CHARACTERIZATION AND DISCRIMINATION OF WHEATS AND FLOURS BY DART-HRMS

BRUNELLA MIANO, ROBERTO PIRO

Istituto Zooprofilattico Sperimentale delle Venezie - Laboratorio di Chimica Sperimentale, Vicenza (VI), Italy

Email address: bmiano@izsvenezie.it

INTRODUCTION

Due to favorable climate condition, Italy is a prominent producer of different wheat varieties. There are huge quantities of wheat by-products, but the most typical Italian food are made of durum and soft wheat flour, like pasta, pizza, bread, biscuits and other bakery products.
Because of the great importance of wheat in Italian food market, authenticity represents an essential quality parameter not only for the producers and regulatory bodies, but also for consumer; one of the most common fraudulent procedure is the soft wheat flour addition to durum flour.

The aims of our study is to test the effectiveness of a new unconventional non-targeted method for a characterization of durum and soft wheat varieties, and to identify small amount of soft wheat in durum wheat flour.

MATERIALS and METHODS

ANALYTICAL METHOD FOR SAMPLE PREPARATION

For the first purpose, a set of 12 wheat cultivars coming from 7 varieties are analyzed: *Triticum aestivum* (soft wheat) , *T. turgidum, T. durum, T. hordeiforme* (durum wheat), *T. dicoccum, T. spelta, T. monococcum* (spelts). Moreover ten durum and ten soft commercial wheat flour are used too and, for the second purpose, mixture at different percentage of durum and soft wheat flours were analyzed .

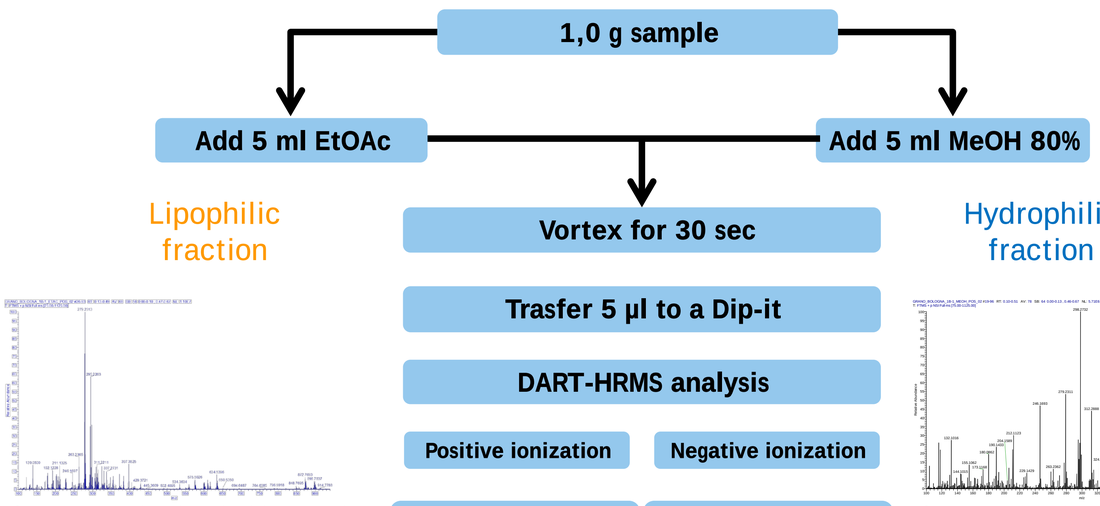

Fig.2 Lipophilic fraction of *Triticum aestivum*, Blasco variety, positive ionization mode.

Fig.3 Hydrophilic fraction of *Triticum aestivum*, Blasco variety, positive ionization mode.

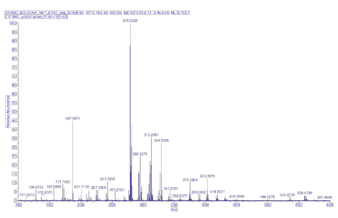

Fig.4 Lipophilic fraction of *Triticum aestivum*, Blasco variety, negative ionization mode.

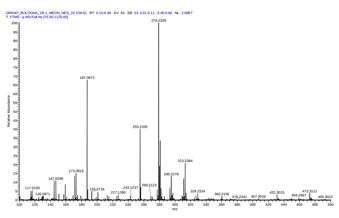

Fig.5 Hydrophilic fraction of *Triticum aestivum*, Blasco variety, negative ionization mode.

