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Abstract

The composition of Serra da Estrela PDO cheeses (total fat, total protein, salt and free amino acids) was assessed using NIR spectrophotometry and UPLC-DAD-MS/MS. In total, 24 cheeses were acquired from 6 certified cheesemakers located in 5 different municipalities within the delimited PDO geographical region. Cheeses were produced from raw ewe milk of two autochthonous Portuguese sheep breeds, between November 2017 and March 2018, and were acquired after 45 days of maturation. The data include the mean (and respective standard deviations) levels of moisture (%), total fat (%), total protein (%) and salt (%), obtained by NIR spectrophotometry. As well the mean (and respective standard deviations) of free amino acids contents (mg/100 g of cheese, in wet basis) evaluated using a UPLC-DAD-MS/MS method are shown. The latter data include information regarding 8 essential free amino acids (histidine, leucine-isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) and 9 non-
essential free amino acids (arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, proline, tyrosine and serine). Leucine and isoleucine, being isomers, were quantified together. Leucine-isoleucine, phenylalanine and serine were the most abundant essential free amino acids and cysteine, proline and asparagine were the most abundant non-essential free amino acids. Free amino acids contents depended on the cheese producer as well as on the production time-period.

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Specifications Table

| Subject | Agricultural and Biological Sciences |
|---------|--------------------------------------|
| Specific subject area | Food Science |
| Type of data | Tables in the text (mean value ± standard deviation) and as Excel files (raw and calculated data) in supplementary material Figures in the text |
| How data were acquired | The moisture, total fat, total protein and salt levels of Serra da Estrela cheeses were determined using a NIRMaster™ spectrophotometer (Buchi NIRSolutions™). The free amino acids contents of Serra da Estrela cheeses were assessed by UPLC-DAD–MS/MS chromatographic system (Dionex Ultimate 3000 UPLC instrument from Thermo Scientific) equipped with a diode-array detector (DAD) and coupled to Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan) with an ESI source. |
| Data format | Raw and analysed data in Excel files |
| Parameters for data collection | Data were collected from Serra da Estrela cheeses (Portuguese PDO cheeses) produced from raw ewe milk by certified cheese producers, according to the legal requirements, between November 2017 and March 2018. |
| Description of data collection | 24 PDO Serra da Estrela cheeses with 45 days of maturation and produced by 6 certified cheese producers, were analysed. Moisture, fat, protein and salt contents were determined by NIR spectrophotometry. Free amino acids profiles were established by UPLC-DAD-MS/MS chromatography, being the MS detection performed in positive mode by multiple reaction monitoring. |
| Data source location | UPLC-DAD-MS/MS data: Instituto Politécnico de Bragança, Escola Superior Agrária Bragança Portugal NIR spectrophotometry based data: Instituto Politécnico de Viseu, Escola Superior Agrária Viseu Portugal |
| Data accessibility | With the article |
| Related research article | Reis Lima M.J., Santos A.O., Falcão S., Fontes L., Teixeira-Lemos E., Vilas-Boas M., Veloso A.C.A., Peres A.M. (2019). Serra da Estrela cheese’s free amino acids profiles by UPLC-DAD-MS/MS and their application for cheese origin assessment. Food Research International, 126, art. no. 108729. https://doi.org/10.1016/j.foodres.2019.108729 |

Value of the Data

- The chromatographic data on free amino acids showed the presence of both essential and non-essential amino acids in Serra da Estrela cheeses.
- Consumers and producers of Serra da Estrela cheeses may use the free amino acids data from a dietary-nutritional perspective.
- Serra da Estrela cheeses, produced by different dairy farms and at different production dates, provide dietary amino acids for human nutrition.
- Free amino acids isomers (leucine and isoleucine) quantification requires optimization of the proposed UPLC-DAD-MS/MS method.
- The free amino acids data allow a preliminary nutritional awareness related to the desirable intake of branched chain essential amino acids due to cheese consumption.
1. Data

The data included refer to moisture, total fat, total protein and salt contents of the 24 Serra da Estrela cheeses (contents determined from 3 to 5 NIR assays for each cheese sample) being the mean values and respective standard deviations shown in Table 1 and Figs. 1–2 and the respective individual contents (per cheese sample and replica), obtained based on the NIR spectra, given in the supplementary material (file: Table_S1_DIB-D-19-02387.xlsx). The multistep gradient program used for free amino acids detection by UPLC-DAD-MS/MS is described in Table 2. The values of the linearity of UPLC-DAD-MS/MS calibration curves (determination coefficients, $R^2$), the respective dynamic concentration ranges, as well as the overall intra-day repeatability values, for each amino acid studied, are given in Table 3. The peak areas ratios raw data, used to calculate the previous parameters are given in the supplementary material file, named Table_S2_DIB-D-19-02387.xlsx, which raw data can be further used to obtain the calibration curves for each amino acid. The free amino acids (FAA) mean contents (for essential and non-essential free amino acids detected in the cheese samples) were chromatographically determined by UPLC-DAD-MS/MS for each of the 24 Serra da Estrela cheeses with 45 days of maturation (mean values and respective standard deviations for triplicate chromatographic injections carried out for 2 independent cheese samples per each cheese) and are shown in Tables 4 and 5 as well as in Figs. 3–6, according to the 6 different certified PDO cheese producers, located in 5 municipalities of the PDO delimited geographical region, and the production time-period (from November 2017 to March 2018). The raw peak areas ratios used to calculate the contents of each free amino acid detected in each experimental assay (3 injections × 2 independent samples × 24 cheeses) and the

