

# Determining Carbohydrates for Increasing Safety: GC-FID Quantification of Lactose, Galactose, Glucose, Tagatose and *Myo*-inositol in 'Maturo' PDO Pecorino Sardo Cheese.

Alessio Silvio Dedola<sup>1</sup>, Marco Caredda<sup>1</sup>, Margherita Addis<sup>1,\*</sup>, Giacomo Lai<sup>1</sup>, Myriam Fiori<sup>1</sup>, Massimo Pes<sup>1</sup>, Andrea Mara<sup>2</sup>, Gavino Sanna<sup>2,\*</sup>

1        Agris Sardegna, Servizio Ricerca Prodotti di Origine Animale, Loc. Bonassai, Sassari, Italy (A.S.D., [adedola@agrisricerca.it](mailto:adedola@agrisricerca.it); M.C, [mcaredda@agrisricerca.it](mailto:mcaredda@agrisricerca.it); G.L., [gialai@agrisricerca.it](mailto:gialai@agrisricerca.it); M.F., [mfiori@agrisricerca.it](mailto:mfiori@agrisricerca.it); M.P.;, [mpes@agrisricerca.it](mailto:mpes@agrisricerca.it))

2        Dipartimento di Scienze Chimiche, Fisiche, Matematiche e Naturali, Università di Sassari, Via Vienna, 2, 07100 Sassari, Italy (A.M., [amara@uniss.it](mailto:amara@uniss.it)).

\*        Correspondence: M.A, [maddis@agrisricerca.it](mailto:maddis@agrisricerca.it); G.S., [sanna@uniss.it](mailto:sanna@uniss.it).

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### **Macro-composition of Pecorino Sardo PDO cheese samples**

Cheese samples were analyzed for: pH using a Crison Basic 20+ pH meter (Crison Instruments S.A., Alella, Spain), moisture content (ISO 5534; ISO, 2004), fat content (Soxhlet, 1879), total nitrogen (TN; ISO 8968–1; ISO, 2014). Protein content was calculated as follows: protein = [(TN) × 6.38]. Sodium chloride was determined by potentiometric titration with AgNO<sub>3</sub> (ISO 5943; ISO, 2006; automatic titrator Mettler-Toledo DL55, Mettler-Toledo GmbH, Schwerzenbach, Switzerland).

### **Supplementary References**

- S1)** ISO. (International Organization for Standardization). 2004. Cheese and processed cheese-Determination of the total solids content. Method number 5534:2004 (IDF 4:2004). ISO, Geneva, Switzerland.
- S2)** Soxhlet, F. 1879. Die gewichtsanalytische Bestimmung des MilCHFettes. Dingler's Polyt. J. 232:461–465.
- S3)** ISO. (International Organization for Standardization). 2014. Milk and milk products-Determination of nitrogen content—Part 1: Kjeldahl principle and crude protein calculation. Method number 8968-1:2014 (IDF 20-1:2014). ISO, Geneva, Switzerland.
- S4)** ISO. (International Organization for Standardization). 2006. Cheese and processed cheese products-Determination of chloride content-Potentiometric titration method. Method number 5963:2006 (IDF 88:2006). ISO, Geneva, Switzerland.

Table S1 Macro-composition of Pecorino Sardo PDO cheeses (mean  $\pm$  sd)

