Journal of Pharmaceutical and Health Sciences 2016;4(1), 23-33.

Original Article

JPHS

Investigation of Physicochemical Properties and Microbial Contamination of Saffron Style

Romina Ahmadian[1*], Fakhry Hosseni[2], Maryam Salami[3]

[1]*Department of Microbiology, Azad University of Pharmaceutical Sciences, Tehran, Iran*

[2]*Department of Microbiology, Alzahra University, Tehran, Iran*

Abstract

Saffron flower (Crocus sativus) is one of the most precious spices in the world. In this respect, the saffron style samples were gathered from the farms of Torbat-Heidariyeh located in South Khorasan province during one year and their preparation was performed based on the common manufacturing standards. Then, in order to considering the international and national standards for saffron and spices evaluation of the desired properties of saffron style was investigated which included the moisture content, total ash, in acid insoluble ash, the cold water soluble extract, recognition of the color additives and the power of color, total counts of micro-organisms, the form and mold, Escherichia coli, Clostridium perfringens, sulfite-reducing clostridium, and enterococcus based on physicochemical and microbial. It was found that the tested physicochemical properties of saffron style such as the total ash, the acid insoluble ash, the cold water soluble extract, recognition of the color additives, and the power of the color were in lower grade than the stigma. The level of Crocin, Picrocrocin, and Safranal available in the saffron style along with stigma were in different amount amounts and very how compared to the style. The style moisture was the only factor which its amount in stigma was greater than style. Statistically, the tests of moisture content, total ash, the acid insoluble ash, and the cold water soluble extract had a significant difference ($p<0.05$). Furthermore, the recognition tests of the color additives was completely negative in all trials in which a significant difference ($p<0.05$) was observed in the power of color test using a UV-Vis spectrophotometer (Crocin, Picrocrocin, and Safranal). In addition, the microbial contamination of samples was investigated and it showed that the factors of intestinal enterococcus, Escherichia coli, the spores of sulfite-reducing anaerobes (clostridia), clostridium perfringens, and Bacillus cereus were negative, while the mold and yeast, coli-form and total count of micro-organisms were in a higher range compared with the microbial negative factors and had a statistical significant difference ($p<0.05$).

Keywords: Saffron style, Physicochemical properties, Microbial quality

***Corresponding author**: *Romina Ahmadian* *Department of Microbiology, Azad University of Pharmaceutical Sciences, Tehran, Iran*

Email address: *Rominaahmadian@ymail.com*

Introduction

Saffron flower belongs to the Iridaceous family and includes eight kinds of species which among them, seven species are wild and only one species, with a scientific name of Crocus Sativus Lis edible and produced by cultivation and its scientific name is Crocus Sativus. The Saffron style is a part of pistil that attaches the stigma to the ovary (Iranian standard organization 5689).

Nowadays, Iran is the main producer of the saffron in the world, which answers more than 90% (200 tons per year) of the global production since, owns the most areal under cultivation. Saffron is recognized as the most precious spice in the world. After Iran, the main producers of saffron are Spain, Greece, Turkey, India, Morocco and some mid-Asia countries (Bisset & Wichtl, 2001).

Khorasan province with an amount of 20 thousand acres of area under cultivation is one of the most important saffron production centers of Iran. Although saffron is frequently used as the food color and flavor, it has been used as a medicine for more 3000 years. Saffron has different biological activities including antispasmodic, carminative, aphrodisiac, and stimulant, hence, the folk medicine uses it for treatment of abdominal pains, mucus, _heart as well as cognitive disorders. Modern pharmacological studies have shown that the saffron extract has the effects of anti-tumor, antiepileptic, anti-depression, anti-inflammatory, anti-high blood fat, antioxidants, and is a free radical scavenger (Abdullaev, 2002).

Just the saffron stigma, the saffron style, which is located at the end of stigma, has been used as a food flavor and an effective medicine in remediation of diseases in the folk medicine. Unfortunately, its consumption is recently associated with some problems due to some fraudulent types and lack of a manufacturing, national, and international standard method to analyze and quality check. There is no publication in scientific literature that describes the saffron style properties; however the performed studies are concerned with the physicochemical and microbial properties of saffron stigma as follows (Salehi et al. 2003).

