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Purification and characterization of mannoprotein extracted from *Saccharomyces cerevisiae*

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Abstract

Five yeast isolates from various origins were employed in this study. The bioemulsifier was most effectively produced by the Super Maya isolate, with an effectiveness of 63.33%. The purified mannoprotein showed an emulsification activity of 60% using the precipitation method with ammonium sulfate at a saturation rate of 80%. Ethanol, methanol, and acetone solvents were used in purifying the mannoprotein via the precipitation method; compared to other solvents, the cold acetone precipitation was the most effective in partially purifying the mannoprotein, with an efficiency reaching 84%. The emulsification effectiveness was measured for all purification stages, along with estimating the protein and carbohydrate content of the bioemulsifier. The total purification of the mannoprotein relied on using the Sephadex G-75 filtration technique. The mannoprotein maintained its effectiveness within a pH range of 2 to 9, while it lost its efficacy at pH 10. The study demonstrated the stability of the bio- emulsifier's effectiveness against varying temperatures ranging from 15, 25, 30, 37, 40, 50, 60, 70, and 100 C0. The efficacy of the mannoprotein was unaffected by the pepsin enzyme, whereas the alpha-amylase enzyme had a slight impact. **Aim:** The study aimed to present the most effective technique for mannoprotein purification. It is necessary to define its components to elucidate the unique qualities that distinguish the bioemulsifier from others.

Keywords: Purification, Mannoprotein, *S. cerevisiae*

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تنقية وتوصيف المانوبروتين المستخلص من خميرة *Saccharomyces cerevisiae*

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الخلاصة

اعتمدت الدراسة خمس عزلات من الخمائر من مصادر مختلفة. تم إنتاج المستحلب الحيوي بكفاءة أعلى من قبل عزلة Super Maya، بنسبة فعالية 63.33%. المانوبروتين المنقى أظهر فعالية أستحلابية بنسبة 60% باستخدام طريقة الترسيب بكبريتات الأمونيوم عند معدل تشبع بنسبة 80%. تم استخدام مذيبات الإيثانول والميثانول والأسيتون في تنقية المانوبروتين بطريقة الترسيب. بالمقارنة مع المذيبات الأخرى، كان ترسيب الأسيتون البارد هو الأكثر فعالية في تنقية المانوبروتين جزئيا، حيث وصلت كفاءته إلى 84%. تم قياس فعالية الاستحلاب لجميع مراحل التنقية، إلى جانب تقدير محتوى البروتين والكربوهيدرات في المستحلب الحيوي. اعتمدت عملية التنقية الكلية للمانوبروتين على استخدام تقنية الترشيح Sephadex G-75. حافظ المانوبروتين على فعاليته ضمن نطاق الأس الهيدروجيني من 2 إلى 9، بينما فقد فعاليته عند الرقم الهيدروجيني 10. بينت الدراسة ثبات فعالية المستحلب الحيوي أمام درجات حرارة متفاوتة تتراوح بين 15، 25، 30، 37، 40، 50، 60، 70، و100 درجة مئوية. لم تتأثر فعالية بروتين المانوبروتين بإنزيم الببسين، في حين كان لإنزيم ألفا أميليز تأثير طفيف.الهدف: هدفت الدراسة إلى تقديم التقنية الأكثر فعالية لتنقية بروتين المانوبروتين. ومن الضروري تحديد مكوناته لتوضيح الصفات الفريدة التي تميز المستحلب الحيوي عن غيره.

الكلمات المفتاحية: تنقية، بروتين المانوبروتين، S. cerevisiae

Introduction:

The inner and outer layers of *S. cerevisiae* cell wall are detectable by electron microscopy. Mannoprotein is present in the outer layer. The inner layer consists of beta-glucan, which is of two types: β-glucan 1 → 3 and β -glucan 6 → 1 and chitin(Molon et al., 2018). Mannoprotein is a bioemulsifier. It consists of protein and sugar, Approximately 14,000 to 15,800 Daltons make up its molecular weight, It is known as Amphiphilic, It has lipophilic properties and Hydrophilic. This bio-emulsifier is able to stabilize oil-in-water emulsions (Alizadeh-Sani et al., 2018 & Abdel Hamid,.2020)

First reported by Cameron et al. in 1988, the mannoprotein of the yeast *Saccharomyces cerevisiae* possesses emulsifying qualities (De Iseppi et al., 2019). About 90% is mannose, while 5–10% is protein, The outer layer of the *S. cerevisiae* cell wall is composed of structural mannoprotein that is scattered inside a glucan net. (Elena et al., 2020). Baker's yeast is a cheap, Source non-toxic utilized to create this bio-emulsifier. It has emulsifying properties that can be of commercial importance, These good properties include low toxicity, Thus, It is becoming increasingly recognized in the scientific and global communities as a viable substitute for several emulsifiers (Raham and Mahmood, 2017 & De Iseppi et al., 2019 & Saleh,.2021).

