

Preparation, Characterization, and Surface and Biological Properties of *N*-Stearoyl Amino Acids

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ABSTRACT: Several lipoamino acids were synthesized, in which *n*-octadecanoic acid (stearic acid) was coupled with the α -amino group of an amino acid. The products were characterized and their identities confirmed by advanced analytical techniques like Fourier transform infrared, ^1H nuclear magnetic resonance spectroscopy, and differential scanning calorimetry. Their surface properties, such as critical micelle concentration (CMC) and foaming properties, biodegradability, and antimicrobial activity were also evaluated. The *N*-stearoyl amino acids (NSA) had low CMC values, and some of them showed good foaming properties. They were screened for antimicrobial activity against the gram-positive bacteria *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus cereus*, the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, and the yeast *Candida albicans*. All the compounds inhibited at least one of these organisms. *N*-Stearoyl proline was the most effective, the order of antimicrobial activity being aromatic NSA > acidic NSA > basic NSA. However, the effective inhibition by all the compounds indicates the desirability of more thorough investigation and suggests that some of these compounds may have potential utility as biostatic additives in commercial products. All NSA are highly biodegradable and can readily be removed under conditions of normal secondary sewage treatment.

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KEY WORDS: Amino acids, antimicrobial activity, biodegradability, critical micelle concentration, foaming property, lipoamino acids, surface activity.

Studies on lipoamino acids have been of great interest in the last few years because of their high degree of biocompatibility. Surfactants have a variety of applications in detergency, solubilization, emulsification, and preservation of biomaterials. Studies on surface-active properties of different types of surfactants derived from lauroyl and palmitoyl derivatives of glycine and alanine have been reported (1). Critical micelle concentration (CMC) and foaming ability can serve to characterize the physicochemical nature of any surfactant molecule. The evaluation of foaming properties of surfactants by the Ross–Miles method was reported in the literature in 1940 (2). This method involves the measurement of foam height for comparison of relative foam stability. The measured value depends on capillary penetration, time, temperature, solubil-

ity, and volume of the surfactant solutions. Thus, there is a need to develop a suitable method to measure the foaming properties of any surfactant molecules of any types, such as lipoamino acids, to keep pace with advances in the surfactant field. There is a correlation between X-ray diffraction (XRD) and differential scanning calorimetry (DSC) data for the polymorphic states of surfactants (3). Hence, an attempt has been made to measure the foaming properties of lipoamino acids by microcalorimetric techniques.

Studies of biological properties of surfactants include screening of antimicrobial efficacy and biodegradability. The quaternary ammonium compounds that are widely used as germicides (4) are strongly bacteriostatic at higher dilutions and have germicidal action in more concentrated solutions.

Earlier reports of *N*-palmitoylated amino acids tested for their inhibitory action against Sendai virus fusion to liposomes composed of egg phosphatidylethanolamine (5). Fatty acids of varying chain lengths are known for their antimicrobial action (6,7), primarily against gram-positive bacteria and yeast at low pH. Sheba *et al.* (8) studied the fungistatic action of oleic acid, which was found to be active against a wide spectrum of saprophytic molds and yeast. It has also been reported (9) in the literature that long-chain fatty acid derivatives of glycolic acid showed antimicrobial activity.

The biodegradability of surfactants has been the subject of numerous investigations in the past few years. The hydrophobic groups in all nonionic surfactants like dodecyltrimethylammonium glycol monoether can be biodegradable, except those with highly branched carbon chains. Matsumura *et al.* (10) studied the biodegradability of *n*-alkyl glucosides, mannosides, and galactosides and found that these surfactants were up to 50% biodegradable. In recent years, there has been worldwide interest in developing surface-active molecules that have both antimicrobial activity and environmental compatibility, or, in other words, that are easily biodegradable. Different kinds of surface-active molecules (11) have been synthesized, and some of them are being used in practical applications, mainly in cosmetic and food applications. Only a few studies of their properties have been reported in the literature.

N-Acyl amino acids based on a C_{18} chain have not yet been thoroughly studied with respect to their physicochemical and biological properties, in particular, their antimicrobial activity and biodegradability. In the present work, we synthesized *N*-octadecanoic acid-based lipoamino acids and investigated their interfacial, antimicrobial, and biodegradability properties.

