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Delivery of Probiotics by Encapsulation Using Spray Drying Technology and Evaluation of Its Powder Qualities

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Abstract

The present study aimed to develop a nutraceutical powder containing Bifidobacterium longum and to assess its viability after spray drying. The nutraceutical powder was prepared using B.longum and prebiotics like inulin and honey, malted Eleusine coracana and whey protein concentrate. The mixture was then incubated for 4 hours and spray dried. The optimum inlet and outlet temperature were regulated at 170oC and 70oC respectively after assessing the viability of Bifidobacterium and the moisture content of the spray dried powder. The viability of the B.longum before and after spray drying were 9.80 ± 0.014 and 8.926 ± 0.017 respectively and the estimated moisture content was 4.85 ± 0.022. The study also assessed the physical and proximate composition of the nutraceutical powder. The survival of Bifidobacterium during spray drying was a challenge during the preparation of the nutraceutical mix and was overcome by adding 5 percent of the inoculum to maintain the probiotic count.

Keywords: *Probiotic encapsulation, spray dried probiotics, synbiotic powders, Millet based powder, Bifidobacterium whey based nutraceutical.*

Introduction

The concept of probiotics was introduced in the early 20th century [1]. The Food and Agriculture Organization of the United States and the World Health Organization described probiotics as: 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'[2]. The probiotic microorganism consists mostly the strains of the genera *Lactobacillus* and *Bifidobacteria*. Bifidobacterial species are common inhabitants of the infant's gut where they form up to 91 percent of the total microflora in breast-fed babies and up to 75 percent in formula-fed infants. Dairy products containing *Bifidobacteria* have potential benefits for infants and adults that are generally related to the inhibition of pathogens, the maintenance and restoration of normal intestinal flora. It has been reported that these organisms are able to exert beneficial effects including improvement of intestinal microflora by preventing colonization of pathogens, amelioration of diarrhoea or constipation, activation of the immune system and increasing protein digestion. Owing to these properties, *Bifidobacteria* are now frequently used to prepare probiotic dietary adjuncts.

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Colonic foods, which encourage the growth of favourable bacteria, are referred as prebiotics. Prebiotics like fructo oligosaccharides and inulin occur naturally in a variety of fruits, vegetables and grains like *Jerusalem* artichoke, onions, garlic, wheat, honey, tomatoes, asparagus, barley and chicory and are of interest because they are neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract and may beneficially affect the growth or activity of desirable bacteria in the colon.

Whey is a biological source of valuable proteins and is rich in minerals and vitamins, especially vitamin B2. Finger Millet (Ragi, *Eleusine coracana*) is rich in protein, iron, calcium, phosphorous, fibre and vitamin content and is used extensively in the preparation of weaning food. Dehydration is a common practice to preserve biological materials so that they remain stable in the long run. Among the many cell preservation methods, spray drying is widely used in the food industry because it is economical, especially on a large scale. This low operation cost technology has been used by various investigators to prepare lactic-fermented products with starter cultures. In the current study, *B.longum* inoculated in a mixture of whey and prebiotics was spray dried and its viability was assessed before and after the drying process.

Materials And Methods

Bifidobacteria longum: *Bifidobacteria longum* isolated from breastfed infant faeces was identified by phenotypic and molecular methods and used in the present study.

Optimized mixture: An optimized mixture of 4 percent whey protein concentrate, 0.4 percent inulin, 3 percent honey and 9 percent malted ragi extract was mixed in whey with 5 percent inoculum of *B.longum*, incubated for 4 hours and spray dried.

Spray drier: Laboratory Model Spray Drier (Jektron Engineers Pvt. Ltd. Pune) was used for spray drying the formulated nutraceutical whey-based malt food. The inlet and outlet temperatures were optimized at 170°C and 70°C respectively.

Physical parameters of the nutraceutical whey-based malt powder were assessed for moisture**,** water activity, texture (Texture Analyser from Stable Micro Systems, U.K. with Powder Flow Analyser Assembly), powder morphology (Table-top microscope 1000 Hitachi High Technologies, Forensic Science Department, Government of Tamil Nadu.)