The materials and methods:

- Collection of isolates of *Saccharomyces cerevisiae*:

Collected different samples of dry yeast from different origins from local markets, as shown in Table-1below:

Table-1: showing the yeast samples that were used in the study and their origin

sequence	Yeast name	Origin
1	Altunsa	Turkey
2	Angel	China
3	Gloripan	Egypt
4	Saf-instant	France
5	Super maya	Iran

- Growth Activation of *S. cerevisiae* yeast isolates:

Dry yeast isolates were activated and grown according to Bruna et al (2022). By taking 1 gm of yeast powder and inoculating it in 10 ml of liquid Sabouraud dextrose broth and incubating it at a temperature of 30°C for half an hour, After the end of the incubation period, and then growth on Sabouraud dextrose agar and then incubated under the same conditions.

- Identification of *S. cerevisiae* yeast:

A morphological examination was used to identify yeast and the identification was confirmed using the Vitek2 system.

● Investigating the ability of *S. cerevisiae* to produce mannoprotein

- Production Mannoprotein:

Yeast isolates were activated on Sabouraud dextrose broth, mixed well, and They are incubated for 24 hours at 30°C. Cooper & Paddoc's medium inoculated with (0.1% KH2PO4, 0.5% MgSO4.7H2O , 0.01% CaCl2 , 0.01% NaCl, 0.5% Yeast extract, 8% Glucose, 5% Waste cooking oil) With yeast isolates, they were incubated in a shaking incubator at 30°C for 4 days (Alcantara et al. 2013).

- Mannoprotein extraction:

The cells were separated by centrifugation at a speed of 10,000 rpm for 20 minutes at 4°C. Phosphate Buffered Saline (PBS) was then used to suspend the cells. The suspended cells were boiled for 5 minutes and Followed by a 10-minute centrifugation at 3000 rpm, and The supernatant phase representing crude mannoprotein was taken (Alcantara et al. 2013).

The emulsifying activity of mannoprotein was investigated by following the steps described by Dhivya et al. (2014), The emulsification activity was then determined using the following equation.:

$$E24 = \frac{\text{Total height of the emulsion (cm)}}{\text{Total height of the aqueous} + \text{emulsion (cm)}} \times 100$$

- Mannoprotein purification

- Precipitation with ammonium sulfate:

Followed the method of Ananthamurthy et al (2021) in purifying mannoprotein. With saturation rates ranging from 30, 50, 60, 70, and 80%, according to the saturation table for precipitation with ammonium sulfate.

- Mannoprotein purification with organic solvents:

Partial purification with organic solvents of mannoprotein was carried out according to Alcantara et al. (2013) using three types of solvents: Acetone, Methanol, and Ethanol.

- Protein estimation:

Protein was estimated according to the method described by Bradford (1976), Figure-1

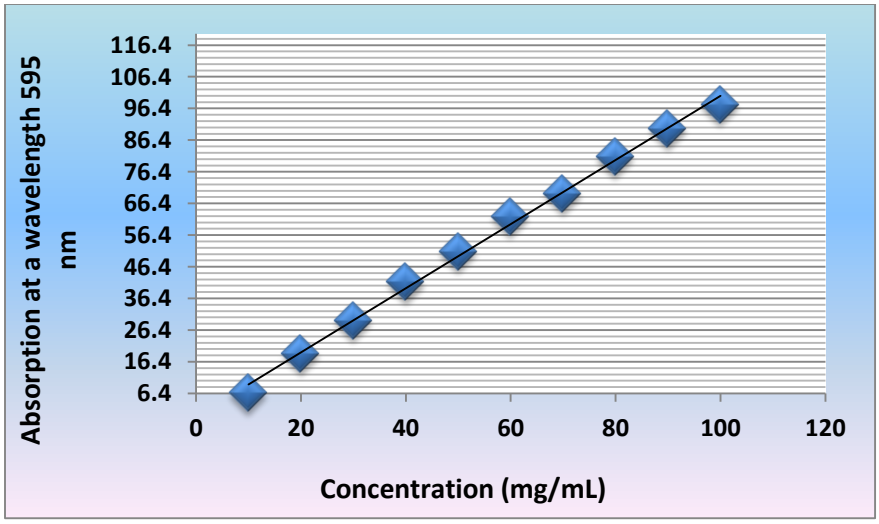

Figure-1 Standard curve for estimating protein concentration using the Bradford method (1976)

- Carbohydrate estimation:

Estimates of carbohydrates were made using the Dubois et al. technique (1956), Figure-2.

