Sustainable utilization and optimization of spray dried fermented pumpkin juice

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Abstract

Pumpkin, a nutrient rich vegetable with approximately 27 million tonnes production globally generates high amount of pumpkin waste. The need of the hour is the utilization of vegetable waste in sustainable way. Production of value added product is the possible solution for this problem. As fruit and vegetables have proved to be promising carriers for probiotics. Hence, in this work, research studies have been carried out on the effective utilization of pumpkin by developing non-dairy probiotic beverage. The extracted pumpkin juice was fermented by *Lactobacillus fermentum* NCDC 141 and microencapsulation of probiotic microorganisms was done by spray drying. Growth conditions of fermenting microorganism were optimized in MRS medium and pumpkin juice. Desirable viable cell count i.e more than 10^6 was observed with 10% inoculum size after 15 hr incubation time. Significant different was seen between properties of fermented and non-fermented pumpkin juice. Optimization of spray drying conditions to obtain maximum viable cells and DPPH scavenging activity and minimum moisture content and hygroscopicity was done by response surface methodology (RSM). Optimal conditions to get desirable characteristics were observed at 20% maltodextrin concentration and 140°C inlet air temperature. Fermented microencapsulated pumpkin powder showed 62.36% DPPH scavenging activity, 7.985 log CFU/ml viable cells, 2.413% moisture content and 16.324% hygroscopicity at optimized conditions. High encapsulation efficiency (89.00 ± 0.91%), water solubility index (87.59 ± 0.89%) and antioxidant activity (64.36 ± 1.28%) was seen for fermented powder. Further, scanning electron microscopy images showed adequate encapsulation of probiotic pumpkin juice with smooth and nearly spherical structure.

1. Introduction

Fruits and vegetables processing has gained popularity in recent years to prevent the wastages of fruits and vegetables. Globally, the production of fruits and vegetable is about 675 million metric tons annually and out of which 42% wastage is produced [1]. Pumpkin is one of the nutrient rich vegetables with good source of valuable nutrients. β- carotene, dietary fiber, vitamins (C, E and B6), minerals, and small amount of fat are the major nutrients present in pumpkin. Other than this, pumpkin is well known for its carotenoids present in the form of β-carotene, α-carotene, lutein, α- and β- cryptoxanthin, antheraxanthin and few other xanthophylls [2]. Owing to the presence of multifarious bioactive components, pumpkin is considered to be used for several health benefits including antidiabetic, antioxidant, anticarcinogenic, and anti-inflammatory [3]. It has also been reported to use as a therapeutic agent for the treatment of disorders such cestodiasis, schistosomiasis, and ascariasis. Regardless of all these benefits, a large portion of this crop is wasted every year which not only cause the economic burden but also leads to a serious threat to the environment by producing toxic gases (methane) during the breakdown of organic matter. So, by processing this crop, conventional approach to deal with pumpkin by landfilling can be changed. It would not only mitigate the pollution problem but also add to the bioeconomy (by a direct use or after further processing in biorefineries) [4].

The utilization of pumpkin for juice preparation and its fermentation with lactic acid bacteria (LAB) helps in value addition and increase in nutritional value of pumpkin. Pumpkin has been found to be a suitable substrate carrier for *Lactobacillus fermentum* due to its natural composition of prebiotics and pH [5]. Sreenivas and Lele, [6] used the probiotic for fermentation of pumpkin slurry and reported a remarkable total short chain fatty acid production indicating the considerable presence of prebiotics in pumpkin. Additionally, processing of pumpkin juice by heat treatment degrades the bioactive compounds present in pumpkin juice nevertheless, fermentation with LAB significantly increases their amount and activity [7]. Moreover, in recent years, the inclination of consumers towards non-dairy probiotic beverage has been observed due to the various health problems associated with the dairy products. Hence, fermented probiotic pumpkin juice is of great interest in the development of probiotic products with non-dairy sources.

However, short shelf-life and requirement of controlled storage has made its market limited. Therefore, drying has been opt to make a non-perishable, convenient and easy to store food. Among different drying methods, spray drying is a commonly used technique for droplet- to-particle transition and microencapsulation of food ingredients owing to its simplicity and cost effectiveness. Powder developed by Spray-drying has been reported to possess desirable powder characteristics such as lower values for moisture content, water activity (aw), hygroscopicity and dissolution time however, higher values for bulk density (ρb), glass transition temperature (Tg) and bioactive retention [8]. Powder characteristics depend upon the microstructure of the microcapsules developed during drying. Spray drying has been reported for drying of various fruit juices such as watermelon, tamarind, mango, blueberry and pomegranate with different carrier agents and in different concentrations.

To our knowledge, no studies has been performed on spray drying of fermented pumpkin juice containing probiotics. Thus, the goal of the study is to evaluate, the effect of fermentation on quality characteristics of pumpkin juice and optimizing spray drying conditions for its encapsulation using maltodextrin as carrier agent. The optimized technology can be simply adapted by industries for production of

functional fermented pumpkin probiotic powder, thus helping in get rid of pumpkin waste and enriching the commercial perspective of pumpkin juice that may aid in improving the economic status of farmers.

2. Materials And Methods

2.1 Preparation of raw material

Pumpkin hybrid variety *Bheema* was purchased from local market of Jalandhar city, India. The seed and peel were physically removed. After cleaning, peeling, and grating, the juice was extracted using mixer grinder. The extracted filtered juice was fltered and pasteurized in a water bath at 85°C for 10 min. After that, cooling of the juice was done at room temperature. Efficacy of pasteurization process was checked as per the method given by Sharma et al., [9].

2.2 Microorganisms and Media

MRS medium (M369) was employed as the culture medium and was procured from Hi-media, India. Medium was prepared as per the instructions given by manufacturer. Steam sterilization was done with the autoclave at 15 psi and a period of 15 min before its usage. In order to accelerate the growth of probiotic strains 0.3 g sterilized L-cysteine was added to the medium. *Lactobacillus fermentum* NCDC 141 was procured from NDRI (National Dairy Research Institute), India.

2.3 Growth profile of probiotic strain in MRS medium

Growth profile of the probiotic strain was determined using the MRS-cysteine medium with static fermentation procedure as suggested by Sharma et al., [9].

2.4 Pumpkin juice fermentation

Seed culture for pumpkin juice fermentation was prepared by suspending the bacterial cells (2.94 ×1010 CFU/ml) in sterilized pumpkin juice [10]. For the optimization of inoculum size, different concentrations (5, 10, and 15 %) of seed culture were inoculated in the pumpkin juice and fermentation was carried out at 37°C for 15 h. During fermentation, samples were aseptically withdrawn at every 3 h interval during the 15 h period. These samples were analysed for cell viability, changes in pH, and TSS.

2.5 Physicochemical analysis

The pumpkin juice was analyzed for its physicochemical characteristics before and after fermentation.

2.5.1 pH, Viscosity and TSS

pH was determined by the digital pH meter (Orion 2 Star pH Benchtop). Brookfield instrument (model DV-1 Pro viscometer, USA) was used to measure the viscosity of the fermented juice. Total soluble solid (TSS) was quantified by digital refractometer (Kruss optronic, Germany).

2.5.2 Titratable acidity, ascorbic acid and total sugar

Titratable acidity (TA), ascorbic acid content and total sugar content were determined by using the lane and Eynon method [11].

2.5.3 Total phenolic content and total Flavonoid content

The total phenolic content was determined by the Folin–Ciocalteau colorimetric method as described by Amjad et al., [12] whereas total flavonoid content was determined by aluminium chloride colorimetric assay procedure, as described by John et al., [13].

2.5.4 DPPH scavenging activity

The antioxidant activity of the extract was determined on the basis of radical scavenging potential as performed by Sharma et al., [10].

2.6 Microencapsulation of probiotic pumpkin juice by spray drying

Pumpkin juice was fermented with probiotic strain *Lactobacillus fermentum* for 15 hr at 37°C and then exposed to microencapsulation. Following probiotic fermentation, the juice was then subjected to microencapsulation by spray drying as conducted by Paim et al., [14], with few modifications. A laboratory scale spray dryer (LabPlant SD-06) was used for spray drying. The nozzle diameter was 0.5 mm and

main spray chamber was of 500 mm × 215 mm. Prior to the spray drying, fermented pumpkin juice was mixed with different concentration of maltodextrin. Drying was performed with drying air speed of 4.8 m/s and compressor air pressure of 0.25 MPa. The feed flow rate used was 0.3 L/h, and the inlet temperature was varied. Afterwards, the obtained pumpkin powder at optimized condition was stored in small glass jars with screw caps for further evaluation.

