PROSPECTIVE ACTION PLAN FOR DEVELOPING PRODUCT CONTAINING MICROENCAPSULATED PROBIOTICS

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ABSTRACT

Probiotic micro-organisms explored for delivering associated proclaimed valuable benefits and its market is expanding in diverse sphere. Probiotics (PRs) are presented as pharmaceutical, dairy, non-dairy, and personal care products. To improve performances and marketplace survival of these products, diverse methods and technology devised. Amongst them microencapsulation (MEC) is widely explored to get product with wished and improved performances. Interest evoke for marketing of product containing microencapsulated probiotics (PCEP) to upkeep performance, reproducible, throughout its life cycle. The review features on prospective action plan for evolution of PCEP including method for combating issues. Presented information will be a helping hand for developers to get PCEP, with excellent feature and performance, and improved marketability.

Keywords: Action plan, development, microencapsulation, probiotics, product.

INTRODUCTION

Nowadays, products containing probiotics (PCP) pushed up for curing illness, and improving health and eudemonia¹⁻³. Alteration in lifestyle, heightened health care cost, alert for personal health and desire for longer life, self-medication, antibiotics resistance, sophistication in technology, and strife overturned their use and marketing⁴. Innovations afoot to make them basis for healthy civilization and overcoming disease, improve safety, get novel products, offer new choice to demand and need of consumers, and so on. Their market and use is expanding systematically in multi direction^{1-3, 5}. Getting consumable of PRs creates difficulties for scientific community and imposing challenges on manufacturers. Global and territorial controls influence their marketing and creating disarray. Table 1 provide important points of territorial and global rule for proclaiming health claim and probiotic use⁷⁻⁹. Viability of probiotic cells (VoPC) within PCP decline with environment of making up; and noncompliance to storage needs, during freight and storage. Following their oral administration, hydrolytic-enzymes, acidic environment of stomach and bile salts in gastrointestinal (GI) tract also declines same. Scientific community and manufacturers are devising approaches to prolong VoPC within PCP and get smart package^{1,3,5}. Diverse approaches undertaken to protect VoPC during processing and storage, and their GI transit. Amongst all, MEC of viable-probiotics cells (VPC) receiving highest interests to conserve VoPC within shelf life and during GI-transit phase of PCP^{1,5,10}. In addition, this sustains VoPC within intestine, improve growth rate of PRs to lifted level referring peristalsis rate and potentiates *in vivo* effect of PCP. In fact, development of product involves multistage and expensive steps and is time-consuming and the process of microencapsulation (PEN) probiotic cell is complicate, challenging, and costly affair^{3,5}. In setting of above, this review focuses prospective action plan on development of PCEP. The informative content will be useful for scientific community of academic and industrial field of PCEP.

Microencapsulation of Probiotics

Microencapsulation, a physicochemical or mechanical process, enables entrapment of PRs in a material to make particles with size in millimetres. PEN, a sequential multiple steps, initiates with scattering of VPC in carrier matrix forming material (CMFM) and ends with cascading PRs with CMFM^{3,5,9}. Consensus on MEC, polymeric, is that it shield VPC from harmful environments of GI tract, and meliorate their intestinal persistency and multiplication. MEC may resulting in an innovative system or impart new role to PCEP. This synergizes efficiency and effectiveness of PCEP. Further, PCEP possess meliorated patient compliance, extended shelf life, and modified and extended release profile^{1,3,5,9,10}. Subsequent gastro-resistant enteric coating (GREC) of prepared microcapsules (MCs) conserves VoPC, on oral administration. This can be either applied as monolayer or layer-by-layer of nanolayers. GREC give gastro-protection to encapsulated VPC side by side potentiates *in vivo* effect¹¹. This also amends shelf life and its amenability for high water contented PCEP^{9,12,13}. On contrary, MEC and GREC affect organoleptic properties of product. Delivered aesthetic features depend on aesthetic characters of encapsulating material. In majority cases they deprives aesthetic features of PCEP while improves same, rarely. Adding prebiotics, protectant, or both in formula of PCEP boost its efficiency and effectiveness^{5,9,11,14}. The quality and quantity of VPC, sustenance of VoPC, and wished quality of PCEP complicates and challenges the PEN. These facts adjudicate efficacy and efficiency of PCEP while PEN monitor shape, size, and size range of MCs. Coating method, GREC, and PEN influence efficiency of VoPC sustenance during processing, storage, and GI-transit phase of PCEP. Co-encapsulating VPC with prebiotics and adding protectant in formula amend stability and performances of PCEP^{5,9,15}.

