ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Studies on Accelerated Fermentation by Encapsulated Microorganism in Porridge

Amol P. Sonone and Dayanand Peter
Department of Food Processing & Engineering, Karunya University, Coimbatore - 641114 (TamilNadu)

Manuscript Info

Manuscript History:

Received: 11 June 2013
Final Accepted: 22 June 2013
Published Online: July 2013

Abstract

Probiotics are feed and food supplements that beneficially affect the host's health. Strain identity is important in order to link a strain to a specific health effect and to enable accurate surveillance and epidemiological studies. Oil emulsion used for microencapsulation of *L.plantarum*, *L.helviticus* with sodium alginate, starch and gelatin with different percentage (w/w) combination. The most common methods of microencapsulation applied to probiotic bacteria are entrapping into a gel matrix by extrusion or emulsion techniques (using ionotropic gel forming mechanism), spray drying, spray chilling or coating and freeze-drying. The most commonly reported microencapsulation procedure is based on the calcium alginate gel capsule formation. The slurry will be subjected to drying that result dried powder of microcapsules. Sodium alginate with gelatin (3%:3%) showing 94% probiotic survivability at 4^0C.

Introduction

The present study was conducted to evaluate the technological characteristics of lactic acid bacteria used as lactic acid starter in the manufacturing of fermented products and which are suitable to local conditions. There is growing scientific evidence supported by mechanistic and clinical studies that probiotic can provide health benefits. Encapsulation defined as a technology of packaging solids, liquids, or gaseous materials in small capsules that release their contents at controlled rates over prolonged periods and under specific conditions.

Material method

Sorghum, Pearl, Finger millet the entire sample were collected are local variety from local market R.S.Puram, Coimbatore. Analytical grade chemicals and reagents used(Sodium alginate, Gelatin, Polysorbitol (tween 80), Xanthan gum, Starch, calcium chloride, MRS agar). The best result for the porridge powder on nutrition basis is sorghum: pearl millet: finger millet in 2:3:2, which was the best against all the other composition.

Chemical analysis

1)**pH**

The pH of the samples was determined according to the method of Pineiro,*et al.*,(1971), by dipping the electrode of the pH meter in the mixture of homogenated fermented slurries after the end of each fermentation period.

2) **Moisture content**

A flour sample is determined by drying at 130°C for 2 hr in an oven to constant mass (Association of Official Analytical Chemists 1984).by using,

$$\text{Moisture (\%)} = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

Where, W_1- Weight of petri dish, W_2- Weight of petri dish with sample before dying,
W_3- Weight of sample with petri dish after drying

3) **Ash content**

The inorganic material, which does not volatilize at that temperature is ash, determined gravimetrically (Association of Official Analytical Chemists 1984). Protein estimation done by Microkjeldahl method (Leroy*et.al*., 2004).

4) **Fat content**

Oil extracted from a sample with a Soxhlet apparatus using hexane and separated by evaporation of the hexane for its estimation (Official and Tentative Methods of the American Oil Chemists' Society 1981).

$$\text{Oil \% in the sample} = ((A - B))/\text{C} \times 100$$

Where, A-weight of flask with sample, B-weight of flask, C- weight of sample taken

5**) Energy value**

Food energy value of the sample was determined according to the method described by Osborn (1972) and O.Chukwu,(2011).

Energy value (kcal/100g) = (4 × protein) + (9 × fat) + (4 × carbohydrate).

6) Calcium Content

Determination of calcium content by Hart and Fisher, (1971) method using the followingformulae.

$$\text{\% Calcium content} = [\text{A} - (8 \times \text{R})](\text{O.08})\left[\frac{\text{M}}{0.0200}\right]$$

7) Estimation of crude fiber

Crude fiber determine by using following formulae,

$$Crudefiber = \frac{\text{loss in wt. on ignition (W1} - \text{W2)}}{wt.ofsample} \times 100$$

Where, W1- weight of petridish with sample before ignition,

W2-weight of petri dish with sample after ignition

8**) Fermentation process**

The flour of Sorghum, pearl millet and finger millet with water was kept in plastic containers at a concentration of 1:3 dilutions (w/v). The slurry was allowed to ferment naturally by natural borne micro organisms in seeds (endogenous microflora on the seeds) at room temperature (20-23^{0}C) for 0, 12, 24, 36 and 48 hrs, in steel pots and then total colony count is done by using formulae (Geetha et al., 2007).

