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Review Article

Surfactant Extraction from Slaughterhouse Offals: Recovery, Characterization, and Biomedical Applications

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Abstract: *The application of surfactants is diverse and extends into biomedical applications, industries, and food science owing to their capacity to lower surface and interfacial tensions. The increasing need for sustainability and superior performance attributes has led to growing interest in the design of natural or biosurfactants that are sourced from animal and microorganism sources. The inedible animal by-products like lungs, skins, and connective tissues can be effectively used to obtain value-added materials like phospholipids, collagen, gelatin, and its derivatives, which exhibit high surface activity. In the biomedical industry, animal-sourced surfactants, especially bovine and porcine surfactants, have exhibited better efficacy in treating respiratory distress syndrome compared to artificial surfactants.*

Also, protein-based surfactants such as gelatin derivatives have impressive properties of emulsification, foam generation, and stabilization; therefore, they can be used in foodstuffs and drugs. Additionally, surfactants can act as nutritional additives for ruminants because they promote the better assimilation of nutrients and increase productivity. Despite the wide range of applications of surfactants, their industrial manufacturing is technologically and financially difficult.

Keywords: *Surfactants, Animal by-products, Biosurfactants, Pulmonary surfactant, Gelatin derivatives, Sustainability*

I. INTRODUCTION

Products made from meat and meat provide essential nutrients in human diets [1]. Yet, their rising consumption poses substantial stress to the environment and the exploitation of resources [2]. Effective and efficient use of animal-derived raw materials after slaughter will contribute to the reduction of greenhouse gases, food wastage, and global hunger issues [3]. There are different value-added products that can be obtained from offals [4], where natural surfactants were found to be surface active components derived from animals, their derivatives, and microorganisms.

A surfactant is a substance which possesses the property of surface activity and has amphiphilic features. A surfactant molecule contains hydrophilic and lipophilic regions in the same molecule. In an oil-in-water system, hydrophilic regions interact with water, while lipophilic regions align themselves in the direction of oil [5]. Surfactants are a type of surface-active agent consisting of organic and organometallic molecules which display varying degrees of polarity and solubility, thus exhibiting surface activity. Most common of all examples of surface activity is their tendency to lower the interfacial tension between two immiscible fluids [6].

Animal products, unfit for consumption, can be used in making surfactants. These include hydrolysate of collagen and gelatin contained in animal by-products that have surfactant activity due to the formation of peptides with the property of emulsification and reduced surface tension. The chemicals are widely used in the food, cosmetic, and pharmaceutical industries thanks to their functional and amphiphilic properties [7].

Natural or modified surfactants extracted from bovine or porcine lungs contain different proportions of surfactant proteins like SP-B and SP-C, and plasmalogens. Nonetheless, artificial surfactants without any of the above substances are equally well-studied and widely used, mainly in the case of preterm infants with RDS [8]. Among such animal products of surfactants, there are beractant (Survanta, Abbott Laboratories Inc., USA), calfactant (Infasurf, Forest Laboratories, USA), and poractant alfa (Curosurf, Dey LP, USA).

Pulmonary surfactant is a lipoprotein complex that performs an important function in preventing alveolar collapse and improving lung compliance [9]. Surfactant therapy is administered through surfactant replacement therapy (SRT) in preterm babies who have not only certain radiographic findings such as small lung volumes, cloudy or ground-glass appearance, air bronchograms, and unclear margins of the heart but also clinical symptoms associated with neonatal respiratory distress syndrome (NRDS) [10]. Treatment of these patients with surfactant reduces their morbidity, mortality, risk of air leaks, and chronic lung disease. With respect to NRDS, early treatment of patients with surfactant leads to better

outcomes. Moreover, better outcomes are achieved in patients who receive surfactant early and then get extubated soon after the treatment, rather than undergoing prolonged mechanical ventilation following surfactant treatment.

These biosurfactants are characterized by various properties making them useful in applications for the food industry. One property attracting recent interest in preventing biofilm formation on contact surfaces is their anti-adhesion properties. The multiple functionalities associated with these biosurfactants include their functions as emulsifiers, anti-adhesion agents, and antimicrobial agents. Despite the various benefits associated with their use in the food sector, biosurfactants have not been widely utilized due to lack of toxicological information and high production costs. The employment of agro-industrial residues as sources of these biosurfactants would considerably reduce their production costs and also minimize the disposal costs of these wastes. Such industries would therefore be able to add value to waste products and at the same time become producers of microbial surfactants [11].

