Fatty acid composition of palatal tissue from denture stomatitis patients

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Palatal biopsy specimens were obtained from patients with denture stomatitis. The fatty acids were extracted from the tissue, then separated, identified, and quantified by a gas-chromatographic technique. The sensitivity of this method enabled analyses of specimens with a wet weight of less than 1 mg. The concentration of the fatty acids C16:1(n-7) and C24:1(n-9) differed significantly between samples from hyperplastic and clinically healthy tissue in the denture stomatitis patients. By comparing specimens from denture stomatitis patients and non-denture subjects, the concentration of seven fatty acids, two saturated and five unsaturated, was found to be significantly different. A multivariate data-analytical method distinguished between the fatty acid composition in specimens from denture stomatitis patients and from non-denture subjects. □ *Biopsy; denture; gas-chromatography/mass-spectrometry; multivariate data analysis*

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The prevalence of denture stomatitis among denture patients has been reported to vary from 9% to 97% (1–4). The term denture stomatitis describes intraoral inflammatory changes restricted to the mucosa covered by a dental prosthesis (5). On the basis of clinical and histopathologic findings denture stomatitis has been divided into an atrophic and a hyperplastic type (6, 7). It has been suggested that denture stomatitis has a multifactorial etiology. Bacteria (8), yeasts (9), and effects arising from denture materials (10–14) are conceivable factors in the etiology. Lipophilic compounds have been shown to leach in vitro and in vivo from denture base materials based on poly(methyl methacrylate) (PMMA) (13–17). The oral mucosal tissue has a potential for uptake of lipophilic xenobiotics (18, 19). Furthermore, lipophilic compounds from dental materials could be absorbed by the lipid bilayer of cell membranes, which consist of fatty acids, and to give rise to structural changes (20–22). Hence, lipophilic compounds leaching from PMMA denture base materials may be assumed to affect the lipid part of palatal tissue.

The aim of the present study was to detect potential differences in the fatty acid composition of clinically healthy and hyperplastic tissue from patients with denture stomatitis and of palatal tissue from subjects without dentures.

Materials and methods

Fourteen patients, nine denture-wearing patients and five subjects without dentures, participated in this study. The denture patients, three men and six women, had hyperplastic denture stomatitis (6, 7) and were having their upper complete denture renewed at the School of Dentistry, University of Bergen. The average age of these patients was 59.4 years (44 to 73 years), and the average period of denture wearing was 14.2 years (3 to 40 years). The subjects without dentures, one man and four women, had an average age of 33.6 years (16 to 49 years).

Written informed consent was obtained from the patients. The procedures involving the patients were approved by the Regional Committee of Ethics in Medical Research.

Sampling procedure

Tissue samples were obtained with a 3-mm diameter biopsy punch (Stiefel Laboratories SRL, Milan, Italy). Epinephrine-free anesthesia remote from the biopsy site was used. A circular incision was made by pushing the punch about 1 mm into the palatal mucosa with a slight rotary movement. The base was then cut with a scalpel (12d, Martin Surgical Blades, Germany). Two tissue samples were taken from each denture patient: one from a region with clinically healthy tissue and one from sites with hyperplasia. All samples were taken in the left part of the palate, towards the alveolar process. A single tissue sample was taken from each non-denture subject, from a region with clinically healthy tissue. It was not necessary to suture the wounds, and no patient had any post-biopsy complications.

The samples were handled aseptically and washed in distilled water. The underlying visible connective tissue

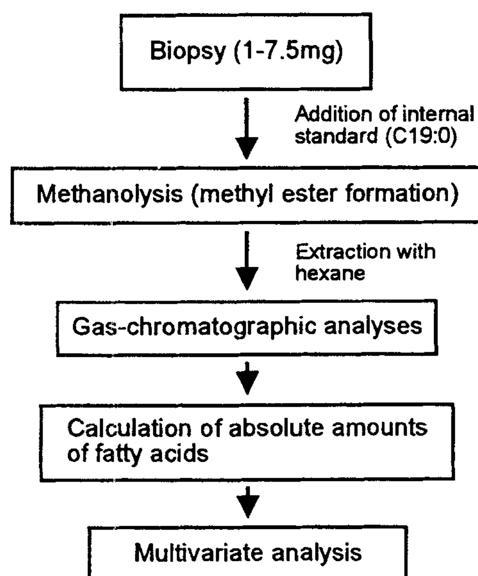

Fig. 1. Procedure for preparation of fatty acid methyl esters from biopsy specimens.

was carefully dissected off, and the samples were weighed (wet weight). The fatty acids were immediately extracted, or samples of tissue were stored at −20°C.

