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Effect of Bulking Agents, Type of Cultures and Compression Pressures on Functional Properties of Probiotic and Starter Culture Tablets

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Abstract

In the current study freeze dried cultures of *Streptococcus thermophilus* MTCC 5460, *Lactobacillus helveticus* MTCC 5463, *Lactobacillus rhamnosus* MTCC 5462 and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCIM 2358 were used for preparation of tablets to be used as either inocula for product preparation or as dietary supplement. Maltodextrin and spray dried lactose as bulking agents were compared for preparation of active ingredients. Selection of bulking agent was based on the viability, activity, micromeritic properties and stability of the active ingredients prepared. Active ingredients of the cultures were mixed with other excipients and subjected to direct compression at different compression pressures *viz.*, low (1-2 kg/cm^2), medium (3-4 kg/cm^2) and high (5-6 kg/cm^2) to prepare tablets. The tablets were analyzed for hardness, disintegration and viability. Viability and activity of the freeze dried cultures were not significantly affected by the bulking agents indicating a good compatibility of cultures and bulking agents used. Between the two active ingredients containing maltodextrin or spray dried lactose as bulking agent, spray dried lactose showed better flow properties, hence it was selected. A significant ($P<0.05$) increase in the hardness and disintegration time of tablets as well as a significant ($P<0.05$) decrease in the viability of all four cultures were seen when the compression pressure was increased from 1-2 kg/cm^2 to 5-6 kg/cm^2. Hence a compression pressure of 1-2 kg/cm^2 was selected for preparation of culture tablets.

Keywords

Lactobacillus helveticus MTCC 5463, probiotic tablets, starter culture, compression pressure, viability

Introduction

Lactic acid bacteria (LAB) as starter cultures are widely used in dairy industry for production of a variety of fermented milk products. Selected strains of LAB have found application as either probiotics or synbiotics and are used as food ingredient or dietary supplements (1-3). The increasing application of starter cultures and probiotics demand them to be made available to the consumers in dosage forms, which can provide ease of handling, ease of addition to food, precise dosage and functionality for specific application and long term preservation. Current marketing strategies of probiotics dispense them as ingredients in foods, nutritional supplements and as pharma products (3). These probiotic products can be taken either directly or along with food and beverages. Freeze dried powder of starter cultures and/or probiotic cultures in dry form are widely used for fermented product preparation or for preparation of various dosage forms such as tablets and capsules. In such dosage forms, live freeze dried bacterial cells are blended with certain protective as well

as bulking agents. Additionally, certain excipients are also incorporated to facilitate ease of preparation and to get desired dosage form characteristics such as flow properties in case of tablets. In such cases, the functionality of the dosage preparations are largely defined by the survival and activity of the starter cultures and/ or probiotic cultures which need to be maintained throughout the shelf life of the dosage form. The processing parameters such as compression pressure applied and the type and characteristics of the bulking agents as well as the excipients used in the tablet formulation has a great influence on survival and functionality of the cultures (4-7). Taking these important aspects into consideration, the present research work was carried out to optimize the active ingredient preparations for probiotic/ starter cultures as well as to select the most suitable compression pressure for preparation of culture tablets to be used as either inocula or food supplement.

Materials and Methods

***Microbial Cultures*:** Starter cultures *viz*., *Streptococcus thermophilus* MTCC 5460 and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCIM 2358 and probiotic cultures *viz*., *Lactobacillus helveticus* MTCC 5463 and *Lactobacillus rhamnosus* MTCC 5462 were used in the current study. All the cultures are indigenous isolates obtained from Dairy Microbiology Department of SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India except NCIM 2358 which is obtained from National Dairy Research Institute, Karnal, India. The probiotic cultures are thoroughly studied for their probiotic potential (8, 9). All the cultures were propagated in sterilized reconstituted skim milk (12% Total solids) by incubation at 37°C for 24 h and stored at 5 ± 2 °C. During the course of study, prior to their use, the cultures were given three successive transfers in the whey medium to activate them.

