RESEARCH ON EXTRACTING PROTEIN AND SOLUBLE FIBRE FROM EDIBLE MUSHROOM *Auricularia auricula-judae*

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ABSTRACT

Some nutrient compounds were isolated from the edible mushroom Auricularia auricula-judae using water, alkali, acid or enzyme extracts. Several factors such as pH, extraction temperature, immersion and extraction time of all methods were studied. Among these methods, akaline and enzymatic extraction were more dominant than the others. In particularly, akaline extraction (sodium carbonate used) showed the highest yield 35.86% (pH10.58, 70 ^{o}C, 85 minutes), enzymatic extraction gave approximate yield 33.01% (pH4.5, 50 ^{o}C, 75 minutes). Besides, recovery yield of protein and soluble fibre is 0.23 g/100ml and 1.33% using akaline extraction. Moreover, according to WHO, sodium carbonate is one of the safe additives for health. Consequently, akaline extraction was found to be the potential method for isolation.

Keywords: Auricularia auricula-judae; Extraction; Recovery yield

1. INTRODUCTION

Wood ‘ear mushroom *Auricularia auricula-judae* has been used for a long time. The reason is that it is not only pure vegetable with a large number of nutrients such as protein, vitamin, soluble fibre, etc. but also treat effectively some diseases such as high blood-pressure, atherosclerosis, cancer and so on [13].

Fig.1: Wood ‘ear mushrooms *Auricularia auricula-judea*

Nowadays, with the development of food and chemical technology, the functions and active mechanism of biological compounds in mushroom have been clarified gradually. However, limited absorption in human is the main problem. It is because that there are not enzymes to break down the cell wall of mushroom in human body and conditions for extraction of these compounds have not be examined clearly. Therefore, the main objective of this reseacrch **"Research on extracting protein and soluble fiber from *Auricularia auricula-judae* mushroom"** is to find optimal conditions to extract most of nutrients as well as biologically active substances in mushroom.
The content of the research: preliminary study about the chemical compositions of raw material; study about the effect of sodium carbonate and citric acid concentrations, pectinase enzyme to the extraction yield.

2. EXPERIMENTALS

Dried mushroom was purchased from Lai Nga, Dinh Quan dist, Dong Nai province. Acid citric, sodium carbonate was from Guangdong Guanghua Chemical Factory Co., Ltd. Pectinex Ultra SP-L (a mixture of pectintranseliminase, polygalacturonase, pectinesterase, cellulase and hemicellulase; optimal conditions at pH of 4.5, temperature of

50 ^{o}C; IU = 9500 PGU/ml) was from Novozymes, Denmark.

Dried mushroom was removed impurities and ground into powder. The powdered mushroom was extracted with solvent at solid/liquid ratio of 2/40 (g/ml) and at T ^{o}C for t mins. Then, solvent was separated for analysing protein, soluble fibre content and dried extract was collected.

In this work, sodium carbonate and citric acid and enzyme solutions were used as solvents which concentrations were altered. Besides, the temperature and time of extractions were changed for investigating to get highest yield.

For changing temperature, the system was kept in water bath. In condition of treating with enzyme, solution was adjusted to pH of 4.5 and kept at 50 ^{o}C.

The extraction yield was calculated as follow equation:

$$\boldsymbol{H = \frac{m_2}{m_1} \times 100} \ (\%)$$

In which m_1 and m_2 were dried weights of the materials and the extracts (g).

The final extracts were evaluated by using analysis standards for contents of water (TCVN 5613-91), ashs (TCVN 5611-91), proteins (FAO 1986, 14/7, P.221), carbohydrates (TCVN 4594-88), crude fibers (TCVN 5714-07) and soluble fibers (AOAC 991.43) at Institute of Hygiene and Public Heath and QUATEST 3.

Each experiment was repeated three times for evaluating reproducibility and the data were processed by the program STATGRAPHICS Plus.

3. RESULTS & DISCUSSIONS

According to Table 1, it was amazing to find that Vietnamese mushroom had very rich nutritional ingredients. The carbohydrate content was about 61.5% which was a high energy source. Moreover, protein also presented in mushroom with high concentration (10.21%). Ragini Madan Bisaria and Mira [22] had found that mushroom contained a full range of essential amino acids for human. Besides, soluble fiber was quantified in the mushroom at concentration of 3.78%. This is a bioactive fiber may prevent heart disease and cancer [18, 20].