| Month of production    | January |   |      |      |   |      | April |   |      |      |   |      | June |   |      |      |   |      |
|------------------------|---------|---|------|------|---|------|-------|---|------|------|---|------|------|---|------|------|---|------|
| Ripening time (months) | 2       |   |      | 4    |   |      | 2     |   |      | 4    |   |      | 2    |   |      | 4    |   |      |
| Dairy                  | A       |   |      |      |   |      |       |   |      |      |   |      |      |   |      |      |   |      |
| pH                     | 4.91    | ± | 0.01 | 5.00 | ± | 0.01 | 5.03  | ± | 0.01 | 5.3  | ± | 0.1  | 5.07 | ± | 0.01 | 5.2  | ± | 0.1  |
| Moisture               | 39      | ± | 4    | 39.3 | ± | 0.5  | 38    | ± | 1    | 35   | ± | 1    | 36.3 | ± | 0.2  | 35   | ± | 1    |
| Fat                    | 31      | ± | 4    | 31.6 | ± | 0.3  | 32    | ± | 1    | 33   | ± | 1    | 33.5 | ± | 0.0  | 35   | ± | 1    |
| Protein                | 23.3    | ± | 0.4  | 23.0 | ± | 0.5  | 24.6  | ± | 0.4  | 24.8 | ± | 0.3  | 23.4 | ± | 0.2  | 24.4 | ± | 0.1  |
| NaCl                   | 1.73    | ± | 0.04 | 1.76 | ± | 0.04 | 1.57  | ± | 0.04 | 1.8  | ± | 0.1  | 1.6  | ± | 0.2  | 1.6  | ± | 0.2  |
| Dairy                  | B       |   |      |      |   |      |       |   |      |      |   |      |      |   |      |      |   |      |
| pH                     | 5.13    | ± | 0.03 | 5.2  | ± | 0.2  | 5.0   | ± | 0.3  | 5.26 | ± | 0.02 | 5.2  | ± | 0.1  | 5.2  | ± | 0.1  |
| Moisture               | 30.0    | ± | 0.4  | 28   | ± | 1    | 31.5  | ± | 0.1  | 29.2 | ± | 0.1  | 31   | ± | 1    | 30   | ± | 1    |
| Fat                    | 36      | ± | 2    | 38.4 | ± | 0.4  | 34.8  | ± | 0.2  | 36.2 | ± | 0.1  | 37   | ± | 1    | 37.2 | ± | 0.3  |
| Protein                | 27      | ± | 1    | 27   | ± | 1    | 28    | ± | 1    | 27.5 | ± | 0.2  | 27   | ± | 1    | 26.9 | ± | 0.1  |
| NaCl                   | 1       | ± | 0    | 1.1  | ± | 0.1  | 1.1   | ± | 0.1  | 1.16 | ± | 0.01 | 1.3  | ± | 0.1  | 1.3  | ± | 0.2  |
| Dairy                  | C       |   |      |      |   |      |       |   |      |      |   |      |      |   |      |      |   |      |
| pH                     | 4.92    | ± | 0.03 | 5.08 | ± | 0.02 | 5.13  | ± | 0.03 | 5.28 | ± | 0.03 | 5.3  | ± | 0.1  | 5.4  | ± | 0.1  |
| Moisture               | 39      | ± | 5    | 33.4 | ± | 0.4  | 37    | ± | 1    | 32   | ± | 1    | 33   | ± | 0    | 31   | ± | 1    |
| Fat                    | 31      | ± | 3    | 34.7 | ± | 0.2  | 31    | ± | 1    | 33.7 | ± | 0.4  | 35   | ± | 1    | 36.1 | ± | 0.2  |
| Protein                | 24      | ± | 1    | 25   | ± | 1    | 25.2  | ± | 0.4  | 26.7 | ± | 0.3  | 25.9 | ± | 0.4  | 26   | ± | 1    |
| NaCl                   | 1.7     | ± | 0.1  | 1.8  | ± | 0.1  | 1.6   | ± | 0.2  | 1.6  | ± | 0.1  | 1.46 | ± | 0.02 | 1.54 | ± | 0.03 |
| Dairy                  | D       |   |      |      |   |      |       |   |      |      |   |      |      |   |      |      |   |      |
| pH                     | 5.0     | ± | 0.1  | 5.1  | ± | 0.1  | 5.12  | ± | 0.04 | 5.1  | ± | 0.1  | 5.13 | ± | 0.01 | 5.01 | ± | 0.04 |
| Moisture               | 34.7    | ± | 0.3  | 32   | ± | 1    | 36.4  | ± | 0.3  | 32.9 | ± | 0.5  | 36.4 | ± | 0.1  | 33.3 | ± | 0.3  |
| Fat                    | 34.2    | ± | 0.1  | 35.9 | ± | 0.2  | 32.0  | ± | 0.2  | 33.7 | ± | 0.0  | 33.1 | ± | 0.1  | 34.7 | ± | 0.2  |
| Protein                | 24      | ± | 1    | 25   | ± | 2    | 25.1  | ± | 0.2  | 26.1 | ± | 0.2  | 24.3 | ± | 0.1  | 27   | ± | 2    |
| NaCl                   | 1.5     | ± | 0.2  | 1.6  | ± | 0.1  | 1.5   | ± | 0.1  | 1.5  | ± | 0.1  | 1.6  | ± | 0.2  | 1.71 | ± | 0.04 |

