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STABILITY TOLERANCE OF SUNFLOWER OIL -IN-WATER EMULSION EMULSIFIED BY SOYA LECITHIN FOR FOOD GRADE ACIDS

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ABSTRACT Oil-in-water emulsion has promised values in food, pharmaceutical, drug, cosmetic and allied industries. The stable emulsion with long shelf life increases its utility. Many flavouring agents are used to increase shelf life as preservative or commercial value. Food grade acids are used to in the emulsion preparation which enhances the taste or flavour. The present study focuses the usage of acetic acid, phosphoric acid and oxalic acid. The emulsions for different acids are prepared ranging concentration from 0.001 to 0.003M using lecithin as emulsifier and sunflower oil. Experiments were done to study organoleptic properties with respect to basicity and dissociation constant values. The colour of the primary emulsion was creamy white and sustainable for acetic acid emulsion to 28 day at experimental temperature 10, 25 and 40 0C. Oxalic acid is recorded a low pH value 1.88 for 0.003 M emulsion solution in compare with acetic acid (3.63) and phosphoric acid (2.32).The relative conductance measurement for oxalic acid shows a very high value 5.6mS to acetic acid 0.257mS and phosphoric acid 0.247 mS. The first dissociation constant value 5.6x10-2 of oxalic acid is larger relatively compare to phosphoric acid 7.5 x 10-3 and acetic acid 1.8 x10-5. Basicity of acids increases from acetic acid (mono basic), oxalic acid (dibasic) to phosphoric acid (tri-basic).These results strongly supports that basicity and dissociation constant values of acids conspicuously influence the stability of the emulsion. Higher value of dissociation constant value and basicity, lower the stability of the emulsion.

KEYWORDS : Sunflower oil, Lecithin, emulsion, acid, basicity, dissociation

INTRODUCTION

The colloidal dispersions containing at least two immiscible liquids having one of them have been dispersed into another in the form of tiny droplets is known as emulsion (1). A common type of emulsion containing oil and water system find lot scope in food, pharmaceutical and cosmetic industries. Emulsion technology is widely employed in the food industry to develop a variety of emulsified foods the as beverages, milks, creams, dips, sauces, deserts, dressings, mayonnaise, margarine, and butter (2, 3). The nature of emulsions contributes to the food stuffs with distinguished characteristic functional attributes, like desirable appearances, textures, mouth feels, and flavour profiles. Emulsions are also widely used means for the encapsulation and supply of bioactive agents, like vitamins and nutraceuticals (4). Moreover, Conventional emulsions are composed of numerous emulsifier-coated fluid droplets dispersed within another immiscible fluid medium (5). Many a time, the stability of the emulsions is limited by characteristic phenomena like coalescence, flocculation, lactation, and Ostwald ripening (6-10). Emulsions like Oil-in-water are thermodynamically unstable dispersions of the oil phase in the water phase. To achieve the expected stability and to make these emulsions kinetically stable, a suitable surfactant or mixture of surfactants is always incorporated, which adsorbs at the oil/water interface and results a strong interfacial film (11). However, a specific emulsifier or surfactant may not be suitable for different crude oils due to variation in physicochemical properties.

A class of additives commonly added to improve the organoleptic properties of oil-in-water food emulsion products is the acidulants that is acid regulators. These food additives control the acidity or alkalinity for safety and stability of oil-in-water food emulsion products. Acidulants confer sharp tastes to food and also act as preservatives. Commonly used food acidulants are acetic acid (12-14), However, food acidulants / acid regulators have tremendous effects on the physical stability of oil-in-water emulsion (15). Other factors having profound influence on the food emulsion stability are emulsifier and oil phase concentration (16) homogenizer type and processing variable (17) and additives such as sodium chloride salt (18). Acetic acid is monobasic acid and can be used to increase the acidity by lowering the pH of food products as well as confer the organoleptic quality through importing to the product an acid flavor, such as salt and vinegar chips. Acetic Acid is known to be a popular preservative as it controls bacterial growth in dressings, sauces, cheese, and pickles. Mahalaxmi Pradhananga and Babita Adhikari (19) had studied the properties the mayonnaise prepared by using sunflower oil and egg yolk in presence of vinegar (food grade acetic acid). The acid value obtained was compared with the mayonnaise prepared using sunflower oil and skim milk power as a substitute for egg yolk. Adeyi, O et.al (20) reported that the influence of vinegar concentrations on the physical stability of sunflower oil-in-water emulsions (40w/w% sunflower oil) stabilized by 7 w/w% Bambara Groundnut Flour (BGNF) was investigated. Oil droplet sizes and emulsion microstructure were measured microscopically. Vinegar significantly affected ($p < 0.05$) emulsion stability of BGNF-stabilized emulsion. Vinegar at all studied concentrations in the emulsion increased droplet size and physical instability of BGNF-stabilized emulsions. The results indicated that the stability of BGNF-stabilized

