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Physicochemical and nutritional properties of *doi* fortified with psyllium husk and basil seed

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Abstract

This work was carried out to evaluate the physical, chemical and microbiological qualities of *doi* fortified with psyllium husk (*Plantago ovate*) and basil seed (*Ocimum basilicum*). Seven different types of *doi* - A (control), B (with 0.4% psyllium husk), C (with 0.8% psyllium husk), D (with 1.2% psyllium husk), E (with 0.7% basil seed), F (with 1.4% basil seed) and G (with 2% basil seed) were prepared. The highest total sensory score (86.67) was obtained in A type *doi* which differ non-significantly with B (81.67) and E (78.67) type *doi*. Addition of higher level of fiber lowered the overall physical score but with a higher total solids content of the *doi*. The higher fat and protein content were found in the *doi* with basil seeds which ranged from 4.88-4.97% and 3.90-4.21%, respectively. The addition of psyllium husk and basil seed significantly increase the crude fiber content of the *doi* and the highest value (0.41-0.48%) being recorded in D and G type *doi*. This fortification also helps to reduce the syneresis of the *doi* both at room and refrigeration temperature without affecting the total viable count. Therefore, *doi* can be fortified with dietary fiber without affecting much of its quality attributes.

Keywords: Doi, dietary fiber, psyllium husk, basil seed, syneresis, nutritional profile

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Introduction

Yogurt is a popular dairy product with live bacteria that are termed "probiotics". It is becoming more popular all over the world as a result of its pleasant sensory qualities and high nutritional content. The food has a high calcium bioavailability and helps those with lactose intolerance. The probiotic yogurt market is stabilizing in Europe and North America, while Asia is seen as having the most potential for growth (Champagne *et al.*, 2018). From 1.39 billion pounds in 1994 to 4.65 billion pounds in 2016, the annual production of plain and flavored yogurt has surged (Chen *et al.*, 2018).

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Where the minimum therapeutic level of probiotics is 10^6 or 10^9 CFU/g per serving is a point of contention (Granato *et al.*, 2010; Karimi *et al.*, 2011). Notably, the addition of

probiotic bacteria to yogurt improves various product qualities (for example, texture), mostly through the creation of pro-bioactive substances such as exopolysaccharides, which are metabolites of bacteria such as *Lactobacillus paracasei* (Champagne *et al.*, 2018).

Doi (yogurt like product) is one of the important fermented milk product consumed throughout the Bangladesh, either as a part of the daily diet along with the meal or as a refreshing item. Doi (known as dadhi in India) is a product obtained by lactic fermentation of cow or buffalo (or other dairy animal) milk or mixed milk through the action of single or mixed strains of lactic acid bacteria or by lactic acid fermentation accompanied by alcoholic fermentation by yeast (Bureau of Indian Standards, 1981). The mixed undefined starter used in the manufacture of doi includes Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris,

Streptococcus salaivarius subsp. thermophilus, Streptococcus bovis, Lactobacillus delbrueckii subsp. bulgaricus and Lactobacillus acidophilus.

Fiber is not found in milk or dairy products. Fruits, vegetables, and cereals have fiber in their cell walls (Trowell, 1976). Fiber from various sources is added to products to enhance cooking yield and waterholding capacity, minimize lipid retention, improve textural characteristics and structure, and lower calorie content by acting as a bulking agent (Larrauri, 1999). Fiber-rich diets may help to prevent or reduce gastrointestinal problems as well as cancer (Elia and Cummings, 2007).

Psyllium husk comes from the *Plantago ovate* plant which is native to India and now grown worldwide. It is used as a dietary supplement and is usually found in the form of husk, granules, capsules or powder. The seeds of this shrub like herb are processed so that the husks can be used as a natural laxative. Psyllium husk has little nutritional value beyond its dietary fiber and it is low in calories and fat content.

The scientific name of basil seed is *Ocimum basilicum*. The different common names of basil seed are Sabja seeds, Falooda, Arabic Falooda seeds, Sabja ginjalu, Thai holy basil etc. The seeds contain many phyto-chemicals and polyphenolic flavonoids like orientin, vicenin and other antioxidants. This seed has a lots of benefits such as cooling and soothing effect on stomach, curing diabetes type-2 and relieves constipation.

