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EMERGING EMULSIFIER: IDENTIFICATION, CHARACTERIZATION AND PERFORMANCE EVALUATION WITH SURFACE ACTIVITY

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ABSTRACT

Emulsifiers are the most indicative material, which is engrossed in immense quantities to a global extent. The food industry depends on using different types of emulsifiers, which increases the search for tailor-made solutions, to the detriment of commercial ones. It can be composed of chemical and biotechnological techniques using sustainable items (monoglycerides and amino acids). The present study tried to combine these two (mono-glycerides= mono-oleate, amino acid=glycine) to produce a specialty class of formulation, amino-acyl glyceride (AAG), that can act as a good emulsifier. The product was characterised structurally using gas chromatography (GC) and Fourier transformation infrared spectroscopy (FTIR) peak assignments. Surface activity was determined by surface tension (ST), interfacial tension (IT) and Critical micelle Concentration (CMC). The structure-property correlation could explain the instance of the deflection of the structure which might lead to excellent surface activity. The foam formation test was determined by foam height and the foamability of solutions. This characterisation depends strongly on the adsorption properties of the surfactants. Studies showed that this newly formulated compound could act as a good food emulsifier due to the significantly low surface activity, low interfacial tension and high foamability. Thus, it can be also an interesting alternative for the food industry concerning other market-friendly emulsifiers.

Keywords: Amino acyl glycerides, Emulsifier, Foamability, Surface Activity.

1. INTRODUCTION

Consumer solicitude towards human health and the environment is reassuring food manufacturers to draw on more natural and viable food ingredients. Principally, the concern is the substitution of synthetic components with natural ones. Increased consumption of natural emulsifiers in food products may lead to a more feasible food supply in a healthier way. Emulsifiers play a decisive role in the development of emulsions, which is recurrently

regarded as an important way to improve the texture and taste of some foods and beverages. Presently the main driving force behind the development of these types of specialty emulsifiers is to add some nutraceutical value to the products; at the same time not diminishing the functional values as well (Maria Rosa Infante et al., 2004). Mono-glycerides and amino acid-based emulsifiers are biocompatible compounds (Pere Clapés et al., 2001) due to their amphiphilic properties, these compounds are extensively used as emulsifiers in food formulations, for example, margarine, low-calorie spreads, and salad dressings, etc.

Nowadays, they are employed in assorted types of cosmetics and cleansing formulations. The possibility of preparing a novel class of emulsifiers by acylation of the hydroxyl groups of mono-oleate with the carboxyl group of amino acid can be explored and the product can be termed as amino acid lipid conjugates. These can be made up entirely from natural food-grade (GRAS) products using a simple esterification reaction which would fulfill the role of the surface-active agent (DJ Mc Clements et al., 2009). They comprise one polar head from glycine, arginine and one or two hydrophobic chains combined with glycerol portion (María Pilar Vinardell et. al., 2004) establishment with ester bonds. The inclusion of an amino group reinstates a free hydroxyl group and, as a consequence, a low hydrophilic molecule will be formed. Acetylated monoacyl glycerides can be an excellent and interesting class of specialty emulsifiers with good surface properties because of their alkyl chain heterogeneity.

The foaming power and foamability of surfactant systems are some of the most important characteristics for the formulation of detergents, cosmetics, and similar compositions. Understanding these factors is essential for developing effective and appealing formulations to evaluate the rate of foam generation in assessing foamability (Shrinivas C. Kothekar & Shamim A. Momin Reader 2007). This leads to an enormous scope for the escalation of the formulations, including the best potential selection of resources.

This work aimed to design and obtain a different kind of amino acid-lipid conjugates identification of the structure through FTIR and understanding the surface-active properties concerning surface tension and CMC, interfacial tension, emulsion stability by foam height and foamability. This study provides information regarding the newly formulated novel emulsifier.

