Study on the Effect of Extraction Process of Moringa Instant Tea on Its Sensory Quality

Kang Dandan[1,2,3, a], Peng Shaodan[2,3], Li Jihua[2,3], Cao Yupo[2,3, *]

[1]College of Food Science & Technology, Huazhong Agricultural University, Wuhan ,430070, China;

[2]Agricultural Products Processing Research Institute, Chinese Academy of Tropical Agricultural Sciences, Zhanjiang, Guangdong, 524001, China;

[3]Key Laboratory of Tropical Crop Products Processing, Ministry of Agriculture, Zhanjiang, Guangdong, 524001, China

[a]Email:1781211568@qq.com; Address: Agricultural Products Processing Research Institute, No. 48 Renmin Avenue South, Zhanjiang, Guangdong, China; Telephone:17635882646,13020015857

Abstract. Using Moringa as the raw material, the single factor and orthogonal experiments of the extraction process were performed to study the effect of the extraction process on the content of free amino acids and soluble sugars and the main volatile components in Moringa oleifera leaves, and finally organoleptic evaluation was performed on the color and odor of instant tea, and the effect of extraction process on its quality was analyzed. The results showed that after single-factor and three-factor three-level experiments, different objects were used as evaluation criteria, and the corresponding optimal extraction scheme was selected, which provided a sufficient theoretical basis for the actual production of Moringa instant tea.

1. Introduction

Moringa oleifera Lam., is a species belonging to the Moringa Branch and Moringaceae family, distributed in tropical and subtropical regions of Asia, Africa[1].The whole plant has edible value and is useful. Studies have shown that Moringa leaves are rich in potassium, phosphorus, iron, and essential amino acids, and having an antioxidant activity of vitamin C, flavonoids and polyphenols substances[2]. Therefore, Moringa has a high nutritional value, and there is not much research on Moringa instant tea

The production of instant tea mainly includes the processing of raw materials, extraction, purification, concentration, drying and packaging. The extraction process is the primary process in the production of instant tea[3]. The extraction temperature, water ratio, time, pH and other factors seriously affect the flavor, physicochemical and decoction color factors of instant tea[4].

In this experiment, the effect of extracting technology from Moringa oleifera leaves on the quality of instant tea was studied. To provide a theoretical basis for the development of Moringa instant tea.

2. materials and methods

2.1 materials

Moringa leaves：fresh leaves of Moringa oleifera was bought from Henan gold M. oleifera Biological Technology Co. Ltd., bake at 80℃ until the moisture content is 5%, Moringa leaves raw material was achieved, then Shatter 60 mesh, placed in the bag to spare.

2.2 method

2.2.1Determination of Extraction Process Conditions of Moringa Instant Tea

2.2.1.1Moringa leaf pretreatment
The Moringa oleifera leaves were dried in an oven at 80° C. to a moisture content of 5%, then crushed, passed through a 60-mesh sieve, and stored in a refrigerator at −20° C. in the dark.

2.2.1.2 single-factor experiment

(1) Selection of extraction temperature
Accurately weigh 5 sets of 60-mesh sieved Moringa leaf powder, 1.0g for each group, and conduct extraction tests at extraction temperatures of 50°C, 60°C, 70°C, 80°C, and 90°C. The extraction time is 40 minutes. The liquid ratio is 1:20. The extract is filtered while hot, and the filtrate is transferred to a 50 ml volumetric flask. The volume is then adjusted to the mark with water. The effects of extraction temperature on GABA content, polyphenol content, free amino acid content, and tea color of the extract were analyzed, and each treatment was repeated 3 times.

(2) Selection of feed-liquid ratio
Accurately weigh 5 sets of 60-mesh sieved Moringa leaf powder, 1.0g for each group, and conduct a leaching test at a ratio of 1:5, 1:10, 1:15, 1:20, and 1:25 respectively. The temperature was 60°C and the extraction time was 40 minutes. The extract was filtered while hot and the filtrate was transferred to a 50 ml volumetric flask. The volume was then adjusted to the mark with water. The effect of feed-liquid ratio on GABA content, polyphenol content, free amino acid content, and tea color of the extracts was analyzed, and each treatment was repeated 3 times.

