STUDIES ON PRODUCTION OF BIOACTIVE PARAPROBIOTIC FOOD PRODUCT

[1]Patewar A. P., [2]Chellamboli C., [3]Devkatte A. N., [4]Chavan Y. V.

[1]M. Tech. student, [2]Associate Professor, [3]Associate Professor, [4]Associate Professor
Department of Ethical Science and Food Technology
MIT College of Food Technology,
MIT-ADT University, Loni Kalbhor, India.

Abstract: Bioactive para probiotic yogurt is a new functional dairy product prepared from pasteurized yogurt and incorporation of Aloe Vera as a bioactive component used to improve health benefit of human immune modulatory properties. Pasteurization of yogurt was done to inactivate the *Lactobacillus* species. The research work was aimed to optimize operational factors of bioactive para probiotic yogurt such as fermentation period, inoculum concentration (*Lactobacillus gasseri: Lactobacillus acidophilus*), bioactive components concentration and pasteurization temperature. The optimized process parameters were incubation time (8 h) at 43 ºC temperature, inoculum concentration (2 %), bioactive components concentration (2 %) and pasteurization temperature (70 ºC for 30 minutes). The quality parameter such as consistency was also optimized. The nutritional compositions of para probiotic yogurt and bioactive para probiotic yogurt were done. The bioactive para probiotic yogurt showed comparatively high protein content (5.60 g/100g) than Para probiotic yogurt.

IndexTerms **- Bioactive, Paraprobiotic, Yogurt.**

I. INTRODUCTION

Probiotic is defined as a live microbial feed supplement that beneficially affects the host by improving the intestinal microbial flora (Fuller, 1989). Bacteria belonging to the genus *Lactobacillus* are the lactic acid bacteria used as a probiotic feed supplements for animals especially poultry. By changing the beneficial bacteria in the avian alimentary tract, probiotics shows the benefits to the host such as improving the growth rate, inhibiting the growth of pathogens and reducing the cholesterol level (Jin *et al.*, 1996, 1998a, b, 2000; Zulkifli *et al*., 2000; Kalavathy *et al*., 2003; Murry *et al*., 2006).

Yogurt is a type of fermented milk product. It is well known for its nutritional composition and health benefits. The word Yogurt derived from Turkey language as 'Yogen' means thick. Yogurt is a one of the functional products made by the process of fermentation of milk and by adding of starter cultures containing *Streptococcus* and *Lactobacillus* species (Akpan *et al*., 2007). McKinley, (2005) have demonstrated that yogurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B), thiamine (vitamin B) and a valuable source of niacin, folate, magnesium and zinc. According to code of Federal regulations of United States Food and Drug Administration (FDA), reported that yogurt is a food product which is produced by fermentation of milk by adding a starter culture and more of the dairy ingredients such as cream, milk, skimmed milk and bacterial cultural that contains lactic acid producing bacteria.

The term Para probiotics has been coined to demonstrate the use of inactivated microbial cells or cell fractions to confer health benefit to host (Taverniti and Guglielmetti, 2011). Para probiotics are well known to modulate the immune system (compounds of cell wall might boost the immune system) (Fujiki *et al*., 2012; Ou, Lin *et al*., 2011) and have adherence to intestinal cells which results in inhibition of pathogens (Grzeskowiak *et al*., 2014). Para probiotics can provide health benefit to host by secreting the metabolites of dead cells (Shin *et al*., 2010). Some of the studies have been shown that Para probiotics can be obtained through various methods such as thermal treatment, high pressure processing, sonication, irradiation and ultra-violet rays and other methods (Ananta and Knorr, 2009; Awad *et al*., 2010; Kamiya *et al*., 2006; Shin *et al*., 2010). The use of Para probiotics in food gives the benefits in relation to probiotics. No internal reaction with the other food component that reflect the increase in the shelf life of food product. Storage and transport simplicity which results in longer in shelf life and act as supplement to immune system compromised individuals (Chuang *et al*., 2007). Para probiotics have been previously referred in literature as "inactivated probiotics" and "ghost probiotics" (Tsilingiri and Rescigno, 2013; Tsilingiri *et al*., 2012). The term "postbiotics" put forward to define the product which has non-viable bacteria or metabolic by products from probiotic microorganisms that have biologic activity in the host.

