Effects of time and temperature of storage on chemical and nutritional characteristics of raw milk for Provolone Valpadana PDO cheesemaking: a multivariate approach

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SUPPLEMENTARY FILE

Materials & Methods

Analytical determinations

Organic acids were extracted according to Park et al. (2006) with some changes: 5 g of milk samples were extracted with 25 mL of H_3PO_4 0.5%, mixed for 30 min on a shaker at 300 rpm, ultracentrifuged (at 4°C, 20.000 rpm for 15 min) and filtered on a 0.2 μm PVDF membrane filter (Phenomenex Inc., Torrance, CA, USA). Organic acid separation and identification were performed by reversed phase-ultra high performance liquid chromatography (RP-UHPLC) through a Nexera UHPLC system (Shimadzu Corporation, Kyoto, Japan) by using a Synergi Polar-RP column (150 x 4.6 mm, 4 μm, Phenomenex Inc. Torrance, CA, USA) and isocratic elution with H_3PO_4 0.5% at 0.5 ml/min. UV-Vis detector was set at 210 nm, except for hippuric and uric acids, detected at 227 and 280 nm, respectively, while the column temperature was set at 25 °C. Identification of each organic acid was carried out by comparing retention times and UV-Vis spectra with those of the analytical standard, while quantification was carried out by means of an external calibration curve.

Compounds of the unsaponifiable fraction were determined according to the method of Panfili et al. (1994), by hot saponification with KOH (60%) for extraction of the compounds, followed by extraction with n-hexane/ethyl acetate (9/1 v/v) and normal phase-high performance liquid chromatography for separation and identification of the analytes. An Alliance 2695 system (Waters, Milford, MA, USA) equipped with a Kromasil column (250 x 4,6 mm, 5μm, Phenomenex Inc., Torranlce, CA, USA), and a gradient eluition of propan-2-ol (1% in n-hexane) and n-hexane at 1,5 mL/min (Panfili et al. 1994) were employed for compound separation. Cholesterol and β-carotene were detected by spectrophotometry (at 208 and 450 nm, respectively), while α-tocopherol (ex. 280nm, em. 325nm) and retinol isomers (ex. 325nm, em. 475) were determined by spectrofluorimetry. Identification of each compound was carried out by comparing the retention time with the analytical standard, while quantification was carried out by means of an external calibration curve.

Degree of Antioxidant Protection (DAP) was calculated as the molar ratio between the molecules showing antioxidant activity, i.e., α-tocopherol and β-carotene, and an oxidation target molecule, which, in the case of milk, is represented by cholesterol (Pizzoferrato et al. 2007).

For the determination of total calcium and total phosphorus, 4g of milk samples were carefully homogenized and weighed into platinum crucibles, then ashed into a furnace at 525 °C for 16 h and finally solubilized with 1mL of HNO_3 (65% v/v), resulting into a final volume of 50 mL (in H2O). Then, total calcium and total phosphorus were determined according to the AOAC method (2002), using an atomic absorption spectrophotometer (A. Analyst 300, Perkin Elmer, Norwalk, CT, USA) for calcium and a visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) set at 400 nm for phosphorus.

The soluble fractions of calcium and phosphorus were analytically determined with the same method (AOAC 2002), prior ultracentrifugation of the milk (8 mL) at 29100 rpm for one hour at 20 °C (Liu et al. 2012), by using an ultracentrifuge Optima Max-XP (Beckman Coulter s.r.l., Cassina De' Pecchi – Milano, Italia).

Refereneces

AOAC - Association of Official Analytical Chemist*s* (2002) *Calcium, Magnesium, and Phosphorus in Cheese* (AOAC 991.25) In: *Official Methods of Analysis*, 17th Ed. Arlington. VA, USA: AOAC International.

Panfili G, Manzi P & Pizzoferrato L (1994) High-performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses. *The Analyst* **119(6)** 1161-1165

Pizzoferrato L, Manzi P, Marconi S, Fedele V, Claps S & Rubino R (2007) Degree of antioxidant protection: a parameter to trace the origin and quality of goat's milk and cheese. *Journal of Dairy Science* **90(10)** 4569-4574

Park Y, Lee J & Lee SJ (2006) Effects of Frozen and Refrigerated Storage on Organic Acid Profiles of Goat Milk Plain Soft and Monterey Jack Cheeses. *Journal of Dairy Science* **89** 862-871.

Figure 1S:

Results of the PCA model calculated on the milk samples before the refrigeration treatments (DATASET-1), collected from 11 producers of Provolone Valpadana PDO cheese and referred to as A1, A2, …A11, in the first year of experimentation (samples labelled in red) and in the second year of experimentation (samples labelled in green): Q residuals *vs.* Hotelling T^2 plot (A), Q residual contribution plot of milk sample A1 of the second year (t0_2) (B); measured values of the variables pH (C), and β-carotene (D) for all the milk samples, where sample A1 of the second year of experimentation is highlighted in magenta color.