emulsion can be controlled and manipulated using vinegar. Food grade phosphoric acid is a tribasic acid and used as an acidulant and flavouring agent in the carbonated beverage industry. Its taste complements the cola flavour in carbonated beverages. It can be added to fats and oils as an emulsifier and to aid the control of fatty peroxides. In addition, it is used as a degumming agent while refining vegetable oil and sugar. Phosphoric acid also acts as an acidulant for baking powders and emulsifying salts in the production of processed cheese.
The effect of basicity and dissociation constant value of an acid on emulsion formation was experimentally studied selecting food grade acetic acid (vinegar), oxalic acid and phosphoric acid in this paper. Sunflower oil- water emulsion stabilized by soya lecithin was prepared in presence of acetic acid, Oxalic acid and phosphoric acid solutions. The stability correlations were done based on basicity of these acids for droplet size, self-life of the emulsion, colour, pH value and conductance.

EXPERIMENTAL

Materials and Chemicals:

Commercial sun flower oil with permitted antioxidants available in the local market was purchased. Lecithin 35% assays, demonized water, Glacial acetic acid, AR grade oxalic acid and Phosphoric acid were used. All chemicals are purchased by Nice chemical suppliers.

Preparation of emulsion:

Emulsion is prepared by dry gum method. One part by weight of lecithin as emulsifier, 4 part by weight of oil and 8 parts by weight of water were used to prepare oil in water (O/W) emulsion. Dry soya lecithin is taken in a mortar and grinded with 2 ml of water. When it forms a homogenized past, 4g oil was added drop wise and grinded. White pasty primary emulsion is formed which is diluted by adding 6ml water and triturated to result the final emulsion.

Organoleptic characteristics.

Organoleptic characteristics like colour, liquefaction and phase separation were studied for freshly prepared primary emulsions. The experiment was conducted at different storage conditions and the variations for colour, liquefaction and phase separation were noted at different intervals, i.e. 0 h, 1 h, 1 day, 3 days, 7 days, 14 days, 21days and 28 days for 28 days

Centrifugation Test

Centrifugation tests were performed for the primary and emulsions immediately after preparation. The same test was repeated for the emulsions after 6 h, 12h, 24h, 2 days, 5 days, 10 days, 20 days and 25 days of preparation. Centrifugation conditions were 10, 25 and 40 °C and 5000 rpm by placing 10 g of sample in the tube.

Conductivity measurements

The conductance of emulsions of different concentrations was determined using scientific equiptronic conductometer having ±0.001mS accuracy. Quantitatively the change in conductivity of the emulsions with time duration was measured for all concentrations. The percentage of stability of the emulsion was calculated by measuring total volume of the emulsion and volume of separation using the formula below. The correlation of conductance, stability and concentrations were studied.

$$\text{Stability (\%)} = \frac{\text{Volume of separation X 100}}{\text{Total volume of the emulsion}}$$

pH measurement

The pH value of the freshly prepared emulsions and the emulsions of different concentrations kept at different conditions were determined by a digital pH-meter. pH measurements were repeated for each emulsion after 1, 3, 7, 14, 21 and 28 days of preparation.

Quantification of droplet sizes and distributions of emulsion by image analysis

Microscopic tests

Multiple emulsions were analyzed under the microscope to confirm the multiple characters. A drop of multiple emulsion was placed on the glass slide, diluted with water and covered by a glass cover. A drop of immersion oil was placed on the cover slide and observed under the microscope (21).

