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Instant Tea Formulation from *Cordyceps militaris* Extract via Convection Drying

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Abstract

The purpose of study was to find out a suitable formulation of instant tea product from *Cordyceps militaris* extract by using the convection drying method. The properties of product were influenced by maltodextrin concentrations (30%, 40%, 50% and 60% w/w), extract concentrations (5%, 6%, 7% and 8% w/w), drying time (15 h, 20 h and 25 h) and drying temperatures (60 °C, 70 °C and 80 °C). Cordycepin content and sensory evaluation were chosen as the main outcomes. The suitable parameters of products were included maltodextrin, *Cordyceps militaris* extract, the drying time and the drying temperature with 40% (w/w), 6% (w/w), 15 h and 70 °C, respectively. The cordycepin content in the product was 97.274 µg/g dry weight (DW) and sensory evaluation studies showed that the product was good with 16.4 of total 20 points according to TCVN 3215-79. Those results showed that using convection method combine maltodextrin as a drying agent get high effects on creating a uniform size powder and could apply in beverage industries.

Keywords: *Cordyceps militaris, Instant tea, Convection drying, Maltodextrin, Cordycepin*.

1. Introduction

Cordyceps militaris is well-known as medicinal caterpillar fungus, which are belonged to the family *Cordycipitaceae* and used widely as the traditional Chinese medicines and nutritious food for centuries [1]. It is parasitized on *Lepidoptera* larvae or pupae in temperate areas such as Europe, Northern Asia, and North America [2]. It is scientifically proven that *Cordyceps militaris* including two parts: the fruiting body and sclerotium (the corpse part of the insect) [3]. Recently, Nguyen Ngoc Quy et al. (2020) conducted the process of extracting extracts from *Cordyceps militaris* in Kon Tum province by using hot extraction with reflux conduction. The author indicated that extraction parameters including the ethanol concentration material/solvent ratio, extraction time, extraction temperature, and number of extraction times with 60%, 1:40, 28 minutes, 70°C and 1, correspondingly [4]. Besides, the fruiting bodies of *Cordyceps militaris* were extracted by maceration and infusion as reported by Pachabadee Marsup et al. (2020) [5]. *Cordyceps militaris* contained many active compounds such as cordycepin (3'-deoxyadenosine), ergosterol, D-mannitol γ-aminobutyric acid (GABA) and polysaccharides [6], which exhibits important biological activities known to protect and improve lung and kidney function [7], immunomodulation [8],

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anticancer [9], anti-diabetic [10], antioxidant [11], antifungal [12], and anti-inflammatory [13]. Because of those benefits it brings, the researchers have developed many commercial products such as drinks, capsules, instant tea and so on; however; these products are quite limited in Vietnam.

In order to meet consumers demand regarding healthy and diversify products, many producers studied forming instant tea products from natural ingredients and plant extracts through different drying methods. For example, Nguyen Phu Thuong Nhan et al. (2020) processed instant tea from *Condonopsis javanica* L. root extract via spray drying technology. The drying yield reached 78.35% at the concentration of maltodextrin as 30% (w/w), the drying temperature of 140°C, and the feed rate as 300 mL/h [14]. Besides, Zeynep Aksit et al. (2018) researched instant tea produced from mint (*Mentha Piperita* L.) via spray drying and freeze drying technology. The results showed that solubility in water of mint tea powders by using freeze drying and spray drying were 96.23; 98.33 and moisture content were 5.56; 0.24% [15].

Many studies on chemical compounds and biological activities of *Cordyceps militaris* have been conducted; however; the product of *Cordyceps militaris* has not been totally researched. Therefore, the objective of this research has been conducted to survey a suitable formulation for instant tea from *Cordyceps militaris* extract by using convection drying. The analyzing parameters consisted of moisture content, cordycepin content, solubility and sensory evaluation.

