

# NANOENCAPSULATION OF FREE GLUTAMIC ACID IN SAN-SAKNG (*ALBERTISIA PAPUANA* BECC.) LEAF EXTRACT BY SPRAY DRYING AND ITS CHARACTERISTIC

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**Abstract:** Nanoencapsulated compound of san-sakng leaf extract from spray dried method can be utilized as food flavoring. The purpose of this study was to produce a spray-dried extract of san-sakng leaf and to determine the effects of different on the structure and physical properties a powder. Four different wall material types were 10% (w/v) maltodextrin; 9.5% (w/v) maltodextrin and 0,5 % (w/v) chitosan; 9% (w/v) maltodextrin and 1 % (w/v) chitosan; 8,5% (w/v) maltodextrin and 1,5 % (w/v) chitosan. Parameters observed in this study based on their physicochemical properties and morphological of the encapsulated powder. Results showed that combination of Mo and Cs nanoparticles have spherical, smooth and a slightly dented surface. The highest amounts of free glutamic acid were found in 9% (w/v) maltodextrin and 1% (w/v) chitosan nanoparticles (21,09%). Color value obtained had the yellowness value, yield of 49,52%, aw of 0,39, bulk density of 0,73 moisture content of 8,44%, and particle size of 3,53 nm.

**Keywords:** *Albertisia papuana* Becc., chitosan, maltodextrin, san-sakng, free glutamic

## I. INTRODUCTION

San-sakng (*Albertisia papuana* Becc.) leaf known as food flavoring in the Dayaks tribe in West Kalimantan. The same plant was also used for the same purpose by the Dayak's from East Kalimantan (Purwayantie, 2009). San-sakng leaf usually used in local dishes such as vegetable soup in the form of fresh or dried, however generally using fresh leaf. In addition, all maturity level of san-sakng leaf contains free amino acid (Mayasari, 2016).

L-Glutamic acid (Glu) is a widespread amino acid present in foodstuffs as the free and protein-bound form. Foods containing large amounts of free Glu (tomatoes, mushrooms and cheese) are traditionally used to obtain savoury dishes (Giacometti, 1979; Yamaguchi & Ninomiya, 2000).

Spray drying is a useful technique for changing liquids into solid powder form which is not only easy handling but also able to extend shelf life and stability of the product. The initial step in drying a colorant involves the selection of a suitable wall material as known as carrier or encapsulating agent (Cai and Corke, 2000; Azeredo *et al.*, 2007).

Encapsulation is the process of trapping a substance in another substance (Zuidam and Shimoni, 2009) which can protect bioactive components from destructive changes and transmute it into the form of flour (Shaikh *et al.*, 2006).

Maltodextrin (Mo) is product derived from starch that has been proved to be popular choices. Mo is a good compromise between cost and effectiveness, as it is bland in flavor, has low viscosity at a high solid ratio and is available in a variety of Molecular weights (Apintanapong and Noomhorm, 2003). However, maltodextrin had deficiencies in term of emulsification property and surface-active features. Therefore, combining other material with maltodextrin is required to stable capsules (Sheu and Rosenberg, 1995).

Cs is a derivative of chitin, a biopolymer found in the shells of crustaceans, cell wall of fungi and other microbial (Pranoto *et al.*, 2005; Anal, 2010). Chitosan has proved to be valuable in wine and juice industry. Liang *et al.*, (2011) prepared by this method chitosan nanoparticles loaded with tea polyphenol extract. The particles have been proved to be good nanosystems for slow release, the polyphenolic material being actively maintained. A comparative analysis about the encapsulation of yerba mate extract was done by Anbinder *et al.*, (2011) who analysed chitosan tripolyphosphate nanoparticles (ionic gelation) and microspheres prepared by spray-drying. Effect of different ratios of chitosan-maltodextrin has influenced characteristics of nanoparticles of coconut shell liquid sMoKe (Saloko *et al.*, 2013).

