

Microencapsulation of Probiotics (Lactobacillus Casei and Bifidobacterium Longum) in Pineapple Jam by Spray Drying and its Comparative Study

Ms. Sharvari A. Surnis
Department of Biotechnology Engineering
T. K. I. E. T. , Warananagar, Kolhapur,
Maharashtra , India

Mr. Kulbhushan B. Huparikar
Mr. Pramod A. Kamble
Mr. Mohsin I. Mulla
Department of Biotechnology Engineering

Abstract— Probiotics are defined as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host. *Lactobacillus casei* and *Bifidobacterium longum* are one of probiotic bacteriums when introduced in sufficient colonies serve as new prophylactic and curing agent. They increase the innate immunity of humans. Non-dairy probiotic foods are becoming popular because they do not pose problems of lactose intolerance and high cholesterol content while they offer an alternative from traditional sources and for personal preferences. The storage of probiotic products requires to maintain viability as high as $> 10^6$ - 10^8 CFU/unit. To tackle these aspects, the microencapsulation of probiotics by spray drying was studied in Pineapple Jam. Encapsulation was performed by spray drying with pectin as polymer. Comparison of nutritional aspects and shelf life of normal jam, multistrain probiotic jam and encapsulated multistrain probiotic jam was performed and it was found that both the parameters were enhanced in case of encapsulated multistrain probiotic jam.

Keywords—Probiotics; *Lactobacillus casei*; *Bifidobacterium longum*; Pineapple jam; Microencapsulation; Nutritional value.

INTRODUCTION

Aerobic and anaerobic bacteria, yeast and fungi thrive into the GI tract. All these organisms in a healthy intestinal tract dwell in a natural balance called symbiosis. There are more than 2000 species of harboring bacterial organisms within our bodies, the vast majority in the gut. In fact the mammalian gut is considered one of the most densely populated ecosystems on earth with a bacterial load in the region of 10^{12} organisms/gram of fecal material in the large intestine. The numerous species of microorganisms in the adult human gut are known as the microbiota. The initial acquisition of intestinal microbiota plays a key role in the development of immune processes and protection against pathogens.

The changing life style has also changed the eating behavior of the people. Now people prefer to consume the food that not only meet their nutritional requirement but also improve their health. This preference leads to the development of functional foods. Probiotics are one of the major constituents of the functional foods.

Probiotics may be defined as the live microorganism which when administered in a sufficient quantity help in improving the health of the host. The minimum concentration of probiotics recommended to attain the desirable health benefits is 10^6 - 10^7 CFU/g or mL of food at the time of consumption.

Bacteria form *Lactobacillus* and *Bifidobacterium* genera are most commonly used as probiotics in food products. [2]

Jam is a very popular food product that can be used as a vehicle to deliver the healthy bacterial cultures to the consumers. It can be manufactured with various concentrations of different ingredients and then mixed with various ratios of probiotic cultures. There are a lot of factors that determine the viability of the probiotic microbes in food products. The factors are total solids, pH, acidity, time and temperature physical damage during mixing, storage time and temperature. [4]

Encapsulation is a process in which tiny droplets or particles are wrapped with a protective coating yielding capsules for countless application. Encapsulation may be defined as a process of entrapping one substance (active agent) within another substance (wall material). The encapsulation technology has been used by the food industry for several years. Microencapsulation in which the cells are retained within an encapsulating matrix or membrane, has emerged as an alternative for protection of probiotics, providing a particular and convenient micro-environment for the encapsulated microorganism, enhancing their viability, and enabling controlled release of cells in the intestinal tract. Spray drying is considered a good long-term preservation method for probiotic cultures. The speed of drying and continuous production capability is very useful for drying large amounts of starter cultures. Spray drying is a unique process in which particles are formed at the same time as they are dried. [8]

MATERIALS AND METHODS

A. Cultivation

Pure lyophilized cultures of *Lactobacillus casei* and *Bifidobacterium longum* were obtained from Unique Biotech, Hyderabad. Lactobacilli MRS (deMan Rogosa and Sharp) and Nutrient agar was used as the selective media for *Lactobacillus* spp. and *Bifidobacterium* spp., respectively.