2.7 Optimization of spray drying conditions

Optimization of conditions for spray drying of fermented pumpkin powder were carried out by using RSM (Response surface methodology). Central composite design (CCD) was used to demonstrate the effect of factors. Maltodextrin concentration (10-20%) and inlet air temperature (140-160°C) were used as independent factors and their effect on moisture content, DPPH scavenging activity, hygroscopicity and viable cells were checked. The parameters and responses were selected on the basis of literature available and low and high values were decided on the basis of preliminary studies.

2.8 Characterization of spray dried probiotic pumpkin powder

2.8.1 Moisture content

The moisture content (%) of the powder was calculated as per the standard oven dry method as given by Saikia et al., [15].

2.8.2 Hygroscopicity

Hygroscopicity of dried pumpkin powder was determined as proposed by Paim et al., [14].

2.8.3 Viable cells

Viable cell count in spray dried fermented powder was checked as per the method suggested by Vivek et al., [16]. MRS agar medium was used to check the viability of cells.

2.8.4 Process yield (%)

Process yield (%) was determined by as described by Santos Monteiro et al., [17] by using formula

$$Process\ yield(\%) = \frac{Mass\ of\ powder\ collected\ in\ cyclone}{Total\ solid\ fraction\ \times\ Sample\ total\ weight} \times\ 100$$

2.8.5 Encapsulation efficiency

Encapsulation efficiency were calculated by the given equation [17]

$$Encapsulation\ efficiency\ (\%) = \frac{N}{N_0} X100$$

where No is the number of cells in the feed solution (cell mL−1), and N is the number of cells in the powder, both measured by calculating CFU/ml.

2.8.6 Solubility and water absorption index

Solubility of dried pumpkin powder and water absorption capacity were determined using the method given by Franco et al., [18].

2.8.7 Density, Cohesiveness and flowability of spray dried powder

Bulk density and tapped density was calculated by as per the method given by Saikia et al., [15]. The cohesiveness and flowability property of the powder samples was calculated by Hausner's ratio and Carr index:

$$Hausner's ratio, HR = \frac{Tapped\ density\ (TD)}{Bulk\ density\ (BD)}$$

$$Carr\ index, CI = \frac{(TD - BD) \times 100}{TD}$$

2.8.8 Morphology of dried pumpkin juice powder

The surface morphology of dried pumpkin juice powder was observed under SEM (Hitachi Se 300H-Tokyo, Japan). With the help of double-sided adhesive tape, samples were mounted on aluminium stubs and coated with a thin layer of gold-palladium. At an accelerating voltage of 15KV, samples were analyzed under the microscope.

Statistical analysis

The results were statistically analyzed by calculating the mean and standard deviation (SD) and presented as the mean±SD of three determinations. Analysis of variance (ANOVA) was conducted using SPSS statistics version 16.0.

3. Results And Discussion

Growth profile of *Lactobacillus fermentum* in MRS medium

Growth profile of *Lactobacillus fermentum* NCDC 141 (**Fig. 1**) indicates the commencement of the exponential phase at the 6th hr of incubation with a continuity till the 8th hr. Afterwards, the growth of probiotic bacteria showed a linear increase from the 10th hour to the 14th hour but from the 18th hour, a decrease was observed. More precisely, the absorbance doubled from 0.259 at the 6th hour to 0.579 at the 8th hour. In accordance with the McFarland scale, at 12-h interval absorbance (0.648) was observed to be indicating the cell density of around 9.00 log CFU/ml. Therefore, 12 h fermentation time has been considered as the optimum fermentation time and was continued for further studies. A similar trend was reported by Nguyen et al., 2019 for the fermentation of pineapple juice using *Lactobacillus acidophilus* La5 strain, with the addition of raftiline, an oligosaccharide as prebiotic. A cell count of 3.35×10^9 CFU/ml was recorded at the starting of the fermentation, but after 8 h, the cell count had increased to 5.46×10^9 CFU/ml, and after 24 h of fermentation, the count had reportedly dropped to 3.99×10^9 CFU/ml. This cell count inclination is attributable to the nutrients available in the matrix of the fermented juice. At the initial stages of fermentation, they are available for the growth and metabolism of the inoculated probiotics but in successive stages, they get diminished with a consequential decrease in the viability count of the fermenting probiotics.

Optimization of Inoculum size

Pumpkin juice was inoculated with the starter culture suspension of *Lactobacillus fermentum* NCDC 141 (5, 10, and 15%) and monitored for growth to the required population of viable cells to meet the specification of a probiotic food (106–107 CFU/ml per serving) [19]. In the present study, 15-h fermentation (**Fig. 2**) was considered in order to obtain the desired properties of probiotic product. Desirable properties as per the probiotic product such as viable count and pH (4.0– 4.5) were obtained with 10% inoculum size with 15 h fermentation time (**Fig. 2**), whereas in 5 and 15% inoculum sizes the viable count and pH were not as per the desired ratio required for probiotic product [20]. Therefore, 10% inoculum size with 15 h fermentation was used for further studies.

Inoculum size and fermentation time are conditions fully known to remarkably affect the viability of probiotics and their eventual population in substrates. Furthermore, especially in food processing, inoculum sizes have been found to related to the sensory attributes of final products produced which would influence their consumer's perception and ultimate marketability. The target output for probiotic food processing is for an optimal population of probiotics in the final product. Wardani et al., [21] investigated the effect of varying inoculum sizes (1, 3, 5, and 10%) on the fermentation efficiency of *Lactobacillus plantarum* Dad 13 for the production of organic acids in milk. The study showed that with increasing inoculum size, the time taken for acid production decreased remarkably. Contrastingly, studies have shown that in excess, probiotics could impart undesirable characteristics to food products. Chen et al., [22] studied the individual effects of the inoculum size of *L. casei* and *L. acidophilus* on the sensory characteristics, viable count, acidity, and pH of fermented goat milk. Inoculum sizes of 1,3,5,7, and 9 % respectively were used for both Lactobacillus species individually and samples were withdrawn for analyses at 1.5, 3.0, 4.5 and 6.0 h respectively. For both bacteria, 7 % inoculum size was found to be superlative with regards to the

desirable attributes examined. The sensory evaluation involving taste, smell, color, and comprehensive evaluation showed that the 7 % inoculum size was most preferred. At the optimal inoculum size and fermentation time of 4.5 h, a total viable count of 2.20×10^9 cfu/mL and 1.69×10^9 cfu/mL were reported to for *L. casei and L. acidophilus*, respectively. In the same order, total acidity and pH for both probiotics were reported as 4.48, 91°T and 4.38, 96°T respectively.

3.3 Effect of fermentation on physicochemical analysis

The microbial fermentation was performed on the optimized conditions (Inoculum size (10%) and fermentation time (15h) for best results. The effect of fermentation on properties of pumpkin juice was shown in Table 1. Initial pH and temperature of fermentation medium has is closely related to the microbial growth and metabolism. The significant decrease in pH (6.90-4.01) was observed after fermentation. The possible reason for decreased pH might be due to conversion of sugars to organic acids such as lactic and acetic acid during fermentation (Dimitrovski et al., 2021). Similar results were reported by Sharma et al., [9] and yang et al., [24].

Viscosity was found to increase from 1.69 ± 0.04 to 1.82 ± 0.03 mPa/s after fermentation of pumpkin juice. These results are in consistent with Zhao et al., 2015 in which pumpkin juice was fermented by the basidiomycetous fungus *Ganoderma lucidum* and viscosity changes from 1.72 ± 0.09 to 1.95 ± 0.06 mPa/s after 4 day of fermentation. Fermentation has been reported to increase the viscosity of pumpkin juice due to protein denaturation by synthesis of organic acids [25]. Though other dissolved solids and additives can spur increases in viscosity, decrease in pH due to fermentation acidity would affect protein configuration and cause changes in the viscosity profile. On another note, pumpkin polysaccharides have been studied to be responsible for its viscosity whether in terms of steady-state flow/ apparent viscosity or the intrinsic viscosity which corresponds to the hydrodynamic volume and which determines the extensional viscosity and polymer drag reduction [26]. Pumpkin fermentation has been reported to possibly initiate the syntheses of exopolysaccharides by the fermenting probiotics.