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EXPERIMENTAL PROCEDURES

Materials. All the amino acids used in this study (L-glutamic acid, L-aspartic acid, L-lysine, L-arginine, L-histidine, L-proline, L-phenylalanine, L-tryptophan, and L-tyrosine) were purchased from Hi Media Laboratories Pvt. Limited (Bombay, India). *n*-Octadecanoic acid (stearic acid) was purchased from Loba Chemie (Bombay, India), acetone from S.D. Fine Chemicals Ltd. (Bombay, India), ethyl alcohol from Bengal Chemicals (Calcutta, India), petroleum benzene from Ranbaxy Fine Chemicals Limited (New Delhi, India), and the deuterated solvent CDCl₃ from Aldrich Chemicals (Milwaukee, WI). Sodium hydroxide and sulfuric acid were obtained from Fischer Inorganic and Aromatics Ltd. (Madras, India). Double distilled water was used wherever necessary. All other chemicals used were analytical-grade reagents.

Synthesis of *N*-stearoyl amino acids (NSA). Synthesis of NSA involves a two-step process, i.e., preparation of the acid chlorides as reported elsewhere (12) followed by preparation of the corresponding NSA. A typical method is as follows.

To an acetone and water mixture (pH 12 with NaOH), 0.24 mol of L-amino acids and 0.2 mol of stearoyl chloride were added with stirring over 25 min at 0°C. After further stirring for 30 min, the mixture was acidified with sulfuric acid to obtain crystalline and semisolid *N*-stearoyl amino acid. Following washing with petroleum benzene, the crystals obtained were recrystallized from an ethanol/petroleum benzene mixture.

All the prepared NSA were characterized by Fourier transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (NMR) spectroscopy. FTIR spectra were recorded using a Shimadzu FTIR 8000 series Spectrometer (Kyoto, Japan); KBr pellets were used for solid samples, and a transmission cell with NaCl windows was used for liquid samples. NMR spectra were recorded using a Bruker 300-MHz FT-NMR spectrometer (Karlsruhe, Germany). Samples were dissolved in CDCl₃, and tetramethylsilane was used as an internal standard.

Determination of CMC. The CMC of all NSA were estimated by a conductivity method (Direct Conductivity Measurement Meter 303; cell constant = 1.0003; Systronic Electronics, Amendabad, India). NSA solutions (10 mM) were prepared in 0.1 N sodium hydroxide and added to a beaker containing 40 mL of double distilled water. The specific conductance was measured during gradual addition of the NSA solution. When differences in specific electrical conductivity were plotted against the concentration of NSA in the beakers, two straight lines were obtained and were extrapolated to obtain the concentration at their intersection, which was defined as the CMC. All the measurements were carried out in duplicate.

Determination of foaming properties by differential scanning calorimetry (DSC). All DSC measurements were performed on a DuPont 2000 Thermal Analyzer, equipped with a DSC cell (DuPont, Boston, MA). The peak areas were estimated by the DuPont Advanced DSC (V. 4.1.C) program. For

analysis, 5–10 mg of the NSA was weighed into an aluminum DSC pan. The samples were analyzed under a nitrogen atmosphere at a program rate of 5°C/min up to 350°C. All samples were analyzed in duplicate. The peak integrations were performed automatically by the computer program.

