ACADEMICIA: An International Multidisciplinary Research Journal
ISSN: 2249-7137 Vol. 13, Issue 5, May 2023 SJIF 2022 = 8.252
A peer reviewed journal

CHEMICAL COMPOSITION OF BITTER WATERMELON AND DETERMINATION OF ANTIOXIDANT ACTIVITY OF FOOD ADDITIVES BASED ON WATERMELON

Ibragim Rahmonovich Askarov*; Tulkin Topvoldiyev;**
Zubayda Odiljonqizi Mirzayeva***

Andijan State University
Doctor of Chemical Sciences, *Professor,
Department of Chemistry, Honored Inventor of the Republic of Uzbekistan,
Chairman of the "TABOBAT" Academy of UZBEKISTAN

**Professor,
Doctor of Biology Sciences,
Andijan Institute of Agriculture and Agrotechnology,
UZBEKISTAN

***Doctoral Student,
Andijan Institute of Agriculture and Agrotechnology,
UZBEKISTAN

DOI: 10.5958/2249-7137.2023.00059.9

ABSTRACT

In this article, the amount of amino acids and vitamins in the juice and seeds of bitter watermelon grown in Andijan region was determined, and the results were presented in the form of tables, diagrams and the antioxidant activity of bitter watermelon was determined using the method of autooxidation of adrenaline in vitro in order to determine the chemical composition, medicinal properties, as well as its use in folk medicine and biological activity of biologically active substances in the bitter watermelon in Andijan.

KEYWORDS: *Bitter Watermelon Seeds, Bitter Watermelon Rind,Bitter Watermelon Juice, Protein, Amino Acid, Vitamins, Ethanol, Biologically Active Substance, Protein, Fats, Linolenic Acid.*

INTRODUCTION

The bitter watermelon plant (Citrullus colocynthis) belongs to the Cucurbitaceae family, the Citrullus family, and is widespread in the Mediterranean and Asia. It is usually considered the ancestor of cultivated watermelon (Citrullus lanatus) [1].

The main fatty acids in Citrullus colocynthus seeds are linoleic (70.7%), oleic (10.9%), palmitic (8.3%) and stearic (7.8%) acids. When analyzing the content of free amino acids, threonine (0.32 μgmL-1), valine (0.26 μgmL-1) and tryptophan (0.19 μgmL-1) were found to be present. In addition, Citrullus colocynthis seed oil contains serine (0.29 μgmL-1), ethanolamine (0.18 μgmL-1), glycine (0.25 μgmL-1) and aspartic acid (0.12 μgmL-1) contains amino acids.[2]

Studies conducted on this species have shown that the characteristic biologically active compound of Citrullus colocynthis is called cucurbitacin glucoside [3-4]. This substance has

anti-inflammatory, as well as cytotoxic and insecticidal effects [5].

When studying the properties of Citrullus colosynthis as a biologically active source, the composition of essential fatty acids and amino acids in the plant helps to determine their nutritional value and promotes their use as food additives[6].

The study of the level of secondary metabolites of plants showed that the seed composition consists of bioactive components. Phytochemical analysis revealed a high concentration of tannins and alkaloids. Some antinutrients such as phytate, cyanogenic glycosides, hemoglutinin, saponins and oxalates have been found to be present in very low concentrations. Flavonoids, steroids, terpenoids, and phenols were detected in high concentrations, while beta-carotene was detected in very low concentrations of 0.92±0.01mg/100g. High concentrations of vitamin C, flavonoids and phenols indicate high antioxidant activity[7].

The bitter watermelon plant (Citrullus colocynthis) belongs to the Cucurbitaceae family, the Citrullus family, and is widespread in the Mediterranean and Asia. It is usually considered the ancestor of cultivated watermelon (Citrullus lanatus) [1].

Citrullus colocynthis has many common names, including Abu Jahl melon, (original name in Turkey) colocynth, bitter apple, bitter cucumber, etc.Citrullus colocynthis contains carbohydrates, proteins, isolated amino acids, tannins, saponins, phenols, flavonoids, flavone glucosides, terpenoids, alkaloids, anthranol, steroids, cucurbitacins, saponarin, glycoloids, and many other chemical groups. It has antioxidant, anti-diabetic, anti-microbial, anti-cancer, anti-inflammatory, analgesic, reproductive, protective and other pharmacological effects [1].

Fruit pulp contains resins, glycosides (up to 2%), pectins and proteins extracted with diethyl ether and chloroform. The strong laxative effect of the fruits of the plant is due to the glycoside colocyntin contained in their pulp, and their genins are elaterins, derivatives of the tetracyclic triterpene cucurbitacin [8] [9].

