



Research Article (Araştırma Makalesi)



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Cereal-based fermented synbiotic instant powders: a dessert practice

Tahıl bazlı fermente sinbiyotik hazır tozlar: tatlı denemesi

* This article is summarized from the first author's thesis.

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ABSTRACT

Objective: This study was aimed to develop synbiotic and probiotic instant powder mixtures that can be used in food products to meet the increasing interest of consumers in functional foods.

Materials and Methods Cereal flours (oat, rice, wheat), sesame, cranberry, chestnut and milled germinated grains (lentil, mung bean) were blended and autoclaved then subjected to fermentation process using the *Lactobacillus plantarum* strain. A part of the porridge was conventionally dried (FPP) and skim milk powder+fructooligosaccharide+inulin mixture as prebiotic sources were added to the other part and subjected to freeze drying (FSP). The viability of probiotics in the gastrointestinal environment and the Angiotensin-converting enzyme inhibition and bile-acid binding capacities of the powders after *in vitro* digestion were analyzed. The sensory acceptability of the desserts was evaluated.

Results: FSP contained a higher number of viable cells than FPP after *in vitro* digestion. Relative bile-acid binding and angiotensin-converting enzyme inhibition capacities of samples were confirmed as their cholesterol-lowering and blood pressure-regulating potential. The panelists rated the dessert samples enriched with 5-15% FPP and FSP as 'liked'.

Conclusion: The integration of these powders into a variety of food products will provide consumers with healthier dietary choices that support their overall health goals.

ÖΖ

Amaç: Bu çalışmanın amacı, tüketicilerin fonksiyonel gıdalara artan ilgisini karşılamak için gıda ürünlerinde kullanılabilecek sinbiyotik ve probiyotik hazır toz karışımların geliştirilmesidir.

Materyal ve Yöntem: Tahıl unları (yulaf, pirinç, buğday), susam, kızılcık, kestane ve öğütülmüş çimlendirilmiş taneler (mercimek, maş fasulyesi) karıştırılarak otoklavlanmış ve *Lactobacillus plantarum* suşu kullanılarak fermantasyon işlemine tabi tutulmuştur. Lapanın bir kısmı konvansiyonel olarak kurutulmuş (FPT), diğer kısmına prebiyotik kaynak olarak yağsız süt tozu+fruktooligosakkarit+inülin karışımı ilave edilip, dondurarak kurutmaya (FST) tabi tutulmuştur. Probiyotiklerin gastrointestinal ortamdaki canlılığı ve *in vitro* sindirim sonrası anjiyotensin dönüştürücü enzim inhibisyonu ve safra bağlama kapasiteleri analiz edilmiş, hazırlanan tatlıların duyusal kabul edilebilirliği değerlendirilmiştir.

Araştırma Bulguları: FST, *in vitro* sindirimden sonra FPT'den daha yüksek sayıda canlı hücre içermiştir. Örneklerin relatif safra asidi bağlama ve anjiyotensin dönüştürücü enzim inhibisyon kapasiteleri, kolesterol düşürücü ve kan basıncını düzenleyici potansiyel etkileri doğrulanmıştır. Panelistler %5-15 FPT ve FST ile zenginleştirilmiş tatlı örneklerini 'beğenildi' olarak değerlendirmişlerdir.

Sonuç: Bu tozların çeşitli gıda ürünlerine entegrasyonu, tüketicilere genel sağlık hedeflerini destekleyen daha sağlıklı beslenme seçenekleri sağlayacaktır.

INTRODUCTION

Recent studies have highlighted the significance of consuming cereals, vegetables, and fruits in reducing the risk of chronic and degenerative diseases. Plant foods are rich in bioactive compounds, including phenolics, fibers, and phytosterols, which have a pivotal role in maintaining oxidative balance, which is a key factor in cancer development and chronic diseases (Samtiya et al., 2021). To enhance the nutritional properties of foods, ancient and cost-effective methods such as germination and fermentation have been employed. *De novo* synthesis and microbial activity, during these processes not only increase the content and quality of proteins, amino acids, lipids, and vitamins but also lead to the development of bioactive compounds with health benefits in humans (Nkhata et al., 2018). Furthermore, dietary fibers' functional properties, such as bile acid sorption and bioactive peptide formation during germination, fermentation, and gastrointestinal digestion, are vital in mitigating the risk of diet-related diseases (Prasadi & Joye, 2020).