Free Glu of san-sakng leaf needs to be protected against deterioration during the process by mean encapsulation. However, as our knowledge there is no reporting the encapsulation of san-sakng leaf extracts. In addition, a little known of combination Mo-Cs into san-sakng leaf powders. The objectives of this study was to carry out on the content of free Glu in spray-dried san-sakng leaf extracts and to determine the effects of diferent on the structure and physical its properties.

## II. MATERIAL AND METHODS

### 2.1 Materials

San-sakng leaf were used in the study was obtained from local people of Dayak ethnic. The leaf were harvested in March 2016 from surround Keranji Paidang village in the Sengah Temila subdistrict, Landak district, West Kalimantan province of Indonesia. Maltodextrin (Mo) with Dextrose Equivalent (DE) 10,8%; Chitosan (Cs) with deacetylation degree 91,5%, moisture 10,43%, ash 0,71%; NaCl.

### 2.2 Sample preparation

The dried leaf obtained from fresh leaf dried in a cabinet dryer at 50° C temperature until 10 % Moisture content. The leaf powder made from dried leaf that ground into powder of 60 mesh in size. The powder sample were stored in the dark at low temperature (-20° C) until used.

### 2.3 Extracts preparation

The extract was prepared from Mayasari, *et al.*, (2017), extraction method carried out by 1 grams of leaf powder were homogenized in 100 ml solution of aquades for 1 minutes by vortex, then boiled (90°C) for 15 minutes too. The mixture was vacuum filtered and the residues were re-extracted with the same process as explained in the previous stage. The collected filtrate was added to 0,6 % NaCl concentration then homogenized for 1 minutes by vortex. Filtrate stored at freezing temperature (-20° C) until used.

### 2.4 Preparation of nanoparticles

The nanoparticles was prepared from Saloko *et al.*, (2013), *san-sakng* leaf extract was undertaken using encapsulating materials 10% (w/v) Mo; 9.5% (w/v) Mo and 0,5 % (w/v) Cs; 9% (w/v) Mo and 1 % (w/v) Cs; 8,5% (w/v) Mo and 1,5 % (w/v) Cs. *San-sakng* leaf extract nanoparticles were made with 10% of the total weight of the solids in 500 ml of nanoparticles solution. The encapsulating materials mixture was homogenized with *san-sakng* leaf extract, this process used a stirrer for 30 minutes, heated in a water bath of 100 rpm up to a temperature of 50° C. The subsequent homogenization process used turrax (homogenizer ultra turrax T50) for 2,5 minutes at 4000 rpm. The homogenous nanoparticles solution was filtered using a vacuum filter with Whatman filter paper no.41.

### 2.5 Drying condition

Drying condition in this study was performed referring to Saloko *et al.*, (2012). The extracts were fed into a Buchi B-290 minute spray dryer (Flawii, Switzerland) for drying. The operating conditions were as follows; aspirator rate 50%; drying inlet air temperature 150° (± 2°C), while the outlet air temperature varied between 82°; feed flow rate was set at 5.1 ml/minute; atomization air rotameter 30 mm (to the bottom of the gauge ball) and the nozzle cleaner set to 4. The system was kept running without heating after the completion of the experiment until the inlet air temperature fell below 70°C. Spray-dried powders were collected, kept in amber bottles and stores under desiccated conditions at room temperature prior to powder characterization.

### 2.6 Production yield

The procedure for determined of production yield following method of Saloko *et al.*, (2012). The percentage of production yield (% w/w) was calculated from the weight of dried microspheres ( $w_1$ ) and the sum of the initial dry weight of starting material ( $w_2$ ) as the following equation :

$$\text{Yield} = \frac{w_1}{w_2} \times 100\%$$

### 2.7 Moisture content and water activity ( $a_w$ )

Moisture content of powder determined according to the AOAC (2006) methods which is measured by hot air oven at 105°C for 16 hours. The water activity is measured using by Decagon Pawkit water activity meter (Emin Tech, Sweden).

