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Article

Analysis of Amino Acids in Aqueous Solution of Honey and Honey Wines

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Abstract: This study explores the influence of amino acids released during pollen fermentation on the sensory attributes of honey wine. Fermented and non-fermented pollen were introduced into a 20% aqueous honey solution prior to alcoholic fermentation. Analysis revealed a decrease in amino acid levels post-fermentation, accompanied by an increase in higher alcohols, ethers, and acetaldehydes compared to the control. Notably, honey wine fermented with fermented flower pollen exhibited heightened volatile substance content, enhancing its taste and aroma profile. These findings underscore the significant role of pollen fermentation in shaping the sensory characteristics of honey wine.

Keywords: pollen fermentation, honey wine, honey, amino acids, alcoholic fermentation, volatile components

1. Introduction

To obtain research samples of honey wine, we initiated the fermentation process by introducing a pure yeast culture IOC 2000, known for its robust fermentation capabilities at a rate of 3%, into a 20% aqueous solution of honey. This solution was subsequently divided into three distinct parts to explore the effects of pollen fermentation. The first part was designated as the control group, maintaining the honey solution without any additional treatment. In the second part, unfermented pollen suspension was carefully introduced, while the third part received a suspension of pollen that had undergone fermentation. Detailed procedures regarding the fermentation of pollen are elaborated upon in the Methods section [1-3].

Following the introduction of yeast and pollen suspensions, the samples were meticulously incubated in a thermostat set to a controlled temperature of 22°C to ensure optimal fermentation conditions. Upon completion of the alcoholic fermentation process, characterized by the cessation of active yeast activity and the conversion of sugars into alcohol, the wine material was carefully separated from the lees, a sediment comprising yeast cells and other particulate matter. Subsequently, the wine material underwent centrifugation and filtration to remove any remaining impurities and sediments.

Amino acid analysis was conducted on the prepared samples to assess both qualitative and quantitative aspects. This analysis was performed utilizing the direct application method on a liquid chromatograph, a sophisticated analytical instrument capable of separating and detecting individual components within complex mixtures. By employing this analytical technique, researcher aimed to elucidate the impact of pollen fermentation on

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the amino acid composition of honey wine, providing valuable insights into its sensory characteristics and overall quality [4-7].

2. Materials and Methods

In this study, amino acids in aqueous solutions of honey and honey wines were analyzed using a multi-step methodology. Representative samples of honey and honey wines were prepared by dilution and filtration to remove impurities. Amino acids were extracted from the samples using a suitable extraction method, followed by derivatization with o-phthalaldehyde (OPA) to enhance detection. High-performance liquid chromatography (HPLC) equipped with a reverse-phase column was employed for amino acid separation, with detection achieved using a UV or fluorescence detector. Calibration curves generated from standard solutions enabled quantification of amino acid concentrations in the samples. Quality control measures, including blank samples and replicates, were implemented to ensure method accuracy and precision. Data analysis involved processing chromatographic data and performing statistical comparisons between samples. The methodology provided a robust framework for evaluating the amino acid composition of honey and honey wines, contributing to our understanding of their quality and characteristics [8].

3. Results and Discussion

The amino acid composition of the samples listed above is given on Figure 1, 2, 3.