Chemicals: Analytical grade chemicals were used during the entire study. L-Ascorbic acid was purchased from Qualigens Chemicals Pvt. Ltd., Mumbai. Maltodextrin was procured from Loba Chemie Pvt. Ltd., Mumbai, India and cryoprotectant Glycerol was supplied by Glaxosmithkline Pharmaceuticals Ltd., Mumbai. Skimmed Milk Powder (SMP) used for the preparation of suspending medium belonged to Sagar brand and was procured from the local market. Other excipients used in the study included Spray dried lactose and Superstarch 200, supplied by DFE Pharma Pvt. Ltd., Klever Strasse; Crospovidone/ Sodium Starch Glycolate/ Cross carmelose sodium, Polyvinyl pyrrolidione K-30, Magnesium stearate and talc supplied by S. d. Fine Chem. Ltd., Mumbai, India.

Preparation of Freeze Dried Cultures and their Active Ingredients (AI): Freeze dried powder of the cultures were prepared using protocol optimized by Jani *et al.* (10). The active cultures were inoculated in the whey medium at 2% rate and grown at 37°C for 24 h. The cells were harvested by centrifugation at 6000 rpm at 4 °C for 20min. Cell pellets were washed twice with saline water (0.85% NaCl solution). The cells were then suspended in 12 % reconstituted skim milk added with 1% Glycerol (as cryoprotectant). The contents were mixed thoroughly and distributed in glass petriplates. The suspension was frozen at -20°C for overnight and freeze dried using freeze dryer model Virtis genesis 25XL. During the entire drying process, vacuum of 100 millitorr was maintained. Temperatures in the drying chamber were gradually increased from -40 °C to 30 °C over a period of 15-16 h looking to the process of drying. The freeze dried cultures were individually mixed with reducing and bulking agents for preparing the active ingredients (AI) to be used for dosage forms. L-Ascorbic acid and spray dried lactose were used as the reducing and bulking agent respectively. The rate of culture, reducing agent and bulking agent in the AIs were fixed as 20, 20 and 60% (w/w) respectively based on study done by Panchal *et al*. (11). The AIs were studied for culture–excipient compatibility and micromeritic characteristics.

***Estimation of Viability, Activity and Micromeritic Properties*:** Serial dilutions of the

active ingredients were made using 2% peptone water as dilution blank. For preparing the initial dilution 0.1 g powder was reconstituted in peptone water and incubated at 37°C for 2 h to recover cell injuries. Subsequent dilutions (as per requirement) were then prepared in peptone water and appropriate dilutions were pour plated using respective selective agar medium. MRS medium was used for lactobacilli and M17 medium was used for streptococci. Once the initial agar layer was set, a second layer (5-8 ml) of the same medium was made to maintain facultative anaerobic conditions. The plates were then incubated at 37±2°C for 72 h. Colony counts were taken with the help of colony counter and the count was expressed as log (cfu/g) (12). To test the activity, all four freeze dried cultures and their AIs were checked for their ability to form curd. The inoculation rate was 0.1g/100ml milk. After inoculation of the samples, the skim milk flasks were incubated at 37°C for overnight (15h). The curd was analyzed for titratable acidity, pH and viable count. Active ingredients (AI) were evaluated for bulk density, tapped density, angle of repose, Carr's index and Hausner's ratio to evaluate micromeritic properties (13).

***Microbiological Analysis*:** Eleven grams of curd sample was aseptically weighed and transferred to 99ml phosphate buffer dilution blank to obtain 1:10 dilution. Subsequently, 1 ml of above dilution was used for making further dilutions in 9 ml phosphate buffer tubes. Suitable dilutions were prepared and poured in a set of sterile petri dishes in duplicates.

***Stability of Active Ingredients*:** Accurately weighed amounts of active ingredients were exposed to relative humidity (RH) conditions of 10, 52, 75 and 92% obtained by using saturated aqueous solutions of lithium chloride, magnesium nitrate, sodium chloride and potassium nitrate respectively, in sealed desiccators at temperature of 25°C. After 7 days, samples were analyzed for moisture gain and classified on the basis of hygroscopicity (14).

***Preparation of Culture Tablets by Direct Compression*:** All the excipients were passed through 80 mesh sieve. Required quantities of AIs and excipients except lubricant and glidant were mixed thoroughly in a double cone blender. The powder blend was mixed with lubricant glidant mixture. This powder mixture was compressed in ten station rotary tablet machine (Rimek, RSB4-1, Karnavati Engg. Pvt. Ltd., Ahmedabad, India) with flat faced punches of diameter 5mm at different compaction forces *viz*., low (1-2 kg/cm^2), medium (3-4 kg/cm^2) and high (5-6 kg/cm^2) (7). The tablets were analyzed for hardness, disintegration time and microbiological assay to assess the effect of compression pressure on the viability of cultures as well on disintegration time.