Table 1: Chemical composition of dried mushroom.

Index	Result
Water content (%)	14.02
Ash (%)	1.96
Protein (g/100g)	10.21
Carbohyfdrate (g/100g)	61.50
Crude fiber (g/100g)	4.20
Solube fiber (%)	3.78

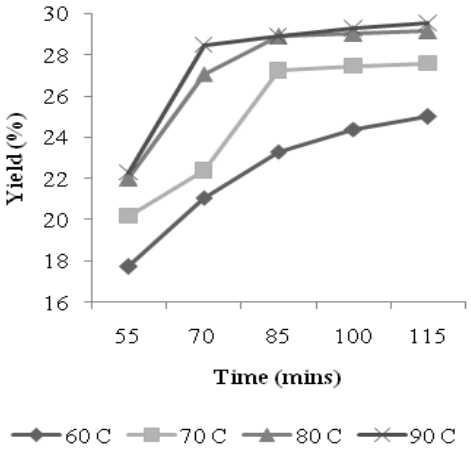

Fig.2: Effects of temperature and time on hot water extraction

The experimental results showed that extraction yield was increased with increasing temperature and time. However, when reaching to certain values, the extraction performance did not change significantly. For example, at a temperature of 80 ^{o}C, difference of the yield changed around 5% after time of 85 mins. Increasing the temperature and time broke up mushroom cell and enhanced tranfer of the soluble polysaccharides and water through the walls. Through the above results, extraction temperature 80 ^{o}C and time 85 minutes were considered optimal in this experiment.

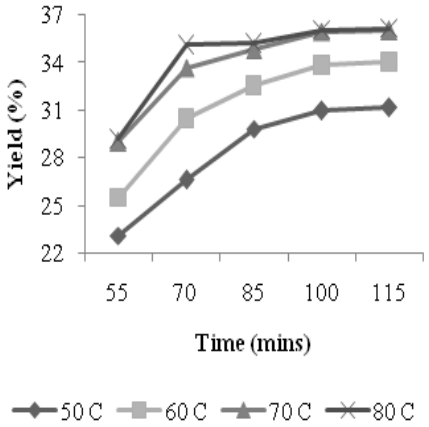

Fig.3 :Effects of temperature and time (sodium carbonate concentration of 1000 ppm)

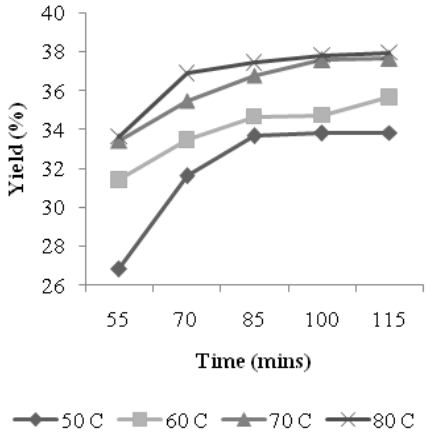

Fig.5: Effects of temperature and time (sodium carbonate concentration of 2000 ppm)

Similarly, when using alkaline solution as solvent, the temperature and time also affected on extraction yield and the results were similar to previous studies [18]. At Na_2CO_3 concentration of 1000 ppm, the extraction reached to yield of 35.93% corresponding to temperature of 70 ^{0}C and time of 100 minutes. When Na_2CO_3 concentration was increased, the extraction yield also increased. Particularly, the yield in concentration of 1500 and 2000 ppm was 36.73% and 36.80% (at *T* of 70 ^{0}C for *t* of 85 min). However, the yield was not significantly change when Na_2CO_3 concentration went up to 2500 or 3000 ppm.

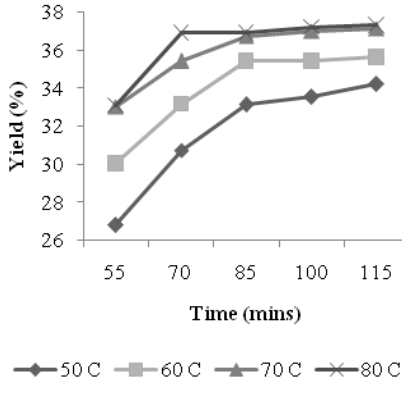

Fig.4: Effects of temperature and time (sodium carbonate concentration of 1500 ppm)

Comparing to experiments using water, alkaline treatment had an remarkable effect on the extraction yield. In these cases, the alkaline agent attacked to cell walls, broke down the network of the chitin/chitosan–glucan complex and the extraction happened easily. However, the presence of alkaline agent also affected to pectin networks and the mixture had high viscosity because of receiving more protein and polysaccharide in extraction fluids [16]. Thus, when the solution concentration was increased, the viscosity of the solution also increased and affected the yield as the experimental results.