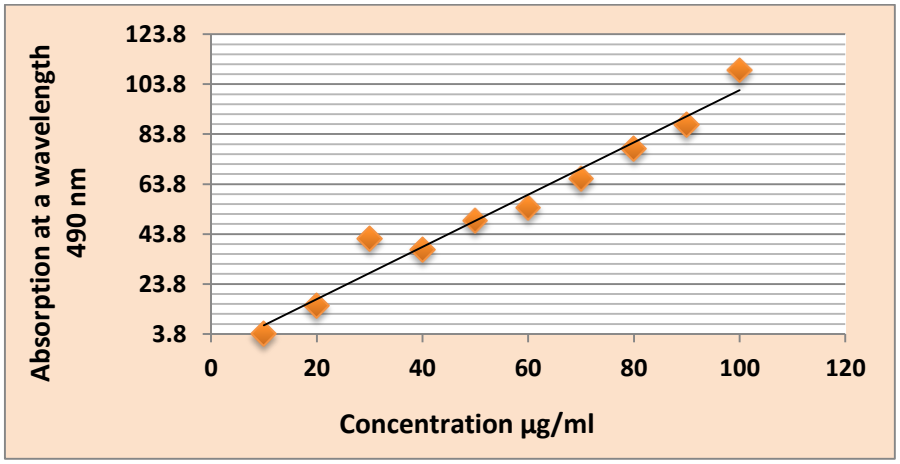

Figure-2 Standard curve for estimating carbohydrate concentration by the method of Dubois et al. (1956)

- Purification of mannoprotein via Sephadex G-75 gel filtration chromatography:

Pass the partially purified extract in an amount of 2 ml along the inner sides of the column near the surface of the gel. Then, the recovery process was carried out with the same buffer solution, and the separated parts of the column were collected in test tubes at a rate of 3 ml/10 minutes. The concentration of both protein and carbohydrates was measured in each tube (Alcantara et al. 2014).

- Mannoprotein characterization

- Test the influence of mannoprotein activity on different pH levels:

The method described by Jin et al. (2019) was followed to test the extent to which the effectiveness of mannoprotein was affected under different pH levels.

- Fastness immutability of mannoprotein activity at uneven levels of temperature:

Temperatures ranging from 15 to 100 degrees C^0 were used to test heat's effect on Emulsifying performance of mannoprotein (Jin et al., 2019).

- Screening of the effect of enzymes on the mannoprotein performance:

Hamel's (2020) method was applied using the enzyme pepsin and alpha-amylase to research their effect on the emulsifying performance of mannoprotein.

Results and discussion:

- Diagnosis of *S. cerevisiae*:

Diagnostic results using the Vitek system display that all isolates used in the research are *S. cerevisiae*(Mohieldin et al., 2015 and Al-Musawi et al., 2016).

-Election of the most effective isolate to generate mannoprotein:

The E24 emulsify action measurement process was used. It is the most delicate process for electing the isolate qualified to produce mannoprotein. Super Maya is the most qualified isolate in generating bio-emulsion, With an emulsifying performance of E24=63.33%, Then, Angle isolated a lower emulsifying performance of 60%, Meanwhile, the gloripan isolate displays a performance of 59.25%. The isolates that displayed the least emulsification of 56.66% were Saf-instant and Altunsa, Figure-3 illustrates this.

