DETERMINATION OF CONTENT OF AMINO ACIDS AND PEPTIDES IN RAW AND SOUS-VIDE COOKED PORK DURING AGEING

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I. INTRODUCTION

Meat is one of the most valuable sources of proteins for human consumption. One main criterion of value of meat is its content of amino acids, especially the essential ones. However, recently other features of meat has come into focus of scientific research, specifically peptides, which are generated by proteolytic degradation during aging [1, 2]. Due to this degradation of proteins and polypeptides a large number of shorter peptides (<3 kDa) is released along with amino acids, and the amount of these peptides increase during ageing of meat. That kind of peptides are of interest because of their high bioavailability [3]. They also might have various bioactive properties, such as anti-inflammatory, antibacterial, antihypertensive [1, 2, 4], making them active participants in the processes in the human digestive tract instead of being only a passive source of amino acids. Here we present an investigation of protein, amino acid and peptide content during ageing in raw and sous-vide cooked pork *Longissimus dorsi* (LD).

II. MATERIALS AND METHODS

Longissimus dorsi (LD) were obtained from both sides of 10 slaughter pigs. Samples were kept at 4 ^{0}C for 1, 3, 6 and 13 days with parallel application of sous-vide cooking at 58 ^{0}C, heating time Tc+17 h (Tc, 3 h – time to reach a core temperature equal to the water bath) [5] for the half of the samples. Each sample of raw and sous-vide cooked LD was vacuum-packaged and stored at -20 ^{0}C till analyzed. Data of protein and amino acid content were obtained by Kjeldahl and HPLC methods. HPLC analysis was performed after hydrolysis of LD samples by HCl (6 M, 110 ^{0}C, 24 h). Isolation of peptides was performed as following – each of LD samples was homogenized with 0.02 M sodium phosphate buffer (pH 7.4), centrifuged at 15000 rpm for 20 min. at 4 ^{0}C, collected supernatant was submitted to ultrafiltration with 3 kDa MWCO membrane. Obtained fraction was lyophilized, the residue was dissolved in 10 ml of water and frozen till further analysis. The contents of peptides were determined using derivatization with o-phthaldehide and 2-aminothioethanol [4] with some modifications.

III. RESULTS AND DISCUSSION

The amount of protein obtained by Kjeldahl method showed no difference between raw and sous-vide cooked LD. However the analysis by HPLC revealed significant drop in protein concentration in the sous-vide cooked LD when compared to those of raw LD ($P<0.001$, Table 1), followed by a non-significant rise as the ageing time was prolonged.

Table 1 Influence of treatment and ageing time to protein concentration of aged raw and sous-vide cooked LD

P-values	Treatment*	Ageing time**	Treatment x Ageing time
Protein concentration	<0.001	<0.257	<0.207

*Treatment: raw and sous-vide cooked (58 ^{0}C, 17 h) LD
**Ageing of LD: at 4 ^{0}C for 1, 3, 6 and 13 days

The drop in protein concentration may be attributed to the formation of water-insoluble aggregates of sarcoplasmic proteins upon heating. HPLC analysis of the samples revealed that the amounts of amino acids in sous-vide LD indeed were lower than in raw LD ones, especially regarding the amounts of lysine and aromatic amino acids – phenylalanine and tryptophan, but the data was not statistically significant (Table 2, data is shown only for essential amino acids).

Table 2 Influence of treatment and ageing time to essential amino acid content in aged raw and sous-vide cooked LD

Amino acid P-values	Val	Leu	Ile	Met	Thr	His	Lys	Phe	Trp
Treatment*	0.819	0.528	0.226	0.279	0.529	0.776	0.304	0.29	0.898
Ageing time**	0.327	0.033	0.029	0.03	0.014	0.586	0.002	<0.001	<0.001
Treatment x ageing time	0.928	0.814	0.962	0.102	0.521	0.801	0.34	0.03	0.142

*Treatment: raw and sous-vide cooked (58 ^{0}C, 17 h) LD
**Ageing of LD at 4 ^{0}C for 1, 3, 6 and 13 days

The concentration of extracted peptides (Figure 1) remained almost constant from day 1 to day 3 of ageing and raised significantly from day 3 to day 6, and continued to raise in the following period up to day 13. The results suggest that degradation of meat proteins proceeds stepwise - at first proteins are cleaved to the large fragments mostly by endopeptidases and only then smaller peptides (<3 kDa) are released with the involvement both endo- and exopeptidases. Contrary for sous-vide cooked LD samples the relative largest increase of peptides was observed from day 1 to day 3 indicating that the protein fragments produced during this period are more prone to hydrolysis at the elevated temperature, possibly due to enhanced activity of endogenous proteolytic enzymes.

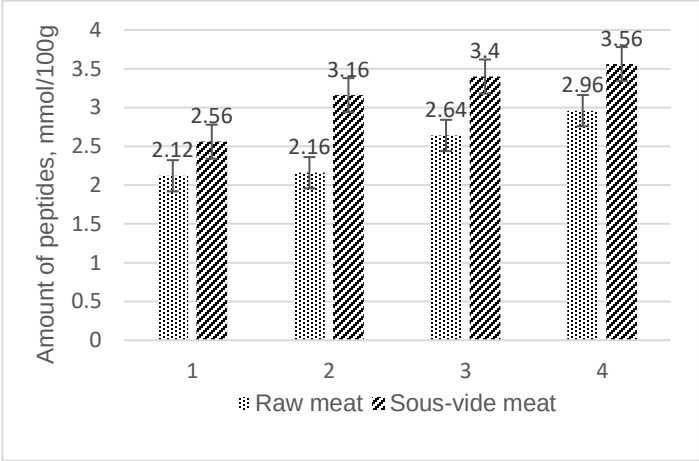

Figure 1. The concentration of peptides in raw and sous-vide cooked LD after 1 day (1), 3 days (2), 6 days (3) and 13 days (4) of ageing

IV. CONCLUSION

The reduced levels of proteins in sous-vide cooked LD indicate that sous-vide cooking method is not ideal regarding the content of amino acids, and yet the increased levels of low molecular weight peptides (<3 kDa) make the sous-vide cooking method attractive concerning easier digestibility thus bioavailability.

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REFERENCES

1. Lafarga, T., Hayes, M. (2014). Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. Meat Science, 98: 227-239.
2. Ryan, J. Th., Ross, R. P., Bolton, D., Fitzgerald, G. F., Stanton, C. (2011). Bioactive peptides from muscle sources: meat and fish. Nutrients, 3: 765-791.
3. Sayd, T., Chambon, C., Sante-Lhoutellier, V. (2016). Quantification of peptides released during *in vitro* digestion of cooked meat. Food Chemistry, 197: 1311-1323.
4. Liu, D., Chen, X., Huang, J., Huang, M., Zhou, G. (2017). Generation of bioactive peptides from duck meat during post-mortem aging. Food Chemistry, 237: 408-415.
5. Christensen, L., Ertbjerg, P., Aaslyng, M. D., Christensen, M. (2011). Effect of prolonged heat treatment from 48 ^{0}C to 63 ^{0}C on toughness, cooking loss and color of pork. Meat Science, 88: 280-285.