Effect of Storage Time and Temperature on Salivary Total Antioxidant Capacity, Total Oxidant Status, and Oxidant Stress Index

Introduction

The use of saliva to measure oxidative stress (OS) is increasing (1), since researchers have demonstrated that saliva contains oxidation biomarkers similar to those in blood (2–5). The analysis of OS biomarkers in saliva is potentially valuable because its collection is simple and may provide a cost-effective approach for the screening of large populations (6).

Nevertheless, sometimes saliva samples are required to be stored for several days and different results can be obtained because salivary constituents may degrade when processed after multiple freeze–thaw cycles or after different time and temperature of storage (7).

Since there is little consistency in the literature regarding the feasibility of saliva storage before OS markers analysis, the aim of this study was to compare the effects of short-term storage in two different temperatures on stability of salivary total antioxidant capacity (TAC), total oxidant status (TOS) and oxidant stress index (OSI).

Material and methods

Saliva samples were collected from twenty healthy volunteers for the study. An aliquot was selected for immediate analysis and the rest was stored at -20°C and -80°C for a period of 120 days, and analyzes were performed at 15, 30, 45, 60, 90 and 120 days. The determination of the TOS and the TAC were performed by colorimetric methods. Results: The results show that the two storage temperatures were able to preserve oxidants and antioxidants up to 60 days with similar levels when compared with fresh samples. When comparing the different storage temperatures at each time point, no significant differences were observed. Finally, the OSI remains constant over time of storage at both temperatures without statistically significant differences between them. The results were expressed as mean ± standard error of mean. Statistical analysis was performed using the repeated-measures analysis of variance (ANOVA), the Bonferroni and t-test. A p-value <0.05 was accepted to be statistically significant. Conclusion: The two temperatures were able to keep salivary TOS and TAC levels similar to fresh saliva samples. Therefore they are reliable for assessing oxidative stress up to 60 days.

Abstract

Objective: The aim of this study was to compare the effects of short-term storage in two different temperatures on stability of salivary total antioxidant capacity (TAC), total oxidant status (TOS) and oxidant stress index (OSI). Material and methods: Saliva samples were collected from twenty healthy volunteers for the study. An aliquot was selected for immediate analysis and the rest was stored at -20°C and -80°C for a period of 120 days, and analyzes were performed at 15, 30, 45, 60, 90 and 120 days. The determination of the TOS and the TAC were performed by colorimetric methods. Results: The results show that the two storage temperatures were able to preserve oxidants and antioxidants up to 60 days with similar levels when compared with fresh samples. When comparing the different storage temperatures at each time point, no significant differences were observed. Finally, the OSI remains constant over time of storage at both temperatures without statistically significant differences between them. The results were expressed as mean ± standard error of mean. Statistical analysis was performed using the repeated-measures analysis of variance (ANOVA), the Bonferroni and t-test. A p-value <0.05 was accepted to be statistically significant. Conclusion: The two temperatures were able to keep salivary TOS and TAC levels similar to fresh saliva samples. Therefore they are reliable for assessing oxidative stress up to 60 days.

Key words

Saliva; Freezing; Antioxidants; Oxidative Stress

Received: August 3, 2018
Accepted: March 29, 2019

Address for correspondence
Prof. Dr. Jose M. Amenabar,
Universidade Federal do Paraná
Stomatology Department, Oral Biochemistry Laboratory – LABIO
Av. Lothário Meissner 632, Jardim Botânico. Curitiba-Paraná-Brazil.
CEP: 80210-170.
Telephone: +55-41-3360-4024.
Fax: +55-41-3360-4053.
jamenaba@ufpr.br

Introduction

Korištenje sline za mjerenje oksidacijskoga stresa (OS-a) u porastu je (1) jer su istraživači pokazali da sadržava oksidacijske biomarkere slične onima u krvi (2–5). Analiza biomarkera OS-a u sline potencijalno je vrijedna jer je njezino prikupljanje jednostavno i može biti ekonomična u slučaju probira u velikim populacijama (6).

