

ORIGINAL ARTICLE

Development of Probiotic Enhanced Prebiotic a Cereal-Based Synbiotic Complex

Divya. J.S¹, Hemamalini.A.J.², Soundararajan³, Priya Iyyer³

^{1,2}Department of Clinical Nutrition, Sri Ramachandra Institute of Higher Education & Research, Chennai, Tamilnadu India.

^cDepartment of Nephrology, Chennai, Tamilnadu India, ^eDepartment of Biotechnology Women's Christian College Chennai.

Corresponding author: hemajanardh@rediffmail.com

ABSTRACT

Probiotics have been defined as the live organisms in food and dietary supplements that upon ingestion, can improve the health of the host. A synbiotic preparation is one that combines probiotics and prebiotics. It is becoming increasingly popular and it is evidenced by rapidly expanding research support and an ever-widening choice of products. Curd is one of the most important fermented milk products in India and have highly beneficial effects on human health. They have been demonstrated to positively modulate the intestinal microflora and when used together they confer a synergistic effect on the host's health. The Aim/objective of this study was development of probiotic enhanced prebiotic A cereal-based synbiotic complex. The curd was prepared in four different kinds of prebiotic sources which were ragi, bajra, oats and barley. The sensory evaluation was done for different food categories of the samples. The food pathogen test was done to check the microorganisms. A taste panel of 30 panelist was undergone for this study were formed. An organoleptic test was conducted and chemical properties of the curd containing prebiotic was found to be acceptable, Kruskal Wallis Test was used to check whether there is any median difference in the ratings among the three samples. For appearance, the mean rating from sample A is 7.1 ± 0.88 , the mean rating for sample B is 7.7 ± 0.99 and the mean rating for sample C is 7.3 ± 1.18 . Statistically significant (p -value = $0.033 < 0.05$) The chemical properties were indicated in terms of pH and acidity and the microbiological properties were indicated by the growth of *Lactobacillus* spp. in the MRS media. The study measured the survival of microorganisms and also to develop probiotic complex over the 28-day storage was examined to check with the colony forming unit (CFU). The pH. control was seen remained viable count of $CFU 10^9$. And finalized with the indigenous synbiotic complex.

Keyword: probiotics, prebiotics, Inulin, microflora, curd, yogurt, gut microbiota, cereals, synbiotic.

Received 15.11.2020

Revised 22.12.2020

Accepted 07.01.2021

How to cite this article:

Divya. J.S, Hemamalini.A.J., Soundararajan, Priya I Development of Probiotic Enhanced Prebiotic a Cereal-Based Synbiotic Complex. Adv. Biores., Vol 12 (1) January 2021: 163-169

INTRODUCTION

Probiotics are widely used in foods, supplements, and drugs in India. Globally, the market for probiotic foods and supplements is considerably larger than the market for probiotics as therapeutic agents. Most probiotics in wide use are derived from human origin strains of intestinal bacteria, while some are from dairy starter cultures. The regulation of probiotics in food and as drugs is a matter of concern primarily because safety needs are to be assured [1].

In 2001 the Food Agriculture Organization (FAO) and World Health Organization (WHO) jointly organized an Expert Consultation on evaluation of Health and Nutritional properties of probiotics in food. This resulted in the FAO/WHO working group report in 2002, which provided guidelines covering the evaluation of probiotics in foods. It is important to keep in mind that a marketable probiotics food has certain desirable characteristics. these characteristics ultimately relate to safety, efficacy, stability of the probiotics during production, storage, and distribution, and to the ease of distribution of a standardized product [2].

During the fermentation process, lactate is converted into cellular energy by the action of bacteria and the glucose is broken down anaerobically to improve the food quality and safety. Another important advantage of this process is it successfully reduces the pH of food materials by holding the action of food poisoning microbes like *S. aureus*, *E. coli*, *Campylobacter coli* and *V. cholerae*. Formation of curd is one of the common food fermentation processes, this process lengthens the life time, quality and safety of the curd when compared to milk [3, 4].