Total soluble solids content of pumpkin juice was found to decrease from 6.8 ± 0.15 to 5.4 ± 0.09 °Brix. This variation in TSS can be related with the expected alteration in sugar content due to metabolism by fermenting probiotics in contrast to the addition of more sugary ingredients which would cause an increase in the TSS value of the juice. Though TSS is commonly used for quality evaluation, the decrease in sugar content as expressed by the TSS study indicates the suitability of fermented pumpkin juice over the unfermented preparation for patients having hyperglycaemia or related metabolic syndrome diseases [27].

The total phenolic content of the pumpkin juice before and after fermentation was expressed as mg gallic acid equivalent per 100 ml sample. Phenolic content of pumpkin juice was found to increase from 47.86 ± 0.90 to 53.74 ± 0.88 mg GAE/100 ml during 15 hr of fermentation. . Fermented pumpkin juice was found to possess higher total flavonoid content than unfermented sample. Total flavonoid value changes from 7.81 ± 0.05 to 10.14 ± 0.55 mg QE/100 ml. This is in agreement with the studied reported by Mauro et al., 2016 in which blueberry and carrot juice blend was fermented with *Lactobacillus reuteri* LR92 and , increase in phenolic content was observed from 112.27 to 120.98 mg GAE/100 ml during 28 days of fermentation. Koh et al., [28] also reported increase in total phenolic content and flavonoid content in fermented pumpkin juice with *Lactobacillus mali* K8. The higher phenolic content might be due to release of bound phenolics due to ferementation. Naturally, phenolic compounds are bounded with sugar which reduces their availability to organism. During fermentation, enzymes from the fermenting organism hydrolyse complexes of phenolics into soluble-free phenols and other simpler and biologically more active ones that are readily absorbed [29]. Such hydrolyses are usually initiated by hydrolytic enzymes secreted by the fermenting probiotics, and their adaptability and syntheses potentials strongly enhance the degradation and depolymerization of complex molecules into simpler phytochemicals. The relatively higher phenolic and flavonoid concentrations in the fermented preparations could also be due to the deglycosylation of glycosylated phenolics in the fresh juice which causes a release of insoluble bounded or soluble conjugated phenolics and simpler flavonols from the cellular matrices of the pumpkin during the fermentation process [30].

DPPH assay has been widely used to determine the free radical scavenging activity of various plant extract. The higher change in colour towards yellow depicts more antioxidant potent of plant extract. DPPH radical scavenging activity of fermented and non-fermented pumpkin juice was found to be 78±3.2 and 52±1.2. the possible reason was might be due to increase concentration of total phenolics after fermentation in pumkin juice. Similar trend was observed by Vivek et al., [16] and Mousavi et al., [31] in Sohiong juice (fermented by *Lactobacillus plantarum* MTCC 2974) and pomegranate juice (fermentation by *Lactobacillus plantarum* and *Lactobacillus acidophilus*) respectively. During fermentation the sugar moieties attached to these phenolics and anthocyanins gets depleted by bacteria and results in increased production of aglycones which are having higher radical scavenging effect. Probiotic bacteria can chelate metal ions and scavenge reactive oxygen species [32]. It has also been reported that probiotic microbiota metabolizing conjugated polyphenols are easily absorbed in the gut and also their bioavailability was increased [33].

3.4 Optimization of spray drying conditions

3.4.1 Model fitting

The design matrix for moisture content, hygroscopicity, DPPH scavenging activity and viable cell count was shown in **Table 1**. The 13 experimental runs were obtained for optimization of pumpkin probiotic drink containing two independent variables. The second order polynomial equation was used to check the significance and adequacy of model. The model F-value for moisture content, DPPH scavenging activity, viable cell count and hygroscopicity were 5.18, 96.50, 23.53 and 38.53 respectively indicating that the model was significant. The p-value of <0.05 for model, linear, interaction and quadratic parameters indicates the significance of model terms. All the response variables showed non-significant lack of fit which implies that polynomial model adequately fits all design points. Higher R^2, adjusted R^2 and predicted R^2 proposed that the quadratic model is statistically significant as well as reliable and precise. The pareto chart were developed to show the effect of each factor on response variables (**Fig. 3**). Inlet air temperature was seen as effective linear variables on moisture content and viable cell count whereas maltodextrin concentration was seen effective against DPPH scavenging activity and hygroscopicity. Interaction factor was also found to quite effective on all response variables whereas quadratic factors were found to be least effective.

3.4.2 Effect of independent variables on response variables

3.4.2.1 Moisture content

Moisture content refers to the total amount of water (free and bound) in food however, water activity refers to the amount of free water available for biochemical reactions, hampering the storage life [34]. Moisture present in spray dried sample is the major factor that affects powder stability. Stickiness and caking are the major drawbacks caused by the presence of water [35]. **Fig. 4 a** showed that independent variable has significant effect on moisture content of spray dried pumpkin juice which decreases with increase the process the variables. Inlet air temperature has more pronounced effect followed by maltodextrin concentration. The highest moisture content (3.02%) was obtained in run number 9 at 135.5°C inlet air temperature and 15% maltodextrin content (Table 2). This negative effect of maltodextrin concentration can be related with the moisture absorbing capacity of maltodextrin. Also, with the addition of carrier agent to the feed before spray drying, the total amount of solid content get increases which leads to decrease in moisture content of the resulting powder (Lee et al., 2017). Similar trend was seen by Ferrari et al., [35] and Gong et al, [36] in which they observed the effect of maltodextrin concentration on drying efficiency. They reported that 17% maltodextrin improves the drying efficiency nevertheless, lower maltodextrin concentration (7%) resulted in a powder with a higher moisture content. The increase in inlet air temperature also decreases the moisture content (Fig. 3a). This might be attributed to the formation of scorching air during drying, which retained the water vapour contained in the supplied probiotic drink and further lowered the moisture content. Additionally, moisture content of powder was also affected by the temperature gradient between the atomized feed and the drying air. A higher temperature gradient increases the heat transfer into the particles, consequently, production of powder with less moisture content.

3.4.2.2 Hygroscopicity

Hygroscopicity is defined as the ability of a substance to absorb moisture when kept under the relative high humid surroundings. It is one of the major parameters to determine the shelf-life studies of food products especially powders as absorption of moisture from surroundings can increase the water activity hence, growth of microorganisms [37]. Product composition and amount of drying aids are the major contributors to the hygroscopicity of food products. A powder with low hygroscopicity, low moisture content, less degree of caking and high solubility is considered a good powder [38].

Hygroscopicity values of spray dried pumpkin powder ranged from 18.84 to 24.43 g/100 g dry matter and effect of interaction of independent variables shown in **Fig. 4b**. The significant effect of both the variables was seen on hygroscopicity. Maltodextrin concentration has a significant negative linear effect depicting decrease in hygroscopicity with increase in maltodextrin concentration. The reason for alleviation in hygroscopicity with increased concentration of maltodextrin is due to low hygroscopicity of maltodextrin which proves its efficiency as a carrier agent during spray drying. However, with increased concentration of maltodextrin quality of powder get compromised owing to dilution of nutrients present in sample [39]. Whereas hygroscopicity increased with increase in inlet temperature. The higher hygroscopicity of dried probiotic drink is might be due to higher moisture gradient between powder particles and atmosphere. Another reason is increased linkages between the hydrogen of water molecules and free aldehyde or ketone group of reducing sugars in amorphous regions [40]. Similar trend was observed by Bringas-Lantigua et al. [41] during the spray drying of honey.