Salehi and coworkers published a research on the saffron pharmacopoeia in which the methods of chemical and quantitative control of saffron were investigated (Aghayi & Gholizadeh, 2011).

All the physicochemical and microbial properties of the saffron were decreased during one-year storage, although Safranal was increased. The saffron style is colorless; the accumulation amount of Safranal is achieved by the decomposition of the Picrocrocin during drying the new harvest of odorless saffron under temperature condition (Haghighi et al. 2007).

In this paper, we want to investigate the physicochemical properties and microbial contamination of saffron to obtain a standard for it.

Materials and Methods

Materials

Hydrochloric acid, Ashless filter paper 42, Whatman filter paper 540, methanol, acetone, ammonia 25%, formic acid applied for physicochemical tests.

Peptone water growth medium, Plate Count Agar growth medium, sulfide iron agar, Lauryl Sulfate Broth growth medium, Bromcresol Purple (growth medium), VRB agar (culture media), KG-MYP growth medium, Cook Meat growth medium, which is provided by Merck KGaA, Darmstadt, Germany were used in microbial tests .

Method of sampling

All the samples were gathered from 10 farms in Torbat-Heidariyeh located in Khorasan province, Iran. Four samples were randomly taken from each form, the petals were firstly detached and the attached stigmas to the style were dried and then were detach from the style.

Then, 10 grams was taken from each sorted samples. After that, all the four samples were mixed and kept in a special vessel (dark glass) away from light and heat. The sample number 1 along with number 9 other samples were taken by this method. The samples were moved to the temprecher environment in laboratory for further preparation and performing the desired tests.

Test method

The physicochemical and microbial tests were performed based on national standards for saffron properties and test methods, national microbiological standard for saffron and national microbiological standards for spice which the tests included moisture content, total ash, acid insoluble ash, water soluble extract, Crocin (color), Picrocrocin (taste), and Safranal (odor), recognition of color additives, total counts of micro-organisms, coliform, mold and yeast, Escherichia coli, Sulfite-reducing clostridium, Clostridium perfringens, Bacillus cereus, and Intestinal enterococcus (Haghighi et al. 2007; James et al. 2010).

The physicochemical properties of saffron were measured using spectrophotometry method based on national standard No 259 In this method, spectrophotometer model an uv/vis GENESYS 5 were utilized at the wavelengths of 440, 330, and 275 nm to measure the concentration of Crocin, Safranal, and Picrocrocin, respectively. The recognition of additive color was carried out using thin layer chromatography (TLC). The moisture content was evaluated in an electrical oven at 103°C for 16 hours based on the national standard No259 (Iranian standard organization 259-1; 259-2).

Measurement of aqueous extract was performed according to national standard No 1619 (Iranian standard organization, 1619). The total ash and acid insoluble ash were evaluated at 550°C furnace according the national standards No.1197 (Iranian standard organization, 1197) and No.1253, respectively ((Iranian standard organization, 1253).

In order to estimate the microbial contamination of saffron style, the total count of micro-organisms were evaluated in accordance to the suggested method in national standard number No.5272 (Iranian standard organization, 5272), determination of mold and yeast contamination were carried out in agreement to with the recommended method in Iranian national standard number No.10899-2 (Iranian standard organization, 10899-1).

The search and total counts of forms were evaluated based on the suggested method in national Iranian standard number No. 9263 ((Iranian standard organization, 9263), In addition, the assessment of sulfite-reducing Clostridium, Bacillus cereus, Intestinal enterococcus, and Clostridium Perfringens were performed via the suggested methods in national standards number No.9432 (Iranian standard organization, 9432), No. 2324 (Iranian standard organization, 2324), No. 2198 (Iranian standard organization, 2198), and No.2197 (Iranian standard organization, 2197, respectively.

Statistical analysis:

The acquired results were analyzed using SPSS with version 19 and on-way analysis of variance (ANOVA) considering the Tukey range test at the level of 0.05.

Results

The physicochemical properties of saffron style

As it is shown in the figure1, the results of the moisture variance analysis indicate that there was no significant difference between the moisture of samples ($p<0.05$). The moisture of style is greater than the total moisture of saffron. As the amount of attached style to the saffron increases, the moisture percent increases. These results are in agreement with the national standard No. 259-2. According to this standard, the maximum value of moisture in the upper part of saffron in cut filament and forth graded saffron are equal to 10 and 12% max respectively.