| Cheese Producer | Geographical origin | Cheese code | Production Date | Mean ± standard deviation contents (%) |
|-----------------|---------------------|-------------|----------------|----------------------------------------|
| Producer #1     | Oliveira do Hospital (OH) | SA01         | November 2017 | 46.9 ± 0.2 29.1 ± 0.1 18.5 ± 0.1   |
|                 |                     | SA02         | January 2018  | 43.4 ± 0.1 31.3 ± 0.1 20.1 ± 0.1   |
|                 |                     | SA03         | February 2018 | 48.4 ± 0.2 26.1 ± 0.2 19.5 ± 0.1   |
|                 |                     | SA04         | March 2018    | 47.3 ± 0.1 27.2 ± 0.2 20.3 ± 0.1   |
|                 |                     | SA05         | March 2018    | 43.0 ± 0.2 30.6 ± 0.2 20.2 ± 0.2   |
| Producer #2     | Celorico da Beira (CB) | CA01         | November 2017 | 47.1 ± 0.4 29.5 ± 0.4 19.0 ± 0.2   |
|                 |                     | CA02         | February 2018 | 45.5 ± 0.1 30.2 ± 0.0 19.4 ± 0.1   |
|                 |                     | CA03         | March 2018    | 47.9 ± 0.1 29.3 ± 0.1 18.7 ± 0.1   |
| Producer #3     | Penalva do Castelo (PC) | CI01         | December 2017 | 52.6 ± 1.0 22.6 ± 0.6 20.0 ± 0.4   |
|                 |                     | CI02         | March 2018    | 50.7 ± 0.0 20.2 ± 0.1 23.5 ± 0.1   |
|                 |                     | CI03         | March 2018    | 51.6 ± 0.0 22.0 ± 0.1 21.5 ± 0.0   |
| Producer #4     | Penalva do Castelo (PC) | QG01         | December 2017 | 47.8 ± 0.1 26.2 ± 0.1 21.6 ± 0.2   |
|                 |                     | QG02         | February 2018 | 47.9 ± 0.1 25.0 ± 0.0 22.0 ± 0.2   |
|                 |                     | QG03         | February 2018 | 43.3 ± 0.1 27.6 ± 0.1 24.0 ± 0.1   |
| Producer #5     | Gouveia (G)         | QS01         | November 2017 | 46.8 ± 0.3 20.5 ± 0.3 26.7 ± 0.3   |
|                 |                     | QS02         | January 2018  | 44.1 ± 0.2 25.1 ± 0.1 24.0 ± 0.0   |
|                 |                     | QS03         | February 2018 | 51.3 ± 0.1 19.6 ± 0.1 23.5 ± 0.2   |
|                 |                     | QS04         | March 2018    | 51.4 ± 0.1 21.1 ± 0.1 21.6 ± 0.0   |
|                 |                     | QS05         | March 2018    | 53.8 ± 0.1 15.9 ± 0.2 24.4 ± 0.0   |
| Producer #6     | Nelas (N)           | QL01         | November 2017 | 53.4 ± 0.2 19.2 ± 0.2 21.9 ± 0.2   |
|                 |                     | QL02         | December 2017 | 51.4 ± 0.6 20.0 ± 0.4 22.9 ± 0.2   |
|                 |                     | QL03         | February 2018 | 50.6 ± 0.1 18.1 ± 0.1 25.4 ± 0.1   |
| Mean ± standard deviation: | | | | 49 ± 3 24 ± 4 22 ± 2 1.2 ± 0.4 |
| Minimum value: | | | | 43 16 18 0.7 |
| Maximum value: | | | | 54 31 30 2.2 |
respective free amino acids contents (per assays) can be found in the supplementary material (file: Table_S3_DIB-D-19-02387.xlsx). As pointed out, the data is provided in text tables and on Microsoft Excel Worksheet. The data contain the names of FAA detected and quantified in the cheeses, the cheese sample code, the producer code (Producer #1 to Producer #6), the production date (November 2017 to March 2018) and the name of the Portuguese municipality where each dairy industry was located (Celorico da Beira, Gouveia, Nelas, Oliveira do Hospital, and Penalva do Castelo).

FAA mean contents (mg FAA/100 g cheese) are provided in wet basis (wb).

2. Experimental design, materials, and methods

2.1. Serra da Estrela cheese production details

Cheeses (~1kg) were acquired from 6 certified producers (Producer #1-#6) of Serra da Estrela PDO cheeses, located at 5 different municipalities within the delimited PDO geographical origin (Celorico da Beira – CB, Gouveia – G, Nelas – N, Oliveira do Hospital – OH, and Penalva do Castelo – PC) and following the legal production regulations [1,2]. The 24 cheeses evaluated were produced between November 2017 and March 2018, using raw ewe milk from “Churra Mondegueira” and “Bordaleira” autochthonous Portuguese breeds, and coagulated using the wild thistle flower (Cynara cardunculus L.). All cheeses had 45 days of maturation (acquired between January and May 2018), being immediately transported to the laboratory, coded, split into different sub-samples and frozen at −40 °C until analysis. The 24 cheeses were acquired as follows:

- Producer #1: 5 cheeses produced in November 2017, December 2017, January 2018, February 2018 and March 2018;
Producer #2: 3 cheeses produced in November 2017, February 2018 and March 2018; 
Producer #3: 3 cheeses produced in December 2017 and March 2018; 
Producer #4: 4 cheeses produced in December 2017, February 2018 and March 2018; 
Producer #5: 5 cheeses produced in November 2017, January 2018, February 2018 and March 2018; 
Producer #6: 4 cheeses produced in November 2017, December 2017, February 2018 and March 2018.