The pulp of colocynth fruits has very pronounced laxative properties, when used in small quantities it causes severe diarrhea, when the dose is exceeded it causes vomiting, colic, enteritis and gastritis [10].

Colocynth fruit powder or extract is used as a strong laxative and liver stimulant. In overdose, they can cause severe acute pain in the intestines with dangerous inflammation. A decoction made from the fruit pulp was also used as an insecticide [11].

The seeds are gray and 5 millimeters long and 3 mm wide. They have a bitter, nutty taste, just like the fruit, and are rich in fat and protein. They are eaten whole or used as an oilseed. The oil content of the seeds is 17-19%, 67-73% linoleic acid, 10-16% oleic acid, 5-8% stearic acid and 9-12% palmitic acid. Oil yield is about 400 L/ha. In addition, the seeds contain a large amount of amino acids, including arginine, tryptophan, and sulfur [12].

Experimental part. Experiment 1. Determination of the amount of free amino acids. The composition and amount of amino acids in the sample was determined by the Cohen method, in the form of FTK derivatives of amino acids. In the determination of free amino acids, the samples are extracted with distilled water, centrifugation of the extracts, precipitation of proteins and peptides in the supernatant using 10% UXSK, removal of the precipitate by centrifugation, and the necessary amount for analysis is separated by lyophil drying, and the dried mass is modified by Cohen's method. FTK derivatives of amino acids were analyzed using YuSSX

(High Performance Liquid Chromatography) method. Amino acid identification and quantification was performed by comparison to FTK derivatives of standard amino acids. Chromatograms of FTK derivatives of free amino acids and chromatography conditions are as follows.

Work Performance: Chromatogram of a mixture of FTK derivatives of amino acids. A) standard amino acid mixture, B) samples (column 75x4.6 mm Discovery HS C18., solution A: 0.14M CH_3COONa + 0.05% TEA rN 6.4, V:CH3CN. Flow rate 1.2 ml/min, wavelength 269nm. Gradient % B/minute: 1-6%/0-2.5 minutes; 30%-40 minutes; 60%-45-50 minutes; 0%-55 minutes).

TABLE 1. AMOUNT OF AMINOACIDS

Name of amino acids	**bitter watermelon juice**	**bitter watermelon seeds**
	Concentration mg/g	
Asparticacid	1,226874	2,970367
Glutamicacid	1,696091	0,895334
Cool	1,616709	3,702966
Glycine	1,370501	0,636648
Asparagine	2,748233	1,217866
Glutamine	4,721946	2,387948
Cysteine	3,759563	6,065574
Threonine	3,119094	1,986804
Arginine	1,376472	1,767593
Alanine	1,374203	0,973799
Proline	3,09135	0,711606
Tyrosine	2,378075	0,805768
Valin	2,138948	0,76315
Methionine	0,746879	0,266795
Histidine	7,390025	0
Isolation	1,395528	0,483166
Leucine	2,646349	0,983435
Tryptophan	2,445752	1,554859
Phenylalanine	0,393912	0,457625
LysineHCl	2,505511	0,806112
Total	**48,14201**	**29,43742**

Experiment 2. Determination of the amount of vitamins in bitter watermelon. The amount of water-soluble vitamins was studied by the method of high-performance liquid chromatography.The water-soluble vitamins in the sample were determined by the method of high-performance liquid chromatography.

Work Performance: 5-10 g is taken from the drawer on an analytical balance and placed in a 300 ml flat flask. 50 ml of 40% ethanol solution is added to it. The mixture was heated under

vigorous stirring for 1 h, equipped with a magnetic stirrer, reflux condenser, and then stirred at room temperature for 2 h. The mixture is cooled and filtered. 25 ml of 40 percent ethanol was added to the remaining part and re-extracted 2 times. The filtrates were combined and filled to the mark with 40% ethanol (5-10%) in a 100 ml volumetric flask. The resulting solution is centrifuged at 7000 rpm for 10 minutes. The resulting solution was taken from the upper part for analysis.

Working solutions of water-soluble vitamins with a concentration of 1 mg/ml were prepared. For this purpose, 50.0 mg of each vitamin standard is taken on an analytical balance and dissolved in a 50 ml volumetric flask in 40% ethanol and filled to the line. Phosphorus, acetate buffer systems, and acetonitrile were used as eluents in the literature for the determination of water-soluble vitamins with the YuSSX. We used an acetate buffer system and acetonitrile.