In terms of nutritional composition and bioactivity of components extracted, the extraction with strongly alkaline treatment can decompose glucan and other constituents. Therefore, the suitable extraction in the alkaline treatment should be carried out with concentration of 1500 ppm (pH 10.58; *T* = 70 ^{0}C; *t* = 85 min).

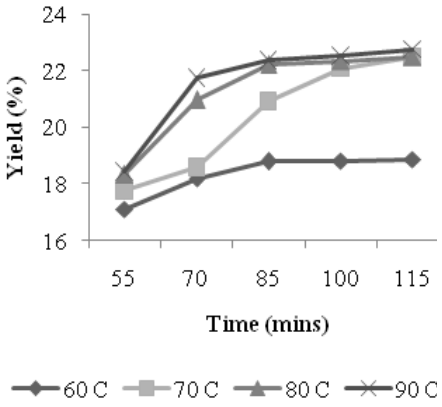

Fig.6: Effect of temperature and time (citric acid concentration of 500 ppm).

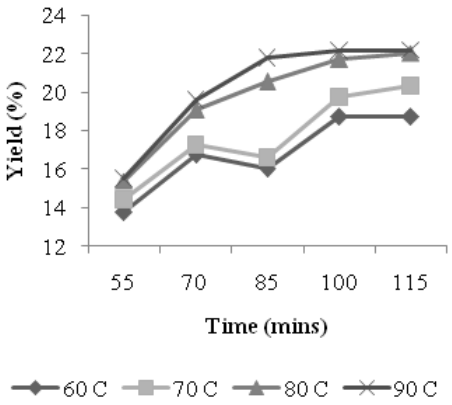

Fig.7: Effect of temperature and time (citric acid concentration of 1000 ppm)

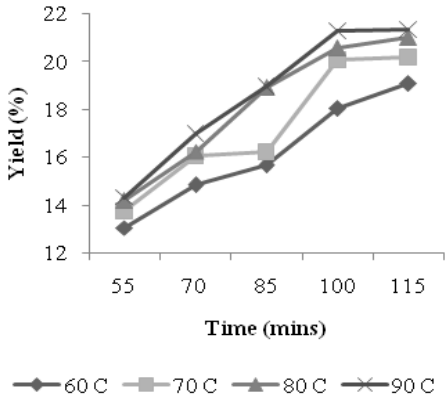

Fig.8: Effect of temperature and time (citric acid concentration of 1500 ppm).

According to Fig 6, 7, 8, it was clearly to realize that the extraction yield tended to decrease when extracted with citric acid solution. The higher citric acid concentration was, the lower extraction yield was. When citric acid concentration increased from 500 to 1500 ppm, the extraction yield decreased respectively from 22.21% down to 20.54%. Because in a acidic environment, chitin/chitosan - glucan complex and pectin were stable and the fungal cell wall could not be broken [2], so the extraction became more difficult. Morever, the denaturation of the protein in acidic environment led to increasing of viscosity and resulted in reducing the extraction yield.

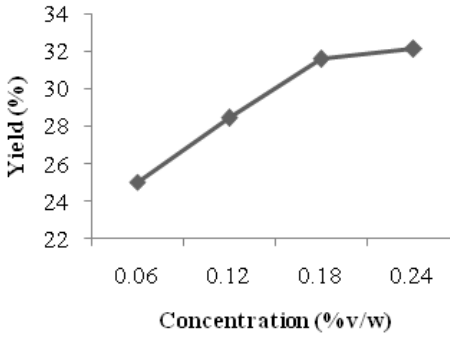

Fig.9: Effect of enzyme concentration.

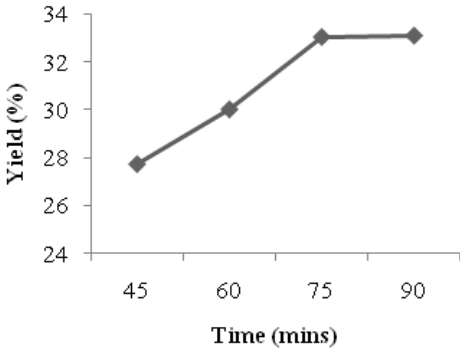

Fig.10: Effect of enzymatic treatment time.