The roughages are part of plant-derived food and beta-glucan is a very common group of dietary fiber present in barley. Beta-glucan stimulates the development of beneficial microbes in food products other than beta-glucan, some other dietary fibers have the same efficiency [5]. Prebiotics is a type of fiber compound and that can stimulate the activity of good gut microbes, when we include prebiotic foods in our diet it helps easy to digest, help to absorb calcium and quickly process carbohydrates in your body [6]. Thus, dried barley and Brewers' Spent Grain (BSG), they have large quantity of beta-glucan [7-9].

MATERIAL AND METHODS

Preparation of the prebiotic mixture

The barley, jowar, oats and ragi was used for the prebiotic mixture of the product and also to analyze the colony forming unit (CFU) microorganisms count present in it. Each sample was completely washed and dried for one day. And then all the dried samples were grinder and made into a powder. The powders were stored at room temperature in a sterile container for the further use in the experiment.

COLONY FORMING UNIT (CFU) OF THE FOOD PRODUCT:

Preparation of the cereal extract:

The cereal extract was finely grounded and was made into a porridge, to get the maximum essence of the barley then the microbial testing and the colony forming unit testing was used to select the appropriate method of obtaining the cereal extract (table 1). Depending upon the consistency taste also the method of obtaining cereal extract was chosen.

The different grain extract was subjected to fermentation using yogurt and curd. The product was kept under 12 hours fermentation for monitoring the effective growth of the probiotic organisms. The mixture of curd and yogurt was kept overnight for fermentation 12 hours to get the final product. The final product was taken to the lab for the testing of microorganisms present in it. The different types of microbial was carried out for analyzing the potential bacteria. It consists of different type of lactobacillus species.

Preparation of the product

- ❖ To grind the raw barley
- ❖ 5g of the powder was boiled in 150ml of water
- ❖ This was kept under cool temperature and then strained.
- ❖ 25ml of curd and yogurt was added
- ❖ And then the final mixture was prepared.

Milk types

Different types of milk were carried out for the preparation of the synbiotic mixture. These were used to check the microorganisms present with that selected milk was taken and then microbiological technique was been done to check the colony forming unit present in it. And then the milk sample was finalized with that selected milk the product fermentation was carried out.

Curd & Yogurt preparation

The inoculum was obtained from the home-made curd for the setting of the curd. The avin green color milk has been used because of its high fat content. The milk was filtered and boiled at 80-90°C for 10-15 minutes. then it was cool down to the temperature of 45-50°C. 500ml of milk was taken of the different vessel 4 tsp of curd and yogurt was added for the setting. CFU during the fermentation of each milk product was mentioned in table 2.

Standardization of the culture microbial testing

Chemical properties of curd samples

pH of the curd samples obtained was measured by using the PH meter.

Acidity of curd samples: the acidity was measured by adding 1ml of pheno.00lphthalein indicator to 9gm of curd & titrating it against 0.1N NaOH.

RESULTS

Preparation of the sample

To select the product of the sample used, and to check for the microbial analyze and the to finalize the product

SAMPLE PREPARATION FOR SENSORY EVALUATION**PREPARATION OF EACH PRODUCT:****Sample 1: barley+ curd**

Barley (5g) was boiled in 150ml water under low flame for 15mins.once cooled to strain it for the thick consistency of the barley and then 25ml of curd was added with it and then kept for overnight fermentation (12hrs) these was tightly closed in the pet containers.

Sample 2: barley curd +yogurt

Barley (5g) was boiled in 150ml water under low flame for 15mins.once cooled to strain it for the thick consistency of the barley and then curd(25ml), yogurt(25ml) was added with it and then kept for overnight fermentation (12hrs) these was tightly closed in the pet containers.

Sample 3: barley+skim-milk powder+curd+yogurt

Skim-milk powder (10tsp) was boiled in 100ml of water and kept under low heat. And in the separate bowl barley (5g) was boiled in 150ml of water under low flame, for 15mins once these both cooled immediately transferred it into the pet container jar, and then curd(25ml), yogurt(25ml) was added with it and then kept for overnight fermentation (12hrs) these was tightly closed in the pet containers.

Organoleptic Properties of Curd Samples

A taste panel of 30 panelists was undergone for this study were formed and an organoleptic test was conducted. The panel included 10 faculty 10 chronic kidney disease patients and 10 were AHS students. A blind test was performed and each member in the panel rated the 3 samples in different categories on a 5-point scale (5= extremely like to 1=extremely dislike) for 5 attributes (appearance, aroma, texture, taste, overall acceptability). Water was provided in between two samples to rinse the mouth. A fresh batch of each curd sample was prepared for this test (table 4).