| E        |       |   |       |       |   |      |      |   |      |      |   |      |      |   |     |      |   |      |
|----------|-------|---|-------|-------|---|------|------|---|------|------|---|------|------|---|-----|------|---|------|
| pH       | 5.0   | ± | 0.1   | 5.2   | ± | 0.1  | 5.2  | ± | 0.1  | 5.2  | ± | 0.0  | 5.2  | ± | 0.1 | 5.3  | ± | 0.1  |
| Moisture | 36    | ± | 1     | 34    | ± | 1    | 36.1 | ± | 0.1  | 33.2 | ± | 0.3  | 34.1 | ± | 0.0 | 31.8 | ± | 0.3  |
| Fat      | 33.6  | ± | 0.3   | 35    | ± | 1    | 32.5 | ± | 0.4  | 34.2 | ± | 0.1  | 35   | ± | 1   | 36   | ± | 1    |
| Protein  | 24.5  | ± | 0.1   | 24    | ± | 1    | 25   | ± | 1    | 26.1 | ± | 0.2  | 23.9 | ± | 0.4 | 25.6 | ± | 0.2  |
| NaCl     | 1.159 | ± | 0.004 | 1.21  | ± | 0.04 | 1.41 | ± | 0.02 | 1.4  | ± | 0.1  | 1.6  | ± | 0.4 | 1.7  | ± | 0.2  |
|          |       |   |       |       |   |      |      |   |      |      |   |      |      |   |     |      |   |      |
| F        |       |   |       |       |   |      |      |   |      |      |   |      |      |   |     |      |   |      |
| pH       | 5.2   | ± | 0.2   | 5.00  | ± | 0.02 | 5.05 | ± | 0.01 | 5.1  | ± | 0.2  | 5.2  | ± | 0.1 | 5.2  | ± | 0.1  |
| Moisture | 36    | ± | 1     | 35    | ± | 1    | 37   | ± | 1    | 34   | ± | 1    | 34   | ± | 1   | 33   | ± | 1    |
| Fat      | 32.9  | ± | 0.5   | 33    | ± | 1    | 30.9 | ± | 0.3  | 33   | ± | 1    | 32.3 | ± | 0.1 | 33   | ± | 2    |
| Protein  | 25    | ± | 1     | 24.8  | ± | 0.2  | 24.9 | ± | 0.1  | 25.9 | ± | 0.1  | 27   | ± | 1   | 27   | ± | 2    |
| NaCl     | 1.6   | ± | 0.1   | 1.83  | ± | 0.03 | 1.6  | ± | 0.1  | 1.7  | ± | 0.3  | 1.7  | ± | 0.1 | 1.88 | ± | 0.03 |
|          |       |   |       |       |   |      |      |   |      |      |   |      |      |   |     |      |   |      |
| G        |       |   |       |       |   |      |      |   |      |      |   |      |      |   |     |      |   |      |
| pH       | 4.89  | ± | 0.04  | 5.1   | ± | 0.2  | 5.1  | ± | 0.1  | 5.18 | ± | 0.01 | 5.0  | ± | 0.2 | 5.2  | ± | 0.2  |
| Moisture | 36.5  | ± | 0.2   | 33.36 | ± | 0.02 | 36.1 | ± | 0.1  | 33   | ± | 1    | 32.9 | ± | 0.5 | 31   | ± | 1    |
| Fat      | 32.7  | ± | 0.2   | 33.93 | ± | 0.02 | 31   | ± | 1    | 33   | ± | 1    | 34.8 | ± | 0.1 | 35.7 | ± | 0.0  |
| Protein  | 24    | ± | 1     | 26.5  | ± | 0.3  | 26   | ± | 1    | 26   | ± | 3    | 26.4 | ± | 0.4 | 26   | ± | 1    |
| NaCl     | 1.2   | ± | 0.3   | 1.27  | ± | 0.03 | 1.3  | ± | 0.1  | 1.2  | ± | 0.1  | 1.2  | ± | 0.1 | 1.2  | ± | 0.1  |

In Table S2 are listed the linearity interval and the coefficient of determination for each carbohydrate quantified in Pecorino Sardo PDO cheese samples.

**Table S2 – Operative linearity interval and the coefficient of determination for the analytes quantified in Pecorino Sardo PDO cheese samples**

| <b>Analytes</b>                       | <b>Linearity range<br/>(ppm)</b> | <b>Coefficient of determination<br/>(R<sup>2</sup>)</b> |
|---------------------------------------|----------------------------------|---|
| <b><math>\alpha</math>-Lactose</b>    | 4 ÷ 182                          | 0.960   |
| <b><math>\beta</math>-Lactose</b>     | 6 ÷ 221                          | 0.959   |
| <b><math>\alpha</math>-Galactose</b>  | 4 ÷ 160                          | 0.968   |
| <b><math>\beta</math>-Galactose</b>   | 7 ÷ 277                          | 0.966   |
| <b><math>\gamma</math>- Galactose</b> | 1 ÷ 62                           | 0.972   |
| <b><math>\alpha</math>- Glucose</b>   | 4 ÷ 163                          | 0.970   |
| <b><math>\beta</math>-Glucose</b>     | 6 ÷ 246                          | 0.972   |
| <b><math>\alpha</math>-Tagatose</b>   | 30 ÷ 139                         | 0.999   |
| <b><math>\beta</math>-Tagatose</b>    | 181 ÷ 811                        | 0.999   |
| <b>myo-Inositol</b>                   | 180 ÷ 1007                       | 0.997   |

Figures S1 – S10 show the Internal standard calibration plots for all the carbohydrates determined in Pecorino Sardo PDO cheese samples.