Atomic Absorption Spectrophotometry was used for the estimation of nutrients. The viability of *B.longum* was assessed before and after spray drying. The data obtained were analyzed statistically [3].

Results And Discussion

Viable count of *B. longum* at different inlet temperatures in control (SMP) and nutraceutical whey-based malted food (BMW)

Table 1 reveals a highly significant difference (P≤0.01) in the viable count of *B.longum* before and after spray drying. A survival percentage of 91.10, 91.10 and 88.9 of *B.longum* was observed in the spray-dried nutraceutical whey-based malt food (BMW) with inlet temperatures of 165°C, 170°C and 175°C respectively. This indicated that the viability of *B.longum* was highest at inlet temperature of 165°C and 170°C and least at 175°C. The reduction in bifidobacterial count from 9.80 ± 0.014 to 8.926 ± 0.017 at 170°C in the present study after spray drying is in agreement with the work of [4] who recorded a reduction of 2.45-3.34 log in the population of *B.pseudocatenulatum* from an initial value of 10^9 cfu/g after spray drying.

The reduction in the viable count numbers at high spray drying temperature is related to the inactivation of critical sites such as ribosomes, loss of magnesium

Table - 1: Viable count of B.longum at different inlet temperatures in control (SMP) and nutraceutical whey based malted food (BMW)

CONTROL (SM)					TREATMENT(BMW)			
Viable Count ($\log_{10}$ cfu/g)					Viable Count ($\log_{10}$ cfu/g)			
Inlet temperature	**Before spray drying**	**After spray Drying**	**% of Survivability**	**t-test**	**Before spray drying**	**After spray drying**	**% of Survivability**	**t-test**
165°C	5.80 ± 0.156	4.11^{A} ± 0.030	70.86	9.77**	9.80 ± 0.014	8.928^{B} ± 0.017	91.10	69.87**
170°C	5.80 ± 0.156	3.98^{A} ± 0.090	68.62	10.63**	9.80 ± 0.014	8.926^{B} ± 0.017	91.10	77.38**
175°C	5.80 ±0.156	3.87^{A} ± 0.051	66.72	12.15**	9.80 ± 0.014	8.716^{A} ± 0.019	88.90	66.90**
	F value	17.92**			F value	56.30**		

Average of six trails.
Capital superscripts shows difference in treatment
** Statistically Highly significant

and denaturation of protein as inferred by [5]. In the present findings adoption of temperature of 170°C is in accordance with [6] who reported that at a constant inlet temperature of 170°C and an outlet temperature of 85-90°C, a significant survival was achieved for the bifidobacterial strains. The survival percent of more than 90 is in accordance with [7] who reported a survival rate of 97 percent with *L. paracasei* during spray drying at an outlet temperature of 70°C. These findings are attributed to the greater thermal tolerance of the strain.

In the present study, there was only a one-log reduction in the viable count of *B.longum* and this could be due to the added carrier prebiotics acting as a protective sheath. The survival rate of *B.longum* was 91.10 percent in the present study and is in agreement with the findings of [8] that *B.longum* B6 was the least sensitive to spray drying and had the highest survival rate of 82.6 percent after drying with skim milk. They also reported that an elevated outlet temperature reduced the viability of bifidobacterial species as observed with the bifidobacterial count in the present finding with 175 °C.

From this table it was concluded that taking viability as a criteria 165°C or 170°C can be used as the optimum inlet temperature for the spray drying of the nutraceutical whey based malt food containing *B.longum*

The viable count of the nutraceutical whey-based malt food containing *B.longum* (8.926 ± 0.017) was in consonance with the recommended level of 10^6 cfu/ml to be called a probiotic food [9].