Ipak, katkad je potrebno pohraniti uzorke sline nekoliko dana, no pritom se mogu dobiti različiti rezultati jer se njezini sastojci mogu razgraditi nakon više ciklusa zamrzavanja i odmrzavanja ili nakon različitih razdoblja i temperatura skladištenja (7).

Budući da u literaturi nema konzistentnosti kad je riječ o izvedivosti skladištenja sline prije analize markera OS-a, cilj ovog istraživanja bio je usporediti utjecaj kratkotrajnog skladištenja na dvjema različitim temperaturama na stabilnost...
total antioxidant capacity (TAC), total oxidant status (TOS) and oxidant stress index (OSI).

Material and methods

Ethics Committee Approval

The study protocol was approved by the Research Ethics Committee of the Federal University of Paraná, Brazil (Project CEP: 872583/2014). All the participants received detailed information concerning the nature and the procedures involved in the study and signed informed consent forms.

This analytic study included saliva samples from 20 healthy volunteers, with an age range of 28–38 years, recruited from February until July 2015.

Saliva Collection and Storage

Saliva production was stimulated by chewing a paraffin gum and total saliva samples were collected in plastic tubes on ice, after 2 hr-fasting, always between 8:00 and 10:00 a.m. Each participant collected saliva for approximately 10 minutes. All the samples were centrifuged at 2,600g for 10 min at 4°C to remove cellular and food debris and none of them were contaminated with blood.

Each saliva sample was divided in 13 identical aliquots (500µL). One aliquot was used immediately to analyze fresh saliva and two series of 6 identical aliquots were used to analyze after 15, 30, 45, 60, 90 and 120 days of storage at -20°C or -80°C.

Saliva Analysis

Measurement of the TAC of the saliva

Saliva TAC was determined using the automated colorimetric measurement method of Erel (8). In this method hydroxyl radicals react with O-dianisidine to produce the bright yellowish brown dianisyl radical and after the addition of saliva, the oxidative reactions are suppressed by the antioxidant components of saliva thus preventing the color change. The results were expressed as millimolar Trolox equivalent per liter (mmol Trolox equiv/L).

Measurement of the TOS of the saliva

Saliva TOS was measured using the fully automated colorimetric method of Erel (9). In this method, the oxidants present in saliva oxidize the ferrous ion –o-dianisidine complex to ferric ion. The results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H₂O₂ Equiv/L).

Oxidative stress index

The percent ratio of the TOS to the TAS gives the oxidative stress index (OSI). To perform the calculation, the result unit of TAS, mmol Trolox Equivalent/L, was changed to µmol Trolox Equivalent/L, and the OSI value was calculated as follows: OSI = [(TOS, µmol/L)/TAS, µmol/L]/100

Statistical analysis

The results were expressed as mean ± standard error of mean. Statistical analysis was performed using the repeated-ukupnog antioksidacijskog kapaciteta sline (TAC-a) i ukupnog oksidacijskog statusa (TOS-a) te na indeks oksidacijskog stresa (OSI).

Materijali i metode

Odobrenje Etičkog povjerenstva

Protokol istraživanja odobrilo je Etičko povjerenstvo za istraživanje Saveznoga sveučilišta u Parani, Brazil (projekt CEP: 872583/2014).

Svi sudionici u istraživanju detaljno su informirani o njegovoj prirodi i postupcima te su potpisali informirani pristup.

Ovo analitičko istraživanje uključivalo je uzorke sline prikupljene od veljače do srpnja 2015. godine od 20 zdravih dobrovoljaca u dobi od 28 do 38 godina.

Prikupljanje i skladištenje sline

Proizvodnja sline stimulirana je žvakanjem parafinske gume, a uzorci su skupljeni u plastične epruvete na ledu, nakon dva sata posta, uvijek između 8.00 i 10.00 sati. Svaki sudionik skupljao je slinu približno 10 minuta. Svi su uzorci centrifugirani na 2,600 g 10 minuta na 4 °C da bi se uklonili ostaci stanica i hrane. Ni jedan nije bio kontaminiran krvlju.

