Bioemulsifying Potential of Exopolysaccharide Produced by an Indigenous Species of *Aureobasidium pullulans* RYLF10

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Abstract

In this study, the bioemulsifying potential of exopolysaccharide produced by an indigenous species of *Aureobasidium pullulans* RYLF10 has been determined and various factors affecting the emulsification activity has been evaluated. The emulsification activity was determined with 8 different vegetable oils (olive, soybean, sesame, sunflower, coconut, mustard, groundnut and almond oil) which are mainly used for various food purposes. The result obtained revealed the emulsification activity (%EA) of the test EPS was quite fair with all the vegetable oils used in the study. However, it was found maximum (56%) with olive oil at the concentration of 1.5% and was very much comparable with the emulsification activity of gum Arabic, the standard emulsifier. Therefore, the olive oil was used for studies related to various factors affecting the emulsification activity of the test emulsifier. The emulsion formed was found to be oil in water (o/w) type which possessed remarkable temperature, pH and salt for 24 hours. Droplet size analysis of the test emulsifier revealed to possess monomodal type of size distribution with droplet size of 105 µm which was responsible for stabilizing the emulsion. The result obtained suggest that the emulsion of the test EPS with olive oil can potentially be used in various food applications where olive oil is used.

Introduction

Exopolysaccharides (EPS) are produced by large number of organisms however, EPS of microbial origin have attracted worldwide attention due to their unique properties. They are long, branched or linear chain of carbohydrates which are linked together by different glycosidic bonds. The complexities in their structure make them to possess important industrial properties like emulsification, flocculation, gelation, biopolymer film formation etc. which are used in various industries like food, cosmetics, paint, oil and personal care. The requirement of bioemulsifiers has been increasing over the past several years, especially in food processing industries and personal care product manufacturing sectors. Biosurfactants are surface active biomolecules produced by microorganisms. These molecules are capable of reducing surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures. High molecular weight biosurfactants produce stable emulsions without lowering surface or interfacial tension and they are called bioemulsifiers (Bognolo, 1999). Bioemulsifiers have higher biodegradability over chemical emulsifiers as high selectivity, higher foaming, lower toxicity and stability at extreme temperatures, pH and salinity. This has lead to the screening of various microorganisms including fungi, bacteria, actinomycetes etc. for their potential of producing bioemulsifiers. Besides, the work on bioemulsifying potential of EPS by fungi is very less though some filamentous and yeasts have been reported to produce the EPS with potential emulsifying activity. Therefore, in present investigation the emulsifying potential of exopolysaccharide produced by an important indigenous black yeast *Aureobasidium*

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pullulans RYLF 10 has been determined. The identification, screening, production, purification and characterization of exopolysaccharide has been done and reported in our earlier publication Yadav *et al.*, (2014).

Keywords: Exopolysaccharides, Indigenous, *Aureobasidium pullulans*, Bioemulsifiers

Materials and methods

Production of EPS

The production of exopolysaccharide was done as per method of Sutherland (1990) and Maziero *et al.*, (1999). For EPS fermentation, the pure culture of *Aureobasidium pullulans* RYLF10 was grown in potato dextrose broth at pH5.6, temperature 30±1ºC for14 days. After respective incubation period the mycelial biomass was separated by filtering the fermentation broth with Whatman filter paper no. 1 and the filtrate was mixed with 5% TCA (tricarboxylic acid), left overnight at 4ºC for precipitation of proteins. Next day the filtrate was centrifuged at 10,000 rpm for 20 minutes at 4°C to remove the protein precipitate and the filtrate was added with 4 volumes of ethanol (filterate: ethanol = 1:4 v/v), stirred vigorously and left overnight at 4°C for precipitation of the exopolysaccharides. Next day, the precipitated exopolysaccharide was separated by centrifuging the solution at 10,000 rpm for 20 minutes at 4°C. The supernatant was discarded and the pelleted precipitate of crude EPS was purified, lyophilized and stored for further use.