The surfactant is now an essential part of modern living, with wide-ranging applications in both the domestic and industrial sectors, as well as in various biomedical applications. The classification of surfactants depends upon the electrical charges of surfactants and their sources, and they include anionic, cationic, non-ionic, and amphoteric surfactants, apart from biosurfactants that occur naturally. It is significant to note that surfactants have biomedical applications that have increased in significance because of their surface activity and multipurpose functionality.

In addition, it should be noted that surfactants can be obtained from animal wastes, like lungs, hides, and fat. Therefore, the use of offal provides an opportunity to produce surfactants sustainably, besides preventing waste and pollution.

A. Isolation of Bovine Pulmonary Surfactant

Freshest possible bovine lungs, along with intact hearts, were procured post-mortem directly from slaughterhouses and put in a big tub. A small rubber vacuum hose (outer diameter 3.0 cm) was inserted into the trachea and attached tightly. This was subsequently attached to a plastic box filled with 16 L of cold saline-salts solution (0.130 M NaCl, 0.010 M $CaCl_2$, 0.008 M $MgCl_2$).

About 8-10 liters of this solution was instilled into the lungs under gravity, until full distention was attained. The solution was allowed to drain out through gravity drainage, aided by light massage. This procedure was done repeatedly until the solution was drained out by suction, using a manually operated suction pump. It should be noted that no overdistension occurred during the procedure, and any bloody or red-colored washout solution was not collected. On the average, approximately 12 liters of white foamy washout solution was collected from each set of lungs. Antimicrobial solution was included in the mixture to prevent bacterial growth. The samples were kept cool during transportation and storage at 4°C prior to subsequent use. In general, 4-6 animals were used for each preparation.

The lavage fluid was strained using a kitchen strainer and then spun down at 3500 rpm (1450 g) by continuous-flow centrifugation to remove cell debris. Supernatants were concentrated about tenfold by using membrane filtration with a 100 kDa molecular weight cut-off. The concentrated suspension was then centrifuged at 8000 g for 60 minutes to obtain a white pellet, which was resuspended in saline containing 0.001 M $CaCl_2$ to achieve a phospholipid concentration of 25–50 mg/mL. This preparation was designated as natural surfactant.

Lipid extracts were obtained by treating the natural surfactant with a chloroform–methanol mixture (1:1). Proteins were removed by centrifugation, and phase separation was achieved by adding 1% KCl following the method of Bligh and Dyer, 1959 [13]. The lower organic phase was collected, evaporated to dryness, and further purified through repeated washing to reduce residual protein content.

B. Analytical Techniques

Neutral lipids were separated from phospholipids using silicic acid chromatography and analyzed as t-butyldimethylsilyl derivatives by gas–liquid chromatography [14] Phospholipids were separated by thin-layer chromatography using a chloroform/ethanol/water/triethylamine solvent system [15] and detected under UV light after Rhodamine 6G staining. Phosphorus content was measured from gel scrapings [16] and fatty acid composition was determined using gas chromatography of methyl esters with internal standards [17]

Specific phospholipids such as phosphatidylcholine and phosphatidylglycerol were isolated and further analyzed for fatty acid distribution using phospholipase A_2 digestion [18] Molecular species were characterized after phospholipase C hydrolysis and separation via thin-layer chromatography [19] Extracted fractions were analyzed for fatty acid profiles following standard procedures [13].

Protein content was determined using the Lowry method with bovine serum albumin as a standard, and further confirmed through amino acid analysis after acid hydrolysis [20]. Protein profiles were evaluated using polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate [21].

II. SURFACTANT ACTIVITY EVALUATION

Functional characteristics of the bovine lung surfactant and its lipid fractions were determined via pulsating bubble surfactometer method [22]. This technique involved introducing an air bubble into a surfactant solution (1% in concentration) containing 0.15 M NaCl and 0.001 M $CaCl_2$. The air bubble was subsequently oscillated between expansion and contraction phases at a temperature of 37°C, and pressure measurements were taken. Surface tension was calculated via the Laplace law, where surface tension is proportional to pressure differential and bubble diameter [21]

To obtain artificial surfactants, the lipid fractions were dried in nitrogen gas and resuspended in saline solution with $CaCl_2$ followed by sonication.

