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SAUSAGES: PROBIOTICS, MICROENCAPSULATION AND POSTBIOTIC INVASION

Prabhjot Kaur Sabharwal*[1] and Satakshi Aggarwal[2]

[1*]Shaheed Rajguru College of Applied Sciences for Women, Delhi University, Vasundhra Enclave, Uttar Pradesh, India.
[2]Amity Institute of Food Technology, Amity University, Noida.

ABSTRACT

Food such as meat is a highly nutritious food but lacks in bioavailability of some nutrients such as iron and folic acid. Consumption of meat sometimes also lead to obesity, cancer etc. In order to enhance the functional properties of the food and to satisfy consumer demands various techniques are employed. Probiotics is one such method which can be stated as 'inoculation of live microorganisms under optimum conditions in a specific amount (as a feature of nourishment), provides medicinal benefits to the host. Strains from types of *Bifidobacterium* and *Lactobacillus* are generally preferred. Several studies have been focued on alive microbes but very less attention is given to intracellular soluble fractions or postbiotics. Main aim of this review is to focus on the prevailing application of conventionally available probiotics in products to enhance the quality, preservation aspects, mandatory regulations and guidelines needed; and introduction to intracellular soluble fractions.

KEYWORDS

Meat, Probiotics, *Lactobacillus* and Postbiotics.

Author for Correspondence:

Prabhjot Kaur Sabharwal
Shaheed Rajguru College of Applied Sciences for Women,
Delhi University,
Vasundhra Enclave, Uttar Pradesh, India.

Email: prabsabharwal@hotmail.com

INTRODUCTON

Meat is a profoundly nutritious food that is rich in protein, amino acids, vitamins (especially vit. B group) and minerals such as zinc and iron. But iron, vitamin B12, and folic acid have less bioavailability in different food products. Customers frequently associate meat with a negative account that meat is high in fat and red meat is considered as a cancer promoting food product (Arihara, 2006)[1]. Hence, meat and meat items is often avoided to restrict the danger of cancer, obesity and other diseases.

Presently, customers demand for meat products with low levels of salt, both nitrites and nitrates,

cholesterol part and fat and whose fatty acid profile has been adjusted. Tertiary functions are the parts of food components in anticipating diseases by innovating physiological structure. Various functions of foods are anti-aging action, anti-carcinogenicity, anti-mutagenicity and anti-oxidative action. Due to increasing problems, endeavors have been made by food industries in numerous nations to grow new foods with tertiary functions. Such foods having tertiary functions are considered as functional foods.

It is not sufficiently enough for food to contain bioactive ingredients in amounts enough for the food to survive processing, handling and storage, however the food should likewise function as a vehicle for conveyance of these components to human amid utilization in amounts enough to have the desired function. For a food to be known as a functional foods its ingredients might originate from regular sources and when consumed it ought to control physiological functions of the human body like retarding aging and supporting the immune system against diseases (Douglas and Sanders, 2008)[2]. Enhanced product shelf life is no longer the main rule to be satisfied with a specific end goal to be successful in the market (Decker and Park, 2010)[3].

Preservation of meat products by fermentation has been utilized for many years. Starter cultures have a vital role in development of high quality meat products as a result of their impact on pH, the desired flavor development, and also giving stability and safety. Subsequently, it is essential to figure out which starter culture or combinations ought to be utilized to fabricate safe and high quality meat products for buyers.

Probiotics is characterized as 'live microorganisms which, when managed in optimum amount (as a feature of nourishment), present a medical advantage on the host (FAO/WHO, 2002)[4]. Probiotics are mostly the strains from types of *Bifidobacterium* and *Lactobacillus*. LAB are fermentative group of microbes that exist as anaerobe facultative, aero-tolerant organisms; present normally in the gut of humans and other animals, raw vegetables, meat and meat products, and cereals (Carr *et al*. 2002)[5].

As indicated by the scientific results for isolation and characterizing probiotic microorganisms a few qualities are vital i.e., human origin , resistance from acid and bile toxicity, adherence to human intestinal cells, colonization of the human gut, threat against pathogenic microbes, creation of antimicrobial substances, immune modulation properties, history of safe use in humans.