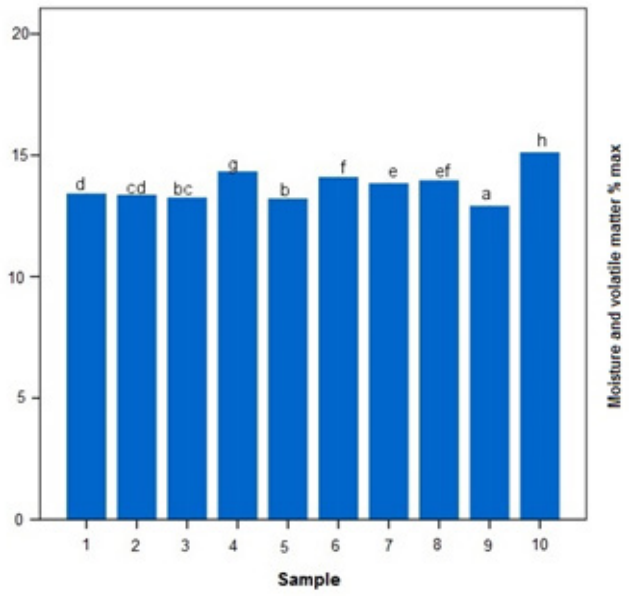


Figure 1: The amount of moisture and volatile material in terms of mass percent of saffron style of samples (p<0.05)

According to the Figure 2, regarding total ash in the all samples showed a significant difference (p<0.05). In accordance to the national standard number 259-2, it was found that with an increase in the amount of style in the fourth grade saffron in filament, the total ash increases.

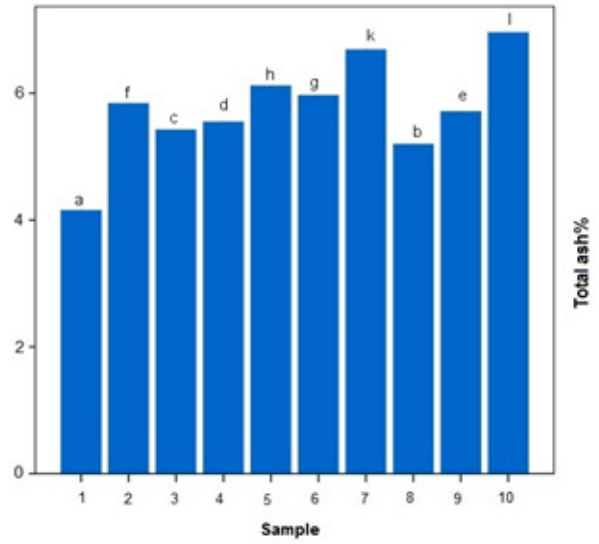


Figure 2: The amount of total ash (mass percent) of saffron style of samples (p<0.05)

As shown Figure 3, there was a considerable difference in acid-insoluble properties of samples (p<0.05). According to the national standard number 259-2, it was illustrated that increasing the style quantity of fourth grade saffron in filament, leads to increase in the level of acid-insoluble ash.

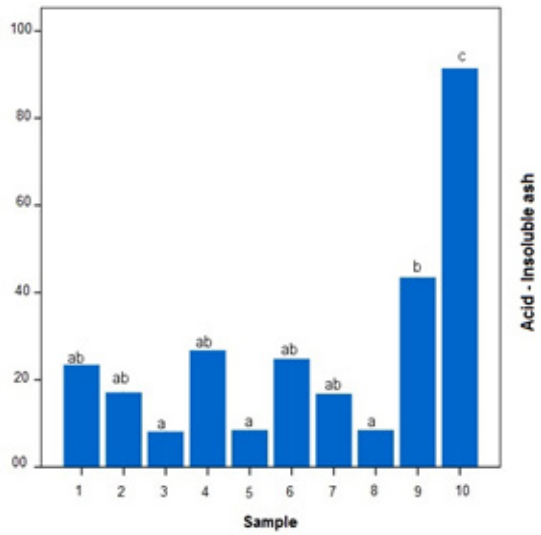


Figure 3: The acid-insoluble ash (mass percent) style of samples (p<0.05)

There was no significant error in the cold water soluble extract of samples in the confidence level of 95% (Figure 4). The results are in agreement with the national standard No. 259.