Fatty acid extraction procedure

The fatty acids were extracted from the tissue samples by a method previously used by Grahl-Nielsen & Barnung (23) (Fig. 1). The samples were kept in 0.5 ml 3 mol/l HCl in methanol in 15-ml tubes. One hundred microliters of a solution of C19:0 (nonadecanoic acid, 10 ng/ml) in methanol was added as an internal standard. The samples were methanolyzed at 110°C for 2 h, 1 ml distilled water was added to each tube, and the fatty acid methyl esters were extracted three times with 1 ml of hexane. The samples were evaporated to 100–200 μl under a stream of nitrogen and transferred to sample vials, and the fatty acids subjected to gas-chromatography.

Analytical procedure

Gas-chromatography (GC) and gas-chromatography/mass-spectrometry (GC/MS). A gas-chromatography system (Hewlett-Packard 5890, Avondale, Pa., USA) equipped with a flame-ionization detector and an autosampler (Hewlett-Packard 7673) was used to separate the fatty acids. The fused silica capillary column used (DB 23) was 30 m × 0.32 mm in inside diameter, with 0.25-μm film thickness. The temperature program was 2 min at 60°C, 30°C/min to 145°C, 1 min at 145°C, 2.8°C/min to 220°C, followed by a final hold time of 3 min. Splitless injection was used, and the injector and detector temperature was 300°C. The detector output was coupled to a Multichrom lab-data system for storage and treatment of the chromatograms (VG Data Systems Ltd., Manchester, UK). The areas of peaks were integrated and converted to mass units (micrograms) by way of an internal standard (C19:0), using the same response factors for all fatty acids. Fatty acid methyl esters were identified by comparing the retention time with a standard solution of fatty acid methyl esters (Nu Check Prep Inc., Elysian, Minn., USA). A GC/MS system (Hewlett-Packard 5970 MDS) with an autosampler (Hewlett-Packard 7673) was used to identify unknown peaks.

Presentation of results and statistics

The absolute amount of fatty acids was determined in micrograms per gram tissue. In the nomenclature of fatty acids, the first number indicates number of carbon atoms. The number after the colon indicates number of double bonds, and the position of double bonds is denoted by (n-x), where n is chain length and x is number of carbon atoms from the last double bond to the terminal methyl group.

The number of peaks in chromatograms from tissue samples were counted by the chromatography software, indicating number of compounds in the samples.

The Wilcoxon test and Kruskal–Wallis one-way analysis of variance were used to test for statistical significance. A significance level of 0.05 was chosen. The Spearman rank correlation coefficient (r_s) was used to compute the correlation between data.

Areas of 12 peaks, representing fatty acids, were used for multivariate data analysis by principal-component analysis (PCA). The resulting matrix was analyzed with the multivariate data program SIMCA (24), implemented in the program package SIRIUS (25). The data were normalized by expressing each peak as percentage of the sum of the peaks for each sample. To prevent an undue influence on the total variation in the data matrix of peaks with high recordings, the data were scaled by logarithmization. The dimensionality of the matrix was then reduced to 2, which represented the directions of the largest and second largest variation among the samples in the original 12-dimensional coordinate system. The samples were plotted by projecting them onto the plane constituting the principal components—that is, a $PC_{1,2}$ plot.

Results

The median wet weight of tissue from the healthy palatal areas was 4.55 mg and that from the hyperplastic areas was 3.75 mg in denture stomatitis patients. The median weight of samples from the non-denture subjects was 4.50 mg. These values were not statistically significantly different ($p > 0.4$).