Fig. 2. Boxplots for physicochemical raw data (moisture, total fat, total protein and salt levels, in %) of 24 Serra da Estrela PDO cheeses determined by NIR spectrophotometry, according to the production date (November 2017, December 2017, January 2018, February 2018 and March 2018). Dairy farms were located in 5 municipalities (Celorico da Beira – CB, Gouveia – G, Nelas – N, Oliveira do Hospital – OH, and Penalva do Castelo – PC) within the PDO geographical region and the cheeses were produced by 6 certified cheese producers (Producer 1 to 6), being acquired after 45 days of maturation (values calculated using the supplementary material provided as Table_S1_DIB-D-19-02387.xlsx).

Table 2
Multistep gradient program applied for free amino acids detection by UPLC-DAD-MS/MS.

| Time (min) | Flow (mL/min) | Mobile phase (%) |
|------------|---------------|-----------------|
|            |               | Solvent A<sup>a</sup> | Solvent B<sup>b</sup> |
| 0.00       | 0.40          | 80               | 20               |
| 0.50       | 0.40          | 80               | 20               |
| 0.75       | 0.40          | 70               | 30               |
| 1.00       | 0.40          | 70               | 30               |
| 5.00       | 0.40          | 5                | 95               |
| 6.00       | 0.40          | 5                | 95               |
| 8.00       | 0.40          | 80               | 20               |
| 10.00      | 0.40          | 80               | 20               |

<sup>a</sup> Solvent A: 0.1% (v/v) formic acid in water.
<sup>b</sup> Solvent B: 0.1% (v/v) formic acid in acetonitrile/water (50:50, v/v).
Table 3
Linearity of UPLC-DAD-MS/MS calibration curves and respective dynamic concentration ranges (values calculated using the supplementary material provided as Table_S2_DIB-D-19-02387.xlsx).

| Free amino acids | Calibration curves dynamic concentration intervals\(^a\) (μmol/L) | \(R^2\) calibration curves | Overall intra-day repeatability interval (CV%\(^b\)) |
|------------------|---------------------------------------------------------------|-----------------------------|-----------------------------------------------|
| **Essential amino acids** |                                                                             |                             |                                               |
| Histidine (His)  | 0.938–7.5 7.5–37.5 150–1200                                      | 0.9987 0.9989 0.9975        | 0.13–9.62%                                    |
| Leucine-Isoleucine (Leu-Ile) | 0.117–15 15–150                                                  | 0.9996 0.9991              | 0.02–7.57%                                    |
| Lysine (Lys)     | 0.117–75 75–150                                                 | 0.9979 0.9946              | 0.03–6.73%                                    |
| Methionine (Met) | 0.117–18.75 18.75–300 300–3000                                   | 0.9997 0.9982              | 0.37–10.10%                                   |
| Phenylalanine (Phe) | 0.117–3000                                                     | 0.9988                      | 0.04–5.71%                                    |
| Threonine (Thr)  | 0.469–37.5 37.5–150                                            | 0.9983 0.9977              | 0.26–9.61%                                    |
| Tryptophan (Trp) | 0.117–7.5 7.5–600 600–300                                       | 0.9994 0.9968              | 0.48–9.09%                                    |
| Valine (Val)     | 0.117–18.75 18.75–150 150–1200                                   | 0.9998 0.9962              | 0.30–6.83%                                    |
| **Non-essential amino acids plus cystine** |                                                             |                             |                                               |
| Alanine (Ala)    | 37.5–900 900–3000                                                | 0.9989 0.9989              | 0.30–7.58%                                    |
| Arginine (Arg)   | 0.469–37.5 37.5–150                                            | 0.9996 0.9988              | 1.01–5.84%                                    |
| Asparagine (Asn) | 0.469–15 15–75                                                  | 0.9995 0.9944              | 0.26–5.02%                                    |
| Aspartic acid (Asp) | 0.117–18.75 18.75–150 150–1200                                  | 0.9987 0.9992              | 0.42–5.23%                                    |
| Cysteine (Cys)   | 3.75–75 75–900                                                  | 0.9931 0.9968              | 0.42–5.23%                                    |
| Cystine (Cys-Cys) | 0.234–18.75 18.75–300                                           | 0.9970 0.9976              | 0.50–8.74%                                    |
| Glutamic acid (Glu) | 0.117–18.75 18.75–150                                           | 0.9998 0.9953              | 0.31–6.25%                                    |
| Glutamine (Gln)  | 0.234–37.5 75–900                                               | 0.9994 0.9938              | 0.03–7.01%                                    |
| Glycine (Gly)    | 18.75–1200                                                      | 0.9981 0.9981              | 0.91–10.31%                                   |
| Proline (Pro)    | 0.117–7.5 7.5–3000                                              | 0.9989 0.9941              | 1.42–9.03%                                    |
| Tyrosine (Tyr)   | 0.117–75 75–150                                                 | 0.9997 0.9905              | 0.44–5.87%                                    |
| Serine (Ser)     | 0.469–18.75 150–900                                             | 0.9987 0.9976              | 0.80–7.30%                                    |

\(^a\) Dynamic concentration ranges of the calibration curves \(\left(\frac{\text{Peak area}_{\text{AA}}}{\text{Peak area}_{\text{IS}}}=\text{intercept}+\text{slope} \times |\text{AA concentration}|\) established for each amino acid (AA).