Moisture per cent in spray dried control (SMP) and nutraceutical whey-based malt food with *Bifidobacteria* (BMW) at different inlet temperatures:

The moisture content in the nutraceutical spray dried malt food with *B.longum* showed the least moisture percent of 4.35 ± 0.022 at 175°C, followed by 4.85 ± 0.022 at 170°C and 4.90 ± 0.025 at 165°C in that order (Table 2). Although the moisture percent at 175°C, was the least, the findings of the previous table showed least viable count of *B.longum* and hence this temperature was not preferred for spray drying. At the inlet temperature of 165°C, though the viable cell count showed no statistical difference with the viability of *B.longum* at 170°C, the equivalent moisture content was not suitable for powder storage. Hence 170°C was the choice of the inlet temperature for spray drying the nutraceutical whey-based malt food containing *B. longum*. The optimized inlet temperature of 170°C in the present study agrees with that of [10] who concluded that an inlet temperature of 171°C improved the viability of cultures and other quality attributes of yogurt.

The moisture content in the nutraceutical powder was less than the findings of [4] who reported a moisture percent of 5.25 to 6.31 when skim milk containing *B.pseudocatenulatum* G4 was spray dried at an outlet temperature of 85 ± 2°C. However, it is in agreement with the findings of [7] who obtained a moisture percent of 4.1 to 4.2 during spray drying skim milk with probiotic *L.paracasei* and *L.salivarius* strains. The increased moisture content in this present study could be due to the hygroscopic nature of the ingredients supplemented.

Authors [6] in their findings reported a moisture percent of 4 with skimmed milk carrier and gum acacia. However, the findings of the present work were well within the stipulated maximum moisture requirement of 5 g/100g of milk cereal-based complementary foods by the Indian standard milk-cereal-based complementary foods- specification

Table - 2: Moisture Per Cent In Control (Smp) And nutraceutical whey based malt food (BMW) at different inlet temperatures

Inlet temperature	Moisture % SMP	Moisture % BMW	t test
165°C	4.90^B ± 0.025	5.33^B ± 0.033	10.28**
170°C	4.46^A ± 0.042	4.85^A ± 0.022	8.03**
175°C	4.35^A ± 0.022	4.75^A ± 0.050	7.30**
F Value	85.57**	71.02**	

Average of six trails.
** Statistically highly significant (P ≤ 0.01)
Capital superscripts shows difference in treatment.

Physical properties of spray dried control (SMP) and nutraceutical whey-based malt food (BMW):

Table 3 shows a highly significant difference (P≤0.01) in the physical properties between spray dried control skim milk powder (SMP) and nutraceutical whey-based food (BMW).

The mean values of loose and packed bulk density of BMW were 0.29 ± 0.003 and 0.40 ± 0.003 respectively. This low bulk density in BMW was due to the moisture content and is concurrent to the findings of [11] who attributed moisture factor with hygroscopic food powders which resulted in lower loose bulk densities. Low bulk density is seen in agglomerated products as noticed in BMW during prolonged storage at room temperature. The present study with nutraceutical whey-based malt food (BMW) showed values lesser than [12] who investigated the physical properties of bifidogenic milk powder for loose and bulk density and reported values of 0.45 g/ml and 0.535 g/ml respectively

Bulk density depends on water content and particle size. A high bulk density is preferred in order to reduce the shipping volume, saving packing material and increasing storage capacity. The findings in this study were lower than the bulk density proposed for non-fat dry milk as 0.56 g/cc, millet at 0.64g/cc and whole milk as 0.51g /cc

The values for loose and packed bulk density in the present study were lower than the loose bulk density (0.42 g/cc) and packed bulk density (0.69 g/cc) of soy-whey beverage powder reported by [13].

The mean values of insolubility (BMW) in the present study had a reasonably high index of 4.00 ± 0.051. The reason for the high insolubility index may be due to a complex combination of whey protein and lactose, bacterial activity and is seen in compounded products like baby foods. The findings in the present study were in accordance with the finding of [14] who formulated instant spray dried kheer powder with an insolubility index of 4.0 ml but was more than the index reported by [12] in bifidogenic milk.