2. MATERIALS AND METHODS

2.1. Materials

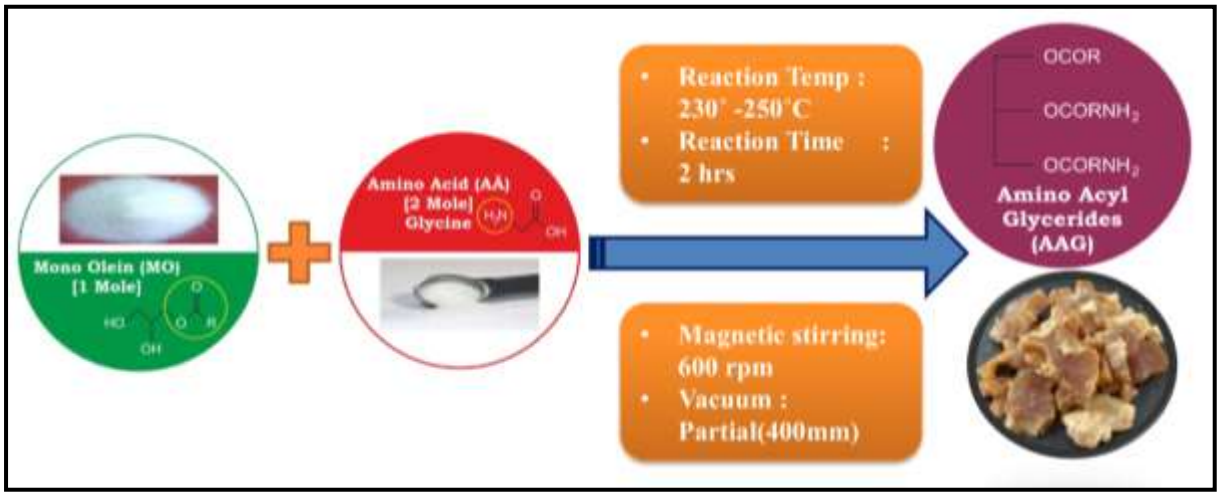
Distilled authentic 1-oleoyl-glycerol [mono-oleate (MO)] was purchased from Sigma Aldrich and determined for fatty acid content by the gas chromatographic (GC) method. Purified amino acid [Glycine (GLY)] was bought from Merck India Ltd., Mumbai, India. All reagent-grade solvents were imposed beyond other purification.

2.2. Methods

2.2.1. Preparation of AAG1

The reaction was conducted with a 1:2 molar ratio of MO: GLY in a flask and stirred at 600 rpm at 230°C -240°C (because the melting point of glycine is 233°C). The reaction was carried out in an area that was completely free from solvents and under a partial vacuum (400

mm) for 2 h **(Scheme 1)**. The amino acid (GLY) was added to the reaction after MO reached its melting point (35°C) and the reactants became homogeneous. After the completion of the procedure, a light brown waxy material was obtained. The product was extracted by chloroform extraction method. Finally, a newly formulated product was obtained, named **Amino Acyl glyceride 1 (AAG1).**



Scheme 1: Formulation of amino acyl glyceride (AAG1) using mono-oleate (MO) and amino acids (GLY)

2.2.2. Structural analysis of MO, GLY and AAG1

The amount of fatty acid and amino acid present in AAG1 was determined by hydrolysing the product with methanolic KOH for 2 h followed by acidification with dil. HCl solution. The product was extracted with petroleum benzene (3 times) to extract the free fatty acids. Then the aqueous part was adjusted to pH 3.7 for precipitation of amino acid. The precipitate was washed with acetone, dried and weighed.

2.2.2.1. Fatty acid percentage by Gas Chromatography (GC)

The fatty acid profiling was carried out by gas chromatographic analysis. The GC (Agilent 6890 N) was equipped with a DB-Wax capillary column (30 m x 0.25 mm x 0.25 µm) and a flame ionization detector (FID). The amount of H_2, N_2 and airflow was kept at 40, 30 and 400 ml/min, subsequently. The front inlet and FID temperatures were maintained at 250°C. The oven temperature was maintained at 150°C. After 5 mins temperature was increased to 190°C with a run time of 13 mins at a rate of 5°C/min. Again temperature was increased to 230°C and maintained for 10 mins at a rate of 5°C/min. The total run time was 31 mins.

2.2.2.2. Analysis of functional groups by Fourier transform infrared spectroscopy (FTIR)

FTIR peak assignments of the samples were documented by Perkin Elmer Spectrum GX Spectrophotometer (range 400–4000 cm^{-1}). The precisely thin layer of samples roofed the NaCl cells (25mmi.d × 4mm thickness) were prepared and determined.

2.2.3. Determination of the physicochemical properties of MO, GLY and AAG1

2.2.3.1. Solubility:

The solubility of compounds was checked at a concentration of 1% (w/v) over three types of the solvent range such as (a) polar solvents (water, ethanol and methanol), (b) dipolar

solvents (acetone, acetonitrile and ethyl acetate) and (c) nonpolar solvents (hexane, chloroform, diethyl ether, petroleum ether) at room temperature (30˚C).