Determination of emulsification activity

Emulsifying activity of the test EPS was determined as per modified method of Cameron *et al*. (1988). In this method 1 ml each of different vegetable oils (Olive, Soybean, Sesame, Sunflower, Coconut, Mustard, Almond and Groundnut oil) was added to 1ml of 1.5% (w/v) the test EPS suspension and vortexed at high speed for 3 min. The emulsification activity (%EA) was determined after 1h whereas the emulsion stability was determined as emulsification index (%EI) after 24, 72 and 96h (or E_{24}, E_{72} & E_{96}). The %EA and %EI were calculated by dividing the measured height of emulsion layer (in cm) by the total height of the mixture (in cm) multiplied by 100. All tests were performed in triplicate.

Determination of optimum concentration of the test EPS for emulsification activity

The optimum concentration of the test EPS for obtaining the best emulsification activity, was determined by taking different concentrations of the test EPS (viz., 0.5, 1, 1.5 and 2% (w/v) with the oil screened out as the best for formation of emulsion with the test EPS and %EA and %E_{24} determined for all the concentrations in triplicates.

Determination of emulsion type

The type of emulsion formed by the test EPS, was determined as per filter paper wetting test of Rieger (1986). In this method, a droplet of the test emulsion (the EPS) was dropped onto the filter paper. A water-in-oil (w/o) type of emulsion, droplet remains as a droplet on the filter paper while in oil-in-water (o/w) type, it disperses rapidly on the filter paper.

Determination of temperature, pH & salt stability of emulsion

The pH, temperature and salt stability of the EPS based emulsion was determined as per method of Dikit *et al*. (2010). For this the lyophilized test EPS (1.5% w/v) was used.

Effect of pH

The effect of pH on stability of the test emulsion (1.5%) was determined in standard buffers within the pH range of 3-9. The pH 3 and 4 were adjusted with citrate buffer, pH 5 and 6 with acetate buffer, pH 7 with phosphate buffer and pH 8 and 9 with Tris-Cl buffers. The emulsification activity was determined and compared with the standard (Gum Arabic) with the same concentration.

Effect of salt concentration

The effect of different salt concentrations on the stability of the test emulsion (1.5%) was determined with the NaCl with the concentration ranging from 0.0 to 3.0% (w/v), $MgCl_2$ and $CaCl_2$ with the concentrations ranging from 0.0 to 0.1% (w/v). The emulsification activity was determined and compared with the standard Gum Arabic with the same concentrations.

Effect of temperature

The effect of temperature i.e., thermal stability on the stability of the test emulsion was studied by incubating the emulsion (1.5% w/v) at different temperature i.e. 63^{o}C for 30min, 100^{o}C for 15min and at 121^{o}C for 15min and then cooled to 30^{o}C. The emulsification activity was determined and compared with the standard Gum Arabic with the same concentrations.

Determination of emulsion droplet size distribution

It is very important to know the size of droplets which is generally formed during the emulsification process because it gives richness and mouth feel. So the droplet size distribution in the test emulsion was determined by laser light scattering technique using particle size analyzer (Malvern Mastersizer MS 2000). The size of the droplet from emulsion was determined after 1 and 24h. This instrument is capable of measuring droplet size ranging from 0.02-2000µm. In this technique, emulsion was placed into measuring unit and deionized water was used as dispersant.

Results and Discussion

Determination of emulsification activity of the test EPS

Some natural, plant-derived, food emulsifiers such as lecithin and gum Arabic are already in the market and some of the constraints associated with the properties and supply of natural, plant-derived emulsifiers have compelled to search for suitable and feasible alternative. Emulsifiers of microbial origin can solve the problem to a large extent and therefore there is need to explore the possibilities of the same in a systematic manner. The screening of emulsifying property of the EPS produced by *A. pullulans* RYLF10, in the present study is an important step in this direction. For this, the emulsion was prepared with different vegetable oils (olive, soybean, sesame, sunflower, coconut, mustard, groundnut and almond oil) which are generally used in various food preparations. These oils were utilized as substrate for the formation of emulsion. The result obtained revealed the emulsification activity of the test EPS was quite fair with all the vegetable oils used in the study. However, it was found maximum (56%) with olive oil and was very much comparable with the emulsification activity (%EA) of gum Arabic, the standard emulsifier. It was observed that the emulsification activity of the gum Arabic was almost better with all the vegetables oils used. However, with sesame oil both the %EA and $\%E_{24\text{-}96}$ of the test EPS was found far better. It was interesting to note that the test EPS showed greater affinity for the olive oil in spite of having the maximum degree of unsaturation in the olive oil as compared to other oils (Table 1). Thus, the result obtained suggest that the emulsion of the test EPS with olive oil can potentially be used in various food applications where olive oil is used. In present study the olive oil was selected for further studies related to various factors affecting the emulsification activity of the test emulsifier.