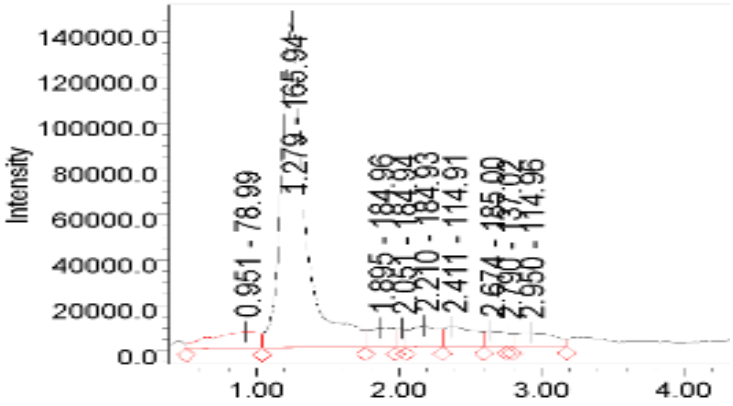

Figure 1. Chromatograms of amino acids of honey wine

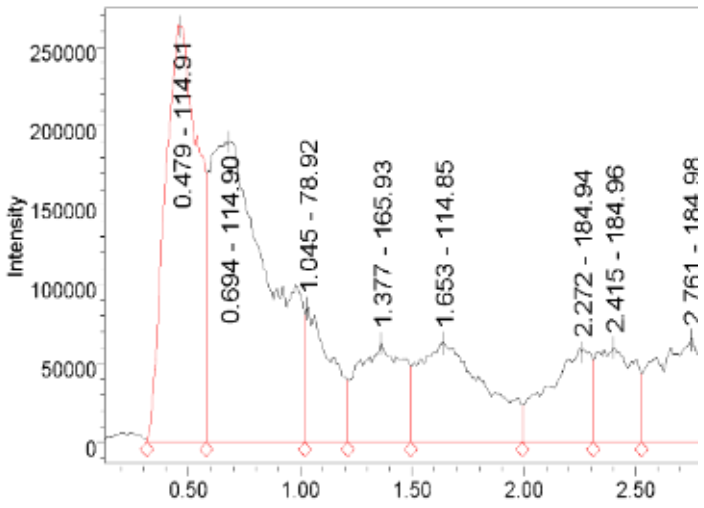

Figure 2. Chromatograms of honey wine fermented with unfermented pollen

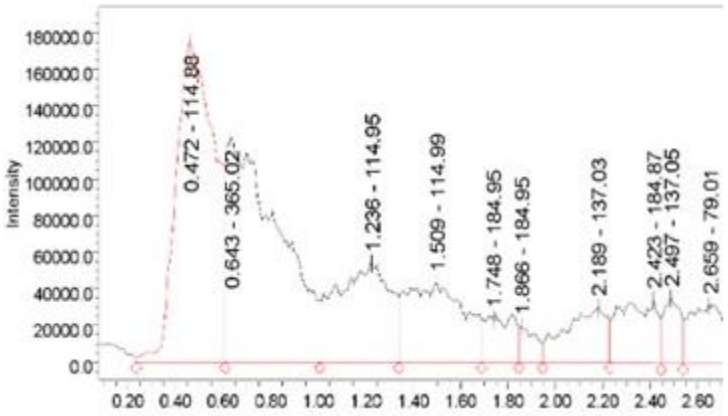

Figure 3. Chromatograms of honey wine fermented with fermented pollen

Honey wine fermented using non-fermented pollen contained higher amounts of amino acids than control honey wine. Wine obtained using fermented pollen contains qualitatively more amino acids than wine fermented with non-fermented pollen suspension.

Model tests were conducted to explore the influence of amino acids released during the decomposition of fermented pollen grains on the taste and aroma of honey wine. During the experiment, we added 3 amino acids (at 2 mg/l) to the sweet prepared for the initial fermentation, which are released in relatively large quantities from the cytoplasm of the flower's enemy cell by breaking down the membrane of the kernel. Amino acids included in the experiment are: phenylalanine, tryptophan and proline. We fermented honey sweet with amino acids separately. After fermentation, the samples were subjected to centrifugation, filtration, and the amount of amino acids in sweet and wine was determined by the method of direct input into the chromatograph. The types and amounts of amino acids in sweets and wine are given in Table 1, 2, 3.

Table 1. In test samples - content of amino acids in aqueous solutions of honey (mg/ml)

No	Sample Names	Amino acid mg/ml		
		Proline	Phenylalanine	Tryptophan
1	Aqueous solution of honey (20%)	0,0531	0,0037	0,0028
2	Aqueous solution of honey (20%) + proline	0,0743		
3	Aqueous solution of honey (20%) + phenylalanine		0,0086	
4	Aqueous solution of honey (20%) + tryptophan			0,0043

Table 2. In test samples - the content of amino acids in honey wine (mg/ml)

No	Sample Names	Amino acid mg/ml		
		Proline	Phenylalanine	Tryptophan
1	Honey wine			
2	Honey wine + proline	0,0642		
3	Honey wine + phenylalanine		0,0049	-
4	Honey wine + tryptophan			0,0019
5	Proline	0,0212		
6	Phenylalanine		0,0012	
7	Tryptophan			0,0015

The aqueous solution of honey contains a small amount of amino acids compared to the first, there are amino acids in a large amount in the aqueous solution added to non-fermented pollen. Compared to the other, there are more amino acids in the aqueous solution added to the fermented flower pollen.