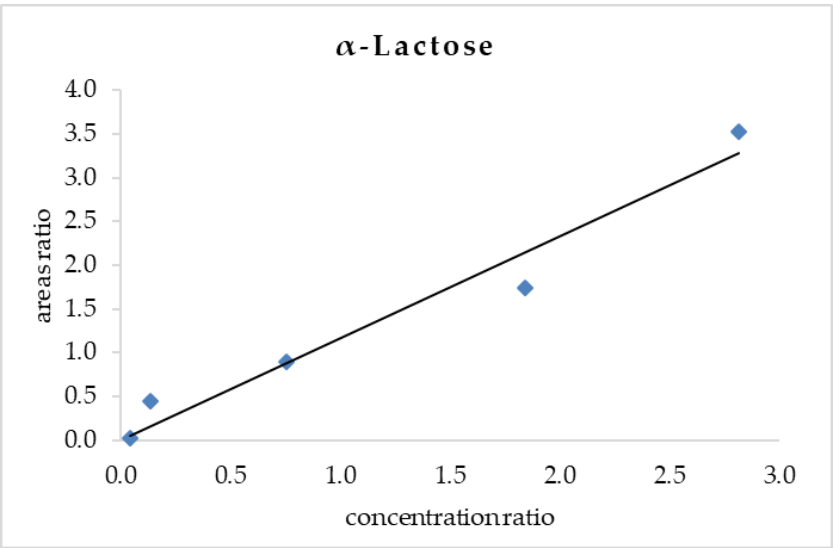


Figure S1 - Internal standard calibration plot for  $\alpha$ -Lactose

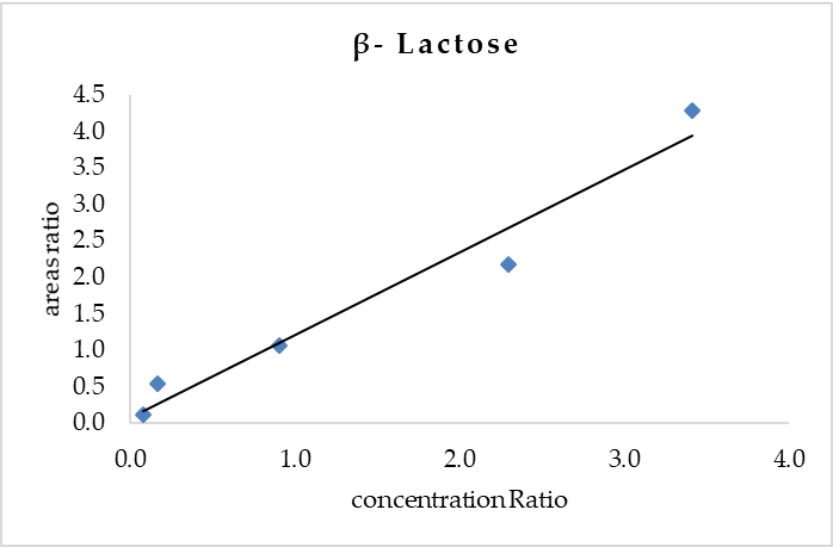


Figure S2 - Internal standard calibration plot for  $\beta$ -Lactose

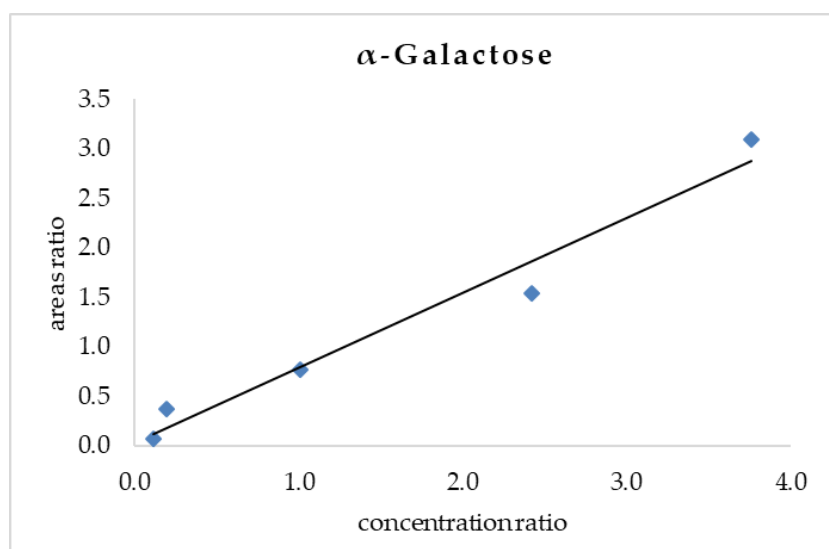


Figure S3 - Internal standard calibration plot for  $\alpha$ -Galactose

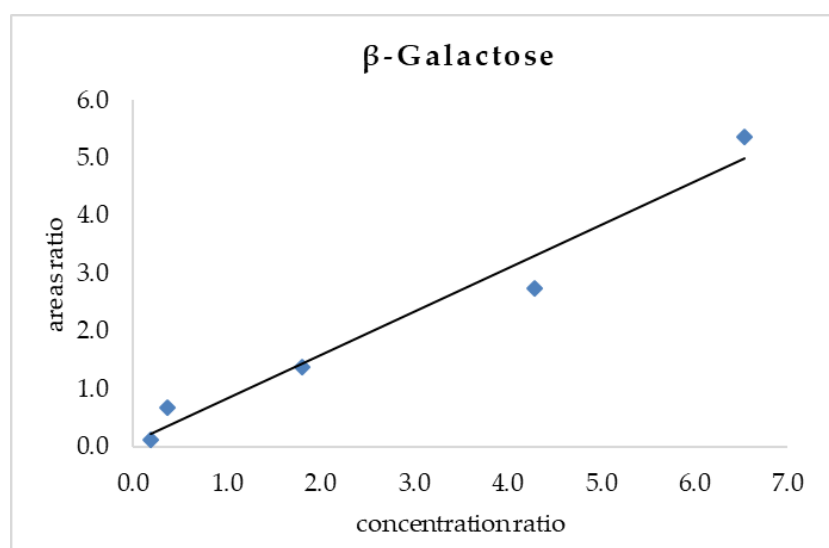


Figure S4 - Internal standard calibration plot for  $\beta$ -Galactose