Table 3: Physical parameters of spray dried control (SMP) and nutraceutical whey based malt food (BMW)

Physical Parameters	SMP	BMW	t-Test
Loose Bulk Density g/l	0.38 ± 0.005	0.29 ± 0.003	8.04**
Packed bulk density g/l	0.54 ± 0.020	0.40 ± 0.003	6.74**
Insoluble index (ml)	1.45 ± 0.056	4.00 ± 0.051	33.39**
Water activity	0.23 ± 0.003	0.33 ± 0.003	22.42**
Cohesion index	20.88± 0.001	23.10±0.002	6.89**
Caking strength (g)	41.755±0.001	83.31±0.002	7.23**

Average of six trails.
** Statistically highly significant ($P \leq 0.01$)

The findings in the present study were related to the work of [15] who concluded that the insolubility index of the spray-dried powders increased linearly with air outlet temperature. They reported an insolubility index of 3.2 ml / 50ml at an outlet temperature of 91°C.

In the present study, the mean water activity of BMW was 0.33 ± 0.003. This was more than the $\mathbf{a}_w$ value of 0.2 reported by [16] for maintaining the viability of *Bifidobacteria*. Most foods have a_w level in the range of 0.2 for very dry foods to 0.99 for moist fresh foods. The $\mathbf{a}_w$ value in the present study is slightly more than the value prescribed for dry foods and less than the value of the enteral formulation prepared by [17] who concluded that the enteral immune enhancing formulation had a water activity of 0.4 percent.

The mean values of the Cohesion index in control (SMP) and BMW were 20.88 ± 0.001 and 23.10 ± 0.002 respectively. Both the powders had high cohesive values (above 20) and thus were unable to flow freely during discharge. This may be due to the high moisture content in the powder as the inlet temperature was reduced. The cohesion test signifies the flow properties of powder which is important in some processing aspects. The index in the present study is above 15 and suggests the cohesiveness nature of particles with an increased tendency to agglomerate with more force**.** High cohesion index is associated with cohesive, poor-flowing powders. [18].

The mean values of cakiness in g of BMW was 83.31 ± 0.002 as against 41.755 ± 0.001 for SM. The high difference (P≤0.01) in mean caking strength suggests that BMW had a high tendency of caking and in the formation of lumps as compared to the control sample due to the hygroscopic nature of its various ingredients. As per the observations of [11] high 'caking strength' indicates the tendency of the powder to form a stable cake during storage (Figure 1&2)

