The Tyrolean Iceman and other Mummies: Comparison of Tissue Samples by Chemometric Methods

Varmuza K. [1*], ***Mader R.*** [2], ***Werther W.*** [1]
Makristathis.A. [3], ***Schwarzmeier J.*** [2], ***Seidler H.*** [4]

Vienna University of Technology (1)
Laboratory for Chemometrics, Institute of Food Chemistry
A-1060 Vienna, Getreidemarkt 9/160, Austria

University of Vienna (2)
Department of Internal Medicine I
A-1090 Vienna, Währinger Gürtel 18-20, Austria

University of Vienna (3)
Department of Clinical Microbiology, Hygiene Institute
A-1090 Vienna, Währinger Gürtel 18-20, Austria

University of Vienna (4)
Institute of Human Biology
A-1090 Vienna, Althanstrasse 14, Austria

* Correspondence author; email: kvarmuza@email.tuwien.ac.at; fax: +43-1-58801-16091

Poster Presentation at **CHEMOMETRICS V**
29 August - 1 September 1999, **BRNO**, Czech Republic

Abstract

In September 1991 a well conserved frozen mummy of a Late Neolithic man has been discovered in a glacial field near the Austrian-Italian border. This approximately five thousand year old *Tyrolean Iceman* (nicknamed *Ötzi* because found in the Ötztaler Alps) has been the subject of several studies [1].

The body of Ötzi exhibits a remarkable preservation. Aim of this work was to characterize tissue samples by the concentrations of fatty acids and to compare the results with those obtained from other well preserved mummies. Also data obtained from samples of fresh corpses have been considered.

Tissue samples were treated by standard procedures to obtain the methyl esters of fatty acids [2]. Quantitative analysis was performed by GC; compounds were identified by GC/MS, followed by spectral library search. The concentrations of 16 fatty acids have been used to characterize the samples.

PCA, *k*-nearest neighbor classification, and other chemometric methods clearly indicate the different types of conservation and degradation in the investigated samples from mummies found in Austrian glaciers, in Peru and Siberia. The Ötzi samples constitute a separate cluster close to other glacier specimens.

[1] Spindler, K.; Wilfing, H.; Rastbichler-Zissernig, E.; zur Nedden, D.; Nothdurfter, H. (eds.): *Human mummies: a global survey of their status and the techniques of conservation.* Springer, Wien (1996).

[2] Makristathis, A.; Mader, R.; Varmuza, K.; Simonitsch, I.; Schwarzmeier, J.; Seidler, H.; Platzer, W.; Unterndorfer, H.; Scheithauer, R.: in [1], p.279 (1996).

Acknowledgment. The authors thank I. Simonitsch (Department of Clinical Pathology, University of Vienna), W. Platzer (Institute of Anatomy, University of Innsbruck), H. Unterndorfer and R. Scheithauer (Institute of Forensic Medicine, University of Innsbruck) for providing samples.

Introduction

In September 1991 a frozen mummy of a Late Neolithic man has been discovered in a glacial field near the Austrian-Italian border.

The body of this *Tyrolean Iceman*
- nicknamed *Ötzi* because found in the Ötztaler Alps -
exhibits a remarkable preservation.

Condition of this corpse can be described as follows:

When Ötzi died he was approximately 45 years old and 160 cm tall; he suffered from some nasty diseases (artery sclerosis, arthritis, intestinal infection by worm eggs).

Mountain winds lead to mummification through desiccation; the epidermis was tanned and thereby protected the tissue underneath.

The tissue is macroscopically well preserved with little formation of *adipocere* (conversion of body fat into a lipid mixture of wax-like consistency and gray-white color).

Samples were available also from mummies that have been found on glaciers, in a mountain lake, in permafrost ground, on mountains at high altitudes, and in a desert. It is expected that the different storage conditions are reflected in the concentration profiles of fatty acids.

Aim of this Work

Tissue samples from Ötzi, other mummies, and a fresh corpse have been characterized by the concentrations of fatty acids.

Standard multivariate data analysis methods have been applied to these data to investigate the different types of conservation, degradation and origin.

