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Oil Meals: A Novel Cheap Source of Prebiotic Food Materials

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ABSTRACT

Oil seed meals are mainly used for animal feedstock but there is a huge potential for using these meals as prebiotic food material. Three oil seed meals, namely ricebran, mustard and sesame have been identified as prebiotic food material in the present study. Three lactobacillus strains (*Lactobacillus plantrum* MTCC 2941, *Lactobacillus rhamnosus* MTCC 1408, *Lactobacillus casei* NCIM 2651) were identified to show significant growth on these oil seed meals in absence of any other usual carbon and nitrogen sources in the growth medium. Prebiotic activity score was calculated for each oil seed meal and compared with Inulin; the most marketed prebiotic material and the results obtained for the oil seed meals are very encouraging. Our study shows that oil seed meal has excellent potential as prebiotic food material as they support the growth of probiotic bacteria along with an antagonistic effecton the pathogenic bacteria like*Eschericchia Coli*.

Keywords: *Escherichia Coli,* lactobacillus, Oil meals, prebiotic food material.

1. INTRODUCTION

Rapid population increase and expanding urbanization demands alternative non-conventional sources of food for all over the world. Edible oil seed meals or cakes obtained after oil extraction can play an important role in this aspect as the meals contain sufficient amount of proteins, dietary fibre, carbohydrates and minerals (1, 2). Till date these oilseed cakes are used mainly as animal feed, plant fertilizer and soil compost. In recent past a strong interest has been observed to utilize these oilseed cakes as food for humans. This is not only due to increasing global population and increase in food prices but also due to the attractive nutritional information recently available on these by-products (3-6).

Sesame (Sesamum indicum) oil meal or oil cake, a protein rich by product along with dietary fibre and carbohydrate has been identified as a valuable source of protein for animals (7, 8) but are used mainly as a feedstock, especially for ruminants and poultry. high level of sulphur-containing amino-acids, methionine and cystine, lack of trypsin inhibiting factors and its pleasant flavour has enhanced its importance as an alternative sources of human food.

Sesame meal is presently used in ready-to-use infant foods like *cholam* and *samai* porridge which has been developed by the ICMR. Fermented foods like *vada* and *dosa*, popular South Indian foods, have also been prepared by utilising sesame flour. The sesame-supplemented *dosa* is organoleptically acceptable food which contains higher levels of methionine than the plain *dosa* (9). Sesame flour is also used for the preparation of bread, cookies, high-protein beverages, high-protein biscuits etc.

Rice bran meal or cake is a by-product generated from rice bran after the extraction of oil from rice bran (10). This cake has been considered as a waste product of rice milling and utilized as a low-cost animal food for poultry, cattle, fish, and also used as fuel for boilers, manufacturing sodium silicate, silica gel, insulation bricks etc.

But due to presence of high amount of protein, dietary fibre and bioactive compounds, it can be converted to value-added foods and their addition in different foods will increase the overall nutritional quality of the processed foods. Different types of snacks such as bread, cakes, noodles and pasta have been prepared by using rice bran cake without hampering the functional and textural properties of the product.

Mustard cake is also produced during extraction of mustard oil. Mustard cake is widely used as a fertilizer for flowering and vegetable plants (11, 12). It is also used in the feeding of cattle and buffaloes. Apart from fertilizer and animal feedstock, mustard cake can be utilized as natural weedicide (13, 14). But mustard cakes are very rich in proteins, carbohydrates and dietary fibre for which it can be used as non-conventional human food. It is also a good source of bioactive components including phenolics, glucosinolates and phytates (15, 16). Mustard meal has its application as a fermentation medium for preparation of enzyme, mushroom and lactic acid production (17-21). Several researchers (22-24) isolated the protein concentrate from mustard cake and used it in different functional food ingredients.