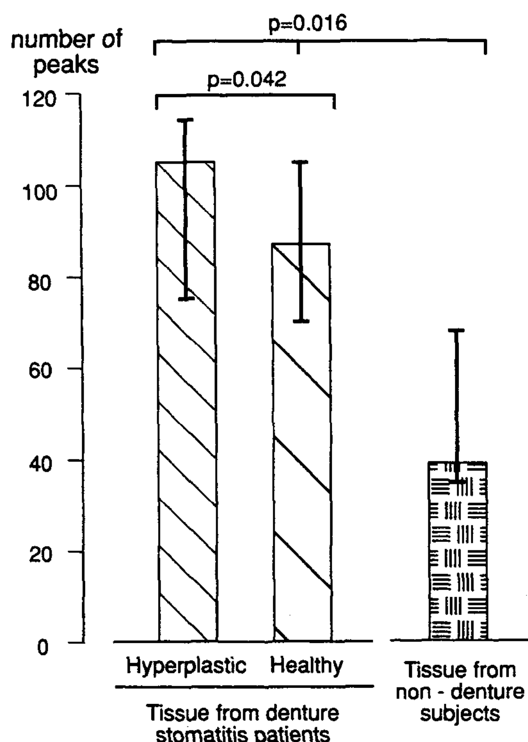

Fig. 2. The total number of peaks in the chromatograms of the tissue from denture stomatitis patients and non-denture subjects.

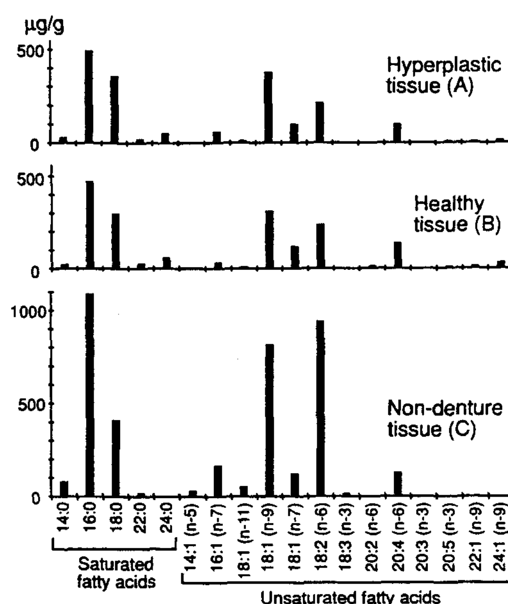

Fig. 3. The concentration of unsaturated and saturated fatty acids from hyperplastic (A) and healthy (B) tissue in denture stomatitis patients and non-denture subjects (C).

The number of peaks, counted from the chromatograms, was highest for the samples taken in hyperplastic areas and lowest for samples from non-denture subjects (Fig. 2).

Correlation analyses between the age of patients (n = 9) and the total amount of fatty acids showed a value of $r_s = -0.325$.

A statistically significant difference was shown between the content of fatty acids from healthy and hyperplastic tissue in denture stomatitis patients with regard to C16:1(n-7) and C24:1(n-9) (Fig. 3A and 3B). Significant differences were recorded for seven fatty acids by comparing the three groups of hyperplastic (Fig. 3A) and healthy tissue (Fig. 3B) from denture stomatitis patients and tissue from non-denture subjects (Fig. 3C). This difference was found for two saturated fatty acids (C14:0, C24:0 and five unsaturated fatty acids (C16:1(n-7), C18:1(n-11), C18:2(n-6), C20:5(n-3), C24:1(n-9)) (p values from 0.01 to 0.049). In this comparison the tissue samples from non-denture-wearing subjects had the highest content of the fatty acids C14:0, C16:1(n-7), C18:1(n-11), and C18:2(n-6) (Fig. 3C).

Tentative MS analyses of two unidentified compounds in chromatograms of healthy and hyperplastic tissue from denture stomatitis patients, with molecular fragments similar to the fatty acids, showed that the content of these substances was highest in the hyperplastic tissue ($p = 0.022$, $p = 0.014$).

Results from the multivariate data analysis using samples from denture stomatitis patients and non-denture subjects showed that the pattern made by 12 fatty acids, 3 saturated and 9 unsaturated, separated the biopsies in 2 classes (Fig. 4). The first principal component (PC_1) accounted for 28.5% of the total variation, and the second (PC_2) accounted for 26.2%.

