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SHORT COMMUNICATION

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Puferski kapacitet probiotičkih jogurta

The Buffering Capacity of Probiotic Yogurt

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Sažetak

Svrha: U ovom se istraživanju želio odrediti u literaturi još neistražen potencijal puferiranja probiotičkih jogurta. Zadaća je bila odrediti pH, titracijsku kiselost određenih probiotičkih jogurta i njihov puferski kapacitet s naglaskom na raspon pH u kojem je puferiranje učinkovito. **Materijali i metode:** Dvadeset i pet mililitara svakog odabranog jogurta titrirano je s 1 mol/L natrijeva hidroksida. Dodavao se postupno po 0,5 mililitara sve dok nije postignuto 10, kako bi se procijenila ukupna titrabilna kiselost – mjera za puferski kapacitet jogurta. **Rezultat:** Početni pH bio je najniži kod jogurta Activia Peach (4,40± 0,14), a najviši kod Danonea natural (5,29± 0,10). Puferski kapacitet analiziranih jogurta može biti sljedeći: Danone natural >Danone peach >Activia plain >Activia strawberry >Activia Peach. Između tih pet jogurta nema statistički značajne razlike (p >0,05). **Zaključak:** Trebalo bi preispitati tvrdnju o puferskom kapacitetu probiotičkih jogurta.

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Ključne riječi
Bifidobacterium; puferi; probiotici; jogurt

Uvod

Probiotičke bakterije živi su dodaci prehrani koji povoljno utječu na domaćina jer mu poboljšavaju crijevnu floru i njezinu ravnotežu (1). Budući da se probiotici često primjenjuju, a činjenica je da svi obično korišteni sojevi bakterija učinkovito stvaraju kiseline, važno je razumjeti njihovu zadaću u oralnoj ekologiji. U sklopu dentalne medicine obavljena su klinička istraživanja s probiotskim sojevima kao što je *Bifidobacterium,* te su dobiveni suprotni rezultati o utjecaju na oralne mikroorganizme. Tako je među važnijim nalazima smanjena kolonizacija streptokoka mutans povezanih s incidencijom karijesa (2-4). Nedavno je ustanovljeno da b*ifidobakterije* testirane u uvjetima *in vitro* djelotvorno stvaraju kiseline iz glukoze i snižavaju pH u usporedbi sa sojem *S.mutans,* a ni jedan soj *Bifidobacterium* nije bio u stanju fermentirati šećerne alkohole (5). Uobičajena je probiotska hrana jogurt te se dnevna konzumacija mliječnih proizvoda čini najprirodnijim načinom za unos probiotičkih bakterija (1). Trenutačno se proučava oralni utjecaj probiotičkih jogurta. Prema našim spoznajama još nije opisan puferski kapacitet probiotika sa sojem *Bifidobacterium* na oralnu mikrofloru. Svrha ovog istraživanja bila je odrediti pH, titrabilnu kiselost različitih probiotičkih jogurta i njihov puferski učinak s naglaskom na raspon pH u kojem je pufer učinkovit.

Introduction

Probiotic bacteria are living microbial food supplements that beneficially affect the host by improving intestinal balance (1). As probiotics are used widely and the most common probiotic bacterial strains produce acids efficiently, their role in oral environment is important to understand. Within dentistry, there are clinical studies about probiotic strains such as *Bifidobacterium* which have revealed confusing results on oral microorganisms. Among the significant findings, a reduced colonization of the caries-associated *Streptococci Mutans* has been suggested (2-4). However, it was recently stated that *Bifidobacterium* strains tested in an *in vitro* study produced acids efficiently from glucose and the decrease in pH was comparable to that caused by *S. Mutans* while none of the *Bifidobacteria* fermented sugar alcohols (5). Yogurt is the archetypical probiotic food and daily consumption of dairy products seems to be the most natural way of ingesting probiotic bacteria (1). At this point, probiotic yogurts are target dairy products to be evaluated regarding their oral effects. To our knowledge, the buffering effects of *Bifidobacteria*-derived probiotics on oral microflora have not been reported. The aim of the present study was to determine the pH, titratable acidity of a selection of various probiotic yogurts, their buffering effects with emphasis on the pH range in which the buffer is efficient.

