EFFECTS OF FERMENTATION PARAMETERS ON THE PRODUCTION OF OPTIMUM PACKED CELL VOLUMES OF ISOLATED *LACTOBACILLUS* SPECIES (H-1) AND (N-2)

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Abstract

Lactobacillus spp. (H-1) and (N-2) were isolated from the samples bought from Kamayut and North Dagon markets using tomato juice agar medium. These lactic acid bacteria were applied in the study of the effects of fermentation parameters on the optimum packed cell volumes of lactic acid bacteria. The experiments were conducted during May 2018 to December 2018 in the Fermentation Department, Pharmaceutical Research Department, Ministry of Industry - 1, Yangon Region. After the isolation and identification of *Lactobacillus* species, the effects of various fermentation conditions were investigated. This study was carried out by preparing the fermentation medium, and then measuring the pH (4 to 7), ages of culture (1 to 5 days) and sizes of inoculum (5%, 10% and 15%) with fermentation period (1 to 7 days). Packed cell volume (PCV) and pH resulted in fermented broth were recorded. According to experimental results, H - 1 and N - 2 strains were found that 3 days of cultured age, 10% size of inoculum and pH - 6.00 with the fermentation period of 3 days showed maximum packed cell volume.

Keyword: *Lactobacillus*, fermentation, broth.

Introduction

Fermentation is a mean by which cells grow anaerobically dispose of excess hydrogen atoms generated during the breakdown of sugar, known as glycolysis in which the terminal hydrogen acceptor is an organic molecule. In lactic acid bacteria, they dump excess hydrogen on to pyruvic acid, the breakdown product of glucose and this produces lactic acid.

The fermentation conditions, such as temperature, pH medium composition, dissolved oxygen tension (DOT) and types of neutralizer greatly influence the growth of *Lactobacilli* (Lim *et al.,* 2007). Inoculum sizes may have effect on pH, acidity, viable counts and kind of fermented milk. The growth of starter culture is affected by many factors such as milk chemical composition, the amount of inoculum, temperature and time of incubation and the cooling time. Addition of 3, 5 and 10% inoculums resulted in the significantly increase population of lactic acid bacteria during milk fermentation (Wardani *et al.,* 2017).

The optimum inoculum concentration in goat milk was 3% with the incubation temperature at 43°C using *L. bulgaricus* and *S. thermophilus* as starter cultures. After 24 h fermentation the pH of fermented milk with inoculum size of 3%, 5%, and 10% drop the pH to 4.41; 3.94, 4.05 respectively (Shu *et al.,* 2014). The present research is to examine optimum pH, age of culture, size of inoculum and fermentation period for collection of cell sediments.

Materials and Methods

In this study, microbiological work was conducted from May 2018 to December 2018 in the Fermentation Department, Pharmaceutical Research Department, Ministry of Industry 1, Yangon Region.

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Instruments

The following instruments are used in this experiment. Autoclave is used to sterilize equipment and supplies by subjecting them to pressurized saturated steam at 121 °C (249 °F) for around 15–20 minutes depending on the size of the load and the contents. Incubator is a device used to grow and maintain microbiological cultures or cell cultures. Hot air ovens are electrical devices which use dry heat to sterilize. Centrifuge is used to separate supernatant and pellet.

Figure 1 Autoclave

Figure 2 Incubator

Figure 3 Hot air oven

Figure 4 Centrifuge

Preparation of fermentation medium

Tomato juice agar medium (tomato juice - 20.00 g, yeast extract - 10.00 g, dextrose - 10.00 g, dipotassium phosphate - 0.50 g, monopotassium phosphate - 0.50 g, magnesium sulphate - 0.20 g, manganese sulphate - 0.01 g, ferrous sulphate - 0.01 g, sodium chloride - 0.01 g, agar - 20.00 g, pH - 6.7) was used both for inoculum and fermentation medium.

Preparation of age of culture and size of inoculums

Lactobacillus spp. (H-1) and (N-2) strains from isolated pure culture in tomato juice agar slant were transferred into another slants and incubated at 37°C for 1 to 5 days. Different ages of culture of *Lactobacillus* spp. (H-1) and (N-2) were used as inoculums. The size of inoculum were 5%, 10% and 15% respectively of fermentation medium.