Considering the above nutritional facts of the *doi*, psyllium husk and basil seeds, the present research was conducted to assess the feasibility of using psyllium husk and basil seed for the production of dietary fiber enriched *doi* and its impact on the quality of the *doi*.

Materials and Methods

Site and period of experiment

The present study was conducted at the laboratory of Dairy Chemistry and Technology, Department of Diary Science, Bangladesh Agricultural University, Mymensingh-2202. In addition, few analyses were also done at the Laboratory of Animal Nutrition, Department of Animal Nutrition; Laboratory of Agricultural Chemistry, Department of Agricultural

Chemistry and Professor Muhammad Hossain Central Laboratory, Bangladesh Agricultural University, Mymensingh-2202.

Table 1: pH, acidity (%) and chemical composition (%) of the raw milk used for *doi* making.

Parameters	Values
pH	6.80
Acidity	0.13
Moisture	86.87
Total solids	13.13
Fat	4.30
Protein	3.20
Carbohydrate	4.90
Crude fiber	Not found
Ash	0.73
Calcium	0.42
Phosphorus	1.51

Collection of raw materials

For *doi* preparation, fresh cow's milk (Table 1) and starter culture were collected from Bangladesh Agricultural University Dairy Farm, Mymensingh-2202. Psyllium husk and basil seed was collected from local market (Swadeshi bazar, Mymensingh-2202, Bangladesh). Sugar was collected from Kamal Ranjit market, BAU campus, Mymensingh-2202, Bangladesh.

Preparation of doi

The raw milk was boiled for about 10 minutes to reduce the volume of milk by about 20% and create optimum condition for the lactic acid fermentation (Chandan and Shahani, 1993). During boiling, 10% table sugar was added to the milk and mixed gently with stirrer. After complete mixing of sugar, the milk was left at room temperature to reduce the temperature to 42°C and then added with 1% mixed undefined starter culture. Aliquots of 50 mL milk was made in plastic cups and eventually for plain doi (A), it has been incubated at 42°C for 4 - 5 hrs. To prepare Psyllium husk doi, 0.4% (B), 0.8% (C) and 1.2% (D) husk was added to different portions of milk after the addition of starter culture. This was followed by blending before pour the milk into the cup for incubation. The same procedure was also followed for Basil seed doi except the inclusion level of the Basil seed (0.7% (E), 1.4% (F) and 2.0% (G)). After incubation, all the seven types of doi samples

(A, B, C, D, E, F and G) were kept in the refrigerator at 5°C until further analyses.

Chemical Analysis

The pH values were determined by using pH meter (HANNA - instrument, model HI 2211 Basic pH/ORP Benchtop Meter, USA). Acidity was estimated by titrating with N/10 sodium hydroxide solution using phenolphthalein as an indicator. Total solids and ash content of doi samples were determined by oven heating and incineration in muffle furnace, respectively as per the method described in the AOAC (2004). Fat content was measured by Babcock method and protein content by Kieldahl procedure. Carbohydrate content was determined mathematically. For crude fiber analysis, 15–20 gm sample was used to digest consecutively by 120 mL of 1.25% H2SO4 and 1.25% NaOH. Each of the digestion lasts for 30 min in a digestion chamber. The residue was oven dried at 105°C and ignited at 600°C. For Ca content, EDTA titrimetric method was used and Phosphorus content was determined by spectrometer (model-T80 UV/VIS Spectrometer) at 660 nm wavelength. For this, 1 g sample in a 100ml volumetric flask was added with 4 mL sulphomolybdic acid and 5 drops of stannous chloride solution. The color intensity was then measured within 15 minutes after the addition of stannous chloride solution.

Sensory analysis

All *doi* samples were judged individually by the faculty members and post-graduate students of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202. Smell and taste (50 points), Body and consistency (30 points), and Color and appearance (20 points) made up the total score of 100 points on the scoring card.

Syneresis

Syneresis of different *doi* samples were done to test the water holding capacity of *doi*. Degree of syneresis expressed as portion of free whey, was measured by a small modification of method of Al-Kadamany *et al.* (2003). A 30-40 g sample of *doi* was placed on a filter cloth resting on the top of a funnel. After 1 hr of draining, the quantity of remained *doi* was weighed and syneresis was calculated as follows:

Syneresis% = {(weight of initial sample-weight of sample after filtration) \div (weight of initial sample)} $\times 100$.

