Generation of dipeptides in low-salted Spanish dry-cured ham

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Objectives: dry-cured ham processing involves a set of biochemical reactions from which proteolysis leads to the release of dipep- tides with relevant taste and bioactivity. In addition, although salting is a crucial step in the production, there is a growing interest in cutting down the amount of salt from foods. However, salt can act as a prooxidant and has inhibitory effects on the proteolytic enzymes, which can lead to flavor modulation. Moreover, these short peptides have high relevance as potential bioactives due to their resistance to be degraded during digestion. Unfortunately, there is not enough data about their generation, role on the typical dry-cured ham flavor development and bioactive properties. For these reasons, research has been done for the identification and quantitation of dry-cured ham-derived dipeptides through the optimization of mass spectrometry-based approaches. Their contribu- tion to flavor and potential cardiovascular health benefits have also been evaluated.

Materials and Methods: Hydrophilic interaction liquid chromatography (HILIC) coupled to a triple quadrupole (QQQ) mass spectrometer was used to quantitate dipeptides in 12 months-aged Spanish drycured hams elaborated with a lower content of salt. The *in vitro* inhibitory activity of angiotensin-I converting enzyme (ACE-I) of the identified peptides was evaluated along with an *in silico* strategy based on bioinformatic tools to find out the potential antihypertensive properties of the identified dipeptides. Finally, the *in vivo* oral administration of the AA dipeptide to spontaneous hypertensive rats (SHRs) was performed to test its potential hypotensive bioactivities.

Results and Discussion: The dipeptides AA, DA, DG, EE, ES, GA PA, and VG were identified and quantitated in dry-cured hams elaborated with a lower amount of salt than standard hams. The resulting amounts were 180.89 and 44.88 μg/g dry-cured ham for AA and GA dipeptides, respectively; and values ranging from 2 to 8 μg/g dry-cured ham for VG, EE, ES, DA, and DG dipeptides. PA showed the lowest concentration with a value of 0.18 μg/g dry-cured ham. Such amounts were not statistically different from those found in traditionally-elaborated 12 months-aged dry-cured hams except for the dipeptides DA, PA, and VG, whose concentrations were significantly higher in traditional dry-cured hams. Although a greater peptide amount was expected for the low-salted samples, the outcomes might be due to the larger salt content in traditionally elaborated dry-cured hams that inhibits peptidases and therefore dipeptides tend to accumulate. Some of these dipeptides are taste active, thus, the differences in flavor between tradition- ally elaborated and low-salted dry-cured hams might partly be due to the dipeptides concentration fluctuations. *In vitro* ACE-I inhibitory activity assays indicated that AA, GA and VG are strong inhibitors with half maximum inhibitory activity (IC_{50}) values of 110.8, 516.88 and 377.67 μM, respectively. The dipeptide AA, with the lowest IC_{50} value, was then orally administered at a dose of 1 mg/kg body weight to SHRs, exerting a significant decrease on the systolic blood pressure (SBP) starting from 4 h after adminis- tration, up to a maximum decrease of 15.97 3.67 mmHg at 8 h. SBP was restored to control-like values after 24 h of administration. Overall, the outcomes from these studies support the high potential of the dipeptides to exert a hypotensive bioactivity. Besides, their higher bioavailability in comparison with longer peptides makes them of relevance for further research.

Conclusions: The dipeptides AA, DA, DG, EE, ES, GA PA, and VG, were quantitated in dry-cured hams elaborated with a lower amount of salt, by means of hydrophilic interaction liquid chromatography coupled to a triple quadrupole mass spectrometer, ex- hibiting concentrations of μg/g dry-cured ham order. The difference of dipeptide concentrations between traditional and low-salted dry-cured hams might contribute to sensory distinctions. The IC_{50} of various dipeptides as well as the *in vivo* outcomes, demon- strate the high potential of the identified peptides to exert hypotensive effects and the multifunctional properties of dry-cured ham in view of its content in bioactive dipeptides.

References:

Heres, A., Yokoyama, I., Gallego, M., Toldrá, F., Arihara, K., & Mora, L. (2021). Antihypertensive potential of sweet Ala-Ala dipep- tide and its quantitation in dry-cured ham at different processing conditions. *Journal of Functional Foods*, 87, 104818.

Heres, A., Gallego, M., Mora, L., & Toldrá, F. (2022). Identification and Quantitation of Bioactive and Taste- Related Dipeptides in Low-Salt Dry-Cured Ham. *International Journal of Molecular Sciences*, 23(5), 2507.

Gallego, M., Toldrá, F., & Mora, L. (2022). Quantification and in silico analysis of taste dipeptides generated during dry-cured ham processing. *Food Chemistry*, 370, 130977.

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