## 2.8 Determination of color values

Colour values are determined according to the methods of AOAC (2006) by using Minolta colorimeter (Minolta Spectrophotometer CR300 and CT310 was used to determine  $L^*$ ,  $a^*$ ,  $b^*$ , chroma ( $C$ ), and Hue ( $h^\circ$ ) values in which  $L^*$  is the lightness of color (100 = white, 0 = black),  $a^*$  value  $+a^*$  = red,  $-a^*$  = green),  $b^*$  value ( $+b^*$  = yellow,  $-b^*$  = blue),  $C = ((a^*)^2 + (b^*)^2)^{1/2}$  and  $h^\circ = (\tan^{-1}(b^*/a^*))$ .

## 2.9 Morphological Examination

The Morphology of the nanoparticles was examined using scanning electron microscope (SEM FEI, Inspect S50, Oregon, USA) and the nanoparticles sizes formed can be determined by using a particle size analyzer.

## 2.10 HPLC analysis

HPLC determination was described from Purwayantie *et al.*, (2015) modified. Liquid samples were diluted to derivatization and HPLC analysis. 4 ml from those samples were centrifuged at 20.000 x g for 20 min. 25  $\mu$ l of supernatant were mixed with 300  $\mu$ l of OPA (o-Phthaldialdehyde) solution. The mixture was vortexed for 1 min and 20  $\mu$ l aliquot was injected and analyzed using HPLC (SHIMADZU LC 10) with a Licrospher 100 RP 18 (5  $\mu$ m) and a 125 x 4 mm column. The separation of OPA-derivatives was performed using a Mobile phase consisting of methanol, 50mM Na-acetate, THF (2:9:2) at pH 6.8 as solvent A and 65% methanol as solvent B. The gradient elution program was held at 100% of A for 0.1 min, ramped at 100% of B for 45 min and stopped at 50 minutes with a flow rate of 1 ml/min. Detection was performed using a fluorescence Shimadzu RF-138 set at 360nm (Ex) and 460nm (Em). Each FAA was identified by using the authentic standard (Sigma-Aldrich) and quantified by the calibration curve of the authentic compound (external standard method).

# III. RESULT AND DISCUSSION

## 3.1 Water activity ( $\alpha_w$ )

The  $\alpha_w$  value of nanocapsule showed range from 0.45 to 0.35. The highest  $\alpha_w$  value were found for 10% (w/v) Mo, and the lowest  $\alpha_w$  value were found for ,5% (w/v) Mo : 1,5 % (w/v) Cs. The using of Mo as encapsulant could increase the content of  $\alpha_w$  value. The higher Mo concentration gave the higher  $\alpha_w$  value. Dursch and Schwarz (2006) found that spray drying resulted in a stable product acquisition of these  $\alpha_w$  according to storage conditions that free from damage and microbial oxidation.

## 3.2 Moisture content and yield

The Moisture content showed at Table 1 that varied from 10,52 – 8,13 %. Variation of Mo and Cs as the wall material for encapsulation influence of the quality parameters for a powder. The lowest value was achieved for the 8,5% (w/v) Mo : 1,5% (w/v) Cs (8,13%), the highest value was 10% (w/v) Mo : 0% (w/v) Cs (10,52%). The higher Mo concentration gave the higher moisture content. The encapsulation yield was determined gravimetrically as

the ratio between the weight of microspheres obtained at the end of the process and the weight of the atomized materials (encapsulation material plus extract). It gives an estimative of the material losses during the process. The encapsulation yield of the spray drying process was around 47,29% - 51,32%. Thus the lower value was achieved for the 10% (w/v) Mo (47,29%), the highest value was 8,5% (w/v) Mo and 1,5 % (w/v) CS (51,32%). Combination Mo-Cs as an encapsulant able to increase the encapsulation yield. In accordance with other published works using Mo and Cs as the encapsulating material referring to Saloko *et al.*, (2012) reported was 8,5% (w/v) Mo and 1,5 % (w/v) Cs as wall materials into liquid smoke solution has provided the highest yield.

