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In vitro thermostability and rumen dissolution evaluation of various rumen protected lysine and methionine products

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Abstract
An *in-vitro* study was conducted to determine the effect of temperature (corresponding to feed mill pelleting condition) on dissolution of rumen protected lysine and rumen protected methionine products microencapsulated by different coating technologies. Each product was prepared by using three different type of technologies:
1) Fluid bed process FBPT
2) Spray congealing SCT
3) Solid dispersion SDT.
Dissolution percentage (in rumen mimic solution) of rumen protected methionine products encapsulated by fluid bed process technology kept at 90 °C for 30 sec, 60 sec, 120 sec varied from 2.44%-10.08%, those encapsulated by spray congealing techniques varied from 6.38%-32.46% while those manufactured by solid dispersion technique varied from 31.32%-78.2%. Dissolution percentage (in rumen mimic solution) of rumen protected lysine products encapsulated by fluid bed process technology kept at 90 °C for 30 sec, 60 sec, 120 sec varied from 3.2%-6.6%, those encapsulated by spray congealing techniques varied from 9.04%-29.41% while those manufactured by solid dispersion technique varied from 36.45%-83.47%. Results from study indicated that release of amino-acids in rumen mimic solution coated with same type of coating agents depends on type of technology employed. Products coated using fluid bed top-spray process technology had lowest coating degradation in rumen mimic solution and thus highest rumen bypass efficacy while products coated with solid dispersion technology had high coating degradation in rumen mimic solution and thus lowest rumen bypass efficacy.

Keywords: Methionine, lysine, dairy cattle, rumen, by-pass, microencapsulation, fluid bed process, dissolution, spray congealing, solid dispersion

Introduction
Methionine ($C_5H_{11}NO_2S$, Mwt 149.21g/mol) and Lysine ($C_6H_{11}NO_2S$, Mwt 146.19g/mol) are widely used amino acids in animal feed. Methionine is commercially available in the form of DL Methionine 99% powder and liquid hydroxyl methionine 88%.
Lysine is commercially available in the form of L-lysine HCl 98.5% powder with lysine 78% and L-lysine sulfate 55% granules. Lysine and methionine are limiting amino acids for dairy cattle [1]. Amino acids have direct impact on feed intake, milk production, true milk protein concentration and true milk yield [2]. Protected form has a definite advantage over unprotected form on performance of lactating cows [3]. In 1970 first rumen-protected methionine supplement was fed to dairy cows [4]. Rumen protected methionine and rumen protected lysine improve milk yield and protein contents of dairy cows and was better than supplying alone [5]. A hypothesis of this paper is that technology of rumen protection play a distinct effect on rumen protection of a product and thus their rumen pass efficacy.

Materials & Methods
Reagents
Analytical grade of Potassium dihydrogen phosphate, Sodium hydroxide, Sodium bicarbonate, disodium hydrogen phosphate decahydrate, Sodium chloride, Potassium chloride, Magnesium chloride, Magnesium Chloride, conc HCl and Methanol were procured from Merck. HPLC water was procured from Rankem. Glacial acetic acid and mercuric acetate were procured from Qualigens. 0.1 N Perchloric acid & formic acid were procured from Fisher scientific. DL methionine and Lysine HCl analytical standard were procured from sigma Aldrich.

Solutions
Mimic rumen solution: McDougall Buffer solution: pH6.5, Each Ltr of McDougall buffer solution contains 7.43g of sodium bicarbonate, 7.0g of disodium hydrogen phosphate, 0.34g of sodium chloride, 0.43g of potassium chloride, 0.10g of Magnesium chloride, 0.05g of calcium chloride.
HPLC Mobile Phase (for DL methionine): Dissolve 3.4g of Potassium dihydrogen phosphate in 500 ml water. To this add 500ml acetonitrile.

Preparation of Standard/sample solution (DL methionine for assay purpose): Grind and weigh 30mg sample in 100 ml volumetric flask and make up volume with HPLC water. Filter through 0.45µ nylon membrane filter paper.