3.4.2.3 DPPH scavenging activity

DPPH scavenging activity is related to the antioxidant potential of food products. Antioxidants are required to scavenge free radicals generated in the body as these free radicals can cause several fatal diseases such as heart disease, stroke, atherosclerosis, diabetes and cancer. **Table 3** showed that interaction of both the independent variables has significant effect on DPPH scavenging activity of spray dried probiotic drink. With the increase in maltodextrin concentration the DPPH scavenging activity increased whereas opposite effect was seen with increase in inlet air temperature (**Fig. 4c**). The possible reason for the low free radical scavenging activity may be the breakdown of the structure of phenolics and their degradation because of the exposure to higher temperatures. Similar results were observed in spray dried amla juice [42] and gac juice powder [43].

3.4.2.4 Viable cell count

For spray dried probiotic pumpkin powder, the viability of probiotic microorganism ranged between 6.328 to 8.342 log CFU/ml. **Fig. 4d** showed that maltodextrin concentration was observed to show significant positive effect on viability of probiotic microorganism depicting increase in viable cells with increase in maltodextrin concentration. Increase in cell viability with increase in maltodextrin concentration can be related with the formation of protective layer around bacterial cells by carrier agent. These results are in accordance with Arepally and Goswami [44] where gum arabic was used as carrier agent and it increases the encapsulation efficiency. On the other hand, inlet air temperature showed linear negative effect on viable cell count. Behboudi-Jobbehdar et al. [45] reported that the higher heat and mechanical stresses during spray drying at higher inlet temperature decrease encapsulation efficiency and probiotic viability. This decrease in viability due to multiple reasons such as denaturation of cellular material (DNA and RNA), rupture and collapse of cell membrane due to water removal. These results are similar to the study reported by Anekella and Orsat [46].

3.4.3 Validation and verification of predicted model

A multi-criteria decision approach i.e. numerical optimization by desirability function was used to optimize the independent variables for spray dried probiotic pumpkin drink. The independent variables were kept within range and optimized conditions for dried drink were 20% maltodextrin concentration and 140°C inlet air temperature. The experimental values for response variables at these optimized conditions were 2.21% (moisture content), 8.18 logCfu/ml (viable cell count), 64.36% (DPPH scavenging activity) and 17.52 g/100g (Hygroscopicity). As expected, under the optimal conditions, the experimental value and predicted value showed no significant difference as confirmed by paired t-test which showed the validity of model and RSM approach is reliable and can be considered acceptable for predicting future results.

Characteristics of spray dried probiotic pumpkin juice at optimized conditions

3.5.1 Yield

Product yield is an imperative parameter during the drying process because it is related to the economic return. The main factors for the low yield are the deposition of the powder on the walls of the dryer and the low efficiency of the cyclone to collect fine particles [17]. The yield of spray dried pumpkin powder at optimized was observed 53.74±1.36% (**Table 5**). The higher yield of dried powder may be due to higher maltodextrin concentration which increases the solid content of juice. Maltodextrin also aids in increasing the glass transition temperature of food ingredients, thereby reducing the powder's stickiness. This is also crucial for enhancing powder yield. Similar results were reported by Wilkowska et al., [47] in microencapsulation of blueberry juice using HP-β-CD and Maltodextrin as carrier agent and got 43.9% and 43.7% product yield respectively.

3.5.2 Encapsulation efficiency

The encapsulation efficiency of *Lactobacillus fermentum* in spray dried pumpkin powder was carried out by microbial plate count method. Under optimized conditions of spray drying, the encapsulation efficiency of probiotic pumpkin powder was 89.00±0.91% (**Table 5**). The higher encapsulation was due to thermos-protective effect on *Lactobacillus fermentum* during spray drying process. The high encapsulation efficiency and viability of bacteria during spray drying might be due to hydrogen bonding interaction of membrane protein of *Lactobacillus fermentum* with hydroxyl group of maltodextrin, preventing protein denaturation and maintaining native integrity [48]. The results are in consistent with probiotic sohiong powder (85.75% (encapsulation efficiency)) [16] and encapsulation of probiotic *Enterococcus* strains where encapsulation efficiency varied from 79-93% [49].

3.5.3 Water solubility index

Water solubility index (WSI) is an important factor for determining the reconstitution quality of the powder. The quick and complete reconstitution of spray dried powder is one of the main quality indicator for customers. The water solubility index of probiotic

pumpkin powder, spray dried at optimized conditions, was 87.59±0.89% (**Table 5**). Higher WSI might be due to higher maltodextrin concentration which decreases powder moisture content and increase in powder solubility. Similar results were observed by Lee et al., [50] and Vivek et al. [16] during the spray drying of mandarin juice and probiotic sohiong juice with WSI values were 90.88-93.42% (maltodextrin concentration 20-25%) and 92.33% (maltodextrin concentration 12%) respectively.

3.5.4 Water absorptive index (WAI)

WAI defines the ability of a sample to rebound with water under limited water conditions. An ideal powder would wet quickly and thoroughly, and sink rather than float [51]. Water absorption index of probiotic pumpkin powder was 1.53±0.06% (**Table 5**). The low value of WAI could be due to the high value of WSI. Additionally, the outer layer of maltodextrin modifies the surface stickiness of dried powder due to transformation into glassy state. This changes stickiness properties of dried reduces the particle cohesiveness resulting in less agglomeration and which leads to lowering in water holding capacity of powder [52].

3.5.5 Bulk and True density

The bulk density in the food production industry is an important variable for the transport, storage and packaging. Bulk density and tapped density of probiotic powder obtained at optimal conditions (Inlet air temperature 140^{o}C, maltodextrin concentration 19.201%) was 0.42±0.02 g/cm^3 and 0.587±0.05 g/cm^3 (**Table 5**). Maltodextrin concentration and inlet air temperature significantly affect the bulk density of spray dried powder. Higher inlet air temperature rapidly forms the dried layer over the droplet surface which causes skinning and casehardening of particles whereas maltodextrin minimizing the stickiness of thermoplastic materials thereby reduces bulk density [53-54]. The Hausner's ratio was 1.39±0.03 and Carr index was between 28.44±0.02%. According to the classification of powder cohesiveness and flowability determined from Hausner's ratio and Carr index, dried pumpkin powder had medium cohesiveness and average flowability. These results are in coherence with the research reported by Saikia et al., [15]. In their study, they reported the bulk and tapped density in the range of 0.259-0.363 g/ml and 0.394-0.569 g/ml for Mandarin, watermelon, pineapple and Carambola dried powder. Also, they also observed the low cohesiveness in pineapple and Mandarin juice powder and bad flowability in watermelon and carambola powders.

3.6 Morphology of spray dried probiotic pumpkin powder

Fig. 5 showed the morphology of spray dried probiotic pumpkin powder at 20% maltodextrin concentration and 140°C inlet air temperature. The two magnification level (2000X and 4000X) were used to determine the microstructure of dried pumpkin powder. Scanning microscope images showed the protective effect of maltodextrin on probiotic pumpkin juice. With a nearly spherical form, the powder particles had a smooth, wrinkled, and folded surface. This is a desired feature of microcapsules since the integrity of the micro particle surface is critical for improved core retention and decreased water vapour and oxygen permeability. Small spherical particles were also observed on the surface without any fracture or cracks. On the surface of spherical globule, the encapsulated cells were appeared as raised mass indicating that adequate encapsulation had occurred. Similar results were reported by Vivek et al., [16] and Bhagwat et al. [49]. Some dents and vacuoles were also appeared on the surface of some particles. This might be due to rapid evaporation of moisture from dried particles due to higher inlet air temperature resulting in vacuole formation due to low gas permeability of the surrounding skin [55].

4. Conclusion

The whole pumpkin is a reservoir of bioactive components and other nutrients. However, lack of processing and larger production end this product in landfill. The effective utilization of this crop can mitigate this problem. Hence, pumpkin based non-dairy probiotic beverage was developed by fermenting it with *Lactobacillus fermentum* NCDC 141. Lyophilized culture of *Lactobacillus fermentum* was revived in MRS medium till tertiary culture to obtain maximum cell density. Inoculum size (10%) with 15 hr fermentation time was found to show desirable characteristics in terms of viable cells (8.933 log CFU/ml) and pH (4.01). During fermentation, increase in phenolics, flavonoids and antioxidant activity was observed. Microencapsulation of probiotic microorganism was done by spray drying. Response surface methodology was used to optimize the inlet air temperature and concentration of carrier agent. The concentration of the carrier agent and inlet air temperature had a significant effect on all of the responses (Moisture content, hygroscopicity, DPPH scavenging activity and viable cells) evaluated. Optimum condition for all the responses were observed at 140^{o}C and 19.201% maltodextrin concentration. Spray dried powder was observed to show medium cohesiveness and average flowability. On the basis of these results it can be concluded that probiotic dried powder with maximum functional properties can be obtained by spray drying and these powders can be used as functional foods.

