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Development of a UPLC-MS/MS method for establishing Serra da Estrela's free amino acids profile

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Serra da Estrela cheese is a high-value and widely appreciated Portuguese cheese, which as a Protected Designation of Origin recognition, being its production legally regulated. The protein portion of foods, as well as its amino acids composition (namely the essential amino acids), represents a fundamental role on the nutritional and technological value of cheese, influencing greatly its flavour [1,2]. A UPLC–MS/MS, comprising a Dionex Ultimate 3000 UPLC instrument equipped with a diode-array detector coupled to a mass detector, was used to establish the free amino acids profile of Serra da Estrela cheeses. The amino acids chromatographic separation was accomplished using a U-VDSpher PUR C18-E column (100 mm×2.0 mm id, 1.8 µm column) at 40 °C. The MS detection was performed in positive mode by multiple reaction monitoring (MRM) using a Linear Ion Trap LTQ XL mass spectrometer (equipped with an ESI source. Mass spectra were acquired by full range acquisition covering 100–1500 *m/z*. The collision energy used varied from 14 to 30 (arbitrary units) depending on amino acid. Cheese, from 6 certified producers, were acquired after 45 days of ripening, being the amino acids extracted using a water-acetonitrile solution, to which N-acetyl-L-tyrosine was added (as an internal standard). In total, 21 amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, valine, methionine, 4-hydroxy-proline, isoleucine, leucine, phenylalanine, tryptophan and tyrosine) were assessed (**Table 1**) and detected in the cheese samples, being the results in accordance with previous works [1]. The chromatographic method developed showed to be very accurate and allowed overcome experimental drawbacks arisen with conventional liquid chromatography.

Table 1. Free amino acids detection by UPLC–MS/MS

Amino acid	Quantification transition (m/z)	Confirmatory transition (m/z)	Amino acid	Quantification transition (m/z)	Confirmatory transition (m/z)
Histidine	156	137, 111, 109, 94	4-Hydroxy-Proline	132	85
Lysine	147	130, 129, 100	Isoleucine	132	120, 114, 104, 86, 85, 71, 68
Glutamine	147	129, 100, 83	Leucine	132	120, 114, 104, 86, 85, 71, 68
Glutamic acid	148	130, 129, 101, 83	Asparagine	133	115, 112, 104, 87, 89, 85
Serine	106	88, 87, 85, 59	Arginine	175	157, 140, 130, 115, 111, 97
Alanine	90	68, 61	Phenylalanine	166	148, 130, 119
Glycine	76	75, 47, 29	Tryptophan	205	187, 159, 132
Threonine	120	101, 99, 83, 73, 71, 55	Cysteine	121	98, 97, 75
Aspartic acid	134	115, 87, 86, 73	Tyrosine	182	164, 135
Valine	117	100, 90, 71	Cystine	241	224, 14, 177, 168, 93, 151
Methionine	150	132, 103, 101, 55			

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