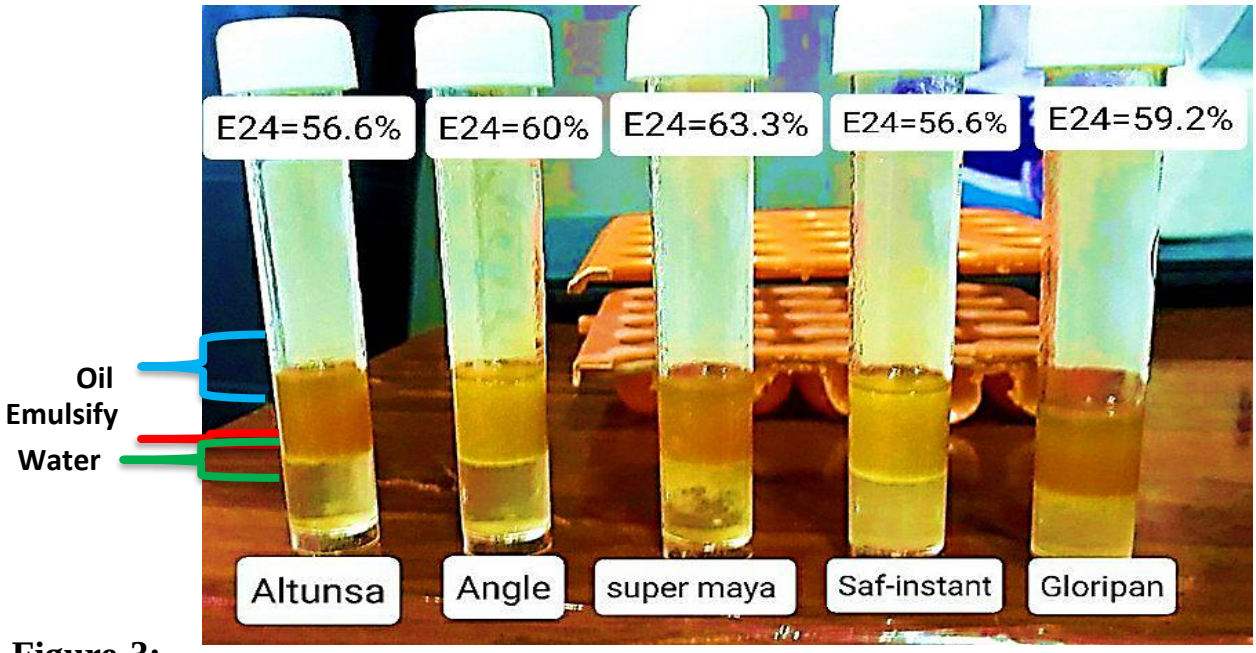

Figure-3:
Emulsifying activity of *S. cerevisiae* yeast isolates of different origins

Industrial strains of *S. cerevisiae* yeast vary in the amount of mannoprotein production, depending on their quality (Gonzalez-Ramos and Gonzalez, 2006). The difference in mannoprotein productivity may be attributed to the cultural conditions, and this is what Giovani et al. (2010) found when they studied three strains of *S. cerevisiae* yeast, Temperature and sugar concentration affect the

production ability of strains to mannoprotein. Orlean (2012) showed, through the results of a survey of Baker's yeast strains, that 1,200 genes, excluding the basic genes, affect the structural components of the yeast's cell wall. A study by Pérez-Través and others (2015) showed that the reason for the difference in the production of this bioemulsifier between isolates is due to the difference in gene expression, which affects the structural structure of the cell wall. Therefore, changes in the cell wall are important factors affecting emulsification activity.

- Precipitation by Ammonium Sulfate:

Mannoprotein purified with ammonium sulfate showed an E24 emulsifying activity for the precipitate of (60%) at 80% saturation. This is similar to what Kazem (2019) found with E24 emulsifying efficiency (66%) at the same degree of saturation. This can be attributed to the small amount of protein that is included in the synthesis of mannoprotein compared to the carbohydrates that constitute the largest part of it. Barriga et al. (1999) concluded that the emulsifying activity of mannoprotein was lost when there were only carbohydrate residues because carbohydrates have a role in stabilizing the formed bioemulsion by reducing the solubility of the protein and preserving it.

- Precipitation using Organic Solvents:

Acetone showed the highest emulsifying effectiveness (E24) (84%), thus superior to both methanol and ethanol, as in Figure-4. Alcantara et al. (2014) concluded, through a study they conducted to purify the bioemulsion from baker's yeast *S. cerevisiae*, that acetone gave the highest emulsifying activity superior to ethanol, and this confirms the results obtained in this study. Alcantara et al. (2014) concluded, through a study they conducted to purify the bioemulsion from *S. cerevisiae*, that acetone gave the highest emulsifying activity superior to ethanol, confirming the results obtained in this study. This is due to the small Dielectric constant for acetone of 21.4 compared to the Dielectric constant for methanol of 33 and ethanol of 24 (Bonner, 2019).