Determination of optimum concentration of the test EPS for emulsifying activity

Determination of optimum concentration of EPS on emulsifying activity was carried out with four different concentrations of the test emulsifier viz., 0.5, 1.0, 1.5 and 2.0% (w/v). The result obtained revealed the 1.5% (w/v) test emulsifier gave the maximum emulsifying activity (%EA) of 56% as compared to other concentrations (Table 2; Figure 1). Therefore, the concentration of 1.5% (w/v) was chosen for further studies.

Type of emulsion formed by the test EPS

The type of emulsion formed by the test EPS determined as per filter paper wetting method revealed that the emulsion formed by the test EPS was oil in water (o/w) type formed with olive oil. The readily dispersion of the test emulsion on the filter paper confirmed it as oil-in-water type emulsion (Figure 3). Since oil-in-water type (o/w) emulsion is generally used in various food preparations, the emulsion formed in present investigation by the test EPS with olive oil may find applications in food.

Effect of temperature on emulsifying activity

The effect of temperature on bioemulsifier (EPS) was studied at wide range of temperature i.e. at 63, 100 and 121^{o}C. These temperature ranges are used in various regular food processing’s such as cooking, pasteurization of food products and where high temperature is required to make the food free from microorganisms, so emulsifier should be stable at these temperatures and does not affect the emulsification activity. The result obtained revealed the stability of the emulsion was not affected by the temperature of 63^{o}C and 100^{o}C and remained almost 47%. While at higher temperature of 121^{o}C, the percent stability of the emulsion decreased to 40% (Figure 4). Thus, the study concluded that the stability of the emulsion was not much affected by higher temperature due to the less hydrolytic action at the higher temperatures.

Effect of pH on the stability of emulsifying activity of the test EPS

The pH stability study was carried out with 1.5% (w/v) concentration of the test EPS. The result obtained revealed the EPS formed emulsion was stable in p pH 3 to 7 (Figure 5). The emulsion was found most active at pH 5 and showed the constant emulsifying activity of 49.2% for 24hours. A slight decrease in emulsifying activity from pH 7 was observed which may be due to precipitation of the EPS in emulsion. A heavy decrease in emulsifying activity was observed with pH 9 and was found to remain about 33.0%. Thus, the emulsion formed in present investigation was found to stable between pH 3 to 7 for 24h. The literatures available also reports different optimum pH values for different emulsifiers. Our investigation is consistent with that of Lukondeh *et al.* (2003) who reported that the bioemulsifier from yeast *Kluyveromyces marianus* showed stability between pH 3-11 for 24 hours. The study conducted by Ameral *et al*. (2006) for the production of bioemulsifier from *Yarrowia lipolytica* also reported the pH stability between pH 3-9 for 24h.

Effect of different salts on emulsifying activity of the EPS

The effect of different salts on emulsification activity of the test EPS was observed. For this, NaCl, $MgCl_2$ and $CaCl_2$ were used with different concentrations i.e., 0.0-3% for NaCl and 0.0-0.1% for $MgCl_2$ and $CaCl_2$. The emulsifying activity against olive oil remained undisturbed i.e., it was stable with different concentration of salts for 24 hours (Figure 6). However, the stability of the emulsion formed decreased with increase in concentration of the salts with increase in time. It is due to the destabilization of emulsion caused by the disturbances in electrostatic forces between droplets. The similar results were obtained by the Klinkesorn and Namatsila (2009) while studying the influence of chitosan and NaCl on o/w emulsion. Thus, the emulsion formed in present study can be used in various food applications where high concentration of salts are involved.

Droplet size distribution in o/w emulsion formed by the test EPS

The droplet size distribution of the emulsion formed by the test EPS with 1.5% (w/v) concentration (Table 3 & 4; Figure 7 a, b & c) was found to be wider and contained large particle of size approximately 105 µm whose frequency was found to be 12% while the frequency of volume having droplet size of 50 µm was observed to be less than 0.5% in emulsion stabilized for 1h (Figure 8). This revealed the mono modal type of droplet size distribution. There was an increase in the size of droplets due to coalescence and flocculation. The droplets size observed in emulsion stabilized for 24h revealed an increase in size which was found to be 185.29µm (Figure 9). Droplet size ranging from 10-150 µm is very ideal in food industry and may possess many applications. Our study revealed the emulsion with droplet size of 105 µm was responsible for stabilizing the emulsion for 24h hence this may find potential food applications especially in emulsions used for making salad dressings.

