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Chemical and Microbiological Quality of Traditional and Industrial Lime Juice Produced in Kashan, Iran

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ABSTRACT

Background: Lime juice is a nutritious drink, which is generally consumed for its' refreshing properties, nutritive value, vitamin content, and health benefits. Therefore, the chemical and microbiological quality of the traditional and industrial lime juice produced in Kashan city was assessed. **Methods:** In this descriptive cross-sectional study, a total of 106 samples were collected and screened for total soluble solid (TSS), pH, acidic value, protein content, mold, yeast, and count of acid-tolerant bacteria according to the Institute of Standards and Industrial Research of Iran (ISIRI). **Results:** Of the total samples, 66 samples (62.26%) were within the Iran's national standard range. Most samples that did not meet the national standard requirements, were related to traditional samples (70.24%) compared to industrial samples (31.82%, $P < 0.001$). No significant difference was found between pH and protein of traditional and industrial lime juice samples ($P > 0.05$), while TSS and acidic values in traditional lime juices were less than those of the industrial lime juice ($P < 0.05$). Additionally, the mold and yeast contaminations in traditional lime juices were more than industrial lime juices ($P < 0.05$). **Conclusion:** In overall, traditional lime juice samples had the most microbial and chemical contaminations. It is necessary to increase the regular monitoring by relevant organizations over quality of the produced lime juices.

Keywords: *Citrus; Food quality; Iran*

Introduction

Lemon, lime, and their juices are highly consumed as popular fruit products that contain high contents of vitamins and phenolic compounds, such as hesperidin, *eriocitrin*, *naringin*, *neohesperidin*, *rutin*, *quercetin*, *chlorogenic acid*, *luteolin*, and *kaempferol* (Khodadadi *et al.*, 2018, Zhou *et al.*, 2017).

The annual universal production of citrus fruits has significantly increased. Furthermore, it has been estimated that lemon and lime, with 100 million tons of annual production, were commercially the most important citrus fruits and accounted for about 6.3 million tons (United

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States Department of Agriculture (USDA)/ Foreign Agricultural Service, 2010).

Previous studies showed that lemon, lime, and their products had various health benefits, such as antibacterial activities, anticancer effect, lipid-lowering effect, protective effects on alcohol-induced liver injury, and cardiovascular diseases (González-Molina *et al.*, 2010, Oikeh *et al.*, 2016, Zhou *et al.*, 2017).

The chemical and microbiological quality of lime juice are strictly maintained in the developed countries under several laws and regulations; unfortunately, in Iran, some of the manufacturers and especially traditional producers are not much concerned about the safety and hygiene of this product due to lack of the related law enforcement. Thus, the adulteration and transmission of some human diseases by drinking juices and other drinks has become a critical problem.

These important adulterations occur in various methods such as addition of water, sugars, pulp wash, as well as substitution of cheaper ingredients and/or synthetic compounds (Khodadadi *et al.*, 2018, Pirsa *et al.*, 2018). Khodadadi *et al.* (2018) showed some extents of adulteration in half of lime juice products (58.3%).

Ogodo *et al.* (2016) demonstrated that various microorganisms were present within the fruits juices. The weak sanitation, raw material contamination and cross contamination, lack of proper heat pasteurization, and sterilization during processing of fruit juices could be the contributory factors for the presence of these organisms in the fruit juices (Ogodo *et al.*, 2016).

In Kashan city, there is a high request for both industrial and traditional lime juices especially during hot seasons. While most manufacturers and some traditional producers produce lime juice in apparently hygienic conditions, in some traditional production centers and in the busy market places, the chemical and microbiological quality of the supplied juices remain questionable unfortunately. Therefore, the aim of this study was to assess the chemical and microbiological quality of the traditional and industrial lime juice produced in Kashan, Iran.

Materials and Methods

Collection of samples: A total of 106 samples of lime juice were collected randomly from shopping centers in Kashan, Iran from May 2016 to April 2018. According to the distribution in different local supermarkets, 84 traditional lime juice samples and based on four popular brands, 22 industrial lime juice samples were chosen in Kashan.