III. SURFACTANT REPLACEMENT THERAPY AND COMPOSITION OF BOVINE PULMONARY SURFACTANT

Surfactant therapy can be viewed as an intervention strategy, which represents one of the most direct and efficient ways of avoiding respiratory failure due to NRDS. The earliest results of experiments conducted on immature fetal rabbits (27–28 days gestation; full term—31 days) showed that surfactant treatment before birth was able to greatly improve lung pressure-volume relationships and alveoli expansion. The same advantages, such as increased survival rate, were obtained with premature rabbits, rhesus monkeys, and sheep. Furthermore, surfactant treatment provided a better gas exchange under artificial respiration conditions and helped avoid bronchial epithelium injury [23].

The first use of aerosolized dipalmitoyl phosphatidylcholine in newborn babies did not have a positive outcome possibly because of the insufficient surface activity of the preparation [24]. But further research carried out by Fujiwara et al.1980 [25], revealed that the semisynthetic surfactant consisting of acetone extract of bovine lung surfactant and dipalmitoyl phosphatidylcholine/phosphatidylglycerol mix had beneficial effect on the function of the lungs in infants suffering from respiratory distress. The similar results were obtained by Smyth et al., 1981[26].

Lipid composition of bovine lung surfactant, however, is similar to the composition of surfactant of other animals [27]. Similar phospholipid composition is observed for surfactant from dogs [28], rabbits [29], sheep [30], pigs [31], and rats [32]. Bovine surfactant differs from canine and rat lung surfactant by its low neutral lipids content (absence of triacylglycerols and small amount of cholesterol ester) and high phosphatidylglycerol concentration, whereas phosphatidylinositol content in bovine surfactant is low [27]. Phospholipids may function in transfer of phosphatidylcholine to the air/liquid interface and stabilization of surfactant monolayer; however, their mechanism of action is not clear yet. Similarities between lipids composition of surfactant are also revealed by comparison with lamellar bodies of rat and human lungs [33].

Pulmonary surfactant is composed of different protein components whose molecular weights range from about 70 kDa, 35 kDa, to 10 kDa in various organisms. It was previously found that the elimination of greater than 99% of these proteins had little or no effect on the surfactant's capability in reducing surface tension [22]. This is because lipids were thought to play an important role while the surfactant proteins merely assist in adsorption and stabilization of the lung surfactant.

It has also been demonstrated that artificial lipid mixtures made up of phosphatidylcholine, unsaturated phospholipids, and phosphatidylinositol or phosphatidylglycerol can display the property of surfactancy, but it is rather unreliable [22]. On the other hand, there are reports which reveal that surfactant-associated proteins can improve the speed of adsorption of lipids at the air-liquid interface reaching an equilibrium surface tension of about 25 dynes/cm [28]. The variation between these studies might be due to different methods used, namely equilibrium or pulsating bubble method [22].

The current results show that almost 90% of the proteins contained in the bovine pulmonary surfactant can be extracted without having any effect on the function of the substance analyzed via pulsating bubble surfactometer test. Moreover, lipid extracts of pulmonary surfactant have displayed biological activities that are similar to those of the whole preparation in terms of lung expansion and survival rates in premature animal models [35]. Clinical evidence has proven the biological activities of lipid extracts in the treatment of humans with NRDS [25]. However, it remains unknown what role is played by the protein component in surfactancy.

IV. NATURAL VS SYNTHETIC-SURFACTANT STUDIES

Fourteen randomized clinical trials conducted in comparing natural and artificial surfactants have been documented [8]. In general, natural surfactants have been proven to be more efficacious when applied during the acute stage of RDS.

This includes rapid improvement in terms of the need for oxygen supply, mean airway pressure, and fewer occurrences of air leak incidents compared with artificial surfactants. Moreover, the mortality rate of neonates using porcine surfactants is less than that of neonates using artificial surfactants such as pumactant [36].

Ainsworth et al. 1997 [36] conducted a randomized controlled trial in which poractant alfa (PA) was compared to pumactant. The study had to be terminated prematurely by the data safety monitoring committee due to a significant difference in mortality. Babies receiving treatment with PA exhibited substantially reduced mortality rates (14.1% vs. 31%; P = 0.006; odds ratio 0.37; 95% CI: 0.18-0.76). This trend persisted even after controlling for several variables including gestational age, birth weight, gender, study centers, plurality, and antenatal steroids usage. Even though this was a secondary endpoint in the trial, this was the first clinical trial providing evidence supporting animal extracts of surfactant compared to synthetic surfactants. No difference was observed in the incidence of bronchopulmonary dysplasia.