Declarations

Conflict of Interest

The authors declare that there is no conflict of interest.

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Authors Contribution

Poorva Sharma: Formal analysis; methodology; writing – original draft; Conceptualization, Piyush Kashyap: writing – original draft, data analysis, Bababode Adesegun Kehinde: Methodology. Sawinder Kaur: review and editing.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Ganesh KS, Sridhar A, Vishali S (2022) Utilization of fruit and vegetable waste to produce value-added products: Conventional utilization and emerging opportunities-A review. Chemosphere 287: 132221.

[2] de Carvalho LMJ, Gomes PB, de Oliveira Godoy RL, Pacheco S, do Monte PHF, de Carvalho JLV, Nutti MR, Nevel ACS, Vieira ACRA, Ramos, SRR (2012) Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. Food Res Int, 47(2): 337-340.

[3] Sharma P, Kaur G, Kehinde BA, Chhikara N, Panghal A, Kaur H (2020) Pharmacological and biomedical uses of extracts of pumpkin and its relatives and applications in the food industry: a review. Int J Veg Sci 26(1): 79-95.

[4] Zema DA, Calabrò PS, Folino A, Tamburino VINCENZO, Zappia G, Zimbone SM (2018) Valorisation of citrus processing waste: A review. Waste manag 80: 252-273.

[5] Valero-Cases E, Cerdá-Bernad D, Pastor JJ, Frutos MJ (2020) Non-dairy fermented beverages as potential carriers to ensure probiotics, prebiotics, and bioactive compounds arrival to the gut and their health benefits. Nutrients, 12(6): 1666.

[6] Sreenivas KM, Lele SS (2013) Prebiotic activity of gourd family vegetable fibres using in vitro fermentation. Food Biosci 1: 26-30.

[7] Ozola L, Kampuse S (2018) Influence of heat treatment methods on bioactive compound concentrations in pumpkin–guelder rose (*Viburnum opulus*) sauces. In *Proceedings of the Latvian Academy of Sciences* 72(2): 97-102.

[8] Tontul I, Topuz A (2017) Spray-drying of fruit and vegetable juices: Effect of drying conditions on the product yield and physical properties. Trends Food Sci Technol 63: 91-102.

[9] Sharma P, Trivedi N, Gat Y (2017) Development of functional fermented whey–oat-based product using probiotic bacteria. 3 Biotech 7(4): 1-8.

[10] Sharma P, Sharma D, Kaur S, Borah A (2021) Optimization of flaxseed milk fermentation for the production of functional peptides and estimation of their bioactivities. Food Sci Technol Int: 1082013220973815.

[11] Ranganna S (2003). Handbook of analysis and quality control for fruits and vegetable products. Tata McGraw-Hill Publishing Company Limited. New Delhi. 11-12.

[12] Amjad L, Shafighi M (2013) Evaluation of antioxidant activity, phenolic and flavonoid content in *Punica granatum* var. Isfahan Malas flowers. Int J Agric Crop Sci 5(10): 1133.

[13] John BIJU, Sulaiman CT, George S, Reddy VRK (2014) Total phenolics and flavonoids in selected medicinal plants from Kerala. Int J Pharm Pharm Sci 6(1): 406-408.

[14] Paim DR, Costa SD, Walter EH, Tonon RV (2016) Microencapsulation of probiotic jussara (*Euterpe edulis* M.) juice by spray drying. LWT-Food Sci Technol 74: 21-25.

[15] Saikia S, Mahnot NK, Mahanta CL (2015) Effect of spray drying of four fruit juices on physicochemical, phytochemical and antioxidant properties. J Food Process Preser 39(6): 1656-1664.

[16] Vivek K, Mishra S, Pradhan RC (2021) Optimization of Spray Drying Conditions for Developing Nondairy Based Probiotic Sohiong Fruit Powder. Int J Fruit Sci 21(1): 193-204.

[17] Santos Monteiro S, Albertina Silva Beserra Y, Miguel Lisboa Oliveira H, Pasquali MADB (2020) Production of probiotic passion fruit (*Passiflora edulis* Sims f. Flavicarpa Deg.) drink using *Lactobacillus reuteri* and microencapsulation via spray drying. Foods 9(3): 335.

[18] Franco TS, Perussello CA, Ellendersen LN, Masson ML (2016) Effects of foam mat drying on physicochemical and microstructural properties of yacon juice powder. LWT-Food Sci Technol 66: 503-513.

[19] Mattila-Sandholm T, Myllärinen P, Crittenden R, Mogensen G, Fondén R, Saarela M (2002) Technological challenges for future probiotic foods. Int Dairy J 12(2-3): 173-182.

[20] Ying D, Schwander S, Weerakkody R, Sanguansri L, Gantenbein-Demarchi C, Augustin MA (2013) Microencapsulated *Lactobacillus rhamnosus* GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice. J Funct Foods 5(1): 98-105.

[21] Wardani SK, Cahyanto MN, Rahayu ES, Utami T (2017) The effect of inoculum size and incubation temperature on cell growth, acid production and curd formation during milk fermentation by *Lactobacillus plantarum* Dad 13. Int Food Res J 24(3): 921-926.

[22] Chen H, Bao C, Wang C (2016) Response surface methodology for optimizing fermentation conditions of goat yogurt with *Bifidobacterium bifidum* and *Lactobacillus casei.* Emir J Food and Agric: 547-553.

[23] Dimitrovski D, Dimitrovska-Vetadjoka M, Hristov H, Doneva-Shapceska D (2021) Developing probiotic pumpkin juice by fermentation with commercial probiotic strain *Lactobacillus casei* 431. J Food Process Preser 45(3):e15245.

[24] Yang F, Wang YP, Zhao H (2019) Quality enhancement of fermented vegetable juice by probiotic through fermented yam juice using Saccharomyces cerevisiae. Food Sci Technol 40: 26-35.

[25] Nazir N, Fiana RM, Yarni, DF (2020) Characteristics of Pumpkin (*Cucurbita moschata*) Fermented Beverage Products With the Addition of a Powder Milk Mixture. IOP Conference Series: Earth and Environmental Science. 515(1): 012054.

[26] Yu G, Zhao J, Wei Y, Huang L, Li F, Zhang Y, Li Q (2021). Physicochemical Properties and Antioxidant Activity of Pumpkin Polysaccharide (*Cucurbita moschata* Duchesne ex Poiret) Modified by Subcritical Water. *Foods 10:*197.

[27] Managa MG, Akinola SA, Remize F, Garcia C, Sivakumar D (2021) Physicochemical Parameters and Bioaccessibility of Lactic Acid Bacteria Fermented Chayote Leaf (*Sechium edule*) and Pineapple (*Ananas comosus*) Smoothies. Front Nutr *8* : 120.

[28] Koh WY, Utra U, Rosma A, Effarizah ME, Rosli W, Park YH (2017) Development of a novel fermented pumpkin-based beverage inoculated with water kefir grains: a response surface methodology approach. Food Sci Biotech 27(2): 525–535.

[29] Adetuyi FO, Ibrahim TA (2014) Effect of fermentation time on the phenolic, flavonoid and vitamin C contents and antioxidant activities of okra (*Abelmoschus esculentus*) seeds. Niger Food J 32(2): 128-137.

[30] Kwaw E, Ma Y, Tchabo W, Apaliya MT, Wu M, Sackey AS, Xiao L, Tahir, HE (2018) Effect of lactobacillus strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice. Food Chem 250: 148-154.

[31] Mousavi ZE, Mousavi SM, Razavi SH, Hadinejad M, Emam-Djomeh Z, Mirzapour M (2013) Effect of fermentation of pomegranate juice by *Lactobacillus plantarum* and *Lactobacillus acidophilus* on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. Food Biotech, 27(1): 1–13.

