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The Use of Fourier-Transform Infrared Spectroscopy to Determine Potential Starch-based Prebiotics

Rafig GURBANOV[1,2] , Sinem TUNÇER[2,3]✉

[1]Department of Molecular Biology and Genetics, Bilecik Şeyh Edebali University, 11230, Bilecik, Turkey, [2]Biotechnology Application and Research Center, Bilecik Şeyh Edebali University, 11230, Bilecik, Turkey, [3]Vocational School of Health Services, Department of Medical Laboratory Techniques, Bilecik Şeyh Edebali University, 11230, Bilecik, Turkey
[1]https://orcid.org/0000-0002-5293-6447, [3]https://orcid.org/0000-0002-8947-8646
✉: sinem.tuncer@bilecik.edu.tr

ABSTRACT

Probiotics play important roles in many crucial functions for maintaining the homeostasis of the host, such as protection against pathogens, immunity and metabolism of dietary compounds. Resistant starch (RS) is the starch or the starch products which cannot be digested through the body, but can be digested by the gut microbiota, producing a variety of metabolites which can provide a range of physiological benefits to the host. An RS can be classified as a prebiotic if it can selectively stimulate the growth and/or activity of the beneficial bacteria. To investigate the bacteria driven structural changes in the prebiotic tapioca starch, we used Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. Principal Component Analysis (PCA) and intensity analyses of individual spectral bands exhibited comprehensive alterations in the polysaccharide composition of the tapioca starch incubated with the probiotic bacteria; the starch samples incubated with the bacteria gained a more amorphous structure with a decrease in the ordered structure. The results suggest that as a fast, cheap, and non-laborious method, FTIR spectroscopy coupled with PCA has a potential to be applied in the research area as well as in the food industry for the analysis of the potential prebiotic activity of starch-based substrates or the investigation of the probiotic potential of a bacterial strain through the examination of its RS hydrolyzing capacity.

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Nişasta Bazlı Potansiyel Prebiyotiklerin Belirlenmesi için Fourier-Transform Kızılötesi Spektroskopisinin Kullanımı

ÖZET

Probiyotikler, konağın homeostazını korumak için patojenlere karşı koruma, bağışıklık ve diyet bileşiklerinin metabolizması gibi birçok kritik işlevde önemli roller oynar. Dirençli nişasta (DN), vücutta sindirilemeyen ancak bağırsak mikrobiyotası tarafından sindirilebilerek, konakçıya çeşitli fizyolojik faydalar sağlayabilen bir grup metabolit üreten nişasta veya nişasta ürünüdür. Bir DN, faydalı bakterilerin büyümesini ve/veya aktivitesini seçici olarak uyarabiliyorsa prebiyotik olarak sınıflandırılabilir. Prebiyotik tapyoka nişastasındaki bakteri kaynaklı yapısal değişiklikleri araştırmak için Zayıflatılmış Toplam Yansıma-Fourier Dönüşümü Kızılötesi (ATR-FTIR) spektroskopisini kullandık. Tekil spektral bantların Temel Bileşen Analizi (PCA) ve yoğunluk analizleri, probiotik bakteri ile inkübe edilen tapyoka nişastasının polisakkarit bileşiminde kapsamlı değişiklikler meydana geldiğini göstermiştir; bakteriler ile inkübe edilen nişasta numuneleri, düzenli yapıda bir azalma ile birlikte daha amorf bir yapı kazanmıştır. Bu sonuçlar hızlı, ucuz ve zahmetsiz bir yöntem olarak, PCA ile birleştirilmiş FTIR spektroskopisinin, nişasta bazlı substratların potansiyel prebiyotik aktivitesinin analizi veya bir bakteri suşunun probiyotik potansiyelinin RS hidrolizleme kapasitesine bakılarak incelenmesi

Araştırma Makalesi

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amacı ile araştırma alanında ve gıda endüstrisinde uygulama potansiyeline sahip olduğunu önermektedir.

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INTRODUCTION

Carbohydrates can be roughly categorized into three large classes based on the number of structural units. Those are monosaccharides, oligosaccharides, and polysaccharides. Polysaccharides containing a single type of monosaccharide units are called homopolysaccharides. Some homopolysaccharides, like cellulose and chitin, have structural functions and some of them, such as starch and glycogen, are used mainly for energy storage (Li et al. 2018).