2.2.3.2. Hydrophilic-Lipophilic Balance (HLB) Value:

HLB values of MO and AAG1 were identified by Griffin's method depending upon the molecular mass of the products according to the equation**:**

$$HLB = 20 * M_h/M$$

Where M_h is the molecular mass of the hydrophilic portion of the molecule, and M is the molecular mass of the whole molecule, giving a result on a scale of 0 to 20. An HLB value of 0 corresponds to a completely lipophilic/hydrophobic molecule, and a value of 20 corresponds to a completely hydrophilic/lipophobic molecule (Griffin 1984).

2.2.3.3. Surface tension and Interfacial tension:

Surface tension was determined using an electronic tensiometer (Data physics DCAT 11, Germany) by the Du Nouy method at 25°C taking the surface tension of water as control. The surface tensions of samples were measured by using an aqueous solution of 1% (w/v) concentration at 25± 0.2°C.

2.2.3.4. Critical micelle concentration (CMC)

The critical micelle concentration of individual products was measured from surface tension measurements on Data physics DCAT 11, Germany tensiometer. Mixtures of predetermined concentrations were serially diluted and tested by the Du Nouy ring method. A circulating water bath was used to maintain the ambient temperature (25 ± 0.2°C). The results were determined by evaluating surface tension versus concentrations of surfactant.

2.2.3.5. Foaming Power and Foamability

Identification of foaming power and foamability was carried out by applying the Bartsch (shaking test) method. The solutions of the products [concentration=1% (w/v)] were dynamically shaken by hand in 50 ml graduated test tubes (20 mm inner diameter). The manual speed i.e. 30 times in 30 seconds was given as uniformly as probable. The foam volume was marked and noted immediately. This marking for foam volume was considered as the 0 minute (initial) foam volume. All the foam volumes were recorded after 5 minutes, 15 minutes and 30 minutes respectively. All the experiments were performed at 30˚C (Shrinivas C. Kothekar & Shamim A. Momin Reader 2007). Foamability is calculated as the percentage of decrease in foam height from 0 min to 30 mins. The formula is:

Foamability= (Foam Height at 30 mins/Foam Height at 0 min) ×100

2.3. Statistical analysis

The results were evaluated in triplicates (n = 3). Findings and observations were demonstrated as Mean ± SEM.

3. RESULTS AND DISCUSSIONS

3.1. Formulation

The synthesis of amino acyl glyceride formulation comprises the reaction with one or two hydroxyl groups of monooleate by the α-carboxyl group of GLY. The preparation of the product was carried out with some modifications. The formulated product consisted of some steps that were presented in **Scheme 1**. The major positive aspect of this type of formulation is that it can be prepared efficiently by technologies without enzymes and chemical catalysts.

3.2. Physical and Chemical Characterisation of AAG1 corresponding to pure MO

Table 1 shows the chemical and physical parameters of MO and AAG1. The fatty acid analysis revealed no significant change in the percentage of total fatty acid content in MO and AAG1. After the reaction also the fatty acid part was unchanged in AAG1, but due to the incorporation of an amino group from GLY, AAG1 contained the desired amount of amino acid as shown in **Table 1**.

TABLE 1: Physical and Chemical characteristics of MO and AAG1

Products	Appearance	Fatty acid* (%w/w)	Amino acid* (%w/w)	Melting point[#](°C)	HLB[α] value
MO	White granulated	33.33	0.0	35.0±.28	3.25
AAG1	The light brown waxy material	33.33	66.02	69.2±.20	14.75

*Fatty acid and amino acid composition of MO, AAG1 are expressed as a percentage (%w/w).

[#]Values are expressed as mean ± S.E.M, n=3

[α]HLB= Hydrophilic-Lipophilic Balance expressed as the molecular mass of the molecule.

The HLB value of MO determined that it is a W/O emulsifier but the newly formulated product (AAG1) showed higher HLB values that determined this could be used as O/W emulsifier and could be used as the promising surfactant. Additionally, higher HLB values can make AAG1 a promising surfactant because it can effectively stabilize oil droplets in a continuous water phase, which is desirable for many applications where O/W emulsions are needed.

3.3 Molecular Characterization

3.3.1. FTIR Analysis:

FTIR analysis was done for the identification or confirmation of a chemical compound through its functional group or functional profile present in MO, GLY and AAG1. The FTIR data of the MO, GLY and newly formulated AAG1 are shown in **Table 2.**

The FTIR data showed peaks at 2916.7 cm^{-1} of significant intensity **C-H stretching** established the presence of long-chain acyl groups in AAG1 similar to MO (C-H stretching at

2917.0 cm^{-1}). Even similar intense carbonyl stretching **(C=O stretching)** was observed at 1730.7 cm^{-1} and 1731.0 cm^{1} in MO and AAG1 respectively.

