

Study on Process Parameters of Extraction of γ -aminobutyric Acid Instant *Moringa oleifera* Powder

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Abstract. To preliminary optimize the extraction of γ -aminobutyric acid instant tea powder from *Moringa oleifera* leaves, taking γ -aminobutyric acid *Moringa oleifera* leaves as raw material and pure water as extraction solvent and GABA content as the main evaluation index, the effects of extraction temperature, ratio of liquid to material and extraction time on biochemical components and sensory qualities of *Moringa oleifera* leaves extractor was studied by using one-factor-at-a-time experiment. The results showed that the optimal extraction conditions for γ -aminobutyric acid instant tea powder were extraction temperature of 60 °C, material-to-water ration of 1:10 and extraction time of 40 min.

1. Introduction

Moringa oleifera Lam. is a species belonging to the *Moringa* Branch and *Moringaceae* family, widely distributed in tropical and subtropical regions of Asia, Africa^[1]. The whole plant has edible value and can be useful^[2]. Its roots, stems, leaves, flowers, seeds, branches and barks are rich in nutrients and medicinal ingredients^[3]. Studies have shown that *Moringa* leaves are rich in potassium, calcium, phosphorus, iron, and essential amino acids, and having antioxidant activity of vitamin C, flavonoids and polyphenols substances^[3]. *Moringa* has antioxidant, anti-cancer, hypoglycemic and lipid-lowering and other biological activities^[4-9].

γ -aminobutyric acid (GABA) is a non protein natural amino acid. It has many physiological activities, such as lowering blood pressure, anti-aging and anti anxiety^[10]. Compared with foreign countries, the domestic amino acid application is very few in the terminal products, and there is not much research on GABA instant tea^[11]. The production of instant tea mainly includes the processing of raw materials, extraction, purification, concentration, drying and packaging^[12]. Extraction process is the primary process in the production of instant tea. The extraction temperature, water ratio, time, pH and other factors seriously affect the flavor, physicochemical and decoction color factors of instant tea^[13]. The extraction process parameters were studied on Black Tea, Green Tea GABA, but there is no study on the extraction technology of *Moringa oleifera* GABA reports.

Therefore, in this experiment GABA *Moringa* leaves as raw material, using water as the extraction solvent, different temperature, solid-liquid ratio and time as the single factor to design experiment, the



Moringa extracts color, GABA ratio, pH value, the yield of flavonoids, polyphenols and free amino acid as test index, to provide theoretical basis for the development of GABA Moringa leaves instant tea.

2. Materials and methods

2.1 materials

GABA Moringa leaves: fresh leaves of *Moringa oleifera* were bought from Henan Gold M. *oleifera* Biological Technology Co. Ltd., concentrated by GABA Technology (monosodium glutamate soaking) after steaming, baked at 80 °C until the moisture content is less than 10 %, Moringa leaves raw material was achieved, then Shatter 60 mesh, placed in the bag to spare.

2.2 method

2.2.1 experimental design

(1) The influence of the extraction temperature on the partition ratio of the extracted solution. Accurately weighed 5 groups of Moringa leaf meal, each 0.5g, extraction temperature was 50、60、70、80 and 90 °C respectively, extraction time was 40 min, solid-liquid ratio was 1:20, extract the hot filtration, the filtrate was transferred to a 50 ml volumetric flask, and then water was fixed to volume. The effects of extraction temperature on GABA ratio, pH value, the yield of flavonoids, polyphenols and free amino acid were investigated, and each treatment was repeated 3 times.

(2) The influence of the solid-liquid ratio on the partition ratio of the extracted solution.

Accurately weighed 5 groups of Moringa leaf meal, each 0.5 g, solid-liquid ratio was 1:5、1:10、1:15、1:20、1:25 respectively, extraction temperature was 60 °C, extraction time was 40 min, extract the hot filtration, the filtrate was transferred to a 50 ml volumetric flask, and then water was fixed to volume. The effects of solid-liquid ratio on GABA ratio, pH value, the yield of flavonoids, polyphenols and free amino acid were investigated, and each treatment was repeated 3 times.

(3) The influence of the extraction time on the partition ratio of the extracted solution. Accurately weighed 5 groups of Moringa leaf meal, each 0.5g, extraction time was 20、40、60、80、100 min respectively, extraction temperature was 60 °C, solid-liquid ratio was 1:10, extract the hot filtration, the filtrate was transferred to a 50 ml volumetric flask, and then water was fixed to volume. The effects of extraction time on GABA ratio, pH value, the yield of flavonoids, polyphenols and free amino acid were investigated, and each treatment was repeated 3 times.

2.2.2 index determination

(1) The content of flavonoids and polyphenols was determined by the method of Vongsak^[15] et al.

(2) The content of free amino acids was determined by GB/T 8314 - 2013^[16].

(3) Method for measuring color difference of extracts by referring to Bian Wei^[17].

(4) Determination of GABA content. Reference Tang Tao^[18] method, but slightly changed.

Chromatography conditions: mobile phase: A is 0.05 mol/L, sodium acetate buffer (pH 6.8, containing 1% N, N- two methyl formamide); B is 50 % acetonitrile water solution (V/V), gradient elution, flow rate was 0.8 mL/min, column temperature was 27 °C and wave length was 360 nm.

