## **Quality Comparison between Stevia, Mint and Lemon Extracts**

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## ABSTRACT

The present study was making to produce highly nutrients extracts from stevia, mint and lemon samples. The physical and chemical properties, sugar profile, bioactive substances (Vit. C, total polyphenol, total chlorophylls, chlorophylls A and B, total carotenoids, antioxidant activity (DPPH), and sensory quality of the extracts were all evaluated. There were slight variations in the values of total soluble solids to acidity ratios, pH, acidity, and TSS. Increasing the concentration of total polyphenol and antioxidant activity (DPPH) in stevia and lemon extracts led to a notable improvement in the sugar profile, bioactive compounds (vitamin C, total polyphenol, total chlorophylls, chlorophylls A and B and total carotenoids), as well as sensory evaluation. The concentrations of total carotenoids increased in lemon extract, but the contents of total chlorophylls, specifically chlorophylls A and B, increased in stevia and mint extract. Lemon and mint extracts have higher vitamin C contents. All quality criteria, including colour attributes, sugar profiles, physical-chemical properties, bioactive components, and sensory evaluation tests, demonstrated that the extracts of stevia, mint, and lemon had good quality and may be suggested for ingestion as dietary supplements. These findings can be used on an industrial basis in the future. Furthermore, stevia has additional health advantages, such as reducing the risk of tooth decay and controlling blood sugar levels, leading to a decrease in calorie intake.

Keywords: Extracts, DPPH, bioactive substances, polyphenols, carotenoids, sugar, sensory.

### INTRODUCTION

Individuals diagnosed with diabetes often confront the challenges posed by medication side effects, contributing to the growing global burden of diabetes. Consequently, a substantial number of diabetics resort to herbal remedies as an alternative solution. The stevia plant has gained considerable recognition for its potential of managing blood sugar levels and addressing various health concerns because of its beneficial health effects, especially its ability to regulate blood sugar levels. This is related to its natural sweetness, which has no caloric content. The stevia plant, scientifically known as *Stevia rebaudiana*, has attracted considerable interest in recent times.

Stevia sweetener is a natural sweetener derived from the steviol glycosides present in its leaves, possesses a sweetness level that is 200 to 300 times higher than that of sugar. Furthermore, the Food Safety and Health Organization (EFSA, 2010) have deemed stevia safe for consumption, making it an attractive option for diabetics who require a calorie-free sweetener.

Egypt is among the countries that have recently started cultivating stevia. Nonetheless, the raw extract of stevia contains bitter substances that necessitate purification to isolate the steviol glycosides. These compounds are the foundation of stevia sweeteners, providing a healthy and safe alternative to sugar (Hellfritsch *et al.*, 2012, Well *et al.*, 2013, Philippaert *et al.*, 2017).

The prevalence of excessive sugar consumption on a global scale has given rise to various nutritional and medical issues, including obesity. As a potential solution, low-calorie sugar alternatives like stevia have gained popularity due to their 200-300 times sweeter taste compared to sucrose, categorizing them as high-intensity sweeteners. However, the widespread use of artificial sweeteners, such as saccharin, has raised concerns about potential health risks. For instance, repeated use has been linked to the potential development of bladder cancer (Jaroslav *et al.*, 2006).

Lemon (*Citrus lemon*), a medicinal plant belonging to the Rutaceae family, has demonstrated antibacterial activity and is rich in alkaloids. Various parts of the plant, such as its leaves, stems, juice, flowers, and peels, contain antioxidants and anticancer substances that are effective against bacterial strains (Kawaii *et al.*, 2000). Citrus flavonoids, which are found in lemons, exhibit a range of biological activities, including antifungal, antidiabetic, 9nd anticancer properties, and have immediate antimicrobial effects upon extraction (Burt, 2004, Ortuno *et al.*, 2006, Nada & Zainab, 2013).

