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**Research Article**

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# Determination of vitamin C and nicotinic acid content in black bean by capillary zone electrophoresis

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**ABSTRACT**

*In this paper, capillary zone electrophoresis method was used for the determination of vitamin C and nicotinic acid content in black bean. 20 mmol/L borax solution and 20% acetonitrile concentration, 20kV voltage, 254 nm UV detection wavelength was chosen for electrophoretic analysis. Measured vitamin C and nicotinic acid content in black bean were 0.333 mg/g (RSD = 3.9%) (n = 5), 0.482 mg/g (RSD = 5.9%) (n = 5), respectively.*

**Key words:** Capillary electrophoresis; black bean; Vitamin C; nicotinic acid

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## INTRODUCTION

The black bean includes rich protein, fat, vitamin, microelement and thick fibre which has high protein, low quantity of heat characteristic property. The protein contents was more than 40%, the composed of indispensable amino acid structure was better than that of soybean, concrete every kind of indispensable amino acid contents was higher than egg. Fat contents was up to 15.9%, the fat gives first place to unsaturation. Black bean was hit by a microelement with the contents that zinc, copper, magnesium, selenium, phosphorus all very high [1]. The black bean had fitness tonic, disease prevention, prolonging longevity, etc [2]. For the determination of cyanidin-3-O-glucoside content in black bean hulls grown in different regions of China by HPLC, the chromatographic method was executed on phenomenex Luna Su $C_{18}$ column by gradient elution using 0.5% phosphoric acid solution as mobile phase A and water: acetonitrile (50:50, *V*/*V*) as mobile phase B at a flow rate of 0.8 mL/min. The detection wavelength was 520 nm [3]. Black bean enriched with selenium was used as carrier to produce black bean sprout; optical methods of deterring selenium content and selenium accumulation were built up by Zhu et al [4]. During germinating process, different selenium concentrations were added by soaking and spraying to make sure the selenium biological accumulation. Polarograph was used to survey selenium content, with which optimum technology of selenium-enriched black bean was optimized. The five soybean isoflavones components containning Genistein, Genistin, Daidein, Daidzin and Glycitin were determined and separated by the established HPLC method, and isoflavones in different tissues and different grow stages of embryo were measured by Zhang et al [5]. The established HPLC condition: an phenomenex $C_{18}$ column was utilized; the mobile phase was MeOH and $H_2O$ (v/v= 30: 70); detective wavelength was 254 nm. Chen et al[6] analyzed various compositions of black beans. The ash, crude fat, total quantity of protein, composition of fatty acid, Vitamin E in black bean flour were determined by using the methods of gas chromatography, HPLC and thermal blast drying oven etc. The contents of four marcoelements, Potassium Calcium and Magnesium and three trace elcments, Iron, Copper, Zine and Manganese in Black-legume, Black-sesame and Black-sesame were determined by Wang et al[7] with Flame-AAS method. It was showed that black bean solution exhibited a s trong DPPH·radical scavenging activity, hydroxyl radical scavenging activity, reducing power and liposome per oxidation inhibiting activity in comparison with ascorbic acid in the literature [8]. The extraction process of flavonoids from the peel of black bean was studied by Zhang et al[9]. The

experiments of single factors, Plackett Burman design and response surface method were utilized to optimize the influence factors (ethanol concentration, temperature, time, the ratio to material of liquid, extraction times) in the experiment. It showed that crude extractings had different bacteriostatic effects on escherichia coli, staphylococcus aureus and bacillus subtilis. For preparing the black bean antioxidant peptides, various peptides in the black bean protein hydrolysates were fractionated with gel filtration by Ren et al[10]. Then, the scavenging capacity to DPPH· free radical and the distribution of molecular weights of fractional peptides were determined and the optimal anti-oxidative peptide fractions were analysed dynamically. Pigment and crude polysaccharide were extracted from the seed of black bean for researching the reather strong antioxidation action by Long et al[11]. The inhibitory effects of pigment and crude Polysaccharide from black bean on whole blood chemiluminescence and on active oxygen had been investigated. The antioxidant effect of water extract from black bean was studied by Xin et al[12] with the methods of pyrogallic autoxidation, salylic acid and DPPH· to assay superoxide, hydroxyl free radical and DPPH· free radical.

Capillary electrophoresis is a new technology of separation and analysis[13-16]. In this paper, a new method for the determination of vitamin C and nicotinic acid content in black bean was estabilished by capillary electrophoresis.

## EXPERIMENTAL SECTION

### Instruments and Reagents

Experimental instruments: CL-1030-type high performance capillary electrophoresis (Beijing Cailu Scientific Instrument Co., Ltd.); HW2000-type chromatography workstation (Nanjing Qianpu Software Ltd.); Capillary (75 μm inner diameter, 59 cm overall length, 50 cm effective length) from Hebei Yongnian Ruifeng Chromatographic Devices Co., Ltd.); Precision pH meter(Shanghai Leici Instrument Factory).