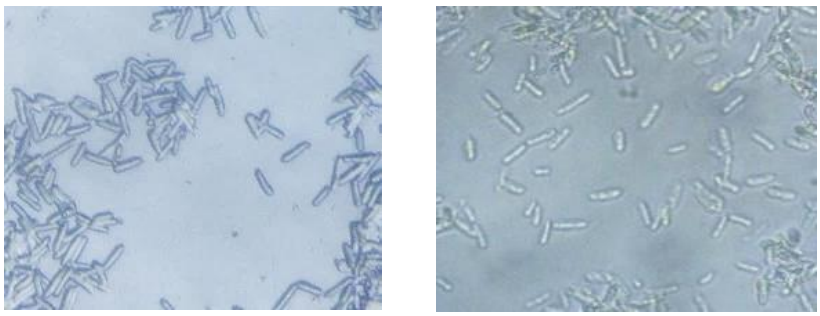
Culture for seed inoculums and fermentation culture of *Lactobacillus* spp. (H-1) and (N-2)

One loopful of 1 to 5 days old culture were aseptically inoculated into a prepared inoculum broth medium (such as pH – 4 to 7) by a sterilized loop and shake gently and inoculums at 37°C for 1 day. Bacteria suspension (5%) was taken from 1 day inoculum of age culture medium and transferred to the 95 ml fermentation medium. At the same time, 10% of inoculums were transferred into their respective flasks containing the 90 ml of fermentation medium. Similarly, 15% of inoculums were transferred into their respective flasks containing the 85 ml of fermentation medium. The experiment procedure was repeated by using inoculums of different age (1 to 5 days).

Determination of packed cell volume and pH

After incubation at 1 to 7 days, 10 ml each of fermentation broth were poured into centrifuge tubes. Then, centrifuged at 2000 rpm for 20 min and the bacterial cell sediments at bottom of the tubes were used for packed cell volume determination. The pH values of fermented broth medium was measured daily by portable pH meter up to 7 days.

Results



H-1 (1000x) N-2 (1000x)

Figure 5 Microscopic characters of *Lactobacillus* species

Determination of optimum conditions of *Lactobacillus* spp. (H - 1) and (N - 2) for fermentation with respect to age of culture, inoculums size and pH

Bacterial growth condition was optimized based on ages of culture, sizes of inoculum and pH of medium. The effects of different ages of culture (1, 2, 3, 4 and 5 days) and sizes of inoculums (5%, 10% and 15%) on the production of packed cell volume of H - 1 and N - 2 (*Lactobacillus* species) were studied. The medium of pH (4, 5, 6 and 7) and fermentation period during 1 to 7 days were also analyzed in this experiment.

In H - 1 strain, the minimum and maximum pH were 4.33 - 6.11 (at pH 4), 5.43- 6.92 (at pH 5), 4.68 - 6.26 (at pH 6) and 5.23 - 6.93 (at pH 7) in 1 day culture aged at 1 to 7 days fermentation period. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.11 ml (at pH 4), 0.12 ml (at pH 5), 0.15 ml (at pH 6) and 0.18 ml (at pH 7) in 10% size of inoculum. These results were exhibited in Table - 1. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.27 - 6.10 (at pH 4), 5.55 - 6.80 (at pH 5), 5.40 - 6.29 (at pH 6) and 5.70 - 7.14 (at pH 7) in 2 days culture aged. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.12 ml (at pH 4), 0.15 ml (at pH 5),

0.20 ml (at pH 6) and 0.20 ml (at pH 7) in 10% size of inoculum. These results were exhibited in Table - 2.

In H - 1 strain, the minimum and maximum pH were 4.20 - 6.17 (at pH 4), 5.31- 6.84 (at pH 5), 5.50 - 6.28 (at pH 6) and 5.43 - 6.85 (at pH 7) in 3 days culture aged at 1 to 7 days fermentation period. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.11 ml (at pH 4), 0.20 ml (at pH 5), 0.24 ml (at pH 6) and 0.21 ml (at pH 7) in 10% size of inoculum. These results were showed in Table - 3. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.38 - 5.70 (at pH 4), 5.41 - 6.69 (at pH 5), 5.50 - 6.42 (at pH 6) and 5.47 - 6.54 (at pH 7) in 4 days culture aged. At pH 5 in 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.12 ml. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.12 ml (at pH 4), 0.20 ml (at pH 6 and 7) in 10% size of inoculum. These results were showed in Table - 4. In H - 1 strain, the minimum and maximum pH were 4.32 - 5.72 (at pH 4), 4.97 - 6.72 (at pH 5), 5.39 - 6.25 (at pH 6) and 5.43 - 6.57 (at pH 7) in 5 days culture aged at 1 to 7 days fermentation period. In 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.10 ml. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.12 ml (at pH 5), 0.18 ml (at pH 6 and 7) in 10% size of inoculum. These results were showed in Table - 5.

