

Sensory and microbiological evaluation of probiotic yoghurt made with different types of probiotic cultures starter *Lactobacillus acidophilus* LA-5[®] and *Bifidobacterium animalis* subsp. *lactis* BB-12[®]

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Article history:

Received: 19 March 2021

Received in revised form: 28 April 2021

Accepted: 10 July 2021

Available Online: 9 March 2022

Keywords:

Probiotic yoghurt,
Lactobacillus acidophilus LA-5[®],
Bifidobacterium animalis subsp. *lactis* BB-12[®],
Direct vat set starter,
Heirloom starter

DOI:

[https://doi.org/10.26656/fr.2017.6\(2\).188](https://doi.org/10.26656/fr.2017.6(2).188)

Abstract

The incorporation of probiotic bacteria into yoghurt is believed could increase its health benefits such as improving intestinal health, reducing the risk of type 2 diabetes mellitus and more. The objective of this study was to evaluate the sensory and microbiological characteristics of probiotic yoghurt (PY) made with 2 types of starter cultures namely direct vat set (DVS) cultures and heirloom (HC) cultures. *Lactobacillus acidophilus* LA-5[®] and *Bifidobacterium animalis* subsp. *lactis* (BB-12[®]) used in this study were obtained from Chr. Hansen. Conventional yoghurt (CY) was made with *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) and *Streptococcus thermophilus* (St). As much as 2% of starter cultures were added into 10% skimmed milk suspension with 10% of sucrose. Lb:St ratio in CY was 1:1, whereas Lb:St:LA-5:BB-12 ratio in PY was 1:1:2:2. The mixture was incubated at 38°C for 6-8 hrs. Total lactic acid bacteria and bifidobacteria, as well as pH value, were determined at 0, 1st, 2nd, 3rd, 4th week of storage. The sensory properties of yoghurt were evaluated at the 0th week and the 4th week. The results showed that after 4 weeks of storage at 4°C, the total lactic acid bacteria were slightly decreased ranging from 0.26 log CFU/mL (DVS-PY) to 0.79 log CFU/mL (CY), while total bifidobacteria was slightly decreased from 0.40 to 0.58 log CFU/mL. The pH value of CY was significantly decreased after 4 weeks of storage, while the pH values of DVS-PY and HC-PY were not significantly different. The result from the Triangle test revealed that DVS-PY has similar sensory properties with CY whereas HC-PY has less thickness. In general, the sensory properties of CY and PY were slightly decreased after 4 weeks of storage at 4°C. In conclusion, incorporation of probiotic bacteria in yoghurt with DVS starter culture give better microbiological and sensory properties compared to HC starter culture.

1. Introduction

The health benefits of probiotics have been known for years. Probiotic was defined as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2002). Dairy products containing probiotic

bacteria have been suggested to have functional effects such as improvement of intestinal health, enhancement of the immune response, reduction of serum cholesterol, cancer prevention (Kechagia *et al.*, 2013) as well as controlling blood glucose level (Ruan *et al.*, 2015). Probiotic addition in fermented food such as yoghurt has increased recently in order to give more health benefits.

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Lactobacillus and *Bifidobacterium* species are the most commonly used probiotics in dairy products. To give health benefits to the host, probiotic bacteria must survive in the upper gastrointestinal (GI) tract and reach the colon where the probiotic will colonize and exert health benefits. In the upper GI tract, probiotic bacteria may undergo several stresses such as low pH, digestive enzymes, and bile salts condition.

There are so many strains of commercial probiotics that can be added to yoghurt. *Lactobacillus acidophilus* LA-5[®] and *Bifidobacterium animalis* subsp. *lactis* BB-12[®] were chosen to be incorporated into yoghurt since there are so many studies using these strains and they are used worldwide with no reported consumer illness or adverse event. *Lactobacillus acidophilus* LA-5[®] and *Bifidobacterium animalis* subsp. *lactis* BB-12[®] could survive in the product during processing and storage as well as in the GI tract (Terpou et al., 2019). Their health benefits have been studied extensively as well (Markowiak and Slizewska, 2017). Several clinical studies regarding the effect of probiotics on type 2 diabetes mellitus have been done. Ejtahed et al. (2012) and Tonucci et al. (2017) has studied the effect of *Lactobacillus acidophilus* LA-5[®] and *Bifidobacterium animalis* subsp. *lactis* BB-12[®] on type 2 diabetes mellitus and they could improve fasting blood glucose and glycemetic control.