Mint, a herb with origins in both Asia and the Mediterranean, is highly nutritious and possesses a pleasant, aromatic aroma. Varieties such as Spearmint and Peppermint have medicinal, culinary, and cosmetic uses. Known for its refreshing and sweet taste, mint is rich in antioxidant properties and has been shown to be resistant to harmful bacteria such as E. coli, Staphylococcus aureus, and Staphylococcus purogens (Minica Dukik et al., 2003, Elansary and Mahmoud, 2015, Eman, 2017). This study aimed to evaluate the quality of extracts from stevia, lemon, and mint by determination of physical and chemical properties, sugar profile, bioactive substances (Vit. C, total polyphenol, total chlorophylls, chlorophylls A and B, total carotenoids, antioxidant activity (DPPH), and sensory properties. It also to explore potential applications for each.

### MATERIAL AND METHODS

#### Materials

Stevia leaves, mint leaves, or entire lemon segments acquired from field experiments conducted at the Agricultural Research Institute in Giza, Egypt's Institute of Agronomy. Samples of stevia leaves, mint leaves, or entire lemon slices were utilized in all processing studies. After being received, the samples were kept at 4°C and processed within a day.

#### Methods

# Preparation and extraction of stevia, mint and lemon extracts:

Stevia leaves, mint leaves and whole lemon extracts were prepared according to Shaaban *et al.*, (2024) as illustrated in Figure (1). The bottled extracts of stevia, mint, and lemon were kept at room temperature.

## Determination of some Physico-chemical properties:

The pH of stevia, mint, and lemon extracts was measured using a digital pH meter (HANNA, HI 902 meter, Germany).. Using a hand refractometer (A TAGO, Japan), to determine the percentage of total soluble solids (TSS), represented as °Brix (0-32). Titratable acidity was assessed using the technique described by Tung-Sun *et al.* (1995).

#### **Sugars Determination:**

The determination of sugar acids using highperformance liquid chromatography (HPLC) was achieved by stirring 3.0g of each sample (stevia, mint, and lemon extracts) with 20 mL of distilled



water, followed by centrifugation at 10,000 g for IO min at 30°C. The residues were washed four times with the same amount of water in order to remove all sugar, and the supernatants were combined. An aliquot of each sample was filtered through 0.22  $\mu$ m Millipore membranes.

For the determining sugars, an Agilent model 1100 Series (Agilent, USA) high-performance liquid chromatography equipped with a quaternary pump, refractive index detector, and Shimpack SCR-101N (300 mm L.  $\times$  7.9 mm I.D., 10µm). The mobile phase was deionized water, degassed under vacuum in an ultrasonic bath. The flow rate was 0.7 mL min–1 at a temperature of 40°C. The quantification was achieved by comparison with analytical curves using glucose, fructose, and sucrose standards.

#### **Colour Characteristics:**

The colour of the samples was measured using the HunterLab a\*, b\*, and L\* colorimeters. Using a spectra-colorimeter (Tristimulus Colour Machine) in the reflection mode and the CIE lab colour scale (Hunter, Lab Scan XE - Reston VA, USA), parameters were measured, very time using the instrument was standardized by the white Hunter Lab Colour Standard tile (LX No. 16379), with the following values: Z = 88.14, Y = 81.94, and X = 72.26(L= 92.46, a\* = -0.86, b\*= -0.16).

Using black and white reference tiles, the device  $(65^{\circ}/0^{\circ}$  geometry, D25 optical sensor,  $10^{\circ}$  observer) was calibrated. The color values were written as follows: b\* (yellowness/blueness), a\* (redness/greenness), and L (lightness or brightness/darkness) according to Sapers and Douglas (1987) and Hunter (1975). The Hue (H), Chroma (C)\*, and Browning Index (BI) was calculated using the Palou *et al.*, (1999) method:

$H^* = tan^{-1} [b^*/a^*].$	.(l)
$C^* = square root of [a2^*+b2^*].$	(2)
BI= [100 (x-0.31)] 10.72	(3)
Where:- $X = (a+1.75L)/(5.645L*+a-3.012b)$	

$$AE = (Aa + Ab2 + AL2)1/2....(4)$$

All values were recorded as the mean of triplicate readings.

