducted.

Chemical tests for ice cream

The percentage of protein in ice cream manufactured was estimated according to indicated by [25] using the formalin method, and the percentage of fat was estimated according to the Kerber method which was mentioned by [26], while the percentage of ash was estimated by the gravimetric method mentioned in [27] which depends on determining the loss in weight of the samples. After incineration at a temperature of 550 °C, the total solids and acidity were estimated as mentioned by [28] . A pH meter from the English company (elle) was also used to measure the pH of ice cream.

#### Physical tests for ice cream

##### .1 Melting resistance rate test

25grams of ice cream samples were placed in the freezer at a temperature of -18°C for half an hour, then placed on a metal clip installed in a funnel that drained into a graduated cylinder placed on top of a scale at a temperature of 22°C and Constant moisture, and after the drop fell The first was to record the weight of the melted ice cream at intervals of (10, 20, 30, 40, and 50) minutes. The mass of melted ice cream was recorded as a function of time during melting and plotted as the percentage of original mass [29– 31].

##### .2 Spreadability test

The spreadability test was conducted according to what was mentioned by [32], by

forming the ice cream sample into cubes with a size of 2 cm<sup>3</sup> in the freezer at a temperature of -30 °C for half an hour, after which they were placed directly on a graduated ruler up to 30 cm in a tray, and a small glass slide was placed on top of the sample and a 100 g weight was placed, after which the distance of spread of the ice cream was measured every two minutes for 12 minutes through the included ruler.

#### statistical analysis

The statistical analysis of the results obtained was conducted using a Completely Randomized Design (CRD). The data were analyzed using an Analysis of Variance (ANOVA) table according to the statistical analysis program SPSS ver. 22 The significant differences between the averages of the coefficients were compared using the least significant difference (L.S.D) at the probability level of 0.05.

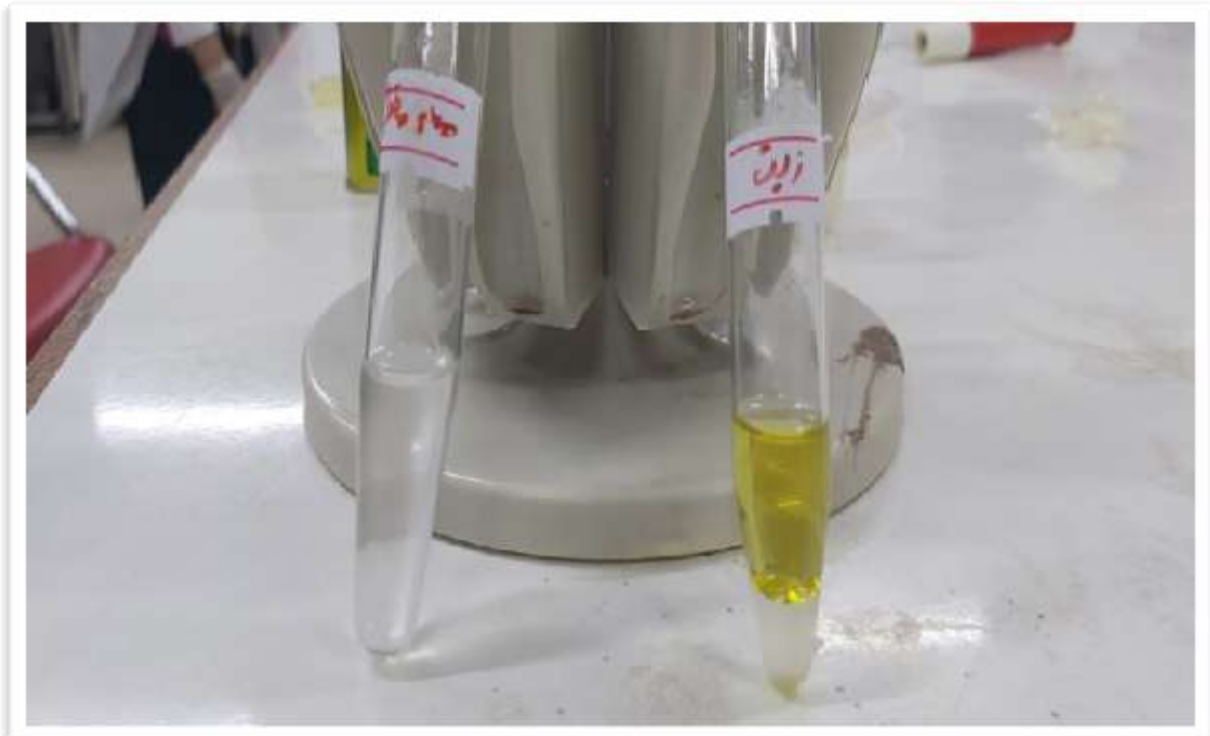
#### Results and discussion

##### Type of bioemulsifier

The results of the test of type the bioemulsifier produced by the yeast of *S.cerevisiae* strain JZT351 confirmed that it is of the oil-in-water (O/W) type, through the dispersion of the drop of the bioemulsifier falling on the filter paper in the Wetting test, which indicates that the hydrophilic part In the bioemulsifier it is more effective than the other lipophilic part, in addition to what was confirmed by the results

of the dilution test when the Mannoprotein bioemulsifier drops were dispersed in the distilled water tube and the appearance of the water became foggy, while the bioemulsifier drops when they fell on the oil medium did not disperse, but were deposited at the bottom of [

the oil tube. Due to gravity, as shown in Figure (3), this indicates that the bioemulsifier is of an oil-in-water (O/W) type, as the solvent with which the emulsifier is dispersed corresponds to the external phase of the emulsion [33- 36



Stability test of Mannoprotein bioemulsifier

Figure (3) results of the dilution test to determine the type of bioemulsifier  
 Stability of Mannoprotein bioemulsifier to change in pH  
 The results of the emulsification effectiveness tests for the Mannoprotein bioemulsifier

shows a wide range of pH numbers ranging from (3-12) , as shown in Figure (4) The highest value for the E24 emulsification index test was recorded at the pH numbers (7,6,5), It reached