Apart from these seed meals have a huge potential as a prebiotic food source where prebiotic activity can be identified with the help of lactobacillus strains.The present study deals to investigate the potential of the three seed meals (mustard, rice ban and sesame) as prebiotic food source using different lactobacillus strains followed by product development from best seed meal based on the experimental results. Sensory and physiochemical characteristic study of the product were also conducted.

2. EXPERIMENTAL

A. Materials

Three oil seed meals namely ricebran, mustard and sesame were collected from G.M. Oil Mills, Mallarhat &Vinayak Oil Mills, Howrah for prebiotic screening. For preparation of mustard meal flour, mustard seed (white variety) purchased from local market, deoiled using food grade hexane and powdered in a kitchen mixer grinder.

Lactobacillus strains like *Lactobacillus casei*(NCIM 2651) was purchased from NCL, Pune whereas *Lactobacillus rhamnosus* (MTCC 1408) and *Lactobacillus plantrum* (MTCC 2941) were purchased from IMTECH, Chandigarh. The cultures were activated and maintained in MRS agar medium. The enteric pathogen *Escherichia coli* (NCIM 2067) purchased from NCL, Pune. The pathogen was cultured in TSB (Trypticase soya broth).Sodium carbonate, HCl, H_2SO_4 and methanol of AR grade were used. Commercial wheat flour, mustard seeds purchased from local market. Food grade quality Hexane and sodium chloride were used in flour and dough preparation.

B. Methods

Proximate analysis for measuring moisture, ash, crude fibre, protein and fat content were done following the methods as described in AOAC,1995 (25).

Estimation of total carbohydrate: Total carbohydrate was measured by hydrolyzing the polysaccharides into simple sugars with acid hydrolysis and estimating the resultant monosaccharide (26). 100 mg sample was taken in a boiling tube and hydrolyzed by keeping it in boiling water bath for 3 h with 5 mL of 2.5 N HCl. The sample was cooled to room temperature and neutralized with solid Na_2CO_3 until effervescence stops. The volume of the mixture was adjusted to 100 mL and centrifuged. About 0.1 and 0.2 mL of the supernatant solution was taken in two separate test tubes and the volume was adjusted to 1 mL with distilled water. 1 mL of 5% phenol solution and 5 mL of 96% $H_2SO_4$was added to each test tube. The solution was stand for 10 min, shaken well and placed in water bath at 25˚C for 20 min. The colour of the solution was measured at 490 nm with the aid of UV-VIS-Spectrophotometer (Jasco, Japan, V630). Standard curve was prepared using standard glucose solution.

Protein Estimation: 2 gm of the sample is taken in a 500 ml Kjeldahl flask to which 0.7 gm of Mercuric oxide, 15 gm of potassium sulphate and 40 mL of concentrated sulphuric acid were added. The flask was placed in an inclined position in the digestion chamber. The flask is gently heated in a low flame until the initial frothing ceases and the mixture boils steadily at a moderate rate. During heating rotate the flask several times. Heating was continued for approximately one hour until the colour of the digest is pale blue. After that, 200mlof water was slowly added to the cooled mixture. Sufficient NaOH solution (450gm/L) was carefully pour down the side of the flask to make the contents strongly alkaline (about110mL) before mixing the acid and alkaline layer.

Then the flask was connected to a distillation apparatus. A delivery tube is connected to the condenser, which dips just below the surface standard acid contained taken in a conical flask receiver. The contents of the digestion flask were boiled until 150mL have distilled into the receiver. The solution was titrated with 0.1N NaOH solution using methyl red indicator. A blank titration was carried out simultaneously.

1ml of 0.1$(NH)_4SO4$ = 0.0014gm Nitrogen
Protein = Nitrogen x 6.25

Culture Media: The ability of the lactobacillus strains to utilize the substrates under study (sesame, mustard and ricebran oil seed meal) were examined in modified MRS medium without Dextrose but supplemented with 2% of the oil seed meals. 0.05% of L-cysteine was also added to maintain anaerobic condition.