Chromatographic conditions:

-Chromatograph Agilent-1200 (equipped with an autodoser)

-Column Exlipse XDB C 18 (obraschenno-faznyy), 5 μm, 4.6 x150mm

-Diode array detector (DAD), 204 nm, 254 nm, 290 nm identified.

-Flow rate 1ml/min

- Eluent acetate buffer: acetonitrile:

0-5 min 96:4,

6-8 min 90:10,

9-15 min 80:20,

15-17 min 96:4,

thermostat temperature 2500C, -5 μl input amount (vkol)

First, working standard solutions and then prepared working solutions were introduced into the chromatograph.

TABLE 2. AMOUNT OF VITAMINS

Vitamin	**bitter watermelon juice**	**bitter watermelon seeds**
	Concentration mg/g	
B-1	0,000	0,0382
B-2	2,151	1,4440
B-6	2,637	3,8146
B-9	148,120	0,0000
PP	0,047	0,1668
C	12,527	0,0784

Experiment 3. Antioxidant activity was determined by phytochemical analysis of samples of products in water and 96% alcohol. The antioxidant activity of bitter watermelon is determined by the inhibition of the autoxidation reaction of adrenaline in vitro and prevents the formation of the free form of oxygen. The method is based on the inhibition of the autoxidation reaction of

adrenaline, it is expressed in percentages (%) due to the formation of the active form of oxygen and autoxidation of adrenaline in vitro.

Work Performance: 2.0 ml of 0.2 M sodium carbonate (Na2CO3-NaHCO3) pH = 10.65 buffer, 56 µl of 0.18% solution of adrenaline (epinephrine) hydrochloride were taken. 30 µL of antioxidant drug (L1 and L2) was added and examined in a spectrophotometer (Cary 60 UV-Vis Agilet Technologies) at 347 nm wavelength for 30 seconds to 10 minutes. The tested amount (concentration of 1 mg in 1 ml of the solution) was used as a standard. As a control, 2.0 ml of 0.2 M buffer and 56 µl (5.46 mM) of 0.18% adrenaline were used.

Antioxidant activity was calculated according to the inhibition of autoxidation of adrenaline with the following formula.

$$AA\% = \frac{D1 - D2 \times 100}{D1}$$

In this, optical density of adrenaline hydrochloride solution added to D_1-buffer;

Optical density of the investigated extract and adrenaline hydrochloride added to D_2-buffer.