Methodology for screening for antimicrobial activity. The NSA prepared in this study were screened for their antimicrobial activity against pathogenic organisms including gram-positive and gram-negative bacteria and a fungal strain. Six organisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Bacillus cereus*, and *Candida albicans*) were used to study the ability of the test compounds (at concentrations of 10 and 20 mg/mL) to inhibit microbial growth. All the bacterial species were grown on nutrient agar (Hi Media Laboratory, Bombay, India); *C. albicans* was cultivated on Sabouraud dextrose media (Hi Media Laboratory). The cultures were incubated at 30°C. The following controls were employed: for *E. coli*, norfloxacin (5 µg); *S. aureus*, tetracycline (20 mg); *P. aeruginosa*, gentamycin (20 µg); *M. luteus*, erythromycin (20 µg); *B. cereus*, doxycycline (10 µg); and *C. albicans*, ketoconazole (500 µg). Twenty-four-hour slant cultures of the microorganism were used to prepare suspensions for plate inoculations. The suspensions served as the inoculum for the determination of antimicrobial activity. Agar plates were inoculated with the appropriate inoculum by placing 3 drops on the agar surface and spreading them uniformly with a sterile, bent glass rod. Filter paper discs 6.5 mm in diameter, made from Whatman No.1 filter paper, were used to evaluate the samples. The paper discs were wetted until they were completely saturated with the test compound (at concentrations of 10 and 20 mg/mL) and then placed on the surface of the agar plates inoculated with the test organisms. *N,N*-Dimethylformamide was used as carrier solvent. A minimum of two experiments was performed at different times, employing duplicate plates for each compound under test. All plates were incubated at the optimal growing temperature for each organism, and readings were taken after 24 h. The zones of inhibition were compared to those of the controls.

Evaluation of biodegradability. The 5-d biochemical oxygen demand (BOD) of NSA was determined by the standard oxygen consumption test (13) using activated sludge obtained from a sewage treatment plant at the Central Leather Research Institute (Adyar, India). All experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Synthesis of *N*-stearoyl amino acids. The NSA used in this study were prepared by conversion of fatty acids to their acid chlorides and further reaction with the L-amino acid under suitable conditions. The lipoamino acids were characterized by ¹H NMR, FTIR spectroscopy and C, H, N analysis. The characteristics of the NSA are given in Table 1.

FTIR spectroscopy. FTIR spectra of all the NSA were recorded. The band at 1460 cm⁻¹, assigned to the CH₂ scis-

TABLE 1
Chemical Characteristics of the Compounds Synthesized

Compound	Amino acid moiety	R in R-CH-COOH NHCO(CH ₂) ₁₆ CH ₃	Yield (%)	MW	M.P. (°C)	Analysis [%; calc. (found)]		
						C	H	N
1	Glutamic acid	Carboxymethylene	92	413.594	154.75	60.11 (61.13)	9.4 (9.27)	3.05 (3.15)
2	Aspartic acid	Carboxymethylene	90	399.567	53.56	59.51 (59.32)	9.3 (9.2)	3.15 (3.20)
3	Arginine	Guanido- <i>n</i> -valeric acid	87	440.668	37.26	58.87 (58.46)	9.8 (9.75)	11.44 (11.24)
4	Lysine	4-Aminobutyl	50	412.654	57.40	34.92 (35.90)	5.86 (5.95)	3.33 (3.31)
5	Histidine	Imidazole propionic acid	93	421.621	43.77	61.53 (61.32)	9.25 (9.55)	8.9 (8.5)
6	Proline	Cyclic	50	381.596	37.94	36.196 (36.25)	5.6 (5.5)	1.8 (1.59)
7	Phenylalanine	Benzyl	63	431.656	49.34	47.33 (46.32)	6.61 (6.59)	2.04 (2.15)
8	Tryptophan	3-Methyleneindole	92	470.693	41.28	68.08 (65.45)	9.06 (10.25)	5.47 (5.35)

soring motion, indicates the presence of methylene groups in the NSA. The absorption bands at around 2960 and 2930 cm^{-1} arise from C-H stretching in methyl and methylene groups, respectively. Absorptions of C=O and amide group were observed at 1657 and 3447 cm^{-1} , respectively.

Proton NMR spectroscopy. The ^1H NMR spectra of NSA were recorded, and the chemical shifts of the constituent protons (14) were as follows. A triplet was observed at a δ value of 0.8, which is characteristic of methyl protons adjacent to methylene protons in long-chain alkyl molecules. The methylene $[(-\text{CH}_2)_{16}]$ proton resonance was observed at δ 1–2 as a multiplet. The resonance of the protons in the amide linkage of the NSA was seen at δ of 4–6 as a doublet. For example, in the case of *N*-stearoyl tryptophan, the amide NH proton resonance appeared as a doublet ($J_{\text{NHCH}} = 5.3$ Hz) with an integrated intensity of 0.75. The NMR signals of the tryptophan protons were spread over δ 6.8–7.8 with a total integrated intensity of 4.92, which is accounted for by the five protons. Similar observations were made for all the samples. The resonances of methylene protons in the amino acid moiety were observed at a δ value of 3.1–3.2. The resonances of all the aromatic and heterocyclic molecules present in the lip amino acid side chains were observed at a δ value of 7–8.

