

Analisis Kuantitatif Asam Amino Histidin sebagai Prediktor Ergothioneine pada Proses Pengeringan dan Penggorengan Jamur Tiram Putih (Pleurotus ostreatus)

Quantitative Analysis of Histidine Amino Acid as a Predictor of Ergothioneine in the Drying and Frying Process of White Oyster Mushrooms (Pleurotus ostreatus)

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ABSTRAK

Latar Belakang: Jamur tiram putih (*Pleurotus ostreatus*) banyak dibudidayakan oleh masyarakat Indonesia karena rasanya yang enak dan bergizi. Jamur tiram putih mengandung 18 asam amino penyusun tubuh dan antioksidan antara lain yaitu senyawa fenolik dan ergothioneine. Ergothioneine sebagai antioksidan kuat adalah asam amino turunan histidin yang memiliki gugus sulfur seperti sistein dan metionin. Peningkatan histidin menunjukkan aktivitas dari ergothioneine.

Tujuan: Penelitian ini bertujuan untuk mengetahui kandungan asam amino histidin sebagai prediktor asam amino ergothioneine pada proses pengeringan dan penggorengan Jamur Tiram Putih.

Metode: Metode ekstraksi yang digunakan pada penelitian adalah maserasi dengan pelarut etanol 90% untuk sampel pengeringan dan etanol 70% untuk sampel penggorengan yang dianalisis menggunakan High Performance Liquid Chromatography (HPLC).

Hasil: Hasil penelitian diperoleh bahwa kandungan histidin meningkat dengan semakin lamanya waktu pengeringan dan menurun dengan semakin lamanya waktu penggorengan. Pengeringan dengan variasi waktu 2 hari, 3 hari, 5 hari berturut-turut yaitu 674788.802 mg/L ; 615302.747 mg/L; 1946113.494 g/L. Penggorengan dengan variasi lama 2 menit, 3 menit, dan 5 menit yaitu 500435.148 mg/L; 232428.391 mg/L; 0.000 mg/L. Sedangkan kandungan metionin menurun dengan semakin lamanya waktu pengeringan dan meningkat dengan semakin lamanya waktu penggorengan. Pengeringan dengan variasi waktu 2 hari, 3 hari, 5 hari berturut-turut yaitu 6673.283mg/L ; 6671.920 mg/L; 1876.358 mg/L. Penggorengan dengan variasi lama 2 menit, 3 menit, dan 5 menit yaitu 2296.698 mg/L; 1243.911 mg/L; 34764.534 mg/L.

Kesimpulan: Berdasarkan penelitian yang telah dilakukan diperoleh bahwa kandungan histidin sebagai prediktor ergothioneine tertinggi pada pengeringan selama 5 hari dan penggorengan 2 menit.

Kata Kunci: Jamur tiram Putih, Histidin, Metionin, Ergothioneine.

ABSTRACT

Backgrounds: White oyster mushroom (*Pleurotus ostreatus*) is widely cultivated by Indonesian people due to its delicious and nutritious taste. *Pleurotus ostreatus* contains 18 amino acids that make up the body and antioxidants, including phenolic compounds and ergothioneine. Ergothioneine as a strong antioxidant is an amino acid derived from histidine which has sulfur groups such as cysteine and methionine. The increase in histidine indicates the activity of ergothioneine.

Objectives: This study aimed to determine the amino acid content of histidine as a predictor of ergothioneine amino acid in the drying and frying process of white oyster mushrooms.

Methods: The extraction method used in this study was maceration with 90% ethanol for drying samples and 70% ethanol for frying samples analyzed using High Performance Liquid Chromatography (HPLC).

Results: The results showed that the histidine content increased with the longer drying time, and decreased with the longer frying time. Drying with a variation of 2 days, 3 days, and 5 days were 674788,802 mg/L; 615302.747 mg/L; and 1946113,494 g / L respectively. Frying with a variation of 2 minutes, 3 minutes, and 5 minutes were 500435,148 mg / L; 232428,391 mg/L; and 0.000 mg / L. On the other hand, the methionine content decreased with the longer drying time, and increased with the longer frying time. Drying with a variation of 2 days, 3 days, and 5 days in a row were 6673,283mg/L; 6671,920 mg/L; and 1876,358 mg / L. Frying with a variation of 2 minutes, 3 minutes, and 5 minutes were 2296,698 mg / L; 1243,911 mg/L; and 34764.534 mg/L.