$$\text{cfu/ml} = \frac{\text{Number of colony count}}{\text{Dilution factor}\mathbf{x}\text{ sample}}$$

9)Isolation and Identification of Lactic Acid Bacteria Isolated from Porridge

The purification of isolates done by transferring Gram +ve rods and cocci shaped bacteria to the plates of selective media MRS agar and sub cultured until pure isolates obtained, in order to protect the typical organoleptic characteristics of traditional porridge preparation.

10) Cultivation of probiotic biomass

*Lactobacillus plantarum*and *Lactobacillus helveticus*was grown in MRS medium (Ziarno*et al.* 2007). A pure 1ml culture of *Lactobacillus plantarum* was suspended with 9 ml sterile peptone water 1% (w/v) and incubated at 37^{0}C for 24 hr. 8 ml MRS broth medium was added, and incubated at 37^{0}C for 24 hr to make the mother solution. It was then centrifuged at 2000 rpm for 5 minutes. The supernatant was centrifuged at 4500 rpm for 30 min and bacterial pellet was collected. It was washed by 10 ml sterile saline water, and centrifuged again at 4500 rpm for 30 min. The pellet obtained after the third centrifugation was suspended in 10 ml sterile saline water and used as the core material for microencapsulation.

Table1. Composition of probiotic encapsulating material for Microencapsulation with the sodium alginate with starch and gelatin with different combination

Composition of microcapsules	A	B	C	D	E	F	G
Starch (g)	0	1	2	3	0	0	0
Gelatin	0	0	0	0	1	2	3
Sodium alginate(g)	3	3	3	3	3	3	3
Probiotic cell pellet(g)	1	1	1	1	1	1	1
$CaCl_2$ 0.01M (ml)	200	200	2000	200	200	200	200
Vegetable oil (ml)	200	200	200	200	200	200	200
Tween 80 %	0.02	0.02	0.02	0.02	0.02	0.02	0.02

11)Emulsion method

Complete procedure for preparation of microcapsule through emulsion shows in flow chart.