Samples / Data Generation

Samples

origin (storing conditions)	*altitude*	*burial time*	*no of samples*	*specimen*
Tyrolean Iceman, Ötztaler Alps, Italy (in ice and melting ice/snow)	3200 m	5000 y	4	skin, nose
Glacier bodies, Tyrolean glaciers, Austria (in ice and water, found uncovered)	2800 m 2700 m	29 y 57 y	9	skin, muscle, lung, liver
Lake Achensee, Tyrol, Austria (found in 50 m depth)		50 y	3	muscle, lung
Permafrost zone, Altai mountains, Siberia (one enclosed in ice, other in soil)	2500 m	2200 y 2500 y	2	skin
High altitude, Mount Ampato, Peru (dry frozen by winds, permanent frost)	6000 m	500 y	2	skin, hair
Desert, Ilo, Peru (dry mummified, no rain in last 1000 y)		1000 y	1	muscle
Fresh corpse as reference			7	skin, muscle, lung, liver, bone marrow
sum			28	

Chemical analysis

Lipid material was saponified and free fatty acids were converted to their methyl esters. Qualitative analysis by GC/MS; quantitative analysis by GC. Evaluation of GC/MS data by spectral library search.

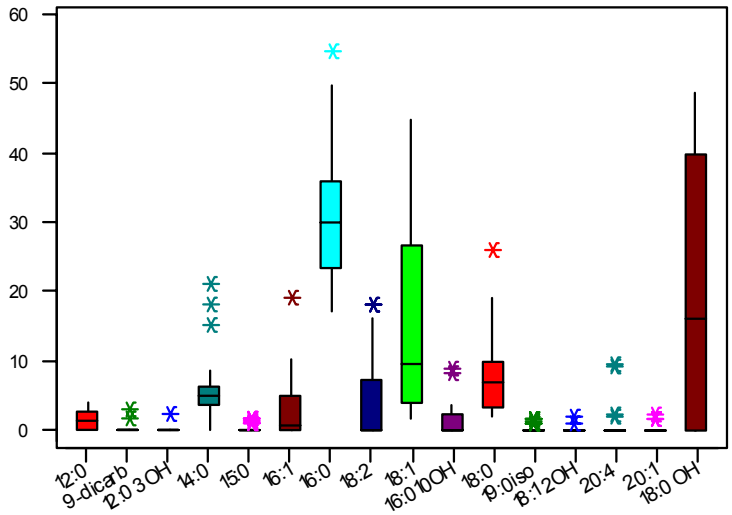
Data selection and preprocessing

From the experimental data **16 most prominent fatty acids** have been selected. Concentrations were normalized to equal sum of the 16 fatty acids.

id	*code*	*name*	*id*	*code*	*name*
1	12:0	lauric acid	9	18:1	oleic acid
2	9-dicarb	acelaic acid	10	16:0 10 OH	10-hydroxy palmitic acid
3	12:0 3 OH	3-hydroxy lauric acid	11	18:0	stearic acid
4	14:0	myristic acid	12	19:0 iso	nonadecanoic acid
5	15:0	pentadecanoic acid	13	18:1 2 OH	2-hydroxy oleic acid
6	16:1	palmitoleic acid	14	20:4	arachidonic acid
7	16:0	palmitic acid	15	20:1	vaccinic acid
8	18:2	linoleic acid	16	18:0 10 OH	10-hydroxy stearic acid