Starters are used for the production of yoghurt and fermented milk. In the traditional method, yoghurt producers usually use heirloom starters that were obtained from the previous batch to inoculate to a new batch. This kind of starter leads to the variable performance of yoghurt. On the other hand, the consistency of yoghurt's characteristics is important in industrial yoghurt. Hence, yoghurt producers prefer to use direct vat set (DVS) or direct vat inoculate (DVI) which is only used one time. DVS cultures usually in the form of freeze-dried cultures powder were added to milk and allowed to incubate. If the cultures will be used for the next batch, the performance might be decreased. On the other hand, there are several cultures that can be used more than one time in the next batch. Such cultures are cheaper than DVS cultures (Cultures for Health, 2019). It is important to evaluate the sensory and microbiological characteristics of the probiotic yoghurt produced with different types of starter. Hence, we can get a good quality probiotic yoghurt before it will be consumed as a therapeutic food. A better sensory characteristic of probiotic yoghurt will increase the compliance of yoghurt consumption. Moreover, if the number of probiotic bacteria in probiotic yoghurt is still high, the health benefit of this probiotic bacteria can be obtained by the patients.

This study aimed to evaluate the sensory and microbiological characteristics of probiotic yoghurt (PY) made with *Lactobacillus acidophilus* LA-5[®] and *Bifidobacterium animalis* subsp. *lactis* BB-12[®] inoculated into yoghurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. There was 2 kinds of probiotic inoculation namely direct vat set (DVS) inoculation and heirloom (HC) cultures inoculation. We conducted triangle and hedonic tests to evaluate the sensory characteristic of probiotic yoghurt as well as microbiological analysis of 1-, 2-, 3-, and 4-week stored yoghurts.

2. Materials and methods

2.1 Starter preparation

Lactobacillus acidophilus LA-5[®] and *Bifidobacterium animalis* subsp. *lactis* BB-12[®] used in this study were obtained from Chr. Hansen Malaysia Sdn. Bhd. These cultures were freeze-dried culture powder that was kept at -40°C prior to use. For direct vat inoculation, we used these cultures powder directly inoculated into the milk. While for heirloom cultures preparation, the freeze-dried cultures were cultivated in the MRS broth medium, incubated at 37°C for 24 hrs, then the pellets were reconstituted in a 10% skimmed milk suspension, incubated at 37-38°C for 18 hrs. The heirloom starter cultures were ready to be inoculated into the milk. *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) and *Streptococcus thermophilus* (St) starters were prepared with the same procedure as described above.

2.2 Yoghurt Production

Conventional yoghurt (CY) was made with *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) and *Streptococcus thermophilus* (St). As much as 2% of starter cultures were added into 10% skimmed milk suspension with 10% of sucrose. Lb:St ratio in conventional yoghurt was 1:1, whereas Lb:St:LA-5:BB-12 ratio was 1:1:2:2. The mixture was incubated at 38°C for 6-8 hrs. The flowchart of yoghurt processing can be seen in Figure 1.

2.3 Sensory evaluation

As many as 25 semi-trained panellists were recruited in this study. Semi-trained panellists were the person who was familiar with sensory evaluation and able to differentiate food classes. They were used to evaluate the acceptability or preference of a final product (Chukkan and Bhadra, 2020). First of all, they were asked to differentiate between three yoghurt samples and determine which yoghurt was not identical to the other two. All samples appeared similar and were given to