Introduction of starter cultures and their roles in fermented meat products (FMP)

Starter culture combinations (*S. xylosus* + *P. pentosaceus, L. plantarum* + *S. carnosus, S. carnosus* + *L. pentosus, S. xylosus* + *P. pentosaceus, P. acidilactici, S. carnosus* + *P. pentosaceus and S. xylosus* + *L. alimentarus*) enhance quality attributes and positive affect on chemical and microbiological qualities of fermented meat items (Gonulalan *et al.* 2004)[6]. *P. acidilactici 413, 419 and 446*, and *P. pentosaceus 416* strains have the best potential for use as fermented sausage starter cultures since these strains are exceptionally versatile to nature found in FMP (Con *et al.* 2001)[7]. Study on the viability of *E. coli O157: H7* amid manufacturing and storage of sausage has also been conducted in fermented sausage prepared with a starter culture following 21 days storage at 25˚C than that accomplished by storage at cooler temperatures (4 and 15˚C) (Calicioglu *et al.* 2001[8], Calicioglu *et al.* 2002)[9].

Role of Probiotics on FMP

Probiotic strains must be achieved from human or animals and perceived as GRAS (Rönkä *et al.* 2003)[10]. Probiotic bacteria ought to have the ability to survive by amid the food production process and going through human digestive system in order to demonstrate their useful impacts.

Probiotics in FMP can be secured by fat and meat particles against human gastrointestinal conditions. It is likewise trusted that a portion of the meat and fat particles might be a source of energy for probiotic microbes in human digestive tract (Tannock, 1999)[11]. Meat gives great conditions to insurance of probiotic LAB organisms against acidic conditions of human digestive system and the

inhibitory impact of bile salts because of its buffering limit. In spite of the fact that raw fermented meat products, for example, sausages contain high quantities of LAB organisms but are not viewed as probiotics (Gänzle *et al.* 1999)[12].

Lactobacilli are of the most significance in meat fermentation due to their ability to provide, within the sight of fermentable sugars, quick and powerful acidification in thus preserving the sausages from the improvement of spoilage and pathogenic microbes. This is the reason they are frequently utilized as starters in dry fermented sausage production.

The utilization of probiotics (*L. acidophilus* and *B. lactis*) in fermented sausage manufacture lessen lipid oxidation, total aerobic bacteria, LAB and *micrococcus/staphylococcus* counts in fermented sausage (Kaya and Aksu, 2005)[13]. In this manner, *B. lactis* and *L. acidophilus* can be utilized together with starter cultures in fermented meat products manufacture as probiotic sources. Probiotic and bio-protective *L.rhamnosus* strains *GG, LC-705* and *E-97800* can deliver high quality dry sausage with diminished risk for *L. monocytogenes* or *E. coli O157:H7(*Erkkilä, 2001)[14].

The utilization of potential probiotic cultures (*L. rhamnosus GG, L. rhamnosus E-97800 and L. plantarum E 98098*) had no adverse impact on sensory and technological properties of fermented sausages (Erkkilä *et al.* 2001[15], Erkkilä *et al.* 2001)[16]. The utilization of *L. paracasei L26* and *Bifidobacteriumlactis B94* with traditional culture had likewise no antagonistic impacts on sensory properties of fermented meat products (Pidcock *et al.* 2002)[17]. Probiotic *L. rhamnosus FERM P-15120* and *L. paracasei* subsp. *Paracasei FERM P-15121* repressed the development of *S. aureus* and its entero toxin production in fermented sausages during fermentation period (Sameshima *et al.* 1998)[18].

Probiotic strains of *L. acidophilus CCDM 476* and *Bifi. animalis 241a* can be utilized as a part of fermented sausage manufacture rather than the traditional starter cultures to produce probiotic sausages with comparative quality parameters with conventional traditional fermented sausage (Holko *et al.* 2013)[19].