2. Materials and methods

2.1. Materials

Fruiting body of *Cordyceps militaris* was obtained from Kon Tum province (Kon Tum, Vietnam), growing on brown rice. The initial ingredient is experienced processes including cleaning, drying, grinding, and sieving to get smooth powder products. Powder *Cordyceps militaris* is extracted in ethanol solution 60% at a temperature of 70°C for 30 minutes. Next, the mixture is filtered in order to remove residue and concentrate at a temperature of 70°C to reduce water until the rest of moisture content as 75.54%. The extraction was repeated many times to get a large amount of extract.

Chemicals: Maltodextrin with a DE value of 12 (China) and distilled water were used in the study is single-distilled water.

2.2. Production of instant tea by using convection drying

Firstly, maltodextrin as carrier is dissolved in distilled water at a certain concentration and keep at room temperature overnight. Secondly, the *Cordyceps militaris* extract was added to the solution in the suitable ratio to create a new mixture. Then, the mixture was poured into on an aluminum tray (29x32 cm) which were covered with baking paper, and a thickness of layer about 1 mm. After that, the tray was put into the convection drying oven at a certain time and temperature. After 15 h, the product was moulded and ground until obtaining uniform size, packed it into the specialized plastic bags. These bags were removed the air and stored at room temperature.

2.3. Determination of Moisture Content

The moisture content of the product was determined using a halogen rapid moisture analyzer (MB90, Ohaus, USA). The samples were dried at a temperature of 105°C until constant weight was obtained [16].

2.4. Determination of Cordycepin Content

The cordycepin content was performed according to the reported method with some modifications (H. Hur et al., 2008). HPLC analysis was performed in a high-performance liquid chromatography equipment with a silica column of 250 mm length and 4.6 mm internal diameter. Mobile phase was chosen methanol:potassium dihydrogen phosphate (KH_2PO_4) with the rate of 15:85 and flow rate was 1 ml/min. Sample injection volume was pumped 10 µl. Detection was performed with a variable-wavelength UV detector (L-4250) at 260 nm.

The standard cordycepin is dissolved, similar to above mobile phase solution at varying concentrations. The data was used to plot a calibration curves of the standard cordycepin [17].

2.4. Determination of solubility (WSI)

The solubility of the powder was determined using the method described by Maruf Ahmed et al. (2010) with some modifications. 2.5 g powder was added 30 ml distilled water, shaken for 1 minute and incubated in a water bath at 30 °C for 30 min, then centrifuged at 5000 rpm for 10 min. After centrifugation, the supernatant was put into a Petri dish and dried at 105

°C until constant weight. The solubility was calculated by using the following equation 1 [18]:

$$\text{WAI (\%)} = \frac{\text{dry sediment weight}}{\text{dry sample weight}} \times 100 \quad (1)$$

2.5. Sensory evaluation

The research of consumers acceptance was evaluated by 10 people and using a 5-point hedonic scale (5 – like very much, 4 – like much, 3 – like, 2 – dislike and 1 – dislike much).

Using TCVN 3215-79 standard to evaluate sensory quality of the final product, including a sensory committee of 10 people. The final product was evaluated in terms of color, appearance, aroma and taste. The scale from 0 to 5 with 0 = very low, poor and 5 = very high, good is used to determine the level of disability of each sensory indicator. The average score of a sensory indicator is the average of the evaluation results for that criterion by a committee. The important factor represents the importance of each sensory criterion. The weighted scores is defined that the average score of each parameter multiply important factor. The last sensory score is the total weighted score of all the sensory indicators.

2.6. Statistical analysis

The data obtained was processed and plotted on Microsoft® excel 2010 software. The experimental results were analyzed using ANOVA by Statgraphics Centurion XV software (Statgraphics Technologies, Inc., USA) at 95% confidential level.

