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Gastrointestinal Mikroflorayı Desteklemek İçin *Bacillus clausii* Sporları İçeren Probiyotik Tofu

Probiotic Tofu with *Bacillus clausii* Spores to Support Gastrointestinal Microflora

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ÖZ

Amaç: Bu çalışma, vegan bireylerin probiyotik tüketimi için diyetlerini değiştirmeden ve ek probiyotik hapları kullanmak zorunda kalmadan yararlanabilecekleri probiyotik *Bacillus clausii* sporları ile zenginleştirilmiş bir tofu ürünü geliştirmek amacıyla yapılmıştır.

Yöntem: *B. clausii*, 2xSG ortamında sporlanacak şekilde kültürlendi. Elde edilen *B. clausii* sporlarının tofuya eklenmesi, probiyotiğin tofu içinde en yüksek miktarda tutulabilmesini sağlamak için prosedürdeki optimum adımı belirlemek için iki farklı yöntemle gerçekleştirildi; *i*) pıhtılaştırıcı $MgCl_2$'den önce probiyotik sporların eklenmesi (P-C tofu), *ii*) pıhtılaştırıcıdan sonra probiyotik sporların eklenmesi (C-P tofu).

Bulgular: P-C tofu ve C-P tofudaki probiyotik sporların verimleri sırasıyla 5,45±0,40 ve 5,33±0,56 log cfu/g olarak hesaplandı ve bu hedeflenen 6 log cfu/g seviyeye oldukça yaklaşmıştır. Probiyotik konsantrasyonu açısından P-C ve C-P tofu yöntemleri arasında anlamlı bir fark gözlenmedi (P=0,36; t testi). P-C tofunun -18°C'de saklanması probiyotik yükü 1 ayda 0,45 log cfu/g azaltmıştır (P=0,003). Soğuk zincir depolamanın bir günlük 20°C'de kesintiye uğraması 0,85 log seviyede anlamlı bir probiyotik azalmasına neden oldu (P=0,01). Sote pişirme uygulaması tofudaki spor yükünü 1,22 log seviyede anlamlı derecede azaltmıştır (P=0,004). P-C ve probiyotik içermeyen tofuların duyusal değerlendirmesinde genel kabul edilebilirlik özelliklerinde anlamlı bir farklılık gözlenmedi (P>0,05).

Sonuç: Probiyotikle zenginleştirilmiş tofu, tüketicilerin sağlığını olumlu yönde etkilerken veganlar ve vejetaryenler için sağlıklı bir alternatif seçim olabilir.

Anahtar Kelimeler: Tofu, *Bacillus clausii*, Probiyotikler, Zenginleştirme.

ABSTRACT

Objective: This study was conducted to develop a tofu product enriched with probiotic *Bacillus clausii* spores that vegan individuals can benefit from without changing their diets for probiotic consumption and without having to use the supplementary probiotic pills.

Method: *B. clausii* was cultured to sporulate in 2xSG medium. The resulting *B. clausii* spores were added to tofu by two different methods to determine the optimum step in the procedure to ensure that the probiotic was able to retain in tofu at the highest amount; *i*) addition of probiotic spores before coagulant $MgCl_2$ (P-C tofu), *ii*) addition of probiotic spores after the coagulant (C-P tofu).

Results: The yields of probiotic spores in P-C and C-P tofu were calculated to be 5.45±0.40 and 5.33±0.56 log cfu/g, respectively, which was slightly lower than the targeted level, 6 log cfu/g. No significant difference was observed between the P-C and C-P tofu methods in terms of probiotic concentration (P=0.36; t test). Storage of P-C tofu at -18°C decreased the probiotic load by 0.45 log cfu/g in 1 month (P=0.003). One-day temperature abuse at 20°C of cold chain storage resulted in a significant 0.85 log reduction (P=0.01). Sauté cooking was found to decrease the spore load in tofu significantly by 1.22 log (P=0.004). Sensory evaluation of P-C and probiotic free tofu did not reveal a significant difference in general acceptability features (P>0.05).

Conclusion: Probiotic enriched tofu may be a healthy alternative choice for vegans and vegetarians while positively affecting the health of consumers.

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Key words: Tofu, *Bacillus clausii*, Probiotics, Fortification.