\(^b\) Intra-day repeatability: range of the coefficients of variation calculated from 3 injections of each standard solution of each amino acid used for establishing the calibration curves (CV% = standard deviation/mean \(\times 100\)). Standard deviations were calculated from the data of the ratios [Amino acids peak area]/[Internal standard (N-Acetyl-L-Tyrosine) peak area] regarding 3 injections for each concentration level and each amino acid. Mean values were calculated from the values of the ratios [Amino acids peak area]/[Internal standard (N-Acetyl-L-Tyrosine) peak area], regarding 3 injections for each concentration level and each amino acid.
**Table 4**

Essential free amino acids contents (mean ± standard deviation of 3 injections × 2 extraction per cheese, mg/100 g of cheese in wet basis) determined by UPLC-DAD-MS/MS, for each of the two independent samples taken from each of the 24 Serra da Estrela PDO cheeses acquired from 6 certified cheese producers, located in 5 municipalities (Celorico da Beira – CB, Gouveia – G, Nelas – N, Oliveira do Hospital – OH, and Penalva do Castelo – PC) within the PDO delimited geographical region and produced from November 2017 till March 2018 (values calculated using the data provided in the supplementary material file Table_S3_DJB-D-19-02387.xlsx).

| Cheese Producer | Geographical origin | Cheese code | Production Date | Essential free amino acids contents (mean ± standard deviation) in mg/100 g of cheese (wet basis) |
|-----------------|----------------------|-------------|-----------------|--------------------------------------------------------------------------------------------------|
| Producer #1     | Oliveira do Hospital (OH) | SA01        | November 2017   | His: 0.23 ± 0.01 ± 0.01, Leu-Ile: 0.35 ± 0.01 ± 0.01, Lys: 1.6 ± 0.3 ± 0.2, Met: 15 ± 1 ± 1, Phe: 0.11 ± 0.01 ± 0.01, Thr: 4.9 ± 0.3 ± 0.1, Trp: 10 ± 1 ± 1 |
|                 |                      | SA02        | January 2018    |                                                                                                  |
|                 |                      | SA03        | February 2018   |                                                                                                  |
|                 |                      | SA04        | March 2018      |                                                                                                  |
|                 |                      | SA05        | March 2018      |                                                                                                  |
| Producer #2     | Celorico da Beira (CB) | CA01        | November 2017   | His: 0.17 ± 0.02 ± 0.01, Leu-Ile: 0.20 ± 0.02 ± 0.02, Lys: 2.1 ± 0.1 ± 0.3, Met: 29 ± 1 ± 1, Phe: 0.19 ± 0.01 ± 0.01, Thr: 0.90 ± 0.09 ± 0.1, Trp: 13 ± 1 ± 1 |
|                 |                      | CA02        | February 2018   |                                                                                                  |
|                 |                      | CA03        | March 2018      |                                                                                                  |
| Producer #3     | Penalva do Castelo (PC) | CI01        | December 2017   | His: 0.32 ± 0.02 ± 0.01, Leu-Ile: 0.5 ± 0.3 ± 0.01, Lys: 1.8 ± 0.2 ± 0.1, Met: 31 ± 1 ± 1, Phe: 0.41 ± 0.02 ± 0.01, Thr: 1.0 ± 0.0 ± 1, Trp: 14 ± 2 ± 1 |
|                 |                      | CI02        | March 2018      |                                                                                                  |
|                 |                      | CI03        | March 2018      |                                                                                                  |
| Producer #4     | Penalva do Castelo (PC) | QG01        | December 2017   | His: 0.11 ± 0.01 ± 0.1, Leu-Ile: 0.54 ± 0.02 ± 0.1, Lys: 1.5 ± 0.8 ± 0.1, Met: 31 ± 1 ± 1, Phe: 0.57 ± 0.04 ± 0.3, Thr: 0.69 ± 0.03 ± 1, Trp: 13 ± 0 ± 3 |
|                 |                      | QG02        | February 2018   |                                                                                                  |
|                 |                      | QG03        | February 2018   |                                                                                                  |
|                 |                      | QG04        | March 2018      |                                                                                                  |
| Producer #5     | Gouveia (G)          | QS01        | November 2017   | His: 0.43 ± 0.05 ± 0.3, Leu-Ile: 0.59 ± 0.5 ± 0.3, Lys: 3.9 ± 0.5 ± 0.5, Met: 52 ± 8 ± 2, Phe: 1.0 ± 0.2 ± 1, Thr: 5.5 ± 0.6 ± 2, Trp: 19 ± 2 ± 2 |
|                 |                      | QS02        | January 2018    |                                                                                                  |
|                 |                      | QS03        | February 2018   |                                                                                                  |
|                 |                      | QS04        | March 2018      |                                                                                                  |
|                 |                      | QS05        | March 2018      |                                                                                                  |
| Producer #6     | Nelas (N)            | QL01        | November 2017   | His: 0.24 ± 0.01 ± 0.1, Leu-Ile: 0.25 ± 0.01 ± 0.1, Lys: 1.7 ± 0.1 ± 1, Met: 31 ± 2 ± 1, Phe: 0.27 ± 0.02 ± 0.1, Thr: 5.4 ± 0.4 ± 1, Trp: 18 ± 1 ± 1 |
|                 |                      | QL02        | December 2017   |                                                                                                  |
|                 |                      | QL03        | February 2018   |                                                                                                  |
|                 |                      | QL04        | March 2018      |                                                                                                  |

**Mean ± standard deviation:**

Minimum value:

Maximum value:

*a* The last number in the sample code refers to the independent cheese sample number of each cheese (1: sub-sample number 1; 2: sub-sample number 2).