Microbiological analysis

Serial dilutions of the samples were made, poured into SPC agar (Himedia, India) plate and the plates were incubated at 32 °C for 48 hrs. Plates with 30–300 colonies were selected for enumeration and dilution factor was used during calculation.

Statistical analysis

Data collected on different variables were subjected to statistical analysis by using one way analysis of variance (CRD). Mean separation was done by using Tukey's HSD test in case of significant differences. All analysis was conducted in Minitab software.

Results and Discussion

Physical parameters

Smell and taste

The doi with no psyllium husk and basil seed obtained the top smell and taste score of 41 out of 50 (Table 2). The first two levels of the added psyllium husk and basil seed doi did not differ significantly (p>0.05) with this score. Arora and Patel (2015) also found higher flavor score in the conventional yogurt than the fiber enriched samples. Tomic et al. (2017) reported a grainy flavor in triticale fiber enriched yogurt and remarked with bitterness. In addition, fiber addition cause a lower overall flavor and texture scores in doi (Fernández-García and McGregor, 1997). These are highly in line with the findings of the present study. Similarly, Hashim et al. (2009) claimed that the different source of fiber affects the flavor score. However, it is noteworthy that Ott et al. (2000) signify the balance of more than 60 compounds of flavor in the yogurt to direct the flavor perception of this product.

Body and Consistency

The highest body and consistency score was attributed to the *doi* without any added fiber which was 27 out of 30 (Table 2). Body and consistency score of *doi* with 0.4% psyllium husk was 2 unit less than that of the control *doi* (p<0.01). The other samples differ significantly from them but among them they were statistically alike (p>0.05). Psyllium, inulin, microcrystalline cellulose and oat fibers blend

did not cause any change in the body and texture score of the yogurt compare to the conventional yogurt but the blend of Psyllium, inulin, oat bran and wheat fiber did so (Arora and Patel, 2015). Hashim et al. (2009) do not found any effect of the addition of 1.5% date fiber to the yogurt on the texture attributes but addition of 1.5% wheat bran cause a

significant alteration. Tomic *et al.* (2017) found pronounced sandiness/grittiness due to the addition of triticale fiber in the yogurt which is insoluble. These variable findings indicates that different sources of dietary fibers incorporated affects differently in the yogurt matrix and they have different water holding capacity.

Table 2: Changes in the physical, chemical and microbial qualities of the *doi* added with different levels of psyllium husk and basil seed.