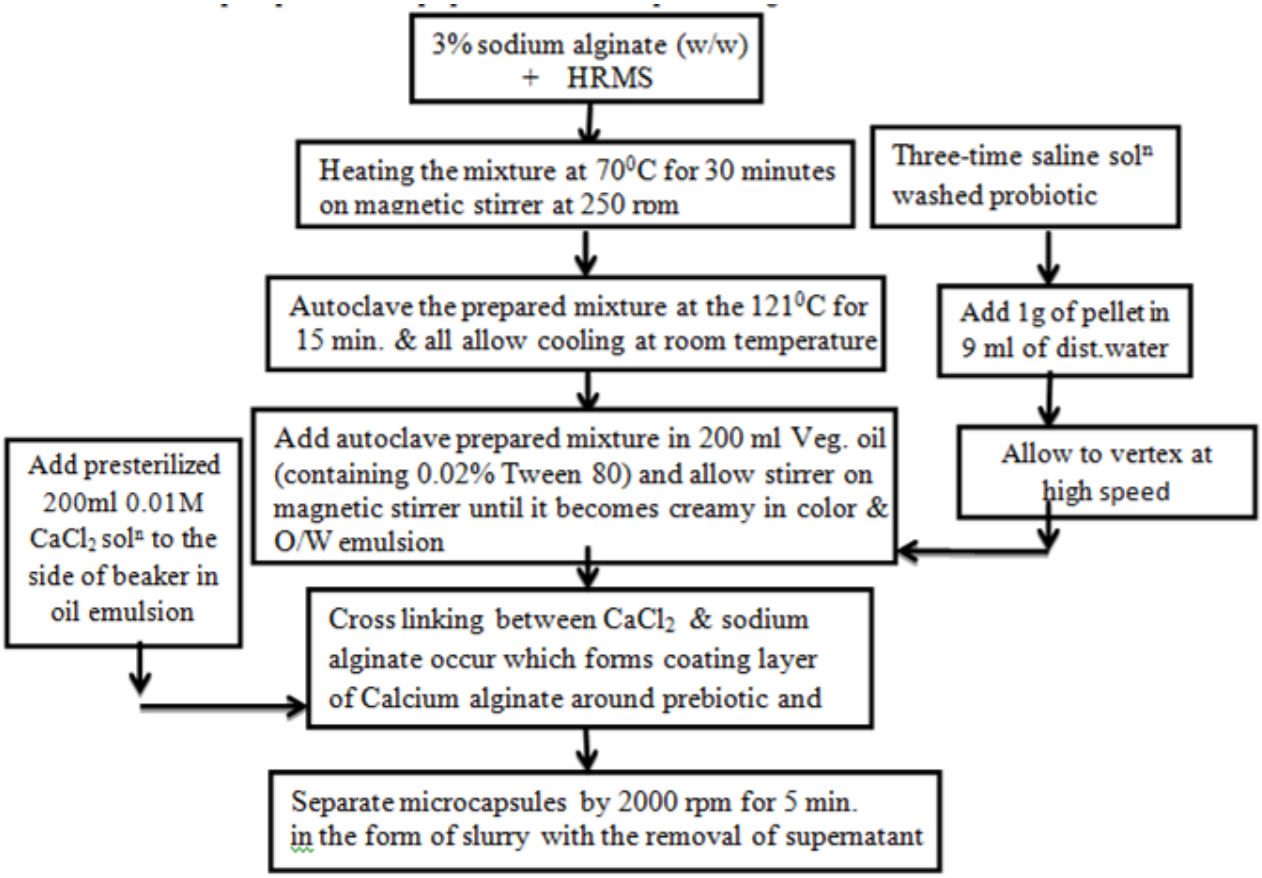


Fig. 1 Flow chart showing steps of oil emulsion microencapsulation for *L. plantarum*and *L. helveticus* (Sultana K, *et al.*, 2000).

Analysis of microcapsules

1) Evaluation of stability of microcapsules in different media

Dried microcapsules of known weight (10 g) in a glass vial containing 100 ml of solution and incubate at 37°C with 110 rpm of shaking for 4hour. The beads were periodically removed, weighed percentage of swelling of the beads calculated from the formula(Xiao et al., 2009).

$$\text{Sw}(\%) = \frac{Wt - Wo}{Wo} \times 100$$

Where, Wo - initial weight of the beads and

Wt- weight of the swollen beads at equilibrium swelling in the media

2) Viability assay of cells in granules and Sodium alginate beads

Granules of sodium alginate beads in dry and wet form was dissolved in phosphate buffer saline (PBS, pH 7.4). A serial dilution of this suspension made until a suitable cell density obtained.

$$\text{Survival } (\%) = \frac{\text{cfu of the dried granuals}}{\text{cfu of the wet granual}} \times 100$$

3) Microcapsule characteristic (morphology, size)

Morphology, size and surface microstructure of microcapsules were studied by the use of scanning electron microscopic (SEM) (Chávarri, 2010)

4)Bulk density

Two grams of powder loosely weighed into 10 ml graduated cylinder. Then the cylinder was tap on a flat surface for a constant volume. The final volume of the powder gives the bulk density as follows(Yahya et al., 2012).

$$\text{Bulk density}\,(g/cm^3) = \frac{\text{Weight of the powder}}{\text{Volume of the sample}}$$

5) Solubility

Solubility of the powder was determined as described by Bhandari*et al.*, (2003) (i.e.,) 2g of powder in 20 ml of water at room temperature (28 ± 2ºC). The mixture was centrifuged at 15000 rpm for 90 sec. The presence of sediment is the indicative factor for the solubility test and the powder was added gradually till the formation of sediment (Sultana et al., 2000).

$$\text{Solubility} = \frac{\text{Weight of powder}}{\text{Weight of water}} \times 100$$

Result and Discussion

1)Soaking effect on sorghum, pearl millet and finger millet.

The table shows the nutritional values of sorghum, pearl and finger millet, after the overnight soaking with water.

Table 2.Soaking effect on sorghum, pearl millet and finger millet.

Table 2.Soaking effect on sorghum, pearl millet and finger millet.