Conclusions

The present work reports a remarkable emulsifying property of the test EPS produced by an indigenous species of *Aureobasidium pullulans* RYLF10 where it opens the possibility to use this EPS based bioemulsifier to prepare stable food emulsions like salad dressing, jellies, jam, sauces etc.

Acknowledgement

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Table 1: Emulsifying activity of the test EPS with different vegetable oils and its stability after 24, 72 and 96 hours.

S. No.	Vegetable Oils	%EA		$\%E_{24}$		$\%E_{72}$		$\%E_{96}$	
		TE	GA	TE	GA	TE	GA	TE	GA
1	Olive oil	56±0.1*	60±0.2	55.9±0.5*	58±0.2	50±0.2*	55±0.3	45±0.2*	45±0.4
2	Soybean oil	40±0.1	58±0.2	40±0.4	55±0.2	35±0.2	50±0.4	30±0.2	40±0.2
3	Sesame oil	45±0.2	-	43±0.2	-	40±0.1	-	35±0.2	-
4	Sunflower oil	40±.02	60±0.2	38±0.1	58±0.5	34±0.1	56±0.2	30±0.5	51±0.2
5	Coconut oil	40±.01	66±0.1	40±0.1	62±0.1	30±0.2	60±0.2	30±0.5	52±0.2
6	Mustard oil	44±.04	60±0.2	44±0.1	59±0.2	40±0.5	58±0.2	35±0.2	50±0.4
7	Almond oil	50±.02	60±0.1	50±0.1	58±0.2	45±0.1	50±0.3	38±0.2	45±0.2
8	Groundnut oil	42±0.01	56±0.6	40±0.1	55±0.1	37±0.1	49±0.4	30±0.2	39±0.1

TE: Test EPS and GA: gum Arabic,
***p** <0.05 vs GA.
Values are mean SD of three observations.

Table 2: Effect of different concentrations of the test EPS on its emulsifying activity with olive oil.

S. No.	Concentration of EPS (%)	Emulsifying activity (%EA)	Emulsification index ($\%E_{24}$)
1	0.5	48.0±0.6	45.0±0.2
2	1.0	52.0±0.1	48.0±0.1
3	1.5	56.0±0.2	55.0±0.3
4	2.0	40.0±0.2	35.0±0.6

Values are mean SD of three observations

Table 3: Summarized values of particle size distribution of emulsion formed by the test emulsifier after one hour.

Samples	D (0.1)μm	D (0.5)μm	D (0.9)μm	D[4,3]μm	D[3,2]μm	Span	Specific surface area m^2/g
1h emulsion	90.203	171.00	302.88	105.41	132.97	1.244	0.0451

Table 4: Summarized values of particle size distribution of emulsion formed by the test emulsifier after 24 hours.

Samples	D (0.1)μm	D (0.5)μm	D (0.9)μm	D[4,3]μm	D[3,2]μm	Span	Specific surface area m^2/g
24h emulsion	92.69	187.45	341.90	185.29	142.47	1.330	0.0421

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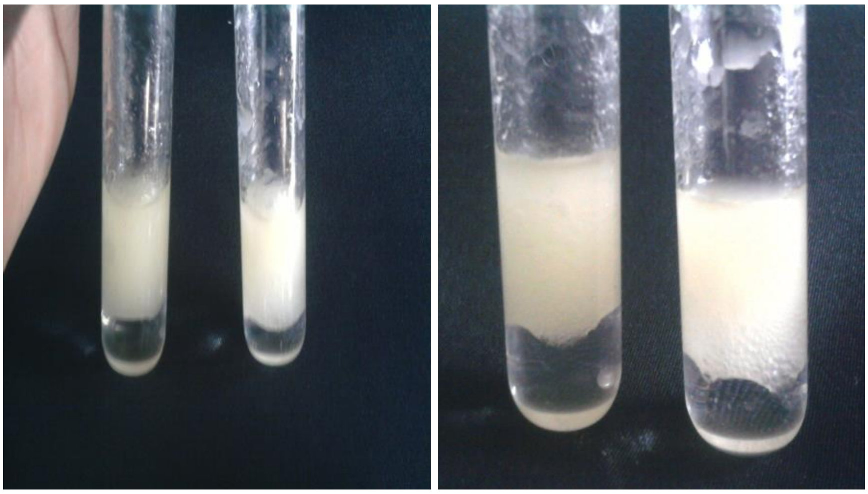

a **b**

Figure 1: The emulsification activity of 1.5% of the test EPS with olive oil. a) after 1hour; b) The stability of emulsion after 24 hours.

