Evaluation of nutritional quality in different varieties of green and purple leaf lettuces

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Abstract. To determine the quality difference in different varieties of leaf lettuces, we investigated the characteristics of 74 varieties of leaf lettuces (55 green and 19 purple varieties). Both domestic and foreign varieties of lettuce were included in this study. We determined six nutritional indicators of these varieties: such as total soluble sugar, starch, cellulose, proteins, and vitamin C. The grey correlation analysis was used to analyze the 74 varieties of leaf lettuces. Thus, the merits of these traits were compared. The results indicate that the purple leaf lettuce P-S23 had grey comprehensive evaluation value of about 0.8, which was the highest among all varieties. For green leaf lettuces G-S68 and G-S10, grey comprehensive evaluation values were less than 0.5. This indicates that nutritional quality and nutritional value of the purple leaf varieties were higher than that of the green leaf varieties. Furthermore, the quality of green varieties of G-S68 and G-S10 were of poor quality, whereas the purple variety P-S23 was most suitable for commercial production.

1. Introduction
Lettuce (Lactuca sativa L.) has high nutritive value and quality, so it has received considerable attention. Lettuce has been considered as a model system for plant biology; moreover, it forms an integral component of diet thanks to its health benefits [1][2]. Lettuce is an important class of green leafy vegetables that contain nutrient-rich protein [3], carbohydrates, vitamins, and minerals. In addition, it offers several health benefits: it helps in preventing anemia; it possesses anti-cancer and anti-aging qualities; it lowers blood pressure and prevents heart rhythm disorders [4][5]. Sugar is one of the major metabolites of higher plants. Sugar content is high in higher plants; moreover, different isomers of sugar can be isolated from these plants. Sugar plays an important role in many physiological processes of plant growth and development as it is either a metabolic intermediate or final product in these processes [6]. Lettuce contains the following vitamins: vitamin B9, vitamin C, and vitamin E [7]; however, vitamin C content in lettuce is lower than that of other leafy vegetables, such as spinach and kale. Vitamin C (ascorbic acid) is a water-soluble antioxidant, and several studies have proved that vitamin C plays an important role as a plant growth regulator [8].
Vegetables stimulate appetite in humans by improving digestion: they regulate pH of stomach and promote intestinal peristalsis. Recently, domestic varieties of vegetables, such as spinach [9], carrots [10], tomatoes [11], and asparagus [12], have been assessed by nutritional quality analysis and evaluation studies. By implementing the fuzzy membership function method, Min Q et al. [13] determined seven quality indicators in 34 types of spinach. Thus, they conducted a comprehensive analysis of different spinach types in terms of nutritional quality; they determined nitrate, tannin, and acid contents of these species.

Grey correlation analysis is a method of treating and analyzing data according to grey system theory. This method can be performed on relatively small amounts of sampling data. With this method, scientists can easily analyze relationships of many factors [14]. Currently, grey correlation analysis is widely used in agricultural science studies. In a previous study, Ma et al. [15] performed grey relational analysis to evaluate 13 main agronomic characters of 10 sugarcane lines. The 13 agronomic characterized were as follows: sprouting rate, seeding emergence rate, tillering rate, stalk diameter, effective stalks, single stalk weight, sugar content, stalk yield, sugar yield, fiber content, reducing sugar, rate of internal blight rate, and dry top rot. Grey relational analysis (GRA) was used to classify dried roselle (Hibiscus sabdariffa L.). All the roselle calyces (undried and dried) were correctly identified and classified according to GRA grades [16].

Loose leaf lettuce is extremely rich in nutrients, especially in vitamins A and C and calcium; however, different cultivars have significantly different proportion of nutrients. Therefore, this study analyzed nutritional quality differences of different varieties of loose leaf lettuce. Then, we screened these lettuce varieties to identify the ones with higher nutritive value and quality. The results served as a theoretical basis for germplasm improvements and quality breeding in future studies.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted in greenhouses of Beijing University of Agriculture, Beijing, China. In this experiment, we included the following varieties of lettuce: G-S1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 18, 19, 20, 21, 22, 23, 24, 25, 26, 30, 31, 33, 34, 37, 39, 41, 42, 43, 45, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 75 and P-S1, 2, 3, 4, 5, 6, 9, 12, 16, 20, 21, 22, 23, 28, 29, 30, 32, 33, and 34 (These were assigned by our laboratory. Green leaf lettuces were marked as G-S, while purple leaf lettuces were marked as P-S.). We used the following product seeds: G-S1 (‘Jinwangjiegai’), G-S3 (‘Aosaidi’), G-S6 (‘Ruibite’), G-S19 (‘Xiangshengcai’), G-S42 (‘Huangshengcai’), G-S45 (‘Jiequshengcai’), G-S49 (‘Jiguanshengcai’), G-S50 (‘Sanyeshengcai’), G-S53 (‘Bolishengcai No.93’), G-S54 (‘Dongshanshengcai’), G-S59 (‘Aijiaoshengcai’), G-S59 (‘Aijiaoshengcai’), G-S63 (‘Luosheng No.3’), G-S68 (‘Yingzi’), P-S20 (‘Meiguziyeshengcai’), P-S21 (‘Zishengcai’), P-S22 (‘Hongsheng No.1’), and P-S34 (‘Dxiangsheng No.1’).

