Comparison Between Traditional and Pattern Recognition (SIMCA) Strategies in Classification of Old Proteinaceous Binders

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Abstract

An application of multivariate analysis to the characterisation of proteinaceous binders in the field of cultural heritage is provided. We compared identification results of protein binders obtained with three classical strategies and a more robust pattern recognition technique as SIMCA. A collection of natural binders prepared according to old recipes was used as reference material (training set) to develop SIMCA models at both class and subclass level. The amino acid profile data was used to classify the origin of binders present in 11 samples (evaluation set) coming from mural and easel paintings, manuscripts and polychrome sculptures from the 15th to 18th century. Results obtained are compared with those obtained using classic strategies.

Introduction

Historically, three types of proteinaceous matter -casein, egg and animal glue- were used to disperse and fix pigments. For different reasons, the identification of binders and organic matter in old works of art in general is a not completely solved issue. This work aims to improve the knowledge of proteinaceous binding media in old works of art and to distinguish them according to their origin. Strategies based on extracting the underlying information in the amino acidic profile of samples are the most reliable. All strategies -Amino acid ratios (*Aa ratios*), two-dimensional amino acid ratio plots (*2D-Plot*), and statistical correlation factor (*SCF*)- use reference proteinaceous standards for to perform identification [1]. We carried out a comparison between these traditional strategies and a pattern recognition technique, SIMCA (Soft Modelling Analogy Class Analysis) [2]. SIMCA results showed a more reliable and less subjective identification (knowing the probability of success). It also made it possible to approach identification on a subclass level (albumin: whole egg, yolk or white; casein: goat, cow or sheep; and collagen: mammalian or fish).

Materials & Methods

Samples. For this study, a natural reference material collection of 151 samples traditionally employed (albumin, casein and collagen-like substances) was prepared according to historical treatises [3]. This collection has variability inherent in different animal species and sample origins. The collection was used as the calibration standard (training set) to perform the protein material identification [4]. Eleven samples (Table 1) belonging to different manuscripts, murals and easel paintings and polychrome sculptures (15^{th} - 18^{th} C.) were used in this study (evaluation set).

Sample treatment. We used a sample treatment procedure described previously [4]. Samples containing protein were extracted in a phosphate buffer solution, purified by removing inert, micro molecular and inorganic charges, hydrolysed, derivatised by Phenylisothiocyanate (PITC), and finally analysed by HPLC in order to determine the amino acid profile. Training set samples were used to define SIMCA class models and then evaluation set samples adjusted to these models.

Results

Eleven samples from cultural heritage works of art were analysed. Using their profile of amino acids along with the SIMCA pattern recognition technique, we identified the origin of the

proteinaceous binders present. Table 1 shows the comparison of the results obtained by SIMCA and other identification strategies mentioned above.

Table 1. Sample origin and protein binder identification according to the four considered identification strategies.

nº	*Samples*	*Aa ratios*	*2D-Plot*	*SCF*	*SIMCA (P>95-99 %)*
1	Manuscript XV C., Catholic king's diploma*, ARCG	K	E	-	None
2	Manuscript XV C., ARCG	K	K, E	-	None
3	Manuscript XVI C., ARCG	K	C	-	None
4	Manuscript XVI C., ARCG	C	C	C	None
5	Manuscript XVI C., ARCG	C	C	C	C, F
6	Easel painting canvas XVIII C. *"Madonna with child"* (unknown) Cathedral of Guadix (Granada)	C	C	C	C, M
7	Polychrome sculpture XVII C. *"S. Sebastian"* Church of Villanueva de Mesía. (Granada)	C	C	C	C, F
8	Easel painting XVIII C. "E*xaltation of Eucharist"* (unknown) Cathedral of Guadix (Granada)	C	C	C	C, F
9	Easel painting 16th - XVII C. "Crucified Christ" (unknown)	C	C	C	C, F
10	Easel painting XVIII C. "Ascent to Calvary's Mount" (unknown) Cath. of Guadix (Granada)	C	C	C	C, M
11	Mural painting XVI C. Santa Paula's Palace Hotel (Granada). Old Convent of Sta. Paula.	C	C	C	C, F

ARCG: Archive of Royal Chancery of Granada. K=Casein, C=collagen, E=Egg, F=Fish collagen, M=Mammalian collagen., *authenticated by the king's "rueda" (device in the form of two concentric circles containing a legend, derived from the rota of the papal chancery, used in Spain for the authentication of classes of beneficial document)

In samples 1 to 3, the classic strategies are non-conclusive. In samples 1 and 2, the *Aa ratios* indicates casein, the *2D plot* suggests egg for the former, and casein and egg for the latter, and the *SCF* makes no conclusion, whereas *SIMCA* conclusively indicates the absence of any considered protein binder. In sample 3, the *Aa ratio* suggests casein, the *2D-plot* collagen, the *SCF* makes no conclusion and *SIMCA* confirms the absence of protein. In sample 4, all three classic strategies conclude the presence of collagen, while *SIMCA* does not find any protein under study (P>95-99 %). The rest of the identified samples (5-11) coincides with the results obtained using *SIMCA*, but SIMCA does offer more information related to the origin of the collagen (mammalian or fish).

Conclusions

The use of the SIMCA pattern recognition technique with the amino acid profile of proteinaceous binders makes it possible to classify the proteinaceous binder used in cultural heritage samples with a good acceptance probability compared with the other more subjective procedures commonly used to classify the kind of binder. Protein aging effect will be considered in next work.

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