1. INTRODUCTION

The majority of the microflora of digestive system are probiotics (1). According to the Food and Agriculture Organization (FAO) probiotic bacteria are viable microorganisms that can have health-promoting effect in humans when consumed at sufficient amounts (FAO, 2002). In addition, probiotics are defined as living microorganisms which are used as nutritional supplements by positively affecting the intestinal microbial balance in order to benefit the health of consumers. According to the U.S. Food and Drug Administration, food containing probiotics at least 10^6 colony-forming-unit (cfu) per milliliter or gram, which is defined as the minimum therapeutic level, are classified as probiotic food (2). Probiotics are proven beneficial and have long-term health benefits on developing immune system, preventing cancer and allergies, and also during early infancy (3). The use of probiotics in plant-based food is common in Asian countries due to cultural, economic, and climatic factors. Also, consumption of dairy products are not common, especially when compared to cereal consumption, including fermented vegetables, fruit, and soybeans (4). Today, there is an increasing demand by the consumers for more non-dairy food products that contain probiotics (5,6) because of the consumer cautiousness on both excess cholesterol intake and the undesired results of dairy product consumption such as lactose intolerance (6).

The indigenous probiotic bacteria that make up the microflora of the digestive system are mostly dairy probiotics *Lactobacillus* and *Bifidobacterium* (7). However, sporeforming *Bacillus* strains are also used as probiotics in drugs and supplements (8). One of the most obvious factors affecting intestinal microflora is diet. Vegetarianism and especially its stricter form veganism are a way of life where people do not consume food items of animal origin (9). Since the vegan diet is limited, the products for vegan consumers pose as a growing market (9). The fecal microbiota of vegan test subjects was investigated and low microbial counts of the *Bifidobacterium* species were observed (10). In a study of probiotic supplementation, the presence of coliforms, *Escherichia coli*, and *Bacterioides* species in gut microflora was observed to decrease due to probiotic consumption (11). Therefore, probiotic intake is important as it may improve the balance of intestinal microflora. In the current study, the spores of probiotic bacteria were aimed to be included in tofu during production process in an effort to provide probiotics to vegan or vegetarian individuals.

Soybeans are one of the most important plant based protein sources in the world that are widely used in food processing. Tofu is the most common example of soybean products. Tofu is composed of approximately 6.0-8.4% protein, 79-87% water, and has almost neutral pH value (5.2-6.2) (12). Tofu is a well-known and highly nutritious food product made from hot soy milk mixed with certain food grade salts like calcium chloride and magnesium chloride, which are used as coagulants (13).

Bacillus strains (*B. cereus*, *B. clausii*, and *B. pumilus*) have potential probiotic effects (14) as they have the potential for colonization, immunostimulation, and antimicrobial activity. *B. clausii* was found to alleviate the symptoms of diarrhea without any side effects (15,16) and it is a commercially used as probiotic. As mentioned above, *B. clausii* has both the ability to create spores and has probiotic characteristics (17). Therefore, *B. clausii* spores were qualified to be a good option to be used in probiotic fortification of tofu in this study.

To induce the ratio of sporulating cells in a bacterial culture, media composition plays an important factor (18, 19). The US Army Research, Development and Engineering Command

conducted a 2 year study for optimization and characterization of spores quality of *Bacillus* species (20). In this study, four types of sporulation media were compared including 2x Schaefer's Glucose (2xSG) media among others. The best sporulation efficiency (>90-95%) was observed in 2xSG media in solid medium and in the shortest time (6 days). In addition, the use of 2xSG media as a liquid medium gave the best results from these four types of media (> 60%). One of the research findings was that the number of spores for 2xSG media is inversely proportional to the number of cells planted. Another finding was that the spores formed in 2xSG sporulation media are the most resistant compared to other media after being subjected to heat shock application at 65ºC and 85ºC for 30 minutes (20).

To have the highest benefit, it is important for the target microorganisms to be able to reach the intestinal flora without acquiring any damage from the gastric acid and before germination to the vegetative form. The main objective of this study was to deliver the probiotics to the intestinal microflora in the highest possible proportion. Instead of taking probiotic supplement pills as a separate dietary supplement, it was aimed to provide them in food to prevent negative effect of taking pills and to provide dose control of probiotics by consuming tofu.