Challenges for probiotics for manufacture of FMP

FMP is altogether influenced by a few factors, for example, pH, H_2O_2, organic acid concentration, the presence of other microbes, temperature, oxygen content, moisture content, salt, sugar and additives. Fermented sausages have negative effect on the viability of probiotic bacteria on account of their high acidic conditions contrasted and conditions in non-fermented meat products (Shah, 2000)[20]. One of the most essential factor influencing the growth and stability of probiotic bacteria in fermented meat products is the pH. For example, probiotic *L. acidophilus* and *Bifidobacteria* require the ideal pH run between 5.5 - 6.0 and 6.0 - 7.0 respectively (De Vuyst, 2000)[21].

Probiotic cultures ought to likewise be able enough for growing fast during the fermentation, be effortlessly developed on an industrial scale, resist to freezing and lyophilization forms, give longer shelf life to the product and in addition add to the sensory quality of the final product. There are some possible methods for developing healthier meat products such as including functional foods i.e., modification of carcass composition, manipulation of meat raw materials, reformulation of meat products for example, minimizing fat content, alteration of fatty acid profile, minimizing cholesterol level, reduction of calories , reduction of sodium content, reduction of nitrites, incorporation of functional ingredients, etc.

Guidelines for the assessment of probiotics

Perceiving the imbalance of products and the absence of any administrative rules, the Argentinian government campaigned the FAO/WHO to have an Expert Consultation to analyze regardless of whether probiotics had demonstrated advantages and assuming this is the case, to set up an arrangement of rules that would guarantee product safety and reliability (FAO/WHO, 2002)[4]. The resultant report not just settled that there were to be sure magnificent information on probiotics, however that an arrangement of Guidelines or Operating Standards was required. The guidelines

make a number of critical points which will now be presented and illustrated in terms of relevance to clinical practice.

Stage one: Recognize the correct strain that is being utilized

The initial phase in an organism(s) or item being alluded to as a probiotic is to recognize and describe the life form to genus and species level utilizing universally acknowledged techniques, for example, DNA-DNA hybridization, sequencing of DNA encoding 16S rRNA. More up to date frameworks are being created, for example, terminal restriction fragment - length polymorphism (T-RFLP), digoxigenin-labelled peptide nucleic acid to distinguish lactobacilli PCR amplicons immobilized on membranes from denaturing gradient gel electrophoresis (Burton *et al.* 2003)[22]. This is essential so as to separate reliable and proven strains and products from those of questionable quality as referenced previously. Next stage is to guarantee that the specific strains utilized are legitimately assigned (e.g., *Lactobacillus acidophilus NCFMTM*).

Every "probiotic" strain is unique. For instance, *uropathogenic p fimbriated E. coli* causing pyelonephritis is absolutely particular clinically from *E. coli 0157:H7* causing gastroenteritis and hemolytic uremic disorder. All together for a strain (or a few strains in a blend item) to be probiotic, every life form must be assigned and their significance in giving the medical advantage reported.

Stage two: *in vitro* proof is deficient to call a strain a probiotic

The choice of suitable strains might be additionally refined by attempted a progression of *in vitro* tests, perpetually to analyze capacity to adhere fast to surfaces, and hinder development and attachment of pathogens. In general*in vitro* information alone isn't adequate to depict strains as probiotic. Two Lactobacillus strains showed comparable development, survival, and adherence properties *in vitro*, yet *L. johnsonii NCC 533* colonized the intestinal lumen and translocated into mucosal lymphoid tissue more adequately than *L. paracasei NCC 2461*, because of an enlistment of various sorts of immune responses (Ibnou-Zekri, 2003)[23].

Stage three: Safety of probiotics

There is chronicled information to show that *lactobacilli* and *bifidobacteria* are safe for human utilization (Marteau, 2002)[24].

The FAO/WHO Rules suggest that, at any rate, probiotic strains be portrayed utilizing a progression of particular tests: (a) Assurance of anti-toxin protection designs; (b) Appraisal of certain metabolic activities (e.g., D-lactate generation, bile salt de-conjugation); (c) Appraisal of symptoms amid human investigations; (d) Epidemiological observation of adverse occurrences in consumers (post-market); (e) If the strain under assessment belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production (one conceivable plan for testing poison generation has been prescribed by the European Union Scientific Committee on Animal Nutrition); (f) If the strain under assessment belongs to species with known hemolytic potential, assurance of hemolytic activity is required; and (g) Evaluation of absence of infectivity by a probiotic strain in invulnerable compromised creatures (including a measure of confidence in the safety of a probiotic).