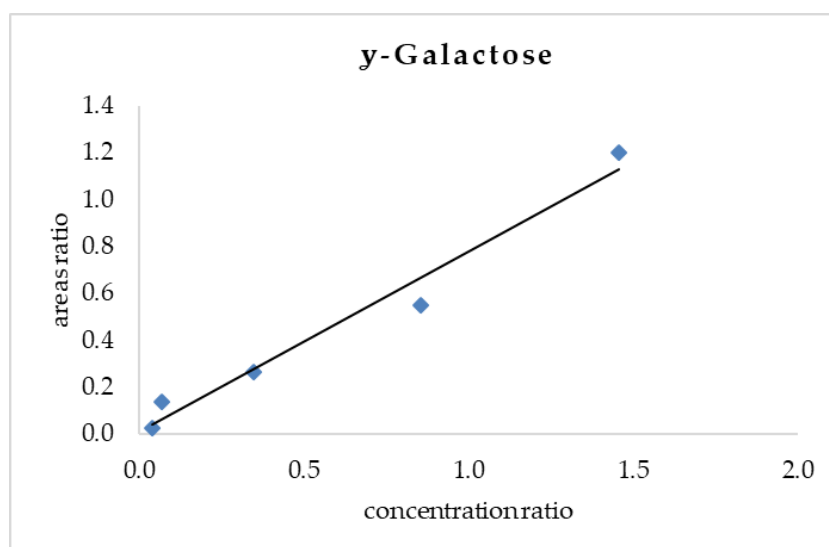


Figure S5 - Internal standard calibration plot for  $\gamma$ -Galactose

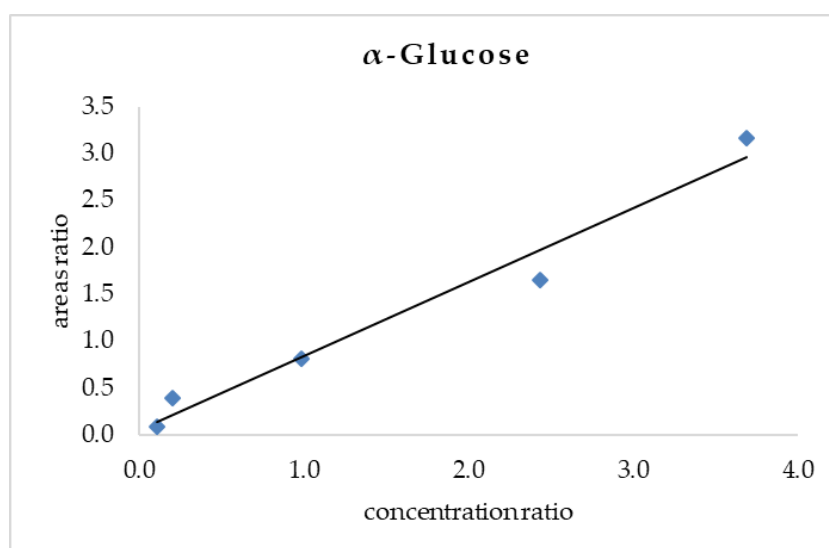


Figure S6 - Internal standard calibration plot for  $\alpha$ -Glucose



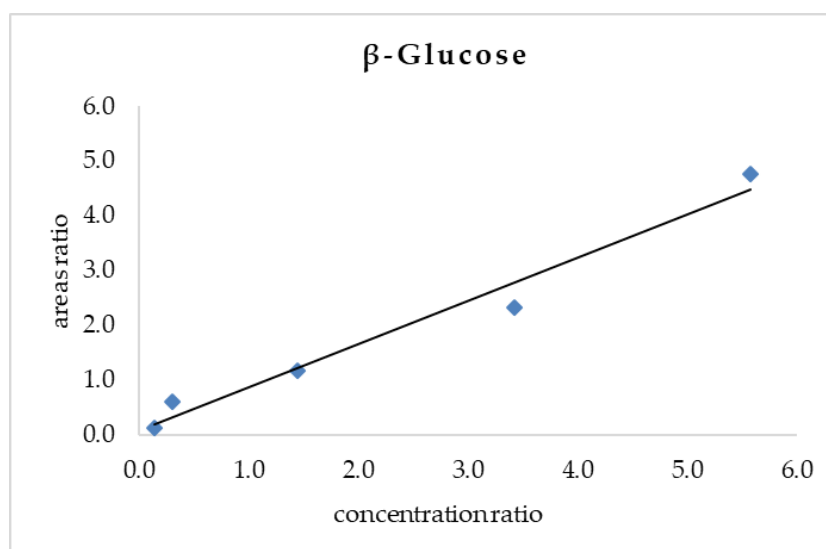


Figure S7 - Internal standard calibration plot for  $\beta$ -Glucose

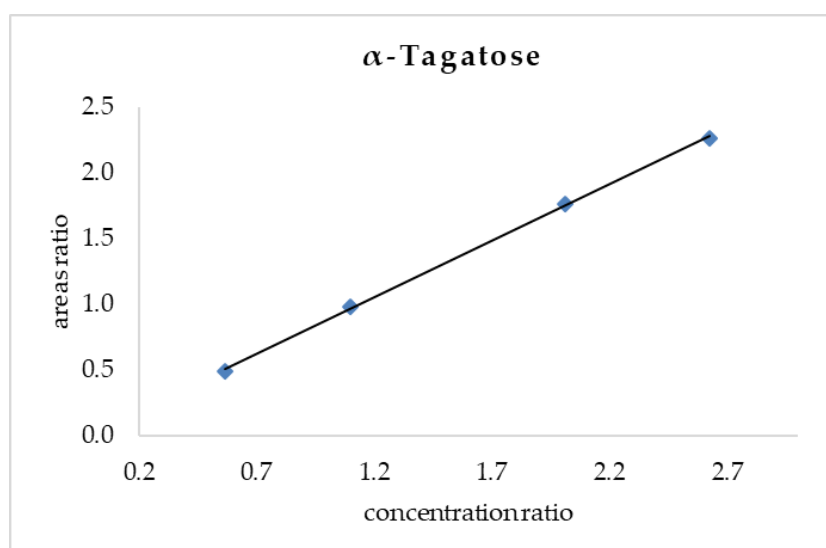


Figure S8 - Internal standard calibration plot for  $\alpha$ -Tagatose

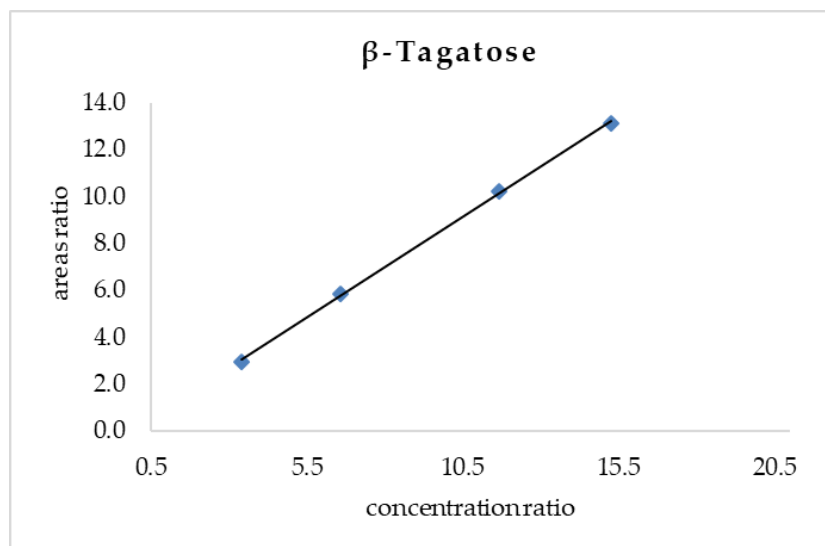