As cereal-based foods keep on being a major source of optimum nutrition in developed and developing countries, the food industry should focus on improving their nutritive value by innovatively formulating composite blends of cereals, grains, and dried foods. Additionally, optimizing daily nutrition can be achieved, by enhancing prebiotic and probiotic properties in a wide range of products (EI & Simsek, 2012; Blandino et al., 2003).

In this study, we aimed to produce probiotic and synbiotic instant powders to achieve optimal survivability. In this respect, cereal flours (oat, rice, wheat) and chopped sesame, cranberry, chestnut and milled germinated grains (lentil, mung bean) were selected to prepare functional cereal-based fermented probiotic and synbiotic instant powders. The fermentation process was carried out using a strain of *L. plantarum* and skim milk powder, fructooligosaccharides and inulin were used as prebiotics. Instant properties of powders were evaluated, and *in vitro* digestion was applied to powders. The ACE inhibition (Angiotensin-converting enzyme) and bile-acid binding capacities of the samples were assayed. Subsequently, these powders were integrated into a commercial ready-to-eat mousse powder at varying ratios for dessert preparation, and sensory evaluation was conducted by a panel.

MATERIALS and METHODS

Ingredients and chemicals

Green lentil (*Lens culinaris*), mung bean (*Vigna radiata*), rice (*Oryza sativa* L.), whole wheat (*Triticum durum*), oat (*Avena sativa* L.), chestnut (*Castanea sativa*), sesame (*Sesamum indicum* L.), dried cranberry (*Vaccinium oxycoccos*), ready-to-eat instant mousse dessert and skim milk powder (SMP) were purchased from the hypermarket in Izmir. Inulin (Beneo Orafti) and fructooligosaccharides (FOS) were obtained from Artisan Food Inc. *Lactobacillus plantarum (Lp)* (Visby-vac Serie 1000, prod-Nr: 40022951) was kindly provided by Ege University, Department of Food Engineering and Biotechnology Section. α -amylase (A1031), pepsin (P7000), bile acids (B 8631), pancreatin (P1750), ACE (angiotensin-converting enzyme, EC 3.4.15.1) (A6778), pefabloc SC (76307), FAPGG/N- (3-[2-furylacryloyl]-phe-gly-gly) (F7131) were purchased from Sigma-Aldrich. MRS agar (1106600) and MRS broth (1106610) were provided from Merck Darmstadt Germany. Bile Acid Diagnostic Kits were provided by Trinity Biotech plc, Bray Co. (Wicklow, Ireland). The Vivaspin 20 model ultrafiltration membrane (MWCO 3000) ultrafiltration membrane was obtained from Sartorius Stedim Biotech Gmb. The study was conducted at Ege University, Food Engineering Department Nutrition Laboratory between 2016 and 2019.

Germination

Green lentils (*Lens culinaris* M.) and mung bean (*Vigna radiata* var.) were rinsed with water containing 0.5% hypochlorite. The seeds were then soaked at room temperature for 6 h at 1:5 v/w, germination was performed in an incubator at 30°C for 3 days. Seeds with sprouts were freeze-dried and milled into flour, then kept at -20°C.

Fermented probiotic powder (FPP) and Fermented synbiotic powder (FSP)

The production of cereal-based fermented probiotic powder (FPP) and synbiotic powder (FSP) is shown in Figure 1. Rice, whole wheat, oat, and chestnut flours in equal amounts (8 g) and germinated mung bean (3 g) and lentil (3 g) flours, chopped cranberry (3 g) and sesame (3 g) were mixed and then water (1:10 g/mL) was added into the mixture. The mixture was placed into the glass jars and autoclaved at 121°C for 15 minutes. After being cooled to room temperature, *Lactobacillus plantarum (Lp)* was inoculated (10⁶ cfu microorganisms/100 g of product). The fermentation period ended after 32 h, at 37°C. The mixture was divided into two parts; one of the parts was mixed with 10 g inulin+FOS+SMP mixture (1:1:2, w/w/w) to 100 g fermented sample. Then the sample was homogenized at 6000 rpm, 6 min and subjected to a freeze-drying process (FSP). The other part (no added prebiotics) was dried at 60°C, 42 h in a conventional oven (FPP). All samples were ground to a particle diameter of 0.3 mm and stored at -20°C until analysis.