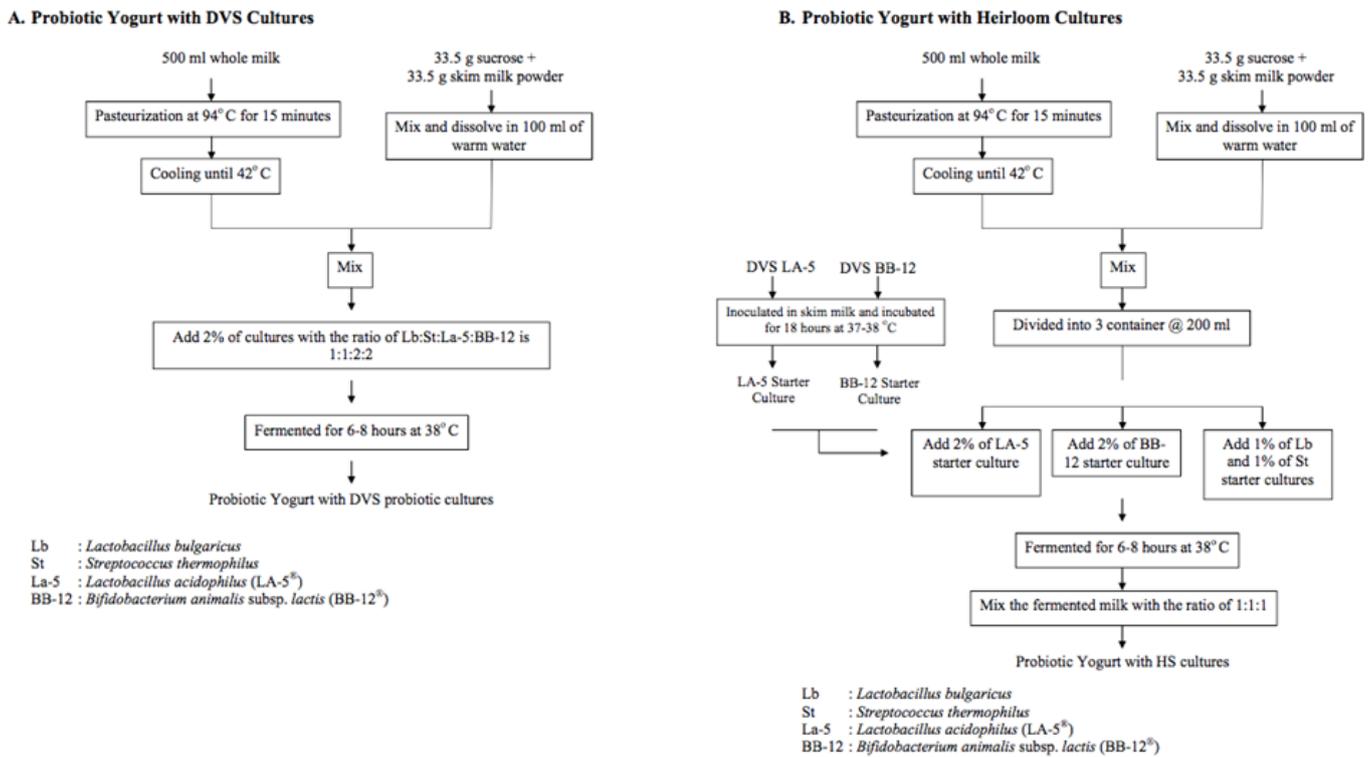


Figure 1. Flowchart of Yogurt Processing

panellists simultaneously. Each sample had a code on it (473, 766, and 987) that the panellists were told to record and state which two samples were the same and which one was different. Then, the hedonic test was conducted to evaluate the yoghurt preferences. They were asked to rate the samples based on a 7-point hedonic scale anchored by: 1 = dislike very much; 2 = dislike; 3 = dislike slightly; 4 = like slightly; 5 = like; 6 = like very much; and 7 = like extremely which modified from Sharif *et al.* (2014). Mineral water was provided as a neutralizer between samples in order to avoid carryover effects. The hedonic test was done twice namely at the 0 and 4th weeks of storage at 4-10°C.

2.4 Microbiological analysis and pH determination

Yoghurt samples were submitted to serial decimal dilutions in NaCl solution (0.85%). The last 3 dilutions namely 10^{-4} , 10^{-5} , and 10^{-6} were then pour plated in MRS agar (Oxoid, Basingstoke, U.K.) for total lactic acid bacteria analysis. The plates were incubated at 37°C for 48 hrs. Plates containing 30-300 colonies were selected, and the colonies were enumerated. The number of colony-forming units per millilitre yoghurt sample was calculated.