Stage Four: Efficacy - Demonstrating that the probiotic gives a noteworthy improvement in health

As a statistically and biologically significant improvement occurs in a patient's condition, symptoms, signs, well-being or quality of life, risk of disease or speedier recovery from illness, then the producers of the probiotic will have important supportive material to convince physicians to consider their proposed remedy.

The greater part of the information demonstrates that strains *L. rhamnosus GG* and *L. reuteriSD2222* can decrease the span of bacterial and rotavirus related diarrhea when joined with oral rehydration treatment. Counteractive action of rotavirus disease isn't anything but difficult to survey since it is hard to guarantee that dynamic and fake treatment bunches are similarly presented to the infection (Guandalini, 2000)[25].

Stage 5: Adequacy

It is important to decide if a probiotic treatment is as good as, or superior to a standard treatment for a specific infection. The *L. rhamnosus GG* strain is for the most appropriated part in dairy item production, however as a freeze dried capsule, it can be enlisted as a drug. Such items must be produced under FDA, European Pharmacopeia or equivalent quality assurance regulations. Not many probiotic makers have achieved this Great Assembling Practices (GMP) standard, with Chr Hansen Research centers, Denmark being the most eminent exemption.

Stage 6: Health Claims and Labelling

An imperative component of the FAO/WHO rules includes marking and utilization of particular health claims. The guidelines suggest this be changed keeping in mind the end goal to furnish the customer with more helpful and exact data. Articulations, for example, 'helps lactose intolerant people better digest milk' are considerably more illuminating than 'helps intestinal prosperity'.

For instance, one site expresses that "*Lactobacillus acidophilus* is a standout amongst the most critical microorganisms found in the small intestine. It is known to embed itself on the intestinal wall, and in the lining of the walls of the vagina, cervix and urethra." obviously there are no companion investigated information to help these cases for this bacterium and no logical prove either that *L. acidophilus* is a standout amongst the most vital intestinal organisms, or that probiotics implant themselves anywhere. Another site recommends that their restrictive probiotic organisms "multiply in the intestines and really compete "unsafe" microorganisms for receptor sites, swarming out the pathogens and having their spot".

Immobilization and Encapsulation

Immobilization/encapsulation of food ingredients especially in probiotics has become a potential field in food technology that has grown expeditiously in the previous decade. Most prominent use of probiotic immobilization method is to control and provide ceaseless conveyance of cells in the gut. Dry fermented sausages with their low pH and water activity; and salts used for curing and competing organisms, would seem to be present in a challenging conditions for the durability of probiotics while processing (Rouhi *et al.* 2013)[26]. Viability of probiotic strains gets eliminated due to thermal methods also. Dairy products are pasteurized prior to culture addition whereas in cooked meat products meat is heated upto 70°C which restricts the use of probiotics.

Immobilization adverts to trapping of the material inside or all through a lattice/matrix whereas encapsulation is a way in which a consistent cover framing is done around that is contain a capsule as a core part of an encapsulated material. Both techniques involves a bidirectional dissemination of particles i.e., nutrients, deluge of oxygen and growth factors, fundamental for cell metabolism and the outward dispersion of waste products ought to be allowed. Cell immobilization gives with several advantages like stability of the immobilized cells and delaying of activity, hence the immobilization supports as a defensive agent against physic-chemical changes such as temperature, pH, bile salts, etc.

Immobilization strategies appear to be the trend of the future making it possible to add probiotics to food matrices where high processing temperatures are used (Perez-Chabela, Lara-Labastid*a et al.* 2013[27], Wang, Ho *et al.* 2015)[28]. Moreover, heat tolerance ability of LAB strains isolated from cooked meat products facilitating their inoculation in heat processed food products which will become effective in cold storage pack under vacuum (Ramírez-Chavarín *et al.* 2013)[29].