Figure S9 - Internal standard calibration plot for  $\beta$ -Tagatose

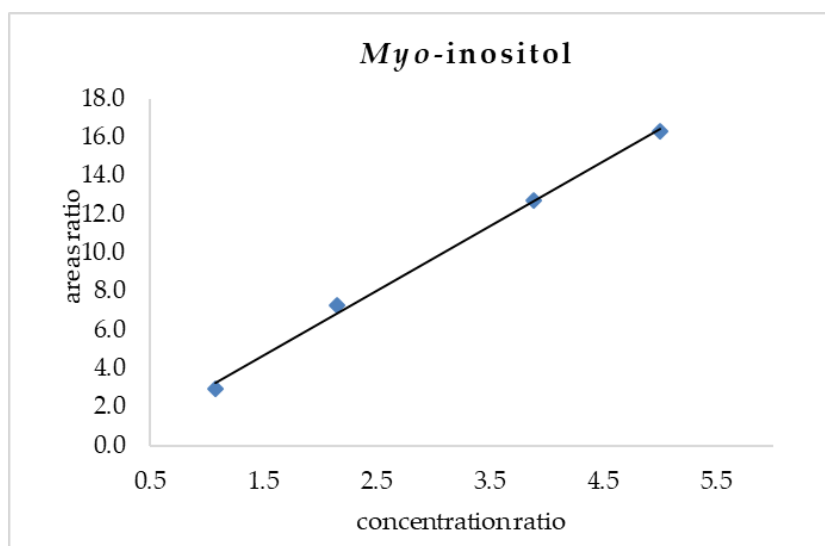


Figure S10 - Internal standard calibration plot for *Myo*-inositol

In Figures S11 – S15 the box plots of saccharides determined in cheese samples from seven Dairies are shown.

The coloured area corresponds to the second and third quarters (50% of samples). The x symbol corresponds to the mean value and the vertical line represent the range of the data.