Conclusions: the study concludes that the content of histidine as the highest ergothioneine predictor is at drying for 5 days and frying for 2 days.

Keywords: White Oyster Mushroom, Histidine, Methionine, Ergothioneine.



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INTRODUCTION

Indonesia as a mega-biodiversity country has an abundance of natural resources which have the advantageous potential. The exploration of unique plants is indispensable to optimize their benefits in the health sector and long-term productivity, and also to contribute in preserving the diversity of natural resources in Indonesia. One of them is the exploration of white oyster mushroom (*Pleurotus ostreatus*), which is widely cultivated by Indonesian people since it can be consumed and increases income. People consume oyster mushroom because it tastes good and delicious. White oyster mushroom contains high protein up to 35% and low fat about 2.2%¹. White oyster mushroom is one type of mushroom which has a higher nutritional content than other mushrooms. Oyster mushroom contains up to 35% protein, 9 kinds of amino acids, 2.2% fat consisting of 72% unsaturated fatty acids and carbohydrates in every 100 gr of white oyster mushrooms¹. Besides, white oyster mushroom (*Pleurotus ostreatus*) has the potential to be an antioxidant since it contains phenolic compounds, ergothioneine, selenium, and vitamin C². Shinta et al. (2018) state that the antioxidant activity of oyster mushroom flavonoid extract is classified as very strong with an IC50 value of 39.89 mg/ml³. This is in line with research stating that the ethanol extract shows very strong antioxidant activity in white oyster mushrooms with an IC50 value of 0.55 mg/ml⁴.

White oyster mushroom also contains 18 types of amino acids needed by human body and also does not contain cholesterol, such as alanine, arginine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine⁵. Furthermore, oyster mushroom also contains a lot of antioxidants ergothioneine^{6,7}. Mushroom ergothioneine is found in mycelium and stem. The content of *Pleurotus ostreatus* which has a major role in the health sector is the antioxidant ergothioneine. Ergothioneine (EGT; 2-mercaptohistidine trimethylbetaine) is an amino acid derived from histidine (*betaine thio-histidine*) with sulfur groups, such as cysteine and methionine, which has strong antioxidant activity⁸. The amino acid ergothioneine can be synthesized by mushroom, such as white oyster mushrooms (*Pleurotus ostreatus*) on the stem and mycelium⁶. There is a study that succeeds in establishing a two-step biosynthesis mechanism for the amino acid ergothioneine using the amino acids histidine, glutamate, and cysteine.⁹ By applying quantitative analysis approach and focusing on histidine amino acid as the initial reagent which has a structure similar to ergothioneine and methionine as amino acids that have a sulfur group, this study aims to determine the amino acid

content of histidine as a predictor of amino acid ergothioneine in the drying and frying process of white oyster mushrooms.

METHODS

This study used an experimental laboratory research study conducted at the Nutrition Laboratory, Faculty of Public Health, Diponegoro University. Analysis of histidine content was carried out at the Diponegoro University Integrated Laboratory. All samples of mushrooms used in this study came from mushrooms cultivated by the people of Banyumeneng Rural Village, Mranggen Sub-district. The independent variables in this study were the drying and heating processes, while the dependent variables were the content of histidine and methionine. The control variable was the mass of mushrooms used in this study.

Tools and Materials

The tools used in this study were digital analytical balance, measuring cylinder, erlenmeyer, blender, mortar stamper, funnel, drop pipette, centrifuge, and the instruments of High-Performance Liquid Chromatography (HPLC). The materials used in this study were white oyster mushrooms (*Pleurotus ostreatus*) cultivated by the people of Banyumeneng Rural Village, Mranggen Sub-district, 90% ethanol, 70% ethanol, water, sulfuric acid, and cooking oil.

Working Procedures

The Preparation of Simplicia

This research phase began with several steps, such as preparing the simplicia, making the extracts, and then analyzing histidine and methionine using HPLC. The white oyster mushrooms (*Pleurotus ostreatus*) were collected from Banyumeneng Rural Village, Mranggen Sub-district, and cleaned of dirt. After that, the mushrooms were washed with running water until they were clean. Mushrooms that had been cleaned were cut into small pieces, and then dried in the open air protected from the sun. Drying was carried out with several variations of time, namely 2 days (P.2), 3 days (P.2), and 5 days (P.5). After they were dry and browned, the mushrooms were mashed using a blender and mortar stamper to obtain dry simplicia. Meanwhile, processed oyster mushrooms were fried with several variations of time, namely 2 minutes (G.2), 3 minutes (G.3), and 5 minutes (G.5). After that, the mushrooms were drained to reduce the oil content. The dried mushrooms were then mashed using a blender.