Table 1 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 1 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
1	5	4.00	4.50	4.82	5.55	5.79	5.93	6.00	**6.11**	0.01	0.08	0.08	0.10	0.10	0.10	0.08	0.08
	10	4.00	**4.33**	4.87	5.07	5.28	5.51	5.65	5.65	0.02	0.10	0.10	**0.11**	0.10	0.10	0.10	0.10
	15	4.00	4.67	4.88	5.13	5.40	5.63	5.78	5.80	0.02	0.08	0.08	0.10	0.10	0.08	0.08	0.05
	5	5.00	5.50	5.81	6.04	6.15	5.97	5.80	5.72	0.01	0.08	0.10	0.10	0.10	0.10	0.10	0.08
	10	5.00	**5.43**	5.87	6.17	6.38	6.31	6.35	6.35	0.02	0.10	**0.12**	**0.12**	**0.12**	**0.12**	0.10	0.10
	15	5.00	5.67	5.98	6.23	6.30	6.53	**6.92**	6.84	0.02	0.10	0.10	0.10	0.10	0.08	0.08	0.05
	5	6.00	6.04	6.10	6.26	6.21	6.13	5.82	5.70	0.01	0.08	0.12	**0.15**	**0.15**	0.12	0.10	0.10
	10	6.00	5.99	6.17	6.19	5.98	5.74	5.40	5.55	0.02	0.10	0.12	**0.15**	0.13	0.13	0.10	0.10
	15	6.00	6.10	6.17	**6.26**	5.93	5.49	5.60	**4.68**	0.02	0.10	0.13	0.13	0.13	0.13	0.10	0.10
	5	7.00	6.71	6.40	6.30	6.02	5.91	5.90	5.96	0.01	0.08	0.10	0.12	0.10	0.10	0.10	0.10
	10	7.00	6.83	6.50	6.29	6.09	5.60	5.55	**5.23**	0.02	0.10	0.15	**0.18**	0.15	0.15	0.12	0.10
	15	7.00	**6.93**	6.45	6.32	6.29	6.33	5.94	5.55	0.02	0.12	0.12	0.13	0.13	0.10	0.10	0.09

Table 2 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 2 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
2	5	4.00	4.55	4.71	5.19	5.44	5.67	5.80	**6.10**	0.01	0.08	0.10	0.10	0.10	0.10	0.10	0.09
	10	4.00	4.55	4.87	5.00	5.31	5.60	5.73	5.95	0.02	0.10	0.10	**0.12**	**0.12**	0.10	0.10	0.10
	15	4.00	**4.27**	4.76	4.96	5.01	5.25	5.58	5.80	0.02	0.10	0.10	0.11	0.11	0.10	0.10	0.10
	5	5.00	**5.55**	5.81	6.19	6.21	6.36	6.08	5.82	0.01	0.08	0.10	0.12	0.12	0.12	0.10	0.09
	10	5.00	5.65	6.00	6.27	6.41	6.50	6.53	6.45	0.02	0.12	0.12	**0.15**	0.12	0.12	0.10	0.10
	15	5.00	5.77	6.06	6.32	6.51	6.65	6.78	**6.80**	0.02	0.10	0.10	0.12	0.12	0.12	0.10	0.10
	5	6.00	5.91	6.13	**6.29**	5.95	5.72	5.53	**5.40**	0.01	0.08	0.11	0.16	0.15	0.13	0.11	0.09
	10	6.00	6.12	6.18	6.23	6.17	6.09	5.85	5.81	0.02	0.12	0.12	**0.20**	0.17	0.15	0.15	0.11
	15	6.00	6.19	6.17	6.23	6.07	5.95	5.88	5.70	0.02	0.08	0.12	0.12	0.12	0.12	0.11	0.10
	5	7.00	6.55	6.32	6.27	6.00	6.12	5.93	5.80	0.01	0.10	0.11	0.15	0.12	0.12	0.11	0.10
	10	7.00	**7.14**	6.69	6.32	6.22	6.05	5.84	**5.70**	0.02	0.12	0.18	**0.20**	0.17	0.15	0.15	0.15
	15	7.00	6.84	6.57	6.39	6.32	6.14	5.96	5.71	0.02	0.08	0.10	0.12	0.12	0.10	0.10	0.10