For Bifidobacteria enumeration, the last 3 dilutions were then pour plated in Bifidobacterium Selective Count Agar Base with the addition of Supplement A and B (Himedia, Mumbai, India). The plates were incubated at 37°C for 48 hrs under anaerobic conditions (Anaerogen, Oxoid, U.K.). Plates containing 30-300 colonies were selected, and the colonies were

enumerated. The number of colony-forming units per millilitre yoghurt sample was calculated. The microbiological and pH analysis was done at 0, 1st, 2nd, 3rd, and 4th week. The pH values were determined using pH meter Ohaus ST300.

3. Results and discussion

3.1 Sensory characteristic

Samples 473 (CY) and 766 (DVS-PY) were revealed to be identical and sample 987 (HC-PY) was the odd sample. As many as 85.7% panelists found that HC-PY was the odd sample and only 14.3% of the panelists found that CY was the odd sample (Figure 2). It means that probiotic yoghurt made with DVS cultures could produce yoghurt that has similar properties to conventional yoghurt. Probiotic yoghurt made with heirloom starter has a sandy texture and less thickness compared to the other. In actuality, DVS-PY and HC-PY

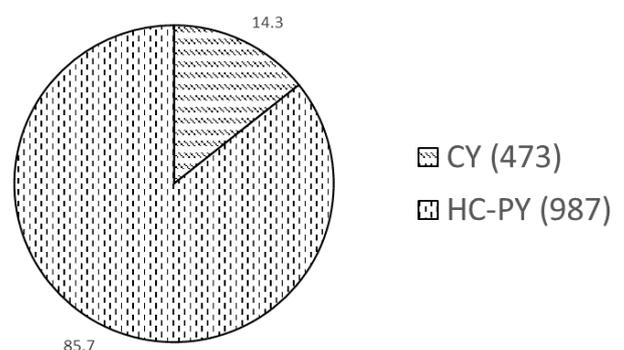


Figure 2. Triangle test to differentiate the characteristics between yogurt samples

were made with the same cultures, however, the yoghurt's characteristic was extremely different.

The hedonic test showed that DVS-PY has similar characteristics with CY both before and after 4 weeks of storage at 4-10°C (Figure 3). This result is in line with the Triangle Test. The sensory characteristics of probiotic yoghurts, both DVS-PY and HC-PY, were decreased after 4 weeks of storage at 4°C, and the highest reduction of the sensory characteristic was found in HC-PY (Table 1). Hence, probiotic starters *Lactobacillus acidophilus* LA-5® and *Bifidobacterium lactis* BB-12® are better to be used as DVS cultures than heirloom culture. The fermentation capability of the heirloom cultures might be decreased because they are used more than one time (Cultures for health, 2019). On the contrary, freeze-dried cultures are usually stable in terms of viability and activity as well as they were convenient in handling, storage, marketing and application. The survival level of LAB cultures was high during freeze-drying and subsequent storage (Ana et al., 2004).

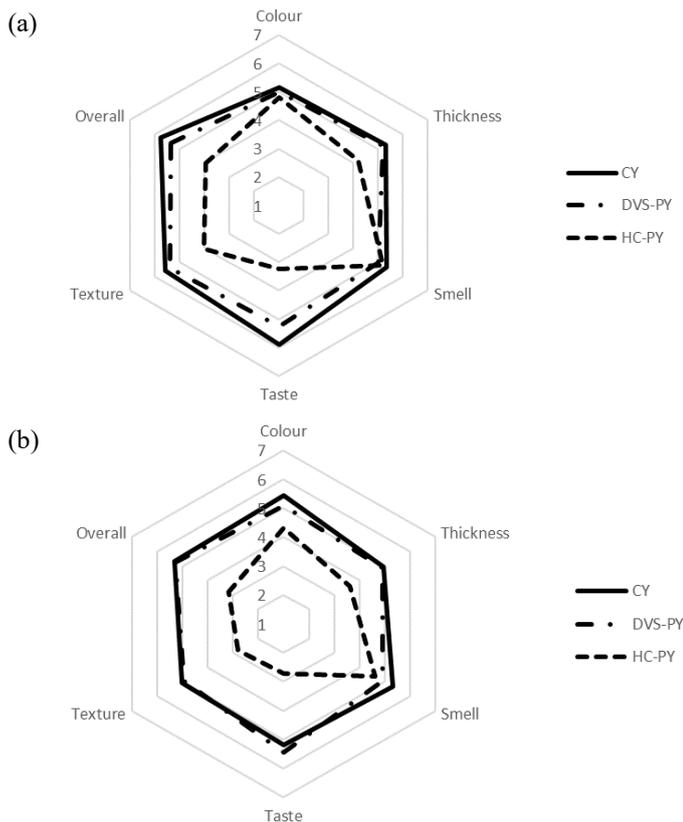


Figure 3. Hedonic test of yogurt samples before (a) and after (b) 4 weeks of storage at 4-10°C