Among researches on synthetic surfactants, only one research by [37] indicated reduced rates of BPD for lucinactant in comparison with colfosceril palmitate. This trial included 1294 very low birth weight premature infants (600–1250g). The trial was conducted as a prophylactic study, however, worked more like early-rescue study owing to some difficulties with administering treatment. Lucinactant requires pre-heating before treatment, and therefore, it had impact on time when patients received treatment. Despite of that, no differences between studied groups were revealed in relation to outcome measures.

Another trial conducted by Sinha et al.,2018 [38] and aimed at non-inferiority of lucinactant to poractant alfa (PA), demonstrated no significant difference. Nevertheless, this trial had numerous methodological issues including premature termination of study, usage of old data on mortality and inconsistent doses. Long-term follow-up showed similar results in relation to survival rate at age of 1 year among infants receiving either lucinactant, BE or PA [39].

According to the American Academy of Pediatrics and Committee on Fetus and Newborn in 2008 [40], more studies need to be carried out before drawing conclusions based on the results of the trials on lucinactant. The reasons cited included premature termination of trials, lack of statistical power, and uncertainty on the metabolism of the drug.

Generally, the scientific literature suggests that animal surfactants perform better than any synthetic surfactant currently under investigation. Currently, there are no synthetically made surfactants approved for routine practice.

V. COMPARISON AMONG ANIMAL-DERIVED SURFACTANTS

A number of animal-derived surfactants have been tested for the management of RDS in premature neonates. Such agents vary with respect to source, isolation method, composition, viscosity, phospholipids content, plasmalogens content, and concentrations of surfactant proteins SP-B and SP-C [41]. Some common formulations include those made from bovine or porcine lungs by either minced tissue method or lung lavage procedure.

The beractant (BE), prepared from minced bovine lung tissue, contains relatively low phospholipids, plasmalogens, and SP-B when compared with the calfactant (CA) prepared from bovine lung lavage and having high phospholipids and protein content. The other formulation made from bovine lung lavage, namely, SF-RI1 (Alveofact), contains low content of plasmalogens. On the other hand, poractant alfa (PA), a porcine surfactant prepared by further purifying process like liquid gel chromatography, mainly consists of polar lipids and has the highest plasmalogens concentration among all these formulations.

An increased plasmalogen content in the tracheal aspirates of preterm babies is linked to the decreased development of bronchopulmonary dysplasia. Eight randomized control trials and two retrospective studies [8] have done comparisons between these natural surfactants. Four trials were conducted with regard to comparing BE and CA, whereas four other trials compared BE with PA. But there are no prospective studies to compare CA and PA yet.

VI. APPLICATION OF SURFACTANTS AS FEED ADDITIVES IN RUMINANT NUTRITION

The role of surfactants in enhancing rumen fermentation as feed additives for ruminant nutrition has been considered in many research studies. Inclusion of non-ionic surfactants into the ration of animals was observed to improve feed efficiency, especially when diets rich in fibers were used, which resulted in improved productivity expressed by milk and meat yield enhancement. Moreover, such feed additives might have positive effects on digestion process and the general productivity of the animals. Nevertheless, despite the potential advantages of surfactants use in feed, it is difficult to optimize its use because of the complexity of the ruminant digestive tract [42].

VII. GELATIN DERIVED SURFACTANT

The development of a new class of surfactants from gelatin hydrolysates possessing surface active properties via the reaction between gelatin hydrolysate and alkyl succinic anhydride was reported. The surfactants showed good functional properties, including reduced surface tension, along with good wetting, foam formation, and emulsification properties. An

increase in surface activity in aqueous solutions was observed with increasing alkyl chain length. In summary, the effectiveness of these surfactants depends to a large extent on their chemical structures, especially the alkyl chain lengths [43].

VIII. SURFACTANT METABOLISM AND FILM FORMATION IN THE LUNG

Surfactant phospholipids (PLs) and related proteins are manufactured in the endoplasmic reticulum in type II alveolar cells (see Fig. 1) [44]. Furthermore, surfactant proteins such as SP-A, SP-B, and SP-D are also synthesized in Clara cells and submucosal cells [45]. The surfactant components are further modified by the Golgi complex and stored in the form of lamellar bodies inside the cell. Surfactant is released from the lamellar bodies by exocytosis into the alveolar hypophase, a fluid phase between the airspace and the alveolar epithelial lining, in the form of heterogeneous aggregates containing phospholipids and tubular myelin [46].

