Amino Acids as Potential Emulsifiers in Stabilizing Oil/Water Emulsions

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ABSTRACT: The potential of some amino acids (leucine, glutamic acid, isoleucine, aspartic acid and tryptophan) as emulsifiers in stabilizing some vegetable oils/water emulsions was investigated. The vegetable oils used for the study were Palm kennel oil, Melon oil, Sesame oil, Coconut oil and Groundnut oil. This was achieved by first forming homogenised emulsions of the oil/water and the time of phase separation was monitored. The amino acids were added to the emulsions and the mixtures homogenised and the phase separation time was studied. In all cases, there was increase in phase separation time, which is an indication of increase in stability. These amino acids can be used as emulsifiers in stabilizing oil/water emulsions.

KEYWORDS: Emulsion, Emulsifier, stabilization, Droplet, Vegetable oil, amino acids.

1   INTRODUCTION

Oils form an important class of food substances as they provide energy and serve several other functions such as delivery of fat-soluble chemicals and food processing. Oils are however not soluble in water, the medium in which much of our food, cosmetics, drugs and many industrial products (pesticides, textiles, detergents/soaps etc) are prepared. As foods, oils give a greasy sticky feel when ingested orally. As a result, much of the oils used as foods drugs or cosmetics are presented in the form of emulsions as exemplified in sauces and salad dressings.

An emulsion is a mixture of two immiscible liquids. It contains tiny droplets of one liquid suspend in another. For example, an oil-in-water emulsion contains droplets of oil dispersed in water. Water and oil are immiscible as such they form two distinct layers when mixed together. Adheeb [1], defined emulsion as a heterogeneous system, consisting of at least one immiscible liquid intimately dispersed in another in form of droplets, whose diameter in general exceeds o.1μm. According to Shoko [2] emulsions are used in many fields which includes cosmetics, medicine and in foods. Such emulsions must be stable to ensure consistency, good texture and physical appeal.

Stable emulsions are produced when an emulsifier is added to the mixture of the liquids. An emulsifier consists of a water-loving hydrophilic head and an oil-loving hydrophobic tail. The hydrophilic head is directed to the aqueous phase and the hydrophobic tail to the oil phase. The emulsifier positions itself at the oil/water interface and reduces the interfacial tension hence stabilising the emulsion. Emulsifiers decrease the average size of the droplets in emulsions produced by homogenization because they reduce the interfacial tension, thereby facilitating droplet disruption. They also improve the stability of emulsions to droplet aggregation by generating repulsive forces between the droplets and/or by forming interfacial membranes around the droplets that are resistant to rupture. Emulsifiers used for some oil/water blends include lecithin, sodium/potassium salts of fatty acids, mono- and diglycerides, spans, polysorbates, polyglycol ethers, proteins and protein residues ([3], [4], [5], [6]). Satoshi [7], summarised the functions of an ideal emulsifiers to include rapid adsorption to the surface of the oil droplets formed during homogenization, lowering the interfacial tension and protecting the droplets against aggregation during emulsion processing, storage, and utilization. Vanessa [6], have also demonstrated the use of proteins and protein residues in the stabilization of edible oil/water emulsions.

Emulsifiers have the ability to interact with other food ingredients. In this way, various functionalities can be obtained, for example interaction with proteins or carbohydrates. The emulsifier may be an aerating agent, starch complexing agent and/or crystallisation inhibitor. For example, fat molecules do not mix well with water and so form globs inside the digestive

tract. Emulsification of the fat breaks the fat droplets into smaller units, thus exposing their surfaces for rapid actions by digestives enzymes (e.g. lipase). These enzymes break up the fat molecules to much smaller fragments that can easily be absorbed by the lining of the intestines. The function of the emulsifying agents is similar to that of detergents used in washing.

Food emulsifiers are molecules, which contain a non-polar unit e.g., aliphatic, alicyclic, or aromatic hydrocarbons; and one or more polar units which contained heteroatoms such as oxygen, nitrogen, and sulfur. The polar functionality makes the emulsifier anionic, cationic, amphoteric, or nonionic. Amino acids like alanine, phenylalanine, leucine and isoleucine are surfactants that contain nonpolar aliphatic and aromatic side chains. While, arginine, lysine and tryptophane, contain amino groups, which promote cationic character to the protein and as such are known as cationic surfactants. Aspartic and glutamic acids possess side chains with carboxyl groups, which contribute to their anionic character hence they are known as anionic surfactants.