Bioactive Para probiotic yogurt is an innovative functional dairy food product which is made by fermentation of milk by adding of starter culture *(Lactobacillus gasseri: Lactobacillus acidophillus)* and by incorporating bioactive component such as Aloe Vera to boost the health benefits of yogurt by hosting the bioactive substance. The probiotic yogurt is pasteurized to get Para probiotic yogurt at suitable temperature and time to inactivate the live cells. Therefore, in this study the optimization of those parameters has been studied i.e., inoculum concentration, cell count, pasteurization temperature, bioactive component concentration and nutritional parameters were optimized to develop a good quality of yogurt.

In Aloe Vera, about 200 active compounds have been recorded including vitamins, amino acids, minerals, enzymes, polysaccharides, fatty acids and more. The most powerful polysaccharide in Aloe Vera is acetylated mannose or acemannan, which is used in European AIDS treatment. Fatty Acids such as gamma-linoleic acid, reduce inflammation, allergic reactions, blood platelet aggregation and improve wound healing.

II RESEARCH MATERIAL AND METHODOLOGY

2.1 Materials

The experiment took place at MIT College of Food Technology, Laboratory of MIT ADT University, Loni Kalbhor, Pune.

2.1.1 Milk

Different categories of milk from various animals were used for production of yogurt. In this project cow milk was chosen particularly "Arogya" brand was used. Arogya cow milk with 3% fat which was launched in 1905 and since then, has won the trust of millions of customers in Tamil Nadu, Karnataka and Andhra Pradesh. To ensure the development of the yogurt culture, the criteria for the raw milk selection was Low bacterial count, free from antibiotics, sanitizing chemicals, colostrum, rancid milk and there should not be any microbial contamination.

2.1.2 Skim milk powder

Govind skim milk powder was used for making the yogurt for adjustment of total solids which contains 0.5% of fat which was purchased from Loni kalbhor market.

2.1.3 Starter culture

The starter cultures were used for making of yogurt was *Lactobacillus gasseri* and *Lactobacillus acidophillus.* The bacterial culture of both the bacteria was purchased from National Chemical Laboratory, Pune. Both the bacteria are Gram positive rod shaped and preserved at refrigeration temperature (4ºC) to slow the physical, chemical and microbiological degradation. For the sub culturing of these bacteria the medium was used as per the recommendation given by NCL, Pune. The inoculum was added in medium and kept in orbital shaking incubator for overnight at 37^0C for activation of culture.

2.2 Methodology

2.2.1 pH

Procedure:

pH is a measurement of H^+ ion activity, this measures active acidity. pH was determined by measuring the electrode potential between glass and reference electrodes; pH meter was standardized using standard pH buffers. Homogenized sample were used for the determination of pH (FSSAI, 2016).

2.2.2 Determination of Moisture

Procedure:

The main principle is that the moisture of the sample is lost by volatilization caused by heat. Amount of material left after the removal of moisture is a dry matter. clean oven dried petri-plates and place them in desiccators for cooling. Weigh the sample in the petri dish and keep in oven at 105º C with lids open until a constant weight loss. Place the dishes in desiccators and weigh after cooling (Ranganna, 2017).

$$\text{Moisture content(\% mc)} = \frac{W1 - W2}{W1} \times 100$$

2.2.3 Fat Content

Procedure:

The sample was weighed (5 g) accurately in thimble and defatted with petroleum ether (40-60°C) in Soxhlet extraction apparatus. The resultant ether extract was evaporated and fat content was calculated (A.O.A.C, 2000).

$$\text{Fat (\%)} = \frac{W3 - W1}{W2} \times 100$$

2.2.4 Protein content

Procedure:

The protein was determined by Micro-Kjeldahl method using 0.2 g of ground sample (moisture and fat free) sample was digested with concentrated sulfuric acid (H_2SO_4) with catalyst mixture for 3-4 h at 100°C. Then it was distilled with 40 per cent NaOH and liberated ammonia was trapped in 4 per cent boric acid and titrated with 0.1 N HCl using mixed indicator (Methyl red: Bromocresol green: 1:5). The per cent nitrogen was calculated and per cent protein was estimated in the sample by multiplying with factor 6.25 (A.O.A.C, 2000).

$$\text{Nitrogen (\%)} = \frac{(\text{Sample titre} - \text{Blank titre}) \times \text{Normality of HCl} \times 14}{\text{Weight of sample} \times 1000} \times 100$$

% Protein = 6.25 x % Nitrogen

2.2.5 Ascorbic acid

The ascorbic acid content was determined by Assay method given by Ranganna (1986) as portrayed below:

Procedure:

Preparation of sample: 10 g/ml of sample was mixed with 100 ml of 3% HPO_3. It was then filtered. An aliquot (10ml) of the sample was measured and titrated against standard dye till pink color was observed as end point, which persisted for 15 s. The Ascorbic Acid content of the sample was calculated by using the following formula:

$$\text{Ascorbic acid(mg/100 g)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of sample taken for estimation} \times \text{Weight of sample taken for estimation}} \times 100$$

2.2.6 Carbohydrates

The carbohydrate was estimated by anthrone method.