Table 3 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 3 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of noculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
3	5	4.00	4.31	4.78	5.20	5.87	6.09	6.13	**6.17**	0.01	0.10	0.10	0.12	0.10	0.10	0.10	0.10
	10	4.00	**4.20**	4.68	4.85	5.02	5.36	5.52	5.73	0.02	0.10	0.11	**0.13**	0.11	0.10	0.10	0.10
	15	4.00	4.40	4.75	4.89	5.04	5.15	5.29	5.40	0.02	0.10	0.11	0.11	0.11	0.10	0.09	0.09
	5	5.00	**5.31**	5.80	6.19	5.73	5.59	5.40	5.38	0.01	0.10	0.10	0.15	0.13	0.10	0.10	0.10
	10	5.00	5.80	6.08	6.25	6.32	6.36	6.30	6.23	0.02	0.12	0.15	**0.20**	**0.20**	0.17	0.15	0.12
	15	5.00	5.60	6.15	6.24	6.69	6.75	6.79	**6.84**	0.02	0.10	0.12	0.13	0.10	0.10	0.09	0.09
	5	6.00	6.14	6.21	6.26	6.15	5.84	5.85	**5.50**	0.01	0.10	0.15	0.17	0.13	0.11	0.10	0.10
	10	6.00	6.10	6.17	6.20	6.11	5.80	5.74	5.55	0.02	0.10	0.20	**0.24**	0.20	0.17	0.17	0.15
	15	6.00	6.06	6.12	**6.28**	6.15	5.92	5.80	5.77	0.02	0.10	0.15	0.20	0.15	0.10	0.09	0.09
	5	7.00	6.79	6.53	6.24	6.20	5.81	5.82	5.75	0.01	0.10	0.12	0.15	0.13	0.12	0.12	0.12
	10	7.00	**6.85**	6.51	6.20	6.11	5.80	5.65	5.55	0.02	0.15	0.18	**0.21**	0.20	0.17	0.15	0.15
	15	7.00	6.80	6.59	6.24	5.92	5.70	**5.43**	5.48	0.02	0.10	0.12	0.20	0.15	0.10	0.10	0.09

Table 4 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 4 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of noculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
4	5	4.00	4.39	4.60	4.98	5.15	5.30	5.53	5.60	0.01	0.10	0.10	0.10	0.10	0.10	0.10	0.08
	10	4.00	4.41	4.76	4.98	5.02	5.22	5.45	**5.70**	0.02	0.10	0.10	**0.12**	0.12	0.10	0.10	0.10
	15	4.00	**4.38**	4.82	4.95	5.04	5.49	5.65	5.60	0.02	0.09	0.10	0.10	0.10	0.10	0.08	0.08
	5	5.00	5.59	5.85	6.28	6.20	6.06	5.73	5.80	0.01	0.10	0.10	**0.12**	**0.12**	0.10	0.10	0.08
	10	5.00	**5.41**	5.76	6.08	6.19	6.20	6.35	6.25	0.02	0.10	0.10	**0.12**	**0.12**	0.10	0.10	0.10
	15	5.00	5.88	6.22	6.32	6.54	**6.69**	6.65	6.60	0.02	0.10	0.10	**0.12**	0.10	0.10	0.08	0.08
	5	6.00	6.18	6.30	**6.42**	6.37	6.25	5.90	5.69	0.01	0.08	0.10	0.12	0.12	0.12	0.10	0.08
	10	6.00	6.11	6.15	6.28	6.23	6.24	5.92	**5.50**	0.02	0.12	0.19	**0.20**	0.18	0.15	0.15	0.15
	15	6.00	6.17	6.26	6.25	5.98	5.75	5.70	5.71	0.02	0.12	0.15	0.15	0.15	0.08	0.08	0.08
	5	7.00	6.52	6.39	6.19	6.04	5.70	5.67	**5.47**	0.01	0.08	0.12	0.15	0.15	0.15	0.10	0.08
	10	7.00	6.44	6.24	6.25	5.96	5.81	5.80	5.67	0.02	0.12	0.15	**0.20**	0.18	0.12	0.12	0.10
	15	7.00	**6.54**	6.38	6.25	6.12	5.83	5.71	5.59	0.02	0.12	0.15	0.15	0.15	0.10	0.08	0.08

