ENCAPSULATION OF PROBIOTICS INTO MILK-BASED FUNCTIONAL FOODS

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by

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CERTIFICATE

This is to certify that the thesis entitled, **"Encapsulation of Probiotics into Milk-Based Functional Foods"** being submitted by **Mr. Asutosh Mohapatra** to the **Indian Institute of Technology Delhi** for the award of **'Doctor of Philosophy'** is a record of bonafide research work carried out by him. He has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis. To the best of my knowledge, the results contained in the thesis have not been submitted, in part or full, to any other university or institute for the award of any degree or diploma.

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ABSTRACT

The ongoing demand of probiocation for developing functional foods to confer an improved health with better nutritional profile has gained significant attention in present scenarios. The recommended dosage of probiotics to confer the desirable health benefits requires a minimum consumption of 10^6-10^7 CFU/g. However, the effectiveness of any probiotics during oral consumption needs to be efficiently maintained because of their gastrointestinal transit, overpassing all the associated potential stresses. Therefore, technological intervention and advancement in probiotic encapsulation are critically required to provide adequate protection with sustained gastrointestinal release. Considering the importance of probiotic functionality, and the current need to include probiotic-enriched foods in daily intake, the research thesis is an attempt to develop an efficient encapsulation technique with a suitable wall matrix for the production of a stable encapsulated probiotic powder with high bacterial viability and sustained release. Further, the research was aimed at formulating probiotic-enriched functional curd with enhanced bioavailability.

An attempt was made to optimize the wall matrix concentration for effective spray-encapsulation of *Lactobacillus acidophilus* (ATCC 11975) at an air inlet temperature of 150°C and feed flow rate of 2 mL/min. The concentration of wall materials viz., maltodextrin (MD) (10-30% w/w), skim milk powder (SMP) (10-30% w/w), and cellulose acetate phthalate (CAP) (2-5% w/w) was optimized with response to survival rate, log reduction, encapsulation efficiency, residual moisture, and powder yield. The response variables were predicted using Artificial Neural Network (ANN) followed by optimizing the wall materials using Genetic Algorithm (GA). The optimized values of the wall matrix using ANN+GA model was SMP: 10.0% (w/w), MD: 11.2% (w/w), and CAP: 2.0% (w/w).

The encapsulated *L. acidophilus* powder showed a cell viability more than 7 log CFU/g during simulated digestion. The behavioral release of *L. acidophilus* from the encapsulated matrix showed the occurrence of ‘Super Case-II’ and ‘Anomalous type’ transport mechanisms. The zeroth order equation was the best-suited model for bacterial release in

SGF, SIF, and pH 4. However, for pH 2 and 3, the best-fitted was Korsmeyer-Peppas model. The encapsulated *L. acidophilus* powder showed improved physicochemical characteristics with enhanced bioavailability and stress adaptation during gastrointestinal transit. The fingerprint region of *L. acidophilus* in the powder was in the range of 900-1400 cm^{-1}. Morphological characterization ensured an improved physical barrier with spherical shape microparticles. The zeta potential and thermogravimetric analysis of the powder confirmed a prolonged storage stability with a bimodal particle size distribution.

Probiotic-enriched functional curd was formulated by incorporating encapsulated *L. acidophilus* powder at 5%, 7.5%, and 10% w/w, followed by studying their physicochemical, textural, biochemical, rheological, and sensory attributes. The textural properties of curd revealed a positive correlation between firmness and stickiness with the powder concentration. *In-vitro* characterization confirmed a presence of at least 8 log CFU/g of lactic acid bacteria in the curd formulations. Rheological characterization demonstrated a non-Newtonian shear thinning behavior; which shifted towards a shear-thickening type by increasing powder concentration. All the curd formulations behaved as soft gels with strong gelling strength. The strongest sensory attributes of probiotic curd were color and texture.

The storage study of *L. acidophilus* powder demonstrated an increased cell viability (> 6 log CFU g^{-1}) up to 180 days. Refrigerated storage at 4°C was proven as the efficient storage with lower values of specific cell death constant. The GAB and Caurie equations were proven best to predict the sorption data with an exhibition of Type-III moisture isotherm.

The thesis demonstrates the feasibility of developing spray-encapsulated probiotic powder and successful formulation of functional probiotic curd in daily dietary intake to strengthen the gut microbiota.

Keywords: *Spray-encapsulation; Lactobacillus acidophilus; Controlled release; Gastrointestinal release kinetics; Mechanistic model system; Probiotic curd; Sensory attributes; Storage viability*