*b* His: Histidine; Leu-Ile: Leucine-Isoleucine; Lys: Lysine; Met: Methionine; Phe: Phenylalanine; Thr: Threonine; Trp: Tryptophan; and, Val: Valine.
Table 5
Non-essential free amino acids contents (mean ± standard deviation of 3 injections × 2 extraction per cheese, mg/100 g of cheese in wet basis) determined by UPLC-DAD-MS/MS, for each of the two independent samples taken from each of the 24 Serra do Estrela PDO cheeses acquired from 6 certified cheese producers, located in 5 municipalities (Celorico da Beira – CB, Gouveia – G, Nelas – N, Oliveira do Hospital – OH, and Penalva do Castelo – PC) within the PDO delimited geographical region and produced from November 2017 till March 2018 (values calculated using the data provided in the supplementary material file Table_S3_DB-D-19-02387.xlsx).

| Cheese Producer | Geographical origin | Cheese code⁴ | Production Date | Non-essential free amino acids⁴ contents (mean ± standard deviation) in mg/100 g of cheese (wet basis) |
|-----------------|---------------------|-------------|----------------|------------------------------------------------------------------------------------------------------------------|
| Producer #1     | Oliveira do Hospital (OH) | SA01        | November 2017  | 0.11 ± 0.01 14 ± 1 0.35 ± 0.03 78 ± 7 0.32 ± 0.01 23 ± 2 0.24 ± 0.01 0.08 ± 0.02 0.50 ± 0.06 |
|                 |                     | SA02        | January 2018   | 0.19 ± 0.00 18 ± 1 0.46 ± 0.04 59 ± 9 0.24 ± 0.02 28 ± 2 0.50 ± 0.02 2.0 ± 0.2 0.67 ± 0.05 |
|                 |                     | SA03        | February 2018  | 0.15 ± 0.01 8 ± 1 0.21 ± 0.02 41 ± 1 0.06 ± 0.00 14 ± 1 0.57 ± 0.02 0.56 ± 0.06 |
|                 |                     | SA04        | March 2018     | 0.15 ± 0.00 4 ± 1 0.17 ± 0.01 27 ± 2 0.05 ± 0.02 11 ± 1 0.26 ± 0.01 1.3 ± 0.1 |
|                 |                     | SA05        | March 2018     | 0.10 ± 0.01 18 ± 1 0.45 ± 0.02 77 ± 8 0.65 ± 0.04 12 ± 1 26 ± 3 0.04 ± 0.00 0.9 ± 0.3 |
| Producer #2     | Celorico da Beira (CB) | CA01        | November 2017  | 0.06 ± 0.00 25 ± 1 0.66 ± 0.07 131 ± 5 0.15 ± 0.01 1.6 ± 0.2 32 ± 4 0.13 ± 0.00 0.28 ± 0.05 |
|                 |                     | CA02        | February 2018  | 0.08 ± 0.00 20 ± 1 0.39 ± 0.02 117 ± 17 0.19 ± 0.04 2.0 ± 0.1 32 ± 3 0.29 ± 0.04 0.45 ± 0.02 |
|                 |                     | CA03        | March 2018     | 0.06 ± 0.01 15 ± 1 0.34 ± 0.02 95 ± 10 0.04 ± 0.00 1.3 ± 0.1 27 ± 3 0.16 ± 0.03 0.34 ± 0.08 |
| Producer #3     | Penalva do Castelo (PC) | CI01        | December 2017  | 0.07 ± 0.01 37 ± 1 1.4 ± 0.1 78 ± 13 0.16 ± 0.01 0.50 ± 0.02 49 ± 7 0.23 ± 0.01 0.24 ± 0.03 |
|                 |                     | CI02        | March 2018     | 0.06 ± 0.00 32 ± 2 1.4 ± 0.2 77 ± 1 0.36 ± 0.02 1.1 ± 0.5 46 ± 1 0.24 ± 0.01 0.24 ± 0.02 |
|                 |                     | CI03        | March 2018     | 0.07 ± 0.00 33 ± 0 1.2 ± 0.1 83 ± 4 0.29 ± 0.01 11 ± 1 51 ± 1 0.37 ± 0.03 0.20 ± 0.03 |
| Producer #4     | Penalva do Castelo (PC) | QG01        | December 2017  | 0.07 ± 0.01 16 ± 2 0.49 ± 0.02 84 ± 9 0.54 ± 0.01 0.9 ± 0.2 25 ± 4 0.29 ± 0.05 0.37 ± 0.03 |
|                 |                     | QG02        | February 2018  | 0.09 ± 0.01 21 ± 1 0.34 ± 0.06 33 ± 1 0.48 ± 0.02 2.7 ± 0.1 28 ± 1 0.63 ± 0.2 0.48 ± 0.03 |
|                 |                     | QG03        | February 2018  | 0.07 ± 0.01 25 ± 3 0.51 ± 0.03 93 ± 13 0.20 ± 0.02 3.5 ± 0.2 35 ± 4 2.1 ± 0.1 0.31 ± 0.08 |
|                 |                     | QG04        | March 2018     | 0.07 ± 0.00 24 ± 4 0.5 ± 0.2 50 ± 7 0.46 ± 0.023 2.2 ± 0.2 33 ± 3 2.6 ± 0.3 0.30 ± 0.02 |
| Producer #5     | Gouveia (G)         | QS01        | November 2017  | 0.16 ± 0.01 76 ± 1 2.4 ± 0.2 99 ± 4 2.1 ± 0.1 9 ± 2 60 ± 4 2.6 ± 0.7 0.75 ± 0.09 |
|                 |                     | QS02        | January 2018   | 0.38 ± 0.11 27 ± 4 0.7 ± 0.4 83 ± 11 0.54 ± 0.11 3.2 ± 0.6 35 ± 6 0.4 ± 0.2 0.8 ± 0.2 |
|                 |                     | QS03        | February 2018  | 0.14 ± 0.01 27 ± 2 0.62 ± 0.03 56 ± 8 0.46 ± 0.02 1.3 ± 0.1 36 ± 2 1.7 ± 0.5 0.59 ± 0.04 |
|                 |                     | QS04        | March 2018     | 0.10 ± 0.00 8 ± 1 0.7 ± 0.3 34 ± 6 0.09 ± 0.02 0.8 ± 0.1 13 ± 2 1.6 ± 0.3 0.50 ± 0.08 |
|                 |                     | QS05        | March 2018     | 0.11 ± 0.01 23 ± 2 1.3 ± 0.1 70 ± 7 0.20 ± 0.02 2.4 ± 0.1 31 ± 3 0.13 ± 0.04 0.54 ± 0.02 |
| Producer #6     | Nelas (N)           | QL01        | November 2017  | 0.10 ± 0.01 33 ± 2 1.9 ± 0.2 93 ± 8 0.24 ± 0.00 0.61 ± 0.06 46 ± 6 0.56 ± 0.04 0.9 ± 0.2 |
|                 |                     | QL02        | December 2017  | 0.10 ± 0.00 28 ± 2 0.7 ± 0.1 73 ± 4 0.13 ± 0.02 0.25 ± 0.02 40 ± 3 0.36 ± 0.04 0.73 ± 0.06 |
|                 |                     | QL03        | February 2018  | 0.07 ± 0.01 25 ± 4 1.4 ± 0.4 52 ± 10 0.24 ± 0.03 0.68 ± 0.20 34 ± 6 0.16 ± 0.03 1.12 ± 0.02 |
|                 |                     | QL04        | March 2018     | 0.08 ± 0.00 25 ± 2 0.4 ± 0.1 107 ± 8 0.07 ± 0.00 0.44 ± 0.01 34 ± 2 0.20 ± 0.03 0.51 ± 0.02 |