Preparation of MRS Broth & MRS agar (Control): 10 g peptone, 10 g meat extract, 5 g yeast extract, 20 g D-glucose, 1 g tween 80, 2 g K_2HPO4, 5 g sodium acetate, 2 g tri-ammonium citrate, 0.2 g $MgSO_4$. $7H_2O$ and 0.05 g $MnSO_4.4H_2O$ were dissolved in 1000ml distilled water and the pH was adjusted at 6.2-6.6 and sterilized. MRS agar was prepared by adding 1.5% agar agar in the culture media and sterilized.

Preparation of MRS Broth without glucose supplemented with only oil seed meals: 20g of oil seed meals,10 g peptone, 10 g meat extract, 5 g yeast extract, Tween 80: 01.0g K_2HPO_4: 02.0g, Sodium acetate: 05.0g, Tri-ammonium citrate 02.0g, $MgSO_4$. $7H_2O$: 0.2g, $MnSO_4.4H_2O$: 0.05g were dissolved in 1000ml distilled water pH was adjusted at 6.2-6.6 and sterilized.

MRS broth without glucose supplemented with deoiled oil seed meals: 20g of deoiled oil seed meals, 10 g peptone, 10 g meat extract, 5 g yeast extract, Tween 80: 01.0g, K_2HPO_4: 02.0g, Sodium acetate: 05.0g, Tri-ammonium citrate 02.0g, $MgSO_4$. $7H_2O$: 0.2g, $MnSO_4.4H_2O$: 0.05g were dissolved in 1000ml distilled water pH was adjusted at 6.2-6.6 and sterilized.

MRS broth without glucose and nitrogen source supplemented with oil seed meals: 30g of oil seed meals mixed in a solution containing in 1000ml distilled water K_2HPO_4: 02.0g, Sodium acetate: 05.0g, Tri-ammonium citrate 02.0g, $MgSO_4$. $7H_2O$: 0.2g, $MnSO_4.4H_2O$: 0.05g and Tween 80: 01.0g. pH was adjusted at 6.2-6.6 and sterilized.

Prebiotic Screening: 1ml fresh culture of each lactobacillus strain grown on MRS broth for 24 hours was added to 50 ml of the above mentioned culture medium in conical flasks. Anaerobic environment was maintained within the flask by purging CO_2 gas into the flask. After 24 hours of growth 1ml culture from each flask was diluted and spread onto the MRS agar plates to give approximately 30-300 colonies. The plates were incubated anaerobically for 24 hours at 37˚C-40˚C. Colonies were counted using a digital colony counter after 24 hours of growth in the agar plate.

Fresh culture of enteric pathogen *E. Coli*(NCIM 2067) grown under similar condition and compared with lactobacillus strains.

CFU/mL = Number of colonies counted / (dilution factor x volume of sample plated for analysis)

Determination of prebiotic activity score
By definition, higher prebiotic score denotes that the prebiotic molecules support the growth of probiotic bacteria and does not support the growth of pathogenic bacteria in the presence of prebiotic molecules. Lower prebiotic score denotes that the prebiotic molecules support the growth of pathogenic bacteria and does not support the growth of probiotic bacteria in the presence of prebiotic molecules.

Calculation of Prebiotic activity score (27) =

$$\frac{[\text{probiotic } \log\frac{\text{cfu}}{\text{ml}} \text{on the prebiotic at 24 hr} - \text{probiotic } \log\frac{\text{cfu}}{\text{ml}} \text{on the prebiotic at 0 hr}]}{[\text{probiotic } \log\frac{\text{cfu}}{\text{ml}} \text{on glucose at 24 hr} - \text{probiotic } \log\frac{\text{cfu}}{\text{ml}} \text{on glucose at 0 hr}]}$$

$$\frac{[\text{enteric } \log\frac{\text{cfu}}{\text{ml}} \text{on the prebiotic at 24 hr} - \text{enteric } \log\frac{\text{cfu}}{\text{ml}} \text{on the prebiotic at 0 hr}]}{[\text{enteric } \log\frac{\text{cfu}}{\text{ml}} \text{on glucose at 24 hr} - \text{enteric } \log\frac{\text{cfu}}{\text{ml}} \text{on glucose at 0 hr}]}$$

Preparation of mustard meal flour: Deoiled mustard seed meals were washed with water to remove any foreign particles. The meal were spread thin in trays and dried at 55˚C using a cross flow drier for 12 h to a moisture content around 10%. The dried meal was powdered and passed through 80-mesh sieve.