INSTRUMENTAL CONDITIONS

The analysis are made with an high resolution mass spectrometer with a DART (Direct Analysis in Real Time,fig.1) ion source and an Orbitrap mass analyzer (Thermo Exactive +). The instrumental condition are showed in the table 1.

Fig.1 DART ion source with ORBITRAP inlet

DART	
Temperature	350°C EtOAc/220°C MeOH
Grid Voltage	250V
Gas	Helium
Polarity	Positive
Speed	0.3 mm/s
Dopant	Ammonium 25%

ORBITRAP	
Polarity	Positive/Negative
Mass Range	From 75 to 1125 m/z
Time analysis	0.66 min
Resolution	70.000 FWHM
AGC	3e6
Capillary Temperature	250 °C
Slens-RF	55
CID	0 eV

Tab.1 Instrumental conditions used for DART ion source analysis and Orbitrap Mass analyzer

RESULTS

Target evaluation with mMass revealed the presence of several compounds. Hydrophilic fraction spectra (fig. 3,5): contain some aminoacids the strongest signals belong to Lysine, Proline, Serine, Leucine.
Lipophilic extract (fig. 2,4) mainly contains free fatty acids (linoleic, oleic, palmitic and linolenic acids), and the corresponding monoglycerides, diglycerides and triglycerides.
Target analysis shows also the presence of other specific compounds (fig.3), like dextrin and isomaltol as starch degradation products, phytosterols as β sitosterol and some alkylresorcinols.

CHEMOMETRIC EVALUATION

All experimental data were processed with MetaboAnalyst, a web application which provides many treatment and normalization procedures, to perform a classification of wheats and flours and to identify the most important discriminating signals. The main results are showed in the figures.

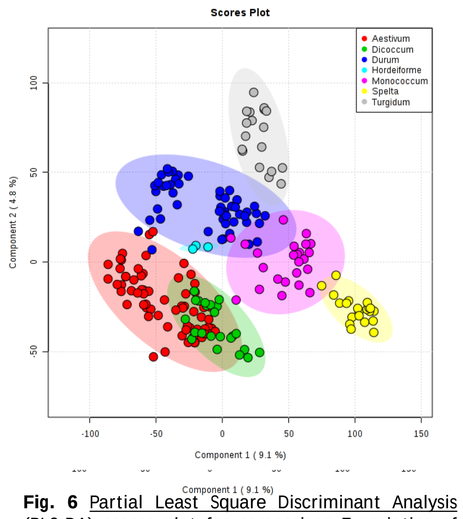

Fig. 6 Partial Least Square Discriminant Analysis (PLS-DA) scores plot for comparison 7 varieties of different cultivars: *Triticum aestivum, T. turgidum, T. durum, T. monococcum, T. dicoccum, T. spelta, T. durum hordeiforme.*

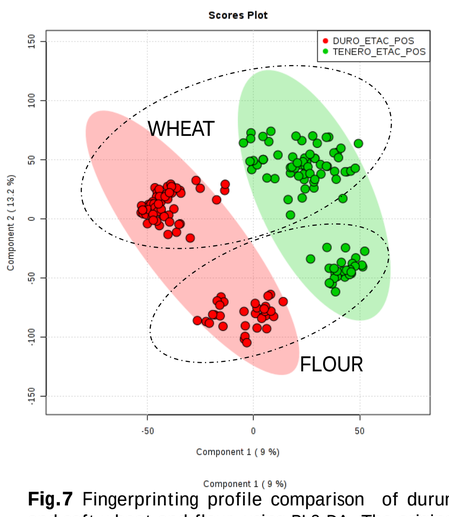

Fig.7 Fingerprinting profile comparison of durum and soft wheat and flour, using PLS-DA. The original data were normalized by sum, treated with log function and Pareto data scaling .

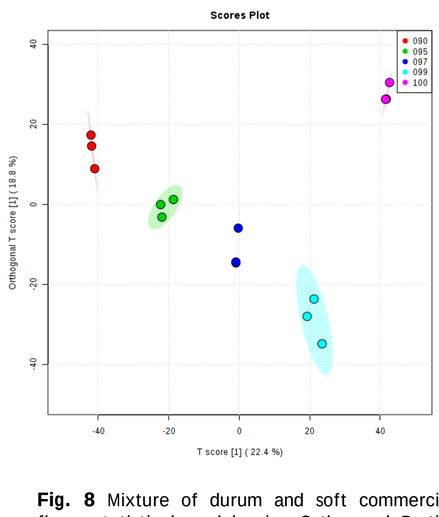

Fig. 8 Mixture of durum and soft commercial flours statistical model using Orthogonal Partial Least Squares Discriminant Analysis (oPLS-DA): 90%durum-10%soft, 95%durum-5%soft, 97%durum-3%soft, 99%durum-1%soft, 100%durum.