3. Results and discussion

3.1. Effect of carrier concentration

Table 1 describes the appearance, and the moisture content of the powder products. Figure 1 presents the effect of carrier concentrations to cordycepin content in powder. At varying concentrations, the obtained-powder is relatively uniform in sharp and the moisture content get a low value under 5%. The result of the ANOVA (analysis of variance) showed that effect of carrier concentrations on cordycepin content was statistically significant ($p<0.05$). Furthermmore, LSD multiple range test pointed out meaning differences among cordycepin content at the four different concentrations ($p<0.05$). The highest cordycepin content (139.85 µg/g DW) was achieved at maltodextrin concentration of 60% (w/w). Generally, the maltodextrin concentration increases from 30% to 60% (w/w), which lead to increase cordycepin content in instant tea product. This can be explained by the effect of surface - active carbohydrate molecules in carriers that could create to the binding with bioactive compounds at the core of products like cordycepin [19]. However, using high carrier concentration leads to a longer drying time due to the increase of the mixture' viscosity which leads to prevent water evaporation [20]. Besides, the sensory evaluation (Figure 2) displayed that carrier concentration of 40% reached the highest sensory score (3.0 of total 5.0). Therefore, carrier concentration of 40% was selected for the subsequent experiments.

3.2. Effect of concentration of Cordyceps militaris extract

Table 2 shows the observable, and the moisture content of the instant tea products. Figure 3 presents the effect of concentrations of *Cordyceps militaris* extract on cordycepin content. Overall, with high extract concentrations give the increase moisture content in the product and creation of smooth structure in powder. When concentration of *Cordyceps militaris* extract increased from 5% to 8% that cause to the decrease of cordycepin content. The effect of concentration extract on cordycepin content is statically difference ($p<0.05$), demonstrated by one-way analysis of variance (ANOVA). Further LSD multiple range test indicated that the difference among cordycepin content at the four different concentrations. The cordycepin content was achieved the highest value as 94.307 µg/g DW at concentration of extract as 5% and concentration of maltodextrin as 40% . This can be explained by the number of bonds which is created by carrier agent in the drying process. The number of bonds is suitable for covering the greatest amount of cordycepin; therefore; when the concentration of extract increases 6%, 7% and 8% (w/w), the ability of cordycepin' storing did not even improve [21]. In addition, the structure of cordycepin contains unsaturated conjugated polyene resulted in easily lose cordycepin content in the product [22]. According to the sensory score (Figure 4), when using extract concentration of 6%, the product reached the highest point (3.3 of total 5.0). Considering the factors, the *Cordyceps militaris* extract concentration of 6% would be chosen for the next experiments.

Table 1: The appearance, moisture content, cordycepin content and sensory score of instant tea at different maltodextrin concentrations

Concentration (%)	Appearance	Moisture (%)	Cordycepin content (μg/g DW)	Sensory score
30%		3.46	87.321±0.470[a]	2.5
40%		3.23	105.067±0.069[b]	3.0
50%		3.22	125.647±0.836[c]	2.8
60%		3.66	139.850±1.360[d]	2.3

*a,b,c,d letters represent the difference between treatments

Table 2 : The appearance, moisture content, cordycepin content and sensory score of instant tea at different extract concentrations

Concentration (%)	Appearance	Moisture (%)	Cordycepin content (μg/g DW)	Sensory score
5%		1.44	94.307±0.420[a]	2.5
6%		1.81	71.000±0.406[b]	3.3
7%		2.22	56.455±0.049[c]	3.1
8%		3.42	48.129±0.465[d]	3.1

*a,b,c,d letters represent the difference between treatments

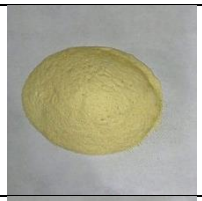
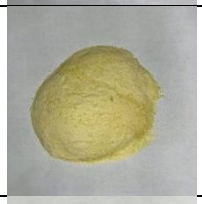
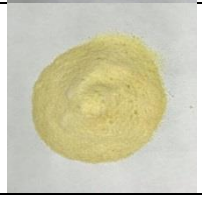
3.3. Effect of drying time