2. METHOD

Preparation of soy milk

Dried soybeans, generously gifted by Vegan İşler Ltd. Şti. (Istanbul, Turkey), were washed, soaked in pure water (1:6, w:v), and stored at 4°C overnight. Soy milk was made from dry soybeans by following the method described by Zielinska et al. (21). Soaked soybean grains were washed, peeled, and mashed with pure water (1:6, w:v), followed by pasteurization at 100°C for 10 min. The pasteurized mixture was filtered through sterile coarse filter paper to remove any solid particles to obtain soy milk.

Production of *Bacillus clausii* spores

The suspension containing 500 µl of *B. clausii* spore suspension (equivalent of ca. 4×10^8 cfu) from Enterogermina® (Sanofi S.p.A., Italy) purchased from a local store was added to Tryptic Soy Broth (TSB) (Merck, 11 10% Na-sesquii carbonate (22), and the spores were germinated to vegetative cells and grown at 37°C. To induce sporulation, 2xSG sporulation medium were used (23). The cells were incubated for 7 days in 2xSG sporulation medium (20) or in TSB with 10% Na-sesquii carbonate as negative control to assess the efficacy of 2xSG sporulation medium. At the end of the incubation, the cell and spore suspension was heat-shocked at 80°C for 20 min to kill the vegetative cells using a dry heat block (Eppendorf, Germany) (24). *B. clausii* spores were recovered from the medium by centrifugation (Eppendorf) at 16,000 ×g for 15 min, and enumerated by serial dilution and spread plating on Tryptic Soy Agar (TSA).

Probiotic enrichment of tofu

Tofu production was performed as previously reported (25). The orders of coagulant magnesium chloride and probiotic spore additions were changed to test if the addition of spores before or after the coagulant addition results in more spore attachment and retention in tofu.

Method 1: Adding probiotics before coagulant (P-C tofu)

B. clausii spores (10^8~10^9) in pure water were added into the hot soy milk (200 ml) at 70-72°C and mixed. Next, coagulant magnesium chloride hexahydrate (MgCl2•6H2O, TK120290, Tekkim, Turkey) was added as 5% (w:v) to the soy milk at 70-72°C and incubated 10 min for coagulation. After coagulation occured, entire mixture was transferred into a sterile press cloth and placed into a mold. Excess liquid was discharged by pressing resulting in the tofu pellet.

Method 2: Adding coagulant before probiotics (C-P tofu)

The coagulant $MgCl_2$•$6H_2O$ was added as 5% (w:v) to the hot soy milk at 70-72°C and incubated 10 min for coagulation. After observing that coagulation had started, *B. clausii* spores (10^8~10^9) were added and mixed. Then, the entire mixture was transferred into a sterile press cloth and placed into the mold. Excess liquid was discharged by pressing the same way as described above, resulting in the tofu pellet.

pH and brix measurement of tofu samples

Soy milk and tofu samples were homogenized with a T18 digital ultra-turrax (IKA-Werke GmbH, Germany) and their pH were measured. Brix (%) values of soy milk samples were also measured by using a digital hand refractometer.

Microbial Enumeration of Liquid Samples

B. clausii vegetative cells or spores in liquid growth media were enumerated using standard serial dilution and spread plate method. Dilutions were carried out in sterile physiological saline (0.9% NaCl) prepared in distilled water. Spread plating was performed on TSA petri plates. The petri plates were incubated at 37°C for upto 2 days and colonies observed were counted. The cell concentration (cfu/ml) was calculated by the following formula:

Cell concentration = (Number of Colonies × (Dilution Factor)^(-1)) / (Volume transferred from dilution tube to petri dish (ml))

Microbial Enumeration of Tofu Samples

Tofu sample (10 g) was homogenized in 90 ml sterile physiological saline. Shortly, the ultra-turrax probe was first cleaned and disinfected in 70% ethanol solution followed by rinsing in sterile distilled water, and the tofu sample was homogenized and serially diluted in physiological saline. Spread plating was performed on TSA petri plates. The petri plates were incubated at 37°C for upto 2 days and colonies observed were counted. Also the effect of storage at -18°C for 1 month on probiotic load in P-C tofu was evaluated.

Effect of Temperature Abuse during Cold Chain Storage on Probiotic Spore Viability

Tofu is a product that needs to be kept at either 4 or -20°C temperature before consumption. Also, it is crucial that the probiotic spores in tofu stay in ungerminated state until consumption as heat application by the consumer during cooking may easily kill germinated probiotic spores. To simulate the effect of a temperature abuse, such as breakage of cold chain

on tofu storage, the tofu samples were left at room temperature (~22°C) for 1 day and the amount of germinated spores were enumerated. Simply, tofu samples were homogenized in sterile physiological saline. One group was heat treated at 80°C for 20 min to kill any vegetative cells formed due to spore germination, whereas other group was not heat treated. Both tofu samples were homogenized and serially diluted in sterile physiological saline and plated on TSA to enumerate the surviving *B. clausii* spores.