Materijali i metode

Odabrani jogurti podijeljeni su prema sastavu u sljedeće skupine: probiotsku (P), prirodnu kontrolu (C), obične (p) i voćne (f). Testirana su tri probiotska jogurta sa sojem *Bifidobacterium* koji se mogu nabaviti u turskim trgovinama: Pp-Activia plain® (Danone, Luleburgaz, Turska); Pf1-Activia strawberry® (Danone, Luleburgaz, Turska), Pf2-Activia Peach® (Danone, Luleburgaz, Turska). Za istraživanje su izabrane i dvije kontrole: Cp-Danone natural® (Danone, Luleburgaz, Turska) i Cf Danone peach® (Danone, Luleburgaz, Turska) (Tablica 1.). Početni pH bio je određen staklenom elektrodom promjera 0,1 milimetar (Ingold U402®, NEL Electronic, Istanbul, Turska) spojenom na pH-metar (PH 900®, NEL Electronic, Istanbul, Turska). Prije svakog mjerenja elektroda je bila kalibrirana korištenjem standardnih otopina od pH 4,0 i 7,0. Dvadeset pet mililitara tek otvorenog jogurta sobne temperature bilo je stavljeno u staklenu posudu IKAMAG RH®, (Janke&Kunkel IKA Labortecknik, Staufen, Njemačka) i pomiješano hladnom, nezagrijanom magnetskom miješalicom dok nije dobivena stabilna vrijednost očitanja pH. Za svaki uzorak zabilježeno je nekoliko vrijednosti kako bi se dobila srednja vrijednost za sve mliječne napitke. Dvadeset pet mililitara svakog jogurta titrirano je s 1 mol/L natrijeva hidroksida (NaOH), a dodavan je po 0,5 mililitara dok nije postignut pH 10 (Edwards i suradnici, 1999.)(6) kako bi se procijenila ukupna titrabilna kiselost kao mjera puferskog kapaciteta probiotskih jogurta. Uzorci su miješani hladnom magnetskom miješalicom dok se nije postigla stabilna vrijednost očitanja pH nakon svakog dodavanja otopine NaOH. Titracije su bile ponovljene tri puta za svaki jogurt iz svake skupine, kako bi se osigurala reprodukcija i dobila srednja vrijednost za svaki jogurt. Procjena puferskog kapaciteta obavljala se na dva načina. Na početku je bio nacrtan grafikon s pH u odnosu prema dodanim mililitrima NaOH. Nagib dobivene krivulje omogućio je usporedbu različitih puferskih kapaciteta. Dodatno se bilježio i ukupan volumen potreban da se pH podigne na 10. Te su vrijednosti uspoređene između skupina probiotskih jogurta i to u programu SPSS Windows® (version 16,0, Chicago, Ill, SAD) odabirom neparametrijskih testova. Granična *p*-vrijednost manja od 0,05 smatrala se statistički značajnom.

Materials and Methods

The tested yogurts were categorized with codes regarding their being probiotic (P) or natural control (C); then plain (p) or fruit (f). Three available probiotic yogurts with *Bifidobacterium* on the Turkish market; Pp-Activia plain® (Danone, Luleburgaz, Turkey); Pf1- Activia strawberry® (Danone, Luleburgaz, Turkey), Pf2- Activia Peach® (Danone, Luleburgaz, Turkey), and two controls: Cp- Danone natural® (Danone, Luleburgaz, Turkey) and Cf Danone peach® (Danone, Luleburgaz, Turkey) were chosen for the study (Table 1).

The initial pH was determined by a glass pH electrode with a diameter of 0.1 mm (Ingold U402®, NEL Electronic, Istanbul, Turkey) connected to a pH meter (PH 900®, NEL Electronic, Istanbul, Turkey). Before measuring, the electrode was calibrated at the start of each session using standard buffers of pH 4.0 and 7.0. A quantity of 25 ml of freshly opened yogurt, which was at room temperature, was placed in a beaker (IKAMAG RH®, Janke&Kunkel IKA Labortecknik, Staufen, Germany) and stirred using a non-heating magnetic stirrer until a stable reading was obtained. Several readings were taken of yogurts from each group to give a mean measurement for each yogurt.

25 ml of each yogurt was titrated with 1 mol/L sodium hydroxide (NaOH), added gradually by 0.5 ml, until the pH reached 10 (Edwards et al., 1999) (6) to assess the total titratable acidity - a measure of probiotic yogurt's own buffering capacity. The samples were again stirred using a non heating magnetic stirrer until a stable pH reading was obtained after each addition of NaOH. Titrations were repeated in triplicate for several yogurts from each group to ensure reproducibility and to give a mean value for that yogurt.