***Analysis of Tablets for Disintegration, Hardness and Viability*:** For estimating the disintegration time**,** the tablets were put in the water maintained at 37°C and the time of complete dissolution was noted. Hardness of tablets was measured using Monsanto hardness tester. For estimating the viability of tablets, serial dilutions of the tablets were made using 2% peptone water as dilution blank. For preparing the initial dilution one tablet was dissolved in 10ml of peptone water. Subsequent dilutions (as per requirement) were then prepared in peptone water and appropriate dilutions were pour plated using respective selective agar medium. Once the initial agar layer was set, a second layer (5-8 ml) of the same medium was made to maintain facultative anaerobic conditions. The plates were then incubated at 37±2°C for 72 h. Colony counts were taken with the help of colony counter and the count was expressed as log cfu/tablet. For checking the activity of the tablets, one tablet was put in 100ml sterile reconstituted skim milk (12%Total solids) and dissolved completely. It was then incubated at 37±2°C for overnight (15h). The curd formed was evaluated for sensory characteristics by expert panel of judges using nine point hedonic scales.

***Statistical Analysis*:** The values of each attribute under study were subjected to statistical analysis using Completely Randomized Design with equal

number of observations using the model proposed by Steel and Torrie (15).

Results and Discussion

***Viability and Activity of Freeze Dried Cultures*:** The average viable counts of fresh freeze dried *Streptococcus thermophilus* MTCC 5460, *Lactobacillus helveticus* MTCC 5463, *Lactobacillus rhamnosus* MTCC 5462 and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCIM 2358 were found to be 10.57±0.08, 10.73±0.12, 10.79±0.15 and 10.67±0.20 log cfu/g respectively. These counts were in line with the viability of strains of lactobacilli and streptococci observed by earlier workers (10, 11, 16). The average rate of acid development in sterile reconstituted skim milk upon inoculation of freshly prepared freeze dried cultures at the rate of 0.1g/100ml milk and incubation at 37°C for overnight (12-14h) is shown in Table 1. A significant ($P < 0.05$) difference in the rate of acid development was observed among the four cultures, which was expected keeping in mind the variation in the milk fermenting ability of the four cultures. Likewise a significant ($P < 0.05$) difference was observed for pH of the curds and the respective culture counts. The curd obtained was good and uniform quality. The set curds were sufficiently firm to hold their shape when poured. There was no whey separation and the curds were organoleptically acceptable. However, as expected, the coagulation time by freeze dried cultures was longer than otherwise traditionally propagated active liquid cultures.

Table 1. Viability and activity of freeze dried cultures

Cultures	Viability (log cfu/g)	Acidity (% Lactic Acid)	pH	Count in Curd (log cfu/g)
Streptococcus thermophilus MTCC 5460	10.57±0.08	0.82±0.07^{a}	4.46±0.07^{b}	9.25±0.03^{a}
Lactobacillus helveticus MTCC 5463	10.73±0.12	1.77±0.06^{b}	3.34±0.10^{a}	9.55±0.03^{c}
Lactobacillus rhamnosus MTCC 5462	10.79±0.15	1.63±0.22^{b}	3.44±0.15^{a}	9.35±0.22^{ab}
Lactobacillus delbrueckii subsp. *bulgaricus* NCIM 2358	10.67±0.20	0.78±0.04^{a}	4.47±0.07^{b}	9.44±0.10^{bc}
CD (0.05)	NS	0.19	0.17	0.19

Each observation is mean±SD of four replications; NS= Not Significant
$^{a\text{-}c}$ Superscript letters following numbers in the same column denote significant difference ($p<0.05$)