[32] Curiel JA, Pinto D, Marzani B, Filannino P, Farris GA, Gobbetti M, Rizello CG (2015) Lactic acid fermentation as a tool to enhance the antioxidant properties of *Myrtus communis* berries. Microb Cell Factories 14(1): 67.

[33] Laparra JM, Sanz Y (2010) Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacol Res 61(3): 219-225.

[34] Tuyen CK, Nguyen MH, Roach PD (2010) Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (*Momordica cochinchinensis*) fruit aril powder. J Food Eng 98(3): 385-392.

[35] Ferrari CC, Germer SP., de Aguirre JM (2012) Effects of spray-drying conditions on the physicochemical properties of blackberry powder. Drying Tech 30(2): 154-163.

[36] Gong P, Zhang L, Han X, Shigwedha N, Song W, Yi H, Du M, Cao C (2014). Injury mechanisms of lactic acid bacteria starter cultures during spray drying: a review. Drying Tech 32(7): 793-800.

[37] Juarez-Enriquez E, Olivas GI, Zamudio-Flores PB, Ortega-Rivas E, Perez-Vega S, Sepulveda DR (2017) Effect of water content on the flowability of hygroscopic powders. J Food Eng 205: 12-17.

[38] Bakar J, Ee SC, Muhammad K, Hashim DM, Adzahan N (2013) Spray-drying optimization for red pitaya peel (*Hylocereus polyrhizus*). Food Bioproc Tech 6(5): 1332-1342.

[39] Quek SY, Chok NK, Swedlund P (2007) The physicochemical properties of spray-dried watermelon powders. Chem Eng Process: Process Intensification 46(5): 386-392.

[40] Thakur M, Pant K, Naik RR, Nanda V (2021) Optimization of spray drying operating conditions for production of functional milk powder encapsulating bee pollen. Drying Tech 39(6): 777-790.

[41] Bringas-Lantigua M, Pino JA, Aragüez-Fortes Y (2016) Influence of process conditions on the physicochemical properties of honey powder produced by spray drying. Revista CENIC Ciencias Químicas, 47: 64-69.

[42] Mishra P, Mishra S, Mahanta CL (2014) Effect of maltodextrin concentration and inlet temperature during spray drying on physicochemical and antioxidant properties of amla (*Emblica officinalis*) juice powder. Food Bioproc Tech 92(3): 252-258.

[43] Kha CT, Nguyen HM, Roach DP (2010) Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (*Momordica cochinchinensis*) fruit aril powder. J Food Eng 98: 385–392.

[44] Arepally D, Goswami TK (2019) Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. LWT-Food Sci Technol 99: 583-593.

[45] Behboudi-Jobbehdar S, Soukoulis C, Yonekura L, Fisk I (2013) Optimization of spray-drying process conditions for the production of maximally viable microencapsulated L. acidophilus NCIMB 701748. Drying Tech 31(11): 1274-1283.

[46] Anekella K, Orsat V (2013) Optimization of microencapsulation of probiotics in raspberry juice by spray drying. LWT-Food Sci Technol 50(1): 17–24.

[47] Wilkowska A, Ambroziak W, Czyzowska A, Adamiec J (2016) Effect of microencapsulation by spray-drying and freeze-drying technique on the antioxidant properties of blueberry (*Vaccinium myrtillus*) juice polyphenolic compounds. Polish J Food Nutr Sci 66(1): 11.

[48] Tantratian S, Pradeamchai M (2020) Select a protective agent for encapsulation of *Lactobacillus plantarum.* LWT-Food Sci Technol 123: 109075.

[49] Bhagwat A, Bhushette P, Annapure US (2020) Spray drying studies of probiotic *Enterococcus* strains encapsulated with whey protein and maltodextrin. Beni-Suef Univ J Basic Appl Sci 9(1): 1-8.

[50] Lee KC, Yoon YS, Li FZ, Eun JB (2017) Effects of inlet air temperature and concentration of carrier agents on physicochemical properties, sensory evaluation of spray-dried mandarin (*Citrus unshiu*) beverage powder. Appl Biol Chem 60(1): 33-40.

[51] Nishad J, Selvan CJ, Mir SA, Bosco SJD (2017) Effect of spray drying on physical properties of sugarcane juice powder (*Saccharum officinarum* L.). J Food Sci Technol 54(3): 687-697.

[52] Srinivas D, Vinoda N, Edukondalu L (2018) Effect of Maltodextrin Concentration on Spray Dried Bitter Gourd Powder. Int J Curr Microbiol App Sci 7(5): 3147-3154.

[53] Mishra P, Brahma A, Seth D (2017) Physicochemical, functionality and storage stability of hog plum (*Spondia pinnata*) juice powder produced by spray drying. J Food Sci Technol 54(5): 1052-1061.

[54] Pui LP, Karim R, Yusof YA, Wong CW, Ghazali HM (2021) Effects of inlet temperature and carrier concentration on spray-dried 'cempedak'(*Artocarpus integer*) fruit powder and its reconstitution properties. Acta Sci Pol Technol Aliment 20(2): 135-148.

[55] Nijdam JJ, Langrish TAG (2005) An investigation of milk powders produced by a laboratory-scale spray dryer. Drying Tech 23(5): 1043-1056.

Tables

Table 1: Effect of fermentation on properties of juice

Properties	Non-fermented juice	Fermented juice
pH	6.9 ± 0.09^{a}	4.1 ± 0.07^{b}
Viscosity (mPa/s)	1.69 ± 0.04^{b}	1.82 ± 0.03^{a}
TSS (°Brix)	6.8 ± 0.15^{a}	5.4 ± 0.09^{b}
TPC (mg GAE/100 ml)	47.86 ± 0.90^{b}	53.74 ± 0.88^{a}
TFC (mg QE/100 ml)	7.81 ± 0.05^{b}	10.14 ± 0.55^{a}
DPPH (%)	52 ± 1.5^{b}	78 ± 3.2^{a}

Table 2: Summary of design of experiment with responses results against factors

RUN	Spray drying conditions			Responses		
	Maltodextrin conc. (%)	Inlet air temp (°C)	Moisture content (%)	DPPH scavenging activity (%)	Viable cell (log CFU/ml)	Hygroscopicity (g/100gm)
1	15	150	2.52	62.49	7.432	23.64
2	15	150	2.51	62.32	7.137	23.75
3	20	140	2.17	65.94	8.342	19.24
4	20	160	0.89	59.94	7.217	23.42
5	22.07	150	1.37	63.43	7.827	18.84
6	15	150	2.38	61.56	7.642	22.58
7	15	164.14	0.97	56.49	6.837	24.43
8	10	160	1.85	58.72	7.148	23.72
9	15	135.85	3.02	70.42	7.947	19.79
10	15	150	2.48	63.23	7.516	22.94
11	15	150	2.43	61.54	7.389	21.95
12	7.92	150	2.62	61.98	6.328	25.52
13	10	140	2.93	68.48	6.784	24.26

Table 3: Analysis of variance (ANOVA) for quadratic model and lack of fit for Moisture content, DPPH scavenging activity, Hygroscopicity and viable cells as per CCD

Source	Moisture content			DPPH scavenging activity			Viable cell			Hygroscopicity		
	F-value	p-value	Sum of square	F-value	p-value	Sum of square	F-value	p-value	Sum of square	F-value	p-value	Sum of square
Model	5.18	<0.0001	5.18	96.50	<0.0001	176.14	23.53	0.0003	3.19	38.53	<0.0001	49.95
A	1.56	<0.0001	1.56	1.05	0.3389	0.3845	64.75	<0.0001	1.75	98.27	<0.0001	25.22
B	2.81	<0.0001	2.81	455.63	<0.0001	166.33	25.07	0.0016	0.6791	54.55	0.0002	14.00
AB	0.0196	0.0307	0.0196	15.39	0.0057	5.62	20.46	0.0027	0.5554	30.22	0.0009	7.76
A^2	0.3649	<0.0001	0.3649	2.01	0.1991	0.7343	7.27	0.0308	0.1970	1.23	0.3044	0.31
B^2	0.5317	<0.0001	0.5317	9.31	0.0185	3.40	0.0324	0.8623	0.0009	9.10	0.0195	2.34
Lack of fit	0.0051	0.7028	0.0051	2.94	0.1620	1.76	5.00	0.0770	0.1497	1.05	0.4634	0.7899
R-squared		0.9964			0.9857			0.9438			0.9649	
Adjusted R^2		0.9938			0.9755			0.9037			0.9399	
Predicted R^2		0.9889			0.9231			0.6663			0.8597	
C.V (%)		2.41			0.96			2.23			2.23	