Microbiological Properties

1mL of curd from the samples was serially diluted up to 10-12 dilutions. Then 0.1 mL from 10-2to 10-12 dilution was used for pour plate method of enumeration of bacterial colonies. The colony count was found higher between 10-4 or 10-6 The media used was nutrient Agar and MRS Agar media which favors the growth of Lactobacillus spp. The growth was fastest in MRS and plate the plates were incubated at 37°C to 48°C for 37 hours.

Statistical analysis:

Kruskal Wallis test was used to test whether there is any median difference in the ratings among the three samples. For appearance, the mean rating from sample A is 7.1 ± 0.88 , the mean rating for sample B is 7.7 ± 0.99 and the mean rating for sample C is 7.3 ± 1.18 (table 6, figure 2). On comparing the mean values, we see that the mean rating is high for Sample B when compared with other two samples and statistically significant (p - value = $0.033 < 0.05$)

Table 1: Colony forming unit (CFU) of different food products

S/N	Food	CFU
1	Barley	20×10^9
2	Oats	16×10^9
3	Bajra	8×10^9
4	Ragi	18×10^9

Table 2:Colony forming unit (CFU) of various milk products

S/N	Food	CFU
1	Curd	65×10^5
2	Yogurt	12×10^6
3	Curd+Yogurt	60×10^9

Table 3: Nutrient analysis of various cereals; Recommended Dietary Allowance (RDA)/100g

S.NO	ENERGY	PROTEIN	CHO	FAT	CALCIUM	PHOS	SODIUM
RAGI	1342	7.16	66.82	1.92	364	210	4.75
BAJRA	1398	9.97	67.68	1.73	27.60	274	5.42
BARLEY	1321	10.14	61.29	1.30	28.64	178	7.56
OATS	1389	16.89	66.28	2.69	54	203	4.00

Table 4: Organoleptic Properties of Curd Samples; SAMPLE A = BARLEY+CURD, SAMPLE B= BARLEY+CURD+YOGURT, SAMPLE C= BARLEY +CURD + YOGURT+SKIMMILK

	Appearance	Texture	Taste	Flavor	Color	Consistency	Overall Acceptability
A	7.10	7.03	6.79	6.67	6.97	7.03	7.07
B	7.70	7.45	7.67	7.60	7.63	7.60	7.97
C	7.30	6.93	6.67	6.57	6.93	6.73	6.73

Table 5: Scoring results of 3 different dairy products, where 1 = not like completely, 2 = not like hugely, 3 = not like fairly, 4 = not like a little, 5 = neither like or dislike, 6 = prefer slightly, 7 = prefer fairly, 8 = like greatly, and 9 = like completely.

Characteristics	Sample	Mean
Appearance	Sample (a)	7.1±0.88
	Sample (b)	7.7±0.99
	Sample (c)	7.3±1.18
Texture	Sample (a)	7.03±0.89
	Sample (b)	7.45±1.24
	Sample (c)	6.93±1.23
Taste	Sample (a)	6.79±1.21
	Sample (b)	7.67±1.21
	Sample (c)	6.67±1.47
Flavour	Sample (a)	6.67±0.92
	Sample (b)	7.6±1.19
	Sample (c)	6.57±1.77
Color	Sample (a)	6.97±0.93
	Sample (b)	7.63±1.1
	Sample (c)	6.93±1.26
Consistency	Sample (a)	7.03±0.85
	Sample (b)	7.6±1.22
	Sample (c)	6.73±1.39
Overall acceptability	Sample (a)	7.07±0.98
	Sample (b)	7.97±1.13
	Sample (c)	6.73±1.48

Table 6: Statistical analysis of organoleptic properties of curd samples(Kruskal Wallis Test)

	Appearance	Texture	Taste	Flavor	Color	Consistency	Overall Acceptability
Chi-Square	6.800	4.532	10.811	10.510	7.474	5.780	16.373
Df	2	2	2	2	2	2	2
Asymp. Sig.	.033	.104	.004	.005	.024	.056	.000