#### **Total phenolic content determination:**

Total phenolic content of the stevia, mint, and lemon extract samples was performed following the methods of Igual *et al.*, (2012) and Amerine &

Ough (1980), with slight modifications 0.25 mL of methanolic extracts were vortexed (WiseMix VM-10, Daihan, Korea) for 15 seconds after 1.25 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany) and 15 mL of distilled water were added. 3.75 mL of 7.5% Na, CO was added to the mixture after it had been incubated in the dark for 8 minutes, and the volume was then adjusted to 25 mL using distilled water. Sample absorbance values were measured at 765 nm using an Optizen 3220 UV, Mecasys, Korea. The samples were kept at room temperature for two hours in the dark. Gallic acid (GA) (Sigma-Aldrich, Germany) was used to produce the calibration curve, and the results were represented as mg GA/100 g on dry weight (d.w.). Extracts were analyzed in triplicates.

#### Antioxidant capacity (DPPH) determination

The antioxidant potential of samples was determined using the DPPH (2,2-Diphenyl-lpicrylhydrazyl) free radical scavenging method of Alothman *et al.* (2009). 0.1 mL of diluted extracts was added to 3.9 mL of a 25 mM methanolic solution of DPPH radical. After giving the mixture a thorough vortex (Wise Mix VM-10, Daihan, Korea), it was allowed to kept at room temperature for 30 minutes in the dark. Measuring each sample's absorbance at 515 nm was done using an Optizen 3220 UV from Mecasys in Korea. The findings were presented in terms of µmol Trolox equivalents (TE) per 1 g dry weight of sample (Merck, Germany). Three duplicates of each extract were tested.

#### **Sensory Evaluation**

Twelve panelists and employees from the National Research Center's Nutrition Research Institute and Food Industries Department of Food Technology made up the panel. Panelists worked together to create standards and descriptors for fragrance and flavour in eleven training sessions. A ten-point scale (10 = great and I = awful) was used to assess the colour, flavour, acceptability, taste, and appearance of the stevia, mint, and lemon extract samples, following the guidelines provided by Garcia *et al.*, (2001) and Bertolini *et al.*, (2008). Samples were provided in a randomized full block design, and each panelist assessed every sample at once. There were four replications finished.

#### **Statistical Analysis**

The acquired results were subjected to statistical analysis utilizing the Least Significant Difference (LSD) and Analysis of Variance (ANOVA with two methods) as stated by Richard & Gouri (1987).

### **RESULTS AND DISCUSSIONS**

### Physicochemical properties of the stevia, mint, and lemon extracts

The pH values recorded for the stevia, mint, and lemon extracts samples were 5.71, 6.72, and 2.01, respectively (Table 1). The increase in pH observed in the stevia and mint extracts resulted in a decrease in total acidity, whereas the decrease in pH in the lemon extract samples correlated with an increase in total acidity.

The Total Soluble Solids (TSS) levels were 0.7, 0.5, and 7 °Brix in the samples of stevia, mint, and lemon extracts, respectively, as detailed in Table (1). The higher TSS in lemon extracts can be attributed to their higher sugar content (glucose, fructose, and sucrose) compared to that in stevia and mint extracts, as shown in Table (3). Table

It was observed that the titratable acidities as citric acid (%) in the stevia, mint, and lemon extract samples were 0.016, 0.012, and 0.907, respectively (Table I). Additionally, the TSS/total acidity ratios, showed a higher values of 44.27 and 41. 73 for stevia b and mint extracts, respectively, but only 7.72 C for lemon extracts (Table 1). A higher TSS/ H total acidity ratio typically indicates superior flavour in the extracts. Consequently, the higher ratios in stevia and mint extracts (44.27 and 41.73, respectively) suggest their N

potential for fresh applications, while the lower ratio in lemon extracts (7.72) may indicate that further processing would be necessary to enhance the quality of the extract. Fellers *et al.* (1988) reported favorable consumer preference scores for grapefruit juice with TSS/acidity ratios above I 1.0 compared to ratios of 7.0, underlining the significance of TSS/acidity ratios in assessing consumer

 
 Table 1: Physicochemical properties of stevia, mint and lemon extracts

Properties	Stevia	Mint	Lemon
pH	5.71	6.72	2.01
TSS	0.700	0.500	7.00
Titratable acidity (as citric acid)	0.016	0.012	0907
TSS: Total acidity ratio	44.27	41.73	7.72

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appeal. They also noted the stability of the pH, total soluble solids, and total acidity of the juice. Similarly, Sohail *et al.* (2013) emphasized that TSS, pH, and acidity are essential factors in the production of high-value processed products like extracts or beverage drinks.