Starch, contains two types of D-glucose polymers amylose and amylopectin, is one of the most abundant carbohydrates in nature. In plants, starch is stored in leaves, tubers, seeds, stems, roots and some fruits, and it constitutes the principal carbohydrate in the human diet (Cummings and Stephen 2007; Navarro et al. 2019). Resistant starch (RS) is attributed to dietary starch and starch degradation products which cannot be digested in the small intestine but digested through microbial fermentation in the large intestine (Giuberti and Gallo 2020). RS is classified into five subtypes, RS1 to RS5 (Birt et al. 2013). RS1 type starch is physically inaccessible to digestion since it is locked within cell walls and food matrixes. RS2 represents native or uncooked starch which cannot be digested because of the ungelatinized crystalline structure of the granule. RS3, also called retrograded starch or crystalline non-granular starch, resists digestion because it is rapidly cooled after gelatinization by heating (Birt et al. 2013; Kasote et al. 2018; Ma and Boye 2018; Navarro et al. 2019). As a consequence of retrogradation, more thermostable structures are formed by amylose rather than amylopectin (Ogbo and Okafor 2015). RS3 is found in cooked and cooled starchy foods (Birt et al. 2013). RS4 refers to chemically modified or repolymerized RS in which the digestibility reduced by chemical modifications (Stewart and Zimmer 2017). RS5 forms when amylose and long branch chains of amylopectin make single-helical complexes with fatty acids and fatty alcohols; therefore, cleavage of starch by amylase is prevented and swelling of starch granules and enzyme hydrolysis is restricted since the amylose-lipid complex entangles amylopectin molecules (Birt et al. 2013). Among these RS types, RS3 collects particular interest in the food industry because it can preserve its nutritional functionality and thermal stability during cooking (Ratnayake and Jackson 2008).

Prebiotics are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria that can improve the host health." Both probiotics and prebiotics have been included as bioactive and functional ingredients in foods because of their health benefits (Saulnier et al. 2009). RS has been gaining attention as a prebiotic (Zaman and Sarbini 2016). Several potential health benefits attributed to RS including but not limited to attenuation of blood glucose and insulin levels in both healthy and diabetic individuals, positive effects on large bowel health along with anti-cancer properties (Al-Tamimi et al. 2010; Alfa et al. 2017; Giuberti and Gallo 2020). RS has been also reported to stimulate the growth of probiotic bacterial species in the gut and modulate gut immune function and microbiota activity by decreasing the concentration of secondary bile acids, ammonia and phenol content, and enhancing the production of short-chain fatty acids (SCFAs) that can provide a range of physiological benefits. Additionally, RS was shown to promote the absorption of zinc, calcium, and magnesium ions and reduce the intestinal pH (Ma and Boye 2018).

Conventional probiotics mostly have intestinal origin such as lactobacilli and bifidobacteria offering relief for gastrointestinal tract related maladies. On the other hand, oral microbiota appears as an important concept since some of the human diseases can be linked either directly or indirectly to the composition of oral microbiota (Hale et al. 2012). For instance, oral bacteria have been implicated in cystic fibrosis, ventilator-assisted pneumonia, hepatic or brain abscesses, endocarditis, and digestive cancers including primary pancreatic adenocarcinoma. Besides, dementia and other mentally impaired diseases were found to be linked with periodontitis that is caused by oral pathogen bacteria. Recently, dysbiosis of colonic microbiota was also suggested to be affected by oral bacteria (Koliarakis et al. 2019) pointing out that oral-colon interaction should be studied in detail, especially for the evaluation of oral bacteria-mediated systemic inflammatory responses. Therefore, the development of functional products capable of fostering a healthy oral microbiota is a special area of interest (Wescombe et al. 2012; Gurbanov and Yıldız 2017). *S. salivarius* M18, also known as Mia or DSM 14685 (Burton et al. 2013), is a bacterial probiotic derived from the oral cavity and designed to be used for oral and dental health (Hale et al. 2012). In 2019, the U.S. Food and Drug Administration (FDA) granted *S. salivarius* M18 as Generally Recognized as Safe (GRAS; Notice No. 807) to be used in foods. Being a Gram-positive, facultative anaerobic bacteria, *S. salivarius* colonizes oral cavity, paranasal sinuses, as well as gastrointestinal and

