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CORN STEEP LIQUOR PREPARATION FOR USE IN THE FERMENTATION PROCESS

Le Ngoc Khanh[1], Dam Thanh Xuan[1], Bui Thi Thuy Luyen[2], Vu Thanh Thao[3], Nguyen Khac Tiep[1,*]

[1]Faculty of Biotechnology, Hanoi University of Pharmacy, Vietnam;

[2]Faculty of Pharmaceutical Chemistry and Technology, Hanoi University of Pharmacy, Vietnam;

[3]Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam

*Corresponding author: tiepnk@hup.edu.vn

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Summary

Corn Steep Liquor Preparation for Use in the Fermentation Process

The primary goal of this work was to develop a laboratory-scale process for producing corn-steep liquor from locally available materials to replace imported commercial products used in microbe culturing media. Additionally, the study aimed to compare the quality of our locally-made corn-steep liquor with commercial products. The findings indicated that corn sourced from Hai Duong Province had more advantages as a starting material for production. The corn-steep liquor was obtained using the process of hot water extraction, wherein 2 kilograms of corn kernel were soaked in water at a temperature of 50°C for 48 hours (water-to-corn ratio of 1.5:1). The resulting liquid was separated and condensed after the extraction process. The locally-produced corn-steep liquor meets the required moisture, pH, and total amino acid content standards, surpassing the imported products in protein concentration. The obtained corn-steep liquor was used for calcium lactate and antibiotics fermentation process, exhibiting a comparable overall yield to commercially available products. The potential use of our corn-steep liquor to substitute imported products in industrial fermentation applications is worth considering.

Keywords: *Corn-steep liquor, Extraction, Amino acid, Total nitrogen, Microorganism media.*

1. Introduction

Corn-steep liquor (CSL) is a product prepared from corn kernels soaked in water and then concentrated to a specific concentration [1],[2]. It is a brown-black viscous concentrate, mildly fragrant, with a pH ranging from 3.7 to 4.7 and a density of about 1.25 g/mL. CSL has a relatively high nitrogen content of 3.85-4.1%, of which 1.45-1.65% is amine nitrogen and 0.15 - 0.30% is volatile nitrogen. It also contains many macro- and micro-nutrient elements and vitamin B complexes [1],[2]. CSL plays an essential role in providing the amino acids and micronutrients needed for the fermentation process, especially in the production of antibiotics. It has been widely used in industrial fermentation processes since the 1950s of the 20th century [1],[2]. In Vietnam, there are various suppliers of CSL for industrial uses; however, all of them are foreign companies.

The quality of CSL is heterogeneous due to its complex chemical compositions, which depend on the corn varieties and the production process. Some essential factors during the production process may include temperature, time, and the solvent ratio used for extraction. This study was conducted with two objectives: (1) to establish a laboratory-scale production process for CSL from corn kernels and (2) to compare the locally-made product with the commercial product on some critical criteria.

2. Material and method

Corn kernel: Corn seeds are collected in different Vietnamese provinces, including Lao Cai City (Lao Cai), Thanh Tri (Hanoi), Hoai Duc (Hanoi), and Nam Sach (Hai Duong). Immediately after harvest, the corn kernel is removed from the cob, dried, and used for the production of CSL.

Bacteria strains: *Lactobacillus acidophilus* ATCC 4356, *Bacillus subtilis* ATCC 6663. *Streptomyces* sp. CND 25 was isolated and stored in the Department of Pharmaceutical Biotechnology.

Commercial product: CSL from Sigma Aldrich (USA).

Locally-produced CSL preparation process: After screening and cleaning, the corn seeds were soaked in hot water for 30-60 hours. To achieve the best finished CSL, the water temperature, time, and water-to-corn ratio were investigated. After soaking, the obtained liquid (steep water) was adjusted to pH 8.0-8.5 with 30% NaOH to precipitate impurity protein. After filtration, the steep water was adjusted to a pH of 4.5-5.0 using 20% HCl and condensed to approximately 50% moisture to obtain the finished CSL [1],[2],[3].

Total nitrogen analysis: The corn seeds were digested in sulfuric acid, and then the total nitrogen was analyzed using Kjeldahl's method. From the total amount of nitrogen, the protein concentration in the sample can be deduced [4].