Extract Making

The extraction method used in this study was an easy maceration extraction method, referring to the study conducted by Lusiana (2015) that had been modified. A total of 25 grams of dry simplicia were macerated using 250 mL of 90% ethanol for variations in drying, and 70% ethanol for variations in frying mushrooms for 24 hours. The extract obtained was then filtered through filter paper. After that, the filtrate was evaporated using rotary evaporator at 50°C to obtain a thick extract.

Analysis Using High Performance Liquid Chromatography (HPLC)

A total of 1 gram of mushroom thick extract was analyzed using the High-Performance Liquid Chromatography (HPLC) to determine the content of histidine and methionine. The mobile phase used were water and sulfuric acid. Meanwhile, the stationary phase used was column C18. Detection was carried out at a wavelength of 254 nm.

RESULTS AND DISCUSSION

White oyster mushroom (*Pleurotus ostreatus*) contains 18 amino acids that can be classified into essential and non-essential amino acids. The essential amino acids are histidine, arginine, cysteine, leucine, lysine, methionine, phenylalanine, tryptophan, and valine. Meanwhile, the non-essential amino acids are alanine, glutamate, aspartate, asparagine, and glutamine⁵. Amino acids which contain thiol and thioether, such as arginine, cysteine, lysine, methionine, and tryptophan have strong anti-oxidant activity^{10,11}. There are several proposed mechanisms to explain the antioxidant activity of amino acids. In particular, amino acids are known to be radical inhibitor by removing single oxygen¹². Amino acids and dipeptides, such as histidine can bind to metals, including transition metals that catalyze the breakdown of hydroperoxides to become free radicals.¹³.

The analysis of amino acid histidine was carried out to qualitatively predict the content of ergothioneine in the drying and frying process of oyster mushrooms. Quantitative analysis of histidine and methionine in the drying and frying process using High Performance Liquid Chromatography (HPLC) is described in table 1.

Table 1. Results of Quantitative Analysis of Histidine

Test indicators	Histidine (mg/L)	Methionine (mg/L)
Drying*		
2 days drying	674788,802	6673,283
3 days drying	615302.747	6671,920
5 days drying	1946113.494	1876,358
Frying**		
Frying 2 minutes	500435.148	2296,698
Frying 3 minutes	232428.391	1243,911
Frying 5 minutes	0.000	34764,534

*Drying using 90% ethanol solvent

**Frying using 70% ethanol solvent

Based on table 1, the highest histidine content is found in the 5-day drying process that is 1946113.494 mg/L, which is indicated by histidine peak at a high retention time of 15-17.5 minutes. The histidine content increases as the drying time takes longer. Based on

qualitative analysis of ergothioneine that was carried out by looking at the chromatograph, there was a peak at retention time of 20-22.5 minutes which showed peak ergothioneine⁹.



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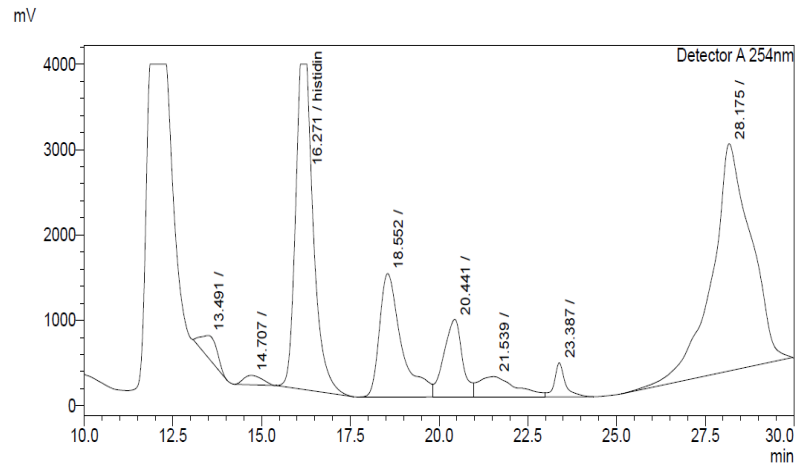