Micrometric Determination of Droplet Diameter

The diameter of the droplet formed was measured by ocular micrometric method. Stage micrometer was utilized to calibrate ocular micrometer (22, 23, 24). One division of the ocular micrometer was calibrated using the formula.

$$\text{one division oculometer} = \frac{d_s}{d_o} D_o$$

dS = Coinciding division on stage micrometer,
dO = Coinciding division on the oculometer (om),
Ds = One division of stage micrometer = 10 m
Diameter of the droplet = d = No. div. across droplet X 1 div.om.

RESULTS AND DISCUSSION

Color

Freshly prepared primary emulsion was creamy white in color. There was no change in color at different storage conditions. This shows that primary emulsion was stable at different storage conditions up to 28 days. There was little change in color of samples kept at 40 oC (in oven) the color became yellowish white. The change in color appeared on the 21st day and persisted up to 28 days. The change in color at the end of the observation period may be due to the oily phase separation which is promoted at elevated temperatures.

Liquefaction

The primary emulsion was stable and has no liquefaction at all storage conditions. For the emulsion, while no liquefaction was observed in the samples kept at 10oC (in refrigerator) and 25oC (in oven) during 28 days, slight liquefaction was observed in the samples kept at 40oC (in oven), on 21st day. Liquefaction is the sign of instability; it may be attributed to the passage of water from the internal phase to external phase as described by many researchers (25, 26).

Centrifugation test

Observations of the centrifugation tests for the primary and diluted emulsions kept at varied storage conditions are given in Table 1. The primary emulsions were not shown any phase separation after centrifugation kept at different storage conditions up to 21th day. However, slight phase separation was determined on the 21st day in the samples kept at 40oCand there was no more increase in the phase separation until the end of study period. No phase separation after centrifugation was seen in all of the emulsion samples kept at different storage conditions up to 14th day. Slight phase separation was seen in the samples kept at different storage conditions after the 21st day. There was no further increase in phase separation in the samples kept at 10oC (in refrigerator) and 25oC (in oven) until the end of 28th day; however, samples kept at 40oC (in oven) showed increase in phase separation until the 28th day.

Table 1: Organoleptic characteristics and centrifugation test results of multiple emulsion at various storage conditions.

a) Acetic acid

Time	Liquefaction			colour			Phase separation			Centrifugation		
	A	B	C	A	B	C	A	B	C	A	B	C
24h	-	-	-	w	w	w	-	-	-	-	-	-
3days	-	-	-	w	w	w	-	-	-	-	-	-
5days	-	-	-	w	w	w	-	-	-	-	-	-
7days	-	-	-	w	w	w	-	-	-	-	-	-
12 days	-	-	-	w	w	w	-	-	-	-	-	-
15 days	-	-	-	w	w	w	-	-	-	-	-	-
21 days	-	-	-	w	w	w	-	-	-	-	-	-
28 days	-	-	-	w	w	w	-	-	-	-	-	-

b) Oxalic acid

Time	Liquefaction			colour			Phase separation			Centrifugation		
	A	B	C	A	B	C	A	B	C	A	B	C
24h	-	-	-	w	w	w	-	-	-	-	-	-
3days	-	-	-	w	w	w	-	-	-	-	-	-
5days	-	-	-	w	w	w	-	-	-	-	-	-
7days	-	-	-	w	w	w	-	-	-	-	-	-
12 days	-	-	+	w	w	w	-	-	+	-	-	+
15 days	-	-	+	w	w	+	-	-	+	-	-	+
21 days	-	+	++	w	+	+	-	+	++	-	+	++
28 days	-	+	++	w	+	++	-	+	++	-	+	++

c) Phosphoric acid

Time	Liquefaction			colour			Phase separation			Centrifugation		
	A	B	C	A	B	C	A	B	C	A	B	C
24h	-	-	-	w	w	w	-	-	-	-	-	-
3days	-	-	-	w	w	w	-	-	-	-	-	-
5days	-	-	-	w	w	w	-	-	-	-	-	-
7days	-	-	-	w	w	w	-	-	-	-	-	-
12 days	-	-	-	w	w	w	-	-	-	-	-	-
15 days	-	-	+	w	w	+	-	-	+	-	-	+
21 days	-	+	+	w	+	+	-	+	+	-	+	+
28 days	-	+	+	w	+	+	-	+	+	-	+	