Making of chapattis/roti: 100 gm mixture of refined flour and deoiled mustard meal flour were taken in different proportions and mixed with 0.05 gm salt & 50 ml of water. A homogeneous mass was prepared and kneaded with hand to get a soft and pliable consistency. The mass is allowed to rest to 20 minutes. After that few balls were prepared from the dough. Each ball was placed on a flat surface and rolled out to a thickness of 0.5 cm with the help of a rolling pin. The chapattis were place a pan on medium flame. A pair of tongs was used to flip over to the other side. The chapattis were cooked from both sides on medium flame till little brown spots appear. Once done, they were wrapped in Aluminium foil and kept for sensory evaluation.

Values are reported as mean ± s.d., where n=3 (n=no of observation).

3. RESULTS AND DISCUSSIONS

C. Analysis of seed meals

Table 1 shows the characteristics of rice bran, mustard and sesame seed meal (100 g each) after extraction of oil. It has been observed from Table 1 that protein content is higher in mustard and sesame seed meal than rice bran meal. Mustard seed meal is also rich in dietary fiber of around 26.78% whereas rice bran and sesame seed meal contained 21.76% and 23.11% respectively. Rice bran meal contained maximum amount of carbohydrate (32.87%) whereas mustard and sesame contained nearly half amounts of carbohydrate e.g. 17.57% and 16.37% respectively.

Table 1: Characteristics of seed meals

Component	Rice bran (100 g)	Mustard (100 g)	Sesame (100 g)
Moisture	3.67±0.11	2.19±0.09	6.54±0.12

Protein	13.78±0.73	21.62±0.17	26.6±0.31
Fat	12.21±0.59	10.23±0.16	7.5±0.13
Crude fiber	7.25±0.22	12±0.43	12±0.27
Dietary fiber	21.76±0.39	26.78±0.53	23.11±0.14
Carbohydrate	32.87±0.31	17.57±0.19	16.37±0.24
Ash	5.07±0.09	6.9±0.11	5.07±0.07
Iron	27.12 mg	11.56 mg	16.56 mg
Calcium	49.67 mg	37.87 mg	36.98 mg

D. Analysis of growth of prebiotic bacteria

Growth of prebiotic bacteria on different seed meal was examined on rice bran, mustard and sesame seed meal for 24 hrs and comparative study of their growth is shown in Table 2. It has been observed from Table 2 that *Lactobacillus plantrum* and *Lactobacillus casei* showed highest growth (cfu/ml) in mustard seed meal but for *Lactobacillus rhamnosus* the growth is lowest in mustard seed meal compared to other seed meals. *Lactobacillus rhamnosus* showed highest growth in sesame seed meal.

Table 2: Comparative analysis of growth of prebiotic bacteria on seed meals after 24 hours of growth

Probiotic bacteria	Glucose (cfu/ml)	Mustard (cfu/ml)	Sesame (cfu/ml)	Rice bran (cfu/ml)
Lactobacillus plantrum	192×10^5	222×10^5	214×10^5	203×10^5
Lactobacillus rhamnosus	198×10^5	184×10^5	224×10^5	191×10^5
Lactobacillus casei	206×10^5	238×10^5	226×10^5	210×10^5

cfu/ml: Colony forming units/ml of growth medium

Prebiotic activity scores were calculated analyzed with respect to the results of growth of prebiotic bacteria on seed meals in comparison to the growth of enteric pathogen as shown in Figure 1. It has been observed that prebiotic activity score for mustard seed meal is highest among all other seed meals under study.