Svaki uzorak sline podijeljen je na 13 identičnih alikvota (500 uL). Jedan alikvot odmah je upotrijebljen za analizu svježe sline, a dvije serije od 6 identičnih alikvota korištene su za analizu nakon 15, 30, 45, 60, 90 i 120 dana skladištenja na -20 °C ili -80 °C.

Analiza sline

Mjerenje TAC-a sline

TAC sline određen je s pomoću automatizirane kolorimetrijske metode mjerenja prema Erelu (8). Pri primjeni ove metode hidroksilni radikali reagiraju s o-dianisidinom kako bi se proizvolio svijetložućkastosmeđi radikal dianisil, a nakon dodavanja sline oksidacijske reakcije potiskuju antioksidacijske komponente sline čime se spriječava promjena boje. Rezultati su izraženi kao milimolarni ekvivalent troloksa po litri (mmol Trolox equiv/L).

Mjerenje TOS-a sline

TOS sline mjeren je uporabom potpuno automatizirane kolorimetrijske metode prema Erelu (9). U ovoj metodi oksidirani u sline oksidiraju kompleks iona o-dianisidina u željezov ion. Rezultati su izraženi u mikromolarnom ekvivalentu vodikova peroksida po litri (µmol H₂O₂ Equiv/L).

Indeks oksidacijskog stresa

Postotni omjer TOS-a prema TAS-a daje indeks oksidacijskog stresa (OSI). U izračunu jedinica rezultata TAC-a, mmol Trolox equiv/L, promijenjen je u µmol Trolox equiv/L, a vrijednost OSI-ja izračunata je na sljedeći način: OSI = [(TOS, µmol/L)/TAS, µmol/L]/100.

Statistička analiza

Rezultati su izraženi kao srednja vrijednost ± standarde pogreška srednje vrijednosti. Statistička analiza obavljena
measures analysis of variance (ANOVA), the Bonferroni and t-test. A p-value <0.05 was accepted to be statistically significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 20.0, SPSS Inc., Chicago, IL, USA).

Results

Compared with fresh saliva samples, salivary TAC and TOS levels were significantly lower after 90 days of storage at both temperatures (Figure 1 and Figure 2). Saliva TOS concentration presented a long term decline during the study at both temperatures. There was a statistically significant effect of time on TOS levels at -20°C, F(11.26, 2.21)=5.09, p=0.002, \( \eta^2_p = 0.253 \); with difference between T0 and T90 (p=0.02) and at -80°C, F(12.08, 2.11)=5.716, p=0.001, \( \eta^2_p =0.276 \); with difference between T0 and T90 (p=0.03).

Saliva TAC concentration also presented a long term decline at both temperatures with statistical significant effect, at -20°C, F(5.71, 2.13)=2.67, p=0.02, \( \eta^2_p = 0.151 \); with difference between T0 and T90 (p=0.012) and at -80°C, F(17.025, 2.50)=6.80, p<0.001, \( \eta^2_p =0.312 \); with difference between T0 and T90 (p=0.02).

On the other hand, no statistical difference was found between the two temperatures of storage at each different time points for TAC and TOS, as shown on Table 1 and Table 2. Also, no statistical difference was found in the OSI level remaining practically constant during the period of the study (Table 3).

Figure 1
The effect of storage at -20°C and -80°C for 120 days on salivary TAC concentration. *p<0.05 is significantly different from the previous month. † p<0.05 is significantly different from day 0.

Figure 2
The effect of storage at -20°C and -80°C for 120 days on salivary TOS concentration. *p<0.05 is significantly different from the previous month. † p<0.05 is significantly different from day 0.