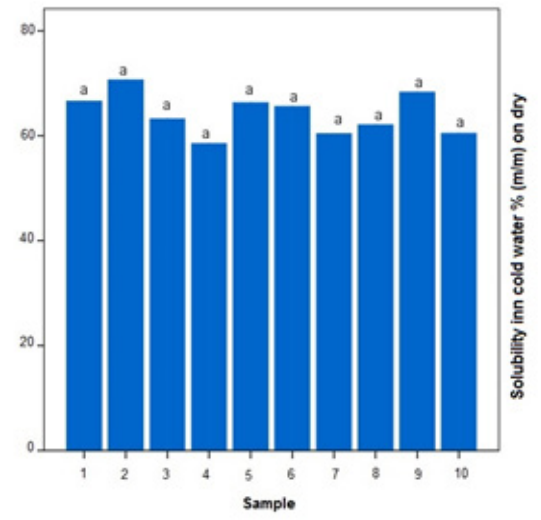


Figure 4: The amount of cold water soluble extract style of samples (p<0.05)

The obtained p-value of Picrocrocin is about 0.0005 which is less than the error level of 0.01. Thus, there is a significant difference in the Picrocrocin average amounts of samples with the confidence level of 95%. Picrocrocin is one of the effective ingredients of saffron (O'Neil & Schwartz, 1992; Rios et al. 1996). Based on the national standard number 259-2, the maximum amount of Picrocrocin is related to the upper parts of saffron in filament. This higher level of Picrocrocin was due to the high amount of stigma in the 5th sample (Figure 5).

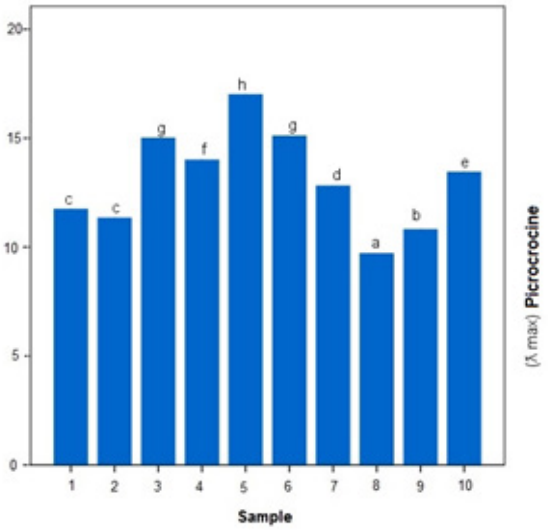


Figure 5: The amount of Picrocrocin in the saffron style of samples (p<0.05)

The results of variance analysis showed that the p-value of Crocin in the confidence level of 95% has a significant difference in all samples (Figure 6). The maximum and minimum amounts of Crocin in the saffron style were observed in samples #5 and #10 and sample #8, respectively, which were in agreement with the national standard 259-2. The results indicate that the attached style can effect on decrease of Crocin amount. The Crocin increase of the samples #5 and #10 was due to the lower numbers of style and higher counts of stigma tail in samples.

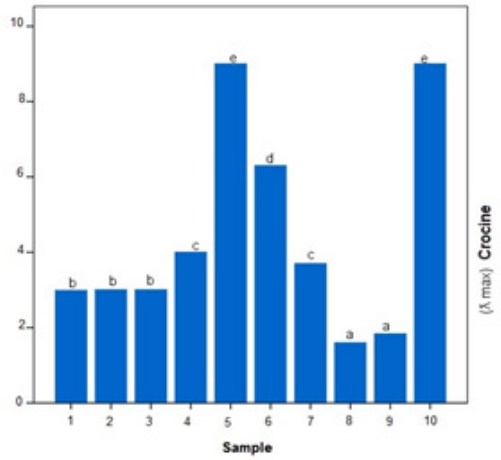
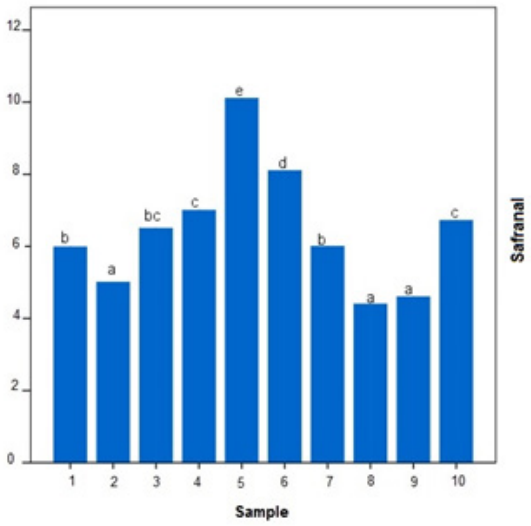