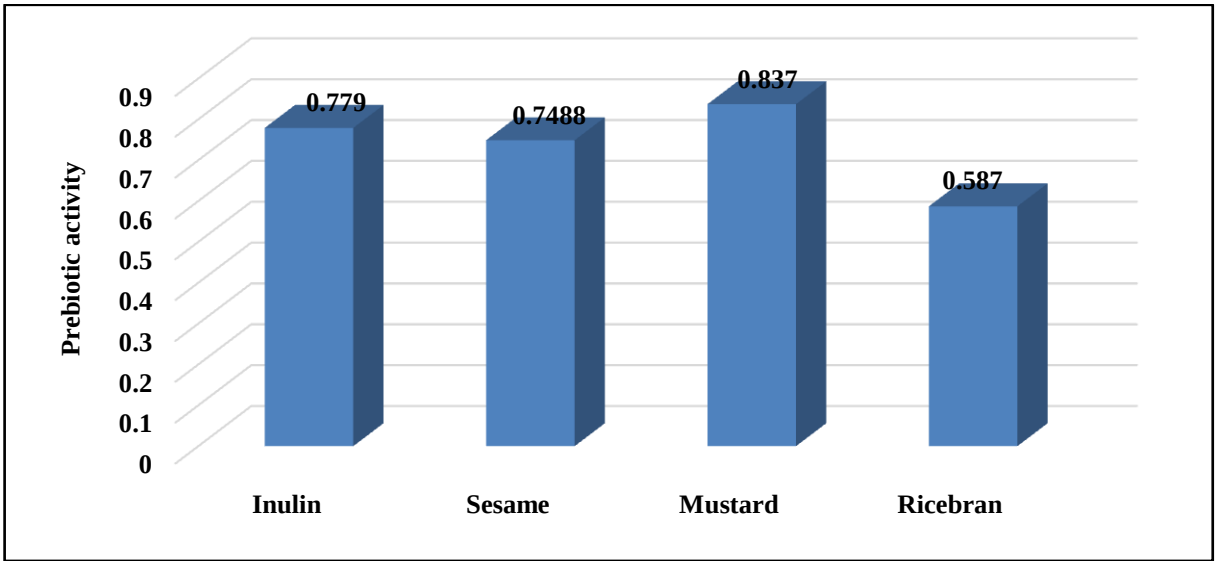

Figure 1. Prebiotic activity score for seed meals

E. Product development

As mustard seed meal have shown the highest prebiotic activity, three flour blends were prepared by mixing normal wheat flour and mustard meal flour in three different ratios as shown in Table 3. Normal wheat flour (A) is taken as control, other three product mixtures B, C and D were prepared on mixing normal wheat flour and mustard meal flour in a ratio of 70:30, 50:50 and 30:70 respectively. Sensory evaluation of the blends was carried out on the basis of appearance, colour, flavor, taste and after taste after preparation of ratios from the blends as shown in Table 4.

It has been found that overall acceptability of product C (50:50) is almost at par with the normal wheat flour A and B. Figure 2 shows the powdered mustard meal flour. A chapatti was prepared from blend C as shown in Figure 3.

Table 3: Flour blends prepared by mixing with wheat flour and mustard meal flour

Flour blends	Wheat flour	Mustard meal flour
A	100	0
B	70	30
C	50	50
D	30	70

Table 4: Mean scores of Sensory Characteristics of products

Blends	Appearance	Colour	Flavor	Taste	Aftertaste	Overall acceptability
A	8.8±0.52	9.1±0.33	8.4±0.39	8.4±0.21	8.7±0.41	8.7±0.36
B	8.3±0.43	8.3±0.42	8.4±0.32	8.4±0.34	8.4±0.43	8.4±0.21
C	8.1±0.31	7.9±0.51	8.3±0.44	8.3±0.11	8.3±0.35	8.2±0.28
D	6.1±0.41	5.1±0.48	6.4±0.50	6.1±0.28	6.0±0.31	6.1.0±0.18