Table 2. Viability and activity of AI1*

Active ingredients of	Viability (log cfu/g)	Acidity (% Lactic Acid)	pH	Count in Curd (log cfu/g)
Streptococcus thermophilus MTCC 5460	9.17±0.09^{a}	0.71±0.03^{a}	4.84±0.36^{b}	9.13±0.06^{a}
Lactobacillus helveticus MTCC 5463	9.80±0.13^{b}	1.00±0.14^{b}	4.31±0.30^{a}	9.37±0.03^{b}
Lactobacillus rhamnosus MTCC 5462	9.79±0.08^{b}	1.02±0.02^{b}	4.28±0.10^{a}	9.23±0.11^{a}
Lactobacillus delbrueckii subsp. *bulgaricus* NCIM 2358	9.71±0.11^{b}	0.81±0.01^{a}	4.54±0.02^{ab}	9.18±0.06^{a}
CD (0.05)	0.16	0.11	0.37	0.11

*AI1 =Freeze dried culture : Ascorbic acid: Maltodextrin (20:20:60)
Each observation is mean±SD of four replications
^{a,b} Superscript letters following numbers in the same column denote significant difference ($p<0.05$)

***Viability and Activity of Active Ingredients*:** Freeze dried cultures and their preparations are meant for longer period of storage and hence maintaining the viability and activity of the cultures in the preparations during the storage period is of prime importance for successful application of the same. If the freeze dried cultures are stored as it is, the viable count may go down and its efficiency to coagulate milk reduces significantly. Hence, it is advisable to incorporate reducing agents and bulking agents for maintaining better viability and activity of freeze dried cells. At the same time, compatibility of these reducing and bulking agents with the cultures in terms of their viability and activity is also important. The incorporation of bulking agents improve the bulk of the lyophilized product, provide an adequate structure to the cake, improve physical characteristics such as flow ability, ease of handling, non-hygroscopicity, etc of the freeze dried preparation which are essential during dosage form making. The incorporation of excipients affect the micromeritic properties of active ingredients such as angle of repose, carr's index and hausner's ratio which are considered important for the flow properties and compaction behavior during dosage form making. The relationship between flow, angle of repose and carr's index is interpreted as per Indian Pharmacopeia (IP)/United States Pharmacopeia (USP). L-Ascorbic acid as reducing agent and maltodextrin and spray dried (SD) lactose as bulking agents were used for preparation of active ingredients (AI) based on the results of earlier study conducted by Panchal *et al*. (11) and other preliminary trials. The viability and activity of the active ingredients prepared using maltodextrin are shown in Table 2.

A significant ($P < 0.05$) difference was observed in the viable counts in the active ingredient preparations of all the four cultures. The acidity, pH and viable counts in curds obtained also showed significant ($P < 0.05$) difference when maltodextrin was used as bulking agent. This may be due to the inherent difference in the fermenting ability of the four cultures used in the study. Andersen *et al*. (17) showed that maltodextrins with a low dextrose equivalent value better preserved the acidifying activity of *Streptococcus thermophilus* compared to maltodextrins with a high dextrose equivalent value. Among the four cultures, the highest count was observed for MTCC 5463 (9.80±0.13 log cfu/g) and the lowest for MTCC 5460 (9.17±0.09). Muller *et al*. (18) reported the protective effect of maltodextrin during the reconstitution of the probiotic powder before its usage. Panchal *et al*. (11) used maltodextrin as bulking agent and ascorbic acid as reducing agent to stabilize probiotic vaginal strain *Lactobacillus helveticus* MTCC 5463 during storage of freeze dried preparation.

The viability and activity of the active ingredient preparation where maltodextrin was replaced with spray dried lactose is shown in Table 3. A significant ($P < 0.05$) difference was observed in the viable counts of the preparation, curd acidity, pH and count among the four cultures. Highest viable count was observed for MTCC 5462 (9.89 ± 0.09 log cfu/g) and the lowest for MTCC 5460 (9.41 ± 0.20 log cfu/g). Zarate and Nader-Macias (19) used lactose in combination with skim milk and ascorbic acid to stabilise probiotic vaginal strains during freeze-drying and storage. Between the two active ingredients, the viable counts were found to be higher in case of AI using spray dried lactose. No significant differences were seen for other parameters such as curd acidity, pH and count.