genitourinary tracts (Cebeci et al. 2015). Several types of nose, mouth, and throat pathogens have been shown to be inhibited by the probiotic *S. salivarius* M18 (Wescombe et al. 2012; Burton et al. 2013; Di Pierro et al. 2015; Poorni et al. 2019). We have also shown that *S. salivarius* M18 was capable of impairing the pathogenic activities of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Tunçer and Karaçam 2020), two common species causing major health-care-associated infections, particularly pneumonia and sepsis (Riquelme et al. 2018). In this manuscript, taking advantage of spectroscopic analysis techniques, we aimed to establish a novel approach and a new method to determine the RS utilization property of the probiotic bacteria *S. salivarius* M18, through monitoring the structural changes that occurred in the tapioca, a prebiotic starch (Wronkowska et al. 2008; Arshad et al. 2018) extracted from the roots of the cassava plant (*Manihot esculenta* Crantz) (Pereira and Leonel 2014). For this purpose, the changes in the amount of ordered/crystalline to amorphous domains in tapioca starch (TS) via the analysis of infrared (IR) spectral bands before and after incubation with *Streptococcus salivarius* M18 were evaluated.

For the determination of the prebiotic property of a substrate, several *in vitro* and *in vivo* methods were reported (Parkar et al. 2010; Rodríguez-Cabezas et al. 2010; Erickson et al. 2018). The quantitative analysis of prebiotic fermentation products such as SCFAs and monitoring the quantitative changes in the bacterial species are the most commonly used approaches for the evaluation of the prebiotic potential of a novel substrate (Vulevic et al. 2004; Mandalari et al. 2007). Even though these analysis methods can offer detailed information about the host's biological response to the prebiotics, the required methodologies aiming to investigate the biological changes are laborious, expensive, and time-consuming. Here we suggest that Fourier Transform Infrared spectroscopy (FTIR), which is a rapid, non-laborious, and low-cost technique, has a potential to be used for the examination of the prebiotic property of a starch and quick screening of the RS sources for prebiotic activities by monitoring the bacteria driven changes in main-spectral bands associated with the characteristic functional groups in polysaccharides.

MATERIALS and METHOD

Materials

Streptococcus salivarius M18 (Blis Technologies, New Zealand) was cultured in reformulated Tryptic Soy Broth (TSB) medium composed of 17 g L^{-1} casein peptone (Merck, Germany), 2.5 g L^{-1} K_2HPO_4 (Merck), 2.5 g L^{-1} D-glucose (Sigma-Aldrich, St. Louis, Missouri, USA), 5 g L^{-1} NaCl (Sigma-Aldrich) and 3 g L^{-1} yeast extract (Condalab, Spain).

Culturing the Bacteria with Tapioca Starch

Bacterial growth medium was supplemented with 0.5% (w/v) or 1.0% (w/v) TS before autoclaving at 121°C for 20 min. For inoculation, optical density (OD) of an overnight culture of *S. salivarius* M18 at 600 nm was adjusted to 0.1. Since *S. salivarius* is a facultative anaerobe (Cebeci et al. 2015), the bacteria ($8x10^8$ colony forming unit-CFU mL^{-1}) inoculated into screw cap conical bottom falcon tubes (15 mL volume) filled (95%) with the bacterial growth medium containing TS and sealed with paraffin film to support fermentation, as described before (Gurbanov et al. 2020). The bacteria were grown for 24 h at 37°C using a shaking incubator (160 rpm). The bacterial growth medium containing 0.5% or 1.0% TS, which has not been inoculated with bacteria, but prepared and incubated under the same conditions, was used as a control to assess the structural changes in the starch in the presence of the probiotic bacteria *S. salivarius* M18.