39.70%, and there was a slight decrease at pH numbers lower and higher than these numbers. The lowest value was recorded at pH (12), where it reached 27.91%, with a remaining effectiveness of 95.49%. As for testing the effectiveness of the emulsifier, although there were significant differences ( $p < 0.05$ ), there were no significant differences between the average values. The highest value for this test was recorded at pH (5), which reached 0.97 nM, and the lowest value was for pH (12), which reached 0.69 nM, with effectiveness. The remaining effectiveness was 90.72%, and with regard to the oil displacement test, there were slight differences between the averages of the test values, and there were no significant differences ( $p > 0.05$ ) for the analysis of variance table. The maximum oil displacement was reached at pH numbers (7, 6, 5), as it reached 8.69 cm per When the lowest displacement was recorded at pH (12), it reached 7.05 cm, with a remaining effectiveness of 90.91%. The results were

consistent with what was reported by [37] in that the bioemulsifier Mannoprotein produced from *Kluyveromyces marianus* yeast was highly stable within a range of pH from (3-11) for 24 hours, and also with what was found by [38] that the highest range of pH stability for the Mannoprotein bioemulsifier produced from *S.cerevisiae* yeast was at pH (5-8). The researcher indicated that the slight decrease in the stability of the emulsifier at low or high levels of pH is due to precipitation the bioemulsifier at these extreme ranges. [39] also noted that the bioemulsifier produced from *Yarrowia lipolytica* yeast was characterized by high stability within a range of pH ranging from (3-9) for a period of 24 hours, and the results also agreed with what was shown [40] showed that the bioemulsifier Mannoprotein produced from *S.cerevisiae* yeast showed high stability towards different pH values ranging from (2-10) and continued to maintain its effectiveness for a month of storage



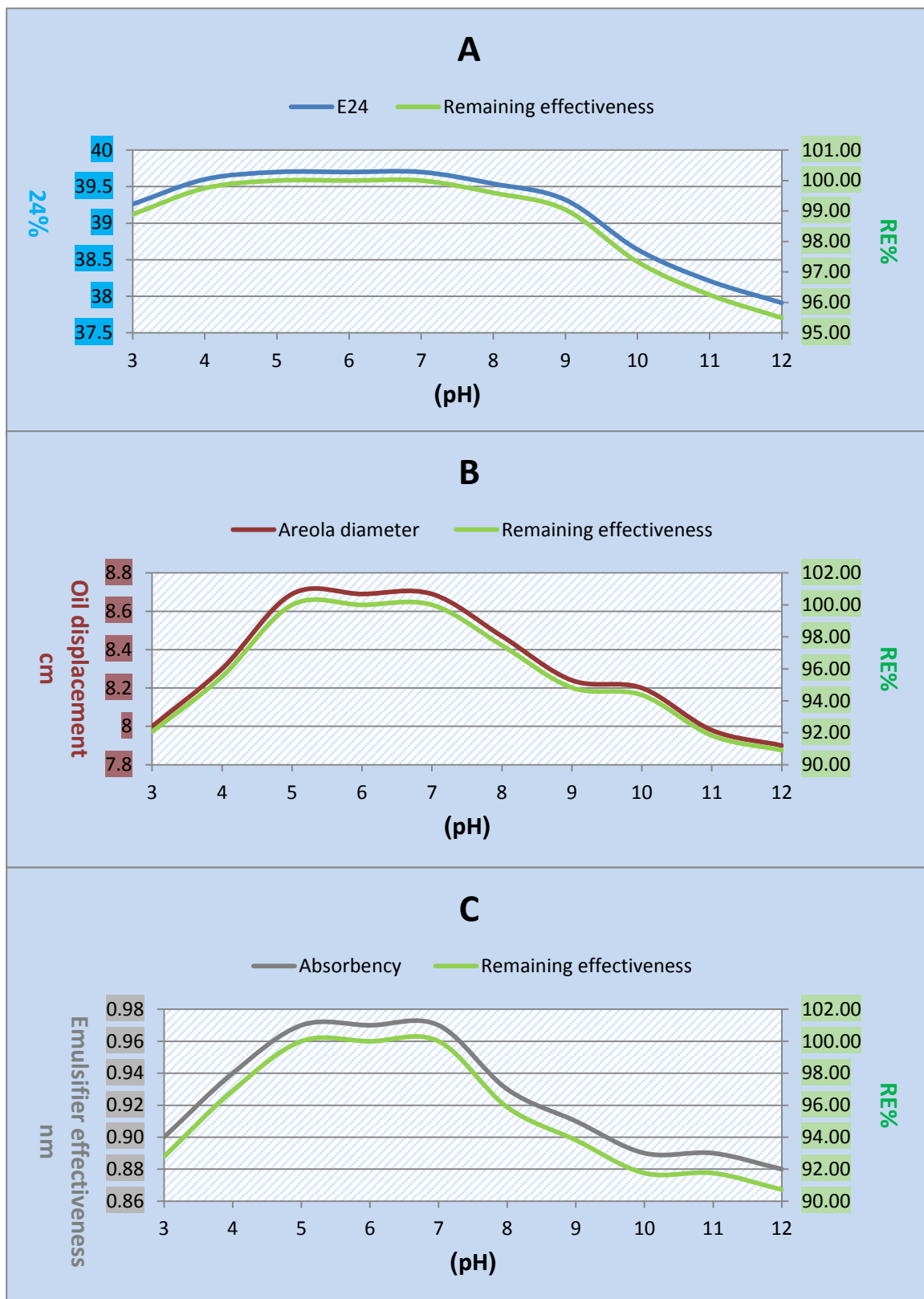


Figure (4) Stability of the Mannoprotein bioemulsifier towards a wide range of pH  
 A: E24 emulsification coefficient, B: Oil displacement, C: Emulsifier effectiveness

## Stability of Mannoprotein bioemulsifier to changes in temperature

The stability of Mannoprotein bioemulsifier to changes in temperature was tested by incubating it at different temperatures, which included 63°C for 30 minutes, 100°C for 15 minutes, and 121°C for 15 minutes. Then, emulsification effectiveness tests were conducted. The results shown in Figure (5) indicated that the values of the emulsification effectiveness tests were close, indicating the high stability of the emulsifier in different temperature ranges. The variance table analysis indicated that there were no significant differences ( $p>0.05$ ) for the emulsification effectiveness tests. The highest value of the emulsification index (E24) was at a temperature of 63°C reached 39.70%, and the lowest value was at a temperature of 121°C reached to 38.53%, with a remaining effectiveness of 97.05%. As for the highest oil displacement, its highest value was recorded at a temperature of 63°C as well, and it reached 8.69 cm, while it was the lowest value. At a temperature of 121 °C, it reached 8.32 cm, with a remaining effectiveness of 95.74%. The results of the effectiveness of the emulsifier were identical to the two previous tests, as its highest value at 63 °C reached 0.97 nM, and its lowest value at a temperature of 121°C reached 0.92 nM, with an effective remaining 94.84%.