Figure 6: the level of available Crocin in the saffron style of samples (p<0.05)

Additionally, there is a remarkable difference between the p-value of the Safranal in the confidence level of 95%. According to the Figure 7, the maximum and minimum amounts of Safranal were observed in sample #5 and sample #8, respectively. In accordance to the national standard of saffron number 259-2, all the samples, including upper parts of saffron in cutting filament and saffron in filament, contain Safranal in a range 20-50 max, Although, fresh saffron style contains a small amount of Safranal, hydrolysis of the Picrocrocin compounds through time leads to release of the volatile aldehyde which are converted to Safranal and therefore, the quantity of Safranal increases.

Microbial contamination of saffron style

The results of analyzing the contamination of saffron style to the Intestinal enterococcus, Escherichia coli, Bacillus cereus, Sulfite-reducing clostridium, and Clostridium perfringens were negative and were in the limit range of national standard of saffron and spice.
Based on the results of Tukey test in the Figure 8, there is a significant difference between the test of mold and yeast of the samples in the confidence level of 95%. The maximum and minimum mold and yeast amounts were observed in the sample #9 and samples #3 and #8, respectively. According to the microbial national standard of saffron number 5689 and standard of mold and yeast number 10899-2, the maximum acceptable amount of mold is 10^3 and according to the national standard of spice number 3677 the maximum allowable limit of yeast is 5*10^3 in gram. The results of the two standards are in an acceptable range. According to the acquired results of the mold and yeast test, the results are in an acceptable limit of the saffron standard.



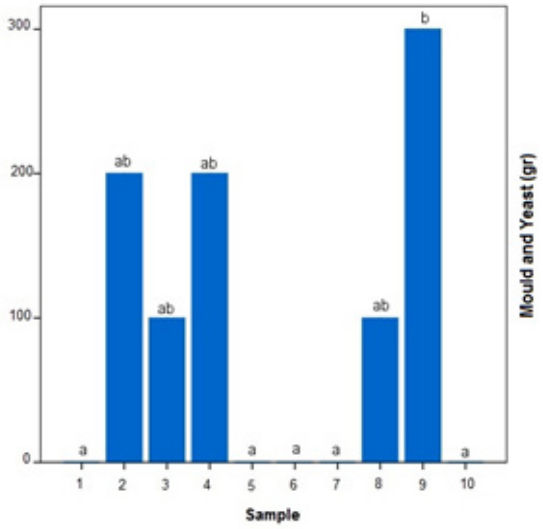


Figure 8: The amount of mold and yeast among the style of samples (p<0.05)

The results of the Tukey test of the total counts of micro-organisms are shown in Figure 9. The results indicate that there is there is a significant difference for this property in the confidence level of 95%. The maximum and minimum values were observed in sample #7 and sample #9, respectively. According to the national standard of spice number 3677, the entire samples are in the acceptable limit of the spice standard.

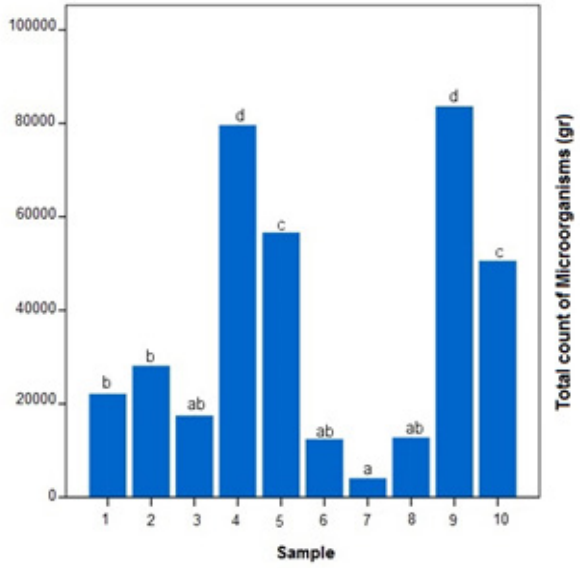


Figure 9: The total counts of microorganism in the style of samples (p<0.05)