Figure 2. Powdered mustard meal Flour

Figure 3. Chapatti from 50:50 wheat flour and mustard meal flour

CONCLUSION

Mustard seed meal has been identified as the cheap source for chapatti preparation after blending with wheat flour. Among the three seed meals e.g. rice bran, sesame and mustard studied in the present research investigation, mustard

seed meal shows best prebiotic activity score based on results of growth of prebiotic bacteria. Initially, Lactobacillus strains like *Lactobacillus casei*(NCIM 2651), *Lactobacillus rhamnosus*(MTCC 1408) and *Lactobacillus plantrum*(MTCC 2941) were utilized for growth of prebiotic bacteria and mustard seed meal showed highest activity for *Lactobacillus casei* and *Lactobacillus plantrum.* So a good and cheap source has been identified in the present study for the preparation of food material after blending with wheat flour. High quality strains may be developed by the future researchers for the betterment of results obtained.

REFERENCES

[1]. S. H. Tan, R. J. Mailer, C. L. Blanchard and S. O. Agboola, "Canola proteins for human consumption: extraction, profile, and functional properties", J. Food Sci., vol. 76 (1), pp. R16–R28, 2011.
[2]. Y. Yoshie-Stark, Y. Wada and A. Wäsche, "Chemical composition, functional properties, and bioactivities of rapeseed protein isolates", Food Chem., vol. 107, pp.32–39, 2008.
[3]. S. S. The and A. D. Bekhit, "Utilization of Oilseed Cakes for Human Nutrition and Health Benefits", In: Hakeem K., Jawaid M., Y. Alothman O. (eds) Agricultural Biomass Based Potential Materials. Springer, Cham. https://doi.org/10.1007/978-3-319-13847-3_10, 2015.
[4]. A. M. Alashi, C. L. Blanchard, R. J. Mailer, S. O. Agboola, A. J. Mawson, R. He, A. Girgih and R. E. Aluko, "Antioxidant properties of Australian canola meal protein hydrolysates", Food Chem., vol. 146, pp. 500–506, 2014.
[5]. K. L. Chee and M. K. Ayob, "Optimization of hexameta phosphate-assisted extraction and functional characterization of palm kernel cake protein", Food Sci. Tech. Int., vol. 19, pp.109–22, 2013.
[6]. L. Sunil, P. Appaiah, P. K. Prasanth Kumar and A. G. Gopala Krishna, "Preparation of food supplements from oilseed cakes", J. Food Sci. Technol., vol. 52(5), pp. 2998–3005,DOI 10.1007/s13197-014-1386-7, 2015.
[7]. R. Hansen, 2011, Sesame. AgMRC, Iowa State University, USA.
[8]. E. S. Oplinger, E. A. Oelke, J. D. Doll, L. G. Bundy and R. T. Schuler, "Sesame. In: Alternative Field Crops Manual", University of Wisconsin-Extension, Cooperative Extension, 1997.
[9]. G. Chopra, S. K. Man, B. L. Kawaira, C. K. Wahal, and S. Bajaj, "Effects of supplementation of sesame seed on the protein quality of dosa", J. Res. Punjab Agric. Univ., vol. 19, pp. 256 – 262, 1982.
[10]. K. W. Chan, M. H. Khong, S. Iqbal and M. Ismail, "Isolation and antioxidative properties of phenolics-saponins rich fraction from defatted rice bran", Journal of Cereal Science, vol. 57, pp. 480-485, 2013.
[11]. M. S. Ullah, M. S. Islam, M. A. Islam and T. Haque, "Effects of organic manures and chemical fertilizers on the yield of brinjal and soil properties", J. Bangladesh Agr. U., vol. 6(2), pp. 271–276, 2008.