The reason for choosing the three temperatures (63,100,121)°C is due to the food being exposed to them during various thermal treatments such as pasteurization, cooking, and sterilization, which require the stability of foods containing emulsifiers. On the other hand, [41] referred to the stability of the bioemulsifier produced from the fungus *Aureobasidium pullulans* RYLF10 was resistant to different ranges of different temperatures. This was attributed to the low hydrolysis at different temperatures, while very high temperatures lead to denaturation of the protein part of the emulsifier and affect its functional properties. The results obtained agreed with what was found by [16] on the stability of the Mannoprotein bioemulsifier produced from *Saccharomyces cerevisiae* KA01 yeast towards different temperatures. This stability was attributed to the presence of effective groups that work to reduce the hydrolysis of the bioemulsifier, as well as to the results reached by [40] A study of the effect of different temperatures, including (4,30,50,70,100,121)°C, on the stability of the bioemulsifier Mannoprotein produced from *S. cerevisiae* yeast, as the researcher noted that the bioemulsifier retained its full effectiveness at all the temperatures studied.

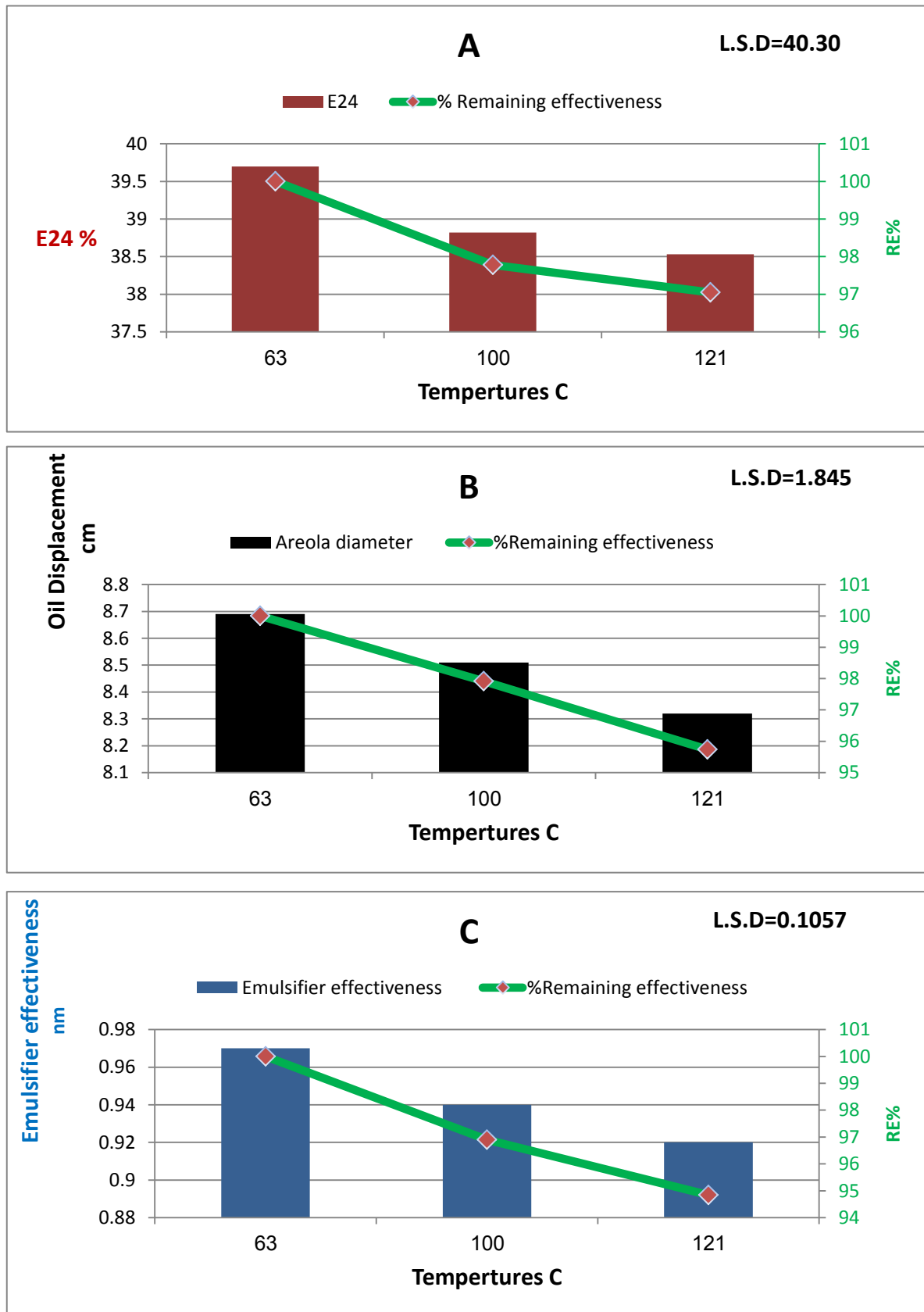


Figure (5) Stability of the bioemulsifier Mannoprotein for a range of thermal treatments

A: E24 emulsification index, B: oil displacement, C: emulsifier effectiveness

Stability of Mannoprotein bioemulsifier to the addition of different concentrations of salts