Content (%)	Sorghum	Pearl millet	Finger millet
Protein	12.015 ± 0.28	11.76 ± 0.07	11.43 ± 0.07
Carbohydrate	64.37 ± 1.67	67.79 ± 0.35	61.30 ± 0.94
Fat	2.64 ± 0.024	3.31 ± 0.081	0.95 ± 0.07
Energy value	392.46 ± 5.83	348.01 ± 2.44	299..49 ± 4.13
Ash	1.25 ± 0.028	0.95 ± 0.036	0.95 ± 0.01
Crude fiber	6.1 ± 0.19	5.1 ± 0.10	6.12 ± 0.26
MC (%)	6.17 ± 0.28	4.48 ± 0.18	5.20 ± 0.21
Initial microbial Load (CFU/g)	133×10^4	186×10^4	167 $\times10^4$

2) Changes in the microbial profile and pH during fermentation of Porridge.

The yeast and mould counts were relatively constant during the fermentation with a rapid decline of 5-log cycles towards the end of the fermentation (42nd hour). This was up to about a value of <1 log10 cfu/ml. There was a steady increase in total viable counts, LAB, and lactococci within the first 24 hours by a value of 1log cycle (shown in graph 1) and the pH was linear with increase in lactic acid percentage of the product. pH deceases from 6.2 to 2.8 indicates lactic acid bacterial growth in low pH

Fig. 2 Growth of microorganism during fermentation.

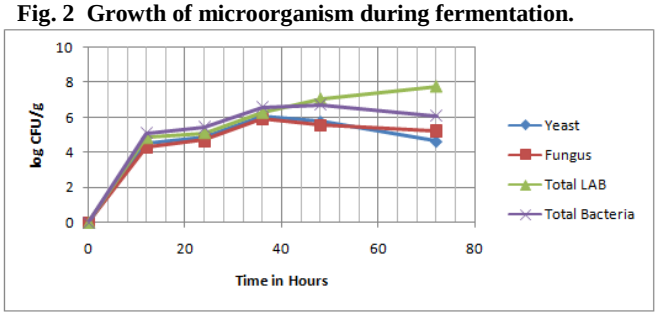


Fig.3 pH profile during fermentation.

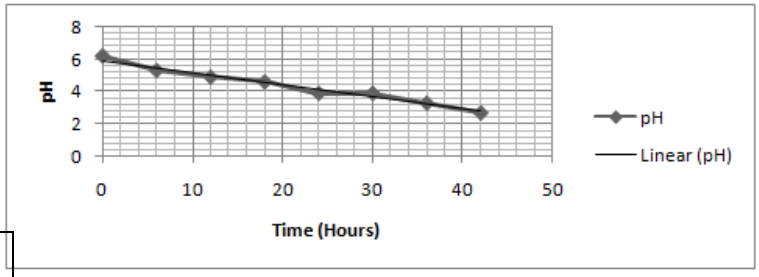


Fig.4 % Survival of free *L. plantarum* and*L. helviticus* in simulating gastrointestinal condition at different times

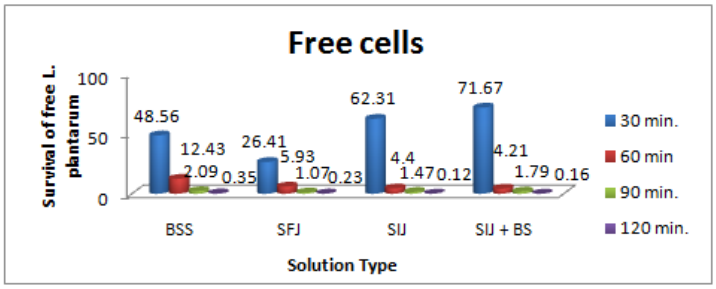


Fig.4 shows that the free cells were 71.67% are survive in the SIJ+BS condition more than other condition.