The supporting of enzyme was one of factors to increase the extraction yield. Pectinex Ultra SP-L was a mixture of enzymes including mainly endopolygalacturonase and a small amount of pectinlyase, pectinesterase, hemicellulase, cellulase, protease and amylase. This enzyme is capable of cutting esterified pectin at high and low level, and it is also able to hydrolyse cellulose, hemicellulose, starch and protein partially [10]. Therefore, this enzyme can break down cell wall radically and release more extracts. The maximum value in the concentration range 0.18 ÷ 0.24% (v/w) was about 32% (Fig. 9).

As enzyme concentration was increased up to 0.24% (v/w), extraction efficiency was not significantly changed because of effect of non-competitive inhibitor (the products of the cleavage reaction of pectin). Mechanism of this inhibition: inhibitor occupied the outside of

the active site of enzyme. As the result of this combination, inhibitor changed the spatial structure of enzyme molecules in the negative direction for the catalytic activity. Thus, the inhibitor reduced the enzymatic activity [1]. Therefore, the enzyme concentration of 0.18% (v/w) was the good condition to obtain the maximum extraction yield.

When the time increased from 45 to 60 minutes, the yield gradually raised from 27.73 to 30% (Fig. 10). The extraction yield was 33.01% in proportion to 75-minute period. If the extraction time continued to increase, the yield did not increase. It can be explained by effect of pectin to viscosity of extraction fluids. Hences, the optimal time to obtain the maximum extraction yield was 75 minutes.

Using various extraction methods influenced to quality of the final extracts. It was obtained that the highest recovery yield of protein was 0.23 g/100ml when using the alkaline treatment and the lowest was 0.09 g/100ml when using acid treatment (Table 2, Fig. 11). However, using the alkaline treatment at high concentration can break protein chain, reducing the quality of the protein. Using alkaline treatment, extraction yield of soluble fiber was the highest (1.33%). It is because that the destruction of the mushroom cell wall made the cell network become less firm, collecting more substances. However, the high concentration of Na_2CO_3 caused the breakdown of soluble fiber, forming the monosaccharide and oligosaccharide units. Therefore, using enzyme to extract was a potential solution.

Table 2: Recovery yield of protein, soluble fiber with various extraction methods

Extraction method	**Protein** (g/100ml)	**Soluble fiber** (%)
Water	0.12	0.81
Base	**0.23**	**1.33**
Acid	0.09	0.67
Enzyme	0.17	0.98

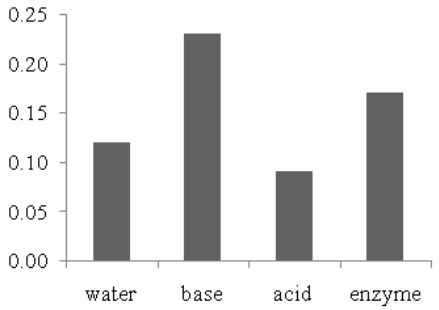

*Fig.*11: Protein contents with various extraction methods.

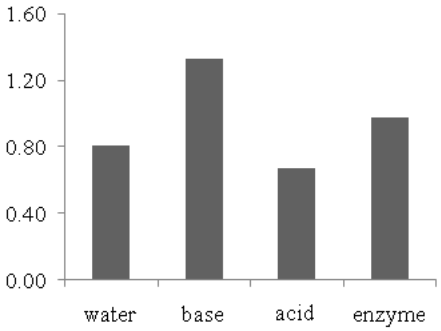

Fig.12: Extracted soluble fiber contents with various extraction methods.

4. CONCLUSIONS

The research on extracting protein and soluble fiber from *Auricularia auricula-judae* mushroom was carried out and many valuable results were achieved. Among these methods, akaline and enzymatic extraction were more dominant than the others. Moreover, according to WHO, sodium carbonate is one of the safe additives for health. Consequently, akaline extraction was found to be the potential method for isolation. The extract mixture can be dried to powder which using residues of jelly ear extraction as a carrier. The dried powder from *Auricularia auricula-judae* mushroom extract is a valuable product and its applications in foodstuffs, and functional foods are very promising.

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