Effect of Cooking Tofu on Probiotic Spore Viability

Tofu samples were diced in 1 cm^3 pieces and sautéed in hot vegetable oil in a frying pan followed by homogenization, serial dilution and enumeration on TSA to find out the number of surviving *B. clausii* spores.

Sensory Analysis

Sensory analysis was conducted as described (26). Shortly, the two tofu samples, probiotic-added and regular probiotic-free, which were encoded by 3-digit numbers were sautéed for 5 min and tasted by 16 untrained volunteering adult panelists. Panelists were asked to rate each tofu sample on color, appearance, taste, and general acceptability levels by line scale method. Panelists were given salt-free crackers and water to neutralize between the samples.

Determination of Consumer Attitudes towards Probiotic Supplements

Fifty university students who study Food Engineering were asked 3 questions to assess their stance on probiotics and probiotic supplements. The questions asked to the subjects were as follows. Do you know what probiotics are? Have you ever used probiotic supplements? If you had the option to take probiotics in the form of a supplement or in the food item that you normally consume, which one would you choose?

3. RESULTS

Productivity of *B. clausii* spore formation

The percentage of spores to total live cell number, which includes the spores and vegetative cells, were found to be 5.01% in TSB with 10% Na-sesquii carbonate medium and 63.10% in 2xSG sporulation medium for 7 days (Table 1). The 2xSG sporulation medium was reported to increase the spore percentage over 60% in 13 days (20). The spores were separated from the media by centrifugation. Supernatant containing waste medium, metabolites, and dead cell debris were removed and spores were recovered in pellet, which was resuspended in pure water to use in tofu production. The mean and standard deviation of spores lost in the supernatant were detected to be 5.30 ± 1.38 log cfu/ml (n=7).

Table 1. 2xSG Sporulation Media to Increase Spore Efficiency (n=3).

Sporulation media used	Total cell concentration (log cfu/ml) ± SD	Spore concentration after treatment at 80°C for 20 min (log cfu/ml) ± SD	Sporulation efficiency (%)
TSB with 10% Na-sesquii carbonate medium	7.7 ± 0.4	6.4 ± 0.7	5.01
2xSG sporulation medium	8.3 ± 0.2	8.1 ± 0.6	63.10

Probiotic tofu production

The regular probiotic-free tofu and probiotic supplemented tofu was produced starting with 200 ml of soy milk. For the probiotic tofu production, the order of coagulant and probiotic addition was changed to find out whether the order has an effect on the retention ability of probiotics in tofu. All three types of tofu were produced at least 3 times. The mean and standard deviation of tofu yield and whey volumes were measured as 46.59 ± 0.59 g and 135.61 ± 2.70 ml, respectively (Table 2). The amount of probiotic spores in tofu was aimed to be 6 log cfu/g; however, the amounts of probiotic spores in P-C tofu and C-P tofu were found to be 5.45 ± 0.40 and 5.33 ± 0.56 log cfu/g, respectively (n=5). No significant difference was observed between the P-C tofu and C-P tofu in terms of spore retention (P=0.36). Whereas, the spores lost in the whey of C-P tofu was significantly higher than that in the whey of P-C tofu (P=0.03). Therefore, it was decided that following steps in this study was conducted on P-C tofu. Storage of P-C tofu at -18°C for 1 month decreased the probiotic load by 0.45 log cfu/g (P=0.003).

Table 2. Quantitative Analysis Data in Tofu Production.

Tofu type	Tofu yield (g) ± SD	Whey volume (ml) ± SD	Probiotic spore load in tofu (log cfu/g) ± SD	Probiotic spore load in whey (log cfu/ml) ± SD	pH ± SD
Probiotic free tofu	45.96 ± 0.57	132.50 ± 10.61	-	-	6.12 ± 0.02
P-C tofu	46.68 ± 3.66	137.00 ± 2.65	5.45 ± 0.40	5.44 ± 0.29	6.13 ± 0.13
C-P tofu	47.12 ± 2.67	137.33 ± 4.62	5.33 ± 0.56	5.98 ± 0.45	6.08 ± 0.08
Average	46.59 ± 0.59	135.61 ± 2.70	-	-	

The pH of soy milk was 6.45 ± 0.16. Coagulation of soy proteins is made possible by reducing the pH of soy milk. While the regular probiotic free tofu has a pH of 6.12 ± 0.02, P-C tofu and C-P tofu have pH values of 6.13 ± 0.13 and 6.08 ± 0.08, respectively. The mean brix (%) value of soy milk was 9.24 ± 1.68 (n = 3).