B. Preparation of modified Jam formulation

A commercial base for jam preparation was used as reference encapsulating material and for jam preparation. Base ingredients, in decreasing order, are: culture powder, Pectin & distilled water. [3]

C. Preparation of probiotics enriched formulation

For the preparation of the probiotic functionalized jam the commercial base was substituted with a 5 % of a modified base enriched with probiotics. The required cellular concentration for the solutions to be spray-dried was calculated based on the following data or hypotheses:

- No vitality loss occurs during spray-drying;
- A final jam with a 10⁸ CFU/g is desired so that a 100 g serving would guarantee the recommended minimum 10⁹ CFU daily intake
- Spray-drying feeding solution has 4% w/v solids of formulation ingredients. [3]

D. Jam preparation

Jam was prepared with a domestic method, according to the process of Figure 1. Commercial fresh pineapple juice and sugar were used. The commercial base has to be used with a “normal process”, which means that it has to be mixed with the other ingredients after preparing jam. The process had to be adapted to laboratory facilities. Juice was pre-heated to 60-65 °C in in a water bath. Sugar was added and mixed with a Citric acid. The mixture was left in the water bath until reaching a 80 °C temperature for 5 minute then added pectin, rapidly cooled in water-ice bath until 10-12 °C and kept at room temperature overnight (12 h).[7]

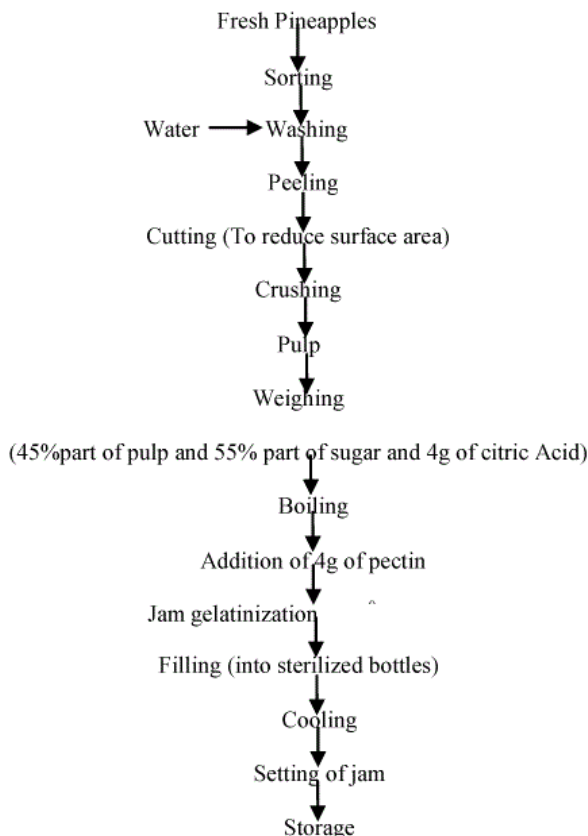


Fig 1. Flow chart of Jam preparation [9]

Assays Performed

A. For culture

1. pH tolerance

The isolated bacterial cultures were inoculated into sterile MRS broth tubes of varying pH, i.e. pH 2, 3, 4, 5, 6, and 7 and incubated at 37 °C for 2-3 days. Then 0.1ml inoculums from each tube was poured to MRS agar medium by pour plate method and incubated at 37 °C for 48hrs. The growth of bacteria on MRS agar were used to designate isolates as pH tolerant.

2. Temperature sensitivity

The selected bacterial cultures were grown at varying temperatures, i.e. 25, 30, 37 and 40°C for 48-72 hrs. Then 0.1ml inoculum was transferred to MRS plates by pour plate method and incubated at 37°C for 48hrs.

3. Anti-microbial screening (well assay)

MRS agar plates were prepared; surface of the MRS agar plates was inoculated with the swab containing 24 hr culture (*V. cholerae*). Wells were punched with the gel puncher 80mg (*L. casei* and *B. longum* Powder) were added to the wells punched in the centre of the plates. Plates were incubated for 24 h at 37°C. *E-coli* and *S. aureus* were also incubated on the MRS agar plates, separately and wells were punched. Wells, with a diameter of 5 mm, were then cut in the agar using sterile gel puncher.