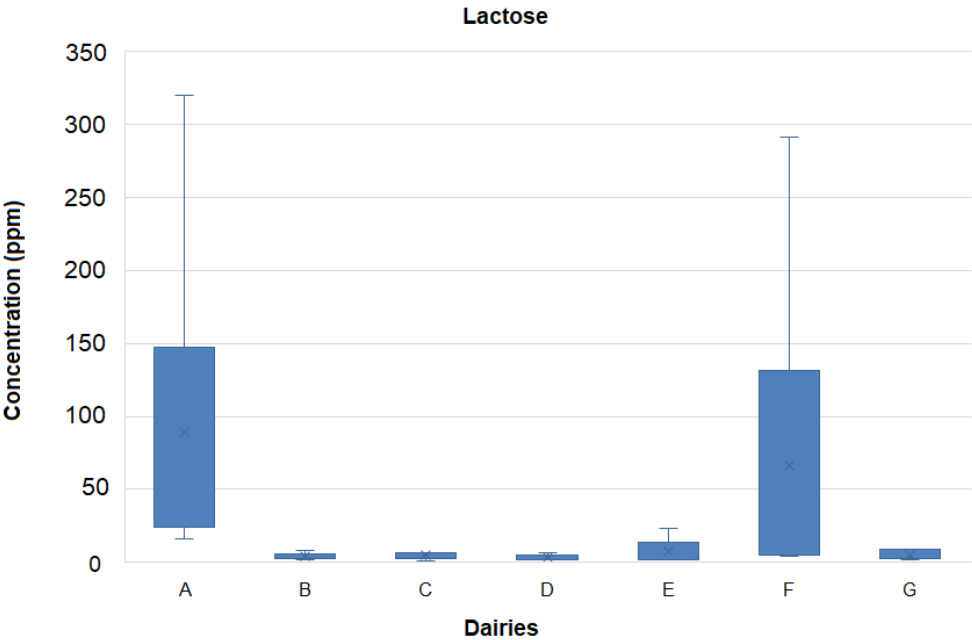


Figure S11 – Box plot of Lactose content in Pecorino Sardo PDO cheese samples after two months of ripening produced in seven Sardinian dairies

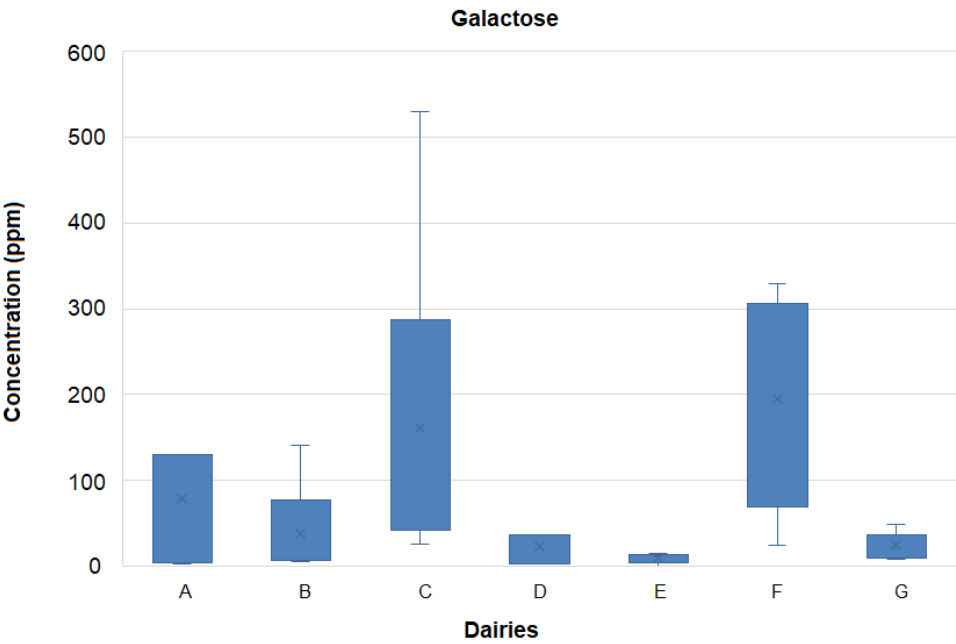


Figure S12 – Box plot of Galactose content in Pecorino Sardo PDO cheese samples after two months of ripening produced in seven Sardinian dairies

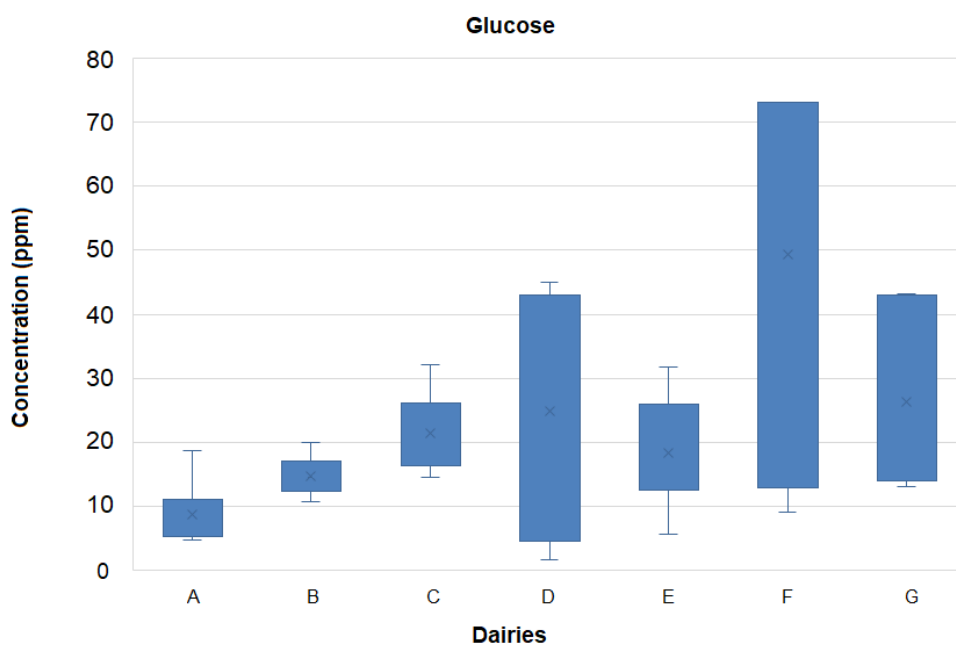


Figure S13 – Box plot of Glucose content in Pecorino Sardo PDO cheese samples after two months of ripening produced in seven Sardinian dairies