The intense signals of **O-H bending and C-O stretching vibrations** in their total range are associated with the glycerol moiety which is structurally quite obvious in MO. **C-O stretching vibrations** are used to identify the presence of glycerol backbones and understand the bonding environment of oxygen atoms in the molecule. The C-O stretching vibrations provide evidence of esterification and confirm the molecular structure of MO or similar glycerides. This total range was also present in the formulated product AAG1, which strongly confirms the fatty acid groups were successfully transferred through the formulation procedure without having any shifts, providing structural information about the ester bond formation.

Table 2: FTIR Peak assignments of MO, GLY and AAG1

Chemical Groups	**Range(cm^{-1})**		
	MO	**GLY**	**AAG1**
C-H bending	1418.1	1436.9	1418.1
C-H stretching	2917.0	-	2916.7
Carbonyl (C=O stretching)	1730.7	-	1731.0
Carboxylate stretching	-	1379.3	1393.7
Hydroxyl (-OH)	3305.4	-	-
O-H stretch	-	2171.1	2485.3
N-H Stretching vibrations	-	3105.2	3308.6
N-H bend	-	1646.2	1646.6
C-N stretch	-	1042.7	1047.3
O-H bending and C-O stretching vibrations	1418.1	1436.9	1418.1
	1311.8	-	1310.0
	1293.7	-	1288.4
	1274.8	-	1266.5
	1255.8	-	1255.5
	1235.9	-	-
	1215.8	-	1220.6
	943.2	-	943.5

In this data Hydroxyl (-OH) group was not present in the range 3305.4 cm^{-1} confirming the removal of this group successfully while synthesis. The presence of the amino group at 3308.6cm^{-1} confirmed the successful incorporation of **N-H Stretching vibrations** to the newly formulated product. Besides, a very broad carboxylate anion stretching associated with the carboxyl group was noted at 1379.3 cm^{-1}, 1393.7 cm^{-1} in GLY and AAG1 respectively. Formations of AAG1 were validated by the existence of the group **N-H Stretching vibrations** at 3308.6 cm^{-1}, which was attributed to the N-H stretching group (amine group). The presence of the **N-H bend** at 1646.6 cm^{-1} in AAG1, confirms the similar bond formation to GLY (N-H bend at) 1646.2 cm^{-1}. This result showed the successful incorporation of amine groups of GLY into the new formulation, AAG1 without changing the glycerol backbone of MO. Thus, **Table 2** depicts the resemblances among the major groups of MO, GLY and AAG1 validating the formation of the novel product and confirming the desired functional groups.

3.4. Physicochemical properties

3.4.1. Solubility

The solubility was studied at different solvent ranges to determine the wide range of solubility of MO, GLY and AAG1 at 30°C shown in **Table 3**.

Table 3: Solubility chart of MO, GLY and AAG1

Solvents	**Solubility (W/V) of the products**		
	MO	GLY	AAG1
Water	I	S	PS
Ethanol	S (at 40°C)	S	PS
Methanol	S (at 40°C)	S	PS
Acetone	S (at 40°C)	I	I
Acetonitrile	PS	I	I
Ethyl acetate	PS	PS	PS
Hexane	S	I	S (at 50°C)
Chloroform	S	I	S
Diethyl Ether	S	I	S
Petroleum ether	S	I	S

*S=Soluble, PS= Partially Soluble, I= Insoluble

Table 3 showed that GLY was soluble in water, MO was insoluble and AAG1 was partially soluble at 30°C. So the AAG1 consists of both of the characteristics of the raw products used for formulation. MO was soluble in polar solvents (ethanol, methanol) at 40°C, Soluble in acetone at 40°C and ethyl acetate at 30°C and readily in non-polar solvents (hexane, chloroform, diethyl ether) at room temperature of 30°C. GLY was soluble in polar solvents (ethanol, methanol) at 30°C, insoluble in acetone and acetonitrile and also in non-polar

solvents (hexane, chloroform, diethyl ether, petroleum ether) at room temperature (30°C). MO, GLY and AAG1 were partially soluble in ethyl acetate. AAG1 showed similar solubility to MO in non-polar solvents as it contains the fatty acid portion of MO already shown in Table 2. The newly synthesized product was partially soluble in polar solvents, insoluble in acetone and acetonitrile and soluble in non-polar solvents (hexane, chloroform, diethyl ether, petroleum ether).

The formulated product had combined characteristics of solubility trends in different solvents. The products abide by one polar head and one or two aliphatic chains i.e. the GLY unified conjointly by glycerol portion i.e. MO. The inclusion of an amino group supersedes a free hydroxyl group resulting in a lesser hydrophilic product being composed.