Table 5 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 5 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
5	5	4.00	**4.32**	4.50	4.95	5.28	5.40	5.57	5.50	0.01	0.08	0.08	**0.10**	**0.10**	0.08	0.08	0.05
	10	4.00	4.56	4.76	4.93	5.19	5.30	5.47	5.65	0.02	0.08	**0.10**	**0.10**	**0.10**	**0.10**	**0.10**	0.08
	15	4.00	4.50	4.89	5.24	5.57	5.64	5.70	**5.72**	0.02	**0.10**	**0.10**	**0.10**	**0.10**	0.05	0.05	0.05
	5	5.00	**4.97**	5.40	5.95	6.28	6.41	6.23	6.29	0.01	0.08	0.08	0.10	0.10	0.08	0.08	0.05
	10	5.00	5.36	5.55	5.83	6.09	6.30	6.47	6.65	0.02	0.08	0.10	**0.12**	**0.12**	0.10	0.10	0.08
	15	5.00	5.50	6.19	6.24	6.67	6.70	6.70	**6.72**	0.02	0.10	0.10	0.10	0.10	0.05	0.05	0.05
	5	6.00	6.02	6.13	6.16	5.70	5.40	5.52	**5.39**	0.01	0.08	0.08	0.10	0.10	0.10	0.10	0.05
	10	6.00	5.97	6.08	6.20	6.14	5.50	5.45	5.57	0.02	0.12	0.12	**0.18**	**0.18**	0.15	0.10	0.10
	15	6.00	6.04	6.24	**6.25**	5.62	5.49	5.67	5.63	0.02	0.10	0.10	0.12	0.10	0.05	0.05	0.05
	5	7.00	6.52	6.20	6.25	6.28	6.13	5.95	5.84	0.01	0.08	0.08	0.10	0.10	0.10	0.08	0.05
	10	7.00	**6.57**	6.48	6.29	6.07	5.77	5.57	5.51	0.02	0.10	0.12	**0.18**	**0.18**	0.15	0.10	0.10
	15	7.00	6.50	6.43	6.25	6.05	5.70	5.61	**5.43**	0.02	0.08	0.10	0.12	0.10	0.08	0.05	0.05

The blue color square indicate the minimum value and the green color square indicate the maximum value.

In N - 2 strain, the minimum and maximum pH were 4.37 - 6.07 (at pH 4), 5.31-6.92 (at pH 5), 5.59 - 6.29 (at pH 6) and 5.65 - 6.93 (at pH 7) in 1 day culture aged at 1 to 7 days

fermentation period. At pH 6 in 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.15 ml. Among the 5%, 10% and 15% inoculum sizes, maximum amount of packed cell volume were 0.11 ml (at pH 4), 0.12 ml (at pH 5) and 0.18 ml (at pH 7) in 10% size of inoculum. These results were showed in Table - 6. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.27 - 5.97 (at pH 4), 5.52 - 6.84 (at pH 5), 5.40 - 6.27 (at pH 6) and 5.80 – 6.91 (at pH 7) in 2 days culture aged. At pH 4 in 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.12 ml. Among the 5%, 10% and 15% inoculum sizes, maximum amount of packed cell volume were 0.15 ml (at pH 5), 0.20 ml (at pH 6 and 7) in 10% size of inoculum. These results were presented in Table - 7. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.26 - 6.07 (at pH 4), 5.21 - 6.83 (at pH 5), 5.70 - 6.29 (at pH 6) and 5.45 - 6.80 (at pH 7) in 3 days culture aged. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.13 ml (at pH 4), 0.20 ml (at pH 5), 0.25 ml (at pH 6) and 0.22 ml (at pH 7) in 10% size of inoculum. These results were presented in Table 8. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.26 – 6.07 (at pH 4), 5.21 - 6.83 (at pH 5), 5.70 - 6.29 (at pH 6) and 5.45 - 6.80 (at pH 7) in 4 days culture aged. In 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.11 ml (At pH 4), 0.12 ml (At pH 5). Among the 5%, 10% and 15% inoculum sizes, maximum amount of packed cell volume was 0.20 ml (at pH 6 and 7) in 10% size of inoculum. These results were exhibited in Table - 9. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.32 - 5.80 (at pH 4), 5.07- 6.77 (at pH 5), 5.45 - 6.25 (at pH 6) and 5.51 - 6.87 (at pH 7) in 5 days culture aged. At pH 4 in 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.10 ml. Among the 5%, 10% and 15% inoculum sizes, maximum amount of packed cell volume were 0.12 ml (at pH 5), 0.18 ml (at pH 6 and 7) in 10% size of inoculum. These results were exhibited in Table - 10.