The assessment of buffering capacity data was carried out in two ways. Initially, a graph was plotted of pH against added ml of NaOH. The slope of the resultant curve allowed comparison of differing buffering capacities. In addition, the total volume of NaOH required to raise the pH to 10 was also noted. These amounts were then compared for the various groups of probiotic yogurts using SPSS software for Windows® (version 16.0, Chicago, Ill, USA) using nonparametric tests. A *p* value less than 0.05 was considered statistically significant.

Tablica 1. Aritmetičke sredine pH-vrijednosti (±SD) za svaku skupinu
Table 1 Mean initial pH values (±SD) for each group.

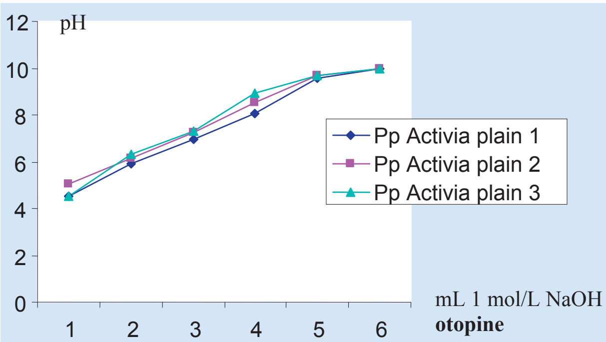
Probiotički jogurt • Probiotic Yogurt	Šifra • Code	pH (mean±SD) • pH (arit.sred.±SD)
Activia plain 1,360.00 mg/L Ca *Bifidobacterium* DN-173 010 bacteria (7x10^{7} cfu/g)	Pp	4.53 ± 0.04
Activia strawberry 1,550.00 mg/L Ca *Bifidobacterium* DN-173 010 (2 x 10^{8} cfu/g)	Pf1	4.47± 0.04
Activia peach 1,600.00 mg/ L Ca *Bifidobacterium* DN-173 010 (2 x 10^{8} cfu/g)	Pf2	4.40± 0.14
Danone natural 1,721.00 mg/L Ca	Cp	5.29± 0.10
Danone peach 1,200.00 mg/L Ca	Cf	4.88± 0.25

Rezultati

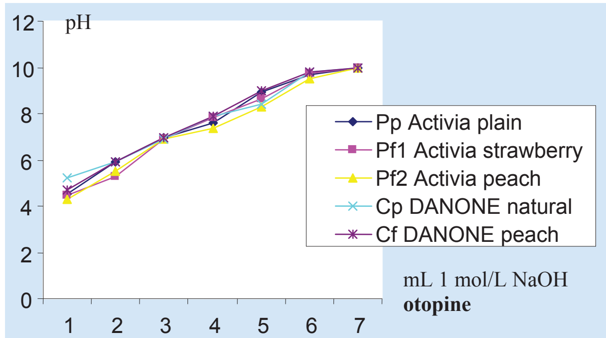
Vrijednosti pH i standardne devijacije nalaze se u Tablici 1. Početni pH bio je najniži za Pf1 (4,40± 0,14) i najviši za Cp (5,29± 0,10). Slika 1. prikazuje procjenu reprodukcije za Pp. Na njoj se vidi da se taj bezalkoholni napitak sva tri puta ponašao gotovo identično. To je bilo tipično za testirane jogurte i upućivalo je na to da se metoda procjene može reproducirati. Kao ilustracija samo jedne skupine, na Slici 2. su krivulje svakog napitka u sklopu Cp-a. Svi ispitani proizvodi ponašali su se slično – trebalo im je prosječno 10 mililitara NaOH kako bi se postigao pH 10. Budući da u uzajamnoj usporedbi nije bilo statistički značajne razlike među analiziranim jogurtima iz bilo koje od pet skupina (p >0,05), Tablica 2. predstavlja p-vrijednost za pojedinačne usporedbe među analiziranim skupinama jogurta za potrebnu količinu NaOH u mililitrima kako bi se pH podignuo na 10. Na Slici 3. (a i b) krivulje su za voćni i običan jogurt. Nije pronađena statistički značajna razlika između bilo kojeg od testiranih običnih ili voćnih jogurta (p >0,05).