For the purpose of knowing the stability of the Mannoprotein bioemulsifier when adding different concentrations of salts, the effectiveness of the bioemulsifier was estimated after adding concentrations (0, 1, 2, 3)% of sodium chloride salt NaCl, and the results shown in Figure (6) the high stability of the Mannoprotein, as the analysis of variance table indicated that there were no significant differences ( $p>0.05$ ) for the emulsifier effectiveness tests, and that the lowest value for this test was at the concentration of 3%, and the remaining effectiveness reached 96.9%. Also, the values were close, and this indicates that the bioemulsifier is not affected by salt concentrations. The results of the E24 emulsification index values did not differ at concentrations (0,1,2)%, reaching 39.70%, while it decreased at a concentration of 3% to 39.56%, with a remaining effectiveness of 99.64%. As for the oil test, it also reached the lowest value at a concentration of 3%, with a remaining effectiveness reached 98.38%, and the results agreed with what was observed by [41] regarding the stability of the bioemulsifier produced from the fungus *Aureobasidium pullulans* RYLF10 when using

different concentrations of salts that ranged between 0-3%, but the emulsifier effectiveness began to decrease as it increased time and salt concentration, as indicated by [37] indicated that the Mannoprotein produced from *Kluyveromyces marianus* yeast was highly stable within a wide range of different concentrations of sodium chloride (NaCl) (2-50) g/L, and also with what [42] mentioned that the Mannoprotein It is stable towards salts at (3-50) g/L, while [21] indicated that adding high concentrations of calcium chloride salts CaCl<sub>2</sub> led to a decrease in the effectiveness of the E24 emulsification index for the Mannoprotein, while the emulsifier effectiveness showed the high stability of the bioemulsifier towards The different concentrations For other salts, and [43] stated that the reason for this decrease is due to the decrease in electrostatic repulsion between the emulsion droplets as a result of the decrease size of the Ca<sup>2+</sup> ion, which leads to a change in the protein adsorbed layer in the emulsion. It also agrees with what was found by [40] in the Mannoprotein bioemulsifier produced from *S. cerevisiae* yeast, as he observed high stability of the emulsifier towards different concentrations of sodium chloride salt, ranging between 2-10% for a period of two weeks.

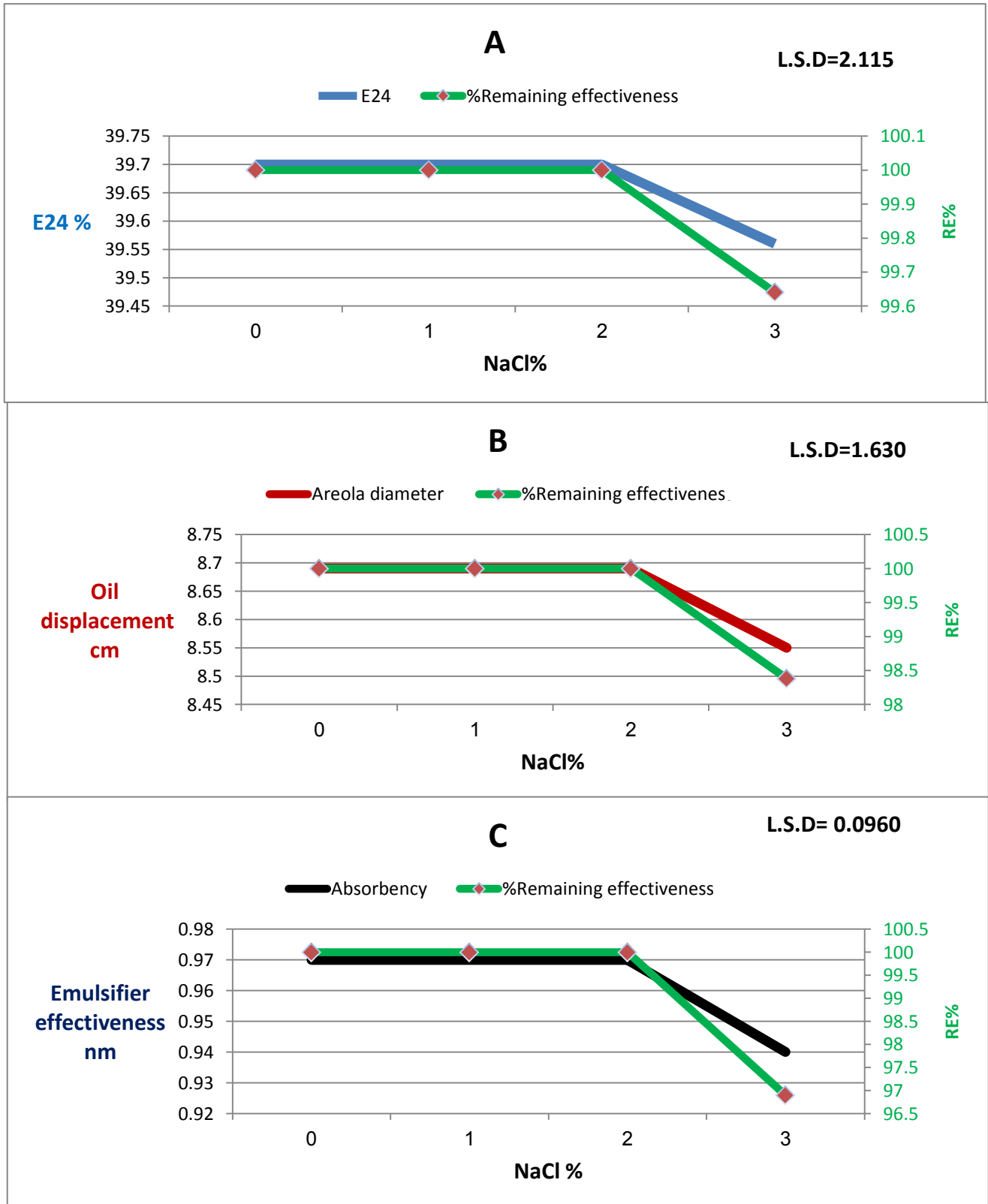


Figure (6) Stability of the Mannoprotein when different concentrations of salts are added

A: E24 emulsification index, B: oil displacement, C: emulsifier effectiveness

Determination of the percentage of proteins and sugars in the bioemulsifier Mannoprotein

The percentage of sugars in the Mannoprotein (on the basis of mannose sugar) and the percentage of protein in the Mannoprotein bioemulsifier produced from the local yeast of *S.cerevisiae* strain JZT351 was estimated. Their percentage was 91% sugars and 3.5% protein. This result was close to what was reported by [20], who found that the percentage of The carbohydrates in the Mannoprotein were 96% and 4% protein, which is not consistent with what was found by [44] when estimating the components of the Mannoprotein, as it was found that the percentage of carbohydrates was 21% and protein was 74%. According to what was shown by [45] the percentages of the components of the Mannoprotein differ depending on For the type of yeast and the extraction method, while [46] indicated that the reason for this difference may be due to the difference in the type of basic material on which the yeast feeds.

