




Comparative Effects of Hibiscus Leaves and Potato Peel Extracts on Characteristics of Fermented Orange Juice

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HIGHLIGHTS

- The results showed no significant mean difference ($p > 0.05$) between samples in acidity.
- FHL₁₀ registered the highest values in total solid solution, vitamin C, antioxidants profile, and sensory evaluation.
- The extracts of hibiscus leaves and potato peel can improve acceptability and shelf life of fermented orange juice.

Article type

Original article

Keywords

Antioxidants
Hibiscus
Fermentation
Colony Count, Microbial
Citrus

Article history

Received: 12 May 2022
Revised: 1 Nov 2022
Accepted: 25 Nov 2022

Acronyms and abbreviations

CFU=Colony Forming Unit
DPPH=2,2-Diphenyl-1-
Picrylhydrazyl
GAE=Gallic Acid Equivalents
LAB=Lactic Acid Bacteria
QE=Quercetin Equivalent
TFC=Total Flavonoids Content
TPC=Total Phenolic Content
TSS=Total Soluble Solid

ABSTRACT

Background: Fermented foods are gaining interest because of their ability to improve health, as well as their good taste, and the desire of many to eat them. This investigation aimed to enhance the chemical and physical properties, sensory evaluation, and shelf life of fermented orange juice by adding liquid hibiscus leave (*Hibiscus sabdariffa*) and potato peel (*Solanum tuberosum*) extracts.

Methods: The extracts of hibiscus leaves and potato peel were added to fermented orange juice, and the samples were divided into five parts. Two parts with different concentrations of hibiscus leave extracts (5%, FHL₅ and 10%, FHL₁₀), and the other two parts with different concentrations of potato peel extracts (5%, FPP₅ and 10%, FPP₁₀). The Fifth part was fermented orange juice without any additives (PC). Then, they were estimated for the acidity, total solid solution, viscosity, carbohydrates, vitamin C, antioxidants profile, color, total microbial count, and sensory evaluation during storage time at 4±2 °C. Statistical analysis was done by IBM SPSS version 25.0 software.

Results: The results showed no significant mean difference ($p > 0.05$) between samples in acidity. FHL₁₀ registered the highest values in total solid solution, vitamin C, antioxidants profile, and sensory evaluation, while FPP₁₀ registered the highest values in viscosity and total microbial count. Also, FPP₁₀ registered the highest values in overall acceptability (8.85±0.24, 8.78±0.26, 8.71±0.26, and 8.78±0.26 at zero, 7th, 14th, and 21st days of storage time, respectively) followed by FPP₅ with no significant mean difference values ($p > 0.05$).

Conclusion: The extracts of hibiscus leaves and potato peel can improve acceptability and shelf life of fermented orange juice summarized in the enhancement vitamin content, antioxidants profile, color, and sensory properties.

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Introduction

Citrus fruits, especially oranges, are among the most popular consumed worldwide. In the recent years, citrus

fruits received considerable attention which has the potential curative benefits associated with high levels

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To cite: El-Hadary A.R.E., Sulieman A.M., El-Shorbagy G.A. (2023). Comparative effects of hibiscus leaves and potato peel extracts on characteristics of fermented orange juice. *Journal of Food Quality and Hazards Control*. 10: 39-50.

of flavonoids, antioxidant, anticancer, as well as anti-inflammatory properties (Benavente-García and Castillo, 2008). World production of oranges is estimated approximately 73,298,838 tons annually. An alternative to waste post-harvest is to process the fruit and produce industrial products such as juices, jams, wines, etc. The use of the fermentation of orange fruits as a substrate for producing high-quality value products has been accomplished; an example is wines obtained by the fermentation of fruit (González et al., 2010).

Lactic Acid Bacteria (LAB) have positive health-promoting effects, e.g. regulating the intestinal flora, decreasing cholesterol level in the blood, strengthening the immune system, and ameliorating oral diseases (Hashemi et al., 2017; Kun et al., 2008). The fermented food with LAB also can prolong its shelf life, enhance the nutritional and organoleptic qualities, improve the sensory properties, and remove undesirable compounds (Kapasob et al., 2017). The traditional study of LAB fermentation focuses on dairy products, which are unsuitable for vegans, lactose, and casein intolerant individuals; and high cholesterol risk individuals (Wu et al., 2020). Thus, the request for the development of non-dairy LAB fermentation food products was pointedly increasing. Fruit juices have been proposed as an alternative vehicle for LAB fermentation due to the high contents of minerals, vitamins, dietary fiber, and antioxidant compounds (Costa et al., 2013; Wu et al., 2020).

El-Hadary et al. (2022) mentioned that pomace and peels (food manufactories by-products) are abundant sources of bioactive compounds. Generally, the by-products of food manufactories did not use, however transaction food manufactories process to environmentally friendly ways becoming a highly important issue. The processing of by-products can generate high food additives value products such as flavonoids (rutin and luteolin), polyphenols (mainly oleuropein and hydroxytyrosol), etc. use in food preparation and manufacturing that increased the shelf life and oxidative stability of stored food products (Canabarro et al., 2019).

The antioxidants are one of most important food additives which used to elongate the shelf life of food products and preserve the nutritional quality of lipid and modify the consequences of oxidative damage in the human body (Canabarro et al., 2019). Natural antioxidants are used instead of synthetic ones to inhibit lipid oxidation in foods to improve their quality and nutritional value. In recent years, several natural antioxidants were used in food preparation and manufacturing which have increased the shelf life and oxidative stability of stored food products (El-Hadary et al., 2022).