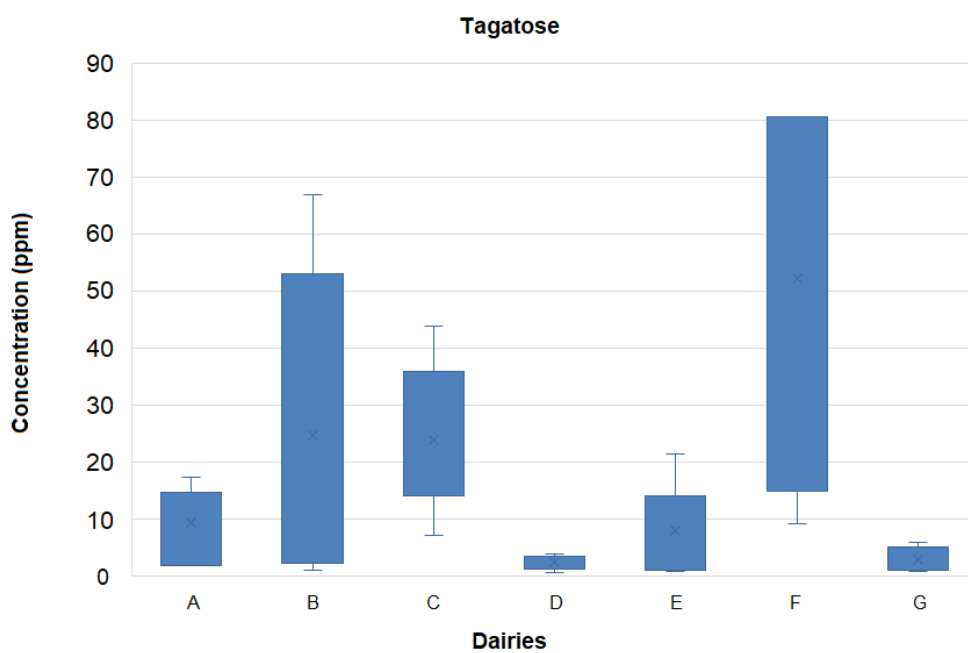


Figure S14 – Box plot of Tagatose content in Pecorino Sardo PDO cheese samples after two months of ripening produced in seven Sardinian dairies

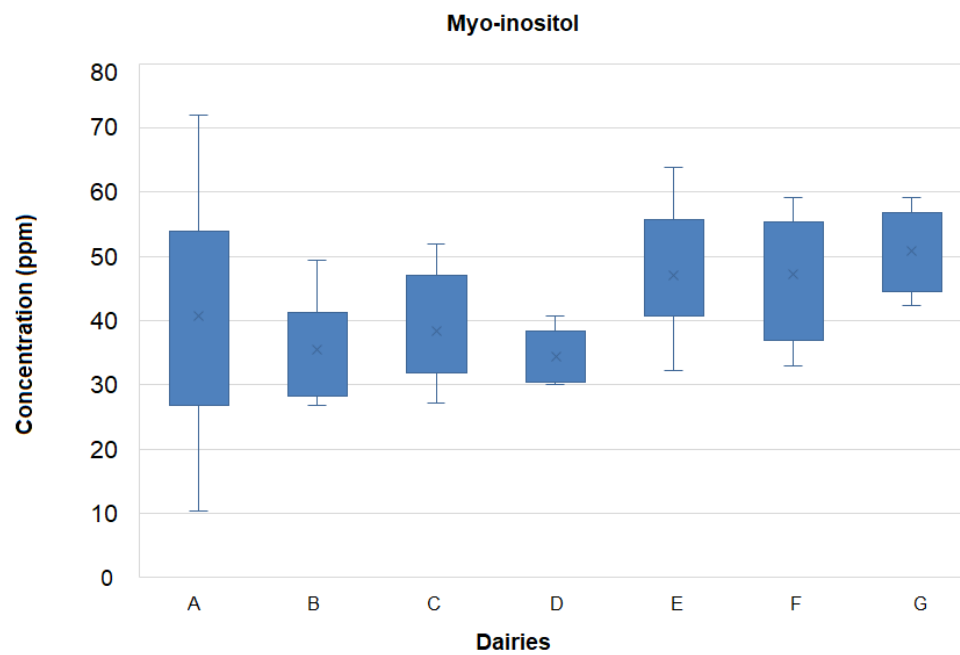


Figure S15 – Box plot of *myo*-inositol content in Pecorino Sardo PDO cheese samples after two months of ripening produced in seven Sardinian dairies