Table 6 **Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 1 day old culture and 5%, 10% and 15% sizes of inoculum (N – 2)**

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	5	4.00	4.42	4.72	5.14	5.59	5.81	6.00	**6.07**	0.01	0.08	0.08	0.10	0.10	0.08	0.08	0.08
	10	4.00	**4.37**	4.87	5.11	5.48	5.51	5.58	5.62	0.02	0.10	0.10	**0.11**	**0.11**	0.10	0.10	0.10
	15	4.00	4.51	4.80	5.13	5.44	5.67	5.79	5.83	0.02	0.08	0.10	0.10	0.10	0.08	0.08	0.06
	5	5.00	**5.31**	5.73	6.07	6.25	6.07	6.10	6.32	0.01	0.08	0.10	0.10	0.10	0.10	0.08	0.08
	10	5.00	5.43	5.87	6.17	6.38	6.31	6.35	6.33	0.02	0.10	**0.12**	**0.12**	**0.12**	0.10	0.10	0.10
	15	5.00	5.47	5.88	6.03	6.30	6.52	**6.92**	6.84	0.02	0.09	0.10	0.10	0.10	0.08	0.08	0.05
	5	6.00	6.07	6.13	6.26	6.21	6.15	5.92	5.80	0.02	0.08	0.12	**0.15**	**0.15**	0.10	0.10	0.10
	10	6.00	6.09	6.17	**6.29**	6.08	5.84	5.80	5.85	0.02	0.10	0.12	**0.15**	0.13	0.12	0.10	0.10
	15	6.00	6.20	6.17	6.26	5.93	**5.59**	5.60	5.68	0.02	0.10	0.10	0.12	0.12	0.10	0.10	0.10
	5	7.00	6.77	6.42	6.30	6.02	5.88	5.90	5.93	0.01	0.08	0.08	0.10	0.10	0.10	0.10	0.10
	10	7.00	6.90	6.50	6.29	6.07	5.90	5.85	5.83	0.02	0.10	0.15	**0.18**	**0.18**	0.15	0.12	0.10

Table 7 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 2 day old culture and 5%, 10% and 15% sizes of inoculum

(N – 2)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
2	5	4.00	4.45	4.71	5.15	5.47	5.60	5.84	**5.97**	0.01	0.08	0.10	0.10	0.10	0.10	0.09	0.09
	10	4.00	4.58	4.77	5.01	5.35	5.60	5.73	5.85	0.02	0.10	0.10	**0.12**	**0.12**	**0.12**	**0.12**	0.10
	15	4.00	**4.27**	4.76	5.11	5.48	5.51	5.63	5.80	0.02	0.10	0.10	**0.12**	0.11	0.10	0.10	0.10
	5	5.00	**5.52**	5.78	6.15	6.41	6.36	6.28	6.32	0.01	0.08	0.10	0.12	0.12	0.10	0.10	0.09
	10	5.00	5.65	6.00	6.27	6.41	6.50	6.53	6.45	0.02	0.12	0.12	**0.15**	**0.15**	0.12	0.10	0.10
	15	5.00	5.53	6.00	6.20	6.51	6.66	6.78	**6.84**	0.02	0.10	0.10	0.12	0.12	0.10	0.10	0.10
	5	6.00	6.16	6.13	**6.27**	5.95	5.81	5.53	**5.40**	0.02	0.10	0.12	0.16	0.15	0.13	0.10	0.10
	10	6.00	6.10	6.18	6.23	6.25	6.09	5.83	5.85	0.02	0.12	0.12	**0.20**	0.15	0.15	0.15	0.11
	15	6.00	6.19	6.17	6.26	6.09	5.95	5.80	5.70	0.02	0.10	0.12	0.12	0.12	0.12	0.11	0.10
	5	7.00	6.59	6.31	6.27	6.00	6.16	5.99	5.80	0.01	0.10	0.11	0.13	0.13	0.12	0.11	0.10
	10	7.00	**6.91**	6.65	6.30	6.22	6.25	5.94	**5.80**	0.02	0.12	0.18	**0.20**	**0.20**	0.15	0.15	0.15
	15	7.00	6.85	6.57	6.36	6.32	6.18	5.96	6.01	0.02	0.10	0.10	0.12	0.12	0.12	0.10	0.10