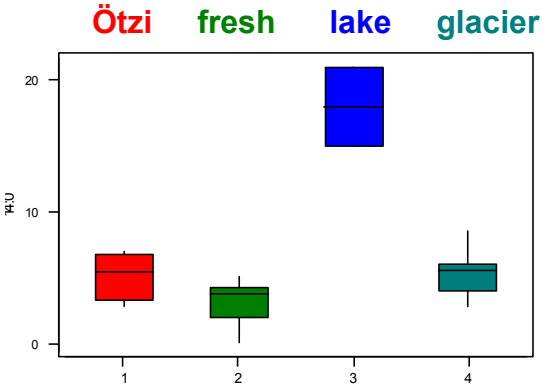
Data Evaluation 1

Univariate Data Analysis

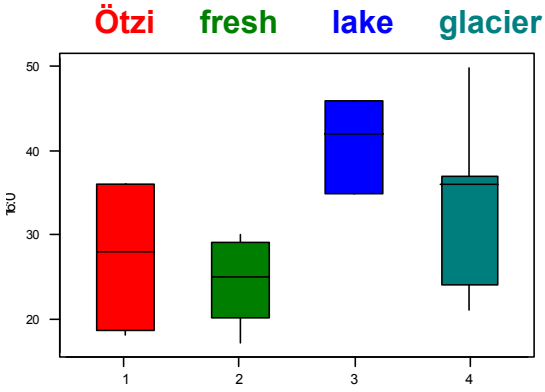


(a) All data

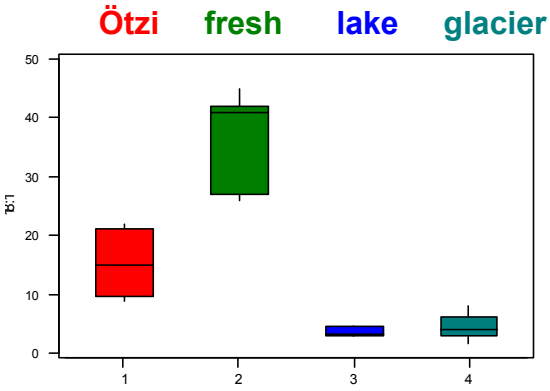
(b) Origin classes



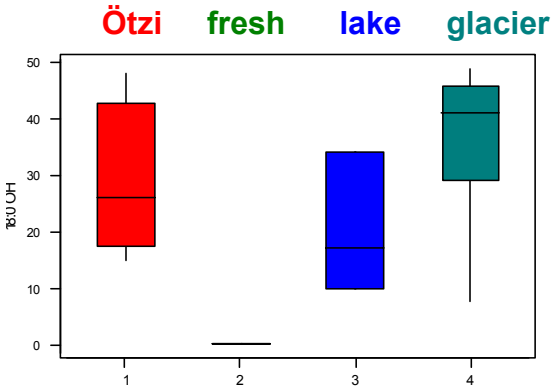
myristic acid **14:0** (id 4)



palmitic acid **16:0** (id 7)



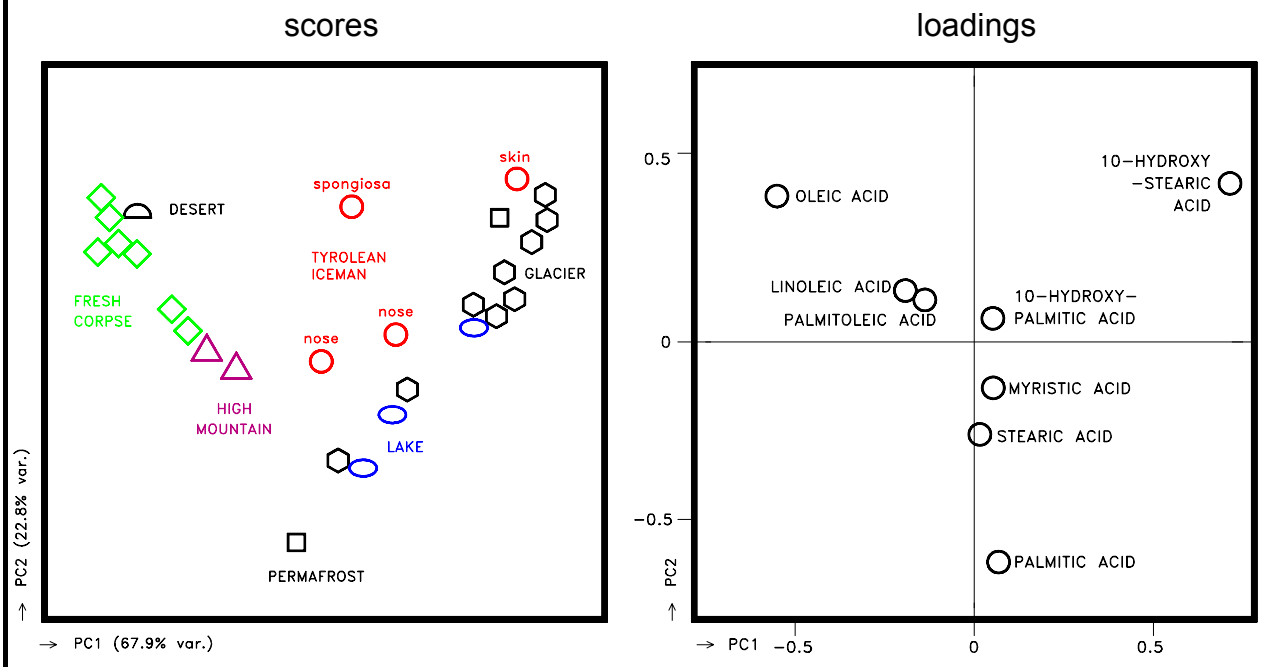
oleic acid **18:1** (id 9)