Vitamin C(Tianjin Chemical Reagent Research Institute); Nicotinic acid(Shanghai Blue Season Science and Technology Development Co., Ltd. ); Black bean(weifang supermarket); Other reagents used in the experiments were all analytical grade; Double-distilled water.

### Experimental Methods

Before the start of the experiment, capillary was successively washed with 0.5mol·$L^{-1}$ hydrochloric acid solution, double-distilled water, 0.5mol·$L^{-1}$ sodium hydroxide solution, double-distilled water, buffer solution, each for 8min. After four times running, capillary was cleaned again using the above method.

Measurements were carded out at 20kV voltage and 28 °C experimental temperature. UV detection wavelength was 254 nm. Injection time was 8s(7.5 cm height difference).

### Sample Preparation

Black beans sample solution: Black beans powder accurately weighed 1.0082 g, added 4 mL 10% methanol-water, cold soak time of 24 h, filtered, washed and set the volume to 25 mL that was the black beans sample solution.

## RESULTS AND DISCUSSION

### Selection electrophoresis conditions

Preparation the concentration of 10, 20, 30, 40, 50 mmol/L borax buffer solution, running the vitamin C, nicotinic acid standard solution and black beans sample solution(20 kV). It is suggested that with the increase of the concentration of borax, vitamin C and nicotinic acid migration time was increased. In 20 mmol/L borax solution, the influence of acetonitrile concentration (10%, 20%, 30%, 40%)(v/v) on sample separation was investigated. It is suggested that 20% acetonitrile concentration was better.

Considering these conditions, 20 mmol/L borax solution and 20% acetonitrile concentration, 20kV voltage, 254 nm UV detection wavelength was chosen for electrophoretic analysis condition.

### Quantitative analysis

#### Standard curve

First, vitamin C standard solution that the concentration were 0.044, 0.022, 0.011, 0.0055, 0.0028, 0.0014 mg/mL was prepared. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. Taking concentration as the abscissa and peak area as the ordinate, the standard curve was drew. Linear regression equation of vitamin C (peak area: y μV•s, density: x mg/mL) and the linear range were as follows: y = -5815.36 +3850000 x (r = 0.999), 0.001~ 0.044 mg/mL.

The same test method, Linear regression equation of nicotinic acid (peak area: y μV•s, density: x mg/mL) and the linear range were as follows: y = -1013.2 +2231200 x (r = 0.999), 0.007~ 0.048 mg/mL.

**Precision test**

Vitamin C standard solution precisely drew and continuously injected for six times under electrophoretic separation conditions, the RSD of vitamin C peak area was 4.69%, indicating good precision.

**Determination of sample content**

Under selected electrophoresis conditions, black beans sample solution were run. Separation chromatogram of the black beans sample solution was showed in Figure 1. Measured vitamin C and nicotinic acid content in black bean were 0.333 mg/g (RSD = 3.9%) (n = 5), 0.482 mg/g (RSD = 5.9%) (n = 5), respectively.

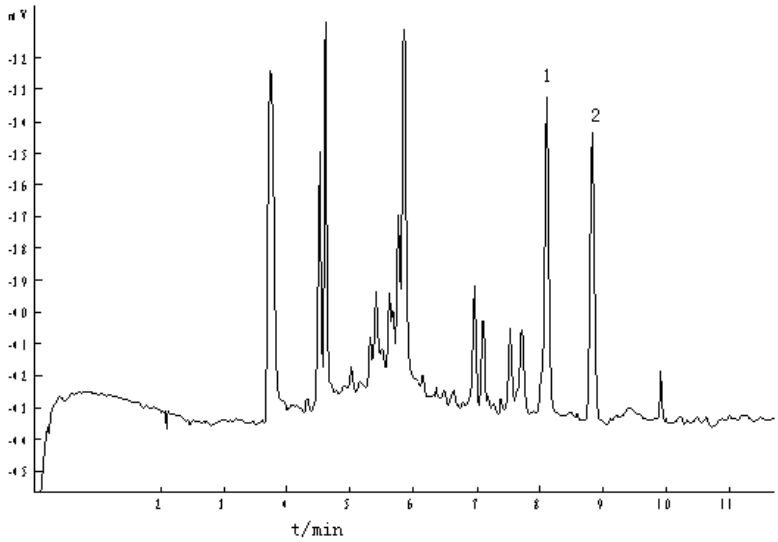


**Fig.1 Electrophorogram of black bean sample solution**
**1-Vitamin C 2-Nicotinic acid**

**Recovery**

After determination for four times, the recoveries of vitamin C in black bean sample were in the range of 88% - 108% (n=4), the recoveries of nicotinic acid were in the range of 87% - 119% (n=4).

## CONCLUSION

A new method for the determination of vitamin C and nicotinic acid content in black bean was established by capillary electrophoresis. Measured vitamin C and nicotinic acid content in black bean were 0.333 mg/g (RSD = 3.9%) (n = 5), 0.482 mg/g (RSD = 5.9%) (n = 5), respectively.

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