Results

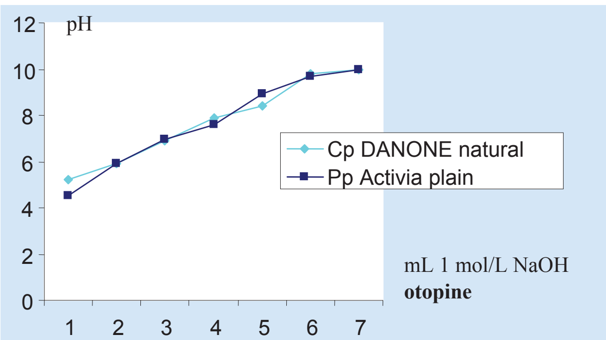
The pH and standard deviation values are shown in Table 1. The initial pH was lowest for Pf1 (4.40± 0.14) and highest for Cp (5.29± 0.10). Figure 1 displays the data for reproducibility assessment of Pp. The plot shows that this soft drink behaved almost identically on all three occasions. This was typical of the yogurt tested and indicated that the method of assessment used is reproducible. As illustration of just one of the groups, Figure 2 displays the plots of each of the drinks within the Cp. All tested products behaved similarly, requiring on average 10 ml of NaOH to bring the pH up to 10. There were no statistically significant differences observed between the yogurts within any of the five groups compared as a whole with one another (p >0.05). Table 2 shows the p values for individual comparisons made between each group of yogurt for the number of ml of NaOH required to raise the pH to 10. Figure 3 a, b displays the plots of the fruit and plain yogurt. There were no statistically significant differences between either of the fruit and plain yogurts tested (p >0.05).



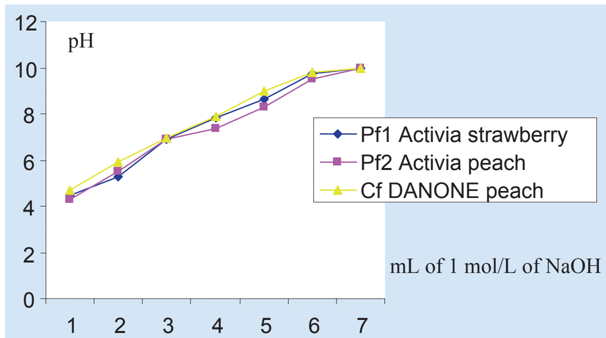
Slika 1. Krivulja ponovljenih titracija običnih probiotičkih jogurta kako bi se osigurala reproduktivnost metodologije
Figure 1 Plots of repeated titrations of a probiotic plain yogurt to ensure methodology is reproducible.



Slika 2. Grafikon titracija za sve jogurte
Figure 2 Graph of titrations for all yogurt



Slika 3a. Grafikon titracija pokazuje sličnosti običnih jogurta
Figure 3a. Graph of titrations to show similarities of plain yogurt



Slika 3b. Grafikon titracija za prikaz sličnosti voćnih jogurta
Figure 3b Graph of titrations to show similarities of fruit yogurt

Tablica 2. p-vrijednost – usporedba broja mililitara NaOH potrebnih da se postigne pH 10
Table 2 p values comparing the number of mL of NaOH necessary to bring the pH to 10

Testirani jogurt • Yogurt tested	Pp	Pf1	Pf2	Cp	Cf
Pp		0.616	0.979	0.707	0.927
Pf1			0.616	0.770	0.924
Pf2				0.770	0.979
Cp					0.924
Cf					

Rasprava

Bifidobacterium, najčešće korišten bakterijski soj u probiotičkim proizvodima, acidogeničan je. Nastanak organskih kiselina poželjno je svojstvo probiotika (7,8). No, stvaranje kiseline od ugljikohidrata jedna je od glavnih značajki kariogenih svojstava. Osim toga, acidogenost i aciduričnost sojeva može inhibirati druge organizme u kolonizaciji i stvoriti lokalni okoliš koji njima odgovara – primjerice zubni plak postaje još kiseliji. Neki od sojeva *Bifidobacterium* mogu imati i pozitivnu ulogu u održavanju zdravlja zbog mikrobiološke ravnoteže u oralnoj šupljini te mogu u sklopu oralne obrane stvarati čimbenike poput sustava peroksidaze koja može inhibirati acidogenost bakterija. Iz tih se razloga, prije nego što se probiotske bakterije mogu preporučiti za oralno zdravlje, mora potvrditi njihova sigurnost i da ne mogu prouzročiti bolesti (8). Vrijednost pH izmjerena u probiotskim prehrambenim proizvodima ne može biti dovoljna za predviđanje mogućega zubnog karijesa ili erozije jer i drugi čimbenici utječu na te destruktivne procese. Oni se svrstavaju u kemijske (pKa vrijednosti, adhezija, kelacijska svojstva, sadržaj Ca, P i F), u navike (prehrana – jela i pića, način života, pretjerano konzumiranje kiselina) i biološke (količina sline, puferski kapacitet, sastav sline, stvaranje pelikule, sastav te anatomija zuba i mekih tkiva) (9). Svrha ovog istraživanja bila je odrediti jedan od kemijskih čimbenika, pa su jogurti svrstani u obične i voćne. U literaturi se od nedavno može naći podatak da voćni jogurti donekle uzrokuju gubitak pokrovne cakline u uvjetima *in vitro*, ali bez vidljivog erozivnog učinka (10). Uloga voća u određivanju puferskog kapaciteta voćnih jogurta važna je (11), a ustanovilo se da je onaj od jagoda blago nezasićen u odnosu prema hidroksilapatitu i zasićen u odnosu prema fluorapatitu, a svi ostali jogurti su zasićeni (12).