(3) Selection of extraction time
Accurately weigh 5 sets of 60-mesh sieved Moringa leaf powder, each group 1.0 g, respectively, 20 min, 40 min, 60 min, 80 min, 100 min extraction time for the extraction test, the extraction temperature is 60 °C, the ratio of material to liquid at 1:10, the extract was filtered while hot, and the filtrate was transferred to a 50 ml volumetric flask and then brought to volume with water. The effects of extraction time on GABA content, polyphenol content, free amino acid content, and tea color of the extracts were analyzed, and each treatment was repeated 3 times.

2.2.1.3 Orthogonal experiment
Based on the results of the best single-factor test, three factors and three levels of the orthogonal test were designed. The scheme is shown in Table 1, and the extraction process parameters were further optimized. Each treatment was repeated three times for the Moringa instant tea powder. The quality is evaluated and the best combination conditions are selected.

Table 1 Parameters of orthogonal experiments

Level	factor		
	(A)Extraction temperature (°C)	(B)feed-liquid ratio	(C) extraction time(min)
-1	50	1:5	30
0	60	1：10	40

1	70	1:15	50

2.2.2 Analysis of Main Flavor Quality Components of Moringa oleifera

2.2.2.1 Determination of free amino acid

(1) The drawing of the amino acid standard curve (refer to GB/T 8314-2013): Weigh 250 mg glutamic acid and dissolve it in an appropriate amount of water, issolve it thoroughly, ilute it to 25 mL and shake well. The standard stock solution mass concentration is 10 mg/mL. Pipette 0.0 mL, 1.0 mL, 1.5 mL, 2.0 mL, 2.5 mL, 3.0 mL of glutamic acid standard stock solution, add water to volume 50 mL, and shake. The series of standard working solution concentrations were 0.2, 0.3, 0.4, 0.5, and 0.6 mg/mL, respectively. Pipette 1.0 mL of the above series of standard stock solutions into five 25 mL colorimetric tubes, add 0.5 mL of phosphate buffer solution (pH 8.0) and 0.5 mL of 2% ninhydrin solution, and heat in a boiling water bath for 15 min. After the addition of water to volume, the absorbance A was measured at 570 nm after 10 minutes.

（2）Sample determination: Weigh 1.0 g (accurate to 0.001g) sample in a 200-mL Erlenmeyer flask, add 80 mL of boiling water, immediately moved into the boiling water bath, leaching for 30min, hot and decompression filtration, rapid cooling and voluming to 200 mL. Pipette 1.0 mL of Moringa leaf extract into a 25-mL colorimetric tube. The rest of the procedure is the same as the standard curve.

（3） Standard curve: According to the amino acid mass concentration X (mg/mL) and the corresponding absorbance value A, a standard curve is obtained, and its linear equation is obtained: A=3.1579X-0.2461, R^2=0.9906.

2.2.2.2 Determination of total soluble sugar content

With reference to Bian Wei's method, soluble sugar content in Moringa oleifera was determined[5].

（1） Preparation of fluorenone reagent: Accurately weigh 0.2g of fluorenone reagent (analytically pure), dissolve in 100 mL of concentrated sulfuric acid, and store in the dark.

（2）Standard curve: Glucose (analytically pure) is baked in an oven at 120°C until constant weight, 0.1 g of standard product is accurately weighed, dissolved in a small amount of pure water, transferred to a 1000-mL volumetric flask, and add water to volume 1000 mL in purified water. Then, the Glucose standard stock solution at a concentration of 100 μg/mL is obtained. According to the scheme of Table 2, the prepared glucose concentration solution was added to a 20 mL stoppered test tube. Immediately, 4.0 mL of the fluorenone reagent was added into cold water and then heated in a boiling water bath for 10 min. The ice-water bath was quickly cooled. One tube was blank and the absorbance (A) of each tube was measured at 620nm. Taking the standard solution concentration X (μg/mL) as the abscissa and the absorbance value A as the ordinate, a glucose standard curve was drawn to obtain a linear equation: A=0.0058X-0.0088, R^2=0.9978.

（3） Sample determination: Weigh 1.0g of Moringa leaf powder, add 60 mL of boiling water, sonicate for 10 min at 300 W, cool at room temperature, centrifuged at 7000 r/min for 10 min, filter by centrifugation, set the volume to 100-mL. Pipette 3mL of the extract into a 100 mL volumetric flask and bring the volume to the mark as a sample test solution. Other operations are consistent with the standard curve drawing operation.

Table 2 standard curve of soluble sugar

Test item	Test tube number						
	0	1	2	3	4	5	6
Stock volume/μL	0	100	200	300	400	600	800

Pure water/μL	1000	900	800	700	600	400	200
Test solution concentration/(μg/mL)	0	10	20	30	40	60	80

2.2.3 Sensory review

Sensory evaluation of fresh leaves of Moringa oleifera after soaking: visual observation by color; odor evaluation by sensory evaluation.