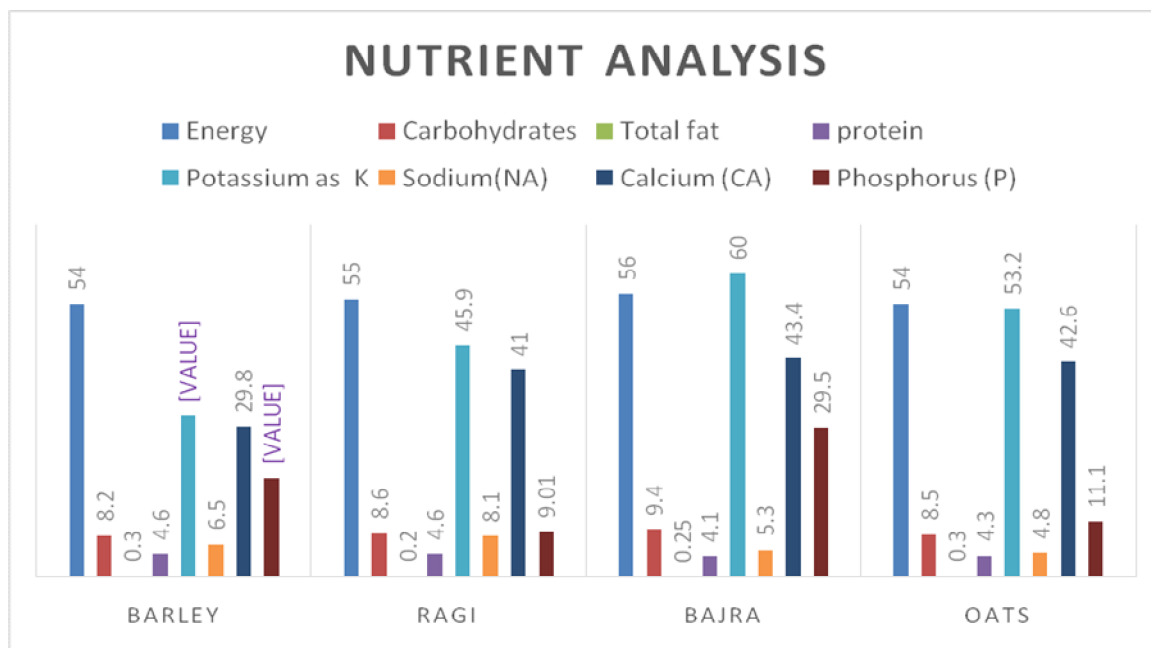


Figure 1: Nutrient analysis of various cereals; Recommended Dietary Allowance (RDA)/100g