Chemical tests: Chemical tests of lime juice samples were evaluated by measurement of Brix, pH, acidic value, and protein according to Iranian national standards No. 2685, 1029 and 117, respectively (Institute of Standards and Industrial Research of Iran, 1985, 2005, 2007, 2013). Total acidity, as citric acid content, was determined by direct titration of one ml of lime juice with sodium hydroxide (0.1 N) using phenolphthalein as indicator. Total soluble solid (Brix) was assessed from the refractive index of a drop of sample at 20 °C using a refractometer (Atago SMART-1, Japan). The pH values were measured using a pH meter (Metrohm 888 Titrando, Switzerland). The protein content was also measured by Kjeldahl method using a Kjeldahl instrument (Turbotherm Gerhardt, Germany) according to the Iranian national standards No. 1029 and 2685 (ISIRI, 2007, ISIRI; 1985).

Determination of microbial load: Microbial tests of the produced traditional and industrial lime juice samples were checked according to the Iranian national standard protocols. Mold, yeast, and acid-tolerant (aciduric) bacteria were counted according to Iranian national standard No. 8788 (ISIRI, 2005).

Briefly, pour plate method was used for enumeration of organisms from lime juice samples. For ten-fold serial dilutions, one ml of lime juice sample was transferred into 9 ml of sterile phosphate buffered saline (PBS) tubes separately. Enumeration of organisms were done at the selective media including yeast extract glucose chloramphenicol agar (Merck KGaA, Darmstadt, Germany) for mold and yeast count and orange-serum agar (Merck KGaA, Darmstadt, Germany) for acid-tolerant (aciduric) bacteria. Inoculated plates were incubated for 3-5 days at 25 °C and 30°C for mold, yeast, and acid-tolerant (aciduric) bacteria, respectively.

Data analysis: Statistical analysis of the obtained data (Mean ± SD) was performed using SPSS version 16. Categorical variables were compared using the chi-square test. One-way analysis of variance (One-way ANOVA) was used to compare the mean differences of chemical and microbial parameters between the study groups. P-values < 0.05 were considered significant.

Results

This study demonstrated that from 106 lime juice samples, 66 samples (62.26%) were out of the Iranian national standard range (**Table 1**). Most of the samples out of the national standard limits were related to traditional samples (70.24 %) compared to the industrial samples (31.82%, $P < 0.001$).

The results of chemical evaluation of lime juice samples from Kashan are represented in **Table 2**. The pH values of 9.52% of traditional samples were out of standard limit. Only one industrial sample had a pH higher than the standard limit. Average pH value of lime juice samples was 2.46 ± 0.21.

Regarding the traditional samples, the acidity values of 23 (27.38%) did not satisfy the standard limit. All industrial samples were within the standard range. Average acidity value of lime juice samples was 5.49 ± 1.23 g citric acid/100 ml.

Protein content of 15 (17.86%) traditional samples were out of the standard limit, while in industrial samples, 6 (27.27%) samples were incompatible with the standard limit. Mean protein content of lime juice samples was 424 ± 215.38 mg/dl.

In traditional and industrial samples, 32.14% and 4.54% of the samples had lower total soluble solid content than the standard limit, respectively. Mean TSS of lime juice samples was 7.11 ± 1.81 g/100 g.

No significant difference was observed between pH and protein of traditional and industrial lime juices ($P > 0.05$). However, TSS and acidic value in traditional lime juice samples were less than those of the industrial lime juice samples ($P < 0.05$).

Acid tolerant bacteria, mold, and yeast contaminations in traditional and industrial lime juice samples are presented in **Table 3**. The mold and yeast contaminations in traditional lime juices were more than the industrial lime juices ($P < 0.05$).

Table 1. Comparative distribution of consumability of lime juice samples based on traditional and industrial process

Lime juice	Standard	None standard	Total	P-value[b]
Industrial	15 (68.18)[a]	7 (31.82)	22 (20.75)	<0.001
Traditional	25 (29.76)	59 (70.24)	84 (79.25)	
Total	40 (37.74)	66 (62.26)	106 (100)	