Effect of temperature abuse during cold chain storage and cooking on probiotic spore viability

To test the effect of temperature abuse on cold chain storage, new batches of probiotic spore added tofu was produced and their spore load was found to be 5.73 ± 0.38 log cfu/g. *B. clausii* spore concentration in tofu that was kept at room temperature for 1 day decreased to 4.88 ± 0.11 log cfu/g indicating a significant 0.85 log (85.87%) reduction (P=0.01). This reduction could be seen as an indication of the amount of probiotic spores that germinates during the 24 h at room temperature pinting out the importance of the continuity of cold chain storage.

Spore concentration in sautéed tofu decreased from 5.73 ± 0.38 to 4.51 ± 0.21 log cfu/g indicating a significant 1.22 log (93.97%) reduction (P=0.004). This level of reduction in probiotic spores was not expected and it is thought that the spores located on or near the surface of the tofu pieces were not tightly fixed in the texture of tofu and easily passed into the oil, considering the tofu pieces were diced in 1 cm^3 pieces. However, the oil used for sauté cooking was not enumerated to validate.

Sensory analysis

The difference between the consumer acceptabilities of the probiotic free tofu and probiotic added P-C tofu was measured as reported by the sensory analysis on 16 untrained volunteers. The answers of the panelists who were asked to grade the two tofu samples by their color, appearance, taste, and general acceptability using a line scale method can be seen in Figure 1. Although the mean color and appearance scores of the probiotic free tofu were higher than that of the probiotic added tofu, the mean taste and general acceptability scores of the probiotic added tofu were higher than the regular tofu. However, none of the differences were significant (P>0.05).

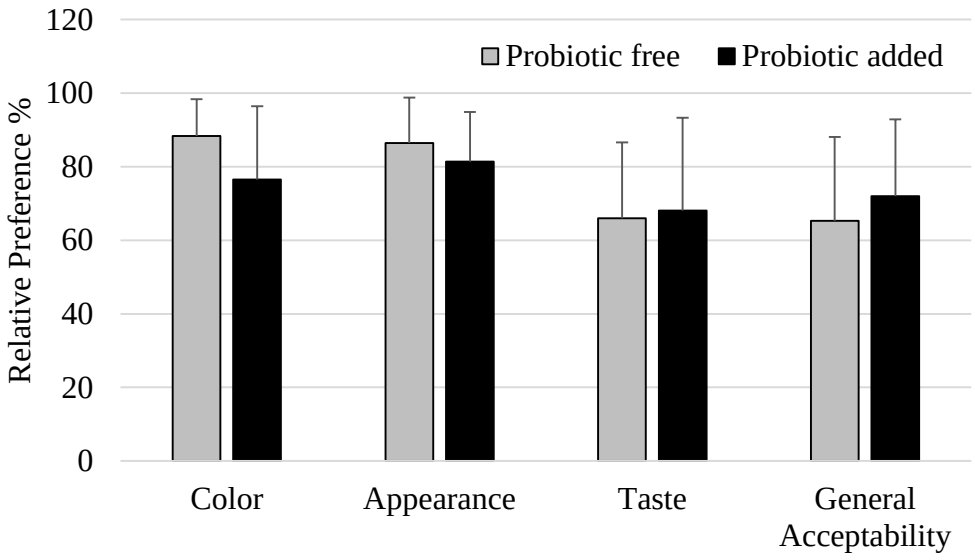

Figure 1. Sensory Analysis Results of Probiotic Free and Probiotic Added P-C Tofu Samples.

Consumer attitudes towards probiotic supplements

A small convenience sample of 50 university students (undergraduate and graduate) were asked to measure their awareness on probiotics and their preference on how to consume them if they had a chance to choose. The group was composed of 13 females and 37 males while 47 of them were undergraduate, only 3 of them were graduate students. Their answers are listed in Table 3.

