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DECIPHERING TASTE ENDOWMENT OF ZANTHOXYLUM BUNGEANUM MAXIM ON SOY SAUCE MARINATED BEEF: DOES THE BINDING OF KEY FLAVONOL GLYCOSIDES TO PROTEIN MODULATE BITTERNESS?

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I. INTRODUCTION

Soy sauce-marinated beef (SSMB) is a traditional Chinese meat product known for its distinctive taste and nutritional value[1]. Among the various spices used in its preparation, *Zanthoxylum bungeanum* is notable for imparting both a tingling sensation and characteristic bitterness[2]. This bitterness has recently garnered increasing research interest, particularly due to its interaction with human bitter taste receptors, such as TAS2R14[3]. Flavonoids, as secondary metabolites, are structurally rich in hydroxyl groups and may activate specific bitter taste receptors[4]. Moreover, prior studies have shown that protein–flavonoid interactions can modulate sensory properties. In meat systems, myofibrillar proteins (MP) are known to interact with volatile compounds[5]. However, the mechanism by which flavonol glycosides from *Z. bungeanum* influence the bitterness of SSMB is not yet elucidated.This study aims to investigate the contribution of *Z. bungeanum* to the overall taste profile of SSMB and to examine how flavonol glycoside–protein binding modulates bitterness perception.

II. MATERIALS AND METHODS

Z. bungeanum (1% w/w) was from Shanxi, China. ST muscles from 2-year-old yellow cattle (~400 kg) were chilled for 48 h at 0–4 °C. Samples were grouped as: EZ (Z. bungeanum only), EBS-1 (salt-boiled ST), EBS-2 (salt-boiled ST + Z. bungeanum), EBS-3 (salt-boiled ST + spices with Z. bungeanum), and EBS-4 (salt-boiled ST + spices without Z. bungeanum).Metabolite Extraction: sample powders were extracted using 80% methanol with zinc acetate and potassium ferrocyanide, following centrifugation (10,000 rpm, 4 °C). Extracts were filtered (0.45 μm) and stored at 4 °C. Non-volatile metabolites were analyzed on a UHPLC-QE-Focus-MS system.
A trained panel (n=11) assessed bitterness using a 0–10 scale. Quinine (0.002%) was used as reference. MP bitter-masking ability was assessed using an electronic tongue (Astree, Alpha MOS).
Protein structures were predicted via AlphaFold3. Ligands (isoquercetin, astragalin) were docked using CB-DOCK2 and analyzed via PLIP and PyMOL for binding energy and interaction profiles.

III. RESULTS AND DISCUSSION

As shown in Table 1, seven key taste compounds—rutin, isorhamnetin, isoquercetin, astragalin, arbutin, chlorogenic acid, and L-tyrosine—were identified as significant contributors to flavor enhancement. Based on the results of binding energy, two flavonol glycosides (isoquercetin and astragalin) were selected to explore bitter masking behavior. As shown in Fig.1, sensory evaluation results showed that the addition of meat significantly reduced the perceived bitterness of the system. Besides, E-tongue analysis suggested that MP contributed to bitterness masking. Molecular docking results indicate that variations in the molecular geometries of isoquercetin and astragalin contribute to their distinct binding interactions with bitter taste receptor TAS2R14 (Figure 2). These findings provide valuable insights into the interplay between taste-active compounds and food components.

Table 1 – The identification of the key taste compounds of SSMB imparted by *Zanthoxylum bungeanum Maxim*.