	Aest	Dicoc	Durum	Hord	Monoc	Spelta	Turgid	Class Error
Aest	68							-
Dicoc		23						-
Durum			50				1	0.02
Hord				3				-
Monoc					24			-
Spelta						21		-
Turgid			3				15	0.17

Tab. 2 Matrix error with error for the 7 varieties of cultivars classification.

The PLS-DA scores plot (fig. 6) and the matrix error (tab. 2) show a good accuracy in the statistic classification: there are overlapping between same of variety but different subspecies.
Durum and soft wheat comparison (fig. 7) reveals differences between unrefined wheat (raw) and flour, to underline the importance of sample texture.
The oPLS-DA (fig. 8) of mixture of soft and durum commercial flours shows difference signals, some of them are presented in fig. 9 and 10.

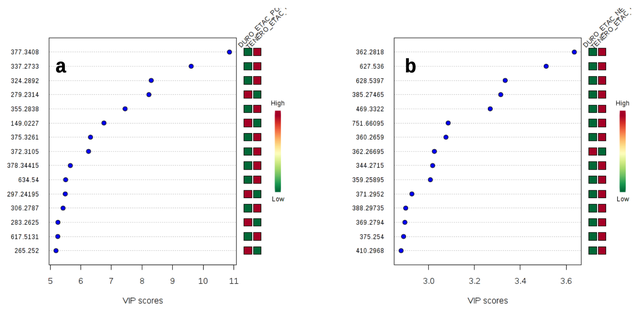

Fig. 9 Variable importance in projection (VIP) using PLS-DA of durum and soft commercial flours, lipophilic fraction, positive (a), and negative (b) ionization mode. Most important signal belongs to 5-(n-Nonadecyl)-resorcinol (377.2408m/z $[M+H]^+$), di-homo-γ-linolenic acid (324.2892m/z $[M+NH_4]^+$), linolenic acid (279.2314m/z $[M+H]^+$), linoleoyl-glycerol (355.2838m/z $[M+H]^+$), octadecenoyl-eicosadienoyl glycerol (627.5360m/z $[M-H_2O-H]^-$), docosapentaenoyl glycerol (385.2746m/z $[M-H_2O-H]^-$), glycyrrhetinic acid (m/z 469.3322m/z $[M-H]^-$)

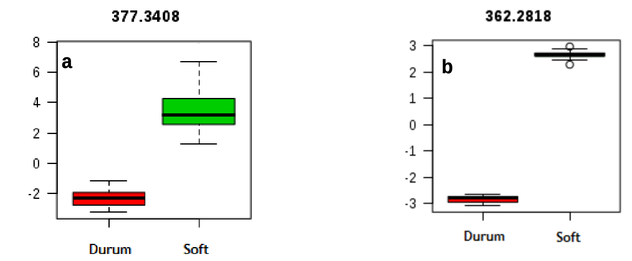

Fig. 10 Box plot of some variables important for discriminate durum and soft wheat flours, using PLS-DA: a) 5-(n-Nonadecyl)-resorcinol with 377.2408 m/z in positive ionization mode, and b) Unknown compound at 362.2818 m/z in negative ionization mode.

CONCLUSIONS

1. **Fast analytical procedure, with a minimum sample preparation;**
2. **DART-HRMS spectra contains all information needed, and representing a great tool to identify nutritional factors and wheat and flour properties;**
3. **Chemometric treatment discriminate different wheat varieties, and reveal low percentage of one flour to the other;**
4. **DART-HRMS screening is a rapid and reliable tool for flours fraud detection;**
5. **We are studying further improvement and standardization of presented method.**

REFERENCE

J.Hajslova, T. Cajka, L. Vaclavik - Challenging application offered by direct analysis in real time (DART) in food quality and safety analysis, TrAC Trends in Analytical Chemistry, Vol 30, issue 2, pages 204-218 , 2011

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