The use of proteins and their residues in stabilization of edible emulsion is significant as such emulsions constitute an important part of our diet. A first line of approach in this study is to examine how amino acids, the building blocks of proteins, affect the stability of the oil-water interface.

2   MATERIALS AND METHODS

The amino acids; leucine, glutamic acid, isoleucine, aspartic acid and tryptophan and the vegetable oils; Palm kennel oil (PKO), Melon oil (MLO), Sesame oil (SSO), Coconut oil (CNO) and Groundnut oil (GNO) were commercially obtained.

2.1   DETERMINATION OF FLOW RATE

A two liter capacity beaker was filled with distilled water at room temperature. 10mL of distilled water was transferred to the un-graduated arm of an Ostwald Viscometer. The Viscometer was suspended into the beaker with the aid of a retort stand and left for about twenty minutes. The water was then pumped by exerting pressure through the un-graduated arm to a level above the upper calibration mark in the graduate arm of viscometer, and then released. The time taken for the meniscus to drop from the upper mark; above the bulb to the lower mark below the bulb in the graduated arm of the Ostwald Viscometer was recorded, five replicate determinations were made in each case. The procedure was repeated using each of the oil samples.

2.2   DETERMINATION OF DENSITY OF THE OIL SAMPLES

A 25mL density bottle was weighed and filled with water and reweighed, the result was recorded. The experiment was repeated using the vegetable oils. The densities of the water and the oil samples were calculated using the mathematical expression density = mass/volume.

From the measured flow times and the densities, the relative viscosities of the vegetable oils were calculated using the mathematical expression. $\dfrac{\eta_o}{\eta_w} = \dfrac{t_o \rho_o}{t_w \rho_w}$

Where η_o = viscosity of oil
η_w = viscosity of water
t_o = flow time of oil
t_w = flow time of water
ρ_o = density of oil
ρ_w = density of water

2.3   DETERMINATION OF THE STABILITY OF HOMOGENISED OIL/WATER MIXTURE

Exactly 1.0mL of oil sample and 9.0mL of distilled water was transferred into a clean dry conical flask and stirred vigorously using a magnetic stirrer for one minutes. The homogenised mixture was quickly transferred into a 25mL measuring cylinder and timing started immediately. The time taken for the oil to clearly separate from water was recorded. Five replicate determinations were made in each case. The experiment was repeated using oil volume fractions from 0.2 to 0.9.

2.4   DETERMINATION OF THE EFFECTS OF AMINO ACID ON THE PHASE SEPARATION OF THE OIL/WATER MIXTURE AT ROOM TEMPERATURE

Oil/water volume ratio of 1:9 was transferred into seven different test tubes labeled from A to G. 0.009g, 0.046g, 0.920g, 0.138g, 0.184g, 0.230g and 0.276g of amino acid samples, corresponding to 1%, 5%, 10%, 15%, 20%, 25% and 30% on the weight of the oil respectively, were transferred into the seven test tubes. The oil/water/amino acid mixtures were stirred vigorously using a magnetic stirrer for five minutes and allowed to stand. The time taken for clear phase separation was recorded. The conductivities of the sample solutions were also measured. Replicate determinations were conducted and the average calculated.

3   RESULTS AND DISCUSSION

The results of density, Table 1.0, indicate that all the vegetable oils were less dense than water. This implies that a mixture of the oils and water will lead to creaming of the oil droplets. Creaming velocity is dependent on density difference between the liquids [8]. The larger the density difference, the faster the creaming velocity. From the result, melon oil with a lower density will cream faster than the rest of the oils. On the other hand groundnut oil with the highest density will have a slower creaming velocity.

Table 1. Density and relative viscosity of the vegetable oils

	Density (g/ml)	Relative Viscosity (pas)
Palm kernel Oil (PKO)	0.93	3.129
Melon Oil (MLO)	0.91	2.865
Sesame Oil (SSO)	0.96	2.866
Coconut Oil (CNO)	0.94	2.711
Groundnut Oil GNO	0.96	2.863
Water	1.04	-

Table 2. Variation in stability of vegetable oils with different oil/water volume ratio