Laboratory applications of Mannoprotein bioemulsifier

Chemical tests of milk ice samples

The chemical tests for the ice cream samples included estimating their basic components individually immediately after their manufacture, as shown in Table (1), The results indicated that there were no significant differences ( $P>0.05$ ) between the average components of the ice cream samples, we also note that there is no noticeable change in the proportions of basic the components of ice cream samples, as the results were consistent with what was indicated by [47] in that there were no significant differences in the percentages of protein, fat, non-fat solids, and ash for the ice cream to which different types of emulsifiers and stabilizers were added. They also agreed The results are based on what was indicated by [24] regarding the stability of the proportions of the components of the ice cream mixture and their unaffected after adding the bioemulsifier Mannoprotein as an emulsifier.

**Table (1) Chemical components of ice cream Samples**

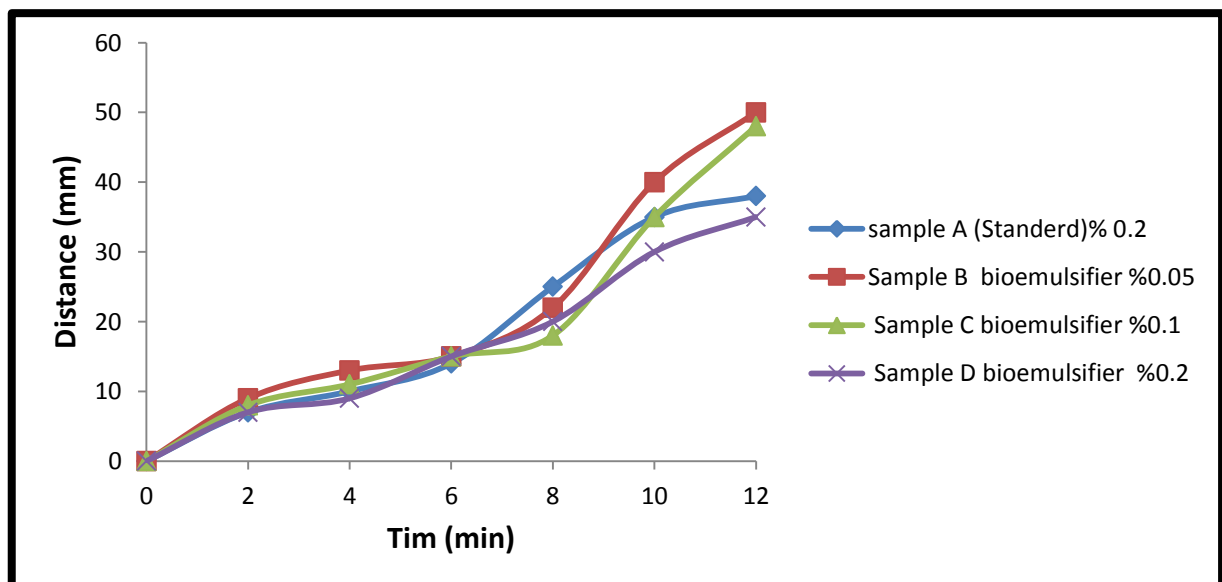
Sample	Protein %	Fat %	solids-not-fat %	Ash %	pH	Acidity
A	4.3	7.3	34	0.88	6.4	0.15
B	4.3	7.1	31	0.86	6.7	0.14
C	4.1	7.4	33	0.91	6.7	0.15
D	4.3	7.3	33	0.93	6.8	0.16

The results also indicated that the acidity and pH were not affected by adding the bioemulsifier Mannoprotein to the ice cream, and this is consistent with the finding of [24] that the acidity and pH of the ice cream were not affected after using the bioemulsifier Mannoprotein. The pH values were recorded immediately after manufacturing ranged between 6.6 and 6.8, and was consistent with many researchers [48– 50] who found that the natural pH of ice cream ranges between 6.6-6.8, and the acidity ranged between 0.14 - 0.16, which matches what [51, 52] observed regarding the pH and total acidity in natural milk. also explained [49] the addition of stabilizers and emulsifiers does not affect the acidity or pH of the milk that used to make ice cream.

Physical examinations of milk ice cream models

Test of spreadability

The results shown in Figure (7) indicated that there was a difference in the ability to spread of the manufactured ice cream samples. The results of the statistical analysis also indicated that there were significant differences ( $p < 0.05$ ) between the averages of the test values, as [53] indicated that increasing the concentration of emulsifier leads to an increase in the viscosity of the milk ice cream and thus a decrease in the rate of its spread. [54] also indicated that emulsifiers play an important role in improving the structure of the milk ice cream by increasing the stability of the fat granules and thus reflecting positively on the appearance characteristics of the product.



### Estimation of melting resistance rate

Figure (8) shows that there is a slight variation in the melting rate of the ice cream samples to which the bioemulsifier Mannoprotein has been added in different percentages. The results of the statistical analysis indicate that there are no significant differences ( $p>0.05$ ) between the averages of the test values. It was also noted that the highest resistance to melting was recorded for the two treatments (D and A), as their results were close, while it was less resistant to melting for the treatment (B) which represents the ice cream to which the lowest concentration of the bioemulsifier

Mannoprotein was added. The results agreed with what was indicated by [24] The rate of melting resistance increases with increasing concentration of the bioemulsifier. [49] showed that the resistance of ice cream to melting is affected by several factors, including amount of fat, amount of trapped air, size of the ice crystals, viscosity of the product, temperature, and the concentration of stabilizers. The results agreed with what It was pointed out by [55] that the bioemulsifier Mannoprotein has good emulsification and stabilization effectiveness.