[12]. S. Ibrahim and E. Mumtaz, "Application of agro-waste products as organic and value added biofertilizer for improving plant growth", J. Pharm. Clin. Sci., vol. 8, pp. 35-41, 2014.
[13]. P. D. Brown, M. J. Morra, J. P. McCaffrey, D. L. Auld and L. Williams, "Allelochemicals produced during glucosinolate degradation in soil", J. Chem. Eco., vol. 17, pp. 2021–2034, 1991.
[14]. R. A. Boydston, T. Anderson and S. F. Vaughn, "Mustard (Sinapis alba) Seed Meal Suppresses Weeds in Container- grown Ornamentals", Hort. Sci., vol. 43(3), pp. 800–803, 2008.
[15]. N. S. Devi and W. V. Devi, "Nutrient Analysis of Some Poultry Feedstuffs Locally Available in Manipur, India", World J. Dairy Food Sci., vol. 6(2), pp. 136 – 139, 2011.
[16]. M. Naczk and F. Shahidi, "Phenolic constituents in rapeseed, In: Plant Polyphenols: Synthesis, Properties, Significance", edited by R. W. Hemingway, P. E. Laks and S. J. Branham, (Plenum press, New York), pp. 895 – 910, 1992.
[17]. A. Ebune, S. AI-Asheh and Z. Duvnjak, "Production of phytase during solid state fermentation using Aspergillusficuum NRRL 3135 in canola meal", Bioresource Technol., vol. 53, pp. 7–12, 1995.
[18]. M. Singh and K. Kakkar, "Enhanced lipase production by solid state fermentation using mustard oil cake as substrate", Int. J. Hum. Genetics Med. Biotechnol. Microbiol. Studies, vol. 1(1), pp. 16 – 23, 2012.
[19]. S. B. Imandi, S. K. Karanam, S. Kumari and H. R. Garapati, "Use of Plackett-Burman design for rapid screening of nitrogen and carbon sources for the production of lipase in solid state fermentation byYarrowialipolytica from mustard oil cake (Brassica napus)", Brazillian J. Microbiol., vol. 44(3), pp. 915 – 21, 2014.
[20]. A. Tuli, R. P. Sethi and P. K. Khanna, "Lactic acid production from whey permeate by immobilized Lactobacillus casei", Enzy. Microbial. Technol., vol. 7(4), pp. 164 – 168, 1985.
[21]. Z. Bano, M. N. Shashirekha and S. Rajarathnam, "Improvement of the bioconversion and biotransformation efficiencies of the oyster mushroom (Pleurotussajor-caju) by supplementation of its rice straw substrate with oil seed cakes", Enzy. Microbial. Technol., vol. 15 (11), pp. 985 – 989, 1993.
[22]. M. A. Sadeghi, A. G. A Rao and S. Bhagya, "Evaluation of mustard (Brassica juncea) protein isolate prepared by steam injection heating for reduction of antinutritional factors", LWT- Food Sci. Technol., vol. 39, pp. 911–917, 2006.
[23]. L. L. Diosady, L. Xu and B. K. Chen, "Production of high-quality protein isolates from defatted meals of brassica seeds", US Patent 6, 905,713 B2, 2005.
[24]. L. A. Mejia, "GRAS Notification for Cruciferin-Rich and Napin- Rich Protein Isolates Derived from Canola/Rapeseed (Puratein@ and SuperteinTM), (GRAS Notice (GRN) No. 327), 2010.
[25]. AOAC, 1995, Official methods of analysis. Association of official analytical chemists of the 16th Ed. International, Washington, D.C.,U.S.A.
[26]. M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Reberts and F. Smith, "Colorimetric method for determination of sugars and related substances", Anal. Chem., vol. 28 (3), pp. 350-356, 1956.
[27]. J. Huebner, R. L. Wehling, A. Parkhurst and R. W. Hutkins, "Effect of processing conditions on the prebiotic activity of commercial prebiotics", International Dairy Journal, vol. 18, pp. 287-293, 2008.