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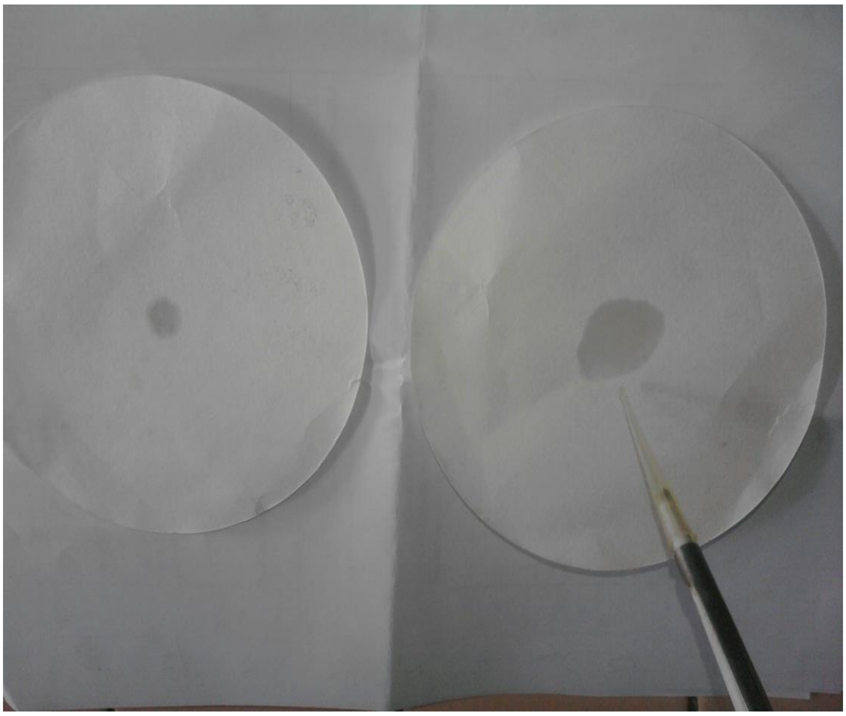
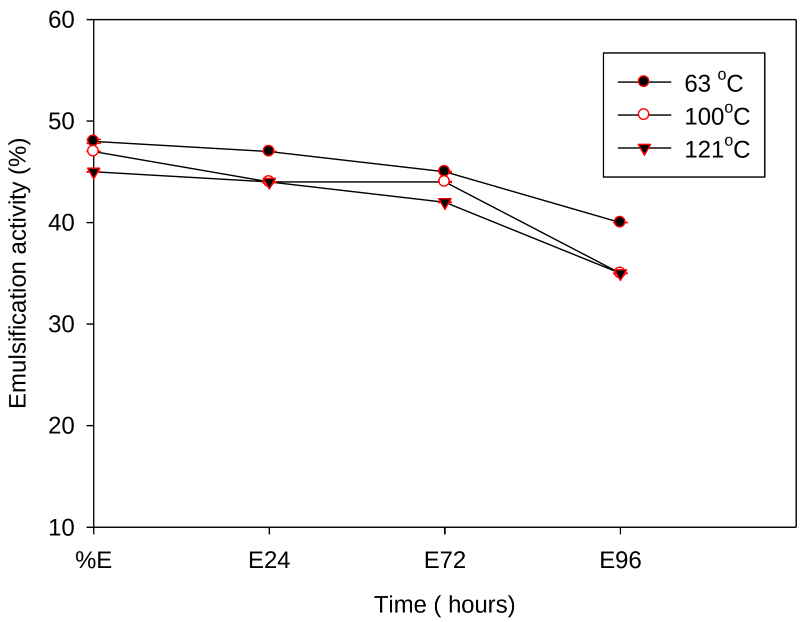
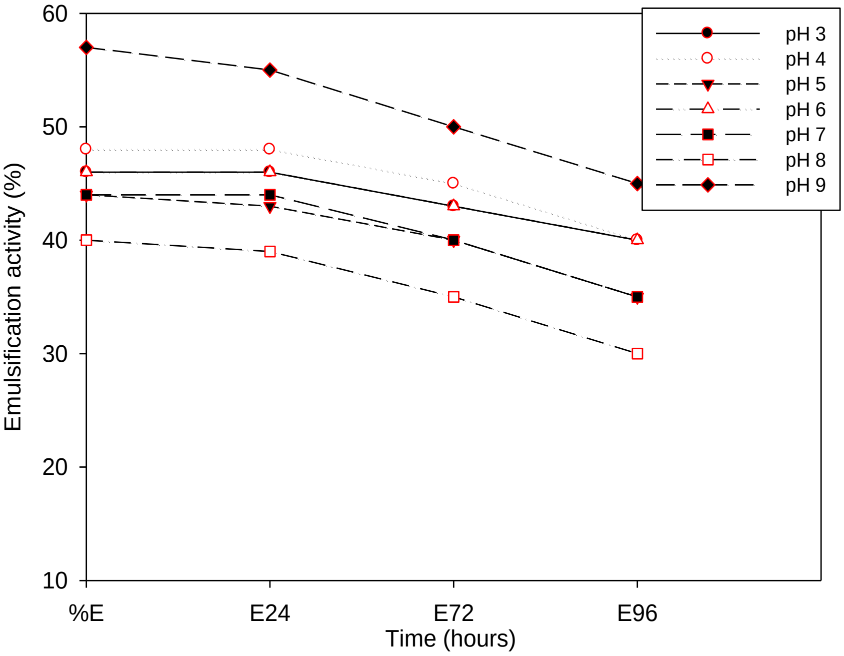