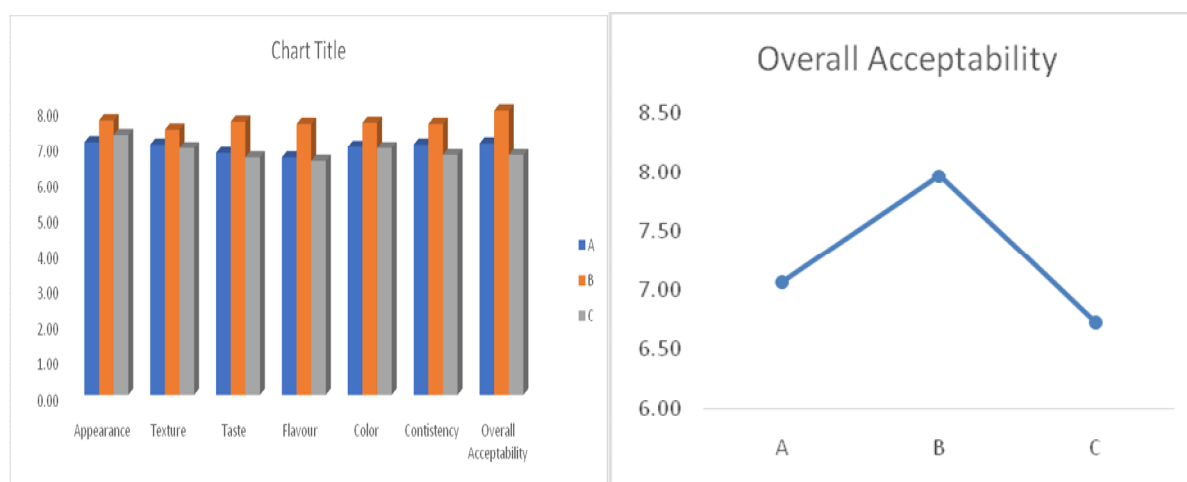
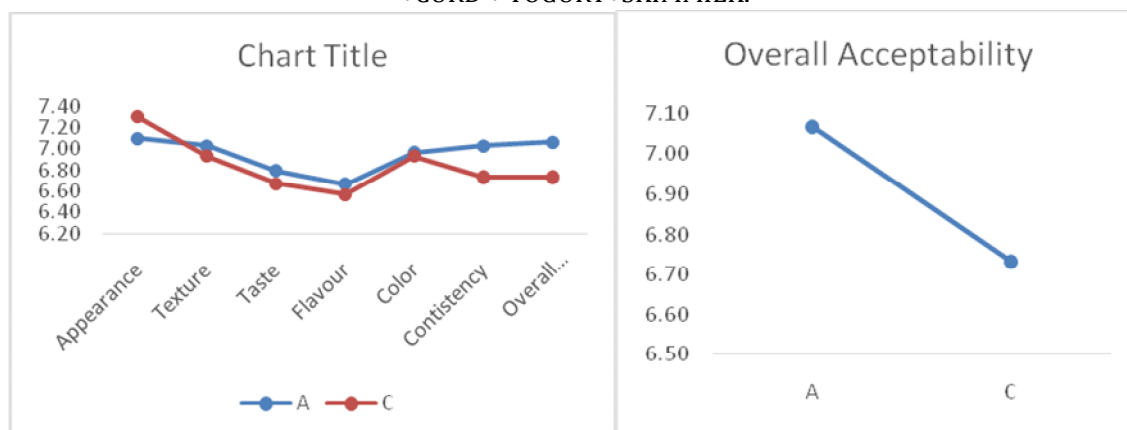


Figure 2: Organoleptic Properties of Curd Samples

Figure 3: Statistical analysis of organoleptic properties of curd samples. A = BARLEY+CURD, C= BARLEY +CURD + YOGURT+SKIMMILK.



DISCUSSION

Bacterial analysis

All the four samples (Ragi, Barley, barley,oats) was successfully yielding with the probiotics. Descriptive statistics of *L.lactis*, *L.acidophilus*, *L.bulgaricus*, *Streptococcus thermophilus* with these samples over all the storage and the colony forming unit (CFU) barley had the highest average of the colony forming unit. And also, it has a good source of inulin a prebiotic. These samples were kept under 4°C to check the shelf life of the colony plate and the microbial growth of the culture medium. the storage was checked since one-month period of time. Day -1, day -7-day 9-day 14 day 28 to check the microbial count of the plate to analyze the shelf life of the prebiotic mixture.

Bacteria count (CFU)

The average tolerability and the S.D for all products are expressed in the descriptive in table 1.

Kruskal Wallis test was used to analyze difference in the ratings among three samples and the attributes of appearance, texture, taste, consistency and overall acceptability.

Data collection

The panelist was requested to taste all food products depends on characteristic of texture, stability, flavor, overall presentation and tolerability based on nine-point hedonic scale. The major indigenous fermented milk products, which is palatable, refreshing, and has characteristic taste which is liked by the people of all age group. People who are lactose intolerant can also consume these products, if not for their therapeutic quality but for the essential and satisfying nutrients required in a daily diet. In addition, it has also been recommended for replenishing the normal flora in the intestine after having heavy doses of antibiotics. Combining curd and yogurt with the prebiotics will be beneficial for human health [8-10]. This will not only enhance the growth of beneficial microorganisms in the gut but also improve the gut health. The study measured the survival of microorganisms and also to develop probiotic complex over the 28-day storage was examined to check with the colony forming unit (CFU). The pH control was seen remained viable count of CFU 10⁹ and finalized with the indigenous symbiotic complex. The consumer's overall acceptability for the product was satisfying and also the pH and acidity was in normal range. The best quality curd was the one containing barley as the prebiotic source. The oats also contain higher percentage of dietary fibers. But the barley contains higher growth of the colony forming unit (CFU) of the microorganisms. Compared to the other food groups like bajra, ragi and oats. Moreover plan to creating fresh and more healthy food materials or just recreating the old ones, through this way we can fulfill the expected nutrient supplement and health beneficial ingredients in our food stuffs [11-14]. Grains are one of the good sources of probiotics but not only the grains there are lots of other natural probiotics containing food products are available they are also support the growth and activity of bacteria to the human gut.