Interfacial properties of NSA. Surface-active properties of NSA were evaluated in terms of CMC and foaming property by DSC.

CMC. Specific conductance of the NSA was measured in alkaline solution. The CMC was determined from a plot of specific conductance vs. concentration of the NSA by extrapolating the two straight lines, the intersection of the lines giving the CMC value of the NSA. The values obtained are tabulated in Table 2. It is inferred that below the CMC, the NSA solutions may behave as ideal solutions. This indicates that surface-active molecules are adsorbed on the surface of the aqueous phase and form a close-packed monomolecular layer. Subsequent addition of surfactant to the system favors molecular aggregation or, in other words, micelle formation. Adsorption and interfacial activity generally increase with increasing concentration up to the CMC. Above the CMC, the surface-active molecules behave nonideally. The shorter the alkyl side chain in the amino acid molecules (10,15), the higher is the CMC value. In the case of NSA (C_{18}) like

N-stearoyl phenylalanine, *N*-stearoyl arginine, *N*-stearoyl proline, and *N*-stearoyl tryptophan, the alkyl side chain of the amino acid is bulky, resulting in restricted molecular mobility in the aqueous environment. Therefore, NSA have the ability to form aggregates at lower concentrations, and thus their conductance becomes low. On the other hand, the overall hydrophobicity of the molecules become larger and the CMC lower.

DSC. The polymorphic states of surfactants are usually studied by XRD, but with the advancement of microcalorimetry DSC techniques, it is possible to identify various polymorphic states of fats and fatty acids by DSC, as reported elsewhere (16–18). The surfactants have four polymorphic states: α , Δ , β , and Ω . In phase identification, XRD and DSC methods can be used as a tool for the assessment of various physical properties related to attributes of the surfactants (17). The following assumptions are made in order to develop this correlation: The α form does not exist under the ordinary conditions of preparation of the NSA (3). In the thermal absorption range below 100°C, the NSA would have the Δ form, and they are assumed to be in a solid-solution or gelatinous state. At 101–180°C, the NSA would have the β form, which indicates that they are in the liquid-crystalline state. The thermal absorption range of NSA between 181 and 300°C is attributed to the Ω form, and the physical nature would be crystalline.

DSC thermal absorption data for the polymorphic states of the NSA are shown in Table 3. All thermal absorptions are re-

TABLE 2
Critical Micelle Concentration (CMC) of Sodium Salts of *N*-Stearoyl Amino Acids (NSA)

Compound	Amino acid moiety	CMC (mM)
1	Glutamic acid	6.00
2	Aspartic acid	2.40
3	Arginine	1.75
4	Lysine	2.40
5	Histidine	2.10
6	Proline	1.75
7	Phenylalanine	2.00
8	Tryptophan	2.00
9	Tyrosine	2.25

TABLE 3
Differential Scanning Calorimetry (DSC) and Lather Value (LV) Data of NSA^a

Amino acid moiety	Δ Phase				β Phase				Ω Phase				LV ^b
	°C	J/g	°J/g	Total °J/C	°C	J/g	°J/g	Total °J/C	°C	J/g	°J/g	Total °J/C	
Glutamic acid	—	—	—	—	154.75	246.6	381.6	381.6	214.75	482.3	1518.03	1518.03	1.2513
Aspartic acid	53.56	83.93	44.95	44.95	—	—	—	—	—	—	—	—	—
Arginine	37.26	8.458	3.151	3.151	134.23	176.0	236.24	236.24	191.67	60.82	116.57	386.61	1.1611
									265.27	101.8	270.04		
Lysine	57.46	62.23	35.72	73.78	—	—	—	—	212.35	117.1	148.66	435.08	1.000
	92.86	40.88	37.96						258.63	72.08	186.42		
Histidine	43.77	53.89	23.58	23.58	156.56	33.54	52.51	72.76	203.05	204.9	416.04	416.04	1.1748
					163.91	12.36	20.25						
Proline	37.94	72.05	27.33	231.77	—	—	—	—	195.04	191.9	374.28	1018.16	1.000
	77.10	17.80	13.72						260.05	247.6	643.8		
	99.39	191.9	190.72										
Phenylalanine	49.34	32.52	16.04	53.15	106.16	18.09	19.20	19.20	—	—	—	—	—
	58.70	63.22	37.11										
Tryptophan	41.28	57.36	23.67	23.67	118.81	197.9	235.1	235.12	—	—	—	—	—
Tyrosine	60.36	115.3	69.59	69.59	—	—	—	—	212.06	160.9	341.20	467.48	1.000
									260.86	48.41	126.28		