Table 8 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 3 day old culture and 5%, 10% and 15% sizes of inoculum (N – 2)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
3	5	4.00	4.43	4.68	5.10	5.54	5.79	5.93	**6.07**	0.01	0.10	0.10	0.12	0.12	0.12	0.10	0.10
	10	4.00	**4.26**	4.58	4.85	5.11	5.46	5.62	5.73	0.02	0.10	0.11	**0.13**	0.11	0.11	0.11	0.10
	15	4.00	4.30	4.65	4.89	5.02	5.19	5.39	5.47	0.02	0.10	0.11	**0.13**	0.12	0.10	0.10	0.10
3	5	5.00	**5.21**	5.60	5.99	5.73	5.59	5.30	5.28	0.01	0.10	0.10	0.14	0.13	0.11	0.10	0.10
	10	5.00	5.50	6.08	6.23	6.32	6.46	6.40	6.31	0.02	0.12	0.15	**0.20**	0.17	0.17	0.15	0.12
	15	5.00	5.38	5.85	6.20	6.59	6.75	6.79	**6.83**	0.02	0.10	0.12	0.13	0.10	0.10	0.10	0.10
	5	6.00	6.13	6.20	**6.29**	6.15	6.04	5.95	**5.70**	0.02	0.10	0.15	0.18	0.13	0.11	0.10	0.10
	10	6.00	6.16	6.20	**6.29**	6.10	5.88	5.74	5.79	0.02	0.12	0.20	**0.25**	0.20	0.20	0.17	0.15
	15	6.00	6.06	6.12	6.28	6.15	5.92	5.80	5.77	0.02	0.10	0.15	0.20	0.15	0.12	0.10	0.10
	5	7.00	6.70	6.53	6.20	6.18	5.85	5.82	5.75	0.01	0.10	0.12	0.15	0.13	0.13	0.12	0.12
	10	7.00	**6.80**	6.51	6.29	6.11	5.89	5.85	5.87	0.02	0.15	0.20	**0.22**	0.20	0.18	0.15	0.15
	15	7.00	6.76	6.59	6.25	5.99	5.70	**5.45**	5.47	0.02	0.10	0.12	0.20	0.15	0.15	0.12	0.10

Table 9 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 4 day old culture and 5%, 10% and 15% sizes of inoculum (N – 2)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
4	5	4.00	4.35	4.65	4.74	5.00	5.34	5.53	5.62	0.01	0.10	**0.11**	**0.11**	0.10	0.10	0.09	0.08
	10	4.00	4.41	4.76	4.98	5.02	5.22	5.45	5.70	0.02	0.10	0.10	**0.11**	**0.11**	0.10	0.10	0.10
	15	4.00	**4.28**	4.72	4.99	5.04	5.49	5.68	**5.79**	0.02	0.08	0.10	0.10	0.10	0.08	0.08	0.08
	5	5.00	5.59	5.85	6.28	6.20	6.05	5.75	5.80	0.01	0.10	**0.12**	**0.12**	**0.12**	0.10	0.10	0.08
	10	5.00	**5.44**	5.76	6.00	6.19	6.27	6.35	6.35	0.02	0.10	**0.12**	**0.12**	**0.12**	0.10	0.10	0.08
	15	5.00	5.88	6.14	6.32	6.50	6.69	6.65	**6.70**	0.02	0.10	**0.12**	**0.12**	0.10	0.10	0.08	0.08
	5	6.00	6.28	**6.37**	6.29	6.33	6.25	5.90	5.78	0.02	0.08	0.10	0.12	0.12	0.10	0.10	0.08
	10	6.00	6.16	6.20	6.28	6.23	6.25	5.99	**5.70**	0.02	0.12	0.19	**0.20**	0.18	0.18	0.15	0.15
	15	6.00	6.15	6.26	6.25	6.18	5.85	5.80	5.81	0.02	0.12	0.12	0.15	0.15	0.10	0.10	0.09
	5	7.00	**6.82**	6.49	6.29	6.06	5.90	5.67	**5.60**	0.01	0.08	0.12	0.13	0.10	0.10	0.10	0.08
	10	7.00	6.74	6.24	6.25	5.98	5.81	5.86	5.77	0.02	0.12	0.15	**0.20**	**0.20**	0.15	0.12	0.10
	15	7.00	6.74	6.68	6.35	6.12	5.88	5.71	**5.60**	0.02	0.12	0.15	0.15	0.15	0.10	0.10	0.08