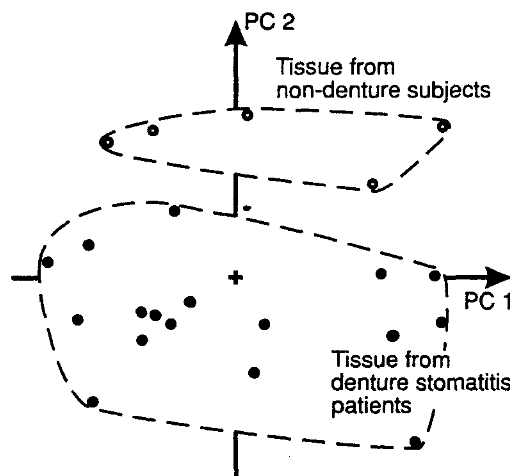

Fig. 4. Principal component (PC) plot of analytical results from denture stomatitis patients and non-denture subjects, on the basis of the first (PC 1) and second principal component (PC 2).

Discussion

Fatty acids are normal constituents of human palatal lipids, and C16:0, C18:0, C18:1, and C18:2 are the dominant fatty acids in healthy tissue (26). The overall fatty acid composition in our study analyzing clinically healthy and hyperplastic palatal tissue from patients with denture stomatatis and healthy tissue from non-denture subjects was in accordance with this previous report.

Biopsy specimens from the denture-wearing patients in our study were taken from the same palatal regions as presented in previous reports from denture stomatitis patients (5, 27). The thickness of our tissue samples was greater than that previously used (5, 28). Care was taken to remove as much connective tissue as possible from the epithelium in the specimens. The effect of residues of connective tissue on total fatty acid composition is minimal, according to Lekholm & Svennerholm (26).

Compared with Lekholm & Svennerholm (26) we have used tissue samples that were one order of magnitude lower in weight. Hence, the analytical sensitivity made it possible to analyze biopsy specimens, not being restricted to autopsy material.

The most commonly applied method for lipid extraction in biologic samples has been the chloroform-methanol method (26, 29, 30). The technique used by us, originally reported by Stoffel et al. (31), was modified to enable rapid analyses of soft-tissue samples (23). The method has previously been used to show the total fatty acid composition of marine soft-tissue specimens in the microgram range (32) and of root substance from individual human teeth in the milligram range (33).

Our study showed an increased level of the unsaturated fatty acids C16:1(n-7) and C24:1(n-9) in the hyperplastic palatal tissue compared with the clinically healthy tissue in specimens from denture stomatitis patients. Several etiologic factors have been suggested in the development of hyperplastic denture stomatitis (9, 34–37). Among them, compounds leaching from the dentures have been considered. Additives (dibutyl phthalate, benzoyl peroxide) have been shown to cause epithelial hyperplasia (38, 39). Moreover, decomposition products from benzoyl peroxide (phenyl benzoate, biphenyl) leach from denture base materials (16, 17, 40). The finding of significantly higher content of seven fatty acids in specimens from non-denture subjects than from denture stomatitis patients (Figs. 3A, 3B, 3C) was in accordance with previous reports. Hence, a significantly higher content of the fatty acids C16:1 and C18:1 has been demonstrated in healthy oral epithelium than in malignant oral epithelium. A higher content of C14:0 was found, although not significant (30). In our study C18:2(n-6), an essential fatty acid, had a lower concentration in specimens from denture stomatitis patients than in tissue from non-denture subjects. This finding may possibly be related to the high content of C24:1(n-9), because an increase of (n-9) fatty acids tends to reflect essential fatty acid deficiency (41). Moreover, essential fatty acids have an indispensable structural function as integral parts of biomembrane phospholipids by regulating the membrane-associated cell functions, such as the enzyme activity (42).

Assuming that the age of the patients does not influence the concentration of total fatty acids ($r_s = -0.325$), we have shown differences in the fatty acid composition by comparing palatal tissue from denture stomatitis patients and non-denture subjects.

Interpretation of the analytical results with regard to the fatty acid composition in healthy and diseased tissue has generally been based on the difference between individual fatty acids (30, 33, 43–45). The multivariate data analysis used here (23, 32) enables us to evaluate the total fatty acid composition of the tissue samples. The pattern in the chromatograms of 12 fatty acids distinguished between specimens from denture stomatitis patients and non-denture subjects (Fig. 4). This method has previously has been used by Kvalheim et al. (46) for data analysis in environmental pollution studies.