3.4.2. Measurement of Surface-active properties

The surface-active properties of MO and AAG1 are presented in **Table 4**.

3.4.2.1. Determination of Surface tension, Interfacial Tension and CMC

The surface tension, interfacial tension, and CMC of the compounds are shown in **Table 4**. It can be observed from the table that the incorporation of the amine group in the glycerol backbone decreased the hydrophobic interaction of the alkyl chains which in turn reduced the surface tension of the compounds and provided pronounced surface activity in these compounds and also increased hydrophobic hydration effects. The surface tension increases with an increase in the molecular weight of the hydrophobic moiety due to cohesive Forces and molecular interaction.

Table 4: Surface active properties by determination of Surface tension, Interfacial Tension and CMC of MO and AAG1.

Products[#]	Surface Tension (mN/m)*	Interfacial Tension (mN/m)*	CMC (µg/mL)*	γ CMC (mN/m)*
MO	35.53 ±.028	5.54 ±.028	9.83 ±.028	34.27 ±.028
AAG1	25.66 ±.026	1.67 ±.028	8.57 ±.029	24.79 ±.027

*Values are demonstrated as mean ± S.E.M (n=3), [#]concentration=1% (w/v)

Table 4 describes the Surface active properties by determination of Surface tension, Interfacial Tension and CMC of MO and AAG1. This reduction in surface tension from MO to AAG1 could be described by a facilitated affinity for emulsifiers with larger hydrophobic molecules favoring aggregation in the solution. AAG1 has a larger hydrophobic region (due to the combination of two groups) resulting in a greater tendency for hydrophobic interactions and aggregation in the aqueous solution. This increased hydrophobicity reduces the free energy at the interface, leading to a more significant reduction in surface tension. It also facilitated the aggregation; the larger hydrophobic surface area of AAG1 allows it to form more stable aggregates or micelles in solution. This aggregation behavior decreases the

overall surface tension more effectively than MO, which has a smaller hydrophobic region and fewer tendencies to aggregate. This would be because of its slow micellization in water. On the other hand, the fast micellization of MO would be the reason for their higher surface tension and interfacial tension values. A decrease of the interfacial tension in AAG1 than MO signifies that these surfactants were absorbed in the interface and became stronger. AAG1 was found to be most efficient in reducing the surface tension and interfacial tension of water. Table 4 also showed AAG1 having lower γ CMC than MO. It indicates a lower γ CMC could be a more effective surfactant, as it achieves significant surface tension reduction at or before reaching the CMC. AAG1 shows a lower γ CMC compared to MO, highlighting its superior surface activity and ability to lower surface tension more effectively, which is valuable for applications requiring efficient emulsification and stabilization at lower surfactant concentrations. AAG1 showed an increase in surface activity in all aspects as evident from its surface tension, interfacial tension, γ CMC compared to MO. These are very important factors in making the most efficient use of this type of surfactant in the bakery industry.

3.4.2.2. Surface active properties by determination of foam formation, stability, foaming power and foamability

Fig 1 and Table 5 show the surface-active properties of surfactants involved in evaluating their ability to form and stabilize foam.

3.4.2.2.1. Foam Formation and Stability

The visual appearance of foam formation and stability at 0 min (immediately after vigorous shaking) and 30 mins of MO (a, c) and AAG1 (b, d) is shown in **Fig 1**. AAG1 was readily involved rapid diffusion of surfactants to the air-water interface, facilitated by mechanical agitation and optimized surfactant properties to lower surface tension and create stable bubbles after vigorous shaking than MO shown in Fig 1.

The observation is that the foam generated by MO decreases significantly after 30 minutes compared to AAG1. It could be attributed to several key differences in their surfactant properties and the mechanisms governing foam stability.