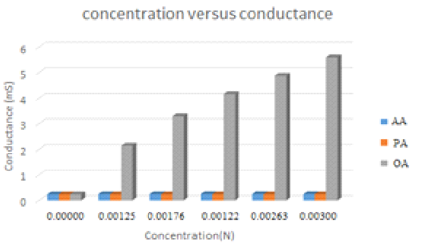
W = White - = No change + = Change ++ = More change A= 10 oC B= 25 oC C=40 oC

Conductance versus concentration and stability of the emulsion The percentage of stability of the emulsion was determined against different concentrations of acidic solution by recording the volume of separation of phases. For oxalic acid emulsion the conductance abruptly increased 5.6mS against 0.003N concentration and limited the stability to 10% as recorded in the Table 2. A graph was plotted conductance in mS against concentration of acid solutions and the trend was observed as shown in the Figure 1.

Table 2: Conductance of the emulsion solutions versus percentage of stability

Acid concentration	Conductance			Percentage of stability		
	Acetic acid	Phosphoric acid	Oxalic acid	Acetic acid	Phosphoric acid	Oxalic acid
0.00000	0.246	0.246	0.246	100	100	100
0.00125	0.253	0.248	2.150	100	100	95
0.00176	0.254	0.248	3.300	100	100	85
0.00222	0.256	0.248	4.160	100	100	60
0.00263	0.256	0.248	4.880	100	100	30
0.00300	0.257	0.249	5.600	100	100	10

Figure 1: Concentration of emulsion acid solution versus Conductance



Conductance of acetic acid and phosphoric acid remains constant up to 0.003N. Oxalic acid shows a large change in conductance with increasing concentration. It was recorded a maximum conductance of 5.6mmho for 0.003N solution of oxalic acid. Stabilization of the emulsion is largely affected by increased conductance which results in creaming of the emulsion. As conductance increases phase separation is conspicuous and finally oily layer separates out from aqueous solution. Conductane of an acid influences the stability of the emulsion considerably to a large extent. 0.00125 N oxalic acid emulsions are quite stable for only 7 to 10 days at the all storage conditions and oxalic acid shown a constant increase in conductance for variable concentrations. 0.003N acetic acid forms a stable emulsion and is 100 % stable for more than 28days in all storage conditions and acetic acid records a very small change in conductance for varying concentrations. The emulsion formed by 0.003N phosphoric acid is 100 % stable to 18 to 21 days in all storage conditions and it shown very small change in conductance for different concentrations. The overall observations confirm that the conductance of acidic solutions largely influence the stability of the emulsion.

pH determination

The pH value of the freshly prepared emulsions and the emulsions kept at different conditions were determined by a digital pH-meter. pH measurements were repeated for different emulsions after 1, 3, 7, 14, 21 and 28 days of preparation. Table 2 shows the pH value of emulsions at different concentrations of acid solutions. Emulsion in acetic acid shows a very small change in pH value (3.28) for the concentration 0.003M even after 28 days in all conditions. Emulsion with oxalic acid gives observable change in pH value (1.22) after 28 days where as phosphoric acid emulsion gives 2.24 for concentration 0.003M. The decreased pH value is largely destabilizing the emulsion which is in turn directly related to the basicity of an acid and their resonance stabilization. As resonance stabilization of ionized molecule increases, the pH value decreases. So, coalesce occurs within 7-8 days in case oxalic acid emulsion.

Table 3: pH value of emulsions with concentration of acids

Acid concentration	pH		
	Acetic acid	Oxalic Acid	Phosphoric Acid
0.0000			
0.00125	3.82	2.07	2.51
0.00176	3.75	1.99	2.44
0.00222	3.70	1.94	2.39
0.00263	3.66	1.90	2.35
0.00300	3.63	1.88	2.32

Acid dissociation constant

In turn, dissociation constant of acid dominantly control the stability of the emulsion. Acetic acid has low value of dissociation constant followed by phosphoric acid and oxalic acid. This explains the anomalies in the stability of emulsion in phosphoric acid as compare to oxalic acid. The trend is as shown in the Table 4.