Mean ± standard deviation:
0.11 ± 0.07 24 ± 14 0.8 ± 0.6 75 ± 27 0.35 ± 0.41 2.0 ± 2.7 33 ± 12 1.0 ± 1.4 0.53 ± 0.28
Minimum value:
0.05 4 0.2 24 0.04 0.2 9 0.04 0.1
Maximum value:
0.49 78 2.6 143 2.2 13 64 7 1.4

⁴ The last number in the sample code refers to the independent cheese sample number of each cheese (1: sub-sample number 1; 2: sub-sample number 2).

*Arg: Arginine; Asn: Asparagine; Asp: Aspartic acid; Cys: Cysteine; Gln: Glutamine; Glu: Glutamic acid; Pro: Proline; Tyr: Tyrosine; and, Ser: Serine.*
Data reported on cheeses’ moisture, total fat, total protein and salt contents were experimentally obtained by NIR spectrophotometry. Data on cheeses’ free amino acids profiles were determined using UPLC-DAD-MS/MS chromatography.

**Fig. 3.** Boxplots for essential free amino acids contents raw data (histidine, leucine-isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, in mg/100 g of cheese wet basis) of 24 Serra da Estrela PDO cheeses (2 independent samples per cheese) determined by UPLC-DAD-MS/MS (triplicate assays), according to the certified cheese producer (Producers 1 to 6). Dairy farms were located in 5 municipalities (Celorico da Beira — CB, Gouveia — G, Nelas — N, Oliveira do Hospital — OH, and Penalva do Castelo - PC) within the PDO geographical region and the cheeses were produced between November 2017 and March 2018, being acquired after 45 days of maturation (values calculated using the data provided in the supplementary material file Table_S3_DIB-D-19-02387.xlsx).
2.2. NIR spectrophotometry: instrumentation, cheese samples preparation and moisture, total fat, total protein and salt contents data