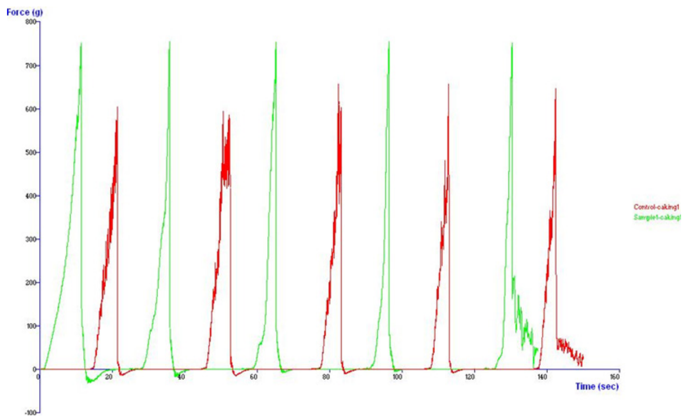

Figure 1: Caking strength of control and nutraceutical whey based malt food

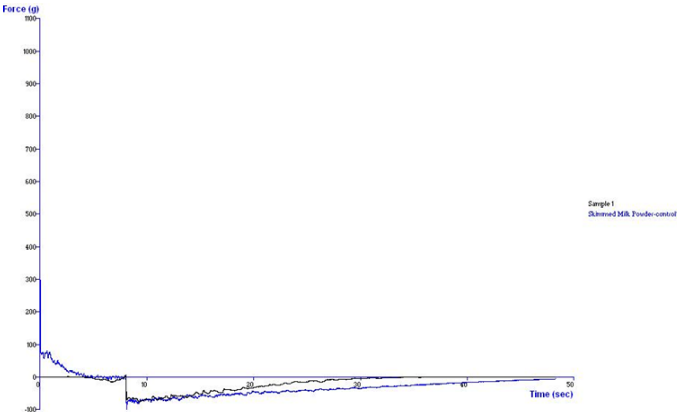

Figure 2: Cohesive index of control and nutraceutical whey based malt food

Plate 2 shows the amorphous particle with typical blow holes due to spray drying. There were differences in particle size, shape and damage to the powder particle structure. Wrinkling of the surface with high porosity was caused by an implosion during the last stage of the drying process or during the cooling of particles that contained relatively large vacuoles. Similar images were reported by [11] who reported that spray drying resulted in hollow amorphous particles of lactose. The porosity of powder particles was in consonance to the observation of [19] who also observed the porosity in spray-dried particles and concluded that there were two mechanisms causing particle porosity; one being the rapid drying due to a large temperature gradient and the other being the mechanical damage to the particles during separation in a cyclone or pneumatic conveyor.

The powder morphology was similar to the observation of [20] who reported that SEM images of spray-dried whey showed smooth skin forming behavior, typical of whey with blow holes particles.

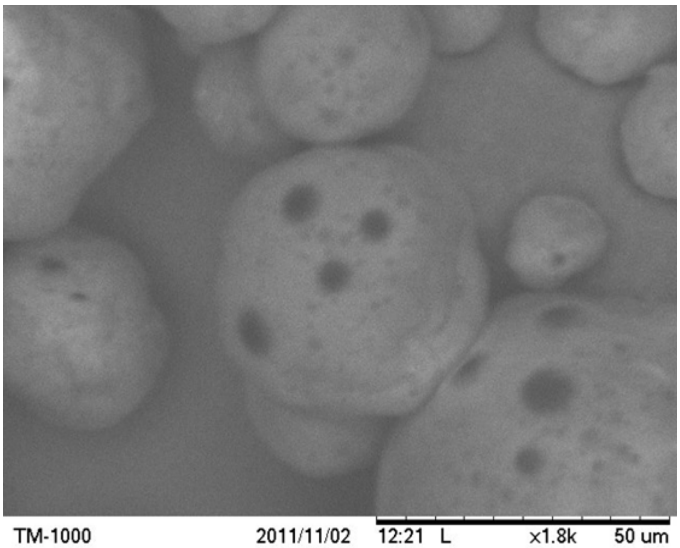

Plate 2: Table top microscopy showing powder morphology of BMW

Proximate composition of the nutraceutical whey-based malt probiotic food:

Protein (22.35g/100g), fat (3.72g/100g), carbohydrate (65.63g/100g) and energy (385.50kcal/100g) in spray dried nutraceutical whey based malt food containing *B.longum* (BMW) was more than the minimum recommended 15 BIS (2007/ level for complementary infant foods as shown in Table 4.

The weaning food made from pigeon pea and millet by [21] had a lower protein content ranging from 8.7 – 10.5 percent but a higher crude fat percent ranging from 9.7 – 11.7 when compared to the present study. The protein content of the present product is also like the findings of [22] who estimated a protein content ranging from 20.87 to 21.81 percent in indigenous food containing, barley, flour, milk, sprouted green gram paste and tomato pulp (2:1:1:1 w/w) and cultured with *L. plantarum* and *S. boulardii*.
The protein and fat content in the nutraceutical whey-based malt food containing *B.longum* (BMW) was more than the nutrients available in the weaning food prepared by [23] using *Eleusine coracana* and *Phaseolus radiatus*.

FAO/WHO has recommended a protein requirement for commercial weaning diet of 15 percent [24] in infant formulation. The protein availability of nutraceutical whey-based malt food containing *B.longum* (BMW) had a significantly higher protein content (22.35 percent).

Vitamin A was less than the recommended [25] level of 350 µg/100 g. This was due to the absence of fat in the preparation of BMW. Vitamins B1, B2 and B3 were almost within the recommended [23] specification. Vitamin C level was negligible. This may be due to the negligible levels of this vitamin in milk. The 0.5mg/100g of Vitamin C may be due to the malting effect of finger millet.