Amino acid composition analysis: The amino acid composition in the CSL was determined using high-pressure liquid chromatography (HPLC) at the National Institute of Nutrition (Hanoi), according to Appendix 10.11, Vietnamese Pharmacopoeia 5th edition [5]. The analysis was performed on both locally made and commercial products to compare their total amino acid composition.

Production yield was calculated as a percentage of the obtained CSL mass and the starting corn kernel mass.

Calcium lactate fermentation: The fermentation process was carried out at 37°C for 96 h. *L. acidophilus* ATCC 4356 was grown in suitable fermentation media, including CSL. The calcium lactate production yield from locally made and commercial products was compared.

Antibiotic fermentation: The fermentation process was carried out at 28°C for 144 h with vigorous shaking. *Streptomyces* sp. *CND 25* was grown in suitable fermentation media, including CSL. The antibiotic production yield was assessed based on an antimicrobial activity test using a disc diffusion test with *B. subtilis ATCC 6633* [5]. The antimicrobial activity of fermented media from locally made and commercial products was compared.

3. Result

3.1. Corn kernel selection

The total nitrogen content and protein concentration of corn seeds from different sources were determined to select the best corn as a starting material for CSL processing. Our preliminary investigation showed that corn from Hai Duong had the highest protein content of 9.11 ± 0,05% compared to Lao Cai (8.73 ±0,036%), Thanh Tri (8.52 ± 0.087%), and Hoai Duc (8.46 ± 0.063%). Therefore, Hai Duong corn was selected as a starting material for the production of CSL.

3.2. Influence of different parameters on the yield of CSL preparation

Table 1. Influence of different parameters on the yield of CSL preparation

Parameter		CSL amount (g)	Yield (%)
Temperature (°C)	40	120.21	6.01
	50	**141.19**	**7.05**
	60	129.94	6.55
Time (h)	24	121.16	6.06
	36	130.81	6.54
	48	**142.20**	**7.11**
	54	140.43	7.02
Water-to-corn ratio	1:1	96.16	4.81
	1.5:1	**140.81**	**7.04**
	2:1	142.20	7.11
	2.5:1	142.18	7.11

Three essential parameters of the CSL preparation process, including temperature, time, and water-to-corn ratio, were investigated, and the results were presented in Table 1. The optimal conditions for the CSL preparation process were determined as corn kernels were soaked in water at 50°C for 48 hours (water-to-corn ratio of 1.5:1).

3.3. Comparison of key quality criteria of locally-produced CSL and imported commercial product

3.3.1. Visual appearance, pH, and moisture content:

Our locally-produced CSL had a slightly darker color than the commercial ones, while the pH and moisture content of both samples were about the same (Table 2). Data was expressed as mean ± SD.

Table 2. Comparison of visual appearance, pH, and moisture content of our locally-produced CSL and commercial product

	Visual appearance	pH	Moisture content (%)
Commercial CSL	Bright brown, mildly fragrant. Viscous concentrated sticky liquid	4.57 ± 0.08	43.5 ± 1.43
Locally-produced CSL	Black brown, mildly fragrant. Viscous concentrated sticky liquid	4.59 ± 0.06	49.89 ± 2.11

3.3.2. Total nitrogen and amino acid composition:

The HPLC analysis result showed that the amino acid composition of our CSL and commercial product was highly identical, with 16 amino acids being the same in both samples (Table 3).

Table 3: Total amino acid content by HPLC in our locally-produced CSL and commercial product (mg/g)

No	Amino acid	Locally-produced CSL	Commercial CSL
1	Acid aspartic	7.02	8.2
2	Serine	2.11	4.05
3	Glutamic acid	9.36	9.5
4	Glycine	4.38	6.27
5	Histidine	4.17	3.7
6	Threonine	2.89	2.2
7	Arginine	6.24	6.5
8	Alanine	6.8	5.69
9	Proline	15.3	12.6
10	Tyrosine	2.64	1.12
11	Valine	3.19	3.92
12	Methionine	1.11	0.72
13	Lysine	2.99	3.81
14	Isoleucine	1.72	2.52
15	Leucine	4.02	5.4
16	Phenylalanine	2.53	3.29
Total amino acid		**76.47**	**79.49**

Table 4 presents the details of total amino acids and total nitrogen content. Data was expressed as mean ± SD. The total amino acid content of the two CSL samples was the same, while our locally produced sample had a higher total nitrogen content than the commercial product.