Procedure:

Get dry and clean test tubes and mark all the tubes as per the protocol. Pipette out 0.1-0.5 ml of glucose standard solution in duplicate test tubes. In a one test tube add only 1 ml of distilled water and mark it as blank. Make the volume up to 1 ml in each test tube by adding distilled water. After that add a 3 ml of anthrone reagent to each one of the test tubes and mix it thoroughly. Heat the test tubes for 8 min in a boiling water bath. Cool rapidly and read the green to dark green color at 630 nm. Draw a standard graph by plotting concentration of the standard on the X-axis and absorbance on the Y-axis. From the graph, calculate the amount of carbohydrate is present in the sample tube.

$$\text{Carbohydrate content in 100 mg of sample} = \frac{\text{mg of glucose} \times 100}{\text{Volume of test sample}}$$

2.2.7 Sensory evaluation

Sensory evaluation of Bioactive Para probiotic yogurt was done by panel of semi trained judges on 9-point hedonic scale.

2.3 Flow chart for preparation of Bioactive Para probiotic yogurt

Raw milk (cow milk)
↓
Modifying the Total solids of milk (23 % of skimmed milk powder)
↓
Pasteurization (63°C for 30 minutes)
↓
Cooling (38°C)
↓
Addition of bacterial activated culture (2% *L. gasseri*: *L. acidophillus)*
↓
Addition of bioactive component (2 % of Aloe Vera)
↓
Incubation (43°C for 8 h)
↓
Pasteurization (70ºC for 30 min)
↓
Refrigeration (4ºC)

Fig. 2.3 Flow chart for preparation of Bioactive Para probiotic yogurt.

2.3.1 Procedure of Preparation of Bioactive Para probiotic yogurt

2.3.2. Raw milk

The cow milk was used for making yogurt which consists of 3 % fat and 5 % of protein.

2.3.3. Modifying total solids of milk

Composition was adjusted to achieve the desired fat and solid content. 23% of skimmed milk powder was added to increase the amount of whey protein to provide a desirable texture.

2.3.4 Pasteurization of milk

The milk was pasteurized at 63ºC for 30 minutes. High heat treatment is used to denaturized the proteins. This allows to form a more stable gel, which prevents separation of the water during storage. Treatment of high heat also further reduces the quantity of spoiling organisms in a milk to provide a best environment for the starter cultures to enhance and grow. Yogurt was pasteurized before the starter cultures was added to ensure that the cultures remain active in the yogurt.

2.3.5. Cooling of Milk

The milk was cooled to 38ºC to bring the yogurt to the ideal growth temperature for the starter culture.

2.3.6 Addition of starter culture

The starter cultures were inoculated into the cold milk. The cultures used are *Lactobacillus gasseri: Lactobacillus acidophillus* (2%).

2.3.7. Addition of bioactive component

Aloe Vera as a bioactive component was added into the cooled milk (2%). The addition of aloe Vera increases the nutritional value of yogurt.

2.3.8 Incubation

After inoculation of starter culture and bioactive compound immediately the milk was incubated at 43ºC in the incubator for 8 hours. This allowed the fermentation to progress to form a soft gel and the characteristic flavor of yogurt.

2.3.9 Pasteurization

Pasteurization was carried out for inactivation of microorganisms to produce Para probiotics. The yogurt was pasteurized at 70ºC for 30 min.

2.3.10 Refrigeration

Bioactive Para probiotic yogurt was refrigerated at 4ºC**.**

III. RESULT AND DISCUSSION

3.1 Treatment details:

3.1.1 Inoculum concentration

By increasing the percentage of inoculum concentration above 2% the yogurt has sour taste due to change in acidity. So, from the trails it was confirmed that maximum starter culture percentage would be 2% (Deepa *et al.*, 2016). (Champagne and Gardener, 2005) analysed that by increasing the starter culture to 2.5% the yogurt forms sour in taste.

Table No 3.1.1.1 Optimization of inoculum concentration of yogurt.