Rezultati

U usporedbi s uzorcima svježe sline, razine TAC-a i TOS-a bile su značajno niže nakon 90 dana skladištenja na obje ma temperaturama (slika 1. i slika 2.). Koncentracija TOS-a u slini dugoročno je pokazala pad tijekom istraživanja na objema temperaturama. Utvrđen je statistički značajan utjecaj vremena na TOS-ove razine na -20 °C, F (11,26, 2,21) = 5,09, p = 0,002, \( \eta^2_p = 0,253 \); s razlikom između T0 i T90 (p = 0,02) i na -80 °C, F (12,08, 2,11) = 5,716, p = 0,001, \( \eta^2_p = 0,276 \); s razlikom između T0 i T90 (p = 0,03).

U koncentraciji TAC-a u slini također je zabilježen pad na objema temperaturama sa statistički značajnim utjecajem, na -20 °C, F (5,71, 2,13) = 2,67, p = 0,02, \( \eta^2_p = 0,151 \); srazlikom između T0 i T90 (p = 0,012) i na -80 °C, F (17,025, 2,50) = 6,80, p <0,001, \( \eta^2_p = 0,312 \); s razlikom između T0 i T90 (p = 0,02).

No, nisu pronađene statistički značajne razlike između dviju temperature skladištenja u različitim vremenskim točkama za TAC i TOS, kao što je prikazano u tablici 1. i tablici 2. Također nije pronađena statistički značajna razlika u razinama OSI-ja koje su ostale praktički konstantne tijekom istraživanja između obiju temperatura skladištenja (tablica 3.).
Table 1

Comparison of the total antioxidant capacity (mmol Trolox equiv /L) of saliva stored at -20°C and at -80°C at different time points.

| Fresh saliva • Svježa slina | 3.53 ± 0.72 |
|----------------------------|-------------|
| Freezing at -20°C • Zamrzavanje na -20°C | Freezing at -80°C • Zamrzavanje na -80°C | P |
| 15 Days • 15 dana | 3.42 ± 1.08 | 3.23 ± 1.07 | 0.665 |
| 30 Days • 30 dana | 3.13 ± 1.47 | 3.15 ± 1.53 | 0.626 |
| 45 Days • 45 dana | 3.05 ± 1.32 | 3.12 ± 1.48 | 0.813 |
| 60 Days • 60 dana | 3.00 ± 1.25 | 3.02 ± 1.53 | 0.852 |
| 90 Days • 90 dana | 2.39 ± 1.29 | 2.50 ± 1.22 | 0.765 |
| 120 Days • 120 dana | 2.09 ± 1.51 | 2.47 ± 1.00 | 0.802 |

Table 2

Comparison of the total oxidant status (µmol H₂O₂ equiv /L) of saliva stored at -20°C and at -80°C at different time points.

| Fresh saliva • Svježa slina | 3.42 ± 1.31 |
|----------------------------|-------------|
| Freezing at -20°C • Zamrzavanje na -20°C | Freezing at -80°C • Zamrzavanje na -80°C | P |
| 15 Days • 15 dana | 3.35 ± 0.96 | 3.40 ± 1.06 | 0.731 |
| 30 Days • 30 dana | 3.21 ± 1.22 | 3.31 ± 1.13 | 0.118 |
| 45 Days • 45 dana | 3.15 ± 1.32 | 3.21 ± 1.02 | 0.191 |
| 60 Days • 60 dana | 3.09 ± 0.87 | 3.10 ± 0.13 | 0.846 |
| 90 Days • 90 dana | 2.24 ± 0.78 | 2.70 ± 1.27 | 0.633 |
| 120 Days • 120 dana | 2.02 ± 0.70 | 2.32 ± 0.76 | 0.796 |

Table 3

Comparison of the oxidative stress index of saliva stored at -20°C and at -80°C at different time points.