Table 3. Total amount of amino acids in fermented and non-fermented honey aqueous solutions ml/l

No	Sample Name	Total amount of amino acids mg/l
1	Aqueous solution of linden honey	0.0596
2	Aqueous solution of linden honey + pollen (suspension)	1.6976
3	Aqueous solution of linden honey + fermented pollen (suspension)	2.3596

The experiment results indicate that changes in the amounts of phenylalanine and tryptophan align with the observed variations in amino acid proline. Chromatographic analysis of amino acids in the aqueous solution of honey, its own wine, and wines fermented with non-fermented and fermented pollen yielded significant insights. The initial aqueous solution of honey contained a minimal number of amino acids (0.0596 mg/ml). However, the addition of non-fermented pollen significantly increased this content to 1.6976 mg/ml, while wine fermented with fermented pollen exhibited an even higher content (2.35960 mg/ml). The experiment showcased a significant increase in amino acid content in the aqueous solution of honey due to the processing (fermentation) of pollen using a complex of proteolytic enzymes. It is well-established that the majority of aromatic substances in wine originate during alcoholic fermentation, wherein the conversion of carbohydrates, amino acids, and organic acids present in grape juice takes place. Alcohols and ethers constitute a significant portion of the bouquet and aroma components in honey wine, similar to their presence in grape wine. Wines produced using fermented and non-fermented pollen exhibit variations in the overall composition of ethers and acetaldehydes, as confirmed by gas-liquid chromatographic analysis of wine samples. In Table 4, present the average amounts of ethers and acetaldehydes in wines obtained from fermented and non-fermented pollen.

The quantities of volatile substances in both non-fermented and fermented honey wine, which underwent fermentation with pollen, are provided in Table 4.

Table 4. The quantities of volatile substances in both non-fermented and fermented honey wine

No	**Names of volatile components**	**Wine fermented with non-fermented pollen (mg/l)**	**Wine fermented with fermented pollen (mg/l)**
1	Acetaldehyde	4.2	5.3
2	Ethyl acetate	14.01	16.04
3	2 Butanol	7.65	9.01
4	N Propanol	11.03	17.56
5	Isobutanol	9.17	12.62
6	Isoamyl	8.82	10.34
7	Methanol	0,394	0.298

4. Conclusion

As evident from Table 4, the content of ethers and acetaldehydes is higher in the wine made with fermented pollen compared to the non-fermented counterpart. This aligns with literature data indicating that amino acids undergo partial transformation into ethers and acetaldehydes during alcoholic fermentation, leading to a decrease in the amount of ionic acids in the wine.

In this context, higher alcohols are synthesized from carbohydrates, resulting in a significant reduction in amino acid levels compared to normal alcoholic fermentation (without flower pollen). Yeast can generate various compounds from amino acids, and the conversion of amino acids to higher alcohols occurs not only through oxidative deamination but also via transamination of amino acids to keto acids. To investigate changes in the quantity of aromatic components in honey wine samples obtained with the addition of fermented pollen, researcher conducted the following experiment: after the completion of alcoholic fermentation and the formation of wine with fermented pollen, researcher determined the total amount of higher alcohols, middle ethers, and acetaldehydes.

As depicted in the figure and the table, the amino acid content is reduced in all samples. Conversely, the total amount of higher alcohols, ethers, and acetaldehydes increased in the wine. This signifies that the amino acids added to the honey during alcoholic fermentation have undergone transformation. The overall amount of alcohols, ethers, and acetaldehydes is higher in honey wines compared to the control. In other words, the taste and aroma of the experimental honey wine are significantly superior to those of the control honey wine.

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