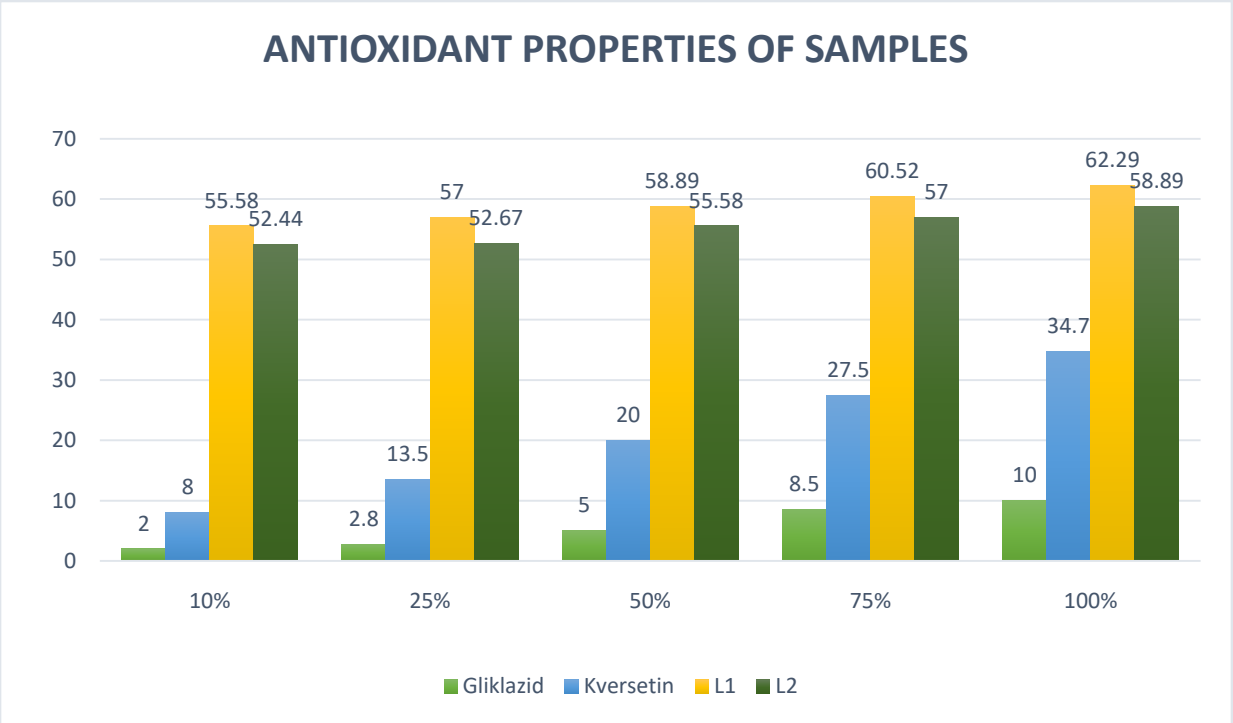
TABLE 1 DRUGS UNDER INVESTIGATION

№	Preparation	Composition	**Solubility**	***In vitro* mkg/ml**
1	As-Mirtol capsule -L1	Bitter watermelon seeds, Sweet watermelon seeds in a ratio of 3:1	Water	100/250/500/750/1000
2	As-Mirtol tea -L2	Bitter watermelon rind, sweet watermelon rind in a ratio of 3:1	Water	100/250/500/750/1000

TABLE 2 RESULTS OF ANTIOXIDANT PROPERTIES OF THE SAMPLES

№	**Substance (Bitter Watermelon Seed, Sweet Watermelon Seed)**	**Control**	**Experience**	**AA%**
1	L1 (10%) 100 mg/ml	0,43052	0,19122	55,58
2	L1 (25%) 250 mg/ml	0,40050	0,17220	57,00
3	L1 (50%) 500 mg/ml	0,43890	0,18041	58,89
4	L1 (75%) 750 mg/ml	0,45905	0,18122	60,52
5	L1 (100%)1000 mg/ml	0,48105	0,18140	62,29
№	**Substance (Bitter Watermelon Peel, Sweet Watermelon Peel)**	**Control**	**Experience**	**AA%**
1	L2 (10%) 100 mg/ml	0,40394	0,19212	52,44
2	L2 (25%) 250 mg/ml	0,3890	0,1841	52,67
3	L2 (50%) 500 mg/ml	0,43052	0,19122	55,58
4	L2 (75%) 750 mg/ml	0,40050	0,17220	57,00
5	L2 (100%)1000 mg/ml	0,43890	0,18041	58,89
№	**Substance (Gliclazide)**	**Control**	**Experience**	**AA%**
1	Gliclazide -(10%) 100 mg/ml	0,02782	0,0235	2,0
2	Gliclazide -(25%)250 mg/ml	0,03895	0,0329	2,8
3	Gliclazide -(50%)500 mg/ml	0,06955	0,0587	5,0
4	Gliclazide - (75%)750 mg/ml	0,11823	0,0998	8,5

5	Gliclazide -(100%)1000 mg/ml	0,13909	0,1174	10,0
№	**Substance (Quercetin)**	**Control**	**Experience**	**AA%**
1	Quercetin - (10%) 100 mg/ml	0,11128	0,0940	8,0
2	Quercetin - (25%)250 mg/ml	0,18778	0,1586	13,5
3	Quercetin - (50%)500 mg/ml	0,27819	0,2396	20,0
4	Quercetin - (75%)750 mg/ml	0,38251	0,3294	27,5
5	Quercetin - (100%)1000 mg/ml	0,67247	0,5348	34,7



Discussion of Results. In the 1st experiment, when the amount of amino acids in the bitter watermelon was studied, it was found that the most common amino acid in the juice of the bitter watermelon grown in Andijan region is histidine, and the seeds contain a large amount of cysteine. Among the rar est amino acids, phenylalanine was found in the juice and seeds.

In the 2nd experiment, when the amount of vitamins in bitter watermelon was studied, it was found that the juice of bitter watermelon grown in Andijan region contains the most common vitamin B-9, and the seeds contain a large amount of B-6. Among the rarest vitamins, it was found that PP is in the juice and B-1 is in the seed.

In the 3rd experiment When the antioxidant activity of the drugs was determined by the inhibition of the autoxidation reaction of adrenaline in vitro, the samples prevented the formation of the free form of oxygen. All samples were compared with standard antioxidant quercetin and gliclazide antioxidants. The obtained results showed that the drugs have antioxidant properties.

The biologically active substances contained in the preparations were analyzed from the literature, and in order to determine this, the antioxidant activity of the preparations was determined using the autoxidation method of adrenaline under in vitro conditions. It was found that the 100% solution of "Asmirtol" capsule has higher antioxidant properties.