Hibiscus (*Hibiscus sabdariffa*) is an important medicinal plant widely distributed in many several areas around the world. Hibiscus characterizes by the presence of phytochemical compounds like flavonoids, phenolic acids, and polysaccharides (Vasudeva and Sharma, 2008). Preliminary phytochemical screening revealed the presence of polyphenols in the leaves extract of hibiscus; also, a recent study indicated that leaves extract of hibiscus had high antioxidant effects (Nade et al., 2010). In addition, potatoes (*Solanum tuberosum*) are peeled during processing. The potato peels are a good source of natural antioxidants. Polyphenols are considered as important groups of natural antioxidants, which are present in potatoes (El-Hadary et al., 2022).

The aims of the present investigation were to the enhancement physical, chemical, and microbiological properties, sensory evaluation, and shelf life of fermented orange juice by hibiscus leaves and potato peel extracts additives.

Materials and methods

Materials

Orange fruits (*Citrus x sinensis*) were obtained from a private farm from Ismailia governorate, Egypt. Hibiscus leaves were obtained from a private farm from Sharqia governorate, Egypt. Potato wastes (*S. tuberosum*) were obtained from farm frits factory.

Chemicals and reagents

Citric acid, sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), phenolphthalein, absolute ethanol alcohol, potassium iodide, sodium thiosulfate, starch, chloroform, acetic acid, and hydrochloric acid were purchased from El-Gomhoria chemical company, Zagazig, Egypt. Thiobarbituric Acid (TBA), β -carotene, linoleic acid, tween 20, folin-ciocalteu reagent, gallic acid, phosphatidyl-choline, potassium chloride, iron chloride, Trichloroacetic Acid (TCA), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), and Butylated Hydroxyl Anisole (BHA) were purchased from Sigma chemical company, Cairo, Egypt. *Lactobacillus plantarum* (EMCC 1027) and *L. bulgaricus* (EMCC 1102) were obtained from the Egyptian Microbial Culture Collection (EMCC) of Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Egypt.

Preparation of samples

Firstly, the different parts of potato factories' wastes (30 kg; water, starch, and peels) were separated. Then, the potato peels were transferred and washed well with

tap water to get rid of the remnants of starch sticking out, and stacked on trays for a 30 min to get rid of excess water before drying. The hibiscus leaves were examined to eliminate the undesirable parts and stacked on trays. Both samples were dried in an oven-dryer at 37 °C for 48 h. The samples were flipping once every hour in the first 4 h. Then, the dried samples were ground to a fine powder, place in plastic bags, wrapped with aluminum foil, and stored at -20 °C until the next procedures (El-Hadary et al., 2022).

Extraction of the antioxidants extracts

The extraction of hibiscus leaves and potato peel extracts of dried samples was conducted using the difference in pressure in 70% concentrations of ethanoic alcohol (El-Hadary et al., 2022). One hundred g of dried weight of hibiscus leaves and potato peel were soaked in 1,000 ml of 70% concentrations of ethanoic alcohol in 2,000 ml conical flask for 6 h with decrease the pressure to 0.6 Pa every 30 min in the sample flask during extraction. The obtained extracts were filtered using paper (Whatman No. 1, England), concentrated using a rotary evaporator (EYELA, Japan) until disappearing the alcoholic odor. Then, they were stored at 4±2 °C until the next procedures (El-Hadary et al., 2022).

Preparation of orange juice and inoculum

The orange fruit was washed with tap water, the pulp was chopped, and the seeds were removed. Then, the orange pulp was crushed using a food-grade juicer and filtered through 80 mesh gauze. Orange juice was divided into five parts. The first part was orange juice with 5% of Hibiscus Leaves extracts (HL₅), the second part was orange juice with 10% of Hibiscus Leaves extracts (HL₁₀), the third part was orange juice with 5% of Potato Peel extracts (PP₅), the fourth part was orange juice with 10% of Potato Peel extracts (PP₁₀), while the Fifth part was orange juice without any additives (PC). Then, the samples were pasteurized at 80 °C for 15 min and cooled to room temperature (25 °C) (Burca-Busaga et al., 2022).

The used cultures were grown at 37 °C separately for 24 h in de Man Rogosa Sharpe (MRS) broth (Difco Laboratories, Detroit, MI, USA) in order to attain approximately 106 Colony-Forming Units (CFU)/ml as inoculating before inoculation into orange juice as 0.5% (V/V).

Enumeration of the cells was performed by plating serial dilutions of bacterial suspensions on MRS agar plates, and incubating at 37 °C, and counting the colonies after 48 h. The inoculation by *L. plantarum* and *L. bulgaricus* was done in the range of CFU/ml, and then incubated at 37 °C for 24 h to make sure the

fermentation operation (Mousavi et al., 2011). After 24 h, the analysis was estimated at zero time. Then, the samples were stored at 4±2 °C for 21st days and analyzed every 7 days.

Determination carbohydrates

Total crude carbohydrates, reduced sugar, and non-reduced sugar of various samples were measured per the methods recommended in AOAC (2019).

Determination of pH

The pH of all samples was measured with a glass electrode of a digital pH meter (Model Mettler Toledo, Switzerland) (AOAC, 2019).

Determination of titratable acidity

The acidity of samples was evaluated by the overall titration method supported the carboxylic acid percentage (AOAC, 2019). A few drops of phenolphthalein were added to 20 ml of homogenized samples in a titration flask. Then, titration with 0.1 M NaOH until a faint pink color persists for 30 s. The concentration of citric acid calculated using the official equation depended on 1 ml of 0.1 M NaOH corresponding to 0.064 mg of citric acid.

Determination of Total Soluble Solids (TSSs)

TSSs and also the index of refraction were assayed using the refractometric method, with an Abbe refractometer and corrected to the equivalent reading at 20 °C (AOAC, 2019).

Determination of viscosity

The viscosity of every sample was resolved at temperature by employing a Brookfield digital viscometer (NDJ-85, Niryn Intelligent Company limited, Shanghai). An acceptable spindle (spindle 2) and rotational speed (60 rpm) were selected for this study (Sun et al., 2006).