Minerals like calcium (507mg/100g) and phosphorus (246mg/100g) were much above the [25] recommended level and were in the ratio of 2:1 while the /ratio of calcium and phosphorus in breast milk is 2:3. The ratio in the present study was above the permitted range in infant formulas of 1.1 to 2.0. The iron content in BMW was 7.3mg/100g which was at par with the recommendations for iron in complementary cereal-based foods by [25] to alleviate anemia. The BMW had 4.28mg/kg of copper and 13.47mg/kg of zinc which was as per the recommendations of

Table 4: Proximate composition of the Nutraceutical Whey based malt Probiotic food

Nutrients	Recommended daily Intake by ICMR (1992) 6-12 months	BIS recommendation for Milk based cereal complementary food	Whey based malt Probiotic food BMW
Protein Min. g/100g	1.65 g/ kg	12	23.35 ± 0.115
Fat g/100g	17-27g/day	7.5	3.22 ± 0.110
Carbohydrate Min. g/100g	--	55	56.63 ± 0.065
Energy kcal/100g	98Kcal/kg b.wt	-	385.50± 0.114
Vitamin A (I.U./ 100g (min)	350	350	122.2 ± 0.026
VitaminB1 mg/100g	55mcg/Kg	0.5	0.25 ± 0.115
VitaminB2 mg/100g	65mcg/Kg	0.3	0.25 ± 0.116
VitaminB3 mg/100g			0.25 ± 0.115
VitaminB6 mg/100g	0.3	3	0.25 ± 0.112
Vitamin C mg/100g	25mg is recommended	25	0.5 ± 0.023
Calcium mg/100 g	500	230	507 ± 0.033
Iron Min mg / 100g	Min -7mg / day	5	73 ± 0.045 mg/kg
Magnesium mg/100 g, Min		22	164 ± 0.016
Phosphorus (mg/100g ,Min)		115	246 ± 0.543
Potassium (mg/100 g, Min)		370	586 ± 0.029
Cu (μg)		280 μg/ 100g (min) to 1500 μg/ 100g(max)	4.28 ± 0.023 mg/kg
Zn (μg)		2.5mg / 100g (min) to 5mg / 100g (max)	13.47 ± 0.174 mg/kg
Mn (μg/100g, Min		20	10.54 ± 0.029 mg/100g

[25] for complementary milk-based cereal foods. Magnesium (164mg/100g), potassium (586mg/100g) and manganese (10.54mg/100g) were above the minimum recommended levels for complementary infant foods as per [25].

The incorporation of probiotics in follow-on foods with the maintenance of the probiotic level for a considerable period will open new vistas in the field of nutraceuticals. Whey, considered as a waste in the Dairy industry is a product of high biological value can be effectively utilised as a vehicle for probiotics. The viability and stability of probiotic products has been both a marketing and technological challenge for industrial producers. Spray drying, a predominant tool used in the dairy industry can be used to preserve these dairy adjuncts and thus maintain their viability in probiotic foods. The functionality of colonic bacteria can be improved by the use of nonconventional prebiotics like millets. The development of new functional ingredients from locally available raw materials also confers the advantage of value addition.

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Conflict of interest

The authors declare that there are no conflicts of interest.

References

[1.] Metchnikoff, E. (1907). Lactic acid as inhibiting intestinal putrefaction. In: 1. The prolongation of life: Optimistic studies. *W. Heinemann*, London: 161-183.

[2.] FAO/WHO Working group Report, Guidelines for the evaluation of probiotics in food. London, Ontario, Canada (2002)

[3.] Snedecor, G.W and Cochran, W.G (1980).. Statistical analytical methods. Oxford and IBM Publishers Co., New Delhi, India.

[4.] Stephenie Wong., Barka Mohammed Kabeir., Shuhaimi Mustafa., Rosfarizan Mohamad., Anis Shobirin., Meor Hussin and Mohd. Yazid Manag. (2010). Viability of B. pseudocatenulatum G4 after spray drying and freezing – *Microbiol. Insights*: 3

[5.] Teixeira P, Castro H, Kirby R. (1997). Spray drying as a method of preparing concentrated cultures of L. bulgaricus. *J. of Appl. Microbiol*, 78 456-462.