3.2 Total lactic acid bacteria, total bifidobacteria, and pH value of yoghurt

Figure 4 shows that after 4 weeks of storage at 4°C, the total lactic acid bacteria were slightly decreased ranging from 0.26 log CFU/mL (DVS-PY) to 0.79 log CFU/mL (CY). Total bifidobacteria was slightly

decreased from 0.40 to 0.58 log CFU/mL. There were a decreasing number of lactic acid bacteria and bifidobacteria, however, the population is still over 10^7 CFU/mL. Hence, it still met the criteria for the probiotic product. More viable lactic acid bacteria can ensure better survival of bacteria in the GI tract. A study conducted by Snyatkoyskii et al. (2004) showed that the yoghurt that incorporated with DVS starter cultures from Chr. Hansen has an extended shelf life. This study is in line with our study that found that the decreasing of total lactic acid bacteria is higher in the conventional yoghurt that did not add with probiotic cultures. Another study by Ahmadi et al. (2014) found that viable counts of free *Lactobacillus acidophilus* LA-5® cells decreased from 9.55 to 7.3 log CFU/g after 60 days of storage, while that of encapsulated cells merely decreased less than 1 log cycle.

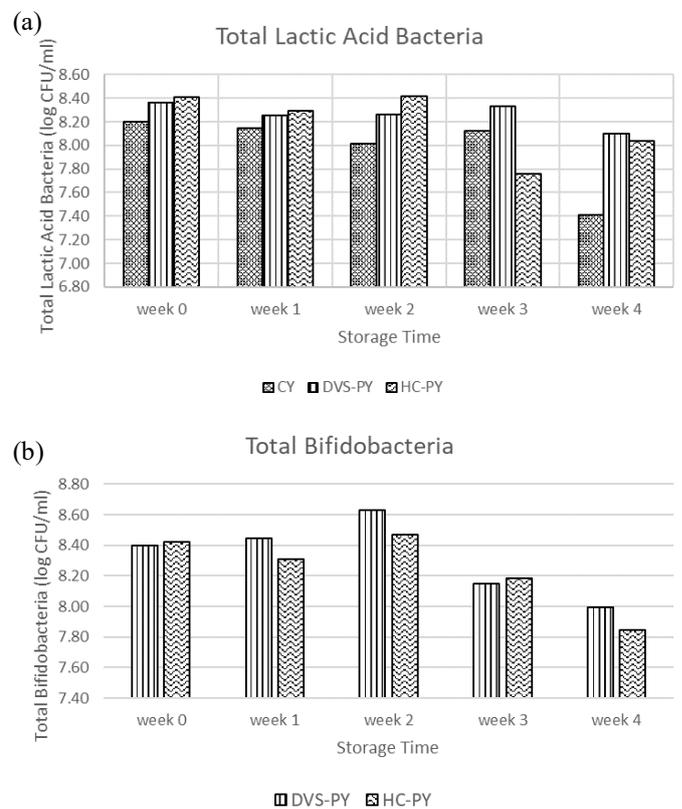


Figure 4. Total lactic acid bacteria (a) and bifidobacteria (b) of yogurt samples before and after 4 weeks of storage at 4-10°C

The finding of our study is in line with a previous study which found that DVS cultures have better viability and activity during processing and storage of yoghurt and yoghurt-ice cream (Snyatkoyskii et al., 2004; Ahmadi et al., 2014). However, since the culture only uses for one time, the yoghurt producers need to purchase a new starter in order to produce yoghurt with stable characteristics. Hence, the production of yoghurt using DVS culture might be more expensive than using heirloom culture.