Determination of color

Orange juice color was measured using the Hunter-Lab (Hunter Lab Color Flex EZ, USA). Color parameter (L*) indicated the degree of lightness to darkness, (a*) indicated the degree of redness to greenness, and (b*) indicated the degree of yellowness to blueness (Zaki, 2022).

Determination of Total Phenolic Contents (TPC)

The content of total phenol concentration of various samples was measured by a UV spectrophotometer (Jenway-UV-VISSpectrophotometer, UK), supported a

colorimetric oxidation/reduction reaction, that described by Škerget et al. (2005). The reagent of oxidizing was Folin–Ciocalteu reagent consistent with AOAC (2019). Five tenths ml of diluted extract (10 mg in 10 ml solvent), 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), and a pair of ml of Na_2CO_3 (75 g/L) were added. The mixture was incubated for five min at 50 °C, and then cooled. For an effect sample, 0.5 ml of water was used. The absorbance was measured at 760 nm. TPC expressed as acid equivalent (Gallic Acid Equivalents (GAE)/g of dried extract) was calculated using the subsequent equation supported the calibration curve (Figure 1) by use the different concentrations of gallic acid (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg GAE/ml):

$$y = 0.2269x + 0.4847$$

$$R^2 = 0.992$$

Where y is that the absorbance; x is that the concentration (mg GAE/g of dried extract); R^2 is correlation coefficient.

Determination of Total Flavonoids Contents (TFCs)

The concentration of TFCs of various samples was measured according Ordoñez et al. (2006) with some modification description by El-Hadary et al. (2022). One point five ml of AlCl_3 ethanolic solution (20 g/L) was added to 0.5 ml of each extract of samples (10 mg in 10 ml solvent), separately and incubated for 1 h at room temperature. The absorbance was measured at 420 nm at room temperature and therefore the yellow color indicated the presence of flavonoids. TFC expressed as Quercetin equivalent (mg QE/g of dried extract) was calculated using the subsequent equation supported the calibration curve (Figure 2) by use the different concentrations of quercetin (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg QE/ml):

$$y = 0.3531x + 0.8191$$

$$R^2 = 0.992$$

Where x is that the absorbance; y is that the concentration (mg QE/g of dried extract); R^2 is correlation coefficient.

DPPH atom scavenging assay

The decolorization of deep purple color of methanolic DPPH[•] solution by various samples was measured according to Gülçin et al. (2004). One tenth ml of every extract (10 mg in 10 ml methanol solvent) was added to 3 ml of 0.1 mM DPPH[•] dissolved within the methanol solvent to every extract, separately, and measured for 2 h every 30 min at room temperature. The control of the assay was prepared consistent with usage to negative control from DPPH[•] solution and only solvent without extracts, and therefore the positive control by exchange

the extracts by BHA synthetic antioxidants. The absorbance resolves against a negative control at 517 nm for each period, separately. Percentage of antioxidant activity of DPPH[•] radical was calculated using the subsequent equation:

$$\text{Inhibition (\%)} = \left(\frac{A_c - A_t}{A_c} \right) \times 100$$

Where, A_c is the absorbance of the negative control; A_t is the absorbance of the sample and/or positive control. IC_{50} is the antioxidant concentration that inhibits the DPPH[•] reaction by 50% under experimental conditions.

Determination of ascorbic acid

Vitamin C (ascorbic acid) content resolves using 2,6-dichlorophenol indophenol reagent (Fluka, Deisehofen, Germany) in step with the strategy described by AOAC (2019). One ml of sample was added to 1 ml of 50 mM solution of oxalic acid (1:1, v/v), mix thoroughly, and leave at room temperature for 30 min. Centrifugation of the tube at 4,000 rpm for 10 min, then the whole of the separated supernatant was collected with a pipette. The standard sample was prepared using 1 ml of the standard solution instead of the analyzed liquid without centrifugation. The absorbance measurements of the test sample (A_x) and standard sample (A_s) were determined at 700 nm against the mixture of 50 mM solution of oxalic acid (1:1, v/v) as a reference sample. The concentration of vitamin C (C_x : μM) in the liquid of different samples was calculated using the formula:

$$C_x = \frac{A_x}{A_s} \times C_s$$

Where, C_s is the concentration of vitamin C in standard solution.

Microbiological analysis

Viable cell counts were determined by serial dilutions and standard plate method after incubation. Dilutions of 10^{-7} and 10^{-8} CFU/ml were prepared of the fermented samples and plated in double plates. Then, sterilized MRS agar (Merck, Germany) medium was poured on them (standard plate count method) (Mousavi et al., 2011). The plates were incubated at 30 °C for 48 h. Then plates containing 30-300 colonies were counted and recorded as CFU per ml of solution (Vinderola et al., 2000). Additionally, the viability of lactic acid cultures was resolved during the cold storage period using the mentioned method and expressed as CFU/ml (AOAC, 2019).

Sensory evaluation

Sensory evaluation was done per Min et al. (2003). Ten panelists were selected (Staff of Food Science

Department, Faculty of Agriculture, Zagazig University, Egypt) without care old or sex. The panelists were asked to point their preference on a 9-point Hedonic scale with a degree of liking: 1=dislike extremely, 2=dislike greatly, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like a great deal, and 9=like extremely. In each session, five different samples got to rate the color, test, flavour, and overall acceptability of the samples.

Statistical analysis

The tests were exhausted triplicate in line with Steel et al. (1997), and therefore the data were analyzed using the means, variance by Microsoft Office Excel(2016), paired sample t-test, and one-way ANOVA variance analysis by IBM SPSS version 25.0 software (SPSS Inc., Chicago, IL, USA) at the extent of probability of ($p < 0.05$).