Analysis

Instant properties

The instant properties of the powders were studied by dispersibility, wettability, and solubility capacities as described by Shittu & Lawal (2007).

Viability in simulated gastric and intestinal fluids

The viability of *Lp* under simulated gastric and intestinal conditions was measured according to the method of Paez et al. (2012) with minor modifications. One-gram powder of FPP and FSP were mixed with the five volumes of simulated saliva and gastric fluids prepared according to the suggestion by Minekus et al. (2014). After incubation at 37°C for 90 minutes in a water bath, the samples were centrifuged ($6000 \times g$, 15 min, 5°C) and resuspended in simulated intestinal juice. *In vitro* intestinal conditions were applied (Minekus et al., 2014) and the samples were taken after 90, 270 and 450 minutes of incubation for *Lp* cell counts cell counts (MRS, 37°C, aerobic incubation, 48 h, 450 min.) as extended duodenal conditions.

In vitro digestion

In vitro static digestion according to the procedure described by Minekus et al. (2014) was used. Also, as participants of INFOGEST, we applied minor modifications to this method. Digestive enzyme activities were assayed and simulated digestive fluids and bile acid solution were prepared according to El et al. (2015). The procedure was applied in oral, gastric, and duodenal phases. After digestion, digesta was ultrafiltered with 10kDa cut-off membrane and then freeze-dried and stored at -18°C.

In vitro bile binding capacity

The bile-acid binding capacities were analyzed according to the method of Kahlon & Smith (2007). A milliliter of 0,01 N HCl was added 100 mg sample in falcon tubes and incubated at 37° C for 1 h in a shaker water bath. The pH was then adjusted to 6.3 with 0.1 mL of 0.1 N NaOH. The working solution of the bile acid mixture (4 mL, 720 µM), 4 mL 0,1 M phosphate buffer (pH 6,3) and 5 mL of pancreatin solution (10 mg pancreatin/mL phosphate buffer) was added and vortexed. After this step, the tubes were incubated for 1 h in a 37° C shaker water bath and at the end of incubation. The mixtures were centrifuged at 10,000xg for 10 minutes. Bile acids in the supernatant were analyzed at 530 nm using the Microplate reader (Thermo Scientific Varioscan Flash, Finland) according to the Trinity Biotech bile acids procedure (Trinity Biotech Distribution, St. Louis, MO). Cellulose and cholestyramine were used as negative and positive controls, respectively. The results were calculated as the percentage of inhibition equivalent to cholestyramine.

ACE inhibition capacity

Angiotensin-converting enzyme (ACE) inhibitory activities of the samples after *in vitro* digestion were performed by Lahogue et al. (2010). The digested sample was mixed with 100 μ L 2,5 μ M FAPGG, in buffer solution in the Eppendorf tube. Five different concentrations were taken, and the reaction was started by adding 25 μ L of ACE enzyme solution (100 mU/mL). Control and contain inhibitor samples were injected into HPLC. The peak area of the FAP reaction product of FAPGG was used to evaluate the degree of ACE inhibition (%).

Dessert practice and Sensory evaluation

A ready-to-eat instant mousse powder, which does not require any heating process, was used for dessert practice. Mousse powder was placed in a container and FPP and FSP were added at different amounts (5, 10, 15 and 20). Then, cold milk was added according to the instructions on the product label and the mixture was whipped with a mixer for 5 minutes. Sixteen panelists were invited to evaluate and rank all FPP and FSP enriched samples according to taste and appearance preferences. A five-point scoring system was applied with scores ranging from 1 (dislike) to 5 (like) (Granato et al., 2012). Samples that received at least 70% "very like" were considered successful.

Statistical analyses

The data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test (p<0.05) using the SPSS, version.

RESULTS and DISCUSSION

Viability of probiotics

The cell counts of FPP (containing no prebiotics) and FSP (containing prebiotics) were presented in Table 1. The initial cell count in FSP was higher than in FPP (p<0.05). During in vitro gastric digestion, both samples had reduced cell viability (p<0.05), with FPP showing the most significant decline after 90 minutes. No change in viability was observed for FPP during small intestinal digestion (270 min) (p>0.05), but there was a notable increase in viability under extended intestinal conditions (450 min) (p<0.05). FSP exhibited a difference in viability between extended incubation (450 min) and intestinal digestion (270 min) (p<0.05). Approximately 81% *Lp* cells survived under extended intestinal digestion (450 min) in FSP.