The tubular myelin, which consists of a cross-linked arrangement of phospholipid layers, allows the constant absorption of surfactant molecules at the air-liquid interface. About half of the surfactant is reused after being absorbed by the cells using endocytosis [46]. Around 10-15% of the surfactant is broken down by alveolar macrophages, whereas about 2-5% is removed towards the air passages.

The surfactant extracted from BALF comprises two types of aggregate particles: large aggregates (LAs) and small aggregates (SAs) [47]. LAs are surface active and have hydrophobic surfactant proteins (SPs), SP-A, and specialized phospholipids, like lamellar bodies and tubular myelin. On the other hand, SAs have SP-D but are less surface active, even though they have similar phospholipid compositions. They are involved in the process of surfactant degradation and recycling, especially when inflammation is present. The LA/SA ratio can change in cases of lung diseases characterized by inflammation [48].

At the interface between air and fluid, the surfactant layer is subjected to compression and expansion cycles during breathing [49]. During exhalation, phospholipids that contain unsaturated fatty acid tails become selectively expelled from the layer, thus leading to a dominance of dipalmitoyl phosphatidylcholine (DPPC). This allows for the development of a compact layer that can generate extremely low surface tension, ensuring that alveoli do not collapse. During inhalation, the excluded phospholipids are again integrated back into the layer.

While DPPC is crucial in lowering surface tension, the ideal function of the surfactant relies on the joint effort of other lipids and surfactant proteins [50]. Unsaturated or shortened fatty acid chains phospholipids provide flexibility to the film, which helps in faster adsorption and spreading. Anionic phospholipids including PG and PI increase the stability of the film and aid in adsorption by their association with hydrophobic surfactant proteins like SP-B [51]. Furthermore, surfactant proteins SP-B, SP-C, and SP-A are essential for proper packaging of phospholipids into lamellar bodies, organization of tubular myelin, and the formation of a functional surfactant film at the air–liquid interface.

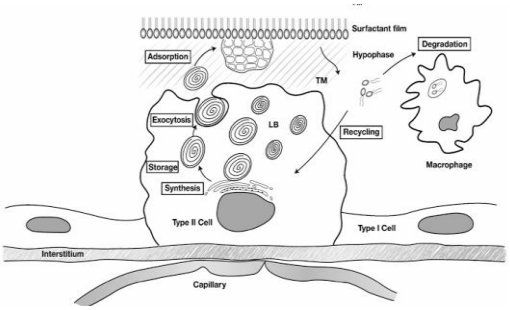

Figure 1: Schematic illustration of surfactant metabolism:

Surfactant is produced by type II alveolar cells and stored in specialized organelles known as lamellar bodies (LB). It is subsequently secreted into the alveolar hypophase, where it forms heterogeneous phospholipid-rich aggregates, including tubular myelin (TM). These components migrate to the air–liquid interface, where they function to reduce surface tension. A substantial proportion of surfactant is taken up and recycled by type II alveolar cells, while a smaller fraction is broken down by alveolar macrophages. [52]

IX. COMPARATIVE EFFICACY OF PORCINE SURFACTANTS AND BOVINE-DERIVED SURFACTANTS

Among the three porcine-derived surfactants currently available—Butantan, poractant alfa, and Surfacen—only poractant alfa is widely marketed internationally and has been directly compared with bovine-derived surfactants in several clinical trials. In this meta-analysis, the effects of poractant alfa were evaluated against all major bovine surfactants using a larger pooled sample size than in previous analyses. This was possible because the included studies compared poractant alfa

with commonly used bovine surfactants such as beractant, bovactant, and BLES, which are considered clinically equivalent. This similarity is consistent with their comparable biochemical and pharmacological characteristics. Additionally, this analysis incorporated several studies that were previously unpublished or not included in earlier systematic reviews [53].

Compared to earlier meta-analyses, such as that by Singh et al.,2011 [54], which included only a limited number of trials focusing on beractant, the present analysis provides a more comprehensive evaluation. The findings indicate that treatment with poractant alfa at a dose of 200 mg/kg is associated with a trend toward lower mortality and a significant reduction in outcomes such as bronchopulmonary dysplasia (BPD), combined BPD/mortality, air leaks, pulmonary hemorrhage, and the need for repeat dosing when compared to bovine surfactants. BPD showed more significant decreases in preterm infants of younger GA, while repeated administrations were required in cases of older GA.

Although these results correlate somewhat with previous literature, they offer a more robust analysis because of the greater amount of data and better methodological approach. Earlier studies suffered from small sample size, fewer surfactant agents considered, lack of comparative analysis, and insufficient controls on potential confounders such as GA, prenatal corticosteroid administration, and different doses used. In this case, both clinical and biochemical/pharmacological perspectives are included into the equation.