Results

Acidity

The acidity of fermented orange juices were shown in Table 1. The results showed no significance means differences ($p > 0.05$) between samples, and no significance means differences ($p > 0.05$) between the values to the same sample during the time of storage. All samples showed low increases in acidity by progress in storage time. The highest value of acidity was 10% of Fermented Hibiscus Leave extracts (FHL₁₀), followed by other samples at 21st days of storage time. While, the lowest value of acidity was 10% of Fermented Potato Peels extracts (FPP₁₀) at zero time of storage.

Effect on TSS and viscosity

The results of TSS assay showed a significant means difference ($p < 0.05$) between almost samples. The highest value of TSS was FHL₁₀ at 21st days of storage time with a significant mean difference ($p < 0.05$) between other samples with no significant mean difference ($p > 0.05$) between FPP₅ and FPP₁₀, and the highest value was FHL₁₀ after both 14th and 21st days of storage time (22.3 ± 0.2 and 22.6 ± 0.1 , respectively) followed by FPP₅ after both 14th and 21st days of storage time (21.3 ± 0.3 and 22.1 ± 0.1 , respectively). While, the lowest values were all samples at zero time of storage with a significant means difference ($p < 0.05$) between PC and other samples (16.36 ± 0.1); and no significant means difference ($p > 0.05$) between FHL₅ and FPP₅ (15.82 ± 0.12 and 15.85 ± 0.1 , respectively); and no significant means difference ($p > 0.05$) between FHL₁₀ and FPP₁₀ (15.35 ± 0.2 and 15.4 ± 0.1 , respectively). The results also showed the

positive effect of the additives hibiscus leaves and potato peel extracts in TSS values of fermented orange juices during the time of storage.

All the samples registered the lowest viscosity values at zero time of storage with no significant means difference ($p > 0.05$) between samples. The results also showed the increases in viscosity values by the progress in storage time with clear improvement in values of samples which have hibiscus leaves and potato peel extracts. All samples registered the highest value of viscosity at 21st days of storage time, and the highest value was FPP₁₀ followed by FHL₁₀ with no significant means difference ($p > 0.05$) between both of them (14.5 ± 0.3 and 14.3 ± 0.2 , respectively), while the lowest value was PC at zero time of storage (10.57 ± 0.57) with no significant means difference ($p > 0.05$) between PC and other samples.

Effect on vitamin C

The impact on the contents of vitamin C during the time of storage was measured and the results are shown in Table 2. The results showed little decrease in values of vitamin C by the progress of storage time to all the samples. The data at 21st registered the lowest value to all the samples and PC was the lowest (51.81 ± 0.21 mg/g of wet weight) with significant mean difference ($p < 0.05$) just with FHL₁₀ (52.87 ± 0.07 mg/g of wet weight). The results also showed a clear effect of hibiscus leaves extracts additives during all the time of storage which showed significant mean difference ($p < 0.05$) between FHL₁₀ and all other samples during storage time.

Effect on carbohydrates

Data in Table 3 show the effect on reducing sugar, non-reducing sugar, and total carbohydrates; respectively. The results showed a decrease in reducing sugar, non-reducing sugar, and total carbohydrates values by progress in time of storage. The results also showed no significant means difference ($p > 0.05$) between all the samples at all the time of storage, and no significance in means differences ($p > 0.05$) between the values to the same sample during the time of storage to reducing sugar, non-reducing sugar, and total carbohydrates. The data at 21st days of storage time registered the lowest values of both reducing sugar, non-reducing sugar, and total carbohydrates to all the samples, while data at zero time was the highest. The reducing sugar represented a high percentage of total carbohydrates, which give 61.68% at the highest value and 60.91% at the lowest value.

Effect on antioxidants activities

The antioxidant activities of different fermented orange

juices were measured by different methods and the results show in the Table 4. TPCs, TFCs, and DPPH scavenging radical activity (IC_{50}) were estimated. The results showed significance in means differences ($p < 0.05$) between samples, and significance in means differences ($p < 0.05$) between the values to the same sample during the time of storage. The results also showed a clear positive effect of hibiscus leaves and potato peel extracts additives by the decreases in IC_{50} value during storage time to all samples, however, the decreases in TPC and TFC values. The highest values of antioxidants activities were at zero time of storage and the highest values were FHL₁₀ to both TPCs and TFCs (448.15 ± 1.15 mg GAE/g and 115.2 ± 0.2 mg QE/g, respectively), and PC in IC_{50} estimated (43.56 ± 0.56 μ g/g of wet weight).

Effect on color

The impact of ethanolic hibiscus leaves and potato peel extracts on the color of different fermented orange juices was measured. Table 5 showed values of lightness (L^*), redness (a^*), and yellowness (b^*), respectively. The results showed decreases in lightness (L^*), redness (a^*), and yellowness (b^*) by the progress in time of storage. However, there was the little significant mean differences ($p < 0.05$) between samples in lightness (L^*) values during storage time, while there was no significant mean differences ($p > 0.05$) between samples at 7th days of storage time. The results registered a clear effect to additives hibiscus leaves extracts on redness (a^*) values which FHL₁₀ gave the highest values of redness (a^*) during storage time (62.62 ± 0.12 , 61.95 ± 0.45 , 61.1 ± 0.1 , and 60.44 ± 0.44 at zero, 7th, 14th, and 21st days of storage time, respectively), and positive effect to additives potato peel extracts on yellowness (b^*) values which FPP₁₀ gave the highest values of yellowness (b^*) during storage time (82.87 ± 0.37 , 80.73 ± 0.23 , 79.52 ± 0.52 , and 78.37 ± 0.37 at zero, 7th, 14th, and 21st days of storage time, respectively).