GREC Materials and CMFMs

CMFM used are gellan gum, xanthan gum, vegetable gums, alginate, alginate-chitosan, sugars, starch, modified starch, dextrans, cellulose derivatives, hypromellose, glyceride

derivatives, waxes, fats, carrageenan, caseinates, gelatine, milk proteins, zein and other proteins, and so on. Commonly used GREC materials are chitosan, carboxymethyl cellulose, hypromellose phthalate, cellulose acetate phthalate, poly-L-lysine, palm oil, and so on. Among these, food proteins and polysaccharides are biodegradable polymer whose use will be worthwhile^{9,16-18}. Biodegradable-biopolymers can deliver VPC at site of action and can alter look, texture, stability, and flavour of PCEP but slightly affect their physicochemical features. Basing on wanted features of PCEP, biopolymer particles crafted with wished features. Biopolymer based particulate conception method is unsuited either because of materials of non-food-grade or increased costs of processing^{9,17,18}. Gellan- or pullulan- or xanthan- gum, and jamilan could extends or modifies release profile. Blend of xanthan or jamilan gum and gellan gum, alginate and xanthan or gellan gum mends gastro-protective feature and achieves colon targeting^{18,19}. Calcium alginate is biocompatible, non-toxic, and cheaper; and requires simple processing steps¹⁸. Hydrogel of alginate yields porous MCs of PRs but requires GREC and creates scale-up problems. Blending alginate with starch or chitosan and its structural modification can eliminate GREC step. Requirements for 60-80°C to prepare its aqueous solution are major drawback^{5,18}. Starch and resistant starches deliver VPC in large intestine while their prebiotics role has synergistic action. Surface feature of resistant starch improves VoPC and results intestinal targeting^{18,20}. Gelatine alone or in blend with gellan gum or other materials is unable to offer gastro-protection¹⁸. Whey proteins and milk proteins improve aesthetic feature^{18,21,22}. Chitosan have inhibitory effects on LAB but result colon targeting¹². Carrageenan needs damaging temperature of 40-45°C, which increases in-process loss of viability of probiotics (iPVLP). It owes approval from several government agencies¹⁸. Hypromellose controls release of PCs, extend their gastric retention time, and improve gut colonization²³. Cellulose acetate phthalate and hypromellose phthalate reckoned as safe and yield MCs, water insoluble one¹⁸.

Method and Procedure

MEC of PCs done following spray drying, spray freeze-drying, impinging aerosol, extrusion, fluidized-bed coating and agglomeration (FCA), and direct compression encapsulation (DCE) method. Substitutes are dispersion and ionic or enzymatic gelification, dispersion and cold gelation or Extrusion (pulsation or vibrational jet nozzle) (EPV). Novel methods are dispersion and interfacial-polymerization or complex coacervation (DIPC), inter-polymer complex encapsulation with supercritical carbon dioxide (IESCF). These have superiority over each other but have limitations like involvement of organic solvents and high -pressure and or -temperature, scale-up problems, and so on. These factors retards lasting of VoPC throughout shelf life or increases cost^{1,5,9}. Succeeding sections provide their leaning and limits. Spray drying method create MCs with quickness, duplicability, and with low production cost. It's nonstop processing and superior scale-up potentiality makes it amenable for industrial scale, but fails in protecting iPVLP. The iPVLP can be improved by adding protectants, such as, whey-protein, and resistant and N-Tack starch, in formula^{5,20,24,25}. Spray freeze-drying method results MCs with controlled size and larger specific surface areas. Referring spray drying method, this employs high-energy and long processing time, and have high production cost (30–50 times)