The pH values of conventional yoghurt and probiotic

Table 1. The aggregate score of hedonic test and the differences of hedonic score before and after 4 weeks of storage at 4-10°C

Sensory properties	Yogurt Variant	Before Storage	After Storage	Delta
Colour	CY	108	114	6
	DVS-PY	105	107	2
	HC-PY	101	91	-10
Thickness	CY	111	104	-7
	DVS-PY	109	103	-6
	HC-PY	88	76	-12
Smell	CY	112	112	0
	DVS-PY	105	103	-2
	HC-PY	109	97	-12
Taste	CY	124	109	-15
	DVS-PY	110	114	4
	HC-PY	68	57	-11
Texture	CY	117	106	-11
	DVS-PY	108	104	-4
	HC-PY	85	59	-26
Overall	CY	121	112	-9
	DVS-PY	113	111	-2
	HC-PY	83	67	-16

CY: conventional yogurt made with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*

DVS-PY: probiotic yogurt made with direct vat set (DVS) cultures of *Lactobacillus acidophilus* La-5[®] and *Bifidobacterium animalis* subsp. *lactis* BB-12[®]

HC-PY: probiotic yogurt made with heirloom (HC) cultures of *Lactobacillus acidophilus* La-5[®] and *Bifidobacterium animalis* subsp. *lactis* BB-12[®]

yoghurt within the same period of storage were not significantly different (Figure 5). The pH value of conventional yoghurt was significantly decreased during 4 weeks of storage. On the contrary, the pH value of probiotic yoghurt both HC-PY and DVS-PY were not decreased during storage. The pH value of the yoghurt reached 3.5 and 3.8, hence it was under the recommended pH value of yoghurt which is between 4.0 and 4.6 (Masulli, 2016). During fermentation, lactic acid bacteria convert lactose into lactic acid, hence it will reduce the pH of milk from 6.7 to ≤ 4.6 . If the pH of milk reached the isoelectric point of casein (pH 4.6), the formation of a three-dimensional network consisting of clusters and chains of caseins occurs (Mulvihill and Grufferty, 1995). Medeiros *et al.* (2015) found that incubation temperature and time, as well as heat pre-treatment of milk, will affect the acidification process

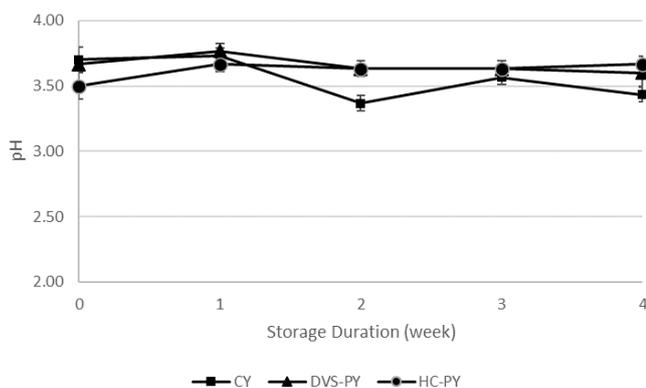


Figure 5. The pH value of conventional yogurt (CY) and probiotic yogurt (HC-PY and DVS-PY) during 4 weeks of storage at 4-10°C

during fermentation. Our yoghurts were incubated at 38°C for 6-8 hrs. The incubation time might be too long so that our yoghurts have a pH value of ≤ 4.0 .

4. Conclusion

Incorporation of probiotics into conventional yoghurt using commercial probiotic starters *Lactobacillus acidophilus* LA-5[®] and *Bifidobacterium animalis* subsp. *lactis* (BB-12[®]) are better to be used as DVS cultures since the sensory characteristic is similar to the conventional yoghurt and microbiological property is better than conventional yoghurt. A DVS culture is very convenient so that consumers can make fermented milk or probiotic yoghurt their own in a simple method.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was partly supported by the Department of Nutrition and Health, Faculty of Medicine, Public Health, and Nursing, UGM for the research funding and Directorate of Research, Universitas Gadjah Mada (UGM) for the publication funds given through the Rekognisi Tugas Akhir (RTA) grant in 2019 granted to Dr Lily Arsanti Lestari (contract number 3152/UN1/DITLIT/DIT-LIT/LT/2019). We thank Chr. Hansen Malaysia Sdn. Bhd. Jakarta representative office and PT. Brenntag for supporting *Lactobacillus acidophilus* LA-

5[®] and *Bifidobacterium animalis* subsp. *lactis* (BB-12[®]).

International Food Research Journal, 22(3), 1030-1036.

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