### 3.3 *The characteristics color*

The characteristic color of the powder derived from different wall material is presented in Table 1. It was found that using Mo gave the highest lightness than Mo and Cs combined. The combination of Mo and Cs gave the highest yellowness than using Mo only.

### 3.4 *SEM of spray-dried*

A representative scanning electron microscopy (SEM) for the granular surface of *san-sakng* powder conducted at magnification 10000x in Figure 2. The result showed that wall materials using Mo as well as combination of Mo and Cs have spherical particle and the bioactive component disposal occurs. 10% (w/v) Mo had a firm dented surface, 8,5% (w/v) Mo : 1,5 % (w/v) Cs had the shrinkage and a lot of dimples surface, while 9,5% (w/v) Mo : 0,5 % (w/v) Cs and 9% (w/v) Mo : 1 % (w/v) Cs had the smooth and a slightly dented surface. Based on the observation of particle morphology using SEM shows that the bioactive component is trapped in encapsulation.

These related Chin *et al.* (2010) reported that the dented surface is caused by shrinkage of particles during the process spray drying. Maltodextrin is more susceptible to shrinkage during drying stage. Patel *et al* (2009) argued that spherical particle has a surface or volume ratio broadly indicating properties suitable for spray drying products.

### 3.5 *Particles size distributions*

The mean particles size distribution of 10% (w/v) Mo, 9,5% (w/v) Mo : 0,5 % (w/v) Cs, 9% (w/v) Mo : 1 % (w/v) Cs, and 8,5% (w/v) Mo : 1,5 % (w/v) Cs were 10,88, 6,62, 3,53, and 6,11 nm, respectively (Figure 2). The average particle sizes of 9% (w/v) Mo : 1 % (w/v) Cs were ~ 2 times smaller than other treatments. The results were supported with the findings of Klaypradit & Huang (2008), they observed for the spray dried tuna oil powder using chitosan, maltodextrin and whey protein isolate as wall materials resulted particle size ranged from  $8,4 \pm 0,3 \mu\text{m}$ .

### 3.6 *Bulk Density*

Bulk density values of were 10% (w/v) Mo, 9,5% (w/v) Mo : 0,5 % (w/v) Cs, 9% (w/v) Mo : 1 % (w/v) Cs, and 8,5% (w/v) Mo : 1,5 % (w/v) Cs were 0,57; 0,65; 0,73, and 0,67, respectively (Table 1). These results founded that powders generated from 9% (w/v)



Mo : 1 % (w/v) Cs had highest bulk density value. Bulk density values associated with powder particle size, Porrarud *et al.* (2010) found that the smallest sizes of particle size can be contained most tightly and represented the highest bulk density value. Reineccius (2004) have concluded that particle in spherical shape had the highest bulk density value, best packing and best flowing ability.

### 3.7 Free Glutamic Acid

The results of free glutamic acid derivatives content derived from different types of wall material as presented in Figure 4 showed that 9% (w/v) Mo : 1 % (w/v) Cs had the highest amount of free glutamic acid, while the lowest was 10% (w/v) Mo : 0% (w/v) Cs. Free glutamic acid is a flavour enhancing properties, therefore it is widely used as a flavour enhancer in the food industry, particularly in the form of the monosodium salt. It gives the typical aroma “umami”, recognized as the fifth basic taste, very similar to “meat aroma” or “broth aroma” (Bellisle, 1999). According to Reineccius (2004) suggests that spherical particles can retain the highest amount of flavoring agents. Mulay (2012), there is a correlation between the chemical structures and the taste compounds. However, the use Mo and Cs as wall materials is the material appropriate to the total amino acid value of the powder *san-sakng* leaf.