Table 4: Predicted and experimental value at optimum spray drying conditions

Factor		Optimum level	
Maltodextrin concentration (%)		20	
Inlet air temperature (°C)		140	
Response	**Predicted values**	**Experimental values**	**Variation (%)**
Moisture content (%)	2.18	2.21	1.49
Viable cell count (log CFU/ml)	8.43	8.18	3.06
DPPH (%)	66.65	64.36	3.35
Hygroscopicity (g/100gm)	17.89	17.52	2.08

DPPH: 2,2-diphenylpicrylhydrazyl

Table 5: Characteristics of spray dried pumpkin powder at optimized conditions (maltodextrin concentration (20%) and inlet air temperature (140°C)).

S. No.	Characteristics	Values
1.	Yield (%)	53.74±1.36
2.	Encapsulation efficiency (%)	89.00±0.91
3.	Water solubility index (%)	87.59±0.89
4.	Water absorption index (%)	1.53±0.06
5.	Bulk density (g/cm^3)	0.42±0.02
6.	Tapped density (g/cm^3)	0.58±0.05
7.	Hausner's ratio	1.39±0.03
8.	Carr index	28.44±0.02

Figures

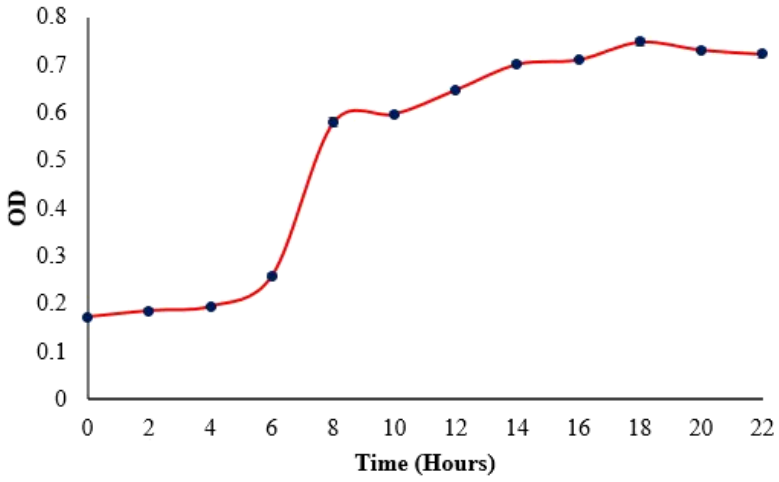

Figure 1

Growth profile of *Lactobacillus fermentum* NCDC 141 in MRS cysteine medium.

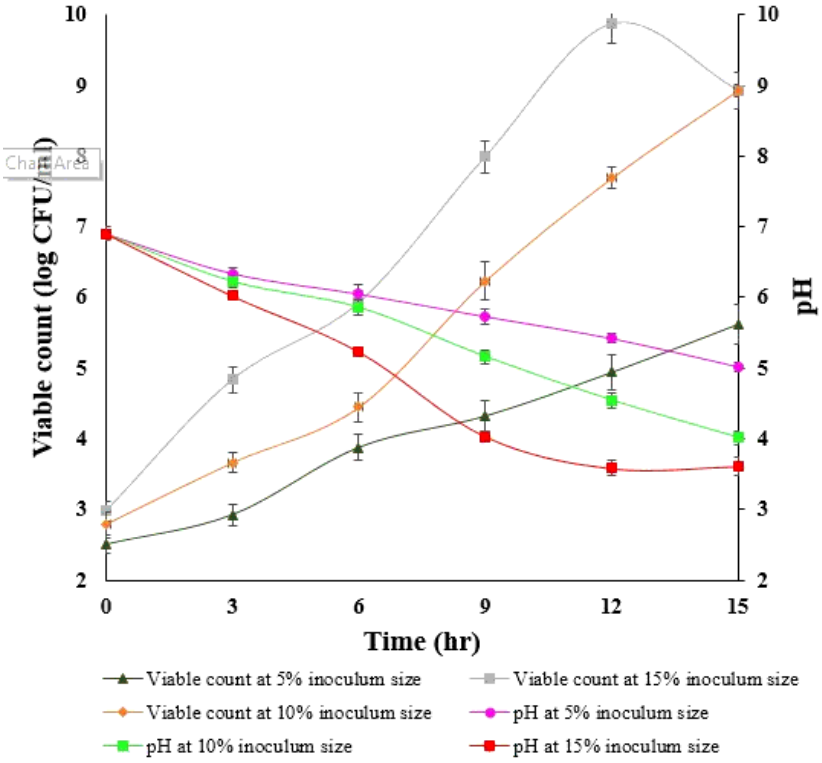

Figure 2

Viable cell count and pH at different inoculum size

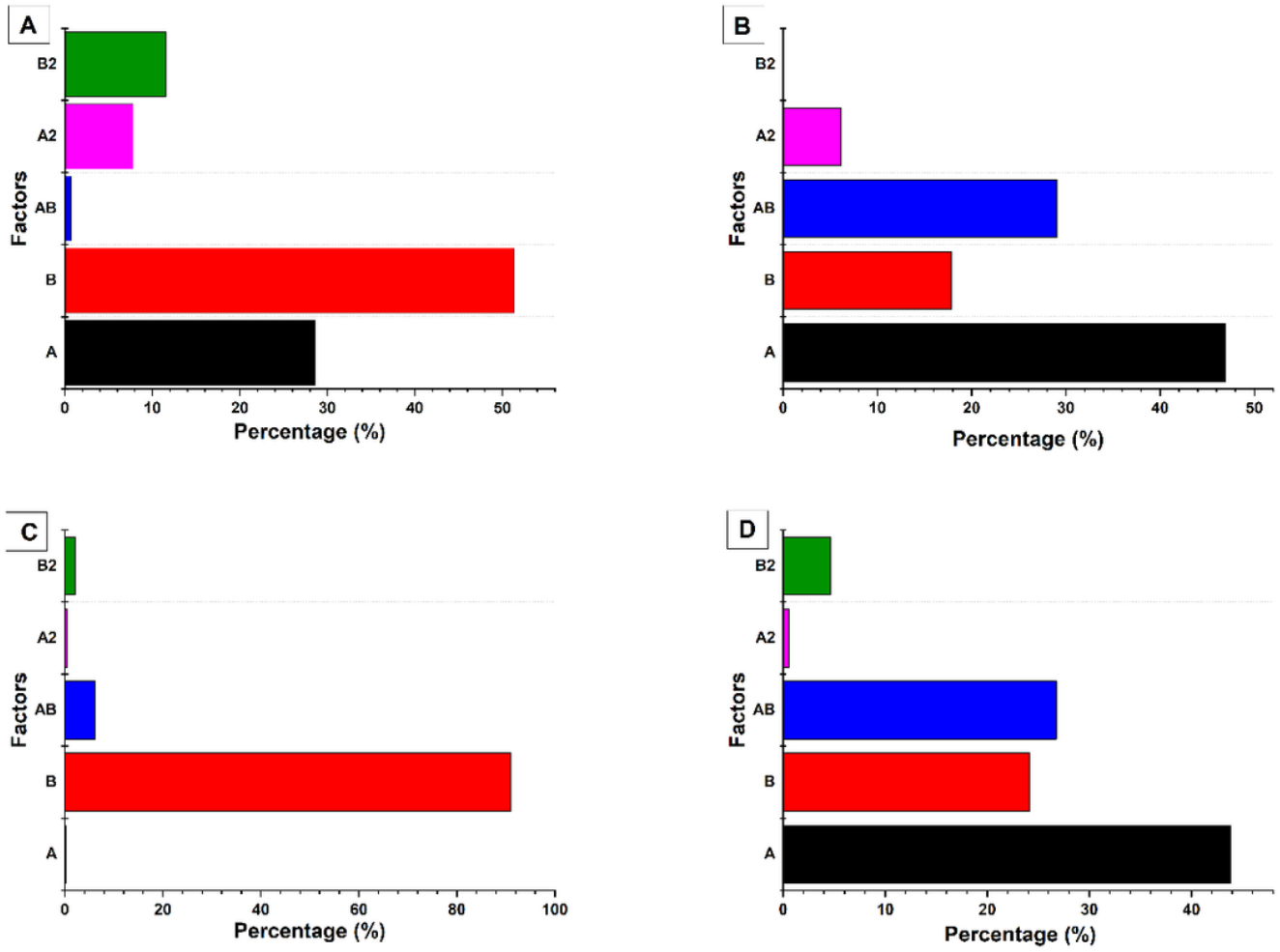

Figure 3

Pareto chart of response variables (A) Moisture content (B) DPPH scavenging activity (C) Viable cell count (D) Hygroscopicity