Preparation of Standard/sample solution (L-lysine HCl for assay purpose): Grind sample and take 100 mg in 250 ml volumetric flask. Analyse it as per USP method.

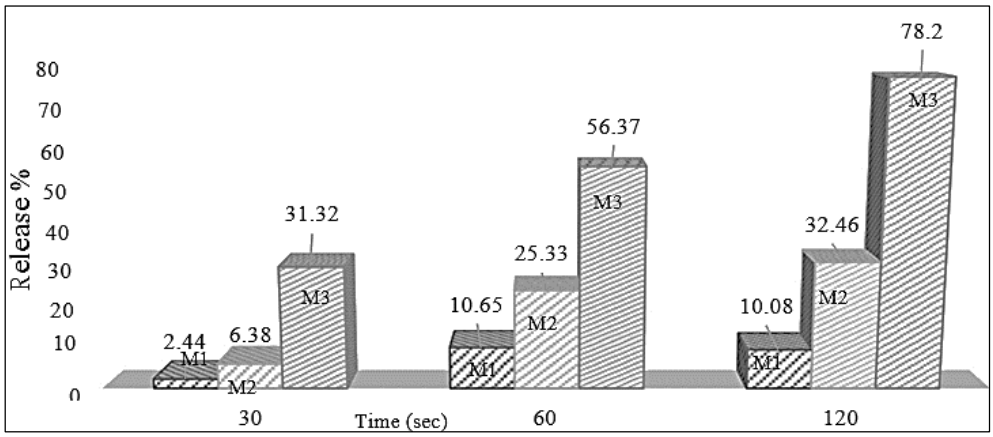
Equipments
Analytical Balance, Mettler Toledo ME204E, Dissolution apparatus, Lab India, Model- DS8000, Waters HPLC Alliance 2695 with UV detector, HPLC column Prodigy LC-18(250 x 4.6mm), 5µm, Column temperature: 300c, injection volume: 20µL. ʎmax: 210nm, Flow rate: 1ml/min, Run time: 10 min, Retention time: 3min Study design: Comparative *in vitro* dissolution studies were done mimicking the *in vivo* condition in dairy cattles. Thermostability conditions were maintained by keeping products in Hot air oven at 900c for 30s, 60s and 120s.

Sample collection
All Sample were developed in-house by different technologies
1) Fluid bed process technology (Core-shell microencapsulation) FBPT
2) Spray congealing technology (Matrix microencapsulation) SCT
3) Solid dispersion technique (Matrix microencapsulation) SDT

Sample identification: Samples were coded as M1, M2, M3, L1, L2 & L3 based on active and technology used Sample M1: DL methionine granulated and encapsulated with hydrogenated Vegetable oil mix by FBPT Sample M2: DL methionine powder mixed with hydrogenated Vegetable oil mix and congealed by SCT Sample M3: DL methionine powder mixed with hydrogenated Vegetable oil mix and congealed in cooled chamber by SCT. Sample L1: L-Lysine HCl granules encapsulated with hydrogenated Vegetable oil mix by FBPT Sample L2: L-Lysine HCl granules mixed with hydrogenated Vegetable oil mix and congealed by SCT Sample L3: L-Lysine HCl granules mixed with hydrogenated Vegetable oil mix and congealed in cooled chamber by SCT. M1, M2 & M3 were prepared at the concentration of 50% while L1, L2 & L3 were prepared at the concentration of 70% determined by HPLC and titrimetric respectively.