and iPVLP. Have improved storage survivability at 4°C and is suitable to control release profile. It's high iPVLP issue solved by adding protectant, in formula, such as whey-protein, resistant starch, skimmed milk, and so on^{18-20,24-26}. Dispersion and enzymatic-gelification method results porous MCs with high entrapment efficiency and insignificant iPVLP and feasible for controlling particle size. Produced MCs are water insoluble; provide gastro-protection and favourable micro-milieu for encapsulated PCs but unsuitable for large-scale. Layer-by-layer coating of nanolayers self-assembly of polyelectrolytes on MCs is done with this method^{5,13,21}. DIPC is unsuitable for commercial scale but is suitable to get mucoadhesive MCs, in small-scale^{5,16,23}. Impinging aerosol method holds continuous processing capacity and high scale-up potentiality, lessens iPVLP and protects VoPC in high acid and bile environment, but costly. IESCF method encapsulates VPC with miserly iPVLP, while hold low scale-up potentiality and costlier^{5,27}. The FCA method adapted to give multilayer coatings to VPC including GREC to prepared MCs in same equipment. It prevents loss of VoPC during processing, storage, and GI transit. The method owes high reproducibility and scale-up potential, and is amenable for commercial scale. High iPVLP, higher processing cost, and improved mastering difficulty are major disadvantages⁵. EPV results MCs with low iPVLP, having loading efficiency up to 96 %. It is considered as a cheap and simple method, and can be carried out under aerobic and anaerobic condition. Involvement for GREC and scale-up problem makes it unalienable for commercial scale. DCE method considered cheapest method that designed for commercial scale production and improving shelf life. It is having low entrapment efficiency and high iPVLP, but retards VPC release in intestinal conditions. These problems can be minimised by using compression pressure below 90 MPa^{5,9}.

Prospective Action Plans

Innovation considered as business mantra or slogan and ability to continue innovation will be only hope for resulting business survival. Thus evolution of PCEP carried over to fulfil consumer's expectancy and wish. This act stepwise thought provoking researchers, performing in both scientific and applied-field^{3,9}. Product design aimed to make best formula through optimization of method. This involves determination of optimal levels of key ingredients essential in getting product with suited sensory and physicochemical characteristics, stability, extended shelf life, and reasonable price. Optimization of method is difficult and challenging task, when several causes needed to be achievable, associated with numerous features^{3,9}. Design of PCEP involves several rudiments including huge fund. Various rudiments crucially supervise their marketplace success and survivability, while consumer acceptance is key one. Manufacturers are responsible for supplying products complying prevailing guidelines (global and territorial), informing consumers relating wholesome diet, and improving availability and affordability of nutritious food. Overall, PCEP have suitable sensory and nutritional appeal, worthwhile properties, and extended shelf life. Consensus on strategy for development of prospering PCEP discussed hereunder and presented with Figure 1, while principal redressing points in development of PCEP provided with Table 1^{3,5,9,28,29}. Microbial strains with approval, and valid identity and genetic stability has to use. Single or combination multiple PRs strains selected basing on projected worthwhile effects^{3,6-9,16,29}.

Figure depicts prospective action plans for developing product containing microencapsulated probiotics

CMFMs, GREC materials, solvents, and additives used should hold consent from regulators. They must comply with specification for food contact substances limit, estimated daily intake limit, and acceptable daily intakes limit. GREC material or CMFM selected depending on wished physicochemical and surface properties of MCs. Besides, incompatibility and thermo-liability issues considered. CMFM and GREC material having release-controlling and synbiotic feature to use preferably, which synergises the effect. Single GREC material or CMFM is unable to result