Fig.5 Survival of free *L. plantarum* in simulating gastrointestinal condition after encapsulating with Sodium Alginate (3%) at different times

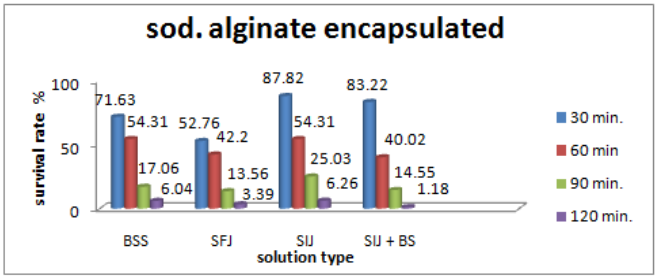
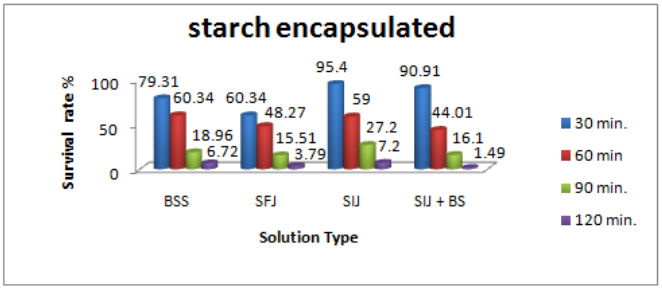


Fig.6% Survival of encapsulated with Starch and sod. Alginate 3:3) *L. plantarum* and*L. helviticus* in simulating gastrointestinal condition at different times



Fig, 7 Survival of free *L. plantarum* in simulating gastrointestinal condition after Encapsulating with Sodium Alginate (3%) and Gelatin (3%) at different time

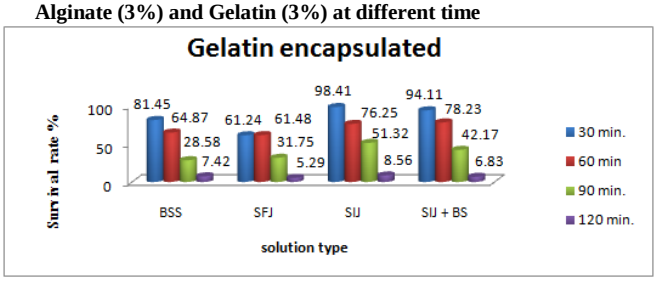


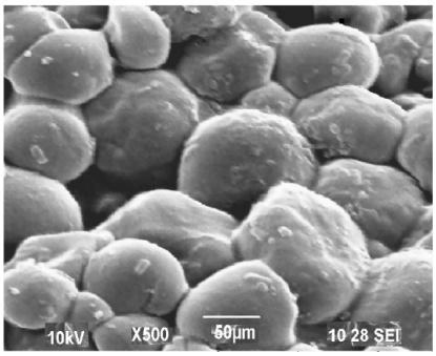
Table.3 Bulk density and efficiency of entrapment of Microcapsules

Combination of media	Ration of media	% of efficiency of entrapment	Bulk density
Sodium alginate	3%:	26.36 ± 0.05	0.49 ± .01
Sodium alginate : Starch	3%: 1%	29.03 ± 2.30	0.49 ± 0.02
	3%: 2%	29.72 ± 0.04	0.51 ± 0.02
	3%: 3%	34.20 ± 0.43	0.54 ± 0.03
Sodium alginate: Gelatin	3%: 1%	28.62 ± 0.39	0.48 ± 0.01
	3%: 2%	33.55 ± 2.54	0.52 ± 0.02
	3%: 3%	38.72 ± 1.31	0.56 ± 0.02

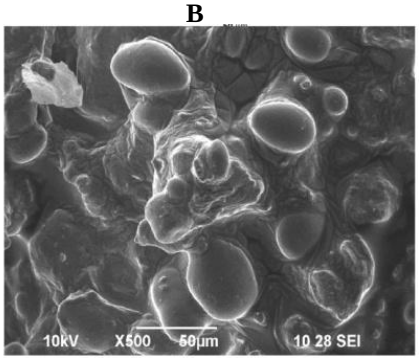
Partical size and morphology of capsule

Partical size of microcapsules are observe by SEM

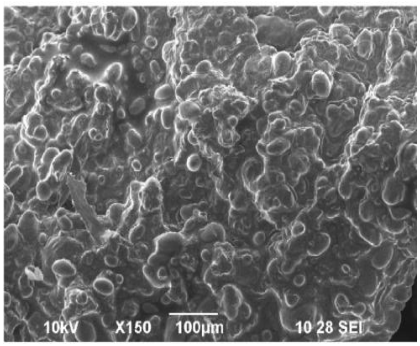
A



B



C



A)Sodium alginate : Gelatin (3%:3%), B)Sodium alginate : Starch (3%:3%), C)Sodium alginate(SA).