***Micromeritic Properties of Active Ingredients*:** A comparative evaluation of the micromeritic properties of the active ingredients prepared using maltodextrin and spray dried lactose is shown in Figures 1, 2 and 3. The results showed that the values for angle of repose and Carr's index differed significantly ($P < 0.05$) between the four cultures in case of AI1 and AI2. The values of angle of repose, Carr's index and Hausner's ratio indicated significant ($P < 0.05$) difference between the micromeritic properties of two active ingredients. For AI1, the values indicated poor to very poor flow property. While for AI2, the

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values indicated better flow properties. Good flow characteristics are a pre requisite for preparation of dosage forms such as tablets. Hence spray dried lactose was selected as bulking agent for the study. Spray dried lactose enables direct compression of formulations in a simple manufacturing process. The narrow particle size distribution is an important factor which affects the appropriate flow properties of spray dried lactose (20).

***Stability Study*:** Active ingredients containing spray dried lactose for all four cultures were further studied for stability at different relative humidity conditions for 7 days at 25°C. The result of the stability study is depicted in Figure 4. The moisture uptake was found to be less than 20% (as per ICH guidelines) in case of all active ingredients except for MTCC 5460 which was slightly higher (20.63). Bora *et al.* (4) has carried out preformulation studies of probiotic *Bacillus*