^aSee Table 2 for abbreviation.

^bSee Equation 2.

ported in degrees Celsius, and the corresponding integrated peak areas are in units of joules per gram (J/g). For the purpose of normalization of these data, the °C value is multiplied by the J/g value and then divided by 100 to reduce the number to manageable values in units of degree joules per gram (°J/g):

$$(\text{°C}) \times (\text{J/g}) / 100 = (\text{°J/g}) \quad [1]$$

The lather values (LV) of the lip amino acids were predicted in the following manner. LV is a dimensionless unit, which represents the potential of foaming. Properties of a surfactant are obtained (3,18) by relating the β phase (responsible for lathering or foaming) with the Ω phase (responsible for insolubility) as described in Equation 2

$$\text{LV} = \text{°J/g} (\Omega) + \text{°J/g} (\beta) / \text{°J/g} (\Omega) \quad [2]$$

On the thermograms of the NSA, the melting points of the samples are observed below 100°C. With further heating, decomposition is observed. For comparison purposes, the higher the LV of a surfactant is, the greater are its surface-active properties. The LV of NSA are given in Table 3. Among all the NSA investigated, *N*-stearoyl arginine has the highest LV and *N*-stearoyl histidine has the lowest.

Evaluation of antimicrobial activity of NSA. The NSA prepared in this study were screened for their antimicrobial activity against several of pathogenic organisms (7–9) including both gram-positive and gram-negative bacteria and fungi. Several of these compounds exhibited a broad spectrum and high level of activity against all or most of the test organisms. Table 4 shows the antimicrobial activity of NSA against the

gram-positive bacteria *S. aureus*, *M. luteus*, and *B. cereus*, the gram-negative bacteria *E. coli* and *P. aeruginosa*, and the fungus *C. albicans*. *N*-Stearoyl proline, *N*-stearoyl phenylalanine, and *N*-stearoyl tryptophan displayed inhibitory activity (+++) against all organisms except *Pseudomonas* sp. *N*-Stearoyl arginine showed inhibition against three microorganisms, *N*-stearoyl lysine and *N*-stearoyl aspartic acid displayed inhibition against two microorganisms, and *N*-stearoyl tyrosine showed inhibition against a single microorganism. Compounds marked + (zone of inhibition less than 0.5 cm) in Table 4 are not necessarily inferior in inhibitory activity to

TABLE 4
Antimicrobial Activity of NSA^a

	Antimicrobial activity ^{b,c}					
	A	B	C	D	E	F
Stearic acid	—	+	—	—	++	—
NS-Glutamic acid	++	++	—	++	++	—
NS-Aspartic acid	—	+	—	+	—	—
NS-Arginine	++	+	—	—	++	—
NS-Lysine	+	—	—	—	+	+
NS-Histidine	—	—	—	—	—	—
NS-Proline	+++	+++	—	++	+++	—
NS-Phenylalanine	++	+++	—	++	+++	+
NS-Tryptophan	++	+++	—	++	+++	+
NS-Tyrosine	—	—	—	—	+	—
Control	+++	+++	+++	+++	+++	+++

^aNS, *N*-stearoyl; see Table 2 for other abbreviations.

^bMicroorganisms: A, *Escherichia coli*; B, *Staphylococcus aureus*; C, *Pseudomonas aeruginosa*; D, *Micrococcus luteus*; E, *Bacillus cereus*; F, *Candida albicans*.