Regarding the NIR analysis [3], from each cheese, 1.5 cm of the rind was removed and a slice of approximately 100 g was placed in a flat-bottom glass cuvette and analysed, in triplicate, on a NIR-Master™ spectrophotometer from Buchi NIRSolutions™ (Flawil, Switzerland). The instrument comprised a polarisation interferometer with TeO2 wedges, an extended range InGaAs detector (with temperature control) that worked in diffuse reflectance within a spectral range of 800–2500 nm (resolution: 8 cm⁻¹). Data were acquired and treated using the NIRWare™ software package (Buchi NIRSolutions™). Blank signals were obtained using an external reference Spectralon®. The internal background was measured with a gold plate reflector. Broad-based calibrations were used and adjusted with Serra da Estrela cheese samples. Each sample was analysed 3–5 times (replicas) and for each assay the spectra were recorded (data not shown), allowing determining the experimental levels of moisture, total fat, total protein and salt contents using pre-established calibration curves, being the raw data provided in supplementary material, as Excel file (file containing the raw physicochemical data determined based on NIR data: Table_S1_DIB-D-19-02387.xlsx). The raw mean data and respective standard deviation values (in %) are provided in Table 1 for each cheese sample, together with the identification of the cheese producer, the geographical origin location of the dairy industry and the cheese production date. Globally, moisture ranged from 43 to 54% (mean value: 49 ± 3%), total fat varied between 16 and 31% (mean value: 24 ± 4%), total protein ranged from 18 to 30% (mean value: 22 ± 2%) and finally, salt level varied within 0.7–2.2% (mean value: 1.2 ± 0.4%).

Fig. 4. Boxplots for non-essential free amino acids contents raw data (arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, proline, tyrosine and serine, in mg/100 g of cheese wet basis) of 24 Serra da Estrela PDO cheeses (2 independent samples per cheese) determined by UPLC-DAD-MS/MS (triplicate assays), according to the certified cheese producer (Producers 1 to 6). Dairy farms were located in 5 municipalities (Celorico da Beira – CB, Gouveia – G, Nelas – N, Oliveira do Hospital – OH, and Penalva do Castelo - PC) within the PDO geographical region and the cheeses were produced between November 2017 and March 2018, being acquired after 45 days of maturation (values calculated using the data provided in the supplementary material file Table_S3_DIB-D-19-02387.xlsx).
Fig. 5. Boxplots for essential free amino acids contents raw data (histidine, leucine-isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, in mg/100 g of cheese wet basis) of 24 Serra da Estrela PDO cheeses (2 independent samples per cheese) determined by UPLC-DAD-MS/MS (triplicate assays), according to the production date (November 2017, December 2017, January 2018, February 2018 and March 2018). Dairy farms were located in 5 municipalities (Celorico da Beira – CB, Gouveia – G, Nelas – N, Oliveira do Hospital – OH, and Penalva do Castelo – PC) within the PDO geographical region and the cheeses were produced by 6 certified cheese producers (Producer 1 to 6), being acquired after 45 days of maturation (values calculated using the data provided in the supplementary material file Table_S3_DIB-D-19-02387.xlsx).
The experimental data (Table 1) imply that the cheese producer/geographical origin and the cheese production date influenced the moisture, total fat, total protein and salt contents determined by NIR spectrophotometry, as can be inferred from the boxplots shown in Figs. 1-2, respectively (raw data can be found in the provided supplementary material, namely in the Excel file: Table_S1_DIB-D-19-02387.xlsx).

2.3. UPLC-DAD-MS/MS chromatography: instrumentation, cheese samples preparation and free amino acids data

Data concerning the free amino acids (FAA) contents of the Serra da Estrela PDO cheeses were obtained by UPLC-DAD-MS/MS [4]. A chromatographic system (Dionex Ultimate 3000 UPLC instrument from Thermo Scientific, USA) equipped with a diode-array detector (DAD) and coupled to Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) with an ESI source was used. An U-VDSpher PUR C18-E (100 mm × 2.0 mm id, 1.8 μm) column (VDS optilab, Germany) was used, at 40 °C, allowing the detection and quantification of FAA. Two solvents were used as mobile phase, namely solvent A (0.1% (v/v) formic acid in water) and solvent B (0.1% (v/v) formic acid in acetonitrile/water (50:50, v/v)). A multistep gradient program was used (Table 2) at a flow rate of 0.40 mL/min, being the injection volume of 5 μL. Each chromatographic assay comprised a 10 min run.
MS detection was performed in positive mode by multiple reaction monitoring (MRM) with nitrogen as the sheath gas (50 psi). The system was operated with a spray voltage of 5.5 kV, a source temperature of 400 °C and a capillary voltage of 18 V. The tube lens offset was kept at a voltage of 25 V. Mass spectra were acquired by full range acquisition covering 100–1500 \( m/z \). For each amino acid the mass spectrometry parameters, confirmation and quantification mass transition \( (m/z) \), and their collision energies were determined [4]. Data acquisition was carried out with Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA). Peaks identification was achieved based on the retention time (min), the quantification transition \( (m/z) \) and the confirmatory transition \( (m/z) \) data. Globally, the developed chromatographic methods allowed establishing calibration curves for 19 amino acids and thus it was possible to detect and quantify 8 essential (histidine, His; leucine-isoleucine, Leu-Ile; lysine, Lys; methionine, Met; phenylalanine, Phe; threonine Thr; tryptophan, Trp; and, valine, Val) and 11 non-essential amino acids (alanine, Ala; arginine, Arg; asparagine, Asn; aspartic acid, Asp; cysteine, Cys; glutamic acid, Glu; glutamine, Gln; glycine, Gly; proline, Pro; tyrosine, Tyr; and, serine, Ser), plus cystine (Cys-Cys). Since the amino acids detection/quantification was based on mass detection, isomers were quantified together, namely leucine and isoleucine. Stock standard solutions (10 nmol/\( \mu L \)) of each amino acid, were prepared using distilled water. Calibration curves were established by linear regression for each of the amino acids for concentration dynamic ranges of 0–3 nmol/\( \mu L \), being established, in general, different linear calibrations (data not shown) for each amino acid considering the concentration intervals for which a linear correlation could be observed between the peak area ratios and the amino acid concentration. The different linear dynamic concentration ranges and the determination coefficients \( (R^2) \) of the calibration curves that can be established for each amino acid and respective concentration interval, are reported in Table 3 (raw data of the peak areas ratios as well as the mean values, standard deviations and respective coefficient of variation can be found in the provided supplementary material, namely in the Excel file: Table_S2_DIB-D-19-02387.xlsx). The internal standard (IS) method was used for calibration purposes using N-Acetyl-\( \alpha \)-Tyrosine as the internal standard since it was not detected in the protein fraction of the cheese samples. The limits of detection and quantification (data not shown, but can be calculated using the raw data provided as supplementary material in file Table_S2_DIB-D-19-02387.xlsx) of the established UPLC-DAD-MS/MS method were previously reported [4] and ranged, respectively, from 0.20 \( \mu \)mol/L, for aspartic acid, to 44.2 \( \mu \)mol/\( \mu L \), for alanine, and 0.62 \( \mu \)mol/L to 134 \( \mu \)mol/L, for the same amino acids. The intra-day repeatability intervals (expressed as the coefficient of variation in percentage, CV%) are given in Table 3, for each amino acid, corresponding to the minimum–maximum values calculated based on 3 injections of each standard solution concentration used to establish the calibration curves, and varied from 0.02 to 10.3%, corresponding, in general, the highest values to the lowest concentrations and/or responses (raw data of the peak areas ratios as well as the mean values, standard deviations and respective coefficient of variation can be found in the provided supplementary material, namely in the Excel file: Table_S2_DIB-D-19-02387.xlsx).