In conclusion, the present report has shown that the total fatty acid composition of palatal specimens from humans may be analyzed with a GC technique. Multivariate statistical analyses show differences in the fatty acid composition between non-denture subjects and denture stomatitis patients. Furthermore, small variations in the fatty acid pattern from hyperplastic and clinically healthy tissue in denture stomatitis patients were also detected. The differences may indicate changes in the cell membrane structure and/or fatty acid metabolism—that is, enzyme activities of palatal tissue.

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References

1. Budtz-Jørgensen E, Stenderup A, Grabowski M. An epidemiologic study of yeasts in elderly denture wearers. Community Dent Oral Epidemiol 1975;3:115–9.
2. Manderson RD, Ettinger RL. Dental status of the institutionalised elderly population of Edinburgh. Community Dent Oral Epidemiol 1975;3:100–7.
3. Smith JM, Sheiham A. How dental conditions handicap the elderly. Community Dent Oral Epidemiol 1979;7:305–10.
4. Pindborg JJ, Glenert U, Andreason JO. Mundslimhindens tilstand hos 478 beboere i De Gamles by. Tandlaegebladet 1985;89:223–7.
5. Jennings KJ, MacDonald DG. Histological, microbiological and haematological investigations in denture-induced stomatitis. J Dent 1990;18:102–6.
6. Bergendal T, Isacsson G. A combined clinical, mycological and