Table 4: Dissociation constant value acids

Acids	Dissociation constant(ka)	Dissociation
CH_3COO_H	1.8 x 10-5	H+ +CH_3COO--
H_2C2O_4	5.9 x 10-2	H+ +HC_2O--
H_3PO_4	7.5 x 10-3	H+ +H_2PO_3--

Basicity of an acid influences the stability of the emulsion considerably to a large extent. 0.00125 N oxalic acid emulsions are quite stable for only 7 to 10 days at the all storage conditions and oxalic acid is a dibasic acid. 0.003N acetic acid forms a stable emulsion and is 100 % stable for more than 28days in all storage conditions and acetic acid is a monobasic acid. The emulsion formed by 0.003N phosphoric acid is 100 % stable to 18 to 21 days in all storage conditions and is a tribasic acid. It is noticed that the basicity and degree of dissociation largely influence the stability of the emulsion. The dissociation constant for oxalic acid is relatively larger to acetic acid and phosphoric acid. The combined effect of basicity and dissociation constant is made acetic acid as good emulsifying solution.

Micrometric analysis of droplet diameter

The emulsion of different acid solutions was studied ocular micrometer. The diameter of the droplet was analyzed by ocular micrometer method. The microscopic image of the droplets was as shown in the figure. Microscopic image was recorded at an optimum concentration 0.003N of acid solutions. All fresh emulsions had shown almost similar droplet size ranging from 13micrometer to 143micrometer and maximum number of droplets whose size were less than 13 micrometers. In case of oxalic acid emulsion, the interface force decreases with basicity and dissociation constant. Hence, the stability of the emulsion is decreased and droplet's size had been increased. This results in separation of oil and aqueous layer which is predominate in EOX

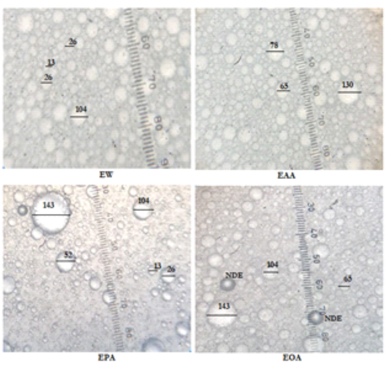


Figure 2: Emulsion of acid solutions with water

EW = Emulsion with water EAA= Emulsion with acetic acid solution EPA= Emulsion with phosphoric acid solution EOE= Emulsion with oxalic acid solution

CONCLUSION

Emulsion technology is widely employed in the food industry to develop a variety of emulsified foods the as beverages, milks, creams, dips, sauces, deserts, dressings, mayonnaise, margarine, and butter. The nature of emulsions contributes to the food stuffs with distinguished characteristic functional attributes, like desirable appearances, textures, mouth-feels, and flavour profiles. The emulsions for different acids are prepared ranging concentration from 0.001 to 0.003M using lecithin as emulsifier and sunflower oil. The influence of basicity and dissociation constant values largely affect the stability of the primary emulsion which could be understood by measuring conductance, pH value, percentage of creaming and morphology of the emulsion. Emulsion with oxalic acid gives observable change in pH value (1.22) after 28 days where as phosphoric acid emulsion gives 2.24 for concentration 0.003M. The decreased pH value is largely destabilizing the emulsion which is in turn directly related to the basicity of an acid and their resonance stabilization. As resonance stabilization of ionized molecule increases, the pH value decreases. So, coalesce occurs within 7-8 days in case oxalic acid emulsion. Stabilizing of the emulsion is largely affected by increased conductance which results in creaming of the emulsion. As conductance increases phase separation is conspicuous and finally oily layer separates out from aqueous solution. Acetic acid is a monobasic acid and 0.003N acetic acid forms a stable emulsion which is 100 % stable for more than 28days in all storage conditions. The emulsion formed by 0.003N phosphoric acid is 100 % stable to 18 to 21 days in all storage conditions and is a tribasic acid. It is noticed that the basicity and degree of dissociation largely influence the stability of the emulsion. Basicity of an acid influences the stability of the emulsion considerably to a large extent. 0.00125 N oxalic acid emulsion is quite stable for only 7 to 10 days at the all storage conditions. Oxalic acid is a dibasic acid. 0.003N acetic acid forms a stable emulsion and is 100 % stable for more than 28days in all storage conditions. Acetic acid is a monobasic acid. The emulsion formed by 0.003N phosphoric acid is 100 % stable to 18 to 21 days in all storage conditions and is a tribasic acid. It is noticed that the basicity and degree of dissociation largely influence the stability of the emulsion.

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