THE QUALITY OF ANALYTICAL PARAMETERS AS A KEY FOR THE ESTABLISHMENT OF CORRECT ASSESSMENTS: INTERPRETATION OF TRANSFORMATIONS DURING THE HAM-CURED PROCESS

García-Garrido, J. A.[(1)], Quiles-Zafra, J. R.[(1)], Tapiador, J.[(1)] and
Luque de Castro, M. D.[(2)]
[(1)]Departamento de Calidad e Investigación, Grupo Navidul, S.A.
[(2)] Departamento de Química Analítica, Facultad de Ciencias, Universidad de Córdoba

1. Background

Standard analytical methods used for measurement of conventional parameters concerning the ham-cured process have been traditionally applied without previous studies of their confidence levels. A systematic study of these methods has been performed in order to establish the analytical quality they provide. The results obtained for some of these methods, in terms of precision, show relative standard deviations higher than 5%, which corroborate the necessity for improvement of the methods before implementing validation studied in order to guarantee the quality of the analytical results.

A number of papers devoted to the study of the evolution of key parameters for the ham-cured procedd have been appeared in the scientific literature in the last few years. The study of these parameters has been carried out either by official, old fashioned method of analysis, or by methods appeared in the literature which have been previously applied to samples with matrices similar or rather different from those of cured ham [1,2]. The use of these methods has not always been supported on an appropriate study of the analytical quality they provide. Example of this assertion is the validation of a mehod within a concentration range, then applied outside the validate range [3]. The use of old-fashioned, complex, slow and expensive methods instead of other which are simpler, faster and cheaper is a clear example of the lack of present information on the field [4].

2. Objectives

The general aim of the research here presented is to give a first step towards validation of analytical methods which are common-place for a specific area in food industrias as food-cured processes. The precision, sensitivity and linear range of the methods for monitoring the transformations that take place along the ham-cured process have been established. Other of the aims of this study is to have a confidence tool as the basis for an in depth study of the evolution of ham during the different steps of the cured process through the change of the key parameters of the process. The last and more important aim is the improvement of the quality of the final product: Spanish dry cured-ham [5].

2. Materials and Methods

Samples

Longissimus Dorsi muscle with 6-h post-mortem time, from 6-month animals was used. After quantitative removal of both fat and connective tissue, the muscle was cut into 100-g pieces, which were freezed to -20 °C. Before use the samples were stored overight in a chamber at 4 °C.

Composition analysis

The moisture, fat, protein and salt contents were determined according to the Official Methods of Analysis edited by the Spanish Health Ministery [6]. The standard method from AOAC [7] was used for the determination of sodium nitrite, whereas potassium nitrate was determined by ion-chromatography using a Perkin-elmer chromatograph equipped with a UV-photodiode array detector [8].

Proteolysis index

The method described by Córdoba et al. [9] was used for the determination of non-proteic nitrogen, after some modifications. In short, 5 g of samples was homogenously suspended in 6 vols of $HClO_4$. After 1 h at 4 °C, the extract was filtered through Whatman No. 6 filter paper, the pH adjusted to 6.00 with 30% (w/v) KOH solution and made to 100 ml with distillated H_2O. Finally, the Kjeldahl method was applied to a Buchi semiautomatic distillator.

The AOAC method [10] was used for the determination of α-amino nitrogen (α-amino-N). 50 ml of neutralized formaldehyde was added to the above-prepared filtered extract. After 20 min, 10 ml of 0.1 N standardized NaOH was added ad the mixture titrated with 0.1 N HCl. For the determination of volatile basic nitrogen (VBN) 50 ml of the previous extract was used, which was distillated in the presence of 5% (w/v) Na_2CO_3. The distillated was collected into 4% (w/v) H_3BO_3 aqueous solution, which was then titrated with 0.1 N HCl [11].

Enzymic activity

The *extract* was prepared as follows: 5-6 g of fat and connective tissue-free sample was suspended in 7 vols of 50 mM sodium acetate buffer of pH 5.00 0.01 containing 1 mM Na_2EDTA and 0.2% (v/v) Triton X-100 in order to obtaing an extract of lisosomal enzymes. The suspension was subjected to magnetic stirring for 1 h at 4 °C and then centrifuged at 32 000 g for 30 min in a Sigma 3K30 centrifuge. The extract thus obtained was filtered through glass-wool after deionisation by rinsing with ultrapure water [12]. The calpains extract was obtained from 3-g samples suspended into 9 vols of 50 mM Tris-HCl buffer of pH 7.50 0.01, which contained 3 mM Na_2EDTA and 150 nM pepstatin. The suspension was homogeneized and stirred for 1 h at 4 °C after adjusting its pH to 7.50 0.01. The suspension was centrifuged at 10 000 g for 10 min ad the supernatat filtered through glass-wool [13].

The assayed enzymes were lisosomal proteases (namely, catepsins B, B+L, H and D) and calcium-dependant neutral proteases (namely, calpains I and II) located in the cytoplasm. The *enzymic activities* were determined as follows: Catepsin D activity was determined at 45°C and pH 3.50 0.01 using a 10% (w/v) hemoglobin (Sigma) solution as substrate [14,15]. Catepsin B, B+L and H activities were determined after separation of fisiological inhibitors (cistatins)

by affinity chromatography. Catepsin B and B+L and H activities were all them determined at 37 °C, pH 6.00 0.01 for the B and B+L catepsin assay, and pH 6.08 0.01 for catepsin H assay. The following fluorescent substrates were used: N-CBZ-L-argynyl-L-arginine-7-amido-4-methylcoumarin (Z-Arg-Arg-NHMec) (Bachem), N-CBZ-L-phenylalanyl-L-arginine-7-amido-4-methylcoumarin (Z-Phe-Arg-NHMec) (Bachem) y N-CBZ-L-arginine-7-amido-4-methylcoumarin (Z-Arg-NHMec) (Sigma) respectively [12, 16]. Finally, total capain activity was determined after separation of its fisiological inhibitors by hydrophobicity chromatography. The enzymic assay was developed at 30 °C, pH 7.50 0.01 using 5 mg/ml casein (Merk) as substrate, 5 mg/ml $CaCl_2.2H_2O$ and 2 mM β-mercaptoethanol [14].

All reagents were of analytical degree.

3. Results and discussion

The results of the evaluation of the methods in terms of precision, are shown in Table 1.

The linearity of the calibration curves of the methods for the determination of enzymic activities has been studied by preparing a concentrated extract, from which and by successive dilutions, solutions of different concentration were obtained. The linearity study involved: a) calculation of the regression coefficient; b) application of ANOVA methods in order to verify assessment between the established linear model and the experimetal values [17]; c) polynomial regression analysis to check the unlikeness of a higher oder model with respect to the linear model [18].

The sensitivity of both the enzymic methods and $NaNO_3$ and KNO_3 methods has been established through the detection and quantitation limits [19].

5. References

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Table 1. Evaluation of the precision of the methods expresses as r.s.d.

PARAMETER	IMPRECISION WITHIN RUN	REPRODUCIBILITY BETWEEN RUN
%MOISTURE	0.33%	0.96%
%FAT	5.72%	10%
%PROTEIN	1.23%	2.85%
%NaCl	0.71%	1.06%
ppm de $NaNO_2$	0.48%	1.63%
ppm de KNO_3	0.26%	0.58%
NNP	2.79%	8.38%
N α-NH_2	3.11%	7.42%
NBV	2.49%	8.15%
cathepsin B	5.12%	7.42%
cathepsin B+L	5.37%	7.89%
cathepsin H	5.23%	7.12%
cathepsin D	4.74%	6.89%