Table.4. Reduction of total microbial count during storage stability.

Combination of media	Ration of microcapsule	Day 1st cfu/g	Day2nd cfu/g	Day4th cfu/g	Day 6thcfu/g	Day 8thcfu/g	Day10thcfu/g
Sodium alginate	3%	57×10^3	43×10^3	31×10^3	18×10^3	11×10^3	6×10^3
Sodium alginate + starch	3 %:1%	89×10^3	68×10^3	43×10^3	27×10^3	16×10^3	8×10^3
	3 %:2%	103×10^3	91×10^3	73×10^3	52×10^3	21×10^3	7×10^3
	3 %:3%	93×10^3	71×10^3	59×10^3	33×10^3	17×10^3	8×10^3
Sodium alginate + Gelatin	3 %:1%	84×10^3	81×10^3	64×10^3	41×10^3	23×10^3	9×10^3
	3 %:2%	97×10^3	78×10^3	58×10^3	43×10^3	27×10^3	8×10^3
	3%:3%	112×10^3	85×10^3	61×10^3	51×10^3	29×10^3	11×10^3

At room temperature microbial count, reduce wildly from 3day itself. However, in case of 4^0C and-30^0C is start from 4th day following for the microencapsulated bacteria the refrigeration temperature is suitable to store up to 8th day.

Conclusion

Overall, the current research introduces a promising technology for microencapsulating not only probiotic bacteria but also other bioactive materials such as, minerals, vitamins, oxidation sensitive lipids etc. Survivability (%) and mortality rate of probiotic composition (sodium alginate, gelatin) containing 3% gelatin contain in microcapsule showed 94% probiotic viability. It proved that more efficient when compared with other combination of sodium alginate and starch.The health benefits of probiotic rich foods are well accepted and clinically proven. The identified isolates used to establish the production of nutritive compounds and to assess their potential as starter cultures for their commercial uses.

References

1. Bhandari B, Krasaekoopt W, Deeth H (2003). Evaluation of encapsulation techniques of probiotics for yoghurt.*Int Dairy J.* 13: 3-13

2. Chavarri, M., Maranon, I., Ares, R., Ibaiez, F. C., Marzo, F. and Villarán, M. C. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro- intestinal conditions. Int. J. Food Microbiol.142:185–189.

3. Geetha, T. Gnanam, G. and Maheswaran, M. (2007). Microbial changes during porridge fermentation of maize and some South Indian millets , School of Biosciences and technology, VIT University, Vellore - 632014, Tamil Nadu, India.

4. Leroy F and De Vuyst L.(2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology.*;15:67-78

5. O. Chukwu, B. A. Orhevba and I. Abubakar (2011), Determination of some physicochemical properties of guinea corn (*sorghum vulgare) (pp. 62-66)* Journal of Innovative Research in Engineering and Science 2(2), April, 2011. ISSN : 2141-8225.

6. Pineiro, M., Stanton, C., 2007.Probiotic bacteria: legislative framework requirements to evidence basis.Journal of Nutrition 137 (3), 850S–853S.

7. Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K (2000). Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt.*Int J Food Microbiol.*62: 47-55.

8. Xiao Yan Li , Xi Guang Chen, Dong Su Cha, Hyun Jin Park and Cheng Sheng Liu (2009) Microencapsulation of a probiotic bacteria with alginate–gelatin and its properties, Journal of Microencapsulation, 2009; 26(4): 315–324

9. Ziarno, M., Sekul, E. &LafrayaAguado, A.(2007). Cholesterol assimilation by commercial yoghurt starter cultures, ACTA

ScientiarumPolonorum-Food Science and Human Nutrition 6(1): 83-94.

10. Yahyashafiei, vadoodrazavilar, afnishjavadi and hamidmirzai, (2012), survivability of free and microencapsulated L. plantarum with alginate and resistant starch in simulated gastrointestinal condition.journal of food agriculture & environmental Vol.10 (3&4) 207-212.6u9.

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