Table 3 presents the appearance and the moisture content of instant tea dried at different drying time. The effect of drying time on cordycepin content is displayed in Figure 5. Data showed in Table 3 indicate that powder dried at different time presented moisture lower than 3%. The obtained-powder was light yellow in appearance with a uniform particle size. Increasing the drying time led to the decreasing in moisture content of products. ANOVA analysis results showed a significant effect of drying time on cordycepin content in powder with 95% confidence ($p<0.05$). In this study, with the drying time of 20 h, the highest cordycepin content was 106.59 μg/g DW. Besides, LSD multiple range test indicated that the drying time of 15 h did not differ significantly from 20 h ($p < 0.05$). Therefore, in terms of economic efficiency, 15 h drying time is the best choice. The longer drying time show the decrease in cordycepin content. This can be explained that cordycepin structure contains long unsaturated conjugated polyene which easily decomposes when rising the drying time [22]. In addition, according to the results of sensory evaluation, as shown in Figure 6, it was found that the 15 h drying sample achieved the highest sensory score (3.4 of total 5.0). For these reasons, a suitable time for the drying process was 15 hours.

3.4. Effect of drying temperature

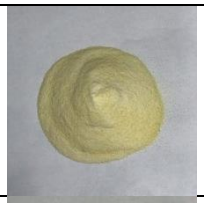
The image of instant tea products and moisture content with different drying temperature is presented in Table 4. Figure 7 shows the effect of drying temperature on cordycepin content. Overall, the products were small particle size and not aggregated together. Moisture content of *Cordyceps militaris* powder ranged from 2.67% to 1.31%. It was observed powder moisture content decreases as drying temperature increases. The results of the ANOVA test indicated that there were a significant effect of drying temperature on cordycepin content at the $p<0.05$ level. Furthermore, LSD multiple range test pointed out meaningfully differences between the drying temperature of 70°C from 60°C and 80°C. The cordycepin content reached the highest value as 108.377 μg/g DW at the drying temperature of 70°C. However, when the drying temperature was increased upwards 80°C, this value tends to decrease. The previous studies indicated that high drying temperature could cause the decomposition of thermo-sensitive substance and thus decreased cordycepin content [23]. Additionally, the results of sensory evaluation (Figure 8) showed that samples dried at 70°C achieved the highest sensory score (3.6 of total 5.0). Therefore, 70°C is the suitable temperature for instant tea drying.

Table 3: The appearance, moisture content, cordycepin content and sensory score of instant tea at different drying time

Drying time (h)	Appearance	Moisture (%)	Cordycepin content (μg/g DW)	Sensory score
15 h		2.32	105.313 ± 0.149^{ab}	3.4
20 h		0.91	106.590 ± 1.113^{a}	2.9
25 h		0.32	104.135 ± 1.078^{b}	2.7

*a,b letters represent the difference between treatments

Table 4: The appearance, moisture content, cordycepin content and sensory score of instant tea at different drying temperature

Drying temperature (°C)	Appearance	Moisture (%)	Cordycepin content (μg/g DW)	Sensory score
60°C		2.67	103.023±0.896[a]	2.8
70°C		2.28	108.377±2.404[b]	3.6
80°C		1.31	101.653±0.374[a]	3.1

*a,b letters represent the difference between treatments

3.5. The physical-chemical properties of instant tea product

Table 5 shows the physical-chemical properties of instant tea products. Instant tea products from *Cordyceps militaris* extract reached a moisture content of 1.713%, which was lower than the maximum level as 6% for instant tea products following by TCVN 9739:2013. The product had a cordycepin content of 97.274 μg/g DW, which was lower than the studies of Hyo-Nam Son (2020) [24] and Luo Wei (2020) [25]. These authors presented the content of cordycepin in the Cordyceps coffee and the milk beverage from *Cordyceps militaris* residues reached 2274.70 μg/g and 1131.1 μg/g, respectively. In addition, the high solubility of 94.843% makes the product easily soluble in water when it is used.