Figure 3: Filter paper wetting test: dispersion of a drop of test emulsion on the filter paper confirming it oil-in-water type of emulsion.



Values are mean SD of three observations

Figure 4: Effect of different temperatures on stability of test emulsion



Values are mean SD of three observations

Figure 5: Effect of different pH on stability of the test emulsion.

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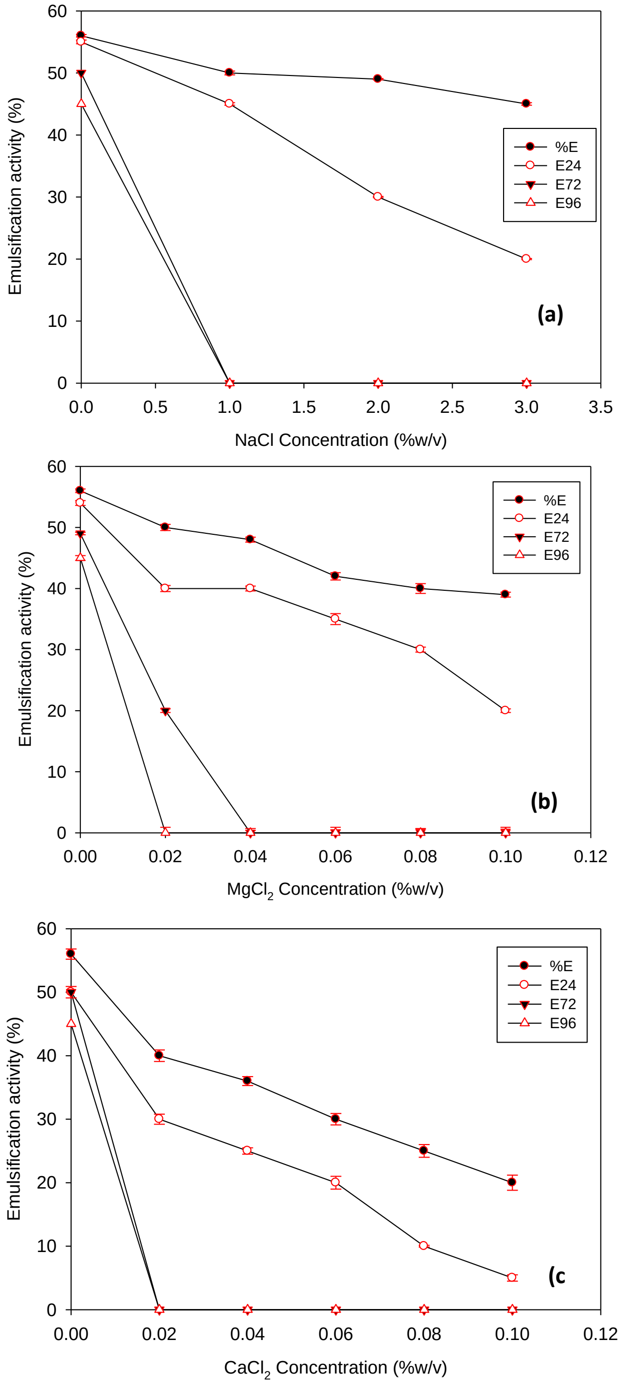