### Colour characteristics, parameters, and non-enzymatic browning of the stevia, mint, and lemon extracts.

In this study, the colour characteristics of stevia extract were quantified using parameters such as 'a\*' (redness), 'L' (lightness), 'b' (yellowness), H (Hue angle), C\* (chroma), BI (browning index), and Delta AE values, which were measured at -11.18, 24.38, 28.30, 68.44, 25.15, 48.82, and 79.36, respectively (Table 2). Notably, these values exhibited a consistent reduction in both mint and lemon extract samples as compared to the stevia extract.

 Table 2: Colour characteristics and non-enzymatic between of stevia, mint and lemon extracts

Properties	Stevia	Mint	Lemon
_*-value	24.38	19.04	16.19
*-value	-11.18	-12.73	-12.17
v*-value	28.30	30.33	25.30
C*-value	25.15	27.78	24.82
I*-value	68.44	67.24	64.30
3I-value	48.82	55.03	42.60
E*-value	79.36	81.13	81.70
Non-enzymatic browning (O.D.420nm)	44.27	41.73	7.72

The chromatic attributes of stevia, mint, and lemon extracts displayed a combination of negative and positive values for 'a\*' and 'b\*', where yellow and red colours are associated with total chlorophylls and total carotenoids. However, it was observed that the intensity of the red component 'a\*' was lower than the green or yellow component 'b\*', indicating a green coloration, especially evident in stevia and mint extract samples compared to the lemon extract sample.

The values for 'L \*', 'b\*', 'C\*', and 'H\*' increased in stevia extract and decreased in lemon extract samples, (Table 2). However, the Delta AE values showed an opposite trend, decreasing in the stevia extract (79.36) and increasing in the mint and lemon extracts (81.13 and 81.70%, respectively), as outlined in Table (2).

Moreover, the non-enzymatic browning (420 nm) values were higher in the stevia extract sample (0.16) than in the mint and lemon extract samples (0.01-0.01), respectively (Table 2). This increase in the stevia extract sample aligns with the heightened potential of browning observed in the present study.

These findings collectively provide insights into the colour characteristics and non-enzymatic browning tendencies of the extracts, highlighting the distinctions between the stevia, mint, and lemon samples.

## Glucose, Fructose and Sucrose Content (mg/ml) of extract samples:

The sugar content of widely consumed extracts, including stevia, mint, and lemon, was analyzed using high-performance liquid chromatography (HPLC). Notably, the glucose content was found to be lower than the fructose and sucrose contents in all extract samples, as presented in Table (3). This observation accords with the results of Sungur & KIIboz (2016).

Interestingly, the results revealed that glucose, fructose, and sucrose were the predominant components of total sugar in lemon extracts, surpassing the concentrations found in the mint extracts. However, none of the stevia extract samples exhibited detectable levels of glucose, fructose, or sucrose.

In lemon extracts, the mean glucose, fructose, and sucrose levels were higher than those in mint extract samples, glucose content was 0.993 mg/ml in lemon extract as compared to 0.565 mg/ml in mint extracts. Similarly, the fructose content in the lemon extract was 1.243 mg/ml, whereas it was 0.703 mg/ml in the mint extract. Notably, the sucrose content in lemon extract was 8.71 mg/ml, whereas it was 0.616 mg/ml in mint extract.

The glucose: fructose ratios, determined under the applied HPLC conditions, were 0.00, 0.804,

 Table 3: Glucose, fructose and sources content (mg/ml) in stevia, mint and lemon extracts
 highlight that the concentration of vitamin C in fresh stevia extract exceeded that in mint

	Glucose (mg/ml)	Fructose (mg/ml)	Sucrose (mg/ ml)	Glucose: Fructose Ratio
Stevia	0.00	0.00	0.00	0.00
Mint	0.565	0.703	0.616	0.804
Lemon	0.993	1.243	8.71	0.799

and 0.799 in the stevia, mint, and lemon extract samples, respectively. It is crucial to highlight that HPLC analysis revealed the absence of glucose, fructose, and sucrose in the stevia extract samples (Table 3). The determination of sucrose, glucose, and fructose contents, along with the glucose: fructose ratio is essential for understanding the sugar profiles of stevia, mint, and lemon extract samples.