B. For Jam

For prepared jam , pH was checked with Digital pH meter after keeping it at room temperature for 48 hrs. Also Brix was measured with digital refractometer after keeping at room temperature for 48 hrs.

C. Nutritional value

Nutritional value and shelf life was checked at laboratory for normal jam, probiotics jam & microencapsulated probiotic jam .

RESULT AND DISCUSSION

A. For culture

Best result obtained for pH tolerance test was at pH 7. For temperature sensitivity test best result was obtained at 37⁰ C. Anti microbial activity test was positive for both the organisms and zone of inhibition was given as below:

| Sr no. | Name of organism | Zone of inhibition | Zone of inhibition |
|--------|------------------|--------------------|--------------------|
| | | <i>(B. longum)</i> | <i>(L. casei)</i> |
| 1 | <i>E. coli</i> | 9 mm | 7 mm |
| 2 | <i>S. aureus</i> | 4 mm | 5 mm |

B. For Jam

| Test | Standard value | Obtained value |
|-----------|----------------|----------------|
| pH test | 3 – 3.5 | 3 |
| Brix test | 68 – 75 % | 71 % |

C. Nutritional value

| Sr No. | Parameter | Normal Jam | Probiotic Jam | Encapsulated probiotic jam |
|--------|-------------------|------------|---------------|----------------------------|
| | Nutritional value | | | |
| 1 | Moisture % | 20.42 | 19.38 | 22.43 |
| 2 | Total minerals % | 00.00 | 00.00 | 00.21 |
| 3 | Crude Protein % | 00.97 | 01.53 | 00.92 |
| 4 | Crude Fat % | 00.04 | 00.04 | 00.04 |
| 5 | Crude Fiber % | 0.00 | 00.00 | 00.00 |
| 6 | Carbohydrate % | 78.57 | 79.05 | 76.04 |
| 7 | Energy,Kcal/100g | 318.52 | 322.68 | 309.64 |
| | | | | |
| | Other Analysis | | | |
| 1 | Shelf Life | 25 | 20 | 26 |

CONCLUSION

As the viability of probiotic cultures is a major challenge in dairy product development, the results of the present study demonstrate the potential of increasing both the technological suitability and expanding the performance of probiotic strains through encapsulation technique. Multistrain probiotic jam is less tedious to prepare and has greater shelf life than other dairy products. Encapsulation by spray drying further enhanced its shelf life. Nutritional value also enhanced when compared with normal jam and probiotic jam

REFERENCES

- [1] Annex J - Summary of current food standards as of 04 APRIL 2013.pdf
- [2] Gildas K. Gbassi et al "Probiotic Encapsulation Technology: From Microencapsulation to Release into the Gut Laboratoire de Conception et Application de Molécules Bioactives" (UMR-7199), Faculté de Pharmacie, UDS-CNRS, Illkirch-Graffenstaden, France, ISSN 1999-4923
- [3] Giorgia Spigno et al , "Spray-Drying Encapsulation of Probiotics for Ice-Cream Application" , Istituto di Enologia e Ingegneria Agro-Alimentare, Piacenza (Italy), Chemical Engineering Transactions Vol. 43, 2015
- [4] Hafiz Shehzad Muzammil et al; "Viability of Probiotics in Frozen Yogurt with different levels of Overrun and Glycerol" , Department of Dairy Technology, Lahore, Pakistan <http://www.fspublishers.org>
- [5] Jeanne-Marie Weathers, "Standards of Identity for Jams, Jellies, Preserves & Fruit Butters" Code Of Federal Regulations Title 21 Part 150
- [6] KnO-100281_Pineapple jam.pdf
- [7] The Science Of Jam And Jelly Making, University of Kentucky , FN-SSB.110
- [8] V. Hazal Ozyurt, Semih Ötles "Properties of probiotics and encapsulated probiotics in food" Acta Sci. Pol., Technol. Aliment. 13(4), 413-424; 2014
- [9] Nwosu JN, Udeozor LO, Ogueke CC, Onuegbu N, Omeire GC et al. "Extraction and Utilization of Pectin from Purple Star-Apple (*Chrysophyllum cainito*) and African Star-Apple (*Chrysophyllum delevoiyi*) in Jam Production." Austin J Nutr Food Sci 2014;1(1): 1003. ISSN: 2381-8980.