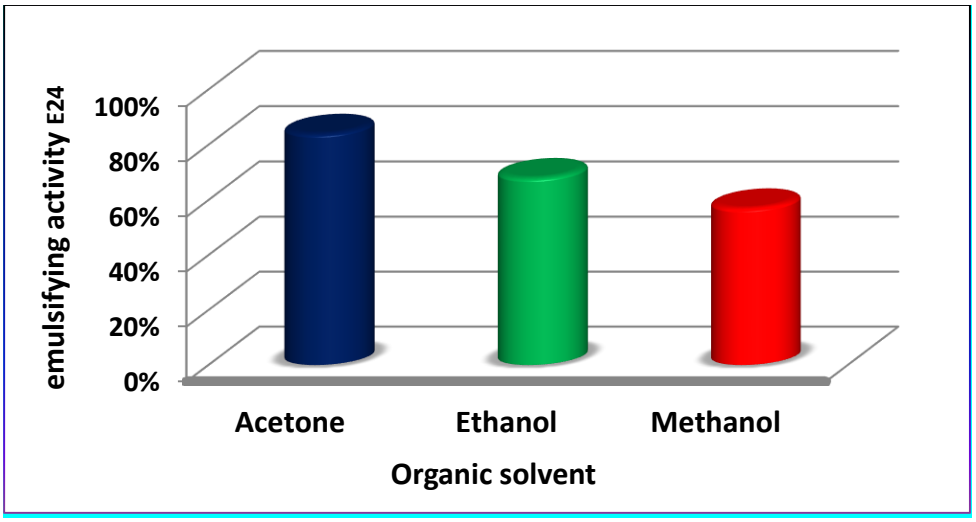

Figure-4: Emulsifying activity of mannoprotein purified using organic solvents

- Purification of mannoprotein via Sephadex G-75 gel filtration chromatography:

The mannoprotein was purified and the separated fractions were read at a wavelength of 280 nm for protein determination. At a wavelength of 490 nm for the determination of carbohydrates, Two separate peaks of protein and one peak of carbohydrates were obtained. I tested the emulsifying activity of E24 for both peaks. Whereas the concentration of carbohydrates is 778.01 µg/ml and protein is 75.21 mg/ml, In addition, the emulsifying effectiveness was estimated at 86.4%, as shown in Table (2). The relationship between the separated parts and the absorbance concentration was drawn in Figure (5).

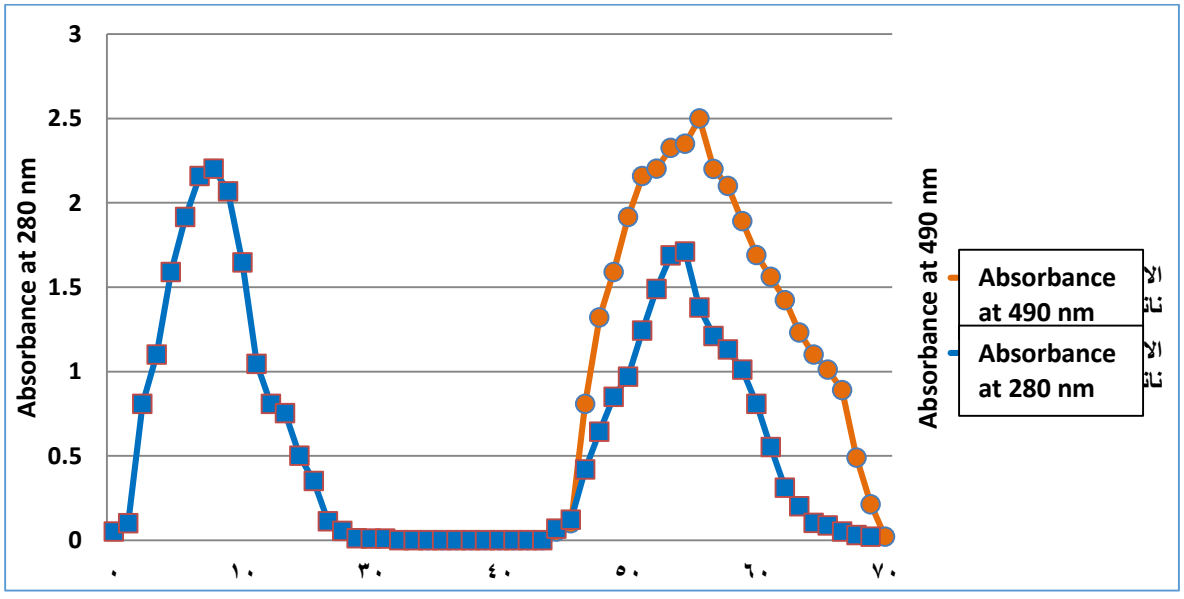

Figure-5: Gel filtration chromatography to purify mannoprotein from *S. cerevisiae* yeast using Sephadex G-75 with dimensions (2.5X70)cm. Balanced with phosphate buffer at a concentration of 0.1 M, pH 7.2. With a flow speed of 3 ml/10 minutes.