U ovom istraživanju nije pronađena statistički značajna razlika među voćnim jogurtima, bili oni probiotički ili ne.

Na kraju, puferski kapacitet probiotičkih jogurta ne bi se trebao isticati, jer u ovom se istraživanju ustanovilo da nema većih razlika između uspoređenih jogurta u bilo kojoj od pet analiziranih skupina.

Discussion

Bifidobacterium, the bacterial genera most often used in probiotic products, are acidogenic. On one hand, production of organic acids is one of the desirable attributes of the probiotics in the intestine (7, 8). On the other hand, the acid production from fermentable carbohydrates is one of the most important cariogenic traits. Furthermore, acidogenic and aciduric species can inhibit other competing organisms and make their local environment, for example, the dental plaque, even more acidic. Yet, some species of *Bifidobacterium* may also have a role in maintaining health by promoting a microbiological balance in the oral cavity and oral defense mechanisms, such as the peroxidase system may inhibit the acidogenicity of bacteria. Thus, before recommending probiotic bacteria for oral health care, their safety, i.e. their inability to cause diseases, must be ascertained (8). At this point, the pH of a probiotic dairy product alone may not be predictive of its potential to cause dental caries or dental erosion since other factors modify the destructive process. These factors were stated as chemical (pKa values, adhesion and chelating properties, Ca, P and F content), behavioral (eating and drinking habits, lifestyle, excessive consumption of acids) and biological (flow rate, buffering capacity, composition of saliva, pellicle formation, tooth composition, dental and soft tissue anatomy) (9). Therefore, this study aimed to point out one of the chemical factors related.

In the present study, yogurts were categorized as plain and fruit. Recently, it has been concluded that fruit yogurts caused material loss of minipig enamel *in vitro* to some degree; however, they had no detectable erosive effects (10). The role of fruit in determining the buffering capacity of fruit yogurt is important (11). Recently, it has been stated that a strawberry yogurt was found to be slightly undersaturated with respect to hydroxiapatite and supersaturated with respect to fluoroapatite while all other fruit yogurts were supersaturated (12). In the present study, there were no statistically significant differences between fruit yogurts, either probiotic or not.

In conclusion, buffering capacities of probiotic yogurt should be undermined; the present study demonstrates that there are no significant differences observed between yogurts within any of the five groups compared as a whole with one another.

Abstract
Aim: The potential buffering role of probiotic yogurt has not been investigated. The aim of the study was to determine the pH, titratable acidity of a selection of various probiotic yogurts, their buffering effects with an emphasis on the pH range in which the buffer is efficient. **Material & Methods:** A quantity of 25 ml of each yogurt was titrated with 1 mol/L sodium hydroxide, added gradually by 0.5 ml, until the pH reached 10 to assess the total titratable acidity - a measure of probiotic yogurt's own buffering capacity. **Results:** The initial pH was the lowest for Activia peach yogurt (4.40± 0.14) and the highest for Danone natural yogurt (5.29± 0.10). The buffering capacities can be listed as follows: Danone natural >Danone peach >Activia plain >Activia strawberry >Activia peach. There were no statistically significant differences observed between yogurts within any of the five groups compared as a whole with one another. ($p > 0.05$). **Conclusions:** Buffering capacities of probiotic yogurts should be undermined.

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Bifidobacterium; Buffers; Probiotics; Yogurt

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