CONCLUSION

The composition and amount of amino acids in the sample was determined by the Cohen method, in the form of FTK derivatives of amino acids. The amount of water-soluble vitamins was studied by the method of high-performance liquid chromatography. The water-soluble vitamins in the sample were determined by the method of high-performance liquid chromatography.

Chemical composition and natural medicinal, biological and nutritional properties of bitter watermelon, nutritional products and chemical composition of bitter watermelon, specific components of bitter watermelon and their use in folk medicine were widely analyzed. As a result, it was determined that it is appropriate to conduct research on the creation of food additives based on bitter watermelon seeds and peel.

The antioxidant activity of preparations was determined by the autoxidation method of adrenalin in vitro. The antioxidant activity of the investigated preparations was evaluated by phytochemical tests.

Chemical composition and natural medicinal, biological and nutritional properties of bitter watermelon, nutritional products and chemical composition of bitter watermelon, specific components of bitter watermelon and their use in folk medicine were widely analyzed. As a result, it was determined that it is appropriate to conduct research on the creation of food additives based on bitter watermelon seeds and peel.

REFERENCES

1. По данным сайта GRIN (см. карточку растения)

2. Yuan, H.; Ma, Q., Ye; L., Piao, G. The Traditional Medicine and Modern Medicine from Natural Products, Molecules, 2016, 21, 559. DOI: 10.3390/molecules21050559

3. Hatam, N. A.; Whiting, D. A.; Yousif, N. J. Cucurbitacin glycosides from Citrullus colocynthis. Phytochemistry 1989, 28, 1268- 1271. DOI:10.1016/0031-9422(89)80230-4

4. Chen, J. C.; Chiu, M. H.; Nie, R. L.; Cordell, G. A.; Qiu, S. X. Cucurbitacins and cucurbitane glycosides: Structures and biological activities. Nat. Prod. Rep. 2005, 22, 386-399. DOI: 10.1039/b418841c

5. Abdelkhalek, A. A.; Sharaf, A. M.; Rabie, M.; El-Subbagh, H. I. Derivatives of cucurbitacin-Eglucoside produced by Curvularialunata NRRL 2178: Anti-inflammatory, antipyretic, antitumor activities, and effect on biochemical parameters. Future J. Pharm. Sci. 2017, 3, 124-130. DOI: 10.1016/j.fjps.2017.04.006.

6. Goldfain, D; Lavergne, A; Galian, A; Chauveinc, L.; Prudhomme, F. Peculiar acute toxic colitis after ingestion of colocynth: a clinicopathological study of three cases. Gut 1989, 30, 1412-1418. DOI: 10.1136/gut.30.10.1412.

7. Igwenyi, I. O. and Akubugwo, E. I. (2010). Analysis of Four Seeds Used as Soup Thickeners in the South Eastern Parts of Nigeria.

Conference Proceedings: International Conference on Chemistry and Chemical Engineering, (ICCCE, 2010), August 1–3, 2010,

Kyoto International Conference Center, Kyoto, Japan. 2010. 426 –430.

8. Dane, Fenny; Liu, Jiarong. Diversity and origin of cultivated and citron type watermelon (Citrullus lanatus) (англ.) // Genetic Resources and Crop Evolution : journal. — 2006. — Vol. 54, no. 6. — P. 1255. — doi:10.1007/s10722-006-9107-3.

9. Talia Ogliore. A seedy slice of history: Watermelons actually came from northeast Africa (англ.). ScienceDaily (24 мая 2021). Дата обращения: 28 мая 2021. Архивировано 26 мая 2021 года.

10. Renner S. S. et al. A chromosome-level genome of a Kordofan melon illuminates the origin of domesticated watermelons (англ.) // Proceedings of the National Academy of Sciences. — 2021. — 1 June (no. 118 (23)). — doi:10.1073/pnas.2101486118. Архивировано 17 февраля 2022 года.

11. Krystyna Wasylikowa, Marijke van der Veen. An archaeobotanical contribution to the history of watermelon, Citrullus lanatus (Thunb.) Matsum. &Nakai (syn. C. vulgaris Schrad.) (англ.) // Vegetation History and Archaeobotany. — 2004. — Vol. 13, no. 4. — P. 213–217. — doi:10.1007/s00334-004-0039-6. Архивировано 9 сентября 2018 года.

12. Узбекистан вышел на четвертое место в мире по выращиванию арбузов. Датаобращения: 21 июня 2020. Архивировано 23 июня 2020 года.