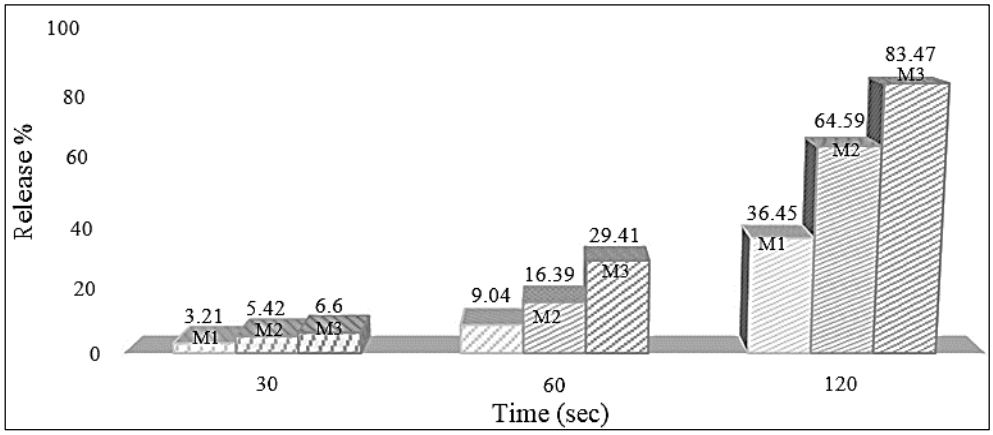
Procedure of evaluation
1 g sample each was initially kept in Hot air oven at 900c for 30s, 60s and 120s and there after loaded to dissolution apparatus. USP-1 type apparatus with six paddle was used for determination of dissolution profile of all samples. Other conditions maintained include temp 39 ℃ and 100 rpm. Mimic rumen solution study was done for 6 hrs. 5ml of solution was taken out after study. The pipetted out samples were filtered and analyzed in HPLC (DL methionine) and titrimetric (L-lysine HCl).

Result & Discussions



Graph 1: Dissolution profile of different rumen protected methionine products (M1, M2, M3)

Dissolution analysis of Rumen protected methionine products (M1: Rumen protected methionine 50% prepared by FBPT, M2: Rumen protected methionine 50% prepared by SCT, M3: Rumen protected methionine 50% prepared by SDT) shows that the in products kept at 900c for 30sec, DL-Methionine release percentage was 2.44%, 6.33% & 31.32% for M1, M2 and M3 respectively. In products kept at 900c for 60sec, DL methionine release percentage was 10.65%, 25.33% & 56.37% for M1, M2 and M3 respectively while in products kept at 900c for 120sec, DL-Methionine release percentage was 10.08%, 32.46% & 78.2% respectively. Dissolution analysis shows M1 samples have lowest release percentage followed by M2 & M3. High dissolution of M2 and M3 directly indicates the vulnerability of active release due to technology employed.



Graph 2: Dissolution Profile of Different Rumen Protected Lysine Products (L1, L2, L3)

Dissolution analysis of Rumen protected Lysine products (L1: Rumen protected Lysine 70% prepared by FBPT, L2: Rumen protected methionine 70% prepared by SCT, L3: Rumen protected Lysine 70% prepared by SDT) shows that in products kept at 900c for 30sec, L-lysine HCl release percentage was 3.21%, 5.42% & 6.6% for L1, L2 and L3 respectively. In products kept at 900c for 60sec, L-lysine HCl release percentage was 9.04%, 16.39% & 29.41% for L1, L2 and L3 respectively while in products kept at 900c for 120sec, L-lysine HCl release percentage was 36.45%, 64.59% & 83.47% respectively. Dissolution analysis shows L1 samples have lowest release percentage in different time length followed by L2 & L3. It indicates that L1 had better coating protection as compared to L2 followed by L3.

Table 1: Comparative dissolution percentage of Rumen Protected Products

Condition	Time (sec)	Dissolution %		
		M1	M2	M3
Mimic rumen solution (6Hr, USP 1, pH 6.5, 39 °C, 100 rpm)	30	2.44	6.38	31.32
	60	10.65	25.33	56.37
	120	10.08	32.46	78.2

Conclusion

DL-Methionine and L–Lysine HCl are widely used amino acid used as supplement in dairy cattle feed for increase milk yield and increased milk protein. Unprotected forms is liable to rumen microbial degradation resulting in less availability at small intestine. Rumen protected products forms are the solution but quality is the major concern. Its shows that rumen protection has a direct relationship with technology employed considering all other factors same. Products (Sample M1, L1) which were coated by FBP (core-shell technology) were having minimal release and thus maximum rumen protection followed by products (M2, L2) coated with spray congealing technology. Products coated with solid dispersion techniques were most liable to rumen degradation and thus are having lowest rumen protection.

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