Parameter	Α	В	С	D	E	F	G	P-value
Smell and taste (50)	41.00a	40.33ª	38.00ª	35.00 ^b	40.00a	37.00 ^{ab}	34.00 ^b	0.00
	±3.61	±1.53	±1.00	±1.00	±1.00	±1.00	±1.00	
Body and	27.67ª	25.33 ^b	22.67 ^c	20.67c	22.67 ^c	21.67c	20.00c	0.00
consistency (30)	±0.58	±0.58	±1.16	±1.16	±2.31	±1.53	±2.00	
Color and	18.00a	16.00a	14.33 ^b	10.00c	16.00a	14.67ab	8.33c	0.00
appearance (20)	±1.00	±1.00	±1.53	±1.00	±1.00	±1.53	±1.53	
Overall physical	86.67ª	81.67ª	75.00 ^b	65.67 ^c	78.67 ^{ab}	73.33 ^b	66.33 ^c	0.00
score (100)	±4.73	±1.53	±1.73	±1.53	±3.51	±4.04	±4.04	
pН	4.53ª	4.24 ^b	4.12 ^c	4.04^{d}	4.23 ^b	4.14 ^c	4.02 ^d	0.00
	±0.02	±0.01	±0.02	±0.03	±0.03	±0.03	±0.01	
Acidity (%)	0.69^{c}	0.74 ^{bc}	0.77 ^{ab}	0.81a	0.74bc	0.78ab	0.80ab	0.00
	±0.01	±0.02	±0.02	±0.02	±0.04	±0.03	±0.03	
Syneresis at Room	30.13ª	19.40 ^c	17.67 ^d	15.33e	22.43 ^b	17.73 ^d	15.60e	0.00
temperature (%)	±0.40	±0.44	±0.12	±0.15	±0.31	±0.45	±0.66	
Syneresis at 4°C (%)	25.37a	14.43c	13.03 ^c	11.23 ^d	18.03 ^b	14.27 ^c	11.20^{d}	0.00
	±0.88	±0.97	±0.21	±0.56	±0.86	±0.15	±0.30	
Total solids (%)	25.52e	25.69 ^d	25.80 ^d	26.55 ^b	26.16 ^c	26.86 ^b	27.53ª	0.00
	±0.05	±0.09	±0.03	±0.23	±0.04	±0.14	±0.10	
Fat (%)	4.87 ^{bc}	4.80 ^d	4.58e	4.44 ^f	4.88 ^{bc}	4.94ab	4.97ª	0.00
	±0.03	±0.02	±0.03	±0.03	±0.03	±0.02	±0.02	
Protein (%)	3.61^{d}	3.53d ^e	3.51 ^e	3.44 ^f	3.90 ^c	4.05 ^b	4.21a	0.00
	±0.03	±0.02	±0.03	±0.03	±0.03	±0.03	±0.06	
Soluble carbohydrate	16.23 ^f	16.35e	16.47 ^d	17.23ª	16.32e	16.60°	16.83 ^b	0.00
(%)	±0.01	±0.02	±0.01	±0.21	±0.01	±0.09	±0.02	
Crude fiber (%)	0.00^{d}	0.15^{c}	0.28 ^b	0.41ª	0.18^{c}	0.30 ^b	0.48^{a}	0.00
	±0.00	±0.02	±0.02	±0.04	±0.03	±0.02	±0.07	
Ash (%)	0.82^{d}	0.88^{c}	0.96 ^b	1.02ª	0.88^{c}	0.96 ^b	1.04ª	0.00
	±0.01	±0.01	±0.01	±0.02	±0.01	±0.03	±0.03	
Calcium (%)	0.14	0.14	0.12	0.12	0.12	0.10	0.11	0.36
	±0.02	±0.02	±0.01	±0.0	±0.04	±0.02	±0.01	
Phosphorus (%)	0.70	0.99	0.92	0.85	2.36	2.12	1.03	0.07
	±0.25	±0.05	±0.12	±0.23	±1.36	±1.28	±0.15	
Total viable count	69.67 ^d	73.00°	75.33bc	80.67ª	73.67 ^c	79.33ab	82.00a	0.00
$(\times 10^4 \text{ cfu/g})$	±1.53	± 2.00	±1.53	±2.08	±1.53	±2.52	±2.65	

A, Control *doi*; B, *Doi* with 0.4% psyllium husk; C, *Doi* with 0.8% psyllium husk; D, *Doi* with 1.2% psyllium husk; E, *Doi* with 0.7% basil seed; F, *Doi* with 1.4% basil seed; G, *Doi* with 2% basil seed; cfu, colony forming unit.

Color and appearance

The *doi* without any dietary fiber had the maximum score for color and appearance which was 18 out of 20 (Table 2). Statistically doi with lower level of psyllium husk and basil seed showed the similar color and appearance index (p>0.05). Tomic et al. (2017) noticed yellowish brown color in yogurt due to the addition of insoluble triticale fiber to the yogurt. Hashim et al. (2009) mentioned that the date fiber and wheat bran incorporation in yoghurt has significant influence on the yogurt color. The yellowness of yogurt mainly depends on the level of fiber incorporated. They also demonstrate a good correspondence between the color appearance. Sanz et al. (2008) reported a yellow greenish color in the yogurt added with asparagus fiber. So, the addition of different fibers and different level of fibers have diverse effects on the yogurt color (Hashim et al., 2009). However, Staffolo et al. (2004) fortified yogurt in his work with commercial wheat, bamboo, or inulin fibers and found no effect on yogurt color.