^c+++ , Zone of inhibition was at least 1 cm beyond disc area at 24 h; ++, zone of inhibition was at least 0.5 cm beyond disc area at 24 h; +, zone of inhibition was below 0.5 cm beyond disc area at 24 h; —, no inhibition detectable.

those rated ++, because the zones of inhibition may be a function of the ability of the compounds to diffuse through the medium beyond the point of contact with the agar surface rather than an indication of their intrinsic ability to retard microbial growth.

The apparently large differences in the inhibitory effects of the compounds could be related to the mode of attachment of the amino acid moiety. The fatty acid chloride reacts with amino acids to form an amide bond. The amide linkages may be responsible for the inhibitory action, such that a stronger hydrogen bond between oxygen of the fatty moiety and the -NH group of the amino acids would result in a higher rate of antimicrobial activity. This effect has been called the hydrogen belt effect in inhibition of antimicrobial action (19). The NSA containing an aromatic amino acid were the most effective, exhibiting a strongly inhibitory action against five of the six organisms.

A simple screening technique was used to obtain general information on whether the compounds prepared had antimicrobial properties that could be useful in commercial products. Further testing of these compounds as biostatic agents should be considered.

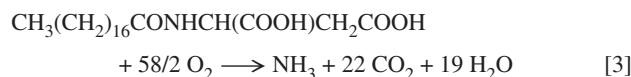
Biodegradability of the lipoamino acids. The biodegradability test is mainly based on a bioassay procedure, involving measurement of O₂ consumed by bacteria while stabilizing organic matter under aerobic conditions. It is necessary to provide standard conditions of nutrient supply, pH, absence of microbial growth-inhibiting substances, and temperature (13). A mixed group of organisms should be present in the sample. If not, the sample has to be seeded artificially. Temperature is controlled at 20°C. The test is conducted for 5 d, as 70–80% of the samples are oxidized during this period.

Table 5 shows the 5-d biochemical oxygen demand (BOD₅) and biodegradability [BOD₅/theoretical oxygen demand (ThOD)] of NSA. The BOD was calculated per the formula reported elsewhere (13). The ThOD values of the NSA were computed from the chemical formula as illustrated for the example of *N*-stearoyl aspartic acid.

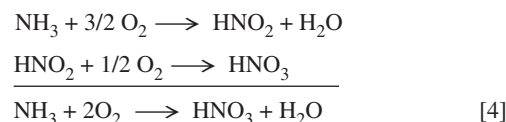
Determination of the ThOD (20) of *N*-stearoyl aspartic acid involves the following assumptions. In the first stage, the

organic carbon and nitrogen are converted to carbon dioxide (CO₂) and ammonia (NH₃), respectively. In the second and third stages, the ammonia is oxidized sequentially to nitrite and nitrate. The ThOD is the total amount of the oxygen required for all three steps.

Balanced reaction for the carbonaceous oxygen demand:



Balanced reaction for the nitrogenous oxygen demand:



Determination of ThOD for *N*-stearoyl aspartic acid:

$$\begin{aligned} \text{ThOD} &= (58/2 + 2) \text{ mol O}_2/\text{mol } N\text{-stearoyl aspartic acid} \quad [5] \\ &= 31 \text{ mol of O}_2/\text{mol } N\text{-stearoyl aspartic acid} \times 32 \text{ g/mol O}_2 \\ &= 839.09 \text{ g O}_2/\text{mol } N\text{-stearoyl aspartic acid} \end{aligned}$$

As illustrated in Table 5 all the NSA tested in this study were >75% in 5 d, suggesting that these compounds are readily biodegradable in the environment.