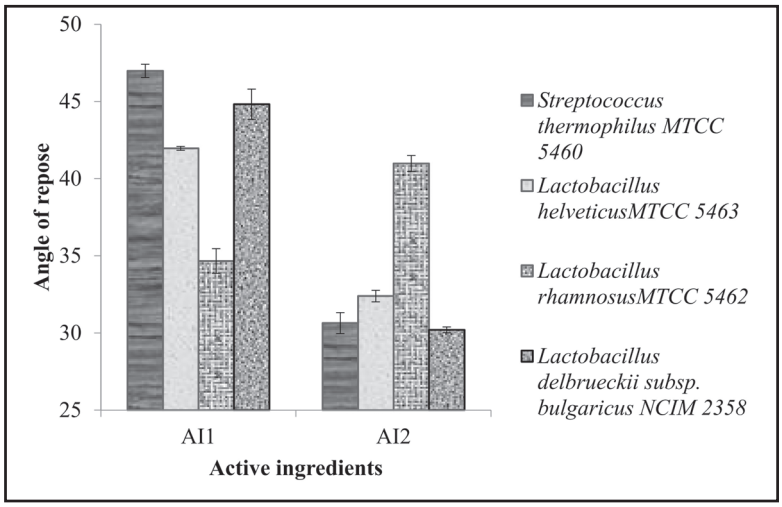

Fig. 1. Comparative evaluation of angle of repose of active ingredients

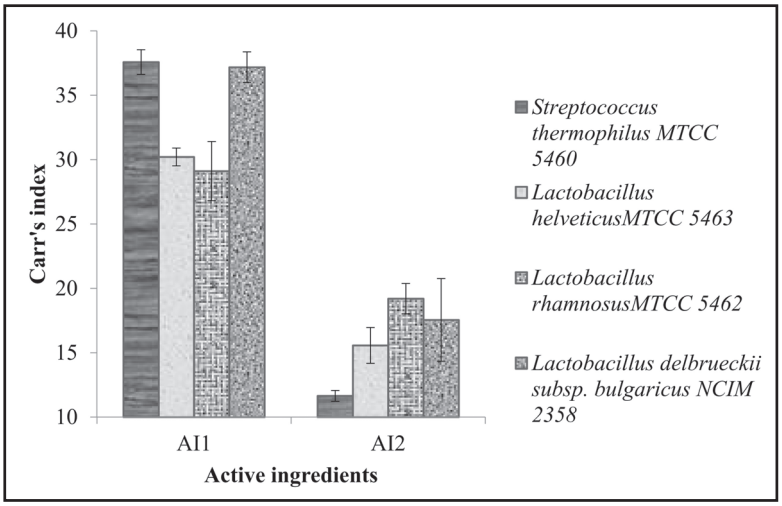

Fig. 2. Comparative evaluation of Carr's index of active ingredients

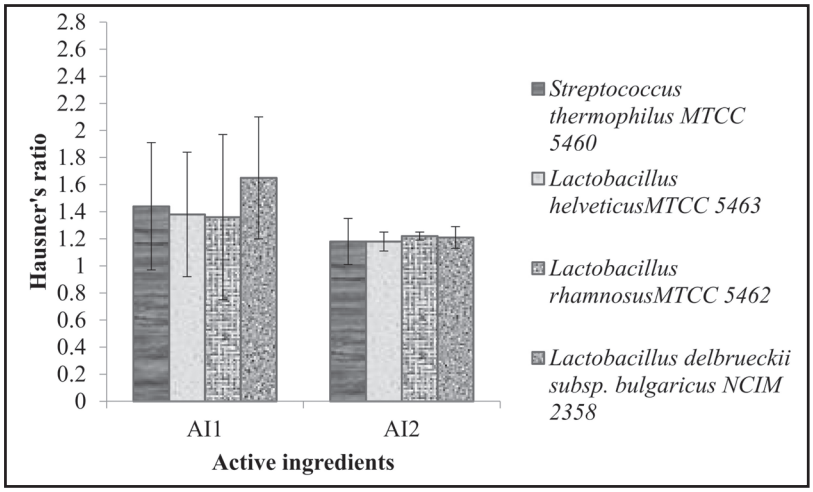

Fig. 3. Comparative evaluation of Hausner's ratio of active ingredients

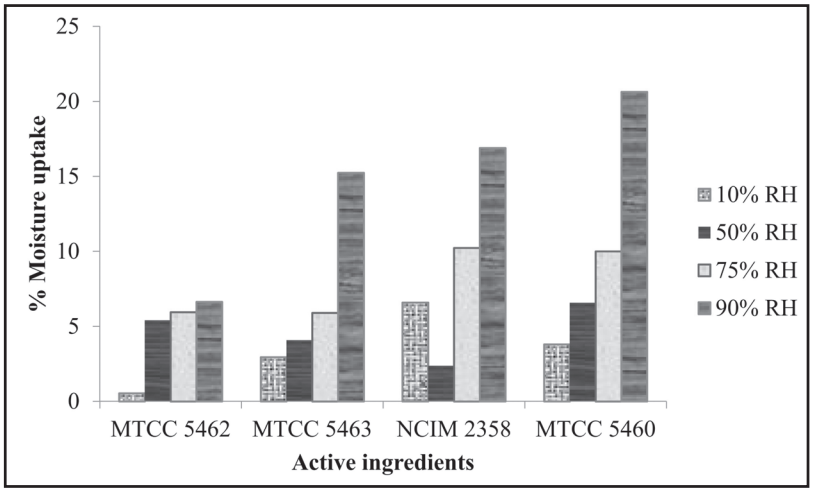

Fig. 4. Stability study of active ingredients containing spray dried lactose

coagulans spores to aid designing of stable formulations. They have studied the excipient compatibility studies of probiotic *Bacillus coagulans* spores and found reduced assay with citric acid monohydrate, meglumine and sodium starch glycolate. The loss of activity seemed to be related to the moisture uptake, free and bound water present in the bulk. The current study results indicated the non hygroscopic nature of active ingredients used and their suitability for preparation of dry dosage forms.

Effect of Compression Pressure on Hardness, Disintegration Time and Viability of Tablets: Preparation of dosage forms such as tablets require compression of all ingredients in a tablet press. In direct compression process active ingredient is blended with a variety of excipients, subsequently lubricated and directly compressed into a tablet. The formulation used for tablets preparation in the current study is shown in Table 4. Formulations varied with respect to type of active ingredients (100mg) only. The effect of

different levels of compression pressure on hardness, disintegration time and viability of tablets is shown in Table 5. A significant ($P < 0.05$) increase in the hardness (n=6) and disintegration time (n=3) of tablets was observed for all four cultures when the compression pressure was increased. This increase was found to be higher (Mean = 3.56) for tablets containing *Lactobacillus helveticus* MTCC 5463 compared to other three culture tablets. Among the cultures, the increase in disintegration time of tablets was found to be not significant. But the interaction effect was again significant (*P<0.05*). The disintegration time of a tablet depends on the hardness of tablet, the kind and amount of disintegrating agents used and how it acts on the tablet formulation (21). Sodium starch glycolate used in the formulation is a superdisintegtant. The extent of cross-linking and degree of substitution in sodium starch glycolate allows for rapid water uptake by the polymer without the formation of a viscous gel (22). Compression pressure had a significant effect ($P < 0.05$) on the viability of cultures in the tablets. Viability decreased linearly with increasing level of compression pressure. This decrease in viability of cultures was found to be not significant among the cultures. The interaction effect was also not significant.