Amino acid derivatization: sample solution 1 mL into 10 ml volumetric flask, add 0.5 mol/L sodium bicarbonate (pH=9.0) 1 mL, mix, then add 0.25 ml 1 % 2,4-dinitrofluorobenzene, vortex oscillation uniformly and 60 °C water bath treatment 60 min, then remove and cool to room temperature, with 0.01 mol/L (pH=7.0) potassium dihydrogen phosphate buffer solution to volume, filter with 0.45 μm membrane.

2.3 data processing

SPSS 22.0 was used to process and analyze the data, and the significance analysis of variance ($P < 0.05$) was carried out. The experimental results were expressed in the form of mean $\pm$ standard deviation.

3. Results and analysis

3.1 The influence of the extraction temperature on the partition ratio of the extracted solution.

Drawn from the analysis of Table 1, with the increase of extraction temperature, the yield of GABA was firstly increased and then decreased, reached the maximum value at 60 °C was 2.94 ± 0.12 mg/g, this is consistent with the results of Zou Fengyang^[20], Xu Yongquan^[21] et al. The GABA component can be better retained at 60 °C. The yield of flavonoids, polyphenols and free amino acids increased first and then decreased. In the range of 50 to 70 °C, the yield of polyphenol increased slowly, and the yield of polyphenol reached the highest at 80 °C. The content of free amino acids reached the maximum at 60 °C. The reason may be that the content of free amino acids is smaller and more content, it is easier to dissolve than polyphenols, while polyphenols are mainly distributed in the deep layer of mesophyll, which has good stability and needs higher temperature to dissolve^[22]. The pH value increased slightly with the increase of extraction temperature. The ratio of phenol to ammonia (tea polyphenols/ amino acid) indicates the taste of tea, the smaller of the value, the higher of the content of amino acid in the tea, the better of the taste of the total extract^[18]. As the temperature increases, the ratio of phenol to ammonia increases gradually, so it is more suitable to choose 50 and 60 °C as the extraction temperature. The greater of the L value, the brighter of the color of the tea. The greater of the -a value, the more green of the soup color; the greater of the b value, the more yellow of the soup color. -a/b value is small, the color of tea is dark^[17]. With the increase of the extraction temperature, the brightness of the extract becomes worse. Considering all the indexes, 60 °C was chosen as the extraction temperature for subsequent test.

Table 1 Effect of extraction temperature on physiochemical of GABA *Moringa oleifera* leaves infusion

Index	50°C	60°C	70°C	80°C	90°C
GABA(mg/g)	2.85 ± 0.11	2.94 ± 0.12	2.7 ± 0.04	2.69 ± 0.08	2.58 ± 0.11
Ph Value	5.78 ± 0.04	5.69 ± 0.03	5.79 ± 0.05	5.90 ± 0.12	5.99 ± 0.08
flavonoids (%)	1.76 ± 0.03	1.82 ± 0.03	1.86 ± 0.02	1.91 ± 0.060	1.85 ± 0.02
polyphenols (%)	1.79 ± 0.03	1.87 ± 0.03	1.90 ± 0.030	2.03 ± 0.07	1.96 ± 0.04
free amino acids (%)	6.46 ± 0.29	6.49 ± 0.11	6.35 ± 0.03	6.16 ± 0.07	5.96 ± 0.09
phenol to ammonia	0.28 ± 0.009	0.29 ± 0.006	0.30 ± 0.005	0.33 ± 0.01	0.33 ± 0.001
L*	86.97 ± 0.24	86.47 ± 0.39	86.31 ± 0.12	86.25 ± 0.46	85.89 ± 0.68
-a*	3.36 ± 0.09	2.49 ± 0.16	2.41 ± 0.04	3.02 ± 0.13	2.47 ± 0.07
b*	45.76 ± 0.62	43.85 ± 0.99	43.9 ± 0.62	46.0 ± 0.34	44.96 ± 0.85
-a*/b*	0.073 ± 0.003	0.057 ± 0.005	0.055 ± 0.001	0.065 ± 0.003	0.055 ± 0.001

3.2 The influence of the solid-liquid ratio on the partition ratio of the extracted solution.

As we can see from table 2, with the increase of the solid-liquid ratio, the pH value increases slightly. The yield of GABA and free amino acids showed an upward trend, which reached the maximum at the ratio of solid-liquid at 1:10 and 1:15, and then decreased with the increase of the ratio of solid-liquid. The yield of flavonoids and polyphenols showed an upward trend, and the range of increase was gradually smaller. On the one hand, the reason may be that the ratio of water is increased, and the amount of dissolved tea is lower, so the concentration gradient between solute and solution in tea is increased, which is more conducive to leaching. But when the increase of the ratio of tea to a certain extent, from the inside to the surface of tea solute transfer resistance has become the leading role, weakened the increase of the ratio of tea to increase the concentration gradient of the role, the dissolution rate increased slowly^[23]. On the other hand, it may be because the two components are fully leached, and increasing the solvent does not increase the amount of extraction^[22]. The ratio of phenol to ammonia was the best and the taste was better, and the color of tea was better at the ratio of solid-liquid ratio was 1:10. The brightness of the extract decreased with the increase of the solid-liquid

ratio. According to the analysis above, the leaching effect was the best when the ratio of solid-liquid was 1:10.