Determination of Colony Forming Units (CFUs)

Representing the number of live *S. salivarius* M18 incubated with or without 1.0% TS, CFUs were counted. For this, the bacteria were grown in the presence or absence of 1.0% TS for 24 h as described above. Followed by 24 h of incubation, 100 µl of the bacterial culture was diluted in the growth medium (10^{-1}-10^{-7}) and spread on the modified Tryptic Soy Agar (TSA) plates composed of 17 g Lt^{-1} casein peptone (Merck), 5 g Lt^{-1} NaCl (Sigma-Aldrich), 3 g Lt^{-1} yeast extract (Condalab) and 1.5% (w/v) agar (Liofilchem, Italy). The Petri dishes were incubated at 37°C for 24 hours and CFUs (on a plate having 30-300 colonies) were counted (Thomas et al. 2015). Three independent replicates were used to obtain mean CFU mL^{-1}.

Sample Collection for ATR-FTIR Spectroscopy Studies

At the end of 24 h incubation, the bacteria cultured in the bacterial growth medium containing TS (0.5% or 1.0%) and the growth medium containing TS (0.5% or 1.0%) without bacteria (control groups) were centrifuged at 100*xg* to precipitate the starch gels. The gelatinized starch was washed in Phosphate Buffered Saline (PBS; Biological Industries, Israel) and the PBS was removed through centrifugation at 100*xg*.

ATR-FTIR Spectroscopy

The spectra of all TS samples were collected using a universal ATR Miracle accessory equipped Frontier FTIR Spectrometer (PerkinElmer, US). The spectrum of air was used as a reference. During the data collection, equal amounts of samples were applied on a ZnSe crystal plate (PerkinElmer, US). The samples were scanned with a resolution of 4 cm^{-1}, in the spectral range between 4000 to 650 cm^{-1} at room temperature. The spectra were collected as an average

of 32 scans. PBS spectrum was also collected ensuring equivalent circumstances and then were excluded from each sample's spectrum. The taken difference (D) spectra (sample spectrum minus PBS) was used in every single analysis (Gurbanov et al. 2018).

Spectral Data Pre-processing and Analysis

The spectral data were further preprocessed by first taking its second derivative followed by vector-normalization with 9 smoothing points in the polysaccharide (1200-800 cm^{-1}) (Cˇerna´ et al. 2003) region using OPUS 5.5 software (Bruker, US). Subsequently, the absolute intensities of spectral bands were quantified from these spectra by peak picking method via OPUS 5.5 software (Bruker, US) as previously described (Gurbanov et al. 2019).

Principal Component Analysis (PCA)

Initially, the spectral data matrix was transformed using Gaussian filter with 19 segment sizes. Subsequently, PCA was performed to the mean-centered matrix in the polysaccharide (1200-800 cm^{-1}) region via The Unscrambler X 10.4 (Camo, NO) software. Full Cross Validation method, Singular Value Decomposition (SVD) algorithm, and Hotelling's T2 statistics were applied to the model, and the results were shown as scores and loadings plots (Gurbanov et al. 2019; Tunçer and Gurbanov, 2020).

Statistical Analysis

The results were presented as mean ± standard error of the mean (mean± SEM). t-test was applied to compare two groups (*$p \leq 0.05$, **$p \leq 0.01$,***$p \leq 0.001$, ****$p \leq 0.0001$) using GraphPad Prism 6.01 (GraphPad, La Jolla, California, USA).

RESULTS and DISCUSSION

Tapioca Starch Exerts a Prebiotic Effect on the M18 Strain of *S. salivarius*

It is known that the food processing steps influence the proportion of RS in a starch. For instance, cooking or ripening decreases the quantity of RS in raw or immature fruits or vegetables such as green bananas and potatoes (DeVries 2004). RS amount in cassava was also presented to vary depending on the applied processes for obtaining products of cassava (Pereira and Leonel 2014). Kasote et al. were showed that autoclaving increases the RS3 content of the tapioca pearls (Kasote et al. 2018). Therefore, as a part of the experimental design of this study, TS was autoclaved for 20 min at 121°C not only for sterilization but also for enhancing the RS proportion before using it as an RS source for the investigation of the prebiotic activity.