[a]: N (%), [b]: Chi esquare test

Table 2. Chemical tests of lime juice types in Kashan, Iran

Tests	Min	Max	Mean ± SD	None standard (%)	Standard
pH					
Traditional	1.99	2.83	2.43 ± 0.21[a]	8 (9.52)	2.3-2.8
Industrial	2.2	2.64	2.48 ± 0.13[a]	1 (4.54)	
Acidity (g citric acid/dl)					
Traditional	3.22	7.59	5.53 ± 1.1[a]	23 (27.38)	> 5.5
Industrial	5.18	6.74	5.9 ± 0.49[b]	0 (0)	
Protein (mg/dl)					
Traditional	87.37	898.25	454.61 ± 217.73[a]	15 (17.86)	> 450
Industrial	171.16	627.41	434.03 ± 107.21[a]	6 (27.27)	
Brix (g/100g)					> 7.5
Traditional	3.12	9.57	7.13 ± 1.67[a]	27 (32.14)	

Industrial	7.45	9.43	8.3 ± 0.62[b]	1 (4.54)	
TSS/A ratio*					
Traditional	0.97	1.26	1.29	-	-
Industrial	1.44	1.4	1.41		

*:TSS/A ratio: Total soluble solid /acidity is called maturity ratio, Different letters a and b in the same column indicate significant differences ($P < 0.05$).

Table 3. Microbial tests of lime juice types in Kashan, Iran

Tests	Min	Max	Mean ± SD	None standard samples*	Standard
Acid tolerant bacteria (CFU/ml)					
Traditional	<1	>10^3	270.58 ± 396.78[a]	30 (35.71)	< 10^2
Industrial	<1	80	67.58 ± 23.06[a]	0	
Mold (CFU/ml)					
Traditional	<1	>10^3	212.58 ± 358.56[a]	23 (27.38)	< 10
Industrial	<1	4	1.25 ± 0.86[b]	0 (0)	
Yeast (CFU/ml)					
Traditional	<1	>10^3	157.51 ± 321[a]	31 (36.9)	< 10
Industrial	<1	4	1.25 ± 0.86[b]	0 (0)	

*: N (%), Different letters a and b in the same column indicate significant differences ($P < 0.05$).

Discussion

Results demonstrated that 25 (29.76%) samples of the traditional and 15 (68.18%) samples of the industrial lime juices were compatible to the standard limits of ISIRI. Taghizadeh *et al.* (Taghizadeh *et al.*, 2014) reported that 23 (51.1%) of lime juice samples produced in Iran were incompatible to the standard limits of ISIRI. They showed that amounts of potassium, polyphenols, total amino acid, and optical rotation were less in none standard (feigned) lime juices than the standard ones ($P < 0.0001$).

The mean pH values for traditional and industrial lime juice samples were nearly similar (**Table 2**). Evaluation of pH by Ansari and Rezaei (Ansari and Rezaei, 2008) as well as Taghizadeh *et al.* (Taghizadeh *et al.*, 2014) showed that the average pH in lime juice samples of Iran were 2.52 ± 0.1 and 2.49 ± 0.1, respectively, which is close to the value obtained in our study. Taghizadeh *et al.* (Taghizadeh *et al.*, 2014) demonstrated that the sensitivity and specificity of pH test for fraud detection in lime juices were 68.96% and 100%, respectively.

According to the obtained results, only 27.38% of the traditional lime juice samples did not meet the acidity standard limit of ISIRI. Ansari and Rezaei (Ansari and Rezaei, 2008) reported that the average of acidity among all lime juice samples from Jahrom, Roudan, and Minab regions varied from 5.5 to 7.2 g/100 g. A previous study showed an acidity value of 6.1 g/100 g for lemon juice samples (Xu *et al.*, 2008). In our study, the mean acidity values for traditional and industrial lime juice samples were 5.53 ± 1.1 and 5.9 ± 0.49 g/100 g, respectively. Total acidity of citrus fruits is an important factor in evaluating the overall juice quality and in determining harvest time (Ansari and Rezaei, 2008).

The mean TSS of industrial lime juice samples (8.3 ± 0.62 g/100 g) was more than the traditional samples (7.13 ± 1.67 g/100 g). A previous study demonstrated that the average values for TSS from Jahrom, Roudan, and Minab regions varied from 7.5 to 9.5 g/100 g (Ansari and Rezaei, 2008). XU *et al.* reported that the TSS value was 10.9 g/100 g for lemon juice (Xu *et al.*, 2008). Taghizadeh *et al.* (Taghizadeh *et al.*, 2014) showed TSS value of 8.58 ± 0.45 g/100 g for industrial lime juice samples. These differences can be mainly ascribed to the differences in the type and variety of the fruits as well as different geographical locations (Ansari and Rezaei, 2008). For juice producing industries, excessive TSS represents a better quality of lime juice, which in turn results in juices with higher consumer acceptance levels.