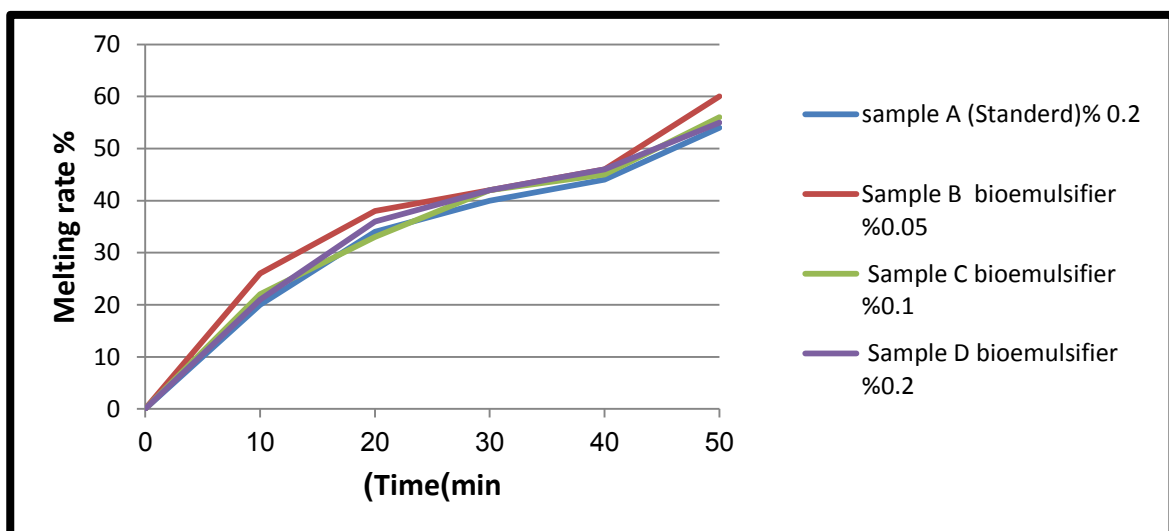


Figure (8): Melting rate for samples of ice cream to which the bioemulsifier Mannoprotein has been added in different proportions.



It was noticed through the results of the sensory evaluation of the ice cream sample manufactured with the addition of the bioemulsifier Mannoprotein at different concentrations that there were significant differences ( $p < 0.05$ ) between the average of treatments, as shown in Table (2) the highest evaluation scores were for sample (D), which is the ice cream manufactured with the addition of 0.2% of the Mannoprotein bioemulsifier, followed sample (A) with an addition 0.2% of the industrial emulsifier (monoglyceride), then Form (C) with a 0.1%, and finally Form B with 0.05% bioemulsifier, which is consistent with what was indicated by

[56] The addition of bioemulsifiers to ice cream improves the consistency, quality, and texture of the ice cream, as it has emulsifying qualities in addition to being a stabilizing substance. It also increases the viscosity of the ice cream, makes it more resistant to melting, and increases the attractiveness of its appearance [57]. Ribeiro et al. (2023) also indicated that bioemulsifiers improve the whipping ability, form small ice crystals, increase the destabilization of fats, and distribute them more evenly in the product, thus increasing the consumer's desire for the product.

**Table (2) Averages of sensory evaluation scores for ice cream added to the bioemulsifier Mannoprotein in different percentages**

Adjective	Sample) A(	Sample) B(	Sample) C(	Sample) D(
Flavor (50)	43.56 <sup>b</sup>	41.72 <sup>d</sup>	43.52 <sup>c</sup>	45.76 <sup>a</sup>
Texture (40)	35.08 <sup>b</sup>	29.36 <sup>d</sup>	32.96 <sup>c</sup>	37.72 <sup>a</sup>
Melting quality (10)	8.32 <sup>b</sup>	7.56 <sup>d</sup>	8.16 <sup>c</sup>	8.4 <sup>a</sup>
total	86.96 <sup>b</sup>	78.64 <sup>d</sup>	84.64 <sup>c</sup>	91.88 <sup>a</sup>

### Conclusions

One of the local isolates of *Saccharomyces cerevisiae* yeast isolated from one of the vinegar production factories in the city of Basra/Iraq was used to produce the mannoprotein bioemulsifier and study some of its characteristics. After conducting two types

of tests to determine the type of emulsion, it became clear that it was an oil-in-water (W/O) type. It was also shown, through conducting three tests of the effectiveness of emulsification, that it has high stability under different conditions of pH, temperature, and

different concentrations of salts. After adding it to ice cream, it improved the consumer's acceptance of the product after conducting a sensory evaluation of the product.

#### Reference

- [1] M. G. J. Shaileshbhai and M. T. Pandya, "Microbial Production Bioemulsifier and its Applications," *Int. J. Res. Appl. Sci. Eng. Technol.*, vol. 11, no. 7, pp. 637–647, 2023, doi: 10.22214/ijraset.2023.54727.
- [2] J. Z. Thraeib et al., "Production and Characterization of a Bioemulsifier Derived from Microorganisms with Potential Application in the Food Industry," *Life*, vol. 12, no. 6, p. 924, 2022.
- [3] Santamaria-Echart et al., "New trends in natural emulsifiers and emulsion technology for the food industry," *Nat. food Addit.*, pp. 1–31, 2021.
- [4] Alizadeh-Sani et al., "Bioemulsifiers derived from microorganisms: Applications in the drug and food industry," *Adv. Pharm. Bull.*, vol. 8, no. 2, p. 191, 2018.
- [5] B. G. Ribeiro, J. M. C. Guerra, and L. A. Sarubbo, "Biosurfactants: Production and application prospects in the food industry," *Biotechnol. Prog.*, vol. 36, no. 5, p. e3030, 2020.
- [6] C. Uzoigwe, J. G. Burgess, C. J. Ennis, and P. K. S. M. Rahman, "Bioemulsifiers are not biosurfactants and require different screening approaches," *Frontiers in microbiology*, vol. 6. Frontiers Media SA, p. 245, 2015.
- [7] E. S. Ethafa and A. J. A. Al-Manhel, "Determination of Optimum Conditions for Ergosterol Production by *Saccharomyces cerevisiae*," *Basrah J. Agric. Sci.*, vol. 35, no. 2, pp. 34–48, 2022.
- [8] S. A. J. Al-Jumaiee, K. S. J. Al-Hussainy, and A. J. A. Al-Manhel, "Effect of different level of  $\beta$ -glucan extracted from baker's yeast *Saccharomyces cerevisiae* and barley bran in the physicochemical properties of fish patties at cooling storage periods," *Basrah J. Agric. Sci.*, vol. 32, no. 1, pp. 79–87, 2019.
- [9] J. A. S. Malabuyoc, V. A. Alcantara, R. E. Arocena, and F. B. Elegado, "Substrate Optimization for Bioemulsification Using *Saccharomyces cerevisiae* 2031 by Response Surface Methodology," *Agro Bali Agric. J.*, vol. 6, no. 1, pp. 1–11, 2023.
- [10] V. Maru, S. Hewale, H. Mantri, and V. Ranade, "Partial purification and characterization of mannan oligosaccharides from cell wall of *Saccharomyces cerevisiae*," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 4, no. 12, pp. 705–711, 2015.
- [11] A. BESLIU et al., "Biochemical composition and antioxidant activity of the mannoprotein preparation obtained yeast

biomass from wine industry waste.,” *Not. Sci. Biol.*, vol. 14, no. 2, 2022.