Effect on the total microbial count

The total microbial count of different fermented orange juices was estimated and the results are shown in Table 6. The results showed a few significant mean differences ($p < 0.05$) between samples during the time of storage with no significant mean differences ($p > 0.05$) between the values to the same sample during storage time, however, the increases in total microbial count values. The results also showed the positive effect to additives hibiscus leaves and potato peel extracts, while PC gave the lowest values of total microbial count with clear significant mean differences ($p < 0.05$) between

samples during the time of storage. All the samples showed no significant mean differences ($p > 0.05$) during storage time accepted PC which gave significant mean differences ($p < 0.05$) between the values at 14th and 21st days of storage time.

Effect on sensory evaluation

Sensory evaluation of different fermented orange juices was estimated and the results are shown in Table 7. The results showed the effect on color, tastes, flavour, and overall acceptability. Potato peel extracts were a positive effect on all sensory evaluations, while additives hibiscus leaves extracts had a negative effect on color evaluation at 10% during all the time of storage. In general, the data showed more acceptability to the FPP₁₀ > FPP₅ > FHL₁₀ > FHL₅ > PC to all sensory evaluations during all the time of storage, and all the degree was ≥ 7 . Almost all panelists were attributing positive effect of potato peel extracts additives to enhance the appearance and flavour, in addition to the decreases in sensory evaluations to PC was attributing to the little bitter taste to the control sample in the last time of storage.

Discussion

The increases in acidity by adding hibiscus leaves and potato peels extracts and also the progress in time of storage may be because of the positive of these extracts on protect citric acid from metabolized by bacterial after fermentation starts, which also explains the decreases of total carbohydrates (both reduce and non-reduce sugar). The appropriate explanation for this phenomenon is the availability of carbohydrates in high concentrations in orange juice. Mousavi et al. (2011) had different results when fermenting a pomegranate juice by probiotic bacteria due to the considerable amounts of organic acid i.e., citric acid in pomegranate juice that was used.

The effect on viscosity, TSS, and a lightness of juice color may be due to the volume of bacterial growth. The spoilage and bacterial growth of food bring about physical changes such as an increase in the viscosity, gelation, sedimentation, and/or color change of the food. First of place, the increases in viscosity by the progress in time of storage and by adding the extracts due to the enhancement of the bacterial growth that has acted to generate the products which have a viscous effect. Also, the increase in TSS by adding the hibiscus leaves and potato peels extracts compared with the control sample attributed to the increases in metabolized resulting from the high volume of bacterial growth. In addition, Quan et al. (2022) attributed the increase in TSS to the ability of bacteria on metabolize organic compounds in fermented orange juices.

Table 1: Acid value of different fermented orange juice samples treated with hibiscus leaves and potato peel extracts

Samples	mg of citric acid/g of wet weight/days-time of storage			
	0	7	14	21
PC	0.93±0.03 ^a	0.96±0.02 ^a	1.05±0.1 ^a	1.2±0.1 ^a
FHL ₅	0.92±0.02 ^a	0.98±0.02 ^a	1.05±0.05 ^a	1.2±0.2 ^a
FHL ₁₀	0.92±0.01 ^a	1±0.05 ^a	1.13±0.13 ^a	1.26±0.06 ^a
FPP ₅	0.93±0.03 ^a	0.97±0.02 ^a	1.05±0.05 ^a	1.2±0.2 ^a
FPP ₁₀	0.91±0.01 ^a	0.98±0.03 ^a	1.07±0.07 ^a	1.2±0.15 ^a

Values mean±SD; n=3. Different letters in the same column indicate significant differences ($p<0.05$).
 PC: positive control
 FHL₅: 5% of fermented hibiscus leaves extracts
 FHL₁₀: 10% of fermented hibiscus leaves extracts
 FPP₅: 5% of fermented potato peels extracts
 FPP₁₀: 10% of fermented potato peels extracts.

Table 2: The contents of vitamin C of different fermented orange juice samples treated with hibiscus leaves and potato peel extracts

Samples	mg/100 g of wet weight/days-time of storage			
	0	7	14	21
PC	52.86±0.36 ^c	52.2±0.2 ^c	52.03±0.3 ^b	51.81±0.21 ^b
FHL ₅	53.22±0.12 ^{ab}	52.91±0.05 ^b	52.46±0.2 ^b	52.2±0.2 ^b
FHL ₁₀	53.73±0.23 ^a	53.52±0.5 ^a	53.08±0.08 ^a	52.87±0.07 ^a
FPP ₅	53±0.5 ^b	52.8±0.3 ^{bc}	52.43±0.43 ^b	52.2±0.2 ^b
FPP ₁₀	52.61±0.11 ^d	52.42±0.42 ^c	52±0.5 ^b	51.85±0.35 ^b

Values mean±SD; n=3. Different letters in the same column indicate significant differences ($p<0.05$).
 PC: positive control
 FHL₅: 5% of fermented hibiscus leaves extracts
 FHL₁₀: 10% of fermented hibiscus leaves extracts
 FPP₅: 5% of fermented potato peels extracts
 FPP₁₀: 10% of fermented potato peels extracts.