Overall physical score

Significantly (p<0.05) higher overall physical score (86.67%) was recorded in the conventional plain doi and addition of dietary fiber tends to reduce the score point (Table 2). Doi with 0.4% psyllium husk and 1.2% basil seed with a range of 78.67-81.67 points score showed statistical similarities with the control doi (p>0.05). Increasing the added fiber level cause a reduction in the score. Hashim et al. (2009) also reported higher overall acceptance of the control yogurt compared to the wheat bran and date fiber added yogurt. The two lower level of date fiber had similarities with the control yogurt (p>0.05), however, the lower level of wheat bran and highest level of date fiber cause a reduction in the score. Tomic et al. (2017) also demonstrated the variation in the overall acceptability of the yogurt sample prepared by using different dietary fibers. All these supports the findings of the present work.

pH and Acidity

Doi without psyllium husk and basil seed had the highest pH value (4.53) which differed significantly (p<0.05) from doi with added fibers (Table 2). The psyllium husk added doi differ among themselves and so for the basil seed added doi (p<0.05). However, the lowest, mid and highest level of psyllium husk and basil seed added doi differ non-significantly between them (p>0.05). Hashim et al. (2009) found significantly lower pH in the plain yogurt when compared with the fiber added

yogurt. The fiber added yogurt differ non-significantly among them. Dabija *et al.* (2018) also found a lower value for the control yogurt compared to the fiber enriched yogurt. This is not in line with the present work.

The acidity content of the two higher level of the psyllium husk and basil seed added doi samples were found significantly more compared to the other doi samples. The lowest acidity value was recorded in the control sample. Raju and Pal (2014) suggested a decrease in the acidity of the fiber added misti doi compared to the doi without fiber. Though Ramirez-Santiago et al. (2010) did not found such result while working on the yam soluble fiber added stirred yogurt. Hashim et al. (2009) also found a non-significant differences among the acidity of the control yogurt and yogurt prepared by adding different dietary fiber. However, Dabija et al. (2018) also evidenced the acidity lowering capacity of added fiber in yogurt. These variations in different works might be attributed to the different sources of dietary fiber used. Because, according to Fernandez-Garcia and McGregor (1997) and Fernandez-Gracia et al. (1998) the proportion of soluble fibers that undergoes microbial fermentation might ended up with the production of organic acids that contributes to the acidity of the product.

Syneresis

Highest syneresis rate at room temperature was found in the plain *doi* which was 30% (Table 2). The range of syneresis in *doi* with 0.4%, 0.8% and 1.2% psyllium husk was 15 - 19%. They differ significantly among themselves and with the plain *doi* (p<0.05). *Doi* with 0.7%, 1.4% and 2% basil seed had syneresis from 15 to 22%. Statistics showed the similar results as it was in psyllium husk. Increased level of fiber addition cause a decreasing trend in the syneresis rate. Lower rate of syneresis was observed at 4 °C than the room temperature. It also showed the similar trend as it was in the syneresis at room temperature.

Raju and Pal (2014) prepared mishti *doi* with the incorporation of inulin, soy and oat fiber in their study and observed a significant variation among the syneresis pattern. Mohamed *et al.* (2014) reported that dried grape pomace addition in yogurt causes 9% lower syneresis compared to the control yogurt. Similarly, addition of yam soluble fiber reduce the syneresis in the stirred yogurt (Ramirez-Santiago *et al.*, 2010). Use of fibers from apple, wheat, bamboo and inulin has non-

significant impact in syneresis process of yogurt (Staffolo *et al.*, 2004). On the other hand, Dabija *et al.* (2018) prepared yogurt with the addition of inulin, oat, pea and wheat fibers and found highest syneresis in the control yogurt and increasing the level of fiber yield a lower syneresis. This agrees with the results of the present work.

Total solids

Highest total solids content (27.53%) was found from 2% basil seed doi (Table 2). Total solids content of doi with 1.4% basil seed and 1.2% psyllium husk were 26.87% and 26.55%, respectively. Doi with 0.7% basil seed has total solids content of 26.16%. Doi without dietary fiber has the lowest value for total solids which was 25.52% and doi with 0.4% and 0.8% psyllium husk have total solids content of 25.69% and 25.80%, respectively. Bhat et al. (2017) reported that only 0.7% psyllium husk added yogurt had significantly higher total solids than the control and 0.1, 0.3 and 0.5% psyllium husk added yogurt. The reported total solids are also very low compared to the present work and this might be attributed to the variation in the yogurt preparation technology (milk volume reduction and sugar addition etc.) and level of added fiber.