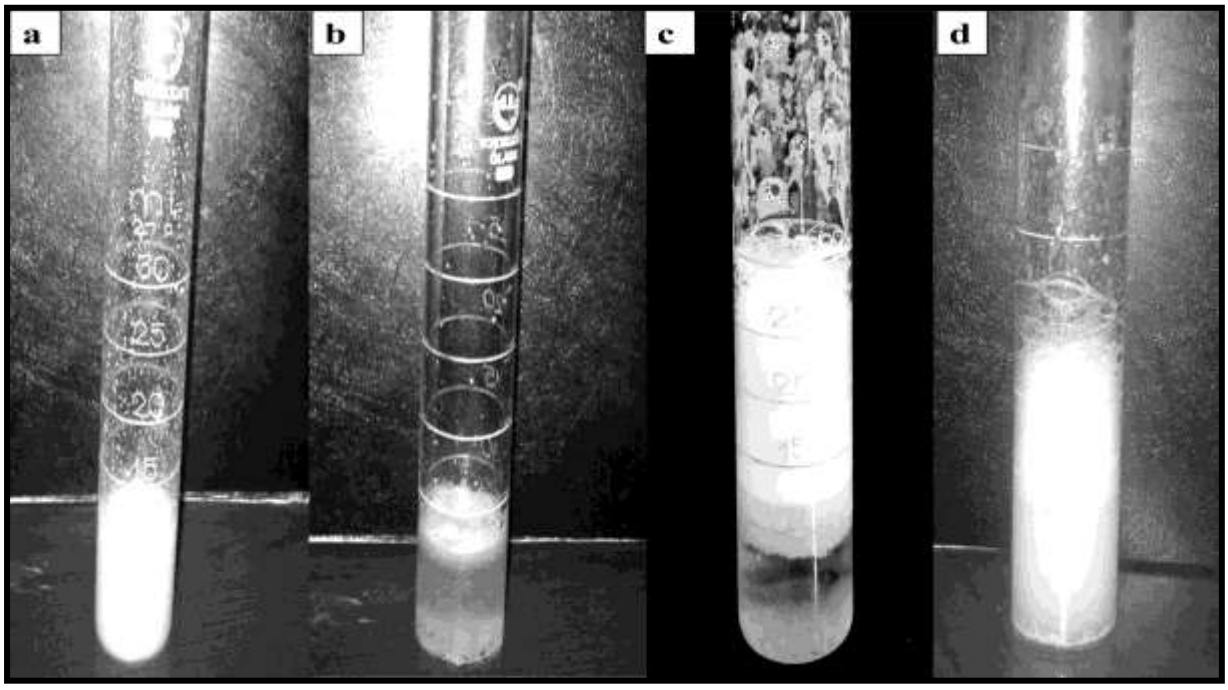


Fig 1: Foam formation and Stability images of MO (a, b), AAG1 (c, d) at 0 min (a, c) and 30 mins (b, d)

AAG1 could resist coalescence and drainage better, maintaining foam stability for a longer duration and enhancing the stability of foam than MO. It may depend on factors like low interfacial tension and low surface viscosity which together prevent bubble coalescence and collapse, ensuring the longevity of the foam structure in AAG1. Effective foam generation requires emulsifiers (surfactants) to quickly diffuse to the air-liquid interface. In the case of AAG1, rapid diffusion ensures that the interface is sufficiently covered by surfactant molecules, promoting bubble formation and stability.

3.4.2.2.2. Foaming power and foamability

These are the classic characteristics of surfactants and the major ability to form stable emulsions. **Table 5** shows the foaming power in the form of foam height and foamability of the formulation. Foaming power was evaluated by the presence of lag period, the amount of diffusion, and the development of foam height at the fluid interface depending upon the time 0 min, 5 mins, 15 mins, and 30 mins respectively.

Table 5: Foaming power and foamability of MO and AAG1

Products[#]	**Foaming power depending upon foam height(cm)**[*]				**Foamability (%)**
	0 min	**5mins**	**15mins**	**30mins**	
MO	10	8	5	4	40.00
AAG1	45	40	35	30	66.66

[*]Error was: Foam height= ±0.1cm, [#]concentration=1% (w/v)

In Table 5 at 0 min, AAG1 shows a foam height of 45cm whereas MO is only 10 cm. The coherence among foaming quality and the dynamics of emulsifier adsorption emerges to be universal for MO and AAG1 but the competent formulation of foams builds on brisk adsorption onto the interface formed better in AAG1. Then as time goes the foam height decreases in both cases. After 30 min the foam height goes down to 30 cm and 4 cm in case of AAG1 and MO respectively. Hence, optimum foaming capability was accomplished under a downward position that diminishes the lag period and induces rapid diffusion of the emulsifiers in contact with the interface to produce tiny bubbles. Then, this ratio, expressed as a percentage, indicates the stability of the foam. A higher percentage of foamability means the foam is more stable over time, retaining a greater proportion of its initial height. The percentage of foamability definite amount of interfacial elasticity were desired to stabilise the bubbles at the time of preparation of the foam.