Oil/water Volume (mL)	Time for Oil/water Phase Separation (Minutes)				
	Palm karnel oil (PKO)	Melon Oil (MLO)	Sesame oil (SSO)	Coconut oil (CNO)	Groundnut Oil (GNO)
1.9	3.99	2.22	4.49	2.43	1.52
2.8	5.81	4.23	6.30	4.53	3.49
3.7	7.35	6.30	7.97	6.40	5.30
4.6	10.00	9.00	9.84	8.41	7.67
5.5	11.50	10.14	12.00	11.50	8.99
6.4	13.62	12.31	14.05	12.62	10.58
7.3	15.83	14.00	15.99	14.51	12.05
8.2	17.51	16.10	16.95	15.99	13.50

Table 3. Variation in stability and conductivity of vegetable oils with different percentages of leucine

Oil/water Volume (mL)	Mass of Leucine	Time for Oil/water phase Separation (Minutes)					Conductivity (mScm)				
		PKO	MLO	SSO	CNO	GNO	PKO	MLO	SSO	CNO	GNO
1:9	1%	11.27	10.46	09.45	07.61	05.61	0.039	0.047	0.035	0.036	0.048
1:9	5%	13.31	12.38	10.36	09.68	06.59	0.035	0.041	0.032	0.034	0.046
1:9	10%	15.28	14.58	12.44	11.49	08.61	0.026	0.039	0.029	0.029	0.035
1:9	15%	17.50	18.42	16.51	14.38	10.64	0.025	0.036	0.026	0.028	0.033
1:9	20%	20.40	21.62	20.33	16.21	12.38	0.023	0.022	0.024	0.025	0.022
1:9	25%	24.62	23.62	23.57	21.55	14.46	0.019	0.020	0.016	0.023	0.021
1:9	30%	27.68	25.50	26.62	23.42	16.41	0.015	0.017	0.014	0.020	0.020

Table 4. Variation in stability and conductivity of vegetable oils with different percentages of glutamic

Oil/water Volume (mL)	Mass of glutamic	Time for Oil/water phase Separation (Minutes)					Conductivity (mScm)				
		PKO	MLO	SSO	CNO	GNO	PKO	MLO	SSO	CNO	GNO
1:9	1%	05.20	07.30	06.57	05.55	04.95	0.0352	0.0435	0.0421	0.0395	0.0334
1:9	5%	07.10	10.03	09.48	07.32	07.87	0.0334	0.0410	0.0415	0.0375	0.0317
1:9	10%	09.55	13.17	12.17	10.05	10.56	0.0299	0.0391	0.0384	0.0330	0.0278
1:9	15%	12.68	17.48	16.38	13.19	11.86	0.0258	0.0372	0.0360	0.0301	0.0249
1:9	20%	15.88	22.58	20.49	17.50	13.46	0.0245	0.0334	0.0320	0.0282	0.0247
1:9	25%	18.98	26.60	24.50	22.60	16.78	0.0242	0.0300	0.0290	0.0255	0.0245
1:9	30%	22.60	31.80	30.62	26.62	20.55	0.0242	0.0250	0.0249	0.0248	0.0242

Table 5. Variation in stability and conductivity of vegetable oils with different percentages of isoleucine

Oil/water Volume (mL)	Mass of Isoleucine	Time for Oil/water phase Separation (Minutes)					Conductivity (mScm)				
		PKO	MLO	SSO	CNO	GNO	PKO	MLO	SSO	CNO	GNO
1:9	1%	5.00	4.38	3.59	3.35	4.0	0.046	0.032	0.032	0.037	0.020
1:9	5%	5.58	4.58	4.50	4.45	4.03	0.043	0.041	0.041	0.060	0.018
1:9	10%	6.59	5.52	5.00	6.00	5.00	0.041	0.037	0.037	0.032	0.012
1:9	15%	8.47	6.47	5.59	7.58	5.59	0.039	0.030	0.030	0.010	0.011
1:9	20%	12.32	8.58	7.56	8.59	8.22	0.038	0.022	0.022	0.008	0.009
1:9	25%	15.00	11.58	9.47	9.58	9.34	0.027	0.018	0.018	0.006	0.007
1:9	30%	15.58	12.80	10.59	30.32	10.53	0.027	0.011	0.011	0.004	0.005

Table 6. Variation in stability and conductivity of vegetable oils with different percentages of Aspartic acid