Moringa extract color difference determination method: using a benchtop spectrophotometer determination, with the use of brightness (L), greenness (a) and yellowness (b) said, L value represents the brightness; a and b are positive, respectively indicates the degree of redness and yellowness, and when it is a negative value, indicates the degree of yellowness and blueness, respectively.

2.2.4 Qualitative Analysis of Volatile Components of Instant Tea Powder

2.2.4.1 Solid-phase microextraction conditions

Weigh 0.2 g of sample into a sample bottle, add 5 mL of pure water, seal and place in a magnetic stirrer in a water bath at 60°C for 10 min. Then 65μm PDMS/DVB solid-phase microextraction head (5min aging at 250°C) was equilibrated at 60°C for 5min, adsorbed for 30min, and finally desorbed for 3.5min.

2.2.4.2 Gas chromatography-mass spectrometry

Chromatographic conditions: HP-5MS elastic quartz capillary column (30 m × 0.25 mm × 0.25 μm), a carrier gas of high purity helium (purity > 99.999%), the flow rate of 1.0 mL/min, and a split ratio of 50: 1.

Temperature program: The program temperature, the initial column temperature is 50 °C, maintain 5 min, to 3 °C per minute rose to 125 °C, maintain 3 min, then 2 °C/min to 180 °C, keep 3 min, and finally to 15 °C/min rose to 230 °C.

Mass spectrometry conditions: ion source temperature 230°C, ionization method EI, electron energy 70eV, electron multiplier voltage 350V, quadrupole temperature 150°C, transfer interface temperature 280°C, mass scan range 35 to 400 amu.

2.2.4.3 Qualitative analysis

The mass spectral data obtained by the GC-MS instrument were analyzed, and the Wiley9 and NIST08 standard library were searched, and their effective volatile components were confirmed according to their matching degree. The peak area normalization method was used to analyze the relative content of volatile components in the sample.

3. results and analysis

Data were processed using Origin 8.5 and SPSS 22.0 software, and the significance of differences between the data in each group was tested using the Duncan multiple comparison methods ($P<0.05$). The experiments were repeated 3 times. The experimental results were expressed as mean ± standard deviation.

3.1 Effects of Different Extraction Processes on Contents of Main Flavor Components in Instant Moringa Tea Powder

Table 3 The results of the content of water extract, amino acid and soluble orthogonal test of instant Moringa oleifera power

No.	A	B	C	Blank column	Free amino acid content (%)	Soluble sugar content (%)
1	-1	-1	-1	-1	6.41	14.45
2	-1	0	0	0	5.59	12.25
3	-1	1	1	1	6.22	14.75
4	0	-1	0	1	6.29	12.48
5	0	0	1	-1	6.38	13.47
6	0	1	-1	0	6.25	10.76
7	1	-1	1	0	6.18	11.97
8	1	0	-1	1	6.52	10.88
9	1	1	0	-1	5.95	9.77
k_1	6.073	6.293	6.393	6.247		
k_2	6.307	6.163	5.943	6.007	Free amino acid	
k_3	6.217	6.140	6.260	6.343		
R_1	0.234	0.153	0.450	0.336		
k_{11}	13.817	12.967	12.030	12.563		
k_{22}	12.237	12.200	11.500	11.660	Soluble sugar	
k_{33}	10.873	11.760	13.397	12.703		
R_2	2.944	1.207	1.897	1.043		

From Table 3 we can see the effect of different extraction factors on free amino acid content in Moringa oleifera leaves. Statistical analysis showed that the extreme values of the three factors A, B, and C were 0.234, 0.153, and 0.450, respectively, that is, the degree of influence of the three factors on the content of free amino acids in the Moringa extract was extraction time > extraction temperature >Liquid-to-liquid ratio, indicating that the extraction time is the main factor affecting the free amino acid content in the Moringa leaf extract, and the correlation with the extraction temperature and feed-liquid ratio is not significant, so select the lower temperature and the smaller feed-liquid ratio for follow-up deal with. After treatment with $A_1B_0C_{-1}$ and $A_{-1}B_{-1}C_{-1}$, the content of free amino acids was relatively high, reaching 6.52% and 6.41%, respectively, while the free amino acid content of the extracted combination $A_0B_0C_1$ was 6.38%, but it was not much different.