Since the beneficial doses of probiotics are measured based on the live cell counts (Hill et al. 2014; Dinkçi et al. 2019), the property of enhancing the growth of a probiotic bacteria is an important phenomenon to evaluate the prebiotic activity of a substrate (Arshad et al. 2018; Önal Darilmaz et al. 2019). We found that after 24 h incubation, the live cell counts for *S. salivarius* M18 grown in the growth medium which has not been supplemented with TS were 0.12 x 10^6 CFU mL^{-1} while incubation with 1.0% TS enhanced the count to 1.32 x 10^6 CFU mL^{-1}. Therefore, in agreement with the previous findings and our recent wet-lab studies emphasizing the prebiotic potential of TS (Gurbanov et al. 2020), these results show that TS has a growth-promoting effect on the probiotic bacteria *S. salivarius* M18.

IR Spectroscopy Indicates Notable Changes in the Structure of the Tapioca Starch Incubated with *S. salivarius* M18

To unveil the consumption of RS by *S. salivarius* M18, we focused on the analysis of polysaccharide marker bands at 1200-800 cm^{-1} spectral region reflecting the structural properties of sugars. Figure 1 provides a PCA model developed on a dataset of 1200-800 cm^{-1} (fingerprint, polysaccharide) IR spectral region. PCA is an unsupervised multivariate data analysis method which displays the similarities within the spectra by taking all the wavenumbers into account. The spectral patterns give information about the characteristic absorption bands based on the molecular similarities of the samples (Capron et al. 2007). To summarize, the PCA scores plot is usually utilized to categorize the samples. For instance, the samples having close scores along with the same PC (Principal Component), which is the eigenvector of the data matrix, are defined as "similar" for common characteristics (Escuderos et al. 2010). As can be seen in Figure 1, the relative scores plot for PC-1 and PC-2 showed clear discrimination of the samples according to the structural properties of sugars along with the first largest component (PC-1). The "only TS" groups (0.5% TS and 1.0% TS) in which TS has not been incubated with the bacteria and the TS groups (0.5% TS+SsM18 and 1.0% TS+SsM18) in which TS has been incubated with the probiotic bacteria *S. salivarius* M18 clearly discriminated from each other along with the 74% variance for PC-1 and 16% variance for PC-2. The "only TS" groups were completely clustered in the positive-bottom side of the plot while TS samples incubated with the probiotic bacteria were chiefly found in negative-top scores. The data shows that FTIR spectroscopy, coupled with the unsupervised pattern recognition technique PCA was able to distinguish TS samples incubated with the probiotic bacteria from the TS samples which have not been incubated with the bacteria (only TS groups as control) with very high PC scores as PC-1+PC-2=90%. This result indicates that significant changes occur in the structure of the TS incubated with the probiotic bacteria *S. salivarius* M18. Of note, "only bacteria"

group SsM18 (*S. salivarius* M18) was also analyzed to evaluate if there was any bacterial contamination in the starch samples. Apparently, the SsM18 group was found to be located in negative-bottom scores of the PCA model excluding the bacterial cell contamination in the analyzed samples.