Sample	Vitamin C (mg/100g)	Fat (% dry Basis)	pH	Moisture (%)	Protein (g/100g)
P-1:1	**6.248**	**6.85**	**5.82**	**75.573**	**5.25**
P-2:1	6.248	6.25	4.98	77.195	5.25
P-1:2	6.248	6.95	5.21	77.992	5.42
P-2:2	6.248	6.2	5.76	76.22	4.55

P- Probiotic
P-1:1 – 1% *Lactobacillus gasseri:1% Lactobacillus acidophilus*
P-2:1 – 2% *Lactobacillus gasseri:1% Lactobacillus acidophilus*
P-1:2 – 1% *Lactobacillus gasseri:2% Lactobacillus acidophilus*
P-2:2 – 2 % *Lactobacillus gasseri:2% Lactobacillus acidophilus*

Table No 3.1.1.2 Microbial analysis of yogurt pasteurized at different temperature (*L. gasseri*)

Pasteurization Temperature (ºC)	Time (min)	Bacteria (10^{-1} cells / ml)	Bacteria (10^{-2} cells / ml)	*L. gasseri* (10^{-1} cells/ml)	*L. gasseri* (10^{-2}cells/ ml)
P-60	30	59	44	46	27
P-70	**30**	-	-	-	-
P-80	30	06	-	09	02
P-90	30	-	12	-	-
P-100	30	-	14	-	-

P- Probiotic

In case of yogurt samples, the lowest count of microorganisms was found in P-70. The Lactobacillus population was observed maximum in P-60 yogurt sample. But it was noted that, there was no yeast growth in all pasteurized yogurt samples. *Miyazawa et al*., 2011 had reported that inactivation of *L. gaserri* at 70ºC for 30 min have immunomodulatory effect. So, from the trails it was confirmed that P-70 was the best.

Table No 3.1.1.3 Microbial analysis of yogurt pasteurized at different temperatures *(L. acidophillus*)

Pasteurization Temperature (ºC)	Time (min)	Bacteria (10^{-1} cells / ml)	Bacteria (10^{-2} cells / ml)	*L. acidophillus* (10^{-1} cells/ml)	*L. acidophillus* (10^{-2} cells/ ml)
P-60	30	13	08	38	05
P-70	**30**	-	-	-	-
P-80	30	-	03	-	-
P-90	30	06	-	-	-
P-100	30	-	-	-	-

P-Probiotic

Initially, the experiment was conducted to optimized pasteurization temperature of yogurt samples. Lactobacillus population was observed maximum in yogurt sample P-60. But it was noted that there was no yeast growth in all yogurt samples. (Ou *et al*., 2011) stated that higher in the pasteurization temperature applied greater the roughness and degree of coarseness of cells which influences their immune modulating properties. So, from these trails it was confirmed that P-70 was the best yogurt sample.

3.1.2 Fermentation Time

Table 3.1.2.1 Nutritional analysis of yogurt for different fermentation time

Sample at different fermentation time (h)	Vitamin C (mg/100g)	Fat (% dry Basis)	pH	Moisture (%)	Protein (g/100g)
PP-0	2.856	4.75	4.81	80.32	3.99
PP-4	2.856	4.90	4.07	79.35	4.13
PP-8	**5.712**	**5**	**4.71**	**78.11**	**4.41**
PP-16	5.712	5.25	4.98	77.80	4.20
PP-20	5.712	5.2	5.01	77.02	4.13
PP-24	5.712	5.5	5.12	75.80	4.34

PP-Para probiotic

These heterogeneous cultures took nearly (4, 8, 16, 20, 24) hours to form yogurt at set incubation period (43ºC). The yogurt sample PP-8 (8 hours) showed good quality and highest protein content (4.41 g/100g). The incubation temperature (43ºC) took minimum time of 8 h. Chandan (1999) have reported similar results for production of yogurt with *Lactobacillus* cultures which took an incubation time of 8.5 h at 43ºC for yogurt formation. So, from this trial it was confirmed that maximum fermentation period would be 8 h.