A number of studies have indicated the effect of compression pressure applied on viability of culture tablets. Fazeli and coworkers (23) reported nearly 1 log cycle reduction in the assay values of tablets of *Lactobacillus acidophilus* after compaction. Durand and Panes (24) demonstrated that different species of probiotic bacteria had different levels of resistance to compaction pressure. The study results of Klayraung *et al*. (5) showed that the proportion of matrix forming excipients in tablets and the compression force affected the properties of probiotic tablets in terms of tensile strength and disintegration as well as the survival of the bacteria. Brachkova *et al*. (25) produced several formulas of mini tablets with or without microcrystalline cellulose and inulin (2.5 mm diameter) and several strains of *Lactobacillus* by applying compaction forces of 1, 2, and 5 kN and reported a decrease of less than 2 log units in the viability of probiotic bacteria. Nagashima *et al*. (21) in their study on development of effervescent tablets with probiotics *Lactobacillus acidophilus* and *Saccharomyces boulardii* reported a decrease in concentration of microorganisms with an increase in hardness when the compression force reached over 20 N. e Silva *et al*. (6) in their research on development of probiotic tablets using microparticles studied the effect of compaction force on viability of the probiotic strain *L. paracasei* L26. Among the compaction forces tested 9.8, 19.6, 29.4, and 39.2 kN, a decrease of 1 log cycle was observed after the compaction with 9.8-kN force. However, the number of viable cells for different compaction forces was of the same order of magnitude showing no increase of detrimental effects for compaction forces higher than 9.8 kN ($p>0.05$).

In the current study, the percent survival of cultures in the tablets decreased significantly (*P < 0.05*) with increasing compression pressure (Figure 5). The survival was found to be nearly 90% (mean=89.75) for all four cultures when the compression pressure applied was 1-2 kg/cm^2. This survival rate decreased from 90% to 76.76% and to 71.75% when the pressure was increased from 1-2 kg/cm^2 to 3-4kg/cm^2 and subsequently to 5-6 kg/cm^2. Between the cultures the difference in survival was found to be non significant. Depending on the pressure applied, the compression of cells may cause damage to the cell walls and membranes or even loss of viability. It is clear that under mechanical stress some cells cannot tolerate such compression. Initially, the increase in the force applied will primarily damage the cell wall and when such pressure is further increased, it will also reach the cell membrane. Therefore, it has been observed that cellular viability decreases almost linearly with the applied compression force (26). Maggi *et al*. (27) also verified a reduction in viability in different strains of lactobacillus during tablet production with other lyophilized strains. Bora *et al*. (4) observed a decline in spore formation in *Bacillus coagulans*

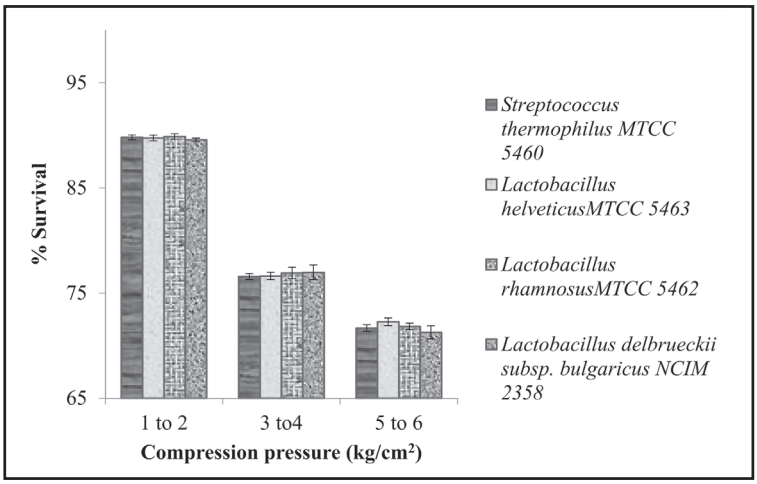

Fig. 5. Percent survival of cultures in tablets made using different compression pressures