No.	Compounds	m/z	Molecular formula	Binding energy (kcal/mol)	Taste
1	isoquercetin	463.0884389	$C_{21}H_{20}O_{12}$	-9.0	bitter
2	rutin	611.1601846	$C_{27}H_{30}O_{16}$	-8.8	bitter
3	astragalin	447.0933874	$C_{21}H_{20}O_{11}$	-8.5	bitter
4	isorhamnetin	315.0513702	$C_{16}H_{12}O_{7}$	-8.4	bitter
5	chlorogenic acid	355.1017956	$C_{16}H_{18}O_{9}$	-8.2	sour

6	arbutin	317.0876769	$C_{12}H_{16}O_7$	-7.1	bitter
7	L-tyrosine	180.0654262	$C_9H_{11}NO_3$	-5.9	bitter

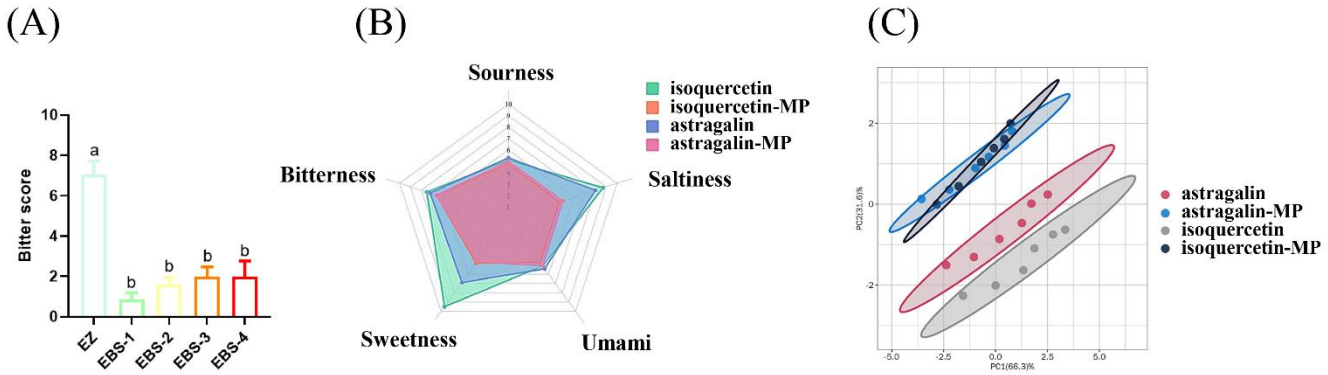


Figure 1. Bitter masking activity evaluation: (A) sensory evaluation result profile; (B) Electronic tongue taste profile of isoquercetin and astragalin; (C) PCA score plot. The significant differences among different groups are indicated by different lowercase letters (P< 0.05).

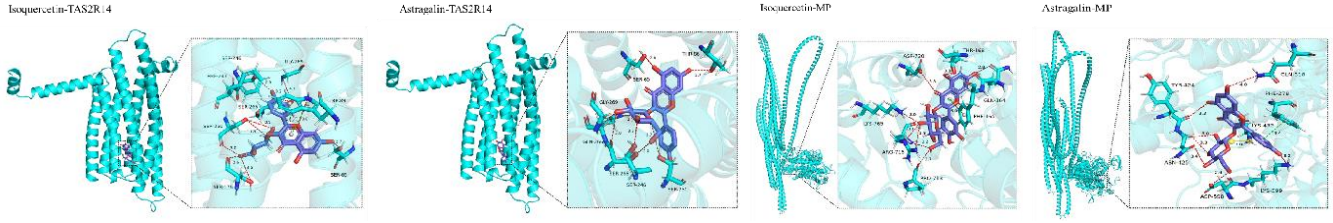


Figure 2. Docking conformations and binding sites of two flavonol glycosides compounds with TAS2R14.

IV. CONCLUSION

This study identified seven key taste compounds from *Z. bungeanum* contributing to the flavor of SSMB, including flavonoids, phenolic acids, and amino acid derivatives. Isoquercetin and astragalin were selected to evaluate the binding behavior with MP. MP showed a bitterness-masking effect. These flavonol glycosides interacted with MP maintaining structural stability and increasing binding with taste compounds. Molecular docking revealed that structural differences influence their interaction with the TAS2R14 receptor. These insights advance the understanding of taste modulation in food systems. Future research could explore other flavonoids and receptor interactions to further improve bitter-masking strategies.

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