As shown in Figure 10, the samples have a significant difference in the confidence level of 95% in the coliform test. Samples #4 and #7 had the maximum and minimum coliform, respectively. Its acceptable limit is 103 in national microbial standard of spice number 3677, thus, the results are in allowable limit of spice standard.

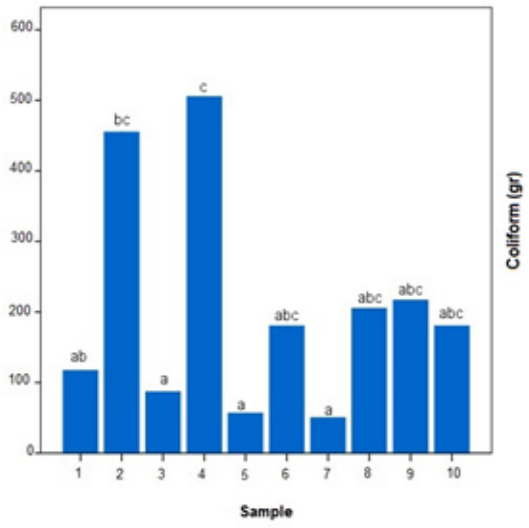


Figure 10: The total amount of Coliform in the style of samples (p<0.05)

Investigation of the relation between the saffron styles properties:

Investigation of the relation among saffron style's properties (parameters) was performed using the Pearson correlation coefficient to understand how each saffron style's property is related with the others. The results are listed in the following table.

Table 1: The comparison of different coefficients of saffron style with each other

Variable	Statistical Index	Moisture and volatile matter % max	Total ash%	Acid- insoluble ash	Solubility in cold water% (m/m)on dry	Crocine	Picrocrocine	Safranal
Moisture and volatile matter % max	Correlation coefficient	1	0.445*	0.588**	-.418*	0.458*	*0.068*	0.101
	p-value	-	0.014	0.001	0.022	0.011	*.722*	0.597
Total ash%	Correlation coefficient	0.445*	1	0.430*	-0.187	0.586**	*0.325*	0.270
	p-value	0.014	-	.018	0.321	0.001	*0.080*	0.149
Acid- insoluble ash	Correlation coefficient	0.588**	0.430*	1	-0.138	0.407*	*-0.075*	-0.066
	p-value	0.001	0.018	-	0.466	0.025	*0.692*	0.728
Solubility in cold water% (m/m) on dry	Correlation coefficient	-0.418*	-0.187	-0.138	1	-0.103	*-0.104*	-0.107
	p-value	0.022	0.321	0	-	0.587	*0.586*	0.574
Crocine	Correlation coefficient	0.458*	0.321	.407*	-.103	1	*0.727***	0.574
	p-value	0	.586**	0.025	0.587	-	*0.000*	**0.808
Picrocrocine	Correlation coefficient	0.068	0.001	-0.075	-0.104	0.727**	*1*	0.000
	p-value	0.722	0.325	0.692	0.586	0.000	-	**0.931
Safranal	Correlation coefficient	0.101	0.080	-0.066	-0.107	0.808**	*0.931***	0.000
	p-value	0.597	0.270	0.728	0	0.000	*0.000*	1

Table 1: The comparison of different coefficients of saffron style with each other

CHARACTERISTICS	Type of saffron			TEST METHOD national standards	TEST METHOD international standards
	Saffron in Filaments Red all	Saffron In Filaments grade:4	Style saffron		
Determination of floral waste content	-	-	-	259-1	-
Determination of foreign matter	0.1	2	-	259-2	-
Moisture and volatile matter % max		12	15.1	259-2	Iso 3632-2
Total ash%	5.5	7	6.96	1197	Iso 930 & 3632-2
Acid- insoluble ash	0.5	1.5	0.9	1253	Iso 930 & 3632-2