4. CONCLUSION

The present study revealed two major findings. Firstly, the incorporation of the carboxyl group of amino acids into simple mono-olein may lead to a novel structure with improved and new properties. The fabricated product entirely made from natural food-grade (GRAS)

products using a simple reaction is a novel approach in the field of the food industry. Secondly, the physicochemical properties of a newly tailor-made formulation, AAG1 played an essential role as an eminent substitute for typical emulsifiers due to their multi-functionality for the food industry. By analyzing the FTIR vibrations, the study can gain valuable insights into the molecular structure, interactions, and properties of compounds containing these types of groups, aiding in the development of new formulations. This versatility makes AAG1 potentially useful in various formulations where emulsion stability. By understanding surface activity by Surface tension, interfacial tension, γ CMC and foaming stability, the formulation can be optimized for applications requiring specific characteristics and stability, enhancing the functionality and effectiveness of the product across various industries.

This product if prepared on a large scale in industries might provide a divergent magnitude to the future technology in the food industry mainly focused on the bakery industry. All these features make the novel formulation an outstanding alternative to conventional specialty surfactants. Therefore this product along with the raw material was chosen for further product formation by different amino acids and characterisation. Future studies on the physicochemical properties and molecular characterization of surfactants like this could provide deeper insights into their behavior and applications.

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Consent for publication: Not applicable

Authorship contribution statement: Sriparna Chakraborty: Writing -original draft, Methodology, Data curation and analysis**. Dipak K Bhattacharyya:** Conceptualization**. Mahua Ghosh:** Supervision, Writing- review and editing. **Sriparna Datta:** Supervision and editing.

Note: In this manuscript author **Dr. Mahua Ghosh** passed away suddenly. However, due to her immense contribution to this manuscript, I would like to keep her name to show respect and gratitude towards her.

REFERENCES:

1. Cabra, V.; Arreguín, R. ; Farres, A. (2008). Emulsifying properties of proteins. *Boletín de la Sociedad Química de México, 2(2)*, 80-89.
2. Clapes P, Infante MR. (2002) Amino Acid-based Surfactants: Enzymatic Synthesis, Properties and Potential Applications. *Biocatal. Biotransform., 20,* 215–233. https://doi.org/10.1080/10242420290004947
3. Eissa, F. M. A (2007). Synthesis and evaluation of some surface active agents from long chain fatty amine. *Grasas Y Aceites, 58 (4),* 379-389. https://doi.org/10.3989/gya.2007.v58.i4.450.
4. Griffin, W.C. (1954). Calculation of HLB values of non-ionic surfactants. *J. Society of Cosmetic Chemists*, 5, p. 259.
5. Golding, M.; Wooster, TJ. (2010). The influence of emulsion structure and stability on lipid digestion. *Current Opinion in Colloid & Interface Science*, 15, 90–101. DOI: 10.1016/j.cocis.2009.11.006.

6. Infante, MR.; Pe´rez, L.; Pinazo, A; Clapes´ P.; Mora´n , MC.; Angelet, M.,; García, MT.; Vinardell, MP. (2004). Amino Acid-based Surfactants, *Comptes Rendus Chimie* volume 7, issues6-7, Pages 583-592. https://doi.org/10.1016/j.crci.2004.02.009.
7. Kothekar, Shrinivas, C.; Momin, Shamim, A. (2007 'a') Prediction of the Properties of a Binary Surfactant Mixture of Arlacel-165 and Myrj-59 in Aqueous System. *Journal of Dispersion Science and Technology*, 28:627–631. https://doi.org/10.1080/01932690701283334.
8. Kothekar, Shrinivas, C.; Ware, Adinath, M.; Waghmare, Jyotsna, T.; Momin, Shamim, A. (2007'b'). Comparative Analysis of the Properties of Tween 20, Tween 60, Tween 80, Arlacel 60, and Arlacel 80. *Journal of Dispersion Science and Technology*, 28:3, 477-484. https://doi.org/10.1080/01932690601108045.
9. Katarzyna, E.; Greber.,Małgorzata.; Dawgul.; Wojciech, Kamysz.; Wiesław, Sawicki.; Jerzy Łukasiak. (2014). Biological and surface-active properties of double-chain cationic amino acid-based surfactants. *Amino Acids,* 46, 1893–1898. DOI 10.1007/s00726-014-1744-9.
10. Kua GKB, Nguyen GKT, Li Z, (2023), Enzyme Engineering for High-Yielding Amide Formation: Lipase-Catalyzed Synthesis of *N*-Acyl Glycines in Aqueous Media. *Angewandte Chemie.* 62 (14). https://doi.org/10.1002/anie.202217878
11. Lundin, L.; Golding, M. Structure design for healthy food. *Australian Journal of Dairy Technology*, 64, 68–74, 2009.
12. Majeed,T; Sølling, Theis, I; Kamal, Muhammad, Shahzad. (2020). Foamstability: The interplay between salt-, surfactant- and critical micelle concentration. *Journal of Petroleum Science and Engineering* 187, 106871. https://doi.org/10.1016/j.petrol.2019.106871
13. Mc, Clements, DJ.; Decker, EA.; Park, Y. (2009). Controlling lipid bioavailability through physicochemical and structural approaches. *Critical Reviews in Food Science and Nutrition*, 49, 48–67. https://doi.org/10.1080/10408390701764245.
14. Mc, Clements, DJ.; Li, Y. (2010). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. *Advances in Colloid and Interface Science*, 159, 213–228. https://doi.org/10.1016/j.cis.2010.06.010.
15. Mc, Clements, DJ.; Decker, EA.; Weiss, J. (2007). Emulsion-Based Delivery Systems for Lipophilic Bioactive Components. *Journal of Food Science*, 72, 109-124. https://doi.org/10.1111/j.1750-3841.2007.00507.x.
16. Mc, Clements, DJ.; Decker, EA.; Park, Y. (2009). Controlling lipid bioavailability through physicochemical and structural approaches. *Critical Reviews in Food Science and Nutrition*, 49, 48–67. https://doi.org/10.1080/10408390701764245.
17. Morán, MC.; Infante, MR.; Clapés, P.(2001). Synthesis of glycero amino acid-based surfactants. Part 1. Enzymatic preparation of rac-1-O-(N_-acetyl-L-aminoacyl) glycerol derivatives. *Journal of Chemical Society, Perkin Transactions 1*, 2063–2070.https://doi.org/10.1039/B103132P.
18. Morán, MC.; Pinazo, A.; Pe´rez, L; Clapes,´ P.; Angelet, M.; García, MT.; Vinardell, MP.; Infante MR. (2004). "Green" amino acid-based surfactants. *Green Chemistry*, 6, 233–240. https://doi.org/10.1039/B400293H.