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REFERENCES

- Paquet, A., Succinimidyl Esters of Fatty Acids for Amino Acid Acylations, *Can. J. Chem.* 54:733–737 (1976).
- Ross, J., and G.D. Miles, Apparatus for Comparison of Foaming Properties of Soaps and Detergents, *Oil Soap* 18:99–102 (1941).
- Gupta, S., The Prediction of Lathering and Solubility Properties of Bar Soaps by Differential Scanning Calorimetry, *J. Am. Oil Chem. Soc.* 68:450–455 (1991).
- Pelczar, M.J., Jr., E.C.S. Chan, and N.R. Krieg, *Microbiology*, Tata McGraw-Hill Publishing, New Delhi, 1998, pp. 73–133.
- Eband, R.F., M.R. Infante, T.D. Flanagan, and R.M. Eband, Properties of Lipoamino Acids Incorporated into Membrane Bilayers, *Biochim. Biophys. Acta*, 1373:67–75 (1998).
- Freese, E., C.W. Sheu, and E. Galliers, Function of Lipophilic Acids as Antimicrobial Food Additives, *Nature* 241:321–325 (1973).
- Lafon-Lafourcade, S., C. Geneix, and P. Ribereau-Gayon, Inhibition of Alcoholic Fermentation of Grape Must by Fatty Acids Produced by Yeasts and Their Elimination by Yeast Ghosts, *Appl. Environ. Microbiol.* 47:1246–1249 (1984).
- Sheba, W.D., R.K. Saxena, and R. Gupta The Fungistatic Action of Oleic Acid, *Curr. Sci.* 76:1137–1140 (1999).
- Bailey, A.V., A.J. De Lucca II, and J.P. Moreau, Antimicrobial Properties of Some Erucic Acid–Glycolic Acid Derivatives, *J. Am. Oil Chem. Soc.* 66:932–934 (1989).
- Matsumura, S., K. Imai, S. Yoshikawa, K. Kawada, and T. Uchibori, Surface Activities, Biodegradability and Antimicrobial Properties of *n*-Alkyl Glucosides, Mannosides and Galactosides, *Ibid.* 67:996–1001 (1990).

TABLE 5
Biodegradability of NSA^a

Compound	Amino acid moiety	ThOD ^b (g O ₂ /mol)	BOD ₅ ^c (g O ₂ /mol)	BOD ₅ /ThOD (%)
1	Glutamic acid	1040	868.5	84
2	Aspartic acid	992	839.09	84.5
3	Arginine	1264	925.26	73.2
4	Lysine	1184	866.75	73.1
5	Histidine	1184	885.36	74.7
6	Proline	1072	801.15	74.7
7	Phenylalanine	1216	906.47	74.5
8	Tryptophan	1328	988.26	74.4
9	Tyrosine	1200	939.96	78.33

^aSee Table 2 for abbreviations.

^bTheoretical oxygen demand.

^cMeasured 5-day biochemical oxygen demand.

11. Takehara, M., Properties and Applications of Amino Acid Based Surfactants, *Colloids Surf.* 38:149–167 (1989).
12. Jungermann, E., J.F. Gerecht, and I.J. Krems, The Preparation of Long Chain *N*-Acylamino Acids, *J. Am. Chem. Soc.* 78: 172–174 (1956).
13. Clescer, L.S., A.E. Greenbery, and A.D. Eaton, in *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, United Book Press, Baltimore, 1998, pp. 5-2–5-6.
14. Willard, H.H., L.L. Merritt, and J.A. Dean, *Instrumental Methods of Analysis*, Van Nostrand, New York, 1966, pp.160–188.
15. Takehara, M., H. Moriyuki, I. Yoshimura, and R. Yoshida, Surface Active *N*-Acyl Glutamate II. Physicochemical Properties of Long Chain *N*-Acylglutamic Acids and Sodium Salts, *J. Am. Oil Chem. Soc.* 49:143–150 (1972).
16. Guth, O.J., J. Aronhime, and N. Garti, Polymorphic Transitions of Mixed Triglycerides, SOS, in the Presence of Sorbitan Monostearate, *Ibid.* 66:1606–1613 (1989).
17. Arishima, T., and K. Sato, Polymorphism of POP and SOS III. Solvent Crystallization of β_2 and β_1 Polymorphs, *Ibid.* 66: 1614–1617 (1989).
18. Busfield, W.K., and P.N. Proschogo, Thermal Analysis of Palm Stearine by DSC, *Ibid.* 67:171–175 (1990).
19. Brockerhoff, H., and E.E. Van Tamelen, *Bioorganic Chemistry*, Academic Press, New York, 1977, pp. 10–15
20. Tchobanoglous, G., and F.C. Burton, *Waste Water Engineering*, Tata McGraw-Hill Publishing, New Delhi, 1995, pp. 82–83.

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