Table 3. Viability and activity of AI2*

Treatments (Cultures)	Viability (log cfu/g)	Acidity (% Lactic Acid)	pH	Count in Curd (log cfu/g)
Streptococcus thermophilus MTCC 5460	9.41 ± 0.20[a]	0.70 ± 0.05[a]	4.75 ± 0.21[c]	9.18 ± 0.07[a]
Lactobacillus helveticus MTCC 5463	9.85 ± 0.09[b]	0.89 ± 0.03[b]	4.49 ± 0.03[b]	9.40 ± 0.06[b]
Lactobacillus rhamnosus MTCC 5462	9.89 ± 0.09[b]	1.09 ± 0.01[c]	4.14 ± 0.01[a]	9.17 ± 0.09[a]
Lactobacillus delbrueckii subsp. *bulgaricus* NCIM 2358	9.76 ± 0.12[b]	0.84 ± 0.02[b]	4.53 ± 0.01[b]	9.18 ± 0.[07a]
CD(0.05)	0.20	0.05	0.16	0.11

*AI2 = Freeze dried culture : Ascorbic acid: Spray dried lactose (20:20:60)
Each observation is mean±SD of four replications
[a-c] Superscript letters following numbers in the same column denote significant difference ($p<0.05$)

Table 4. Formulation used for preparation of tablets

Ingredients	Quantity (mg)
Active ingredient*	100
Spray Dried Lactose	195
Super Starch 200	100
Sodium Starch Glycolate	50
Talc	20
Magnesium Stearate	15
Total weight of Tablet (mg)	480

*Formulations varied with respect to type of active ingredients.

probiotic with an increase in the compression force, indicating that survival also depends on the probiotic species and on the excipients used in the formulation. Overall, our study results were found in agreement with that of the previously discussed research works where it was reported that the compression pressure had a significant effect on the hardness, disintegration time and viability of probiotic tablets. An increased compression pressure lead to tablets with increased hardness, longer disintegration time and decreased viability.

Table 5. Effect of different compression pressures on hardness, disintegration time and viability of tablets

Active ingredients of	Compression Pressure (kg/cm^2) 1-2	3-4	5-6
	Hardness		
Streptococcus thermophilus MTCC 5460	1.32 ± 0.14	3.34 ± 0.14	5.40 ± 0.13
Lactobacillus helveticus MTCC 5463	1.44 ± 0.17	3.23 ± 0.1	5.41 ± 0.12
Lactobacillus rhamnosus MTCC 5462	1.26 ± 0.12	3.14 ± 0.06	5.23 ± 0.03
Lactobacillus delbrueckii subsp. *bulgaricus* NCIM 2358	1.26 ± 0.12	3.16 ± 0.02	5.23 ± 0.03
CD (0.05) A=0.09, P=0.08; AxP=NS			
	Disintegration time (minutes)		
Streptococcus thermophilus MTCC 5460	1.31 ± 0.17	2.10 ± 0.14	3.14 ± 0.05
Lactobacillus helveticus MTCC 5463	1.24 ± 0.13	2.23 ± 0.06	3.13 ± 0.09
Lactobacillus rhamnosus MTCC 5462	1.13 ± 0.07	2.10 ± 0.04	3.21 ± 0.09
Lactobacillus delbrueckii subsp. *bulgaricus* NCIM 2358	1.19 ± 0.04	2.13 ± 0.06	3.07 ± 0.05
CD (0.05) A= NS, P=0.06; AxP=0.12			
	Viability (log cfu/tablet)		
Streptococcus thermophilus MTCC 5460	8.92 ± 0.02	7.61 ± 0.04	7.12 ± 0.02
Lactobacillus helveticus MTCC 5463	8.93 ± 0.03	7.63 ± 0.04	7.19 ± 0.03
Lactobacillus rhamnosus MTCC 5462	8.95 ± 0.01	7.65 ± 0.03	7.15 ± 0.04
Lactobacillus delbrueckii subsp. *bulgaricus* NCIM 2358	8.92 ± 0.01	7.66 ± 0.08	7.09 ± 0.05
CD (0.05) A= NS, P=0.03; AxP= NS			

Each observation is mean±SD of four replications; NS= Not Significant

Conclusion

The study results clearly indicate the significant effect of bulking agents, cultures and compaction forces on properties of probiotic and starter culture tablets. Also the effect of compression pressure is largely decided by the kind and proportion of matrix forming excipients in tablets. Hence along with proper compression pressure, proper selection of the matrix forming excipients is vital for survival of bacterial cultures in dosage forms such as tablets. As the functionality of such culture tablets are greatly depended on the viability and activity of the cultures used, strain to strain variation should be taken into account while optimizing the various parameters.

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