Table 3: The contents of carbohydrates of different fermented orange juice samples treated with hibiscus leaves and potato peel extracts

	Samples	g carbohydrates/100 g of wet weight/days-time of storage			
		0	7	14	21
Reducing sugars	PC	7.76±0.66 ^a	7.75±0.25 ^a	7.63±0.13 ^a	7.6±0.3 ^a
	FHL ₅	7.72±0.22 ^a	7.7±0.2 ^a	7.61±0.11 ^a	7.52±0.42 ^b
	FHL ₁₀	7.63±0.13 ^b	7.63±0.2 ^b	7.5±0.4 ^b	7.43±0.1 ^c
	FPP ₅	7.7±0.2 ^a	7.71±0.21 ^a	7.62±0.32 ^a	7.52±0.22 ^b
	FPP ₁₀	7.56±0.25 ^c	7.55±0.55 ^c	7.4±0.3 ^c	7.31±0.2 ^d
Non-reducing sugars	PC	4.82±0.16 ^b	4.71±0.21 ^{ab}	4.69±0.19 ^b	4.6±0.1 ^b
	FHL ₅	4.78±0.08 ^b	4.71±0.51 ^{ab}	4.69±0.09 ^b	4.66±0.24 ^a
	FHL ₁₀	4.83±0.13 ^b	4.69±0.02 ^b	4.7±0.2 ^b	4.62±0.05 ^b
	FPP ₅	4.82±0.62 ^b	4.75±0.67 ^a	4.73±0.68 ^{ab}	4.68±0.42 ^a
	FPP ₁₀	4.88±0.65 ^a	4.75±0.85 ^a	4.76±0.46 ^a	4.69±0.3 ^a
Total carbohydrates	PC	12.58±0.5 ^a	12.46±0.46 ^a	12.32±0.32 ^a	12.2±0.2 ^a
	FHL ₅	12.5±0.3 ^b	12.41±0.31 ^a	12.3±0.2 ^a	12.18±0.18 ^a
	FHL ₁₀	12.46±0.26 ^c	12.32±0.22 ^b	12.2±0.2 ^b	12.05±0.15 ^b
	FPP ₅	12.52±0.42 ^b	12.46±0.46 ^a	12.35±0.35 ^a	12.2±0.2 ^a
	FPP ₁₀	12.44±0.4 ^c	12.3±0.3 ^b	12.16±0.16 ^b	12±0.5 ^b

Values mean±SD; n=3. Different letters in the same column indicate significant differences ($p<0.05$).
 PC: positive control
 FHL₅: 5% of fermented hibiscus leaves extracts
 FHL₁₀: 10% of fermented hibiscus leaves extracts
 FPP₅: 5% of fermented potato peels extracts
 FPP₁₀: 10% of fermented potato peels extracts.

Table 4: The antioxidants activities–Total Phenolic Contents (TPCs; concentration mg GAE/g of wet weight) and Total Flavonoids Contents (TFCs; concentration mg QE/g of wet weight) of different fermented orange juice samples treated with hibiscus leaves and potato peel extracts

Samples		Days/time of storage			
		0	7	14	21
TPC	PC	431.25±1.25 ^c	430.24±1.24 ^c	428.45±2.45 ^c	428.1±1.1 ^c
	FHL ₅	442.73±1.23 ^b	440.42±0.42 ^b	439.52±0.52 ^b	437.24±2.24 ^b
	FHL ₁₀	448.15±1.15 ^a	446.45±0.45 ^a	445.05±1.05 ^a	443.12±0.12 ^a
	FPP ₅	435.46±0.46 ^d	433.52±0.52 ^d	432.04±1.04 ^d	430.43±0.43 ^d
	FPP ₁₀	438.73±0.73 ^c	437.19±1.19 ^c	435.53±0.53 ^c	433.16±0.16 ^c
TFC	PC	111.82±0.82 ^c	109.13±1.13 ^c	106.25±0.25 ^c	101.46±1 ^c
	FHL ₅	113.45±0.45 ^b	112.05±1.05 ^b	110.59±0.59 ^b	108.25±0.25 ^b
	FHL ₁₀	115.2±0.2 ^a	114.57±0.57 ^a	112.73±1.23 ^a	110.81±0.81 ^a
	FPP ₅	110.12±1.12 ^d	108.94±0.94 ^d	105.81±0.81 ^d	99.84±0.84 ^d
	FPP ₁₀	108.73±0.73 ^c	106.44±0.44 ^c	104.82±0.32 ^c	98.43±0.43 ^c

Values mean±SD; n=3. Different letters in the same column indicate significant differences ($p<0.05$).

PC: positive control

FHL₅: 5% of fermented hibiscus leaves extracts

FHL₁₀: 10% of fermented hibiscus leaves extracts

FPP₅: 5% of fermented potato peels extracts

FPP₁₀: 10% of fermented potato peels extracts.

Table 5: Color of different fermented orange juice samples treated with hibiscus leaves and potato peel extracts

Samples		Days/time of storage			
		0	7	14	21
Lightness (L*)	PC	42.64±0.14 ^a	40.4±0.1 ^a	39.55±0.15 ^a	39±0.5 ^a
	FHL ₅	42.1±0.1 ^c	40.2±0.1 ^c	39±0.5 ^b	38.32±0.32 ^c
	FHL ₁₀	42.1±0.1 ^c	40.13±0.13 ^c	38.73±0.23 ^c	38±0.5 ^d
	FPP ₅	42.26±0.26 ^b	40.26±0.26 ^b	39.6±0.3 ^a	39.01±0.5 ^a
	FPP ₁₀	42.3±0.3 ^b	40.2±0.2 ^c	39.05±0.5 ^b	38.62±0.12 ^b
Redness (a*)	PC	52.43±0.43 ^c	52.21±0.21 ^c	51.62±0.12 ^c	51.13±0.13 ^c
	FHL ₅	57.4±0.1 ^b	56.82±0.12 ^b	56.04±0.5 ^b	55.3±0.2 ^b
	FHL ₁₀	62.62±0.12 ^a	61.95±0.45 ^a	61.1±0.1 ^a	60.44±0.44 ^a
	FPP ₅	50.93±0.43 ^c	50.31±0.31 ^d	49.85±0.25 ^d	49.05±0.5 ^d
	FPP ₁₀	51.02±0.52 ^d	50.1±0.1 ^e	49.67±0.17 ^e	48.72±0.22 ^e
Yellowness (b*)	PC	79.77±0.27 ^c	79.21±0.21 ^c	78.65±0.15 ^c	77.92±0.42 ^c
	FHL ₅	77.01±0.51 ^d	76.3±0.2 ^d	75.62±0.12 ^d	74.73±0.23 ^d
	FHL ₁₀	73.82±0.12 ^e	73.03±0.5 ^e	72.01±0.5 ^e	70.69±0.1 ^e
	FPP ₅	80.22±0.22 ^b	79.99±0.49 ^b	78.75±0.25 ^b	78.09±0.5 ^b
	FPP ₁₀	82.87±0.37 ^a	80.73±0.23 ^a	79.52±0.52 ^a	78.37±0.37 ^a

Values mean±SD; n=3. Different letters in the same column indicate significant differences ($p<0.05$).