Our results suggest that extended intestinal conditions enhanced the survival of Lp in both FPP and FSP. The differences in the number of live bacteria numbers between the beginning and the digestive stages of the FPP and FPS could be attributed to the conventional drying method (60°C, 42 h) used for FPP production and the freeze-drying method used for FSP production. Moreover, the addition of prebiotics in FSP production may play a vital role in protecting and promoting the growth of Lp as compared to FPP. The inclusion of prebiotics significantly enhanced Lp survival under acidic (pH 2.0-3.0) gastric conditions and bile conditions during intestinal digestion, as compared to their levels before digestion (p<0.05). Probiotic enrichment offers various health benefits by modifying the microbial balance in the gastrointestinal tract (You et al. 2022). This can result in positive effects on commensal bacteria or the disruption of the binding of pathogens in the gastro-intestinal tract. Probiotics can also produce nutrients, microbial products, cofactors, and metabolites including polysaccharides which compete with pathogens for binding sites (Nazir et al., 2018).

Maintaining probiotics viability during gastro-intestinal transit and food processing is crucial to provide their health benefits. Typically, 1.0x10 ⁸ cfu g⁻¹ of intestinal contents are required for probiotics to reach their intended site of action. it must contain viable cells from probiotic cultures of at least 10⁶–10⁷

CFU/g or in the portion to be consumed and for beneficial action to occur in the intestine, they must be able to survive processing and storage conditions, be ingested in adequate quantities, reaching the viable number of microorganisms. The viability of microorganisms is notably affected by gastric conditions, primarily due to high acidity (pH 2.0-3.0). Additionally, high bile salt concentrations in the intestine can lead to significant losses in viability (Cook et al., 2012; Zubaidah & Akhadiana, 2013; Kent & Doherty, 2014; Wendel 2021). Jagannath et al. (2010) comparatively examined the cryoprotective properties of skimmed milk, calcium alginate encapsulation, or 0.85% physiological saline and distilled water during the freeze-drying process. It has been reported that skimmed milk exhibits cryoprotective properties during freeze-drying and the colloidal structure contributes to the protection of microorganisms by increasing the glass transition temperature.

 Table 1. Cell counts of L.plantarum in samples before and after in vitro digestion

Çizelge 1. Örneklerin in vitro sindirii	m öncesi ve sonrası L.plantarum sayıları
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	Cell counts (log CFU/g)				
	Before digestion	After digestion			
	-	Gastric Intestinal			
		90 min	270 min	450 min	
FPP	7.70±0.42 ^{a,A}	4.31±0.22 ^{b,A}	4.35±0.2 6 ^{b,A}	5.29±0.25 ^{c,A}	
FSP	8.56±0.33 ^{a,B}	$5.67 \pm 0.43^{b,B}$	6.14±0.28 ^{bc,B}	6.92±0.33 ^{c,B}	

Data are presented as mean values ±standard deviation.

FPP, without prebiotics and conventionally dried sample; FSP with prebiotic and freeze-dried sample

a-c Cell counts in rows with different superscripted letters are significantly different (p<0.05)

A-B Cell counts in columns with different superscripted letters are significantly different (p<0.05)

Instant properties

The reconstitution properties of food powders, including wettability, dispersibility, and solubility play a crucial role in their overall performance (Fang et al., 2008). In our study, we assessed these properties for both FPP and FSP samples, and the results are presented in Table 2. It is noteworthy that instant sugar-cocoa mixtures generally exhibit improved wettability with particle sizes larger than 0.4 mm (Shittu and Lawal, 2007). Our average particle size of the samples was 0.3 mm, which may have influenced the observed differences in wetting time between FPP and FSP (p<0.05).

Various factors can affect wetting time, such as particle size, porosity, surface charge, density surface area, presence of amphipathic substances, and surface activity of particles (Kim et al., 2002).