Figure 1. Histidine Chromatogram on 5 Days Drying Sample

The frying process affects the histidine and methionine content. Table 1 shows that the highest histidine content in the 2-minute frying process is 500435,148 mg/L, which is indicated by the peak at the retention time of 16,480 minutes. It is shown that the frying process reduces the histidine content. This is in

accordance with the prediction that the length of the frying process results in reduced amino acids¹⁴. There is a peak at the retention time of 20-22.5 minutes which indicates the peak of ergothioneine⁹. This indicates that in the frying process, ergothioneine is still present in oyster mushrooms.

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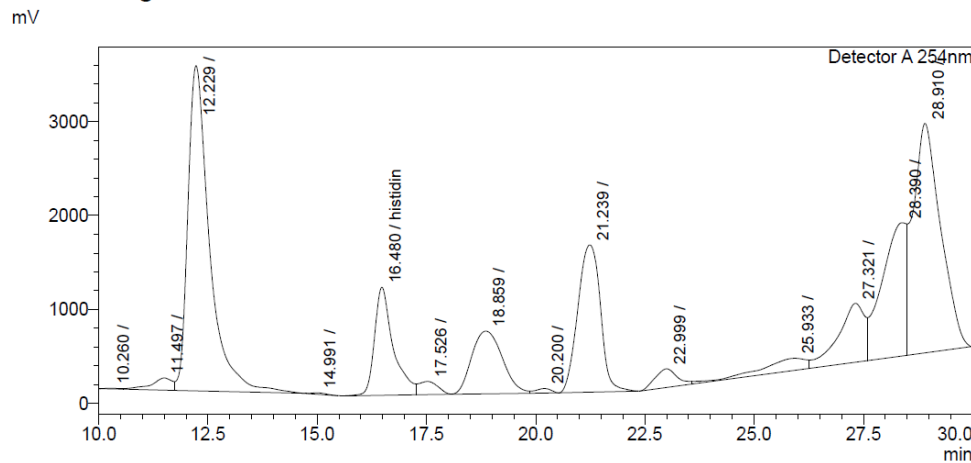


Figure 2. 2 Minutes frying Histidine Chromatogram

The methionine content in oyster mushrooms decreases based on the length of drying time described in table 1. The highest methionine content in the 2-day drying time is 1,876,358 mg/L as shown in Figure 3. Meanwhile in the frying process, the methionine content increases. The frying process for 2 minutes and 3 minutes produce methionine as much as 2,296,698 mg/L and 1,243,911 mg/L, respectively. Meanwhile in the frying

process for 5 minutes, 34,764,534 mg/L of methionine amino acids is produced as shown in Figure 4. The increase in the drying process occurs because each amino acid has different characteristics depending on the group bound. The amino acid structure of methionine which binds to sulfide atoms increases the strength of intermolecular bonds since strong sulfide bonds are formed.



