INVESTIGATING THE EFFECTS OF LOW-SALT PROCESSING ON THE UMAMI PEPTIDES OF DRY-CURED HAM USING PEPTIDOMICS TECHNIQUES

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I. INTRODUCTION

Salt contributes to the hydrolysis of muscle proteins in dry-cured hams, forming peptides and other small molecules that impart sweet, sour, salty, fresh, and bitter flavors, enriching and improving the sensory characteristics of the hams. The salt reduction can change the complex protein hydrolysis system, causing product variations [1]. The aim of this study was to compare the differences in umami peptides between low- and full-salt dry-cured hams using peptidomics.

II. MATERIALS AND METHODS

2.1 Dry-cured ham preparation

Dry-cured hams were processed as previously described [2]. Briefly, 24 bone-in fresh hind legs (10-12 kg for each) from Landrace pigs were purchased from a local slaughterhouse (Yanji, China) and they were equally assigned to two groups. The low-salt group was cured using a mixture of 70% NaCl, 18% potassium lactate, and 12% lysine, and the full-salt hams were cured with 100% NaCl. The amount of curing agent was 6% of each leg weight. *Biceps femoris* (BF) muscles from two groups at the end of processing were collected in marked aseptic bags and stored at -80 ℃.

2.2 Qualitative and relative quantitative analysis of peptides using peptidomics

Three samples were randomly selected from each group to extract peptides, and the method was referred to Zhang et al. [3]. Peptides were separated by the EASY-nLC 1000 ultra-high pressure liquid chromatography and then analyzed by a Q-Exactive mass spectrometer.

2.3 Identification of umami peptides

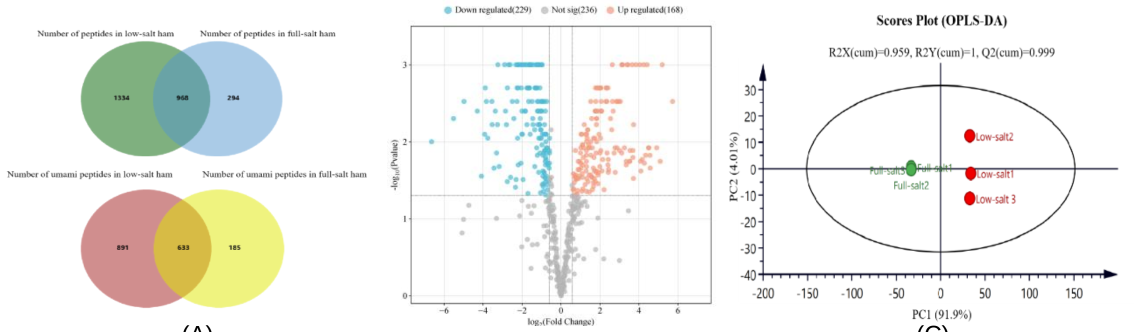
Peptide sequences were imported into the iUmami-SCM (https://camt.pythonanywhere.com/iUmami-SCM) to predict umami peptides in the low- and full-salt groups [4].

2.4 Data analysis

Orthogonal partial least squares-discriminant analysis (OPLS-DA) was performed using the software SIMCA version 14.1.0 (Umetrics, Umea, Sweden), and the significant significance was set as $P < 0.05$. Significantly different umami peptides (SDUPs) between the two groups were identified using variable importance in projection (VIP > 1.0) based on the weighted sum of the squares of the OPLS-DA.

III. RESULTS AND DISCUSSION

According to Figure 1A, 2,302 and 1,262 peptides were identified below 3 kDa in the low- and full-salt groups, respectively, among which 968 peptides were shared in both groups. According to the prediction results from the iUmami-SCM website, 633 umami peptides were shared by both low-salt hams (1,524 umami peptides) and full-salt hams (818 umami peptides). Besides, 62.72% (397/633) of the shared umami peptides showed significant changes in relative abundance. Specifically, the relative abundances of 168 and 229 umami peptides were significantly up- and down-regulated (fold change > 1.5 and $p < 0.05$) in the low-salt group compared with the full-salt group (Figure 1B). The OPLS-DA model was developed to discover characteristic umami peptides (Figure 1C). Finally, a total of 1,011 SDUPs were screened. Table 1 lists the protein sources of umami peptides with VIP score > 1.0 (Top 5). The difference in the relative abundance of umami peptides in low- and full-salt dry-cured hams was significantly affected by the hydrolysis of proteins such as creatine kinase M-type (CKM), fast skeletal muscle troponin T (TnTf) and myosin-1.



(A) (B) (C)

Figure 1. (A) Amounts of peptides and umami peptides in low- and full-salt dry-cured hams; (B) Volcano map; (C) OPLS-DA score plot: PC1 = 91.9%, PC2 = 4.01%, R2X(cum) = 0.959, R2Y(cum) = 1, Q2 = 0.999.

Table 1 – Precursor protein of the SDUPs with VIP score > 1.0 between the low- and full-salt groups.

Number	Protein names	Gene Names	Total VIP>1 SDUPs	Low-salt SDUPs	Full-salt SDUPs
1	Creatine kinase M-type	CKM	163	140	66
2	Fast skeletal muscle troponin T	TNNT3	149	147	23
3	Myosin-1	MYH1	113	90	53
4	Myosin light chain 1	MYL1	74	65	25
5	Phosphopyruvate hydratase	ENO3	63	54	13

Only up to five precursor proteins were shown.

IV. CONCLUSION

Overall, low-salt processing altered the umami peptide profiles of dry-cured hams, producing more unique umami peptides. There were 1,011 SDUPs in the low- and full-salt groups, which accounted for 59.36% of the total umami peptides. Meanwhile, 36.18% and 26.54% of the shared umami peptide abundance was down- and up-regulated in the low-salt group compared to the full-salt group. The SDUPs were mainly derived from differential hydrolysis of CKM, TnTf, and myosin-1. This study offers preliminary evidence for investigating flavor alterations in low-salt dry-cured hams.

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