सारांश

बेहतर पोषण प्रोफ़ाइल के साथ बेहतर स्वास्थ्य प्रदान करने के लिए कार्यात्मक खाद्य पदार्थों को विकसित करने के लिए प्रोबायोकेशन की चल रही मांग ने वर्तमान परिदृश्यों में महत्वपूर्ण ध्यान आकर्षित किया है। वांछनीय स्वास्थ्य लाभ प्रदान करने के लिए प्रोबायोटिक्स की अनुशंसित खुराक के लिए प्रति सर्विंग 10^6-10^7 सीएफयू/जी की न्यूनतम खपत की आवश्यकता होती है। हालाँकि, मौखिक सेवन के दौरान किसी भी प्रोबायोटिक्स की प्रभावशीलता को उनके गैस्ट्रोइंटेस्टाइनल पारगमन के कारण सभी संबंधित संभावित तनावों को पार करते हुए कुशलतापूर्वक बनाए रखने की आवश्यकता होती है। इसलिए, निरंतर गैस्ट्रोइंटेस्टाइनल रिलीज के साथ पर्याप्त सुरक्षा प्रदान करने के लिए प्रोबायोटिक एनकैप्सुलेशन में तकनीकी हस्तक्षेप और प्रगति की अत्यधिक आवश्यकता है। प्रोबायोटिक कार्यक्षमता के महत्व और दैनिक सेवन में प्रोबायोटिक-समृद्ध खाद्य पदार्थों को शामिल करने की वर्तमान आवश्यकता को ध्यान में रखते हुए, शोध थीसिस उच्च बैक्टीरिया के साथ एक स्थिर इनकैप्सुलेटेड प्रोबायोटिक पाउडर के उत्पादन के लिए एक उपयुक्त दीवार मैट्रिक्स के साथ एक कुशल एनकैप्सुलेशन तकनीक विकसित करने का एक प्रयास है। व्यवहार्यता और निरंतर रिहाई। इसके अलावा, अनुसंधान का उद्देश्य बढ़ी हुई जैवउपलब्धता के साथ प्रोबायोटिक-समृद्ध कार्यात्मक दही तैयार करना था।

150°C के एयर इनलेट तापमान और 2 एमएल/मिनट की फ़ीड प्रवाह दर पर लैक्टोबैसिलस एसिडोफिलस (एटीसीसी 11975) के प्रभावी स्प्रे-एनकैप्सुलेशन के लिए दीवार मैट्रिक्स एकाग्रता को अनुकूलित करने का प्रयास किया गया था। दीवार सामग्री की सांद्रता, जैसे माल्टोडेक्सट्रिन (एमडी) (10-30% w/w), स्किम मिल्क पाउडर (एसएमपी) (10-30% w/w), और सेलूलोज़ एसीटेट फ़ेथलेट (CAP) (2-5%) w/w) को जीवित रहने की दर, लॉग में कमी, एनकैप्सुलेशन दक्षता, अवशिष्ट नमी और पाउडर उपज की प्रतिक्रिया के साथ अनुकूलित किया गया था। कृत्रिम तंत्रिका नेटवर्क (एएनएन) का उपयोग करके प्रतिक्रिया चर की भविष्यवाणी की गई थी, जिसके बाद जेनेटिक एल्गोरिदम (जीए) का उपयोग करके दीवार सामग्री को अनुकूलित किया गया था। ANN+GA मॉडल का उपयोग करके वॉल मैट्रिक्स का अनुकूलित मान SMP: 10.0% (w/w), MD: 11.2% (w/w), और CAP: 2.0% (w/w) था।

इनकैप्सुलेटेड एल. एसिडोफिलस पाउडर ने सिम्युलेटेड पाचन के दौरान 7 लॉग सीएफयू/जी से अधिक सेल व्यवहार्यता दिखाई। एनकैप्सुलेटेड मैट्रिक्स से एल एसिडोफिलस की व्यवहारिक रिहाई ने 'सुपर केस- II' और 'एनोमलस प्रकार' परिवहन तंत्र की घटना को दिखाया। एसजीएफ, एसआईएफ और पीएच 4 में बैक्टीरिया रिलीज के लिए शून्य क्रम समीकरण सबसे उपयुक्त मॉडल था। हालांकि, पीएच 2 और 3 के लिए, कोर्समेयर-पेप्पास मॉडल सबसे उपयुक्त था। एनकैप्सुलेटेड एल. एसिडोफिलस पाउडर ने गैस्ट्रोइंटेस्टाइनल पारगमन के

दौरान बढ़ी हुई जैवउपलब्धता और तनाव अनुकूलन के साथ बेहतर भौतिक रासायनिक विशेषताओं को दिखाया। पाउडर में एल. एसिडोफिलस का फिंगरप्रिंट क्षेत्र 900-1400 सेमी-1 की सीमा में था। रूपात्मक लक्षण वर्णन ने गोलाकार आकार के माइक्रोपार्टिकल्स के साथ एक बेहतर भौतिक अवरोध सुनिश्चित किया। पाउडर की जीटा क्षमता और थर्मोग्रैविमेट्रिक विश्लेषण ने द्विमोडल कण आकार वितरण के साथ लंबे समय तक भंडारण स्थिरता की पुष्टि की।