Fat

The highest fat content (4.97%) was found in the *doi* prepared with 2% basil seed. The basil seed added yogurt showed more fat content than the psyllium husk added samples (Table 2). The increase in the psyllium husk resulted in the reduction of fat content in the *doi*. The control samples were found similar to the 0.7 and 1.4% added basil seed *doi* (4.87-4.94%). Bhat *et al.* (2017) found similar fat content in the control, 0.1% and 0.3% psyllium husk added yogurt but yogurt with 0.5 and 0.7% psyllium husk had significantly higher fat. The lowest psyllium husk level used in this work was 0.4% and results agrees with the present findings.

Protein

Highest protein content was observed in the *doi* with 2% basil seed which was 4.21% and second highest was found in *doi* with 1.4% basil seed which was 4.05%, which were significantly different (p<0.05) from each other (Table 2). Lower protein scores ranged from 3.44-3.51%, found in *doi* with 1.2% and 0.8% psyllium husk, respectively. The control yogurt showed significantly (p<0.05) higher protein content than

the yogurt with added psyllium husk. The protein content of all *doi* samples matches the protein content range of 3.99-4.74% (Rashid and Miyamoto, 2005) and the protein content range 4.24-3.25% as observed by Bhat *et al.* (2017).

Carbohydrate

Table 2 showed that carbohydrate and crude fiber content of the different experimental samples significantly (p<0.05). The highest carbohydrate content was found in the 1.2% psyllium husk added doi followed by the doi with 2% added basil seed. The control doi has the lowest value and the carbohydrate content of the doi with lowest level of psyllium husk and basil seed were placed before the control doi. With regards to the crude fiber, the trend was found same. Where the highest score was recorded as 0.41-0.48% in the doi with highest level of added psyllium husk and basil seed, and control sample was null. Mohamed et al. (2014) reported an increased trend of fiber content in the buffalo milk yogurt with the increase of the level of added fiber. This agrees with the findings of the current study.

Ash, Calcium and Phosphorus

The highest ash content were found 1.04% and 1.02% in 2% basil seed doi and 1.2% psyllium husk doi, respectively (Table 2). Doi with 0.8% psyllium husk and 1.4% basil seed had the same ash content which was 0.96%, significantly different (p=0.00) from other doi samples. Doi with 0.4% psyllium husk and 0.7% basil seed also had the same ash content which was 0.88%. Doi without any added fiber had the lowest ash content which was 0.82%, significantly different (p<0.05) from all other doi samples. The ash content of control doi sample was in line with the range of 0.73-0.91% as reported by Haj et al., (2007). However, the present findings of all doi samples were lower than the ash content range (1.08-1.50%) found by Rashid and Miyamoto (2005) in plain doi. Fiber addition cause a reduction in the ash content of the yogurt (Bhat et al., 2017).

Both the calcium and phosphorus content of the control doi and doi with added psyllium and basil seed showed non-significant differences (p>0.05). The calcium content of the different doi samples ranged from 0.10 to 0.14% which was 0.70 to 2.36% for phosphorus content.

Total viable count (×10⁴ cfu/g)

The total viable count per g of the doi with 2% basil

seed was highest and that was 82 (Table 2). Bacteria count for 1.2% psyllium husk *doi* and 1.4% basil seed *doi* were 2 and 3 unit less than the *doi* with 2% basil seed. The *doi* prepared with 0.8% psyllium husk had total viable count 75 which was 5 unit less than the *doi* with 1.2% psyllium husk. Bhat *et al.* (2017) also reported an increased total viable count in the higher level of psyllium husk added yogurt. This increase of the total viable count in the *doi* may be attributed to the increased water activity in the product due to added fiber and prebiotic effect of the added fiber (Hassan *et al.*, 2006).

Conclusion

Fortification of *doi* is of great interest to improve the functionality and create foods with health benefits. The results of the present study suggests that 1.2% psyllium husk and 2% basil seed could be added with the *doi* as a source of dietary fiber. However, instrumental texture, sensorial, physicochemical and color profile should be done to make the addition more precise.

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Conflict of Interest: The authors declares that there is no conflict of interest.

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