ACKNOWLEDGEMENT

This study is funded through "Founder – Chancellor, shri N.P.V. Ramaswamy Udayar Research fellowship" Grant of full time Ph.D. Programme in Sri Ramachandra Institute of Higher Education and Research.

Abbreviations

CFU: colony forming unit, QOL: Quality of life MRS: Man, Rogosa, Sharpe, RDA: Recommended dietary allowances.

REFERENCES

1. Ranganathan N.,B. Pechenyak, U. Vyas, P. Ranganathan, S. DeLoach, B. Falkner, A. Weinberg, S.J. Saggi and E.A. Friedman (2013). Dose Escalation, Safety and Impact of aStrain-Specific Probiotic (Renadyl™) on Stages III and IV Chronic Kidney Disease Patients. *J Nephrol Ther*, 3:3, 141.
2. Gibson, G.R. and M.B. Roberfroid (1995). Dietary modulation of the human colonic microbiota introducing the concept of prebiotics. *J nutr1*, 25:1401-1412.
3. Niwa, T. (2011). Role of indoxylsulfate in the progression of chronic kidney disease and cardiovascular disease: experimental and clinical effects of oral sorbent AST-120.*Therapeutic Apheresisand Dialysis*, 15(2):120–124.
4. Meijers, B. K. I., H. De Loor, B. Bammens, K. Verbeke, Y. Vanrenterghem and P. Evenepoel (2009). p-cresylsulfate and indoxylsulfate in hemodialysis patients. *Clinical Journal of the American Society of Nephrology*, 4(12):1932–1938.
5. Taki, K., Y. Tsuruta and T. Niwa (2007). Indoxylsulfate and atherosclerotic risk factors inhemodialysis patients. *AmericanJournal of Nephrology*, 27(1):30–35.
6. Meijers, B. K. I. and P. Evenepoel (2011). The gut-kidney axis: indoxylsulfate, p-cresylsulphate and CKD progression. *NephrologyDialysis Transplantation*, 26(3):759–761.

7. Lin, C.J., H. H. Chen and C. F. Pan (2011). p-cresylsulfate and indoxylsulfate level at different stages of chronic kidney disease. *Journal of Clinical Laboratory Analysis*, 25(3):191-197.
8. Takayama, F., K. Taki and T. Niwa (2003). Bifidobacterium in gastroresistant seamless capsule reduces serum levels of indoxylsulfate in patients on hemodialysis. *American Journal of Kidney Diseases*, 41(3): S142-S145.
9. Taki, K., F. Takayama and T. Niwa (2005). Beneficial effects of Bifidobacteria in a gastroresistant seamless capsule on hyperhomocysteinemia in hemodialysis patients. *Journal of Renal Nutrition*, 1(51):77-80.
10. Nakabayashi, M., Nakamura, K., Kawakami, T., Ohta, I., Kato, K., Uchida, K. and M. Yoshida (2011). Effects of synbiotic treatment on serum level of p-cresol in haemodialysis patients: a preliminary study. *Nephrology Dialysis Transplantation*, 26(3):1094-1098.
11. Bammens, B., P. Evenepoel, K. Verbeke and Y. Vanrenterghem (2004). Impairment of small intestinal protein assimilation in patients with end-stage renal disease: extending the malnutrition-inflammation-atherosclerosis concept. *American Journal of Clinical Nutrition*, 80(6):1536-1543.
12. Bammens, B., K. Verbeke, Y. Vanrenterghem and P. Evenepoel (2003). Evidence for impaired assimilation of protein in chronic renal failure. *Kidney International*, 64(6):2196-2203.
13. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Cordoba, Argentina, 1-4, 2001.
14. Cannon, J.P., T.A. Lee, J.T. Bolanos and L.H. Danziger (2005). Pathogenic relevance of Lactobacillus: a retrospective review of over 200 cases. *Eur J Clin Microbiol Infect Dis*, 24:125-6.

Copyright: © 2021 Society of Education. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.