Microencapsulation is considered as an innovation that provides a protection to delicate cultures from freezing, high oxygen contents, manufacturing and storage and amid travel through the human gastrointestinal tract. Extrusion is an another stablished approach which develops capsules from hydrocolloids and can be completed by just dropping a fluid form of probiotics into a gelling bath (Doleyres and Lacroix, 2005[30], Muthukumarasamy, 2006)[31].

Another important challenge for probiotic encapsulation is to reduce the particle size as it can effect the sensorial and textural properties of the

final product (Gouin, 2004[32], Mortazavian *et al.* 2008)[33]. Customers find such grainy texture in yogurts containing encapsulated *bifidobacteria* (estimate run particles around 22– 50 µm), objectionable. *L. reuteri* is generally incapable to ferment meat batter adequately. Hence, the probiotic organisms that are weak lactic acid producer i.e. *L. reuteri*, their value as probiotic in the given product depends upon their capability to sustain in the sausages during processing.

Postbiotics - A new call

Postbiotics are also known as paraprobiotics or inactivated probiotics or also known as non-viable probiotics or ghost probiotics. Postbiotics can be referred as those inactivated microbial cells which are induced in food products under certain condition in a particular serves with benefits to consumers.

These ghost probiotics may confer many health benefits in comparison to conventionally termed probiotics as they can increase inflammatory responses and reduce the risk of microbial translocation which were found to be compromised in some clinical cases of probiotics.

Since many years studies have revolved around live microbes or their membranes and components of cell wall but very little consideration has been gained by intra-cellular soluble fractions or primarily call postbiotics.

These metabolites can be a released from bacterial lysis or produced from alive bacteria in the host and contributing other physiological advantages by modifying the metabolic pathways and cellular processes. These non-viable probiotics are a result of cell- disruption method that includes other techniques as well such as centrifugation, dialysis, solvent extraction, enzymatic treatment, column purification, etc.

As per studies conducted by Sawada *et al.,* (1990)[34], polysaccharide-glycopeptide complex was obtained from *L.casei*YIT9018 by dialysis using distilled water contained in a porous membrane.

Also centrifugation can be used for intracellular component extraction from different microbial strains such as *Lactobacillus* spp., *Bifido bacterium* spp. and *Streptococcus* spp. (Amaretti *et al.,* 2013[35], Ou *et al.,* 2006[36], Zhang *et al.,*2011)[37].

Mass spectrometry was used to characterize LTA which is produced by *L. plantarum*K8 (KCTC10887BP) (Kim *et al.,* 2011)[38].

Postbiotics are also known for inhibition of pathogenic bacteria against pathogenic microbes such as *E.coli* E-30, *Salmonella enterica* S-1000, *Listeria monocytogenes* L-MS, and vancomycin resistant *Enterococci* while using cell free supernatants culture achieved from *L. plantarum*RI11, RG11, RG14, TL1, UL4 and RS5 strains.

CONCLUSION

Most commonly used microbes i.e., *B. lactis* and *L. acidophilus* are utilized indivisallyor together with starter cultures in fermented meat products are considered as probiotic sources. Use of probiotic immobilization technique is the controlled and ceaseless conveyance of cells in the gut. The acquired capsule have a small diameter, yet the principle drawback of this technique is that it gives large size range, shape particles and sensitivity during acidic conditions. Important challenge observed for probiotic encapsulation is to lessen the size of the particle, since it can contrarily influence the textural and the sensorial properties of the product. Another issue is the presence of residual oil on capsule surface which is delivered by emulsification that hinders to the texture and the organoleptic properties of the item. After such studies, intracellular soluble components, primarily called as postbiotics has gained attentation in research. These are induced in food products under specific conditions in a particular amounts which serves benefits to consumers. These are either secretions of alive bacteria or released components from bacterial lysis. These components can be extracted from dialysis, characterization can be studied by mass spectroscopy. Centrifugation, solvent extraction and column purification provide assistance in extraction of postbiotics.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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