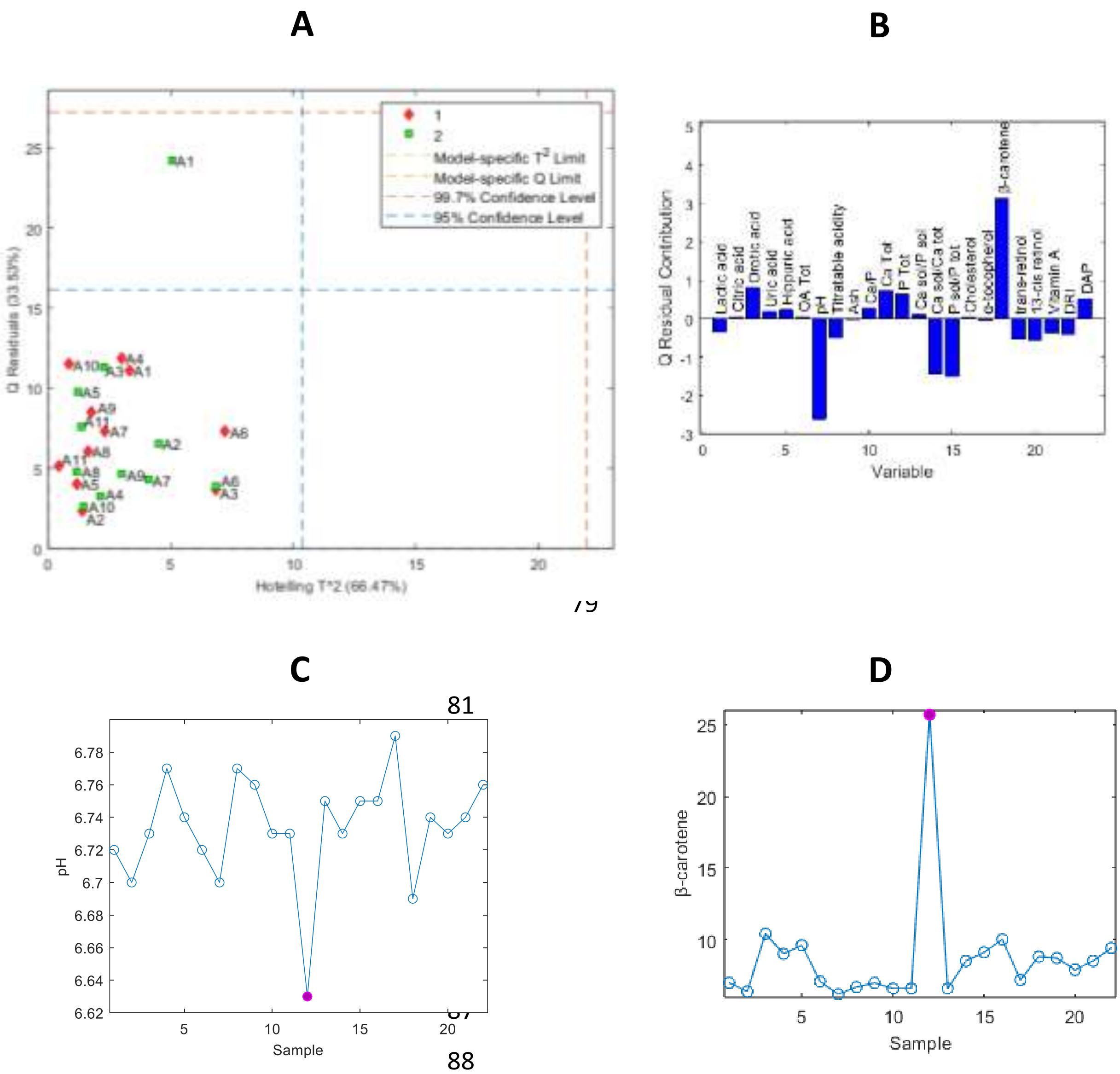

Figure 2S:

Results of the PCA model calculated on the milk samples of the first year of experimentation (DATASET-2), collected from 11 producers of Provolone Valpadana PDO cheese and referred to as A1, A2, …A11, at arrival at the laboratory (t0_1), after storage for 60h at 6°C (T60-6) and 12°C (T60-12): Q residuals *vs.* Hotelling T^2 plot (A), Hotelling T^2 contribution plot of milk sample A6 (T60-12) (B); measured values of the variables lactic acid (C), pH (D) and soluble calcium (E) for all the milk samples, where sample A6 (T60-12) is highlighted in magenta color.