19. Mora´n MC,.; Pinazo A.; Clape´s P, Infante, MR; Pons R (2005) The Effect of Molecular Shape on the Thermotropic Liquid Crystal Behavior of Monolauroylated Amino Acid Glyceride Conjugates. *J. Phys. Chem. B*, 109, 22899-22908. https://doi.org/10.1021/jp053093l
20. Pe´rez, L.; Pinazo, A.; Garcı´a, MT.; Mora´n, MC.; Infante, MR. (2004). Monoglyceride surfactants from arginine: synthesis and biological properties. *New Journal of Chemistry*, 28, 1326–1334. https://doi.org/10.1039/B405733C.
21. Pinazo,A.; Lozano N.; Pe´rez L.; Mora´n MC.; Infante MR.; Pons R. (2011). Arginine diacyl-glycerolipid conjugates as multifunctional biocompatible surfactants, *Comptes Rendus Chimie*,14, 726–735 doi:10.1016/j.crci.2010.10.004
22. Reis, P.; Holmberg, K.; Watzke, H.; Leser, ME.; Miller, R. (2009). Lipases at interfaces: a review. *Advances in Colloid and Interface Science*, 147–48, 237–50. https://doi.org/10.1016/j.cis.2008.06.001.
23. Sayed, R. El. (2008) Surface active properties and biological activity of novel nonionic surfactants containing pyrimidines and related nitrogen heterocyclic ring systems. *GRASAS Y ACEITES*, 59 (2), 110-120. https://doi.org/10.3989/gya.2008.v59.i2.498.
24. Suaad, M.H.; Al, Majidi.; Haider, MM.; Al-tamimy. (2017). Synthesis, Identification and Surface Active Properties of Some Nonionic Surfactants Containing Quinazolinone Ring. *IOSR Journal of Applied Chemistry (IOSR-JAC)*, Volume 10, Issue 5 Ver. I, PP 37-46. DOI: 10.9790/5736-1005013746.
25. Singare, Pravin, U.; Mhatre.; Jyoti, D. (2012). Development of Arginine Based Monopeptides as Cationic Surfactants from Pure Amino Acid. Science and Technology; 2 (4): 55-60. doi:10.5923/j.scit.20120204.01
26. Singare, Pravin, U.; Mhatre.; Jyoti, D. (2012). Cationic Surfactants from Arginine: Synthesis and Physicochemical Properties. *American Journal of Chemistry*, 2(4), 186-190.DOI: 10.5923/j.chemistry.20120204.02.
27. Valderrama, JM.; Juan, Mand, Patino, R. (2010). Interfacial rheology of protein–surfactant mixtures. *Current Opinion in Colloid & Interface Science*, 15, 271–282. https://doi.org/10.1016/j.cocis.2009.12.004.