Solubility in cold water% (m/m)on dry	65	65	70.63	1619	Iso 941
Bitterness expressed as direct reading of the absorbance of Picrocrocine at about 257 nm	85	70	17	259-2	Iso3632-2
Safranal expressed as direct reading of the absorbance at about 330 nm on dry basis. All categories.	20-50	20-50	4-11	259-2	Iso3632-2
Coloring strength expressed as direct reading of the absorbance of Crocine at about 440 nm	220	140	1.6	259-2	Iso3632-2
Thin-layer chromatography: Identification of artificial colorants	neg	neg	neg	259-2	Iso3632-2

Table 3: The comparison of the average counts of microbes in the saffron stigma and style (the analyzed samples by Mashhad food and drug administration)

Specification	Index	Stigma	style
total count of microorganisms	Number	2	25
	Average	55500	32032
	Standard deviation	62932/50	27181/35
Coli form	Number	2	26
	Average	52/5	187/46
	Standard deviation	67/17	167/1
Yeast	Number	1	25
	Average	100	108
	Standard deviation	0	119/059

Table 4: The microbial results of saffron style based on the national standard of spice

Specification	results released	Nearly acceptable	Defects	Test Method in
total count of microorganisms (gr)	32032	105×5	Major	5272
Coli form bacteria (gr)	52/2	103	Major	9263
E.Coli (gr)	0	negative	Critical	2946
Bacilos serou (gr)	0	102	Critical	2324
Closterodiom perferjens (gr)	0	102	Critical	2197
Mold (gr)	108	103×5	Major	10899-2

Discussion

The fresh saffron contains lower amount of Safranal (the special odor of the saffron) and therefore, the fresh saffron or its style is usually odorless but through the time it increases. It is because of the fact that during drying the saffron, the Picrocrocin compounds are hydrolyzed and consequently the Safranal volatile aldehyde is released. It should be mentioned that if the petal of the saffron flower is detached right at the picking the flower, there will no migration of effective material of saffron to the style, thus the style will not contain any effective material. If the stigma is not detached from the style after the process of drying, there would be enough time for the effective materials to release from stigma to the style. Thus, in this condition, there

will be partial amount of effective materials in the style. The color power test has been shown the level of effective materials of each sample. The mixing degree of each prepared sample was depends on the level of attached style to the stigma. Because of this, different levels of effective materials are observed in samples which are called as white saffron in saffron commerce.

The test color power in determination of the Crocin, Picrocrocin, and Safranal values in style showed that there is no color in style. The accumulated Safranal in the dried and picked saffron, which has no odor, is evolved from Picrocrocin degradation in the temperature condition of drying.

The investigation of the effect of ambient temperature (25°C) and relative moisture (20-30%) during the preservation period on the qualitative properties of saffron has shown that through time, the amount of Picrocrocin, and Crocin decreases and the amount of Safranal increases. Since picrocrocin is hydroly and converted to free Crocetin. Safranal belong to the (C10H16) terpenes group, which is in the form of non-volatile Picrocrocin in fresh saffron, and is degraded and converted to the volatile aldehyde of Safranal over the time period Thus, the amounts of Picrocrocin and Safranal are inversely related. Therefore, through time the amount of Safranal is increased.

Conclusion

In this paper, the physicochemical properties of saffron style were investigated via determination of the commercial style's components, which is called as white saffron in commerce and industry.

The main components of saffron include Picrocrocin, Safranal, and Crocin, which are responsible for taste, odor, and color of the saffron, respectively.

The results showed that the moisture level of saffron samples is higher than that in the national standard of saffron and averagely, it does not have a significant difference between the confidence level of 95% ($p<0.05$). The total ash and acid-soluble ash were in the acceptable limit range determined by the national standard of saffron and the samples in the confidence level of 95% ($p<0.05$).

The results of the material analysis indicated that there is significant difference between the Crocin, Picrocrocin and Safranal in the confidence level of 95% which is in agreement with the national standard of saffron. The increase of the Crocin in the samples is due to the low amount of style, since the style does not have Crocin and high amount of tail of stigma. All the cut filament of saffron samples as well Saffron in filament had the Safranal level in range of 20-50 gr. Generally, the amount of safranal is low in the fresh saffron. Over the time, the Picrocrocin compounds undergo hydrolysis and thus volatile aldehyde is released, and converted to the Safranal. Therefore, the amount of Safranal increases while the amount of Picrocrocin decreases in a period of time and the level of Safranal increases. In other words, they are inversely related. The test for additive color by the thin layer chromatography was negative which is in agreement with the study of Salari et al.