Nevertheless, the TSS/acidity ratios called maturity ratios for traditional and industrial samples were 1.29 and 1.41, respectively. According to the reports by Ansari and Rezaei (Ansari and Rezaei, 2008), the TSS/acidity ratios for the limes obtained from Jahrom, Roudan, and Minab regions were 1.33, 1.33, and 1.36, respectively. XU *et al.* (Xu *et al.*, 2008) showed a maturity ratio of 1.8 for lemon juices obtained from China, indicating that such values can vary with a variety of citrus fruits. In addition, different weather conditions should be considered due to different geographical locations. Davis and Albrigo (Davies and Albrigo, 1994) showed that the respiration rates increased at higher temperatures possibly causing less storage of acids in the vacuoles and their faster utilization in plant metabolisms. They reported that the rate of decrease in the acidity was positively correlated with the mean temperature during each season.

Fellers *et al.* (Fellers *et al.*, 1988) showed that an increase in maturity ratio increased the consumer acceptance level of the processed grapefruit juice and consumer perception of sweetness. Instead, some factors such as tartness, bitterness, and aroma decreased these factors.

Our results showed that protein amounts, as a golden standard method to determined adulteration in traditional and industrial lime juice samples, were 454.61 ± 217.73 and 434.03 ± 107.21 mg/100 ml, respectively. A previous study demonstrated that protein content in natural lime juices was 550.04 ± 86.66 mg/100 ml, while in fake lime juices, it was 348.68 ± 87.43 mg/100 ml (Taghizadeh *et al.*, 2014).

The spoilage of acidic food, such as lime juices is mostly owing to contamination of the food with aerobic acid tolerant bacteria, yeasts, and molds (Olorunjuwon *et al.*, 2014). Our results showed that especially in traditional lime juice samples acid-tolerant bacteria, molds and yeasts were identified.

The reason for growth of food spoilage bacteria in traditional lime juices could be attributed to the fact that the most juice producers were lacking special training in food hygiene (Olorunjuwon *et al.*, 2014). Furthermore, situation under which the lime juice was prepared, pasteurized and stored might have contributed to the betterment of the product (Olorunjuwon *et al.*, 2014, Perez-Cacho and Rouseff, 2008).

These products with low pH ranged from 1.99 to 2.83 and high acidity 3.22 to 7.59 g citric acid/100 ml did not inhibit the proliferation of acid tolerant bacteria, yeasts and molds, and these allowed their growth to count > 10^3 CFU/ml.

This is similar to result of Olorunjuwon *et al. (Olorunjuwon et al., 2014)* in their study on microbiological quality of some locally-produced fruit juices in Ogun State, South western Nigeria. They reported that yeast count was highest in orange juice (3.5×10^4 CFU/ml) and lowest in grape juice (2×10^4 CFU/ml). Papaya and grape juices recorded the lowest mold count (2.7×10^4 CFU/ml) while avocado juice recorded the highest (4×10^4 CFU/ml). In a previous study, a total fungal count of 4×10^4 CFU/ml was observed in a lemon juice (Ogodo *et al.*, 2016).

Aneja *et al.* (Aneja *et al.*, 2014) showed that yeasts and molds were the main cause of spoilage of juices and 5 yeast isolates, and 11 mold isolates were isolated from juices.

Conclusions

Obtained results of the present study demonstrated 66 (62.26%) of the total lime juice samples were contrary to standard limit of ISIRI. Also, there was a significant difference between usability and non-usability of conventionally lime juice samples and the industrially lime juice samples. Therefore, it is especially suggested that traditional manufacturers of lime juices pay attention to the Good Hygiene Practice (GHP), the use of pasteurization temperatures, appropriate packaging and Good Manufacturing Practices (GMP) to reduce secondary contamination and enhance the quality of the final product. In addition, it is necessary to increase regular monitoring by relevant organizations over produced lime juices' quality.

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Authors' contributions

Sharafati chaleshtori R. designed the study. Arian A, Alizadeh E and Mazroii N collected the samples and carried out the experiments. Authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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