Table 3. Consumer Awareness and Attitudes Towards Probiotics and Supplements.

Questions	**Yes** **n (%)**	**No** **n (%)**
Do you know what a probiotic is?	48 (96.0)	2 (4.0)
Have you ever used probiotic supplement products?	8 (16.0)	42 (84.0)
	as supplement **n (%)**	**within food** **n (%)**
If you had the option to take a probiotic within food or as a supplement, which one would you choose?	0 (0.0)	50 (100.0)

4. DISCUSSION

The 2xSG sporulation medium was reported to increase the spore percentage over 60% in 13 days (20). In this study, percentage of spores to total live cell number was calculated to be 63.10% in 2xSG sporulation medium after 7 days.

It has been reported that commercial probiotic *Bacillus cereus* spores exhibit better adhesion to high molecular weight and heavily glycosylated proteins such as mucin in the cell membrane compared to vegetative cells (27). Also, polysaccharides such as chitosan, carrageenan, and guar gum are used to modify the texture and extend shelf life of tofu (28–30). In addition, the formation of soybean protein isolate and 70 kDa dextran conjugates has also been reported to reduce the particle size of soy milk and the gel strength of tofu and increase the water-holding capacity of tofu (31). Dextran is composed of d-glucose molecules that are connected through α-(1→6) glucosidic bonds. Due to its neutral charge, dextran is commonly used in protein glycosylation (32). Therefore, it is predicted that the addition of polysaccharides such as dextran, chitosan, carrageenan, and guar gum into soy milk before the coagulation step in the process of tofu making may increase spore retention.

The pH of the soy milk in the current study shows similarity to the pH of 6.6 ± 0.08 measured in a study where the soy milk was prepared at a ratio of 1:6 (soybean:water) (33). Also in another report, the pH of soy milk is suggested to be between 6.4 to 6.6 for higher level of soy protein extraction (34).

Since there is no similar study in the literature, different results have been obtained regarding the effect of storage on probiotics in tofu. Ng et al. (2008), reported that *Lactobacillus bulgaricus* FTCC 0411 and *Lb. fermentum* FTD 13 bacteria counts in tofu were at the level of 7-8 log CFU/g during the 9-day cold storage, while total viable bacterial count decreased by 1

log and the pH decreased slightly (35). However, another study in which probiotic *Lb. rhamnosus* was added to soy cheese, a slow decrease in pH and in the number of added probiotic bacteria were observed after 30 days of storage at 10°C. The period between 5th and 15th days was indicated to be important as the most striking changes occurred during that time of storage (36).

Many studies in the literature have shown that the addition of lactic acid bacteria to soy elevates the consumer acceptance of the products. The "grassy" and "floury" taste of soy, which is unliked by many consumers, is masked by the "acid" taste produced by these lactic acid bacteria (37–40). In our study, similar to the results of these studies, the addition of probiotics to tofu increased the taste and acceptability of tofu.

Table 3 shows that the majority of the university students questioned did not use any probiotic supplement products. With the same point of view, all of the students declared they would prefer consuming probiotics within food rather than as supplement.

5. CONCLUSION

As a result, soybean is a high protein, low energy and valuable raw material for production. Considering its benefits, it is increasingly used for production not only in Asia but also around the world. The fermentation process used in soy increases the nutritional value of soy products and helps to eliminate unacceptable soy flavor. In our current study, we developed a new functional tofu containing probiotic spores, unlike the studies in the literature. Taking into account the specific characteristics of the probiotics or the spores used, adding probiotics to tofu can be an alternative for vegetarians and vegans and can positively affect the health of people who consume these foods.

Conflict of interest statement

No conflict of interest was declared by the authors.