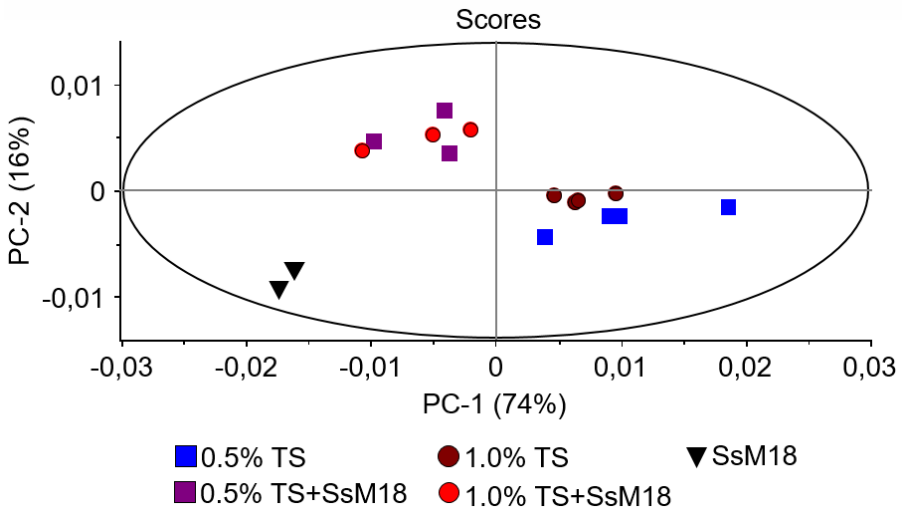

Figure 1. PCA scores plot of the TS samples. In the particular polysaccharide region (1200-800 cm^{-1}), PCAs were applied to the mean-centered and Gaussian filter-transformed spectra obtained from the TS samples either incubated with the probiotic bacteria *S. salivarius* M18 (0.5% TS+SsM18 and 1.0% TS+SsM18) or not (0.5% TS and 1.0% TS). The spectrum of bacteria alone (SsM18) was used for comparison.

Şekil 1. TS örneklerine ait PCA skor grafiği. PCA'lar bir probiyotik bakteri olan S. salivarius M18 ile inkübe edilmiş (0.5% TS+SsM18 and 1.0% TS+SsM18) ya da edilmemiş (0.5% TS and 1.0% TS) TS örnekleri için 1200-800 cm^{-1} polisakkarit bölgesinde, ortalama merkezli ve Gauss filtresi ile transforme edilmiş absorbans spektrumlarına uygulanmıştır. Bakteri (SsM18) spektrumu karşılaştırma için kullanılmıştır.

In this study, we have obtained the main interpretation of RS utilization by the probiotic bacteria from the discriminators of the loadings plot (Figure 2A). On a particular PC, if the score of a sample and the loading of a variable (wavelength/wavenumber) have the same sign, this refers that the sample has higher than the average value for that variable and vice-versa. The larger the scores and loadings, the stronger that relation (Martin et al. 2014). For the analyzed samples, loadings plot revealed 7 positive discriminators detected approximately at 1110 cm^{-1}, 1080 cm^{-1}, 1047 cm^{-1}, 1032 cm^{-1}, 995 cm^{-1}, 980 cm^{-1}, and 960 cm^{-1} along PC-1 (Figure 2B). For these discriminators, we considered the correlation between the signs of scores and the signs of discriminators. For all TS groups where TS has not been incubated with the bacteria, the discriminators were found higher than the average (positive-to-positive pattern) while these discriminators were negatively correlated with bacteria-incubated TS groups (positive-to-negative pattern).

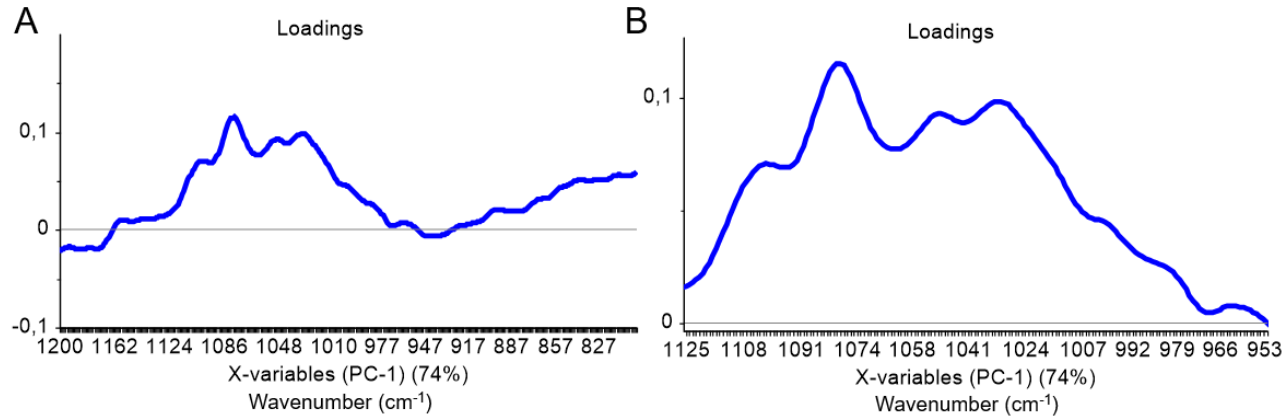

Figure **2.** Loadings plot of PC-1 from the PCA model. (A) PC-1 loadings plot in 1200-800 cm^{-1} spectral region. (B) PC-1 loading plot in the 1125-953 cm^{-1} spectral region.