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mV

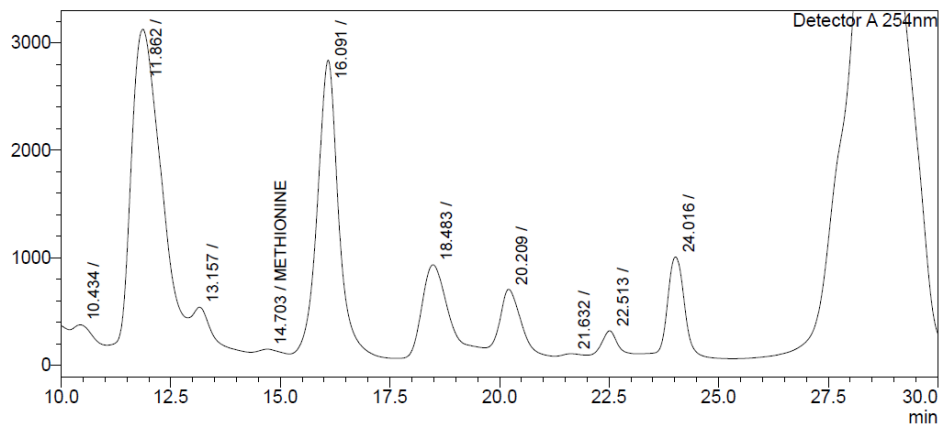


Figure 3. 2 days drying methionine chromatogram

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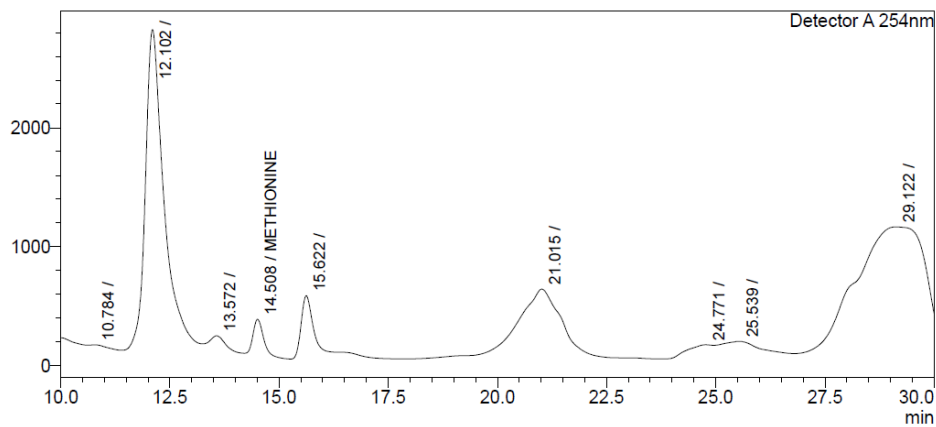


Figure 4. 5 minutes frying methionine chromatogram

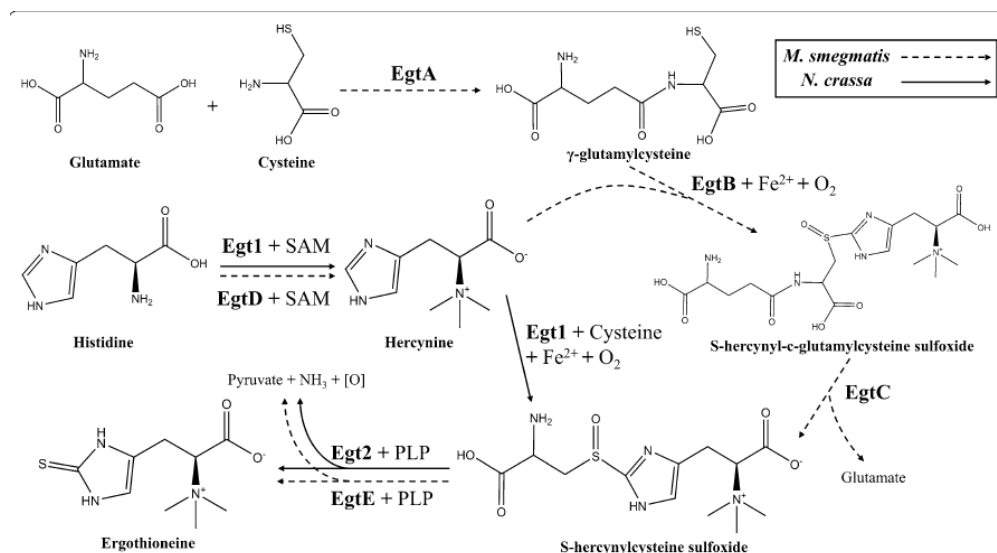


Fig. 1 Two different pathways for EGT biosynthesis

Figure 5. Ergothioneine Biosynthesis⁹

Figure 5 describes the 2-step synthesis of ergothioneine from the amino acids histidine, glutamate, and cysteine⁹. Ergothioneine (EGT; 2-mercaptohistidine trimethylbetaine) is an amino acid that is derived from

histidine (*betaine thio-histidine*) with sulfur groups, such as cysteine and methionine which has strong antioxidant activity⁸. Histidine analysis can predict the qualitative content of ergothioneine.