histological study of denture stomatitis. Acta Odontol Scand 1983;41:33–44.
7. Morimoto K, Kihara A, Suetsugu T. Clinico-pathological study on denture stomatitis. J Oral Rehab 1987;14:513–22.
8. Theilade E, Budtz-Jørgensen E. Predominant cultivable microflora of plaque on removable dentures in patients with denture-induced stomatitis. Oral Microbiol Immunol 1988;3:8–13.
9. Budtz-Jørgensen E. The significance of Candida albicans in denture sore mouth. Scand J Dent Res 1974;82:151.
10. Crissey JT. Stomatitis, dermatitis, and denture materials. Arch Dermatol 1965;92:45–9.
11. Rossbach A. Ein beitrag zur Ätiologie der Prothesenstomatopathien. Dtsch Zahnaerztl Z 1975;30:148–9.
12. Guinta J, Zablotsky N. Allergic stomatitis caused by self-polymerizing resin. Oral Surg 1976;41:631–7.
13. Koda T, Tsuchiya H, Yamauchi M, Hoshino Y, Takagi N, Kawano J. High performance liquid chromatographic estimation of eluates from denture base polymers. J Dent 1989;17:84–9.
14. Koda T, Tsuchiya H, Yamauchi M, Ohtani S, Takagi N, Kawago J. Leachability of denture-base acrylic resins in artificial saliva. Dent Mater 1990;6:13–6.
15. Stafford GD, Brooks SC. The loss of residual monomer from acrylic orthodontic resins. Dent Mater 1985;135–8.
16. Lygre H, Solheim E, Gjerdet NR, Berg E. Leaching of organic additives from dentures in vivo. Acta Odontol Scand 1993;51:45–51.
17. Lygre H, Klepp KN, Solheim E, Gjerdet NR. Leaching of additives and degradation products from cold-cured orthodontic resins. Acta Odontol Scand 1994;52:150–6.
18. Siegel IA, Izutsu KT. Permeability of oral mucosa to organic compounds. J Dent Res 1980;10:1604–5.
19. Pink DA, Foong W-C, Jones D, Gates KA, Mezei M, Farrell K. Plasticizer permeation across mucosal tissue: an experimental and theoretical study. J Appl Biomat 1991;2:41–8.
20. Fujisawa S, Kadoma Y, Masuhara E. A calorimetric study of the interaction of synthetic liposomes with vinyl monomers, acrylates and methacrylates. J Biomed Mater Res 1984;18:1105–14.
21. Fujisawa S, Kadoma Y, Masuhara E. A calorimetric study of the interaction of synthetic phospholipid liposomes with lipid-soluble small molecules used as dental materials and devices. J Biomed Mater Res 1987;21:89–98.
22. Fujisawa S, Kadoma Y, Kodoma Y. 1H and 13C NMR studies of the interaction of eugenol, phenol, and triethyleneglycol dimethacrylate with phospholipid liposomes as a model system for odontoblast membranes. J Dent Res 1988;67:1438–41.
23. Grahl-Nielsen O, Barnung T. Variations in the fatty acid profile of marine animals caused by environmental and developmental changes. Mar Environ Res 1985;17:218–21.
24. Wold S, Sjöström M. SIMCA: a method for analyzing chemical data in terms of similarity and analogy. Am Chem Soc Symp Ser 1977;52:243–82.
25. Kvalheim OM, Karstang TV. A general-purpose program for multivariate data analysis. Chemometrics Int Lab Systems 1987; 2:235–7.
26. Lekholm U, Svennerholm L. Lipid pattern and fatty acid composition of human palatal oral epthelium. Scand J Dent Res 1977;85:279–90.
27. Sharma AK, Mirza FD. Palatal mucosa under dentures: a qualitative histologic and histochemical analysis. J Prosthet Dent 1986;56:574–82.
28. Watson IB, MacDonald DG. Oral mucosa and complete dentures. J Prosthet Dent 1982;47:133–40.
29. Folch J, Less M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 1957;226:497–509.
30. Macleod RI, Cassidy D, Laker MF, Soames JV. Fatty acid composition of clinically healthy and malignant oral epithelium. J Oral Pathol Med 1990;19:149–51.
31. Stoffel W, Chu F, Ahrens EH. Analysis of long chain fatty acids by gas chromatography. Micromethod for preparation of methyl esters. Anal Chem 1959;162:307–8.
32. Ulvund KA, Grahl-Nielsen O. Fatty acid composition in eggs of Atlantic cod (Gadus morhua). Can J Fish Aquat Sci 1988;45:898–901.
33. Lygre H, Solheim E, Gjerdet NR, Skaug N. Fatty acids of healthy and periodontally diseased root substance in human teeth. J Dent Res 1992;71:43–6.
34. Bastiaan RJ. Denture sore mouth. Aetiological aspects and treatment. Aust Dent J 1976;21:375–82.
35. Arendorf TM, Walker DM. Oral candidal populations in health and disease. Br Dent J 1979;147:267–72.
36. Bergendal T. Treatment of denture stomatitis (thesis). Stockholm: University of Stockholm, 1982.
37. Budtz-Jørgensen E, Theilade E, Theilade J. Quantitative relationship between yeasts and bacteria in denture induced stomatitis. Scand J Dent Res 1983;91:134–42.
38. Slaga TJ, Klein-Szanto AJP, Troplett LL, Yotti LP. Skin tumor-promoting activity of benzoyl peroxide, a widely used free radical-generating compound. Science 1981;213:1923–4.
39. Harsanyi BB, Foong WC, Howell RE, Hidi P, Jones DW. Hamster cheek-pouch testing of dental soft polymers. J Dent Res 1991;70: 991–6.
40. Lygre H, Solheim E, Gjerdet NR. Leaching from denture base materials in vitro. Acta Odontol Scand 1995;53:75–80.
41. Levy E, Thibault L, Garafalo C, Messier M, Lepage G, Ronco N, et al. Combined (n-3 and n-6) essential fatty acid deficiency is a potent modulator of plasma lipids, lipoprotein composition, and lipolytic enzymes. J Lipid Res 1990;31:2009–17.
42. Hagve TA. Effect of polyunsaturated fatty acids on membrane functions (invited review). Scand Clin Lab Invest 1988;48:381–8.
43. Mimata H, Kawano S, Ogata J, Takeshita M, Shimada T. Lipid composition of the plasma membrane isolated from normal and precancerous rat bladder epithelium. Urol Res 1987;15:345–8.
44. Nishida T, Hiwa H, Shigematsu A, Yamamoto M, Iida M. Increased arachidonic acid composition of phospholipids in colonic mucosa from patients with active ulcerative colitis. Gut 1987;28:1002–7.46.
45. Pacheco S, Hillier K, Smith C. Increased arachidonic acid levels in phospholipids of human colonic mucosa in inflammatory bowel disease. Clin Sci 1987;73:361–4.
46. Kvalheim OM, Øygard K, Grahl-Nielsen O. Simca multivariate data analysis of the blue mussel components in environmental pollution studies. Anal Chem Acta 1983;150:145–52.

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