DETERMINATION OF THE PROPORTION OF PORK AND CHICKEN IN FRESH SAUSAGE BY REAL-TIME PCR

Yamashita, D. M.[1*], Kumata, B. M.[1], Aoki, M. V. C[1], Haguiwara, M. M. H.[1], Lemos, A. L. S. C.[1]

[1] Ital, Campinas, Brasil.
*Corresponding author email: daniela@ital.sp.gov.br

I. INTRODUCTION

This work aimed to use the real-time PCR (qPCR) technique to quantify the quantity of chicken and porcine simultaneously in samples of fresh chicken sausage, which may contain pork protein in its composition due to the use of pig parts with high fat content, important for contributing to the specific flavor, aroma, and consistency of raw sausages [1]. The analyzed samples served as study material to test the qPCR technique to quantify the DNA of the two species in a meat product since it contains several ingredients, including salt, fat, and sugar, among others, which can make DNA extraction more laborious. The work becomes relevant since the definition of a method that can differentiate and measure the levels of different animal species simultaneously, using the multiplex technique, which makes quantification more agile, can be applied in cases of suspected adulteration in meat products, whether intentional or not.

II. MATERIALS AND METHODS

The samples of fresh chicken sausage were prepared in a pilot plant at the Meat Technology Center of the Institute of Food Technology (CTC-Ital). In sample 125*, the chicken cut used was the chicken breast with skin, and in sample 126*, cuts of chicken thighs and drumsticks with skin. In both samples, pork jowl was added in a smaller quantity. The remainder of the bulk from both samples comprised the basic ingredients for the preparation of fresh sausage.

DNA extraction was carried out using the ReliaPrep™ gDNA Tissue Miniprep System column extraction kit, Promega. The selection of primers used for this study, as well as the hydrolysis probes, was based on the study by Fröder (2022) [2]. Table 1 presents information about the primers and probes used. Two control samples were also tested to confirm the reliability of the results. These samples were prepared in the laboratory with fresh pork and chicken meat, in different proportions (Table 2).

Table 1 – Primers and hydrolysis probes used in this study.

Name	Reference gene	Sequence	Target
Porcine-97bp-F	Beta actin (DQ452569.1)	5′-CGTAGGTGCACAGTAGGTCTGAC-3′	*Sus scrofa domesticus; S. scrofa*
Porcine-97bp-R		5′-GGCCAGACTGGGGACATG-3′	
Porcine-97bp-P		5′-[FAM]-CCAGGTCGGGGAGTC-[NFQ-MGB]-3′	
Chicken-77bp-F	TGF-β3 (AY685072.1)	5′-CAGCTGGCCTGCCGGC-3′	*Gallus gallus domesticus; G. gallus*
Chicken-77bp-R		5′-GCCCAGTGGAATGTGGTATTCA-3′	
Chicken-77bp-P		5′-[FAM]-TGCCACTCCTCTGCACCCAGTGC-[TAMRA]-3′	

The qPCR technique was applied to quantify the two species under study, and the standard curve method was used, which provides the results of the absolute quantification of the samples. To carry out the method, the QuantiStudio™ 3 Real-Time PCR Instrument – Applied Biosystems, Thermo Fisher Scientific, was used as a thermoclycler. The standard curves for chicken and pork species were constructed separately, creating a series of 5 dilutions in a proportion of 1:10 for each.
For amplification of the curve points and samples, GoTaq® Probe qPCR Master Mix, Promega, was used for a 10 µL reaction, with 1 µL of sample for 9 µL of the master mix, primers and hydrolysis probes, and amplification conditions were: initial cycle at 95 °C/2 min; 40 ciclos: 95 °C/15 s e 54 °C/1 min.

III. RESULTS AND DISCUSSION

Table 2 presents the results obtained from qPCR for the samples of fresh chicken sausage with pork jowl and for the two control samples. Comparing the results of the calculated proportion with the initial proportion of the control samples, it is perceived that they are very similar to the prepared proportion, indicating the reliability of the results. When comparing the content of each species of the sausage samples with the initial proportion column (normalized), it is observed in sample 125* that the result was also very similar to what was expected; however, sample 126* presented a slightly greater deviation than expected for the initial content used in the preparation of the meat product. This can be explained by the difference in the cuts used to prepare the sausages, which may present DNA variation throughout the body structure of the animals studied. It can also be assumed that the equipment used in the preparation of the products may have remnants of the product fabricated previously and may have contributed to the increase in the DNA content of the chicken species in this sample.

Table 2 – Results obtained from qPCR for fresh sausage and control samples.

Sample identification	Proportion of species (%)				DNA (ng/reaction)		Calculated proportion of species (%)(3)	
	Initial(1)		Initial normalized(2)					
	Chicken	Porcine	Chicken	Porcine	Chicken	Porcine	Chicken	Porcine
SF50 (control)	50,00	50,00	50,00	50,00	15,997	15,592	50,64	49,36
SF80 (control)	80,00	20,00	80,00	20,00	6,289	23,051	21,43	78,57
125*	73,85	8,00	90,23	9,77	15,397	1,915	88,94	11,06
126*	73,85	8,00	90,23	9,77	17,432	0,915	95,01	4,99

(1) Proportion of mass used in sample preparation. In the case of sausage samples – 125 e 126* - this proportion is related to the total mass prepared, including the rest of the ingredients*

(2) Proportion of the mass used in sample preparation, normalized for sausage samples – 125 e 126* - and excluding the rest of the ingredients, considering only the mass of porcine and chicken protein*

(3) Proportion calculated from the DNA result provided by qPCR

IV. CONCLUSION

The study's findings indicate that the qPCR standard curve technique was found to be effective in quantifying DNA from chicken and porcine species in fresh sausage samples. Additionally, the technique's ability to calculate the proportion of each species was found to be relatively simple, suggesting that it could be a useful tool for identifying fraud in meat products. It is important that the study continues, including the investigation of other species and other meat products.

ACKNOWLEDGEMENTS

I am grateful to the CTC for the opportunity to conduct the research as well as for the funding to participate in the congress.

REFERENCES

1. Lemos, A. L. S. C.; Yamada, E. A.; Haguiwara, M. M. H. Processamento de embutidos cárneos. Campinas: Ital, Centro de Tecnologia de Carnes, 2008. 213p.
2. Fröder, H. Detecção de espécies animais utilizando PCR em tempo real (qPCR) para verificar fraudes em produtos cárneos processados. (Tese - Doutorado em Ciência e Tecnologia de Alimentos). Encantado, Universidade Estadual do Rio Grande do Sul, 2022. 69p.
3. BRASIL. MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO - MAPA. Secretaria de Defesa Agropecuária - SDA 2000. **Instrução Normativa nº 4, 31 de março de 2000**. Regulamento técnico de identidade e qualidade de linguiça. Disponível em: http://www.agais.com/normas/carne/carnes_linguica.htm. Acesso em: 03 jul. 2023.
4. Bustin, S. A. et al The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. **Clinical Chemistry**, Oxford, v. 55, n. 4, p. 611-622, 2009.