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REFERENCES

1. Cutting, S. M. (2011). Bacillus probiotics. *Food Microbiology*. *28*, 214–220
2. Akan, E., and Kınık, Ö. (2015). Gıda üretimi ve depolanması sırasında probiyotiklerin canlılıklarını etkileyen faktörler - factors effecting probiotic viability during processing and storage of food. *Celal Bayar Üniversitesi Fen Bilimleri Dergisi*. *11*, 155–166
3. Thomas, D. W., Greer, F. R., American Academy of Pediatrics Committee on Nutrition, & American Academy of Pediatrics Section on Gastroenterology, Hepatology, and Nutrition (2010). Probiotics and prebiotics in pediatrics. *Pediatrics*, *126*(6), 1217–1231.
4. Rivera-Espinoza, Y., and Gallardo-Navarro, Y. (2010). Non-dairy probiotic products. *Food Microbiology*. *27*, 1–11
5. Pimentel, T. C., da Costa, W. K. A., Barão, C. E., Rosset, M., and Magnani, M. (2021). Vegan probiotic products: A modern tendency or the newest challenge in functional foods. *Food Research International*. *140*, 110033
6. Panghal, A., Janghu, S., Virkar, K., Gat, Y., Kumar, V., and Chhikara, N. (2018) Potential non-dairy probiotic products – A healthy approach. *Food Bioscience*. *21*, 80–89
7. Crittenden, R., Laitila, A., Forssell, P., Mättö, J., Saarela, M., Mattila-Sandholm, T., et al. (2001). Adhesion of bifidobacteria to granular starch and its implications in probiotic technologies. *Applied and Environmental Microbiology*, *67*, 3469–3475
8. Patrone, V., Molinari, P., and Morelli, L. (2016) Microbiological and molecular characterization of commercially available probiotics containing Bacillus clausii from India and Pakistan. *International Journal of Food Microbiology*. *237*, 92–97
9. Ploll, U., Petritz, H., and Stern, T. (2020) A social innovation perspective on dietary transitions: Diffusion of vegetarianism and veganism in Austria. *Environmental Innovation and Societal Transitions*. *36*, 164–176
10. Zimmer, J., Lange, B., Frick, J. S., Sauer, H., Zimmermann, K., Schwiertz, A., et al. (2012). A vegan or vegetarian diet substantially alters the human colonic faecal microbiota. *European Journal of Clinical Nutrition*. *66*, 53–60
11. Rajkumar, H., Mahmood, N., Kumar, M., Varikuti, S. R., Challa, H. R., and Myakala, S. P. (2014) Effect of Probiotic (VSL#3) and Omega-3 on Lipid Profile, Insulin Sensitivity, Inflammatory Markers, and Gut Colonization in Overweight Adults: A Randomized, Controlled Trial. *Mediators of Inflammation*. *2014*, 1–8
12. Kovats, S. K., Doyle, M. P., and Tanaka, N. (1984) Evaluation of the microbiological safety of tofu. *Journal of Food Protection*. *47*, 618–622
13. Jubayer, M., Uddin, M., and Faruque, M. (2014) Standardization parameters for production of tofu using WSD-Y-1 machine. *Journal of the Bangladesh Agricultural University*. *11*, 307–312
14. Duc, L. H., Hong, H. A., Barbosa, T. M., Henriques, A. O., and Cutting, S. M. (2004) Characterization of Bacillus probiotics available for human use. *Applied and Environmental Microbiology*. *70*, 2161–2171
15. Horosheva, T. V., Vodyanoy, V., and Sorokulova, I. (2014) Efficacy of Bacillus probiotics in prevention of antibiotic-associated diarrhoea: a randomized, double-blind, placebo-controlled clinical trial. *JMM Case Reports*. *1*, 3–8
16. Sudha, M. R., Bhonagiri, S., and Kumar, M. A. (2013) Efficacy of Bacillus clausii strain UBBC-07 in the treatment of patients suffering from acute diarrhoea. *Beneficial Microbes*. *4*, 211–216
17. Turnbull, P. C. B. (1996) Bacillus. in S. Baron (Ed.), *Medical Microbiology*, 4th Ed., University of Texas Medical Branch at Galveston, Galveston, TX

18. Schaeffer, P., Millet, J., and Aubert, J. P. (1965) Catabolic repression of bacterial sporulation. *Proceedings of the National Academy of Sciences*. *54*, 704–711

19. Leighton, T. J., and Doi, R. H. (1971) The stability of messenger ribonucleic acid during sporulation in Bacillus subtilis. *Journal of Biological Chemistry*. *246*, 3189–3195

20. Smith, L. S., Wallace, L., and Rastogi, V. K. (2011) *Studies on Sporulation Optimization and Chracterization of Bacillus subtilis Spore Quality, Edgewood Chemical Biological Center, Maryland.* https://apps.dtic.mil/sti/citations/ADA553789

21. Zielinska, D., Kaminska, A., and Kolozyn-Krajewska, D. (2015) Development of tofu production method with probiotic bacteria addition. *Journal of Microbiology, Biotechnology and Food Sciences*. *04*, 485–490