प्रोबायोटिक-समृद्ध कार्यात्मक दही को 5%, 7.5% और 10% w/w पर इनकैप्सुलेटेड एल एसिडोफिलस पाउडर को शामिल करके तैयार किया गया था, इसके बाद उनके भौतिक रासायनिक, बनावट, जैव रासायनिक, रियोलॉजिकल और संवेदी गुणों का अध्ययन किया गया था। दही के बनावट संबंधी गुणों ने पाउडर की सघनता के साथ दृढ़ता और चिपचिपाहट के बीच एक सकारात्मक संबंध का खुलासा किया। इन-विट्रो लक्षण वर्णन ने दही फॉर्मूलेशन में कम से कम 8 लॉग सीएफयू/जी लैक्टिक एसिड बैक्टीरिया की उपस्थिति की पुष्टि की। रियोलॉजिकल लक्षण वर्णन ने गैर-न्यूटोनियन कतरनी पतलेपन के व्यवहार को प्रदर्शित किया; जो पाउडर की सघनता को बढ़ाकर कतरनी-मोटा करने वाले प्रकार की ओर स्थानांतरित हो गया। सभी दही फॉर्मूलेशन मजबूत जेलिंग ताकत के साथ नरम जैल के रूप में व्यवहार करते हैं। प्रोबायोटिक दही के सबसे मजबूत संवेदी गुण रंग और बनावट थे।

एल एसिडोफिलस पाउडर के भंडारण अध्ययन ने 180 दिनों तक बढ़ी हुई सेल व्यवहार्यता (> 6 लॉग सीएफयू जी$^{-1}$) का प्रदर्शन किया। 4°C पर प्रशीतित भंडारण विशिष्ट कोशिका मृत्यु स्थिरांक के कम मूल्यों के साथ कुशल भंडारण के रूप में सिद्ध हुआ है। टाइप-III नमी इज़ोटेर्म की प्रदर्शनी के साथ सोर्शन डेटा की भविष्यवाणी करने के लिए जीएबी और कौरी समीकरण सर्वोत्तम साबित हुए थे।

थीसिस आंत माइक्रोबायोटा को मजबूत करने के लिए स्प्रे-एनकैप्सुलेटेड प्रोबायोटिक पाउडर विकसित करने और दैनिक आहार सेवन में कार्यात्मक प्रोबायोटिक दही के सफल निर्माण की व्यवहार्यता को प्रदर्शित करती है।

Keywords: स्प्रे-एनकैप्सुलेशन; लेक्टोबेसिल्लुस एसिडोफिलस; नियंत्रित रिलीज; गैस्ट्रोइंटेस्टाइनल रिलीज कैनेटीक्स; यंत्रवत मॉडल प्रणाली; प्रोबायोटिक दही; संवेदी गुण; भंडारण व्यवहार्यता

CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

%	Percent
<	Less than
>	Greater than
≥	Greater than or equal to
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of variance
ANN	Artificial Neural Network
ATCC	American Type Culture Collection
a_w	Water activity
BET	Brunauer-Emmett-Teller
CFU	Colony Forming Unit
$CFU.g^{-1}$	Colony Forming Unit per gram
$CFU.mL^{-1}$	Colony Forming Unit per milliliter
°C	Degree Celsius
$°C\ min^{-1}$	Degree Celsius per minute
cm	Centimeter
DSC	Differential Scanning Calorimetry
d.b.	Dry basis
E_a	Activation energy
eg.	example
EMC	Equilibrium moisture content
Eq.	Equation
Ev	Electronvolt
FAO	Food and Agriculture Organization
Fig.	Figure
FTIR	Fourier Transform Infrared Spectroscopy
FSSAI	Food Safety and Standards Authority of India
g	gram
g/L	gram per litre
G′	Storage modulus

G″	Loss modulus
GA	Genetic Algorithm
GAB	Guggenheim-Anderson-de Boer
GRAS	Generally recognized as safe
h	hour
J	Joule
K	Kelvin
kg	Kilogram
$kg.cm^{-2}$	Kilogram per centimeter square
$kg.m^{-3}$	Kilogram per meter cube
$kg\ s^{-1}$	Kilogram per second
kJ	Kilo Joule
$kJ\ mol^{-1}$	Kilo Joule per Mole
$kJ\ mol^{-1}\ K^{-1}$	Kilo Joule per Mole per Kelvin
kPa	Kilo Pascal
k_T	Specific rate of inactivation constant
kW	Kilo watt
kWh	Kilo watt hour
log	logarithmic
µm	micrometer
µL	microlitre
mg	milligram
mL	milliliter
$mL\ min^{-1}$	milliliter per minute
mV	millivolt
Meq	milli equivalent
MPa	mega Pascal
min	minute
mol	Mole
MSI	Moisture sorption isotherm
MS	Mass Spectroscopy
no.	number

nm	nanometer
O_2	Oxygen
°C min^{-1}	Degree Celsius per minute
pA	picoampere
Pa	Pascal
Pa.s	Pascal second
R^2	Coefficient of determination
rad s^{-1}	Radian per second
RMSE	Root mean square error
rpm	rotation per minute
s	Second
SC-CO_2	Super-critical Carbon dioxide
SCF	Sub critical fluid
SD	Standard deviation
SEM	Scanning Electron Microscopy
T_g	Glass transition temperature
TGA	Thermogravimetric analysis
v/v	volume/volume
V	Volt
viz.	videre licet
WHO	World Health Organization
w	weight
w.b.	wet basis
w/w	weight/weight
w/v	weight/volume
τ	shear stress
γ	shear rate
η	viscosity