[6.] Simpson, P.J., Stanton, C., Fitzgerald, G.F and Ross, R.P (2005). Intrinsic tolerance of Bifidobacterial species to heat and oxygen and survival following spray drying and storage. *J. of Appl. Microbiol*, 99 493-501.

[7.] Gardiner, G.E, Sullivan, E.O, Kelly, J., Auty, M.A.E., Fitzgerald, G.F,, Collins, J.K., Ross, R.R and Stanton, C. (2000). Comparative survival rates of human derived probiotic. L. paracasei and L.salivarius strains during heat treatment and spray drying. *Appl. Environ. Microbiol*, 66 (6) 2605-2612.

[8.] Wen- Chian Lian, Hung Chi- Hsiao and Cheng Chun Chou, (2002). Survival of bifidobacteria after spray drying. *Int J. of Food Microbiol*, 4(1-2) 79–86.

[9.] European Union Official Announcement: *Milk and Dairy Products* (2003) No 77/2003

[10.] Banu Koc, Melike Sakin Yilmazer, Pinar Balkr, Figen Kaymak Ertekin, Spray drying of Yoghurt: Optimization of process condition for improving viability and other quality attributes. *Drying Technol.*, 28 (4) (2010) 495-507.

[11.] Gustavo V Barbosa-C' anovas, Enrique Ortega-Rivas, Pablo Juliano and Hong Yon, Food Powders, (2005). Physical Properties, Processing and Functionality. Kluwer, Academic Plenum Publishers, New York.

[12.] Suresh Subramonian B, (2000). Studies on preparation of dietetic milk powder with added bifidogenic properties. Ph.D. Thesis submitted to Tamil Nadu Veterinary and Animal Science University, Tamil Nadu, India

[13.] Patil, G.R (1980). Manufacture of high protein beverage from soyabeans and whey. Ph.D. Thesis, Kurukashetra University, Kurukashetra, India.

[14.] Jha, A., Patel, A.A and Singh, R.R.B (2002). Physico-chemical properties of instant kheer mix. *Lait*, 82 501–513.

[15.] Kieran Keogh, Cathriona Murray, Fames Keley, Brendan O' Kenndey, Effect of the particle size of spray dried milk powder on some properties of chocolate. *Lait*, 84 (2004) 375–384.

[16.] Pisecky IJ, (1997). Handbook of Milk powder Manufacture. Copenhagen Denmark: Niro A/S. ISBN 87-986606-0-8

[17.] Dhruti Bal., Kamal Nath G., Radhakrishnan D., Indriamma, A.R and Vijayalaksmi, N.S. (2009). Storage behaviour of immune-enhancing enteral formulation from natural sources. *Int. J. of Food Sci. and Nutr,* 60(6) 59.

[18.] Landillon, V., Cassan, D., Morel, M.H and B. Cuq. (2008). Flowability, cohesive and granulation properties of wheat powders. *J.Food Eng,* 86 178–193.

[19.] Buma, T.J and Hensera, S. (1971). Particle structure of spray dried milk products as observed by SEM. *Neth.Milk.Dairy J,* 2575-80.

[20.] Kassem al Hakim and Andrew Stoply GF. (2004). Morphology of Spray dried and spray freeze dried whey powders drying. Drying–Proceedings of the 14th International Drying Symposium (IDS 2004), São Paulo, Brazil, 22-25 C 1720-172.

[21.] Onweluzo, J.C and C.C Nwabugwu. (2009). Development and evaluation of weaning foods from pigeon pea and millet. *Pak. J. of Nutr,* 8 (6) 725–730.

[22.] Sangeeta, C and Sindhu Neelam Khetarpaul. (2005). Development, acceptability and nutritional evaluation of an indigenous food blend fermented with probiotic organism. *Nutr. and Food Sci*, 35(1) 20–27.

[23.] Malleshi, N.G and Amla, B.L. (1988).Malted Weaning Foods in India. IDRC, Ottawa, ON, CA. 340-348.

[24.] FAO/WHO Working group Report, Guidelines for the evaluation of probiotics in food. London, Ontario, Canada

[25.] Bureau of Indian Standards, IS1 656: 2007 – Milk Cereal based complementary Foods – Fourth Revision