## Bioactive compounds in stevia, mint, and lemon extracts

The analysis of the total phenols content (TPC) is shown in Table (4). It in the stevia and lemon extract samples was comparable to that in the mint extract, with TPC values recorded at 133.19, 134.21, and 117.73  $\mu$ g/100 gm of gallic acid equivalents (GAE)/liter for stevia, lemon, and mint extracts, respectively. These findings align with those of previous studies that identified stevia rebaudiana as an excellent source of phenolic compounds. Stevia rebaudiana has been reported to contain phenolic and flavonoid compounds, emphasizing its antioxidant properties (Tadhani *et al.*, 2007, Kim *et al.*, 2011, Mwanda *et al.*, 2011, Carbonel-Kabila *et al.*, 2019).

## Table 4: Bioactive compounds content in stevia, mint and lemon extracts

Nutrients	Stevia	Mint	Lemon
Vitamin C (mg/100gm)	1.88	0.563	0.810
Total polyphenols (µg/100gm)	133.19	117.73	134.21
Total Carotenoids (µg/100gm)	0.418	0.536	2.677
Chlorophyls A (µg/100gm)	0.914	1.715	0.357
Chlorophyls B (µg/100gm)	1.849	1.215	0.687
Total Chlorophyls AB (µg/100gm)	2.763	2.931	1.044
DPPH (Scavenging) (%)	30.986	15.660	39.842

Stevia leaves, which are known for their glycosides as non-nutritive substitutes to sugar, also exhibit antioxidant properties, in addition to their sweetening attributes. The results in Table (4)

> in fresh stevia extract exceeded that in mint and lemon extracts, recording values of 1.88, 0.563, and 0.81 mg/100 g for stevia, lemon, and mint extracts, respectively. This aligns with previous research emphasizing the significant concentration of vitamin C in stevia leaves (Khiraoui, 2017).

Regarding carotenoid content, the results Ta in Table (4) show that lemon extract had the highest carotenoid content as compared to the other two extracts, with values of 2.677, 0.536, Sa and 0.418  $\mu$ g/100 g in lemon, mint, and stevia extracts, respectively. The concentrations of chlorophyll A, chlorophyll B, and chlorophyll LS AB in the mint extract were 1.715, 1.215, and M 2.931  $\mu$ g/100g, respectively, compared to LS those in the stevia extract (0.914, 1,849, and 2. Le 763  $\mu$ g/100g) and lemon extract (0.357, 0.687, and 1.044  $\mu$ g/100g, respectively) (Table 4).

Carbonell-Capella *et al.* (2019) reported no carotenoids in Stevia-derived products, our results are consistent to those of Muanda *et al.* (2011), indicating the presence of carotenoids in Stevia rebaudiana water extracts. Discrepancies in results may stem from variations in the extraction methods or other experimental conditions.

There was variation in the antioxidant capacity of stevia, mint, and lemon extracts as determined by the DPPH technique (Table 4). Lemon and stevia extracts exhibited higher antioxidant capacities (39.842 and 30.986  $\mu$ g/100 g, respectively) than the mint extract (15.660  $\mu$ g/100 g), with no difference between the antioxidant activities of lemon and stevia extracts.

This study suggests a positive correlation between Total Phenols Content (TPC) and antioxidant activity influenced by stevia content. This trend aligns with that reported by Carbonell-Capella *et al.* (2019), who observed increased, antioxidant activity in different beverage types with added stevia. Additionally, this study provides insights into the phenolic compounds present in stevia leaves, which contribute to hydrophilic antioxidant activity and therapeutic properties (Kim, 2009 & Myint, 2020).

In conclusion, the use of stevia rebaudiana as a natural, non-caloric sweetener and vegetable based sweetening additive in health drinks and various food products is a promising source of bioactive compounds.