Şekil 2. PCA modelinden PC-1 yükleme grafiği. 1200-800 cm^{-1} spektral bölgede (A) PC-1 yükleme grafiği. (B) 1125-953 cm^{-1} spektral bölgedeki PC-1 yükleme grafiği.

IR Band Intensity Variations Suggest for the Metabolization of Starch by *S. salivarius* M18

Since the loadings of the PC-1 indicated the spectroscopic origin of the discrimination, we analyzed the second derivative spectra at 1200-800 cm^{-1} region for more precise quantification of the changes in the RS content of TS either incubated with bacteria (0.5% TS+SsM18 and 1.0% TS+SsM18) or not (0.5% TS and 1.0% TS). The spectra given in Figure 3 show the unique patterns for all the analyzed samples.

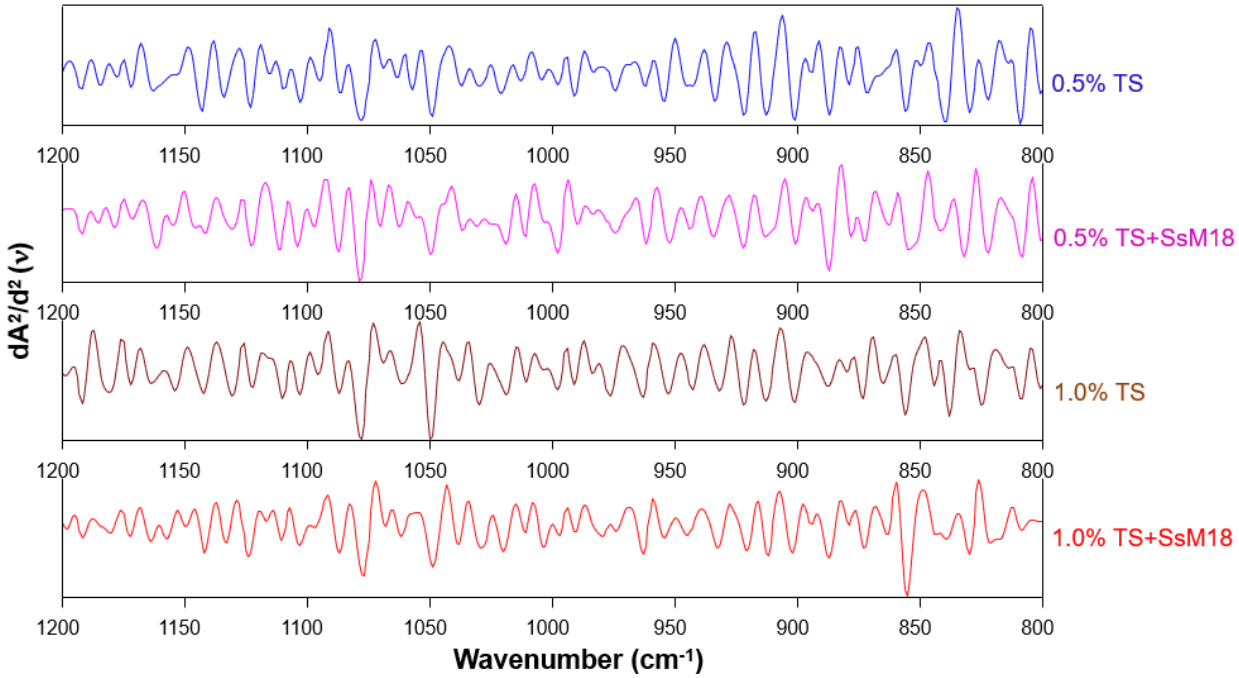

Figure 3. The representative second derivative and vector-normalized IR spectra of TS samples. The IR spectra of the TS samples were shown in the 1200-800 cm^{-1} spectral region.