| Fresh saliva • Svježa slina | 9.71 ± 1.64 |
|----------------------------|-------------|
| Freezing at -20°C • Zamrzavanje na -20°C | Freezing at -80°C • Zamrzavanje na -80°C | P |
| 15 Days • 15 dana | 10.10 ± 1.53 | 10.50 ± 1.88 | 0.673 |
| 30 Days • 30 dana | 10.24 ± 1.55 | 10.49 ± 1.78 | 0.233 |
| 45 Days • 45 dana | 10.31 ± 1.94 | 10.30 ± 2.05 | 0.795 |
| 60 Days • 60 dana | 10.30 ± 1.89 | 10.29 ± 1.83 | 0.581 |
| 90 Days • 90 dana | 9.36 ± 1.18 | 10.77 ± 1.03 | 0.443 |
| 120 Days • 120 dana | 9.65 ± 1.6 | 9.39 ± 1.23 | 0.445 |

Discussion

Saliva has been increasingly used as diagnostic medium because its collection method is easy, non-invasive, can do repeated sampling and suitability for research single analyte or complex measurements (10). Saliva has also been reported to be suitable to detect the body’s oxidative stress levels, helping with diagnosis, prognosis, and therapeutic response of human diseases (10-12). However, some saliva analytes are not stable at room temperature and in many cases, storage of samples for a day prior to analysis is necessary (10). As far as we know, this is the first study to evaluate the stability of the TAC, TOS and OSI of saliva during freeze storage at 2 temperatures (–20º and –80ºC), assessing the variations occurring during 120 days of storage.

According to our results, storage periods led to some changes in both TAC and TOS, regardless of the temperature of storage. It seems that saliva can be frozen and stored at –20ºC or –80ºC for two months, in order to have similar results to the ones found in fresh saliva samples.

Rasprava

Slina se sve više koristi kao dijagnostički medij jer je metoda prikupljanja jednostavna i neinvazivna, uzorkovanje se može ponoviti i prikladna je za istraživanje pojedinačnih analita ili složenih mjerenja (10). Također se navodi da je slina prikladna za otkrivanje razine oksidacijskog stresa u tijelu te može pomoći u dijagnozi, prognozi i terapijskom odgovoru kad je riječ o bolestima ljudi (10 – 12). Neki analiti sline nisu stabilni na sobnoj temperaturi i često je potrebno čuvanje uzoraka prije analize (10). Koliko znamo, ovo je prvo istraživanje koje procjenjuje stabilnost TAC-a, TOS-a i OSI-ja sline tijekom skladištenja na dvjema temperaturama (–20 ºC i –80 ºC), procjenjujući varijacije koje se događaju svih 120 dana skladištenja.

Prema našim rezultatima, razdoblja skladištenja potaknula su određene promjene TAC-a i TOS-a, bez obzira na temperaturu skladištenja. Čini se da se slina može zamrznuti i pohraniti na -20 ºC ili -80 ºC dva mjeseca kako bi se postigli slični rezultati kao i u uzorcima svježe sline.
In this study, the saliva TAC decreased with a similar pattern, regardless of the storage temperature. This decrease can be explained by the fact that some of the antioxidants comprise proteins and it is a known fact that temperature has a strong influence on the activity of proteins in which lower temperatures result in lower activity. One limitation of the present study is that we did not quantify proteins in the samples; therefore we cannot confirm that this did not interfere with the results. One of the reasons for the decrease in TAC measurements may be the loss of protein activity or protein degradation. Hubel et al. (13), on a review evaluated various fluids, including urine, saliva, blood, among others, shows that different conditions of collection and storage have great effects on the stability of proteins, leading to misinterpretation of the results and invalid conclusions. Emekli-Alturfan et al. (7, 14) evaluated the levels of GSH and lipid peroxidation in saliva samples stored at -20°C, in 30, 60, 90, 120, 150 and 180 days after collection. They suggest that saliva can be stored for 30 days at -20°C. Also, Ng et al. (15) analyzed saliva IgA and lysozyme and they observed that the concentrations remain stable for up to 3 months when stored at -30°C.

The evaluation of oxidative status during storage has been made in other body fluids such as urine, breast milk and blood. Remer et al. (16) used urine which was stored for a period of 15 years and they found that some substances have high stability at -22°C, such as uric acid, a non-enzymatic antioxidant. However, other substances such as oxalate had losses over time and to prevent this, the use of preservatives has been proposed. Nevertheless, adding preservatives can interfere with other saliva compounds.

