Insight into ultrasound-induced modifications of the proteome and flavor-related proteins of unsmoked bacon by applying label-free technology

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Objectives: As people highly value the conception of healthy consumption, unsmoked bacon has been considered as an alternative. However, when compared with smoked bacon, unsmoked one has inferior flavor characteristics. Ultrasound is a novel technology that has the potential to be applied in the meat field (Alarcon-Rojo et al., 2015). A recent study found that the application of ultra- sound in unsmoked bacon could also commendably enhance the taste profile mainly by promoting the generation of free amino acids derived from protein degradation (Zhang, Zhang, & Xing, 2021). However, the effects of ultrasound on the changes of proteome and specific proteins closely related with taste improvement in unsmoked bacon are still unclear. Therefore, label-free quantitation (LFQ) approach combined with bioinformatics analysis was carried out to explore the effects of ultrasound on proteins changes.

Materials and Methods:

1. Sample preparation: referring to Zhang Zhang, and Xing (2021).
2. LFQ determination: referring to Fu et al. (2021).
3. Analysis of differentially abundant proteins (DAPs): by using bioinformatics methods.
4. Western blot: referring to Hou, Liu, Zhang, and Zhou (2019).

Results and Discussion:

1. Protein identification A total of 391 proteins were recognized by using LFQ and 378 proteins were shared in all groups of un- smoked bacon. Results showed that ultrasound mainly changed the protein intensities.
2. Screening DAPs A total of 106, 137 and 80 proteins were selected as DAPs between the 0 W group and the ultrasonic groups (250, 500 and 750 W), respectively. Considering that the 500 W group possessed the highest number of DAPs compared with the 0 W group, the DAPs between the 0 and 500 W groups were thus used for further analysis. To be specific, 111 DAPs were significantly up-regulated while 26 DAPs were significantly down-regulated in the 500 W group in comparison to the non-ultrasonic group, which indicates that the intensities of most proteins were improved after ultrasound treatment.
3. Bioinformatics analysis of DAPs The subcellular localization analysis showed that most DAPs (>50%) were distributed in cyto- plasm and cytosol, mitochondrion and nucleus, suggesting that the proteins located in these three positions were more susceptible to ultrasound treatment. The results were also confirmed by the protein-protein interaction network analysis.

4 Analysis of flavor-related proteins Based on the retrieval of published literatures, twenty proteins within the DAPs were chosen as the ones linked with flavor. These proteins could be mainly divided into myofibrillar proteins and metabolic enzymes which were mainly involved in signaling and cellular processes and environmental information processing. Moreover, more than half of the fla- vor-related proteins had an increasing trend in response to ultrasound within 500 W, most of them showing pronouncedly higher intensities in the ultrasound groups than the non-ultrasonic group.

5. Verification of flavor-related protein expression by WB Creatine kinase M-type (CKM) and troponin-T (TNT) were selected as representatives for the verification of protein expression. Results showed that the band intensity of CKM was the lowest in the non- ultrasonic group while the highest in the 500 W group. Compared with the 0 W group, its intensity was significantly increased in the 250 or 500 W group. The same tendency was also observed for the band intensities of TNT. The above results indicate that ultrasound did distinctively change the expression level of flavor-related proteins, which had a high coincidence with the findings by LFQ.

Conclusions: : Ultrasonic treatment could significantly change the protein profiles of umsmoked bacon as analysed by the proteomics data. Twenty proteins closely related to the flavor formation of unsmoked bacon were obviously influenced by ultrasound.

References:

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