## IV. CONCLUSION

The result of this research showed that 9% (w/v) maltodextrin and 1 % (w/v) chitosan in free glutamic acid powder is the best powder based on physicochemical properties and morphological. This is proven by free glutamic acid of 21,09%, color value obtained had the yellowness value, yield of 49,52%,  $\alpha_w$  of 0,39, bulk density of 0,73, moisture content of 8,44%, and particle size of 3,53 nm.

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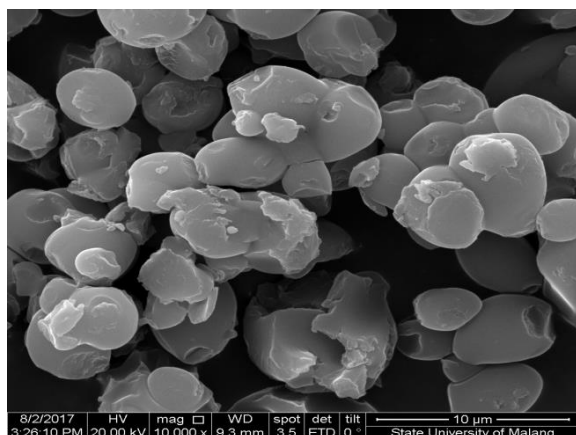
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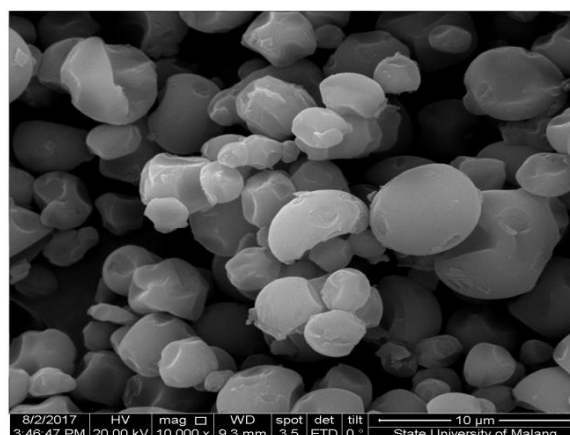
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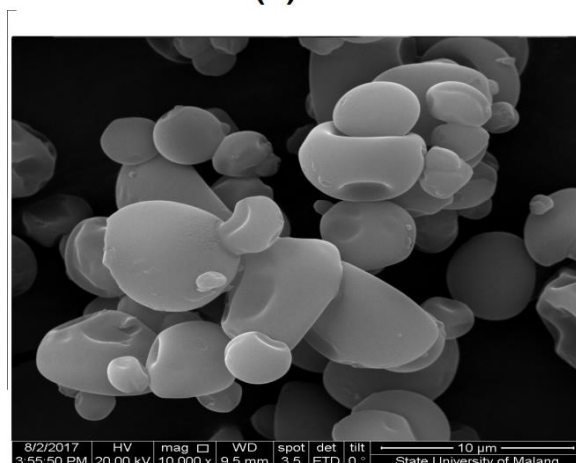
## List of Figures



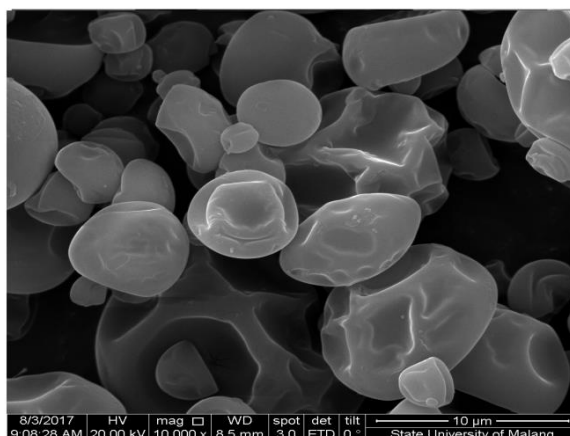
(a)



(b)



(c)



(d)

Figure 1. Scanning Electron Microscopy (SEM) images of san-sakng powder based nanoparticle at (a) x1000 and (b) x10.000 magnification : (a) 10% (w/v) Mo; (b) 9.5% (w/v) Mo : 0,5 % (w/v) Cs; (c) 9% (w/v) Mo : 1 % (w/v) Cs ; (d) 8,5% (w/v) Mo : 1,5 % (w/v) Cs.