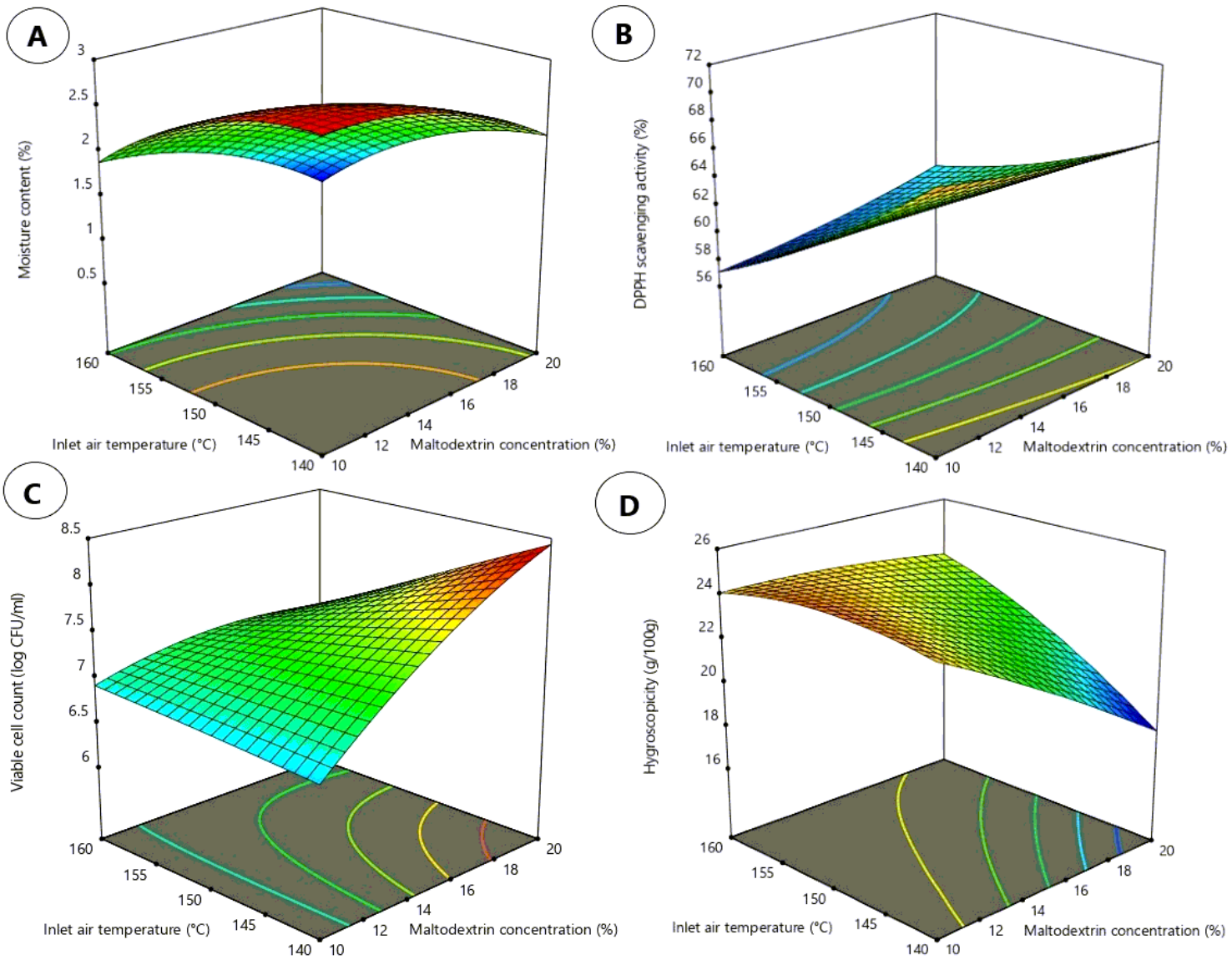

Figure 4

Response surface plots of response variables ((A) Moisture content (B) DPPH scavenging activity (C) Viable cell count (D) Hygroscopicity) of spray dried probiotic pumkin juice powder

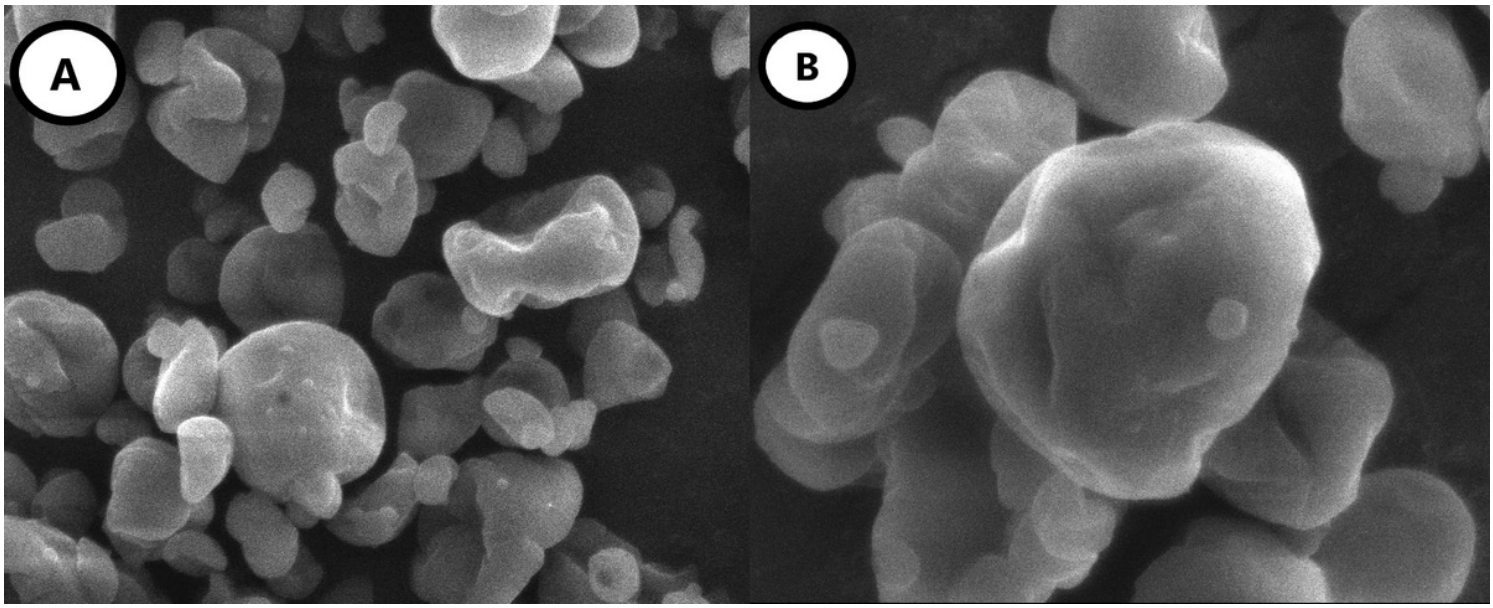

Figure 5

Morphology of spray dried probiotic pumkin juice powder at two magnification (A) 2000X (B) 4000X