Oil/water Volume (mL)	Mass of Aspartic acid	Time for Oil/water phase Separation (Minutes)					Conductivity (mScm)				
		Palm karnel oil	Melon Oil	Sesame oil	Coconut oil	Groundnut Oil	Palm karnel oil	Melon Oil	Sesame oil	Coconut oil	Groundnut Oil
1:9	1%	10.58	6.50	11.14	4.10	11.10	0.055	0.039	0.025	0.041	0.042
1:9	5%	20.14	6.52	14.48	4.14	12.12	0.032	0.039	0.023	0.031	0.041
1:9	10%	40.18	7.10	15.12	6.16	13.20	0.021	0.029	0.014	0.030	0.040
1:9	15%	50.20	7.50	18.20	10.15	15.21	0.025	0.021	0.013	0.020	0.031
1:9	20%	70.30	8.14	20.22	10.18	17.48	0.021	0.005	0.010	0.011	0.031
1:9	25%	80.80	10.15	22.54	10.48	18.50	0.011	0.003	0.010	0.010	0.021
1:9	30%	90.50	10.55	29.40	10.42	20.51	0.010	0.001	0.001	0.001	0.001

Table 7. Variation in stability and conductivity of vegetable oils with different percentages of Tryptophan

Oil/water Volume (mL)	Mass of Tryptophan	Time for Oil/water phase Separation (Minutes)					Conductivity (mScm)				
		Palm karnel oil	Melon Oil	Sesame oil	Coconut oil	Groundnut Oil	Palm karnel oil	Melon Oil	Sesame oil	Coconut oil	Groundnut Oil
1:9	1%	5.00	4.38	3.59	3.35	4.00	0.046	0.052	0.032	0.037	0.020
1:9	5%	5.58	4.58	4.50	4.45	4.03	0.043	0.041	0.027	0.036	0.018
1:9	10%	6.59	5.52	5.00	6.00	5.00	0.041	0.0037	0.018	0.032	0.012
1:9	15%	8.47	6.47	5.59	7.58	5.47	0.039	0.030	0.016	0.010	0.011
1:9	20%	12.32	8.58	7.56	8.59	8.22	0.038	0.022	0.012	0.008	0.009
1:9	25%	15.00	11.58	9.47	9.58	9.34	0.027	0.018	0.009	0.006	0.007
1:9	30%	15.58	12.80	10.59	10.32	10.53	0.018	0.011	0.005	0.004	0.005

The viscosity of a liquid is its resistance to flow due to strong intermolecular forces of attraction existing with the liquid [9]. Viscosity is directly proportional to density. The results (Table 1.0) indicate viscosity of the vegetable oil to range between 2.711pas to 3.129pas. The viscosity of emulsion is dependent on the droplets concentration, size, shear rate and the electrostatic repulsion between droplets [10]. Therefore, if the concentration of the oil droplet is increased, the viscosity will also increase, since droplets become closely packed together such that their movement is restricted. Generally, the vegetable oils used are more viscous than water as such, increased in volume fractions of the oils increases viscosity and hence increase in stability of the systems.

Table 2.0 indicates the volume ratio of oil/water at difference intervals with the time taken for the two phases to be separated. At a lower fraction of the oil, the time of phase separation for the highly viscous oils was very short e.g. groundnut oil and melon oil. This implies that at lower fraction of the oil, the oil/water emulsion is very unstable and cream easily, increasing the volume fraction of the oil with a corresponding decrease in the continuous phase fraction (water) increases the stability of the emulsion. This can be observed by the increase in phase separation time.

Table 3.0 shows result of stability of the emulsion formed by the various vegetable oils on addition of the amino acids at different percentages. There is significant increase in phase separation time even at the least percentage of the amino acid added. This is an indication of increase in stability.

The phase separation time for groundnut oil on addition of leucine was lower than observed for the other vegetable oils. This is due to the high viscosity of groundnut oil when compared to the other vegetable oils. Generally, increasing the percentages of amino acids increases time of phase separation hence increase stability of the emulsion (Table 4.0- 7.0).

The conductivity of the emulsion was observed to decrease as the percentage of amino acid was increased. This is because the amino acids which are amphiphilic substances reduce the interfacial tension of the oil and water thereby enhancing the formation of more stable interfaces which disrupt the free movement of ions in solution and hence lower the conductivity.

4   CONCLUSION

The results of the study show an increase in phase separation time when the amino acids were added to the emulsions, this is an indication of increase in stability of the emulsions. This implies that amino acids can be used as emulsifiers in oil/water emulsions.

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