Figure 2: Poractant alfa

Table 1: Comparative Profile of Animal-Derived and Synthetic Pulmonary Surfactants: Composition, Protein Content, and Dosage Parameters

Type	Surfactant (Brand Name)	FDA Approval / Availability	Origin / Composition	Phospholipid Concentration (mg/mL)	Phospholipid Dose (mg/kg)	SP-B (µg/µmol)	SP-C (µg/µmol)	Dose (mL/kg)
Animal-derived	Beractant (Survanta®)	July 1991, Available	Minced bovine lung extract + DPPC, TPG, PA	25	100	0–1.3	1–20	4
	Calfactant (Infasurf®)	July 1998, Available	Bovine (calf) lung lavage	35	105	5.4	8.1	3
	Poractant (Curosurf®)	November 1999, Available	Minced porcine lung extract	80	200 (initial), then 100	2–3.7	5–11.6	2.5 (initial), then 1.25
	SF-RI1 (Alveofact®)	Not Available	Bovine lung lavage	40	50	2–5.6	1–12	1.25
Synthetic	Colfosceril (Exosurf®)	August 1990, Available	DPPC, hexadecanol, tyloxapol	13.5	67.5	None	None	5
	Pumactant (ALEC®)	Not Available	DPPC, PG	40	100	None	None	1.2
Synthetic (Protein analog)	Lucinactant (Surfaxin®)	FDA approval pending	DPPC, PG + KL-4 peptide	30	175	KL-4 (19.8)	None	5.8

DPPC: Dipalmitoylphosphatidylcholine; TPG: Tripalmitin; PA: Phosphatidic acid; PG: Phosphatidylglycerol; SP-B: Surfactant protein B; SP-C: Surfactant protein C; KL-4: Synthetic peptide analog of SP-B.

X. CONCLUSIONS

Surfactants play an important part in various biological mechanisms and technological applications, including respiratory physiology, food technology, and the recycling of by-products. Due to their amphiphilic nature, surfactants can decrease surface and interfacial tension, which contributes to such processes as stabilization of emulsions and maintaining alveoli stability in the respiratory system. Surfactants produced by type II alveolar cells create a mobile layer between the air and liquid interfaces, which prevents alveolar collapse.

The use of animal-based surfactants in neonatal RDS therapy has proven to be more effective than using man-made analogs. Poractant alfa, an artificial surfactant extracted from the lungs of bovine and porcine species, provides better results in terms of mortality rates, occurrence of bronchopulmonary dysplasia, and retreatment rate. The difference in components, including phospholipid content, plasmalogens, as well as surfactant proteins SP-B and SP-C, is what affects their efficiency.

Besides applications in medicine, surfactants that are obtained from animal wastes offer another significant path toward value addition and sustainable resource management. Wastes that cannot be consumed, such as lungs, skins, and connective tissues, may be converted into functional compounds like phospholipids, collagen, gelatin, and their derivatives that possess surface activity. The possible uses of these compounds include various industries, such as food, pharmaceuticals, and others. Moreover, surfactants based on gelatin and protein hydrolysates have been identified for emulsification and foaming effects.

Surfactants may also be used in ruminant feeding practices, whereby they contribute to improved rumen fermentation and utilization of fibrous feedstuff, thus promoting greater productivity. Nonetheless, caution should be exercised when using surfactants owing to the intricate nature of the ruminant gastrointestinal tract.

In general, the combination of surfactant science and by-products' application is an interdisciplinary area where scientists have an opportunity to benefit human health, animal welfare, and environmental protection. It is advisable to pay attention to improving techniques of extraction and formulation, understanding the structure-property relation of the surfactants and developing economically advantageous and environmentally friendly surfactants.

XI. FUTURE ASPECTS/TRENDS

In the area of surfactants, the following developments will be seen as major milestones in the future:

- Environmental approach: The use of byproducts and wastes from animals for the manufacture of environmentally friendly surfactants.
- Medical applications: Enhanced synthetic surfactants with proteins for treating respiratory diseases more efficiently.
- Biosurfactants on the rise: Importance of microbial surfactants because of their biodegradable and non-toxic nature.
- Applications in foods and agriculture: Using proteins in surfactants for preserving food and increasing agricultural productivity.
- Innovations in technology: Use in drug delivery devices and creation of new surfactants.

XII. REFERENCE

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