Table 4. Total amino acids and total nitrogen content of our locally-produced CSL and commercial product

	Total amino acids content (%)	Total nitrogen content (%)
Commercial CSL	0.7949	1.70 ± 0.04
Locally-produced CSL	0.7647	3.27 ± 0.08

3.3.3. Bioactive compound fermentation using our locally-produced CSL and commercial product

In fermentation, CSL was commonly used as an essential organic nitrogen source to support the synthesis of many microorganism-derived bioactive compounds. Therefore, to compare the quality of our locally-produced CSL and commercial product, calcium lactate, and antibiotic fermentation processes were performed using fermentation media supplemented with different organic nitrogen sources. The amount of calcium lactate in *Lactobacillus* fermentation and the inhibition zone diameter of *Streptomyces* fermented media was used to assess the production yield (Table 5). Data was expressed as mean ± SD.

Table 5. Fermentation results using different sources of inorganic nitrogen

Organic nitrogen sources in fermentation media	Amount of calcium lactate (g)	Inhibition zone diameter (mm)
Not supplemented	2.46 ± 0.29	No inhibition
Yeast extract	6.99 ± 0.3	16.8 (±1.6)
Commercial CSL	7.1 ± 0.32	19.9 (±1.14)
Locally-produced CSL	6.97 ± 0.16	20.0 (±0.98)

The amount of calcium lactate produced when using media supplemented with yeast extract and both CSL products was approximately the same after fermentation. Adding both CSL yielded a better antimicrobial activity than yeast extract supplements, and no difference was observed between our locally produced and commercial samples.

4. Discussion

After corn selection for starting material, the preparation process parameters are critical to yielding a quality CSL, especially the temperature, time, and water-to-corn ratio. In terms of temperature, when extracted at 40°C, the solubility of the corn nutrient was hindered, while at higher temperatures of 60°C, the precipitation of proteins also limited the production yield. Regarding preparation time, 24-hour and 36-hour immersions are too short to extract the corn nutrients completely, resulting in lower yields. At 54 hours, the extraction yield was not higher than at 48 hours, while the diffusion of some impurities might increase, making it difficult for the next step. Increasing the water-to-corn ratio from 1:1 to 1.5:1 showed a remarkable rise in CSL production yield. At the 2:1 and 2.5:1 ratios, the production yield remained the same as at the 1.5:1 ratio. However, the next condensed step will be more time-consuming, so the water-to-corn ratio of 1.5:1 is optimal for the preparation process.

In order to be considered an alternative to imported commercial products, our locally produced CSL must meet their essential criteria. According to the supplier, the commercial product is a bright brown, mildly fragrant, and viscous, concentrated, sticky liquid with a pH of 3.7-4.7 and a moisture content of 40-60%. There is no publication on the total nitrogen and amino acid composition of CSL. Our locally produced CSL matches the imported product in terms of visual appearance, pH, and moisture content. Our CSL is similar to the commercial sample in total amino acid content and surpasses the commercial product in total nitrogen content, suggesting that it is an easy-to-use nitrogen source for microorganism fermentation.

Our CSL application capability in industrial processes is assessed through its use as a fermentation medium for microorganisms. CSL is essential for *L. acidophilus* growth and biosynthesis, and when fermented media contains low-quality CSL, the microbes develop poorly and produce less calcium lactate. In *Streptomyces* sp., CSL provides an easy-to-use source of organic nitrogen, vital for the growth, development, and antibiotic production of bacteria [1],[3]. The results showed that our CSL was better than yeast extract and about the same as commercial CSL as an amino acid source in the biosynthesis of calcium lactate and antibiotics from *L. acidophilus* and *Streptomyces* sp. The fermentation yields of both CSL are equivalent, suggesting the possibility of substituting commercial CSL with our locally produced CSL for industrial applications.

5. Conclusion

The study effectively demonstrated an easy approach for producing CSL on a laboratory scale, using maize kernels sourced from Hai Duong. The extraction procedure was conducted at a temperature of 50°C for 48 hours, using a water-to-corn ratio of 1.5:1. Subsequently, the liquid was condensed to get a finished CSL that had comparable visual characteristics, pH level, moisture content, and total amino acid content as commercially available products. Our laboratory fermentation experiments have shown that our CSL generates a comparable yield of biosynthesis compounds to commercial products. Further detailed research might be conducted to obtain domestically produced corn-steep liquor that can be employed as a substitute for imported products.

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