The presence of SMP, FOS, and inulin in FSP could be responsible for the longer wetting time compared to FPP. Additionally, the chemical composition of the milk powder, particularly the crystallization of lactose, can influence wettability. Moreover, the fat content of particles, due to its hydrophobic properties, can reduce wettability. A wide angle of contact between the powder surface and penetrating water can also lead to deteriorated wettability (Kim et al., 2002; Shittu & Lawal 2007; Fang et al., 2008). Previous studies on similar food powders have reported wettability and dispersibility values. For instance, Audic et al. (2003) studied the ready-to-reconstitute form of a cereal-based traditional Indian dessert, kheer mix powder, and found its wettability and dispersibility to be 2.0 minutes and 75.38%, respectively. Kim et al. (2002) studied the distribution of components of the outer layer of industrial spray-dried dairy powders, highlighting the presence of free fat globules protected by protein during the freeze-drying process. They concluded that the outer surface of powders was largely covered by free fat globules protected by protein during the freeze-drying process. In our study, both FPP and FSP demonstrated high dispersibility, with values of 93.48 and 92.33%, respectively. These values were

similar to those reported for cocoa mixtures by Shittu & Lawal (2007). Unlike fruit drink powders that require complete dissolution, our goal was to provide functional benefits in instant desserts, making dispersibility more important than solubility for our products. The dispersibility of our samples is crucial since they are intended as a functional instant ingredient in instant desserts.

Table 2. Instant properties of samples

Çizelge 2. Örneklerin instant özellikleri

	Time (s)	C	%
	Wettability	Dispersibility	Solubility
FPP	362±15.68ª	93.48±0.042ª	50.19±1.34ª
FSP	2894±14.49 ^b	92.33±0.208ª	54.34±2.06 ^b

Data are presented as mean values ±standard deviation.

Interpretation of symbols is as stated in Table 1.

a-c Values in columns with different superscripted letters are significantly different (p<0.05)

Bile-acid binding activity and potential cholesterol-lowering effects

The relative bile-acid binding values of both FPP and FSP were compared to cholestyramine, with values of 80.33% and 72.97%, respectively (Table 3). Although there were no differences between the two (p>0.05), it is essential to note that FPP and FSP are highly concentrated powders designed for use in formulations, as demonstrated in this study. Even under these conditions, it is evident that both FPP and FSP can be considered functional ingredients with the potential to lower cholesterol levels and support health claims. Kahlon et al. (2007) evaluated the cholesterol-lowering effect of foods and food fractions by determining their bile-acid binding potential. They found that the relative binding of cholestyramine, in vitro bile acid in dry matter, ranged from 1-18% in commonly consumed vegetables. In vitro bile-acid binding capacities of lentil snack raw formulations and extruded formulations were determined to range from 0.6-69%, with lentils exhibiting a binding capacity of 100% (Kahlon et al., 2014). Furthermore, Dziedzic et al. (2012) performed the influence of buckwheat goat diet fractions on bile-acid binding ability, with the highest bound bile acids o hull and bran due to their high total dietary fiber content. While cholesterol reduction through bile-acid binding is beneficial to human health, Simsek et al. (2014) highlighted in their study that sufficient bile salt concentration in the digestive system is essential for efficient absorption of lipophilic compounds. Bile acids are crucial for fat digestion and absorption, synthesized from cholesterol in the liver through the enterohepatic pathway. After absorption, bile acids are reabsorbed by the terminal ileum. Binding bile acids with food fractions, leads to their fecal excretion and stimulates the conversion of liver cholesterol into bile acid (Kahlon et al., 2014; Naumann et al. 2020). Considering Regulation (EC) No. 1924/2006 of the European Parliament and of the Council on nutrition and health claims made on foods, certain food components can be approved for a label health claim for lowering cholesterol. In this regard, our products exhibit the potential to lower cholesterol in a dose-dependent manner and may support health claims related to cholesterol management.