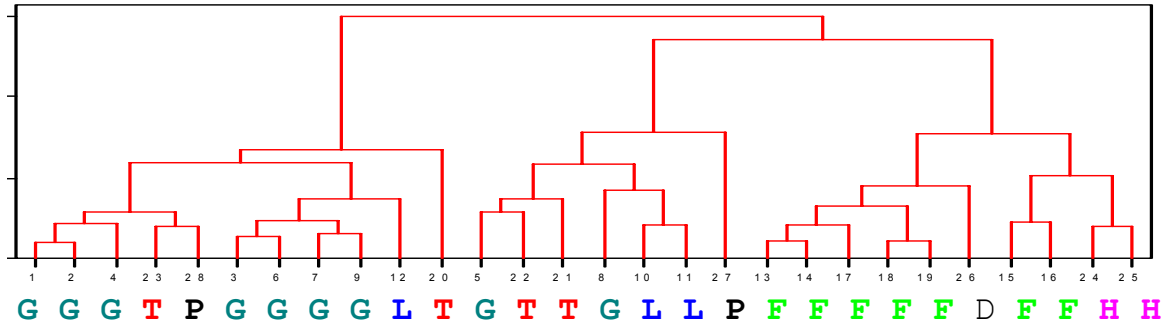
10-hydroxy stearic acid **18:0 10 OH** (id 16)

Data Evaluation 2

PCA



HCA



D: Desert F: Fresh G: Glacier H: High mount.
L: Lake P: Permafrost T: Tyrolean Iceman

KNN

id	***Tyrolean Iceman***	***5 nearest neighbors*** *(excluding Tyrolean Iceman)*
20	bone (spongiosa)	permafrost, glacier, glacier, glacier, glacier
21	nose (cavum nasi)	glacier, high mountain, high mountain, lake, fresh
22	nose (sinus maxilaris)	glacier, glacier, glacier, lake, lake
23	skin	permafrost, glacier, glacier, glacier, glacier

Conclusions

Characteristic fatty acids

glacier	10-hydroxy stearic acid
lake	myristic acid, palmitic acid
Ötzi	palmitic acid, 10-hydroxy stearic acid, unsaturated fatty acids
fresh reference	oleic acid, unsaturated fatty acids

Multivariate methods

PCA, HCA, and KNN gave consistent results.

Fresh reference samples constitute the most compact cluster.

Concentration patterns of fatty acids are much more dependent from environmental conditions than from the origin of samples.

The two samples from permafrost sites (Altai) are very different. This corresponds to the different storage conditions (ice versus soil).

The desert sample is very similar to the fresh reference samples.

Summary

- ☞ **Environmental storage conditions are well reflected in the fatty acid concentration patterns.**
- ☞ **Classification of environmental and storage conditions is possible from concentration data of selected fatty acids by the application of multivariate methods.**
- ☞ **The 5000 year old Tyrolean Iceman is better conserved than corpses buried in glaciers for a considerable shorter time. This can be explained by an initial desiccation of the body.**

Software: SCAN 1.1 (1995)