## List of Table

Table 1. Physicochemical properties of san-sakng leaf extract spray-dried

Physicochemical properties	10% (w/v) Mo	9.5% (w/v) Mo : 0,5 % (w/v) Cs	9% (w/v) Mo : 1 % (w/v) Cs	8,5% (w/v) Mo : 1,5 % (w/v) Cs
Colour Value				
Lightness ( $L^*$ value)	91,01 ± 3,54a	82,21 ± 3,22b	82,11 ± 4,12b	81,76 ± 4,56b
Greenness ( $-a^*$ value)	0,93 ± 3,24a	0,56 ± 2,21b	0,77 ± 2,56c	1,22 ± 2,35a
Yellowness ( $b^*$ value)	8,82 ± 2,12a	10,65 ± 2,31b	10,74 ± 1,76b	11,39 ± 1,89b
Hue ( $h^\circ$ )	83,98 ± 2,10a	86,49 ± 2,08b	85,89 ± 1,87b	83,88 ± 1,54a
Yield <sup>3</sup> (%)	47,29 ± 3,56a	48,32 ± 2,65a	49,52 ± 2,16ab	51,32 ± 2,43b
$a_w$	0,45 ± 0,03a	0,42 ± 0,04a	0,39 ± 0,01b	0,35 ± 0,02c
Bulk density	0,57 ± 0,02a	0,65 ± 0,04b	0,73 ± 0,02c	0,67 ± 0,03b
pH	4,80 ± 1,12a	5,20 ± 0,89ab	5,50 ± 0,76b	6,20 ± 0,32c
Moisture content (% db)	10,52 ± 1,43	9,65 ± 1,42	8,44 ± 2,01	8,13 ± 2,13
Particle size distribution, D(0.5)	10,88 ± 1,84a	6,62 ± 2,78b	3,53 ± 3,54c	6,11 ± 1,69b

Reported means ( $\pm$  standard deviations) derived from 3 replications with 3 samples per replication. Means within a same row followed by the same letter were not significantly different ( $P < 0.05$ ).

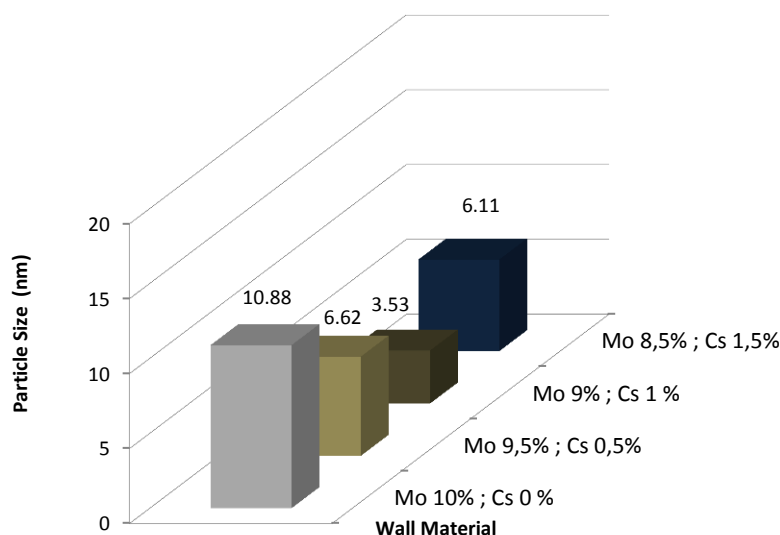


Figure 2. Average Particle Size Nanoparticles Generated from (a)10% (w/v) Mo; (b) 9.5% (w/v) Mo : 0,5 % (w/v) Cs; (c) 9% (w/v) Mo : 1 % (w/v) Cs ; (d) 8,5% (w/v) Mo : 1,5 % (w/v) Cs.