Values are mean SD of three observations

Figure 6: a) Effect of sodium chloride on the stability of test emulsion; b) Effect of magnesium chloride on the stability of test emulsion; c) Effect of calcium chloride on the stability of test emulsion.

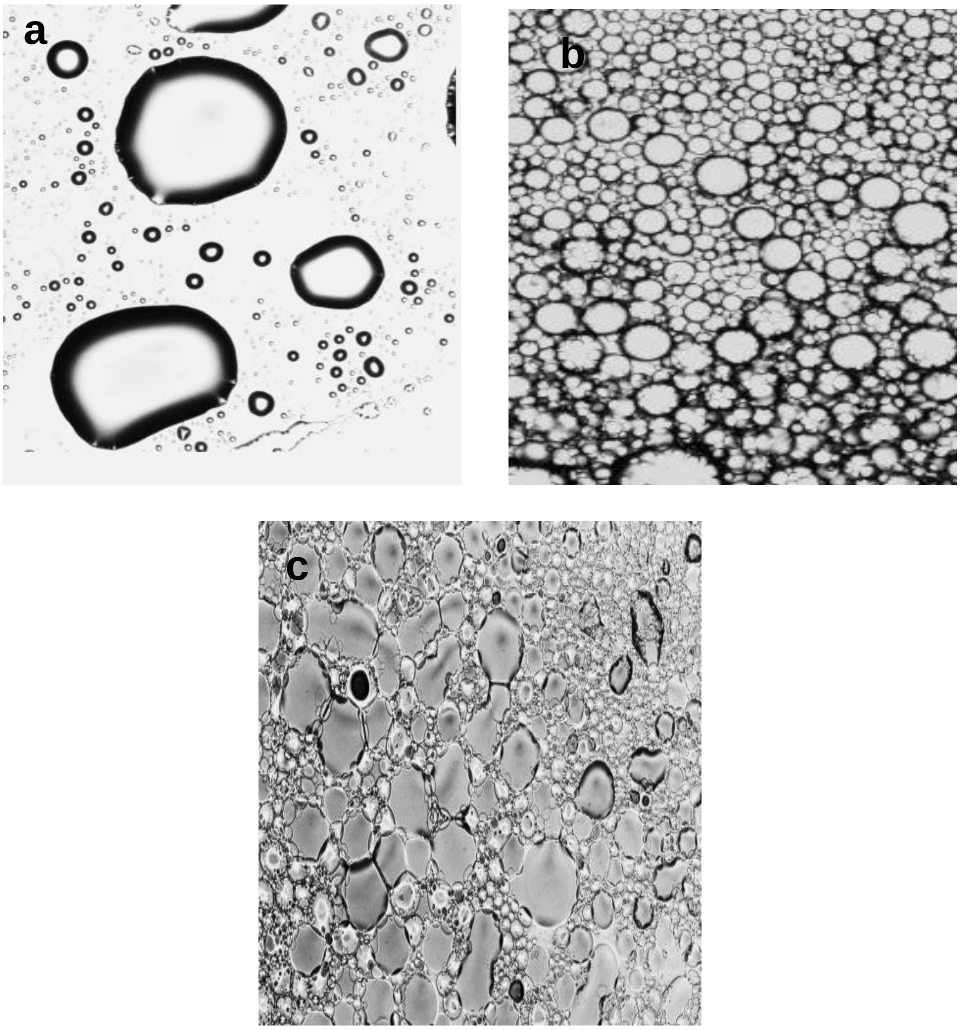

Figure 7: Micrographs at 10X showing the size and distribution of droplets of emulsion in oil-in-water (o/w) type of test emulsion with olive oil; a) Control, emulsion of olive oil and water only (without test emulsifier) after one hour; b) Emulsion with the test emulsifier after 1 hour showing the droplets of emulsion which were approximately of 100 µm in diameter (c): Emulsion with test EPS after 24h showing the droplets of emulsion which were approximately of 185µm in diameter.