[12] A. Bzducha Wróbel et al., “Antimicrobial and prebiotic activity of mannoproteins isolated from conventional and nonconventional yeast species—the study on selected microorganisms,” *World J. Microbiol. Biotechnol.*, vol. 38, no. 12, p. 256, 2022.

[13] S. Wang et al., “Mannoproteins interfering wine astringency by modulating the reaction between phenolic fractions and protein in a model wine system,” *Lwt*, vol. 152, p. 112217, 2021.

[14] N. Oktaviani, K. A. Sarwono, and G. lara Utama, “Bioconversion rice bran and cassava peel into yeasts cell walls mannoprotein as environmental friendly antioxidant,” in *E3S Web of Conferences*, EDP Sciences, 2021, p. 3004.

[15] D. R. Cameron, D. G. Cooper, and R. J. Neufeld, “The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifier.,” *Appl. Environ. Microbiol.*, vol. 54, no. 6, pp. 1420–1425, 1988, doi: 10.1128/aem.54.6.1420-1425.1988.

[16] P. Dikit, S. Maneerat, H. Musikasang, and A. H-kittikun, “Emulsifier properties of the mannoprotein extract from yeast isolated from sugar palm wine,” *Sci. Asia*, vol. 36, no. 4, pp. 312–318, 2010, doi: 10.2306/scienceasia1513-1874.2010.36.312.

[17] W. A. S. Alwaely, A. K. Ghadban, and I. M. N. Alrubayae, “Production and Properties of Biosurfactant from the Local Isolation of *Candida* spp,” *Drug Inven. Today*, vol. 12, no. 5, pp. 948–953, 2019.

[18] H. Torabizadeh, S. A. Shojaosadati, and H. A. Tehrani, “Preparation and characterisation of bioemulsifier from *Saccharomyces cerevisiae* and its application in food products,” *Lwt*, vol. 29, no. 8, pp. 734–737, 1996, doi: 10.1006/fstl.1996.0114.

[19] J. R. Patil and B. A. Chopade, “Studies on bioemulsifier production by *Acinetobacter* strains isolated from healthy human skin,” *J. Appl. Microbiol.*, vol. 91, no. 2, pp. 290–298, 2001, doi: 10.1046/j.1365-2672.2001.01382.x.

[20] P. Dikit, S. Maneerat, and A. H-kittikun, “Mannoprotein from spent yeast obtained from Thai traditional liquor distillation: Extraction and characterization,” *J. Food Process Eng.*, vol. 35, no. 1, pp. 166–177, 2012.

[21] P. Dikit, “Extraction , Purification and Composition of Bioemulsifier from Spent Yeast Obtained from Thai Traditional Liquor Distillation and Its Application Paweena Dikit A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Phil,” 2010.

[22] M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. t Rebers, and F. Smith, “Colorimetric method for determination of

- sugars and related substances,” *Anal. Chem.*, vol. 28, no. 3, pp. 350–356, 1956.
- [23] O. Classics Lowry, N. Rosebrough, A. Farr, and R. Randall, “Protein measurement with the Folin phenol reagent,” *J Biol Chem*, vol. 193, no. 1, pp. 265–275, 1951.
- [24] A. E. A. AMER, S. A. SHERIF, I. M. ROUSHDY, and M. N. EL-MAGDOUB, “The use of extracellular and intracellular emulsifiers from yeasts in ice cream production,” *Egypt. J. Agric. Res.*, vol. 87, no. 2, pp. 579–589, 2009, doi: 10.21608/ejar.2009.194121.
- [25] G. T. Pyne, “The determination of milk-proteins by formaldehyde titration,” *Biochem. J.*, vol. 26, no. 4, p. 1006, 1932.
- [26] A. Togola, “Determination of fat content of milk and milk products (Gerber method)—,” *Methods Chem. Anal. Liq. milk cream. BSI Bull. no 1741*, pp. 5–8, 1989.
- [27] .A. Chemists, *Official methods of analysis (17th ed.)*. 2000.
- [28] U. Abubakar, “Determination of the Chemical and Proximate Properties of Fresh Milk Samples From Small Holder Dairy Cows and Large Mechanized Dairy Cows in Parts of Kaduna State , Determination of the chemical and Proximate properties of milk,” pp. 1–11, 2023.
- [29] C. Clarke, *The Science of Ice Cream*. 2004. doi: 10.1039/9781847552150.
- [30] H. Douglas Goff and R. W. Hartel, *Ice cream*, 7th edition. 2018.
- [31] C. Fiol, D. Prado, C. Romero, N. Laburu, M. Mora, and J. Iñaki Alava, “Introduction of a new family of ice creams,” *Int. J. Gastron. Food Sci.*, vol. 7, no. December 2016, pp. 5–10, 2017, doi: 10.1016/j.ijgfs.2016.12.001.
- [32] C. Fiol, D. Prado, C. Romero, N. Laburu, M. Mora, and J. Iñaki Alava, “Introduction of a new family of ice creams,” *Int. J. Gastron. Food Sci.*, vol. 7, pp. 5–10, 2017, doi: 10.1016/j.ijgfs.2016.12.001.
- [33] A. I. A. Mohamed, A. S. Sultan, I. A. Hussein, and G. A. Al-Muntasherī, “Influence of Surfactant Structure on the Stability of Water-in-Oil Emulsions under High-Temperature High-Salinity Conditions,” *J. Chem.*, vol. 2017, 2017, doi: 10.1155/2017/5471376.
- [34] H. E. Bergna, *Colloid science*. 2005. doi: 10.1524/zpch.1975.97.3\_4.223b.
- [35] R. Pichot, “Pichot12PhD,” Thesis, no. November, pp. 1–219, 2010.
- [36] M. Destribats et al., “Pickering emulsions: What are the main parameters determining the emulsion type and interfacial properties?,” *Langmuir*, vol. 30, no. 31, pp. 9313–9326, 2014, doi: 10.1021/la501299u.
- [37] T. Lukondeh, N. J. Ashbolt, and P. L. Rogers, “Evaluation of *Kluyveromyces marxianus* FII 510700 grown on a lactose-

based medium as a source of a natural bioemulsifier,” *J. Ind. Microbiol. Biotechnol.*, vol. 30, no. 12, pp. 715–720, 2003.