For free amino acids quantitation in Serra da Estrela PDO cheeses, the following experimental procedure was implemented. From each cheese two independent samples of approximately 5 g were collected and inserted into flasks containing 10 mL of water:acetonitrile (50:50) (\( v/v \)) solution with 3.0 mM of \( N \)-Acetyl-\( \alpha \)-Tyrosine (used as the IS). The mixture was shaken using a vortex (LBX V05 series, LABBOX LABWARE S.L., Barcelona, Spain) at 500 rpm for 5 min and then sonicated for 10 min at room temperature (20 °C). The samples were immediately centrifuged at 4 °C and 10000 rpm during 10 min (Heraeus Multifuge X1R, Thermo Fisher Scientific). The supernatant was filtered, under vacuum, through a 0.2 \( \mu \)m Nylon membrane filter (Whatman PURADISC 25 NYL) and stored at −4 °C until analysis.

The UPLC-DAD-MS/MS allowed detecting and quantifying in the cheese samples 8 essential free amino acids (histidine, leucine-isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine; being the isomers leucine and isoleucine quantified together) and 9 non-essential free amino acids (arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, proline, tyrosine and serine). Alanine and glycine in the free amino acids forms were not detected in any cheese sample (if present their contents were lower than the respective limits of detection: 0.787 and 0.073 mg/100 g of cheese in wet basis), neither cystine (which, if present, the contents were lower that the limit of detection: 0.024 mg/100 g of cheese in wet basis). The raw data regarding the ratios between the free
amino acid peak area versus the internal standard peak area, per cheese independent sample and per chromatographic assay (each sample was analysed in triplicate), withdrawn from each of the 24 Serra da Estrela PDO cheeses, as well as the respective free amino acids contents (mg/100 g of cheese, wet basis) calculated using the experimental peak area ratios for each sample (provided as supplementary material in the file Table_S3_DIB-D-19-02387.xlsx) and the calibration curves that can be established using the information provided as supplementary material (Table_S2_DIB-D-19-02387.xlsx), are also reported in the supplementary material, as an Excel file (Table_S3_DIB-D-19-02387.xlsx). The final mean amino acids concentrations and the respective standard deviation values, in mg FAA per 100 g of cheese (wet basis) are reported in Tables 4 and 5, for the essential and non-essential free amino acids, respectively (as mentioned, these data can be calculated using the raw data provided in the supplementary material: Table_S2_DIB-D-19-02387.xlsx and Table_S3_DIB-D-19-02387.xlsx). Free amino acids data are reported for each cheese sample plus the identification of the cheese producer, the geographical origin location of the dairy farm and the cheese production date. The data pointed out that among the essential free amino acids detected in the evaluated cheeses, leucine-isoleucine, phenylalanine and serine were the most abundant ones. Similarly, within the detected non-essential free amino acids, cysteine, proline and asparagine were the most abundant ones. The experimental data (Tables 4 and 5) pointed out that the essential and non-essential free amino acids mean contents, assessed by UPLC-DAD-MS/MS (2 independent samples × 24 cheeses × 3 chromatographic analysis) were influenced either by the cheese producer/geographical origin and the cheese production date (as well as, in some cases, among samples of the same cheese), which can be checked by observing the boxplots shown in Figs. 3–4 and Figs. 5–6, respectively (data can be found in the provided supplementary material, namely in the Excel file Table_S3_DIB-D-19-02387.xlsx).

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.104908.

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