The effect of different extraction factors on the total soluble sugar content in Moringa oleifera leaves is shown in Table 3. Statistical analysis showed that the extreme values of the three factors of A, B, and C were 2.944, 1.207, and 1.897, respectively. Comparing the size of R, ie, extraction temperature> extraction time> solid-liquid ratio, the results showed that the extraction temperature is the main factor affecting the soluble total sugar content in the extract of Moringa oleifera leaves, and has little relationship with the extraction time and the ratio of solid to liquid, so choosing a treatment method with a lower time and a smaller ratio of the material to the liquid is beneficial to reduce the cost and increase the production efficiency. The content of soluble sugar after $A_{-1}B_1C_1$ extraction was relatively high, reaching 14.75%, slightly higher than 13.47% of the extracted combination $A_0B_0C_1$.

3.2 Effect of Different Extraction Processes on the Appearance Color of Instant Moringa Tea

Table 4 The results of color parameters of an orthogonal test of instant Moringa oleifera power

No.	A	B	C	Blank column	L	-a	b

1	-1	-1	-1	-1	83.64	2.61	44.91
2	-1	0	0	0	84.02	2.68	46.19
3	-1	1	1	1	84.36	2.7	46.04
4	0	-1	0	1	86.6	2.34	43.03
5	0	0	1	-1	86.37	2.84	44.98
6	0	1	-1	0	86.13	2.37	45.48
7	1	-1	1	0	86.55	3.56	41.28
8	1	0	-1	1	85.93	3.34	45.46
9	1	1	0	-1	86.04	3.04	44.93
k_1	84.007	85.597	85.233	85.350			
k_2	86.367	85.440	85.553	85.567		L	
k_3	86.173	85.510	85.760	85.630			
R_1	2.360	0.157	0.527	0.280			
k_{11}	2.663	2.837	2.773	2.830			
k_{22}	2.517	2.953	2.687	2.870		-a	
k_{33}	3.313	2.703	3.033	2.793			
R_2	0.796	0.250	0.346	0.077			
k_{111}	45.713	43.073	45.283	44.940			
k_{222}	44.497	45.543	44.717	44.317		b	
k_{333}	43.890	45.483	44.100	44.843			
R_3	1.823	2.470	1.183	0.623			

The effect of different extraction factors on the color of Moringa extract is shown in Table 4. The greater the L value, the better the brightness of the tea soup. From the L value of the extract, the extreme values of the three factors of A, B, and C were 2.360, 0.157, and 0.527 respectively, that is, the degree of influence of the three factors on the L value of the tea soup was the extraction temperature, extraction time, the ratio of solid to liquid indicates that the extraction temperature is the main factor affecting the L value of Moringa oleifera leaf extract, and the effect of extraction time and solid-liquid ratio is small. From the k value, the brightness of the tea soup after $A_0B_{-1}C_0$ extraction combination was the best, the L value was 86.6, slightly higher than the 86.37 of the extracted combination $A_0B_0C_1$, but the difference was is relatively small, the brightness was also better, and the extraction time of combination $A_0B_0C_1$ is shorter than that of $A_0B_{-1}C_0$ and the ratio of material to liquid is small., and it is more suitable for extracting physical and chemical constituents of Moringa oleifera leaves.

3.3 Study on the Ingredients of Instant Moringa Tea Powder and Volatile Components of Moringa oleifera

Table 5 Relative contents of volatile components of instant Moringa oleifera power

No.	Keep time	Name	Peak area (%)	
			Moringa leaves	Instant powder
1	2.239	Dimethyl silanediol	3.8	2.81
2	2.308	3-methylbutanenitrile	2.25	-
3	3.431	Isopropyl isothiocyanate	2.34	0.78
4	3.679	2-Hexenal	2.55	1.41
5	4.843	2-Amino-5-methylbenzoic acid	13.27	10.04
6	5.601	2-Butylisothiocyanate	3.78	0.78
7	6.224	1,1-Dimethylethyl isothiocyanate	1.88	0.54
8	6.46	Benzaldehyde	1.68	0.55
9	8.007	(2E,4E)-Hepta-2,4-dienal	0.22	2.16
10	9.178	Phenylacetaldehyde	5.55	1.61
11	10.421	Hexamethylcyclotrisiloxane	7.58	6.47