Table 10 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 5 day old culture and 5%, 10% and 15% sizes of inoculum (N – 2)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5	5	4.00	**4.32**	4.60	4.95	5.38	5.50	5.67	**5.80**	0.01	0.08	**0.10**	**0.10**	**0.10**	**0.10**	0.05	0.05
	10	4.00	4.46	4.87	5.03	5.29	5.40	5.47	5.65	0.02	0.08	**0.10**	**0.10**	**0.10**	0.08	0.08	0.08
	15	4.00	4.50	4.69	5.04	5.57	5.65	5.70	5.72	0.02	0.08	0.08	**0.10**	**0.10**	0.08	0.05	0.05
	5	5.00	**5.07**	5.40	5.91	6.26	6.41	6.23	6.21	0.01	0.08	0.08	0.10	0.10	0.08	0.08	0.08
	10	5.00	5.46	5.58	5.83	6.07	6.30	6.57	6.65	0.02	0.08	0.10	**0.12**	**0.12**	0.10	0.08	0.08
	15	5.00	5.50	6.02	6.24	6.57	6.70	**6.77**	6.75	0.02	0.08	0.10	0.10	0.08	0.05	0.05	0.05
	5	6.00	6.07	6.13	6.20	5.98	5.60	5.52	**5.45**	0.02	0.08	0.10	0.10	0.10	0.10	0.10	0.08
	10	6.00	5.97	6.08	6.20	6.14	5.70	5.75	5.69	0.02	0.12	0.15	**0.18**	0.15	0.15	0.10	0.10
	15	6.00	6.04	**6.26**	6.25	5.62	5.79	5.67	5.63	0.02	0.10	0.10	0.12	0.12	0.10	0.10	0.06
	5	7.00	6.52	6.32	6.25	6.28	6.20	5.95	5.89	0.01	0.08	0.08	0.11	0.10	0.10	0.08	0.08
	10	7.00	**6.87**	6.50	6.30	6.07	5.97	5.74	**5.51**	0.02	0.10	0.12	**0.18**	**0.18**	0.14	0.12	0.10
	15	7.00	6.80	6.53	6.25	6.07	5.80	5.61	5.63	0.02	0.10	0.10	0.12	0.10	0.08	0.08	0.05

The blue color square indicate the minimum value and the green color square indicate the maximum value.

Discussion and Conclusion

In H-1 strain, packed cell volume (0.24 ml) was the maximum amount in 3 day cultured age and 10% size of inoculum at pH-6. In N-2 strain, packed cell volume (0.25 ml) was the maximum amount in 3 day cultured age and 10% size of inoculum at pH-6. The maximum yield (0.24 ml in H-1 strain) and (0.25 ml in N-2 strain) were recorded in the fermentation period of 3 day.

In 2007, Mar stated that *Lactobacillus* species in 3, 4 and 5 days of age of cultures with the fermentation period of 72 and 96 hours showed the best packed cell volume. The present result agreed with those reported by Mar. Win (1981) reported that 24 to 48 hours old *Lactobacillus casei* culture might be suitable for the effective growth of bacterial cells accompanied by the higher yield of lactic acid in M.R.S. medium. Shu *et al.,* 2016 showed that the optimum incubation temperature was 37°C and the optimum inoculum size was 5%, for growth of *Bifidobacterium bifidum*, *Lactobacillus acidophilus*. The optimum inoculum size of *L. acidophilus* and *L. casei* were all 7% on fermentation of goat milk (Chen *et al.,* 2015). According to these literatures, the results of present work were somewhat different.

Wang *et al.,* 2015 reported that the optimal inoculum size for *L. casei* was 10%. In 2017, Wardani *et al.,* stated that inoculum size may has effect on pH, acidity, viable counts and flavor of fermented milk. Addition of 3, 5 and 10% inoculums resulted in the significantly increase population of lactic acid bacteria during milk fermentation. The data of present research were found to be in agreement with above authors.

The fermented broth medium pH were between 4.20 and 6.17 (at initial pH-4),4.97 and 6.92 (at initial pH-5), 4.68 and 6.42 (at initial pH-6), 5.23 and 7.14 (at initial pH-7) during the fermentation period in H-1 strain. In N-2 strain, the fermented broth medium pH were between 4.26 and 6.07 (at initial pH-4), 5.07 and 6.92 (at initial pH- 5), 5.40 and 6.37 (at initial pH-6), 5.45 and 6.93 (at initial pH-7) during the fermentation period. According to these results, the pH values for maximum packed cell volume was assumed to be in acid side.

Tomas *et al.,* 2002 described that the MRS broth with a pH of 6.5 and a temperature of 37°C yield the highest growth and are the optimal conditions. Yang *et al.,* 2018 stated that the optimal condition for lactic acid bacteria (LAB) was determined in MRS broth, pH 6.2 at 37 °C. Cachon and Divie`s (1994) found that for growth of *L. lactis*, the optimal pH was 6.5. This reported data was in agreement with those mentions.

In the investigation of optimizing the fermentation, H-1 and N-2 strains were found that 3 day of cultured age, 10% size of inoculum and pH-6 with the fermentation period of 3 day showed maximum packed cell volume. It could be concluded that the maximum yield of packed cell volume was produced by *Lactobacillus* species; age of culture (3 days), size of inoculum (10%) at 3 days of fermentation periods and the optimum pH of medium was 6. It is aimed to conduct further study which deal with the application of obtained packed cell volume of lactic acid bacteria in the feed of chicken as probiotic activity.

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