ACE inhibition activity and potential hypertension management

The inhibitory activities of angiotensive converting enzymes (ACE) based on the IC₅₀ values of the samples after *in vitro* digestion are given in Table 3. The Angiotensin I Converting Enzyme (ACE, EC 3.4.15.1) catalyzes the conversion of angiotensin I to II, which shows a crucial responsibility in regulating blood pressure by inactivating bradykinin, a potent vasodilator. Current medical ACE inhibitors in the-form of pills are used to treat hypertension, however, they may have some side effects. As a result, researchers are exploring natural inhibitory peptides and phenolics in food as potential ingredients to help control hypertension (Simsek et al., 2014; Manzanares et al., 2019). These bioactive peptides are

released from the inactive parent proteins through processes such as germination, fermentation, or gastro-intestinal digestion. In our study, the IC50 values of FPP and FSP were found to be 8.52 and 8.29 µg protein/mL, respectively (p>0.05). Kancabas & Karakaya (2013) reported the ACE inhibitory activity of boza, a traditional fermented cereal beverage, with IC50 values of 7.2 µg protein/mL. Another study by Torino et al. (2013) demonstrated that green lentil flour fermented by *L. plantarum* had a lower IC50 value (200 µg protein/mL) compared to the unfermented sample (440 µg protein/mL).

Lahogue et al. (2010) reported that the synthetic ACE inhibitor Captopril and the fish hydrolysate had IC_{50} values of 0.19 ng and 43µg protein/mL, respectively. It is insignificant that ACE inhibitory activity can increase in fermented vegetable juice due to the release of bioactive peptides during fermentation and *in vitro* digestion, as explained by Simsek et al. (2014). These different IC_{50} values observed among samples are indicative of the minimum amount of sample required to inhibit 50% of enzyme activity.

As we continue to explore natural alternatives for managing health conditions, these findings open new avenues for incorporating fermented synbiotic instant powders into various food products to harness their health-promoting potential holistically and effectively.

 $\textbf{Table 3. } \textit{In vitro bile-acid binding and Angiotensin-converting enzyme (ACE) inhibitory capacities based on IC_{50} by samples.$

	Bile binding capacity		ACE inhibition	
	µmol/100 mg sample	Relative to cholestyramine (%)	mg protein/g sample (µg /mL)	IC ₅₀ values*
FPP	7.40±0.07 ^b	80.33±0.80	0.58±0.32° (8.52±0.29)	14.72±0.49ª
FSP	6.73±0.09 ^b	72.97±0.82	0.60±0.31° (8.29±0.20)	13.88±0.33⁵
Cholestyramine	9.23±0.02 ^a	100±0.21	-	-
Cellulose	0.07±0.03	0.76±0.20	-	-

Çizelge 3. Örneklerin in vitro safra aside baglama ve anjiyotensin dönüştürücü enzim (ADE) inhibisyon kapasiteleri (IC_{50})

Data are presented as mean values ±standard deviation.

Interpretation of symbols is as stated in Table 2.

^{a-c} Values in columns with different superscripted letters are significantly different (p<0.05)

*The concentration of the sample required to produce a 50% inhibition of the initial rate of reaction (IC50).

Sensory evaluation

The sensory evaluation of the samples enriched with FPP and FSP revealed a remarkable overall consumer acceptance. However, it was observed that the preference for dessert samples enriched with 20% of FPP and FSP was comparatively lower. Despite this, no significant differences were recorded between FPP and FSP samples across all enrichment ratios (p>0.05). With our results, we can conclude that panelists favor both desserts enriched with 5-15% FPP and FSP due to their outstanding taste, appealing appearance, and delightful texture properties. This optimal enrichment range demonstrates the potential for creating functional foods that combine health benefits with sensory satisfaction. The positive outcome of the sensory evaluation highlights the suitability of FPP and FSP as valuable ingredients in the development of functional food products. These enriched powders offer an opportunity for the food industry to create innovative and health-promoting desserts that align with consumer preferences.

CONCLUSION

In summary, our study indicates that dessert formulations enriched with 5-15% FPP and FSP hold promise for functional food production. As consumers increasingly seek healthier food options without compromising on taste and indulgence, these synbiotic instant powders open possibilities for developing functional desserts that cater to both health-conscious and taste-driven consumers. Our study highlights

the importance of incorporating prebiotics in synbiotic powder production to enhance probiotic viability and maximize health benefits during gastrointestinal transit and food processing. Furthermore, understanding and optimizing the instant properties of cereal-based fermented synbiotic instant powders are essential for enhancing their performance and ensuring their seamless integration into a wide range of food applications. Overall, the bile-binding activity demonstrated by FPP and FSP, along with their potential cholesterol-lowering effects, reinforces their functional properties and further justifies their incorporation as beneficial ingredients in various food formulations aimed at promoting health.

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