Akdag et al. (17) assessed the stability of TAC in breast milk stored at -80°C for 3 months and found that it remains stable without significant losses. Silvestre et al. (18) evaluated the activity of glutathione peroxidase and the concentration of MDA in breast milk in 2 temperatures (-20°C and -80°C), at 15, 30 and 60 days of storage. They found that freezing induces losses in the antioxidant properties of breast milk and that such losses increase with duration of storage and differ in intensity according to temperature. They suggest that in order to maximally preserve the antioxidant properties of breast milk, the latter should be stored at -80 °C degrees for a period of less than 30 days, rather than for shorter time periods at usual temperature of -20°C degrees (18).

In the present study, regardless of the storage temperature, the saliva TAC and TOS decreased with a similar pattern. However, saliva stored at -80 °C seems to be more stable than saliva stored at -20°C. Consequently, the general and widely accepted rule of storing at the lowest temperature possible seems to apply to the saliva TAC and TOS, as might be expected. On the other hand, if saliva cannot be stored at -80°C, it can be stored at -20°C for 60 days without interfering with the TAC, TOS and OSI levels.

The limitations of our study are a relatively small sample size and the fact that only TAC and TOS were analyzed. Although specific biomarkers should be examined to clearly identify oxidative stress, the assessment of oxidative stress through the evaluation of the TAC and TOS may be sensitive to elucidate the oxidant status and the preservation of the samples.

U ovom istraživanju TAC sline smanjio se prema sličnom obrascu, bez obzira na temperaturu skladištenja. To se može objasniti činjenicom da neki od antioksidansa sadržavaju proteine, a poznata je činjenica da temperatura snažno utječe na aktivnost proteina u kojima niže temperature rezultiraju manjom aktivnošću. Jedno od ograničenja ovog istraživanja jest da nismo kvantificirali proteine u uzorcima i zato ne mogemo potvrditi da to nije utjecalo na rezultate. Jedan od razloga za smanjenje TAC-a može biti i gubitak aktivnosti proteina ili njihova degradacija. Hubel i suradnici (13) na temelju ocjene različitih tekućina, uključujući urin, slinu i krv, ističu da različiti uvjeti skupljanja i skladištenja imaju velik učinak na stabilnost proteina, što rezultira pogrešnim tumačenjem rezultata i nevaljanim rezultatima. Emekli-Alturfan i suradnici (7, 14) procijenili su razine GSH-a i lipidne peroksidacije u uzorcima sline pohranjenima na -20 °C, 30, 60, 90, 120, 150 i 180 dana poslije prikupljanja. Oni sugeriraju da se slina može čuvati 30 dana na -20 °C. Ng i suradnici (15) također su analizirali salivarni IgA i lizozim te su uočili da koncentracije ostaju stabilne do tri mjeseca ako se čuvaju na -30 °C.

Procjena oksidacijskog statusa tijekom skladištenja obavljena je i u drugim tjelesnim tekućinama, kao što su urin, majčino mljecko i krv. Remer i suradnici (16) koristili su se urinom koji je čuvan 15 godina i otkrili su da neke tvari zadržavaju visoku stabilnost na -22 °C, kao što su mokračna kiselina i neenzimski antioksidans. No za druge tvari, kao što je oksalat, zabilježen je gubitak tijekom vremena i, kako bi se to spriječilo, predložena je upotreba konzervansa. No dodavanje konzervansa može utjecati na druge spoeve u slini.