Table 2 Effect of ratio of material to water on physiochemical of GABA *Moringa oleifera* leaves infusion

Index	1:5	1:10	1: 15	1:20	1:25
GABA(mg/g)	3.01±0.07	3.14±0.06	2.97±0.13	2.88±0.09	2.74±0.05
Ph Value	5.69±0.14	5.65±0.07	5.66±0.02	5.77±0.14	5.81±0.09
flavonoids (%)	1.65±0.04	1.8 ±0.03	1.89±0.02	1.95±0.05	1.99±0.06
polyphenols (%)	1.79±0.08	1.87±0.06	1.92±0.05	1.95±0.09	1.97±0.050
free amino acids (%)	6.11±0.09	6.47±0.18	6.52±0.09	6.45±0.18	6.18±0.12
phenol to ammonia	0.29±0.01	0.28±0.01	0.29±0.009	0.30±0.006	0.32±0.011
L*	86.38±0.37	85.79±0.11	85.66±0.25	85.63±0.16	85.57±0.19
-a*	2.92±0.06	2.54±0.01	2.46±0.05	2.45±0.12	2.44±0.09
b*	40.99±0.16	44.63±0.28	42.32±0.34	44.32±0.18	42.35±1.26
-a*/b*	0.071±0.001	0.057±0.003	0.058±0.001	0.055±0.003	0.057±0.002

3.3 The influence of the extraction time on the partition ratio of the extracted solution.

From the table 3 showed that with the prolonging of extraction time, pH values have no significant changes. The yield of GABA and free amino acids increased first and then decreased, and reached the maximum at 40 min and 80 min, respectively. The yield of flavonoids showed a downward trend, which indicated that flavonoids had been completely extracted in 20min, probably due to long exposure to air, which led to the oxidation reaction, resulting in lower content. In the range of 20 min to 40 min, the extraction rate increased significantly and with the extraction time prolonged significantly decreased, which may be due to the extraction of the polyphenols had been basically complete, and with the prolonging of the extraction time, the polyphenols could be degraded, transformed or complexed^[22]. The ratio of phenol to ammonia was the smallest at 80 min and 100 min, and the values were basically the same, followed by was 60 min. L* values gradually decreased, brightness became worse, -a*/b* gradually became smaller, and the soup became dark. Although when the extraction time was 40 min, the ratio of phenol to ammonia was large, brightness was not enough, but the yield of GABA and polyphenol was the highest, and the yield of flavonoids remained at a larger value. Therefore, the extraction time was 40 min, and the extract was of the best quality.

Table 3 Effect of extraction time on physiochemical of GABA *Moringa oleifera* leaves infusion

Index	20min	40min	60min	80min	100min
GABA(mg/g)	2.76±0.04	2.99±0.06	2.62±0.12	2.54±0.06	2.5±0.11
Ph Value	5.78±0.12	5.76±0.11	5.77±0.09	5.71±0.12	5.79±0.13
flavonoids (%)	1.85±0.02	1.75±0.08	1.73±0.07	1.60±0.05	1.57±0.01
polyphenols (%)	2.04±0.04	2.25±0.18	2.02±0.04	1.96±0.02	1.94±0.01
free amino acids (%)	6.20±0.05	6.24±0.03	6.27±0.07	6.39±0.04	6.19±0.05
phenol to ammonia	0.33±0.01	0.36±0.03	0.33±0.01	0.31±0.005	0.31±0.002
L*	87.87±3.25	86.11±0.18	85.65±0.097	85.54±0.31	85.54±0.15
-a*	2.95±0.23	2.79±0.03	2.37±0.07	2.41±0.096	2.44±0.12
b*	43.39±0.76	43.07±0.71	43.10±0.72	42.43±0.43	43.73±0.50
-a*/b*	0.068±0.006	0.064±0.001	0.055±0.002	0.057±0.003	0.056±0.003

4. Discussion

With the increase of the extraction temperature, the extraction rate of GABA, flavonoids, polyphenols and free amino acids increased first and then decreased, and the pH value increased gradually, the ratio

of phenol to ammonia increased gradually, and the taste was poor, and the brightness of the extract was decreased. With the increase of water content, pH value increased slightly, GABA and the extraction rate of free amino acids increased at first and then decreased, the yield of flavonoids and polyphenols increased gradually, the phenol to ammonia ratio was increased, taste better, and brightness increased. With the prolonging of extraction time, pH value had no significant difference, GABA, polyphenols and free amino acid was increased first and then decreased, the extraction rate decreased with the prolongation of time, the ratio of phenol to ammonia was decreased, the brightness of the extract was reduced, and the color was deepened. Comprehensive index analysis, the optimum extraction process parameters of γ -aminobutyric acid instant moringa oleifera powder was the extraction temperature was 60 °C, solid-liquid ratio was 1:10 and extraction time was 40 min.

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