MCs which will excel potential for marketability of PCEP. Thus, correct combination of CMFMs, GREC materials, and additives is needed^{2,9,30}. Physicochemical properties of CMFM and GREC material, properties of PCs, concentration of CMFM solution and solution of GREC material, influence efficiency of encapsulation and effectiveness of GREC, need attention. They influence size and size distribution of MCs and iPVL. Early concentration of PCs in formulation slurry, and limits and conditions of PEN influences all these parameters. Cell loading-efficiency of MCs meliorates with increase in early PCs concentration in dispersion. Thus all of these rudiments to selected, adopted, or fixed, in a judicious way^{5, 25}. Single strain or their combination, single prebiotics or their combination, additives, PEN, and coating method having backing from regulators are to select. Simple and continuous PEN and coating method having low processing cost and time, low iPVL, superior scale-up potentiality is to adopt. Among all FCA considered being convenient followed by extrusion and DCE. Spray drying method with inclusion of suitable protectant in formula can be wiser. GREC be applied following fluidized-bed coating⁹. In IESCF method low molecular weight alcohol and or poloxamer and or ethylene oxide-propylene oxide tri-block copolymer used as solubilizer for improving solubility of complementary polymers and inter-polymer complex. Glyceryl monostearate improves viability protecting efficiency of MCs while ethylene oxide-propylene oxide tri-block copolymer and polycaprolactone decreases the same^{5, 9,27}. In alginate-starch system glycerol added in formula to augments survival of VPC during processing and storage while Hi-Maize starch added to improve encapsulation efficiency. Gelatine or gelatine-maltodextrin system requires GREC step. GREC of freeze-dried LAB with fatty acids protects encapsulated PCs from harsh effects of temperature, gastric pH, and compression. Alternately, dual coating of LAB with soy peptides followed by cellulose and gum can be followed^{9,20}. None of MEC method results shelf-stable MCs loaded with required numbers of VPC having lasting viability throughout shelf life. Available instrumentation for PEN is unable to give large quantities of uniform sized MCs, with dwindled iPVL, having aptness for industrial applications⁵. Handling issues and laws on contamination and food safety is mandatory while marketing food product. Contamination and safety to addressed through constitution and execution of hazard analysis critical control point regulations. Balancing inactivation of contaminants and maintenance of sensory properties as well challenges the processes^{3,31}. Chemiresistive immunosensors applied for direct detection of viruses with high sensitivity and specificity. Contamination of food borne pathogenic bacteria to detected with high-performance impedance bacteria biosensors. Substitute is optical chemical sensors^{9, 32}. Sensory analysis is a prerequisite for customer espousal and a decisive step in evolution of PCEP, which links evolution of product with marketplace. Quantitative descriptive analysis, free choice profile, discriminative tests, affective tests, time-intensity analysis methods followed to follow with preference. Open-ended question, free-listening, check-all-that-apply approach, sorting, multivariate adaptive regression splines, survival analysis method, and internal preference mapping methods are alternatives. Among them quantitative descriptive analysis is put-up with preference^{3,14}. Control limits to confirmed and followed, in judicious and scientific way, to get product with ideal and wanted performance.

Table 1: Principal Redressing Points during Development of PCEP

Area	Redressing points
Global and territorial rule	Microbial strains with valid identity and genetic stability to be incorporated as probiotics in the product containing probiotics. Viability of probiotic cells within them. Gut colonization of contained VPC be above 10^9 CFU. Their safety issues, even at the end of shelf life.
Development of PCEP	Getting microcapsules (water-insoluble) that will preserve its integrity within PCEP and can get incorporate in a wide range of products. Having product with projected therapeutic effect and adequate shelf life. Sustaining and releasing VPC (above 10^9 CFU), metabolically active, in intestine. Passing on binding, complexing, or adherence ability to released VPC with target site, molecule, or micro-organism or absorbing target molecule or micro-organism in intestine.

Where: PCEP stands for product containing microencapsulated probiotics and VPC for viable-probiotics cells.

PCEP presented as drug, cosmetic to complying prevailing rules on Drugs, and Cosmetics, while that presented, as food to comply Food rules^{9,31}. Besides, evaluation of finished product involves assessment of microbial and physical stability, safety, sensory acceptance, cost of health benefit, and other essential sensible properties^{4,14,28}. The storage conditions to decide in scientific way and shelf life to assign with valid stability data in proposed packages⁵. Labelling want on strain specificity and number of VPC at end of shelf life to comply according to prevailing norm, supported with encouraging clinical, safety, and stability data^{7,9}. PCEP packed, by tradition, in glass containers, as PCs survival in glass bottles ought to be superior to in plastic bags. Biopolymer coated papers will be worthwhile over conventional synthetic paper coatings. Chemical or biosensors based smart-packages, will help in solving safety issue but increase cost of the product. These packing can signal food spoilage, contamination and packaging failure^{9,32,33}. Packaging system had to be used after carrying exposure assessments of leach food contact substances, complying specifications and limit²⁸.

CONCLUSION

Professionals of this field had to take effort for improving availability of cost-effective PCEP. This can be resulting through evolution of low-cost probiotic strain or strains, and synbiotics combinations. Ascribe are necessary to design and develop novel PCEP with lessened iPVL while upholding sensory and nutritional quality. Future will evidence novel PCEP that will deliver projected benefit in a cost-effective and sustainable way.

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