Şekil 3. TS numunelerinin temsili ikinci türevi ve vektör normalize edilmiş IR spektrumları. TS numunelerinin IR spektrumları 1200-800 cm^{-1} spektral bölgesinde gösterilmiştir.

The IR spectral band at 1047 cm^{-1} is known to be sensitive to the amount of ordered/crystalline starch while amorphous starch is characterized by a band around 1022 cm^{-1}. Therefore, the 1047 cm^{-1}/1022 cm^{-1} intensity ratio displays the amount of ordered starch to amorphous starch (van Soest et al. 1995; Capron et al. 2007). Though the higher ratio of 1047 cm^{-1}/1022 cm^{-1} has been defined to indicate the higher relative crystallinity, the lower ratio of 1022 cm^{-1}/995 cm^{-1} has been assigned to the higher molecular order of double helices of starch granules where the band at 995 cm^{-1} is associated with the ordered structure of starch (Zhang et al. 2014; Wang et al. 2015). Although we could not detect the band at 1022 cm^{-1} from the loadings plot, we were able to obtain the band at 1022 cm^{-1} along with 1047 cm^{-1} and 995 cm^{-1} bands from the second derivative spectra since the second derivative permits a more specific identification of small and neighboring bands (Sanden et al. 2019). Table 1 shows the 1047 cm^{-1}/1022 cm^{-1} and 1022 cm^{-1}/995 cm^{-1} intensity ratios given as fold changes respect to corresponding control groups (0.5% TS or 1.0% TS). In the starch samples incubated with *S. salivarius* M18 (0.5% TS+SsM18 and 1.0% TS+SsM18), the decreased intensity ratio of 1047 cm^{-1} to 1022 cm^{-1} together with the increased intensity ratio of 1022 cm^{-1} to 995 cm^{-1} were found. In other words, the starch samples incubated with the bacteria gained a more amorphous structure with respect to the control counterparts which were not incubated with the probiotic bacteria. Thus, these results indicate that RS utilization ability of a probiotic bacteria can be detected through monitoring the changes in the starch structure by analyzing the variations in the specific IR band intensities.

Table 1. IR band intensity ratios (mean±SEM; sample size N=4) 1047 cm^{-1}/1022 cm^{-1} and 1022 cm^{-1}/995 cm^{-1}.

Çizelge 1. 1047 cm-1/1022 cm^{-1} and 1022 cm^{-1}/995 cm^{-1} IR bant yoğunluk oranları (mean±SEM; örnek sayısı N=4).

TS sample	Intensity ratio 1047/1022 (cm^{-1})	Intensity ratio 1022/995 (cm^{-1})
0.5% TS	1.0	1.0
0.5% TS + SsM18	0.7229 ± 0.03966 ***	1.343 ± 0.06677 **
1.0% TS	1.0	1.0
1.0% TS + SsM18	0.6371 ± 0.06595 ***	1.863 ± 0.4089 *

CONCLUSION

Depending on the growing demand for supplements along with rising consumer perception regarding the health benefits of the prebiotics, the market of RS from various starch sources is growing. Thus, investigation, screening, and characterization of novel RS sources from a variety of starch are important for the development of the prebiotic industry (Zaman and Sarbini 2016). Although there are several different approaches to assess the prebiotic potential of a starch, such as *in vitro* analysis of the bacterial growth rate in the presence of the potential prebiotic starch, stimulation of growth, metabolism, and activities of the certain probiotic bacteria species, and the using of animal models to study changes in the gut microbiota composition and pH, feces humidity, and SCFAs production. However, these methods are costly, time-consuming, and labor-intensive especially for RS source screening purposes (Aquino et al. 2017).

Not only in the research area, but also for the industry, the development of fast and cheap techniques that provide a large amount of information is of big interest. The data presented here suggest that as a novel approach, FTIR spectroscopy has a potential to be applied in the research area as well as in the food industry for the *in vitro* analysis of the potential prebiotic activity of starch-based substrates or for the *in vitro* investigation of the probiotic potential of a bacterial strain through the examination of its RS hydrolyzing capacity.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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