## Sensory evaluation of stevia, mint, and lemon extracts

As presented in Table (5), the sensory acceptance of all tested extracts was notably influenced by the processing or extraction methods employed. The stevia and mint extract samples consistently received superior sensory scores for colour, odour,

Regarding carotenoid content, the results **Table 5: Sensory evaluation of stevia, mint and lemon ex**able (4) show that lemon extract had the **tracts** 

amples	Colour	Taste	Odour	Texture	Overall acceptability
tevia	10ª	9.67 <sup>⊾</sup>	10ª	10 <sup>a</sup>	10ª
SD	0	0.5	0	0	0
lint	10 <sup>a</sup>	8.67 <sup>d</sup>	10 <sup>a</sup>	10 <sup>a</sup>	10 <sup>a</sup>
SD	0	1.32	0	0	0
emon	9.33 <sup>b</sup>	9.0 <sup>b</sup>	10 <sup>a</sup>	9.0°	9.0°
SD	0.5	0	0	0	0

texture, and overall acceptability, earning perfect scores of 1 0 across these attributes. However, the taste score for the stevia extract and mint extract were slightly lower at 9.67, and 8.67, respectively. In contrast, the lemon extract samples received commendable scores for colour (9.33), odour (9), texture (10), and overall acceptability (9) (Table 5).

The initial assessment at time zero revealed that both stevia and mint extract samples achieved the highest sensory scores, surpassing the scores of lemon extract samples at the same time point (Table 5).

Surprisingly, despite the absence of glucose, fructose, and sucrose in the stevia extract samples, as determined by HPLC (Table 3), the sensory taste of stevia extracts was considerably better than that of mint or lemon extracts. This suggests that factors beyond simple sugar composition, such as the presence of bioactive compounds and specific flavour profiles in stevia, contribute to its enhanced sensory appeal.

In summary, the sensory evaluation results highlight the favorable taste profile of stevia extracts as compared to mint and lemon extracts, emphasizing the complex interplay of various compounds influencing overall sensory perception.

### CONCLUSION

In conclusion, the stevia, mint, and lemon extract samples exhibited superior performance across various parameters, including the maximum TSS/acidity ratio, pH, TSS, colour characteristics, sugar profile (glucose, fructose, and sucrose), bioactive compound contents (vitamin C, total polyphenols, total carotenoids, total chlorophylls, and antioxidant activity), and sensory evaluation. These extracts not only demonstrated enhanced nutritional qualities but also met consumer taste preferences. As a result, the utilization of stevia, mint, and lemon extracts holds promising potential for addressing malnutrition challenges in both developing and developed countries, such as Egypt, offering a source of comprehensive and affordable nutrition. The positive outcomes observed in this study suggest that these extracts can be applied on an industrial scale in the future.

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## مقارنة الجودة بين مستخلصات الاستيفيا والنعناع والليمون

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أجريت هذه الدراسة لانتاج مستخلصات عالية القيمة الغذائية من عينات الأستيفيا والنعناع والليمون. حيث تم فحص المستخلصات من حيث اللون والخصائص الفيزيائية والكيميائية وأنواع السكريات (الجلوكوز، الفركتوز، السكروز) والمواد النشطة بيولوجيًا (فيتامين ج، عديدات الفينول الكلية، والكلوروفيل الكلي، والكلوروفيل، A وB، والكاروتينات الكلية) ونشاط مضادات الأكسدة والجودة الحسية.

أوضحت النتائج وجود اختلافات طفيفة في قيم كل من نسبة المواد الصلبة الكلية الذائبة إلى الحموضة والـ pH والمواد الصلبة الذائبة الكلية. وكذا زيادة تركيز عديدات الفينول الكلية (في مستخلصات الأستيفيا والليمون إلى تحسين ملحوظ في صورة السكر والمركبات النشطة بيولوجيًا (فيتامين ج، عديدات الفينول الكلية، والكلوروفيل الكلي، والكلوروفيل A وB والكاروتينات الكلية)، والنشاط المضاد للأكسدة وكذلك التقيم الحسي، زادت تركيزات الكاروتينات الكلية في مستخلص الليمون، لكن محتويات الكلوروفيل الكلي، وتحديدًا الكلوروفيل A وB، زادت في مستخلص الليمون، لكن محتويات الكلية على وتحديدًا تركيز أعلى من فيتامين ج، تحتوي على

أظهرت جميع معايير الجودة بما في ذلك اللون وأنواع السكريات والخصائص الفيزيائية والكيميائية والمكونات النشطة بيولوجيا، واختبارات التقييم الحسي، أن مستخلصات الأستيفيا والنعناع والليمون ذات نوعية جيدة ويمكن وصفها كمكملات غذائية.