Table 5 :The physical-chemical properties of instant tea product

No.	Parameters	Value
1	Moisture content (%)	1.713±0.104
2	Solubility (%)	94.843 ±1.475
3	Cordycepin content (μg/g DW)	97.274±0.423

3.6. The result of sensory evaluation of instant tea product

Table 6 presents sensory score of instant tea product. The powder was light yellow, not clumped, a good stability when it is dissolved into water. The taste of the product is harmony between the sweetness of sugar and the slightly bitter taste of *Cordyceps militaris*, the characteristic *Cordyceps militaris* smell and no strange smell. With the total score 16.4 of a maximum 20 points, the product is in good according to TCVN 3215-79.

3.7. Evaluation of product properties according to Vietnam standards (TCVN)

Samples of instant tea products from *Cordyceps militaris* extract were sent for quality assessment at New Century Corp with criteria according to TCVN (table 7). The analysis results showed that toxic metal, such as Lead, Cadmium and Arsenic were all below the acceptance limit. Total aerobic microorganisms and total yeast spores, molds were not detected. In addition, pathogenic microorganisms such as *Coliforms*, *E. coli*, *S. aureus*, and *Salmonella* do not present in the product. Besides, the total ash was 0.24% lower than the maximum limit (8%) and ash insoluble in hydrochloric acid was not detected. Therefore, the product meets the toxic metal and microorganism criteria of instant tea product according to the TCVN.

Table 6: Sensory score of instant tea product

No.	Criteria	Total	Average score without the important factor	The important factor	Value
1	Color	47	4.7	0.8	3.76
2	Appearance	38	3.8	0.8	3.04
3	Aroma	39	3.9	1.2	4.68
4	Taste	41	4.1	1.2	4.92
Total					16.4

Table 7 : Quality criteria of instant tea according to TCVN

No.	Evaluation criteria	Analytical method	Results	Units
1	Total aerobic microorganisms	TCVN 4884 – 1 : 2015	< 10	CFU/g
2	*Escherichia coli*	TCVN 7924 – 2 : 2008	< 10	CFU/g
3	*Coliforms*	AOAC 975.55	< 10	CFU/g
4	Total yeast spores, mold	TCVN 8275 – 2 : 2010	< 10	CFU/g
5	*Salmonella*	TCVN 10780 – 1 : 2017	Not detected	in 25g
6	Lead (Pb)	AOAC 999.11	< 0.06	mg/kg
7	Cadmium (Cd)	AOAC 999.11	< 0.005	mg/kg
8	Arsenic (As)	AOAC 986.15	Not detected (LOD = 0.05)	mg/kg
9	*Staphylococcus aureus*	TCVN 4830 – 1 : 2015	< 10	CFU/g
10	Total ash content	TCVN 5611 : 2007	0.24	%
11	Ash insoluble in hydrochloric acid	TCVN 5612 : 2007	Not detected (LOD = 0.05)	%

4. Conclusion

The results have given the appropriate parameters for the research on the production of *Cordyceps militari*s instant tea as follows: the concentration of maltodextrin used as a carrier material 40% (w/w), the concentration of *Cordyceps militaris* extract 6% (w/w), drying conditions at 70ºC for 15 h. The instant tea produced by this method is light yellow, not clumped, harmony between the sweetness of sugar and the slightly bitter taste of *Cordyceps militaris*, the characteristic *Cordyceps militaris* smell. Moisture content, solubility, and cordycepin content are 1.713%, 94.843%, and 97.274 µg/g DW, respectively. These results presented that using instant tea from *Cordyceps militaris* extract could bring users many benefits, contribute to improving the ability of application *Cordyceps militaris* extract in beverage industries; and rise potential for commercialization of the products from the natural original.

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6. References

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