12	11.223	1-Nonanal	2.02	1.28
13	15.419	Octamethylcyclotetrasiloxane	2.21	2.39
14	16.086	Dodecamethylcyclododecanehexasiloxane	1.88	1.73
15	19.415	Benzyl isothiocyanate	1.12	0.53
16	19.881	Decamethylcyclopentasiloxane	1.55	2.4
17	20.469	Methyl eugenol	2.3	-
18	23.972	Dihydroactindiolide	1.26	0.47
19	25.539	1,3-Pentanediol,2,2,4-trimethyl-,diisobutyrate	1.73	-
20	28.373	hexadecamethyloctasiloxane	0.88	2.7
21	31.159	Diisobutyl phthalate	0.98	0.95

Based on the ion maps of Moringa oleifera and Moringa instant tea powder, the Wiley9 and NIST08 standard libraries were searched, and the area normalized method was used to calculate the relative content of the volatile components in the sample. Table 5 is an analysis of 22 volatile components with relatively high content according to the spectral library. The volatile components in Moringa oleifera were mainly composed of esters (8 species), acids (1 species), aldehydes (5 species) and hydrocarbons (6 species). The aldehydes were mainly 2-hexenal (2.55%), benzaldehyde (1.68%), phenylacetaldehyde (5.55%), and furfural (2.02%). The esters were mainly dimethyl silanediol (3.8%), isopropyl isothiocyanate (2.34%), butyl isothiocyanate (3.78%), tert-butylisothiocyanate (1.88%), Benzyl isothiocyanate (1.12%), dihydroacturoactone (1.26%), 2,2,4-trimethyl pentanediol isobutyl ester (1.73%), diisobutyl- phthalate (0.98%). Hydrocarbons are mainly hexamethylcyclotrisiloxane (7.55%) and methyl eugenol (2.3%). The acid species was 2-amino-5-methylbenzoic acid (13.27%). The volatile components in Moringa instant tea powder are mainly composed of esters (7 kinds), acids (1), aldehydes (5 kinds) and hydrocarbon (4 kinds). The aldehydes were mainly 2-hexenal (1.41%), benzaldehyde (0.55%), phenylacetaldehyde (1.61%), and furfural (1.28%). The esters are mainly dimethyl silanediol (2.81%), isopropyl isothiocyanate (0.78%), butyl isothiocyanate (0.78%), tert-butylisothiocyanate (0.54%), Benzyl isothiocyanate (0.53%), dihydroacturoactone (0.47%), diisobutyl phthalate (0.95%). The hydrocarbon material is mainly hexamethylcyclotrisiloxane (6.47%). The acid species was 2-amino-5-methylbenzoic acid (10.04%).

Benzaldehyde has a special almond odor that is widely present in plants[6]. Methyleugenol, also known as clove oil, is a colorless to pale yellow liquid at room temperature. It has a low volatility and a relatively weak and long lasting eugenol aroma. It is insoluble in water and soluble in alcohol, ether, chloroform, and oils. Methyleugenol is mainly found in extracts of natural plants such as Asarum and Clove and can be used to prepare various aroma flavors. Chen Rongrong et al. also found that Moringa oleifera contains three volatile components, 2-hexenal, benzaldehyde, and dihydro-kiwifruit lactone [7], which is consistent with the results of this experiment. The three volatile components are characteristic volatile substances of Moringa oleifera.

From the above analysis, the loss of volatile components was severe during the processing of Moringa oleifera to Moringa instant tea powder. The content of benzaldehyde dropped from 1.68% to 0.55%, and the Dihydro-kiwifruit lactone decreased from 1.26% to 0.47% with a large loss, while Methyl eugenol and 2,2,4-trimethylpentanediol isobutyl ester contained in Moringa oleifera leaves were not detected in instant tea powder.

4. Discussion

The extraction time was the main factor affecting the content of free amino acids in the extract, and the free amino acid content after combined treatment of $A_1B_0C_{-1}$ and $A_{-1}B_{-1}C_{-1}$ was relatively high, reaching 6.52% and 6.41% respectively. The main factor affecting the soluble sugar content is the extraction temperature. After the $A_{-1}B_1C_1$ extraction combination treatment, the soluble sugar content is relatively high and can reach 14.75%. In addition, the extraction temperature has the greatest effect on the color of tea soup. The extraction combination $A_0B_0C_1$ is most suitable for extracting. The research on the odor of

tea soup shows that the volatile components are mainly composed of esters, acids, aldehydes, and hydrocarbons, and the loss of volatile components is serious from the processing of Moringa leaves to Moringa instant tea powder.

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