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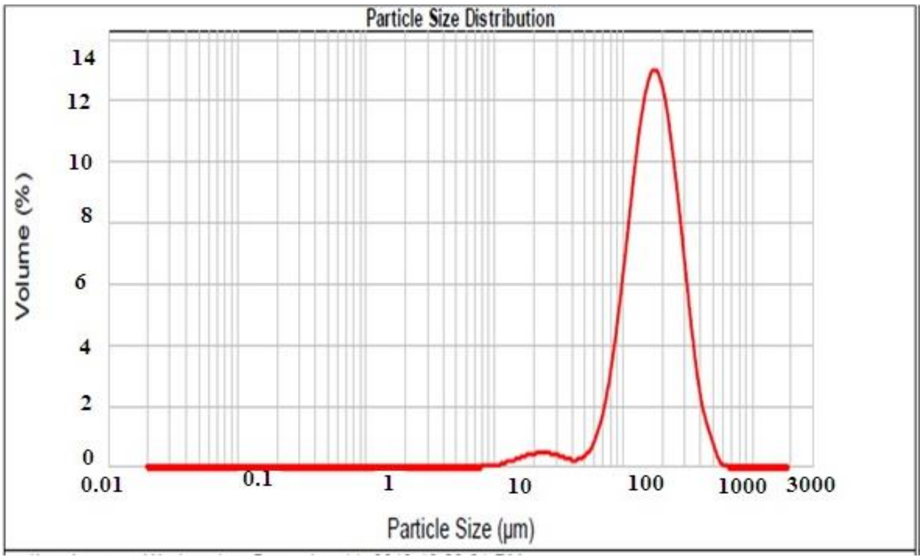

Figure 8: Particle size distribution of droplets formed in emulsion by the test emulsifier with olive oil. Graph shows the monomodal type distribution curve after 1hour with average droplet size of 105.41µm.

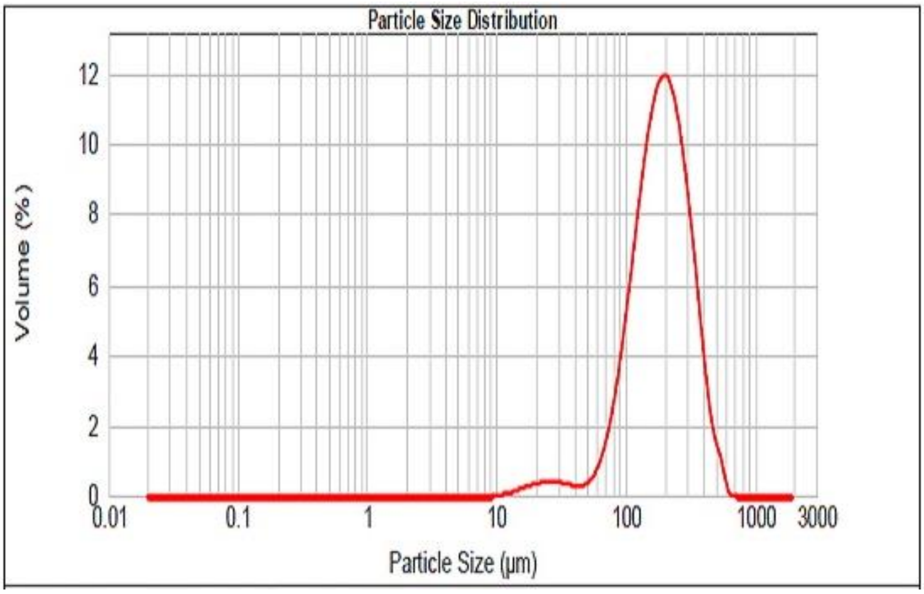

Figure 9: Particle size distribution of droplets formed in emulsion by the test emulsifier with olive oil. Graph shows the monomodal type distribution curve after 24 hours with average droplet size of 185.29 µm.