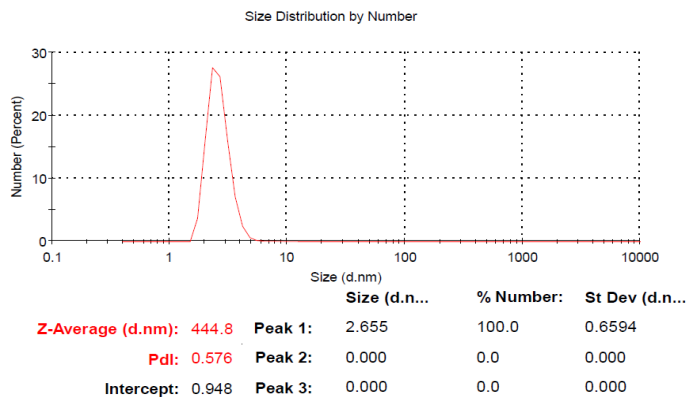


Figure 3. Particle Size Distribution Nanoparticles Generated from 9% (w/v) Mo : 1 % (w/v) Cs

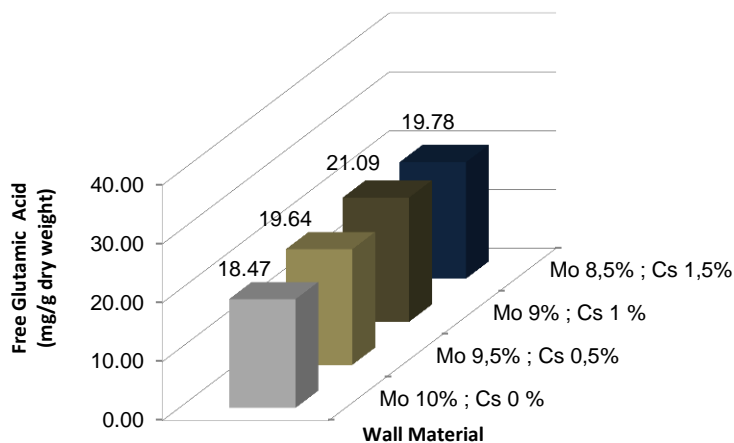


Figure 4. Average Free Glutamic Acid Content Generated from (a)10% (w/v) Mo; (b) 9.5% (w/v) Mo : 0,5 % (w/v) Cs; (c) 9% (w/v) Mo : 1 % (w/v) Cs ; (d) 8,5% (w/v) Mo : 1,5 % (w/v) Cs.

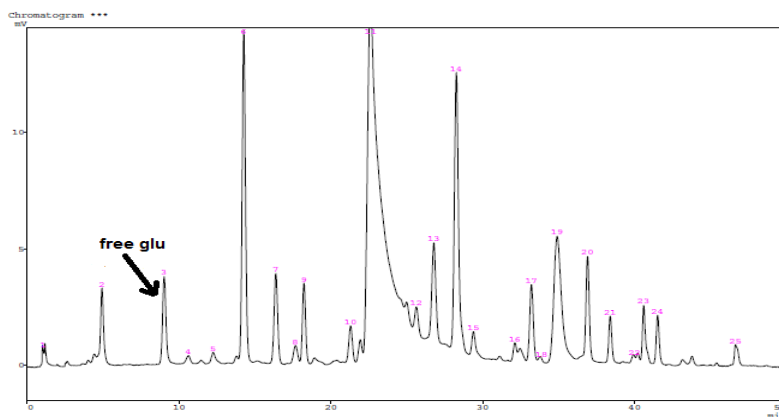


Figure 5. HPLC Chromatogram of 9% (w/v) Mo : 1 % (w/v) Cs nanoparticles