PC: positive control

FHL₅: 5% of fermented hibiscus leaves extracts

FHL₁₀: 10% of fermented hibiscus leaves extracts

FPP₅: 5% of fermented potato peels extracts

FPP₁₀: 10% of fermented potato peels extracts.

Table 6: Total microbial counts of different fermented orange juice samples treated with hibiscus leaves and potato peel extracts

Samples	CFU/g×10 ⁷ /Days-time of storage			
	0	7	14	21
PC	7.3±0.3 ^c	7.5±0.5 ^c	7.6±0.3 ^c	6.43±0.4 ^d
FHL₅	8.1±0.1 ^b	8.39±0.3 ^b	8.3±0.3 ^b	8.26±0.2 ^c
FHL₁₀	8.4±0.4 ^{ab}	8.62±0.6 ^{ab}	8.83±0.53 ^a	8.7±0.5 ^b
FPP₅	8.53±0.5 ^a	8.91±0.5 ^a	8.86±0.56 ^a	8.73±0.53 ^b
FPP₁₀	8.62±0.6 ^a	9.1±0.1 ^a	9.3±0.3 ^a	9.2±0.2 ^a

Values mean±SD; n=3. Different letters in the same column indicate significant differences ($p<0.05$).

PC: positive control

FHL₅: 5% of fermented hibiscus leaves extracts

FHL₁₀: 10% of fermented hibiscus leaves extracts

FPP₅: 5% of fermented potato peels extracts

FPP₁₀: 10% of fermented potato peels extracts.

Table 7: Sensory evaluation of different fermented orange juice samples treated with hibiscus leaves and potato peel extracts

Samples		Days/time of storage			
		0	7	14	21
Color	PC	8.5±0.53 ^c	7.93±0.85 ^d	8±0.5 ^c	7.85±0.95 ^c
	FHL ₅	8.14±0.83 ^d	8.14±0.55 ^c	8.07±0.77 ^b	7.71±1.02 ^d
	FHL ₁₀	7.57±0.53 ^b	7.64±0.76 ^c	7.5±0.55 ^d	7.35±1.52 ^e
	FPP ₅	8.5±0.95 ^c	8.5±0.76 ^b	8.43±0.4 ^a	8.43±1.64 ^b
	FPP ₁₀	8.64±0.75 ^a	8.71±0.55 ^a	8.43±0.43 ^a	8.57±2.03 ^a
Taste	PC	8.07±1.07 ^d	7.78±0.78 ^e	7.43±0.43 ^d	7.21±1.53 ^d
	FHL ₅	8.28±0.58 ^b	8.35±0.55 ^c	7.85±0.85 ^c	7.64±1.64 ^d
	FHL ₁₀	8.14±0.44 ^c	8.07±1.07 ^d	7.97±0.95 ^b	7.78±1.64 ^c
	FPP ₅	8.64±0.65 ^a	8.5±0.55 ^b	8.35±0.35 ^a	8.28±0.52 ^b
	FPP ₁₀	8.64±0.64 ^a	8.71±0.71 ^a	8.35±1.05 ^a	8.5±1.31 ^a
Overall acceptability	PC	7.85±0.89 ^d	7.85±1.13 ^d	7.43±0.95 ^e	7.14±2.3 ^e
	FHL ₅	8.35±0.95 ^b	8.07±1.24 ^c	7.85±0.67 ^d	7.57±1.5 ^d
	FHL ₁₀	8.21±1.12 ^c	8±0.69 ^c	8.14±0.67 ^c	8.43±0.95 ^c
	FPP ₅	8.35±1.06 ^b	8.42±0.87 ^b	8.5±0.55 ^b	8.64±1.02 ^b
	FPP ₁₀	8.71±0.83 ^a	8.71±0.64 ^a	8.85±0.92 ^a	8.78±1.02 ^a
	PC	7.71±0.26 ^e	7.57±0.44 ^e	7.28±0.26 ^e	7±0.28 ^d
	FHL ₅	8.43±0.88 ^c	8.14±0.55 ^d	7.71±0.7 ^d	7.93±0.89 ^c
	FHL ₁₀	8.14±1.2 ^d	8.21±0.59 ^c	8±0.25 ^c	7.93±0.1.02 ^c
	FPP ₅	8.78±0.26 ^b	8.78±0.26 ^b	8.57±0.34 ^b	8.5±0.4 ^b
	FPP ₁₀	8.85±0.24 ^a	8.78±0.26 ^a	8.71±0.26 ^a	8.78±0.26 ^a

Values mean±SD; n=3. Different letters in the same column indicate significant differences ($p<0.05$).