3.1.3 Bioactive component

Table No 3.1.3.1 Nutritional analysis of yogurt for different concentration of bioactive component (Aloe Vera)

Sample (%)	Vitamin C (mg/100g)	Fat (% dry Basis)	pH	Moisture (%)	Protein (g/100g)
PP-0	5.712	6.0	4.48	76.38	4.025
PP-1	5.712	6.3	4.95	77.31	5.42
PP-2	**5.712**	**6.0**	**5.03**	**77.01**	**5.60**
PP-3	5.712	5.5	5.10	77.48	5.82
PP-4	5.712	5.7	5.17	77.83	6.0

PP-Para probiotic

Initially this experiment was conducted to optimize the concentration of bioactive component in yogurt sample. This was done by varying the concentration of bioactive component (1, 2, 3, 4 %). The yogurt sample P-4 showed maximum content of protein (6.0 g/100g). Chauhan *et al*., (2007) reported that Aloe Vera more than 2% recorded lowest sensory score. So, from this trail it was confirmed that the maximum percentage of bioactive component would be 2%. Due to increase in the bioactive component concentration above 2% the yogurt formed bitter in taste**.** so, from this trail it was confirmed that yogurt sample PP-2 was the best.

Table No 3.2 Optimized parameters

Parameters	Results
Inoculum concentration	2%
Incubation time	8 hours
Pasteurization temperature	70^0C
Bioactive component concentration	2%

3.3 Sensory evaluation of yogurt

Table 3.3.1 Sensory evaluation of yogurt

Sr. No.	Sample Code	Appearance/ Color	Texture	Flavor	Taste	Overall acceptability
1	C	7	7	7.5	8	7
2	PP	7	8	7	8	8.5
3	BPP	7	8	7	8	8

C – Control
PP- Paraprobiotic
BPP- Bioactive Paraprobiotic

3. 4 Proximate analysis of selected Bioactive Para probiotic yogurt

Table 3.4.1 Proximate analysis of selected Bioactive Para probiotic yogurt

Sr. no.	Properties	Result (Per 100g)
1	Protein (g/100g)	5.60
2	Fat (% dry basis)	5.0
3	Carbohydrate (g/100g)	4.7
4	Moisture (%)	78.11
5	Vitamin C(mg/100g)	5.7

IV. SUMMERY AND CONCLUSION

This research work on "Studies on Production of Bioactive Para Probiotic Food Product" was to develop the nutritional quality of yogurt. This research was aimed for production of probiotic, Para probiotic and bioactive Para probiotic yogurt shown variable results. For the preparation of Bioactive Para probiotic yogurt, the parameters were optimized such as inoculum concentration (2%), Pasteurization temperature(70^{o}C), fermentation period (8 hours) and Bioactive component concentration (2%). Therefore, from this research work it was concluded that functional foods play an important role for future development in human diet. Several health benefits are related to the ingestion of probiotics foods. The health benefits related to Para probiotic are inhibition of pathogens, recovery of intestinal injuries, immune modulation, reducing lactose intolerance and cholesterol reduction.

REFERENCES

[1] Akpan, U. G., Mohammed, A. D. and Aminu, I. (2007). Effect of preservative on the shelf life of yoghurt produced from soya beans milk. *Leonardo Electronic Journal of Practices and Technologies*, *11*, 131-142.
[2] Ananta, E. and Knorr, D. (2009). Comparison of inactivation pathways of thermal or high pressure inactivated Lactobacillus rhamnosus ATCC 53103 by flow cytometry analysis. *Food microbiology*, *26*(5), 542-546.
[3] AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washington DC.
[4] Awad, H., Mokhtar, H., Imam, S. S., Gad, G. I., Hafez, H. and Aboushady, N. (2010). Comparison between killed and living probiotic usage versus placebo for the prevention of necrotizing enterocolitis and sepsis in neonates. *Pakistan journal of biological sciences: PJBS*, *13*(6), 253-262.