Table-2: Protein and carbohydrate concentrations for purification steps with emulsifying activity

Type of purified extract	Protein concentration (mg/mL)	Carbohydrate concentration (µg/ml)	E24 emulsifying activity
Crude extract	62.95	405.60	67.74%
Ammonium sulphate 80%	31.86	347.93	60%
Acetone	150.52	801.56	84%
Ethanol	79.89	728.96	67%
Methanol	76.88	617.74	57%
Gel filtration chromatography	75.21	778.01	86.4%

In comparison with previous studies, Rahm (2017) achieved, by purifying mannoprotein via gel filtration chromatography, three peaks of protein and one peak of carbohydrates with an emulsifying efficiency of up to 84%. While Kazem (2018) found two peaks of protein and one peak of carbohydrates, with an emulsification efficiency of 70%.

Mannoprotein characterization

- Investigation of the Impact of pH on Mannoprotein:

Mannoprotein showed highly stable emulsifying activity at pH 2, 4, 6 and 7, It also showed stable emulsifying activity at basic levels of 8 and 9, Although mannoprotein loses its emulsifying property at pH 10. Cameron et al. (1988) and Torabizadeh et al. (1996) concluded that the *S. cerevisiae* yeast bioemulsifier exhibits a relatively stable response to pH values ranging from 2 to 11. While Alcantara et al. (2014) found that the bioemulsifier showed the highest emulsifying effectiveness at pH 2, 8, and 10, but its effectiveness decreased significantly at pH 4 and 6. The results reached by Dikit (2010) and Kazim (2018) showed that the

mannoprotein extract showed emulsifying activity at pH 2 - 8, and the emulsifying activity decreased with increasing pH above 9.

- Thermal stability of mannoprotein:

The efficiency of mannoprotein did not seem to be affected by temperature, Mannoprotein gave highly stable emulsifying activity at temperatures that ranged from (15-100°C) with an emulsifying efficiency of 74%. The results of this study were consistent with the results of previous studies. Studies on the effect of heat treatment on mannoprotein conducted by Alcantara et al. (2010), Dikit et al. (2010), Alcantara et al. (2014), Kazem (2018), and Li and Karboune (2019) discovered that the temperatures typically utilized to prepare... foods, Food preparation techniques, including pasteurization, heating, and heat treatment, don't significantly affect the emulsification activity. This high stability of mannoprotein may be attributed to certain chemical groups that protect it from degradation. Many commercial applications requiring emulsifying agents in formulations subjected to high-temperature treatments can benefit from this heat-stabilizing capability, Additionally, in sectors of industries where heating is crucial (Dikit et al., 2010).

- The effect of enzymes on mannoprotein:

The pepsin enzyme did not have any effect on the effectiveness of mannoprotein, It showed an emulsifying effectiveness of 59.21%. The emulsifying activity of mannoprotein was marginally affected by the alpha-amylase enzyme, as the effectiveness reached 57%. These results may be attributed to the components involved in the synthesis of mannoprotein itself. The pepsin enzyme did not have an actual effect on mannoprotein due to the lack of proteins included in the formula compared to the high concentration of carbohydrates that characterize mannoprotein (Li and Karboune, 2019).

Conclusion:

The most effective method for mannoprotein purification is to use organic solvents. The stability of mannoprotein emulsification efficacy for temperature, pH, and enzyme resistance. This is because the amount of carbohydrates is higher than the amount of protein. As a result, the solvent-based precipitation technique proved more effective than the ammonium sulfate method, Because the protein is the primary factor that governs the ammonium sulfate precipitation principle, Consequently, it was unable to attain a high level of emulsification efficacy. The resistance of mannoprotein emulsification to changes in pH, temperature, and enzymes, It is credited to the function of carbohydrates and how they combine with the protein to give the mannoprotein its distinct features.

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