Akdag i suradnici (17) procijenili su stabilnost TAC-a u majčinu mljeaku pohranjenom tri mjeseca na -80 °C i utvrdili da je stabilan i bez značajnih gubitaka. Silvestre i suradnici (18) procijenili su aktivnost glutation peroksidaze i koncentraciju MDA-a u majčinu mljeaku na dvjema temperaturama (-20 °C i -80 °C) nakon 15, 30 i 60 dana skladištenja. Otkrili su da zamaravanje uzrokuje gubitak antioksidacijskih svojstava majčina mljeaka i da se takvi gubitci povećavaju s trajanjem skladištenja i razlikuju se u intenzitetu prema temperaturi. Predlažu, kako bi se maksimalno sačuvala antioksidacijska svojstva majčina mljeaka, da ga treba čuvati na -80 °C u razdoblju kraćem od 30 dana, a ne u kraćim razdobljima na uobičajenoj temperaturi od -20 °C (18).

U ovom istraživanju, bez obzira na temperaturu skladištenja, TAC i TOS sline smanjili su se prema sličnom uzorku. No čini se da je slina pohranjena na -80 °C stabilnija od one pohranjene na -20 °C. Prema tome, čini se da je općeprihvaćeno pravilo o skladištenju na najnižoj mogućoj temperaturi primjenjivo i na TAC i TOS u slini, kao što se moglo i očekivati. Ako se slina ne može pohraniti na -80 °C, može se 60 dana čuvati na -20 °C bez promjena u razinama TAC-a, TOS-a i OSI-ja.

Ograničenja našeg istraživanja razmjerno su mala veličina uzoraka i činjenica da su analizirani samo TAC i TOS. Iako bi se trebali ispitati specifični biomarkeri kako bi se jasno identificirao oksidacijski stres, njegova procjena na temelju analize TAC-a i TOS-a može biti indikativna za rasvjetlanje oksidacijskog statusa i kvalitetu čuvanja uzoraka.
Conclusion
The present results confirm that salivary TOS, TAC and OSI are preserved in samples stored up to 60 days, regardless of temperature. Further research is needed to determine if specific antioxidants are also preserved during this period of storage at both temperatures.

Acknowledgements
This study was supported by the Coordination for the Improvement of Higher Level Education Personnel (CAPES)

Conflict of interest
The authors declare no conflict of interest.

Zaključak
Ovi rezultati potvrđuju da se TOS, TAC i OSI u slini za-

Storage on Saliva Oxidative Status

Sažetak
Cilj: Cilj ovog istraživanja bio je usporediti utjecaj kratkotrajnog skladištenja sline na dvjema različi}-
tim temperatura na stabilnost ukupnog antioksidacijskog kapaciteta (TAC) i ukupnog oksidacijski-
skog statusa (TOS) te na indeks oksidacijskog stresa (OSI). Materijal i metode: Uzorak sline za ispi-
tivanje prikupljen je od 20 zdravih dobrovoljaca. Alikvot je odabran za trenutačnu analizu, a ostatak}
je bio pohranjen 120 dana na temperaturama od -20 °C i -80 °C. Analize su obavljene nakon 15, 30, 45, 60, 90, 120 dana. Određivanje TOS-a i TAC-a provedeno je kolorimetrijskim metodama. Rezulta-
ti: Rezultati pokazuju da se su na obje temperature skladištenja mogli očuvati oksidansi i antioksi-
dansi do 60 dana i da su im, u usporedbi sa svježim uzorcima, razine ostale slične. Pri uspoređivanju
različitih temperatura skladištenja u svakoj vremenskoj točki, nisu uočene statistički značajne razli-
ke. Naposljetu, OSI je ostao konstantan tijekom skladištenja na objema temperaturama, bez sta-
listički značajnih razlika. Rezultati su izraženi kao srednja vrijednost ± standardna pogreška. Stati-
stička analiza obavljena je korelaciono varijance (ANOVA) te Bonferronijevim i t-testom. P-vrijednosti
< 0,05 prihvaćena je kao statistički značajna. Ključne riječi: Objek temperature bile su u stanju održati slič-
ne razine TOS-a i TAC-a u uzorcima svježe sline. Zato su pouzdani za procjenu oksidativnog/oksida-
cijskog stresa do 60 dana.

Sukob interesa
Autori ne navode sukob interesa.

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