[5] Champagne, C. P., Gardner, N. J. and Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. *Critical reviews in food science and nutrition*, *45*(1), 61-84.
[6] Chandan, R. C. (1999). Enhancing market value of milk by adding cultures. *Journal of Dairy Science*, *82*(10), 2245-2256.
[7] Chauhan, O. P., Raju, P. S., Khanum, F. and Bawa, A. S. (2007). Aloe vera: Therapeutic and food applications. *Indian food industry*, *26*(3), 43-51.
[8] Chuang, L., Wu, K. G., Pai, C., Hsieh, P. S., Tsai, J. J., Yen, J. H. and Lin, M. Y. (2007). Heat-killed cells of lactobacilli skew the immune response toward T helper 1 polarization in mouse splenocytes and dendritic cell-treated T cells. *Journal of agricultural and food chemistry*, *55*(26), 11080-11086
[9] Deepa, J., Preetha, P. and Rajkumar, P. (2016). Development of yoghurt with bioactive molecules. *International Journal of Food and Fermentation Technology*, *6*(2), 397-403.
[10] Fujiki, T., Hirose, Y., Yamamoto, Y. and Murosaki, S. (2012). Enhanced immunomodulatory activity and stability in simulated digestive juices of Lactobacillus plantarum L-137 by heat treatment. *Bioscience, biotechnology, and biochemistry*, *76*(5), 918-922.
[11] Fuller, R. (1989). Probiotic in man and animals. *J. Appl. Bacteriol.*, *66*, 131-139.
[12] Grześkowiak, Ł., Collado, M. C., Beasley, S. and Salminen, S. (2014). Pathogen exclusion properties of canine probiotics are influenced by the growth media and physical treatments simulating industrial processes. *Journal of applied microbiology*, *116*(5), 1308-1314.
[13] Jin, L. Z., Ho, Y. W., Abdullah, N, and Jalaludin, S. (1998). Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing Lactobacillus cultures. *Poultry science*, *77*(9), 1259-1265.
[14] Jin, L. Z., Ho, Y. W., Abdullah, N. and Jalaludin, S. (2000). Digestive and bacterial enzyme activities in broilers fed diets supplemented with Lactobacillus cultures. *Poultry science*, *79*(6), 886-891.
[15] Jin, L. Z., Ho, Y. W., Abdullah, N. and Jalaudin, S. (1996). Influence of dried Bacillus substillis and lactobacilli cultures on intestinal microflora and performance in broilers. *Asian-Australasian Journal of Animal Sciences*, *9*(4), 397-404.
[16] Kalavathy, R., Abdullah, N., Jalaludin, S. and Ho, Y. W. (2003). Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *British Poultry Science*, *44*(1), 139-144.
[17] Kamiya, T., Wang, L., Forsythe, P., Goettsche, G., Mao, Y., Wang, Y. and Bienenstock, J. (2006). Inhibitory effects of Lactobacillus reuteri on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut*, *55*(2), 191-196.
[18] Mckinley, M. C. (2005). The nutrition and health benefits of yoghurt. *International journal of dairy technology*, *58*(1), 1-12.
[19] Miyazawa, K., He, F., Kawase, M., Kubota, A., Yoda, K. and Hiramatsu, M. (2011). Enhancement of immunoregulatory effects of Lactobacillus gasseri TMC0356 by heat treatment and culture medium. *Letters in applied microbiology*, *53*(2), 210-216.
[20] Murry, A. C., Hinton, A. and Buhr, R. J. (2006). Effect of botanical probiotic containing lactobacilli on growth performance and populations of bacteria in the ceca, cloaca, and carcass rinse of broiler chickens. *International Journal of Poultry Science*, *5*(4), 344-350.
[21] Ou, C. C., Lin, S. L., Tsai, J. J. and Lin, M. Y. (2011). Heat-killed lactic acid bacteria enhance immunomodulatory potential by skewing the immune response toward Th1 polarization. *Journal of food science*, *76*(5), M260-M267.
[22] Ranganna, S. (1986). *Handbook of analysis and quality control for fruit and vegetable products*. Tata McGraw-Hill Education.
[23] Ranganna, S. (2017) *Handbook of analysis and quality control for fruits and vegetable products*. 2nd edition. Tata McGraw-Hill Publishing Company Ltd., New Delhi.
[24] Shin, H. S., Park, S. Y., Lee, D. K., Kim, S. A., An, H. M., Kim, J. R. and Lee, K. O. (2010). Hypocholesterolemic effect of sonication-killed Bifidobacterium longum isolated from healthy adult Koreans in high cholesterol fed rats. *Archives of pharmacal research*, *33*(9), 1425-1431.
[25] Taverniti, V. and Guglielmetti, S. (2011). The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). *Genes & nutrition*, *6*(3), 261.
[26] Tsilingiri, K, and Rescigno, M. (2013). Postbiotics: what else? Benef Microbes 4: 101–107.
[27] Tsilingiri, K., Barbosa, T., Penna, G., Caprioli, F., Sonzogni, A., Viale, G. and Rescigno, M. (2012). Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. *Gut*, *61*(7), 1007-1015.
[28] Zulkifli, I., Abdullah, N., Azrin, N. M. and Ho, Y. W. (2000). Growth performance and immune response of two commercial broiler strains fed diets containing Lactobacillus cultures and oxytetracycline under heat stress conditions. *British poultry science*, *41*(5), 593-597.