CONCLUSION

Based on the research that has been conducted, it can be concluded that the histidine content increases as the drying time takes longer, and decreases as the frying time takes longer. The results obtained from the drying process with variations in time of 2 days, 3 days, and 5 days are 674788,802 mg/L; 615302.747 mg/L; and 1946113.494 g/L, respectively. Frying process with variations of 2 minutes, 3 minutes, and 5 minutes are 500435.148 mg/L; 232428.391 mg/L; and 0.000 mg/L, respectively. Meanwhile, the methionine content decreases with longer drying time and increases with longer frying time. Drying with variation of time 2 days, 3 days, and 5 days in a row are 6673,283 mg/L; 6671,920 mg/L; and 1876,358 mg/L. Frying with variations of 2 minutes, 3 minutes, and 5 minutes are 2296,698 mg/L; 1243,911 mg/L; and 34764,534 mg/L. Based on this research, it is suggested that the drying process should be in 5 days, and the frying process should be in 2 minutes in order to maintain the best histidine content.

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Conflict of Interest and Funding Disclosure

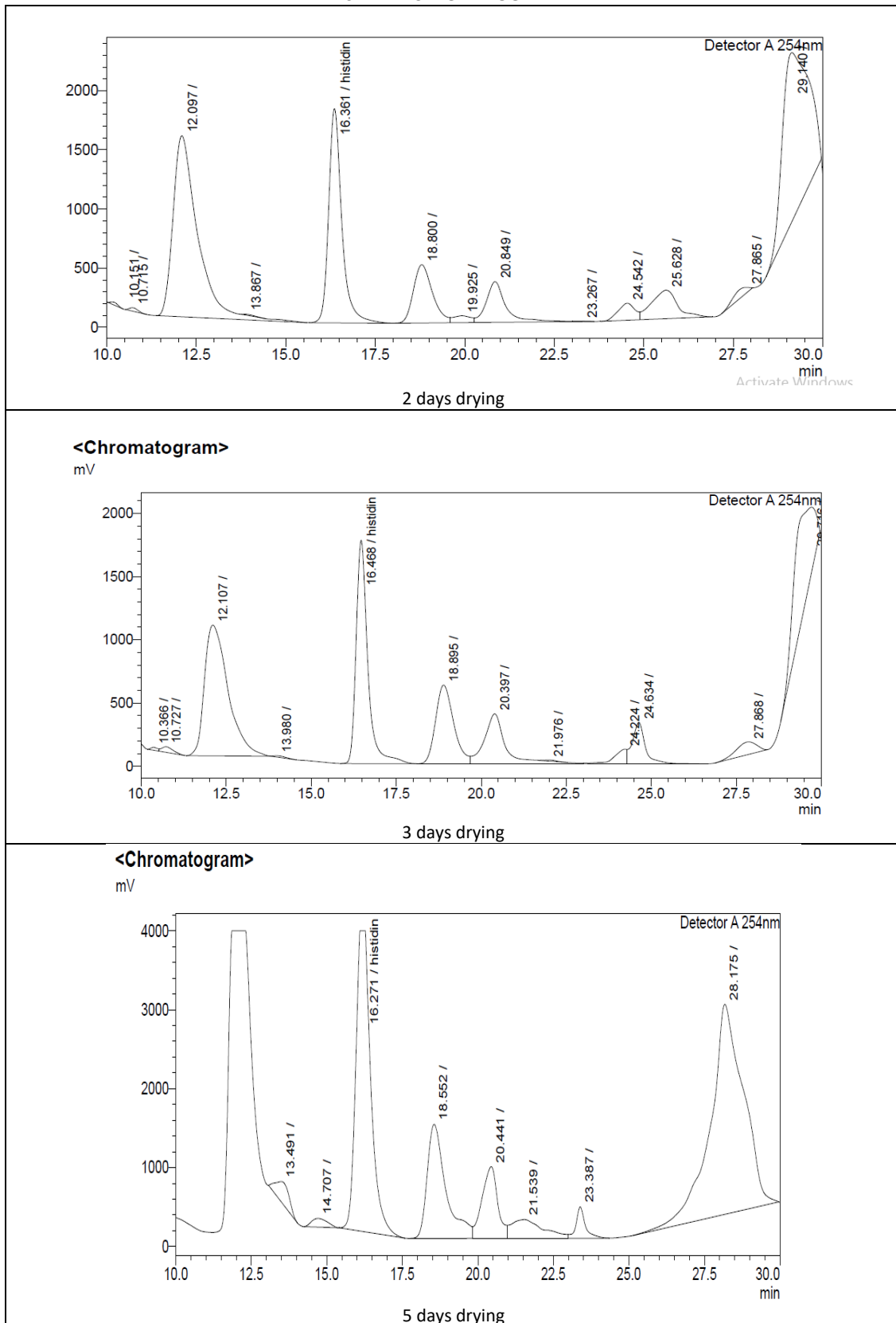
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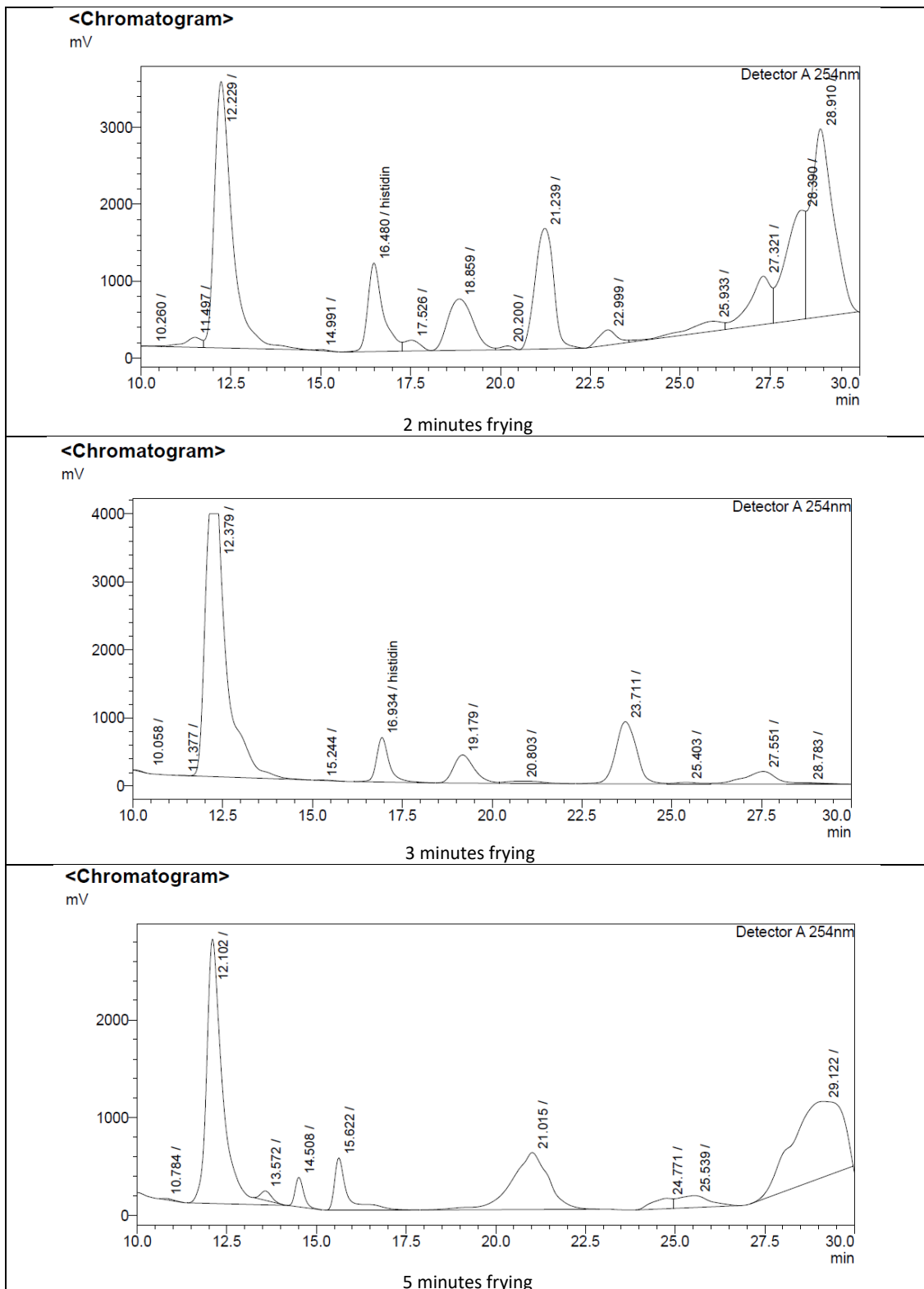
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HISTIDINE CHROMATOGRAM DATA





METHIONINE CHROMATOGRAM DATA