[38] P. Dikit, S. Maneerat, H. Musikasang, and A. H-kittikun, “Emulsifier properties of the mannoprotein extract from yeast isolated from sugar palm wine,” *ScienceAsia*, vol. 36, no. 4, pp. 312–318, 2010, doi: 10.2306/scienceasia1513-1874.2010.36.312.

[39] P. F. F. Amaral et al., “Production and characterization of a bioemulsifier from *Yarrowia lipolytica*,” *Process Biochem.*, vol. 41, no. 8, pp. 1894–1898, 2006.

[40] Y. A. Elsaygh, M. K. Gouda, Y. Elbahloul, M. A. Hakim, and N. M. El Halfawy, “Production and structural characterization of eco - friendly bioemulsifier SC04 from *Saccharomyces cerevisiae* strain MYN04 with potential applications,” *Microb. Cell Fact.*, pp. 1–17, 2023, doi: 10.1186/s12934-023-02186-z.

[41] K. L. Yadav, D. K. Rahi, and S. K. Soni, “Bioemulsifying potential of exopolysaccharide produced by an indigenous species of *Aureobasidium pullulans* RYLF10,” *PeerJ Prepr.*, vol. 2, p. e726v1, 2014.

[42] H. Torabizadeh, S. A. Shojaosadati, and H. A. Tehrani, “Preparation and characterisation of bioemulsifier from *saccharomyces cerevisiae* and its

application in food products,” *LWT-Food Sci. Technol.*, vol. 29, no. 8, pp. 734–737, 1996.

[43] U. Klinkesorn and Y. Namatsila, “Influence of chitosan and NaCl on physicochemical properties of low-acid tuna oil-in-water emulsions stabilized by non-ionic surfactant,” *Food Hydrocoll.*, vol. 23, no. 5, pp. 1374–1380, 2009.

[44] V. A. Alcantara, I. G. Pajares, J. F. Simbahan, N. R. Villarante, and M. L. D. Rubio, “Characterization of biosurfactant from *saccharomyces cerevisiae* 2031 and evaluation of emulsification activity for potential application in bioremediation,” *Philipp. Agric. Sci.*, vol. 93, no. 1, pp. 22–30, 2010.

[45] N. H. Hyder, “Production, characterization and antimicrobial activity of a bioemulsifier produced by *Acinetobacter baumannii* AC5 utilizing edible oils,” *Iraqi J. Biotechnol.*, vol. 14, no. 2, 2015.

[46] J. Li, S. Karboune, J. Sedman, and A. Ismail, “Characterization of the structural properties of mannoproteins isolated from selected yeast-based products upon the enzymatic treatment,” *LWT*, vol. 131, p. 109596, 2020.

[47] M. A. Murtaza and M. A. Shabbir, “Quality Evaluation of Ice Cream Prepared with Different Stabilizers/Emulsifier Blends Pesticides View project Date palm View project,” pp. 65–67, 2004, [Online]. Available:

<https://www.researchgate.net/publication/237686321>.

[48] M. G. Hayes, A. C. Lefrancois, D. S. Waldron, H. D. Goff, and A. L. Kelly, “Influence of high pressure homogenisation on some characteristics of ice cream.,” 2003.

[49] M. Moeenfarid and M. M. Tehrani, “Effect of Some Stabilizers on the Physicochemical and Sensory Properties of Ice Cream Type Frozen Yogurt,” *J. Agric. Environ. Sci.*, vol. 4, no. 5, pp. 584–589, 2008.

[50] C. S. Favaro-Trindade, J. C. De Carvalho Balieiro, P. F. Dias, F. A. Sanino, and C. Boschini, “Effects of culture, pH and fat concentration on melting rate and sensory characteristics of probiotic fermented yellow mombin (*Spondias mombin* L) ice creams,” *Food Sci. Technol. Int.*, vol. 13, no. 4, pp. 285–291, 2007, doi: 10.1177/1082013207082387.

[51] M. O’Mahony, *Sensory evaluation of food: statistical methods and procedures*. Routledge, 2017.

[52] C. B. O’connor, “Rural Dairy Technology ILRI Training Manual I,” *Int. Livest. Res. Institute*, Addis Ababa, Ethiop., 1995.

[53] Q. Abbas Syed, “Effects of different ingredients on texture of ice cream,” *J. Nutr.*

*Heal. Food Eng.*, vol. 8, no. 6, pp. 422–435, 2018, doi: 10.15406/jnhfe.2018.08.00305.

[54] M. M. Warren and R. W. Hartel, “Effects of Emulsifier, Overrun and Dasher Speed on Ice Cream Microstructure and Melting Properties,” *J. Food Sci.*, vol. 83, no. 3, pp. 639–647, 2018, doi: 10.1111/1750-3841.13983.

[55] L. Zhong et al., “Quality Characteristics of Reduced-Fat Emulsified Sausages Made with Yeast Mannoprotein Enzymatically Prepared with a  $\beta$ -1,6-glucanase,” *Foods*, vol. 12, no. 13, pp. 1–14, 2023, doi: 10.3390/foods12132486.

[56] A. Liaqat et al., “Applications of biosurfactants in dairy industry,” in *Applications of Next Generation Biosurfactants in the Food Sector*, Elsevier, 2023, pp. 509–526.

[57] A. T. Mahajan, G. L. Khatik, and S. Chaudhary, “Antibiofilm properties of biosurfactants: A tool against the food pathogens,” in *Applications of Next Generation Biosurfactants in the Food Sector*, Elsevier, 2023, pp. 91–110.

[58] B. G. Ribeiro, J. M. Campos Guerra, and L. A. Sarubbo, “Biosurfactants: Promising Biomolecules in the Food Industry,” in *Microbes for Natural Food Additives*, Springer, 2023, pp. 147–168