The results of the microbial analysis showed that all the factors were in the limits of national standards of saffron and spice. Thetest of intestinal Enterococcus, Escherichia coli, Bacillus cereus, sulfite-reducing Clostridium, Clostridium Perfringens were negative however, total counts of micro-organisms, mold and yeast were remarkably different in confidence level of 95%. The results for the mold and yeast were in the acceptable limit of the saffron and spice standard. The results of the coli form and total counts of the micro-organisms were in agreement with the standard of spice. According to the results of acquired coliform the saffron style analysis, the draft of the saffron style's standard is written.

References

Abdullaev, FI, Cancer Chemopreventive and Tumoricidal properties of saffron (Crocus sativus L.), Experimental Biology and Medicine, National Institute of Pediatrics, Mexico City 04530, Mexico 2002 227(12): 20–5.

Aghayi M, Gholizadeh R. Evaluation of comparative advantage on production and export of saffron. J. Agri Econ and Dev 2011; 25(1):121-132.

Bolandi M, investigation of Chemical and biological properties of saffron stigma by spectrophotometer, PhD thesis of food industry, Mashhad, Ferdowsi University of Mashhad 2007; 87-91.

Bisset NG, Wichtl M, Herbal drugs and phytopharmaceuticals. A handbook for practice on a scientific basis, (2ed.) London: Med. Pharm. Scientific practice on a scientific basis 2001; 167-169.

Haghighi B, Feizi J, Hemmati-Kakhaki A, Identification of colored styles as one of the saffron adulterations with HPLC, J. Res of Food Sci and Tech 2007; 201(4): 62-65.

Iranian standard organization, saffron microbiology-properties- standard number: 5689.

Iranian standard organization, saffron-properties-standard number: 259-1.

Iranian standard organization, saffron-test methods-standard number: 259-2.

Iranian standard organization, Spices and condiments-measuring the cold water soluble extract, standard number. 1619.

Iranian standard organization, methods to determine the total ash in spice and condiments, standard number.1197.

Iranian standard organization, methods to determine acid insoluble ash, standard number: 1253.

Iranian standard organization, Microbiology of food and animal feed- a Comprehensive method for searching for and total cantet method of the most probable detection method: 5272.

Iranian standard organization, Microbiology of food and animal feed-a Comprehensive method for the enumeration of molds and yeasts-first part: method of counting of colony in products with aqueous activity more than 95%. Standard number: 10899-1.

Iranian standard organization, Microbiology of food and animal feed-a Comprehensive method for the enumeration of molds and yeasts-second part: method of counting of colony in products with aqueous activity equal to or less than 95%. Standard number: 10899-2

Iranian standard organization, Microbiology of food and animal feed- a Comprehensive method for searching for and Coliform forms method of the most probable detection method: 9263.

Iranian standard organization, Microbiology of food and animal feed- a Comprehensive method for enumeration of sulfite-reducing bacteria in Anaerobic condition, standard number: 9432.

Iranian standard organization, Microbiology of food and animal feed- a Comprehensive method for Bacillus cereus, standard number: 2324.

Iranian standard organization, Microbiology of food and animal feed-searching for identification and enumeration of intestinal enterococci in food, standard number: 2198

Iranian standard organization, Microbiology of food and animal feed- a Comprehensive method for the enumeration of clostridium perfringens, standard number: 2197.

James A, Duke Z, Mohagheghzadeh A, Shams Ardekani MR, Culture medicinal plants. Tehran ideas emerge 2010; 12; 48-90.

O'Neil CA, Schwartz SJ. Chromatographic analysis of cis/trans carotenoid isomers, Jour. Geol. 1992; 624. 235-252.

Rios JL, Recio MC, Giner R, Manez SJ. Update review of saffron and its active constituents, Phytotherapy Research, Jour. Geol. 1996; 10, 45, 189-193.

Salehi MH, Amin GH, Kaveh SH, Saffron Pharmacopoeia, Faculty of Pharmacy, Tehran University of Medical Sciences 2003.