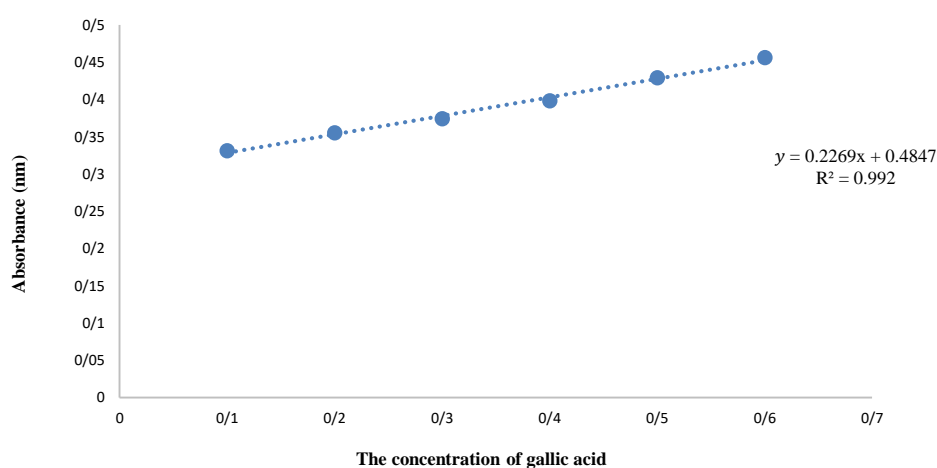
PC: positive control

FHL₅: 5% of fermented hibiscus leaves extracts

FHL₁₀: 10% of fermented hibiscus leaves extracts

FPP₅: 5% of fermented potato peels extracts

FPP₁₀: 10% of fermented potato peels extracts.

**Figure 1:** The stander curve of gallic acid

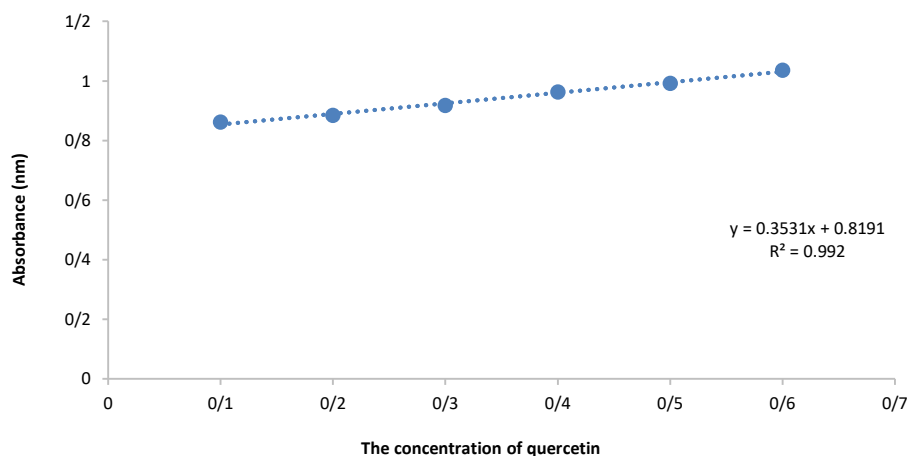


Figure 2: The stander curve of quercetin

The different concentrations of vitamin C between the control sample and the samples which had hibiscus leaves and potato peels extracts may be attributed to the concentrations of vitamin C in these extracts. In addition, the decrease in vitamin C values during storage time may be attributed to the consumption in the nurture of the microorganisms. Also, the increase in vitamin C concentration of samples that had hibiscus leaves and potato peels extracts is due to the protective effect of these extracts on vitamin C consumption by oxidative process and/or bacterial metabolized. Quan et al. (2022) attributed the increase in vitamin C concentration in samples of fermented orange juice to the ability of probiotic bacteria to generation vitamin C.

The results of antioxidants activity by different methods shown the hibiscus leaves and potato peels extracts exhibited high potential antioxidant. In addition, fermentation has a positive affect that shown from the increase in DPPH[•] scavenging radical activities (IC₅₀) during storage time. The data was similar to a previous study on the effects of *L. plantarum* fermentation on phenolic characteristics and various polyphenol compounds, even though different raw materials (Li et al., 2021). The increases in DPPH[•] scavenging radical activities (IC₅₀) during storage time, however the decreases in TPC may be because of production enzymes or some other bioactive compounds which had an antioxidants effect or by the decreases in TFC which had a negative effect on antioxidants activities. Orange juice in different fermentation stages had different antioxidant activities, but overall, the antioxidant activity continued to

increase.

The turbidity of juice during the progress in time of storage, and the difference of color by the different additives of extracts may be attributed to the effect of these extracts on bacterial growth. The decreases in lightness (L*) values by the progress in time of storage may be because of the effect of bacterial cells, while the increases in redness (a*) values by additives hibiscus leave extracts, and yellowness (b*) values by additives potato peel extracts because the red color to hibiscus leaves extracts and yellow color to potato peel extracts. The data was similar to a previous study on the effects of *L. plantarum* fermentation on color characteristics which found a positive effect on turbidity by adding *L. plantarum* to mulberry juice, also the fermentation effect on both of redness and yellowness (Kwaw et al., 2018).

The little increase in the total microbial count during storage time indicated the positive effect of hibiscus leaves and potato peel extracts additives due to the contents of these extracts from important compounds use in microbial metabolized. Also, the protective effect of these extracts gave more validity to the products during the time of storage and more quality not only to microbial growth but also protected the important compounds which had a positive effect on human health.

Conclusion

The extracts of hibiscus leaves and potato peel can improve acceptability and shelf life of fermented orange juice summarized in the enhancement vitamin content,

antioxidants profile, color, and sensory properties.

Author contributions

A.R.E.E.-H. and A.M.S.: Conceptualization; A.R.E.E.-H. and A.M.S.: Formal analysis; A.R.E.E.-H. and A.M.S.: Methodology; A.R.E.E.-H.: Statistical analysis of manuscript; A.R.E.E.-H. and G.A.E.-S.: Writing original draft and writing review and editing. All authors read and approved the final manuscript.

Conflicts of interest

All the authors declared that this is no conflict of interest in the study.

Acknowledgements

This work was supported by Food Science Department, Agriculture Faculty, Zagazig University (Egypt).

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