22. Nielsen, P., Fritze, D., and Priest, F. G. (1995) Phenetic diversity of alkaliphilic Bacillus strains: proposal for nine new species. *Microbiology*. *141*, 1745–1761

23. Leighton, T. (1974) Further studies on the stability of sporulation messenger ribonucleic acid in Bacillus subtilis. *Journal of Biological Chemistry*. *249*, 7808–7812

24. Yano, K., Masuda, K., Akanuma, G., Wada, T., Matsumoto, T., Shiwa, Y., et al. (2016). Growth and sporulation defects in Bacillus subtilis mutants with a single rrn operon can be suppressed by amplification of the rrn operon. *Microbiology*. *162*, 35–45

25. Dey, A., Prasad, R., Kaur, S., Singh, J., and Luwang, M. D. (2017) Tofu: technological and nutritional potential. *Indian Food Industry Magazine*. *36*, 8–24

26. Chavan, S. M., and Verma, R. C. (2017) Textural and Sensory Characteristics of Freeze Dried Tofu. *International Journal of Current Microbiology and Applied Sciences*. *6*, 119–128

27. Sánchez, B., Arias, S., Chaignepain, S., Denayrolles, M., Schmitter, J. M., Bressollier, P., et al. (2009). Identification of surface proteins involved in the adhesion of a probiotic Bacillus cereus strain to mucin and fibronectin. *Microbiology*. *155*, 1708–1716

28. Chang, K. L., Lin, Y. S., and Chen, R. (2003). The effect of chitosan on the gel properties of tofu (soybean curd). *Journal of Food Engineering*. *57*, 315–319

29. Li, M., Chen, F., Yang, B., Lai, S., Yang, H., Liu, K., Bu, G., et al. (2015). Preparation of organic tofu using organic compatible magnesium chloride incorporated with polysaccharide coagulants. *Food Chemistry*. *167*, 168–174

30. Shen, Y. R., and Kuo, M. I. (2017) Effects of different carrageenan types on the rheological and water-holding properties of tofu. *LWT*. *78*, 122–128

31. Li, L., Wang, C., Li, K., Qin, W., Wu, D., Hu, B., et al. (2021). Influence of soybean protein isolate-dextran conjugates on the characteristics of glucono-δ-lactone-induced tofu. *LWT*. *139*, 110588

32. Spotti, M. J., Martinez, M. J., Pilosof, A. M. R., Candioti, M., Rubiolo, A. C., and Carrara, C. R. (2014) Influence of Maillard conjugation on structural characteristics and rheological properties of whey protein/dextran systems. *Food Hydrocolloids*. *39*, 223–230

33. Lakshmanan, R., de Lamballerie, M., and Jung, S. (2006) Effect of soybean-to-water ratio and pH on pressurized soymilk properties. *Journal of Food Science*. *71*, E384–E391

34. Lambrecht, H. S., Nielsen, S. S., Liska, B. J., and Nielsen, N. C. (1996) Effect of soybean storage on tofu and soymilk production. *Journal of Food Quality*. *19*, 189–202

35. Ng, K. H., Lye, H. S., Easa, A. M., and Liong, M. T. (2008). Growth characteristics and bioactivity of probiotics in tofu-based medium during storage. *Annals of Microbiology*. *58*, 477–487

36. Liu, D. M., Li, L., Yang, X. Q., Liang, S. Z., and Wang, J. S. (2006). Survivability of Lactobacillus rhamnosus during the preparation of soy cheese. *Food Technology and Biotechnology*. *44*, 417–422

37. Dalev, D., Bielecka, M., Troszynska, A., Ziajka, S., and Lamparski, G. (2006) Sensory quality of new probiotic beverages based on cheese whey and soy preparation. *Polish Journal of Food and Nutrition Sciences*. *15*, 71–77
38. Kandiah, J., and Laird, J. (2002) Sensory attributes and acceptance of flavored soy nuts by college students. *Nutrition Research*. *22*, 405–410
39. Miguel, D., Valdez, G., and Rossi, E. (2004) Sensory and chemical aspects of frozen soy yogurt fermented with Enterococcus faecium and Lactobacillus jugurti. *Alimentos e Nutrição Araraquara*. *15*, 197–201
40. Wenrich, T., and Cason, K. (2004) Consumption and perceptions of soy among low-income adults. *Journal of Nutrition Education and Behavior*. *36*, 140–145