In a greenhouse, germination of seeds was carried out in cell trays filled with soil. A variety of seeds were sown in a cell tray with 50 cells, each cell containing one seed. The daytime temperature of the greenhouse was fixed at 25 °C, while the night time temperature was maintained at 20 °C. Loose leaf lettuce was sampled 50 days after sowing, and the same parts of each lettuce. After removing main veins, we measured the weight of shredded leaves on an analytical balance (BSA224S, Sartorius, Germany); then, they were frozen in liquid nitrogen. The samples were stored at –80°C in an ultra-low temperature freezer for further analysis.

2.2. Method of determination

In this experiment, we measured five nutrition indicators of vegetables, namely, total soluble sugar, starch, cellulose, proteins, and vitamin C. A titrimetric method was used to measure the content of vitamin C; the indicator 2,6-dichrophenolindophenol was used in titration. Anthrone colorimetry was used to measure soluble sugar, crude fiber, and starch content. Coomassie brilliant G-250 method was
used to calculate protein content in samples. The above tests were carried out using UV-visible spectrophotometer (UV-5200, METASH Shanghai, China).

Grey correlation analysis was performed to assess the quality of loose leaf lettuces. The specific method of analysis can be summarized as follows:

A. Determine the comparison sequence \( x_i = \{x_i(k) | k = 1, 2, \ldots, n\} (i = 1, 2, \ldots, n) \) and the reference sequence \( x_0 = \{x_0(k) | k = 1, 2, \ldots, n\} \). The maximum value of each nutrient index is selected as the reference sequence.

B. Dimensionless treatment:

\[
X_i = \frac{X_i(k) - \min X_i(k)}{\max X_i(k) - \min X_i(k)}
\]

\( i = 0, 1, 2 \ldots m; \quad k = 0, 1, 2 \ldots, n \)

C. Coefficient of correlation:

\[
\zeta(k) = \frac{\min \Delta(k) + \rho \max \Delta(k)}{\Delta(k) + \rho \max \Delta(k)}
\]

\[
\Delta(k) = |X_0(k) - X_i(k)|
\]

the distinguish coefficient(\( \rho \)) is 0.5 generally.

D. Relationship of degree

\[
\gamma = \frac{1}{n} \sum_{x=i}^{n} W_k \zeta(k)
\]

\( W_k \) is the weight value of the \( k^{th} \) quality index. In this study, \( W_k \) is 0.2.

E. Ranking of related degrees

2.3. Statistics and data analysis

The experiment was arranged in a randomized block design and was performed in three replicates. Data were analyzed with OriginPro software (OriginLab Corporation, Northampton, USA) and SPSS 10.0 software (International Business Machine, Chicago, IL, USA).

3. Results

3.1. Comparison of soluble sugar content of different varieties of leaf lettuces

As shown in Figure 1, total soluble sugar content (0.97±0.03%.FW) was highest in the variety G-S66. On the other hand, total sugar content (0.03±0.01%.FW) was lowest in the variety G-S12. There was a difference of 0.94% between the maximum and minimum values of total sugar content. In the purple leaf lettuces, the highest total soluble sugar content was P-S20 (0.72±0.18%.FW), and the lowest soluble total sugar content was P-S23 (0.09±0.01%.FW).

In 31 varieties (41.89%), total soluble sugar content was in the range 0–0.3%.FW, these include 10 purple leaf lettuce varieties. In 27 varieties (36.49%), total sugar content was in the range 0.30-0.60%.FW, these include 5 purple leaf lettuce varieties. In fifteen varieties (20.27%), total sugar content was in the range 0.60–0.90%.FW, these include 4 purple leaf lettuce varieties. Total sugar content of the variety G-S66 was the highest, ranging from 0.90 to 1.00%.FW. In most varieties, total soluble sugar content was in the range 0–0.30%.FW; therefore, these varieties were more abundant than the varieties containing sugar contents in the range 0.90–1.00%.FW.

3.2. Comparison of starch content of different varieties of leaf lettuces

As shown in Figure 2, the variety G-S68 had the highest starch content at 0.98±0.04%.FW. On the other hand, the variety G-S12 had the lowest starch content at 0.04±0.01%.FW. There was a difference of 0.94%FW between the maximum and minimum values of starch content. In the purple leaf lettuces,
the highest starch content was P-S32, 0.91±0.02%.FW, and the lowest starch content was P-S12, 0.08±0.01%.FW.

Among the 74 lettuce varieties, the starch content of 44 varieties (59.46%) was in the range 0–0.30%.FW, these include 6 purple leaf lettuce varieties. Starch content of 22 varieties (28.73%) was in the range 0.30–0.60%.FW, these include 7 purple leaf lettuce varieties. Starch content of seven varieties (9.46%) was in the range 0.60–0.90%.FW, these include 4 purple leaf lettuce varieties. Only the variety G-S66 had starch content in the range 0.90–1.20%.FW. In most varieties, starch content was in the range 0–0.60%.FW; therefore, these varieties were more abundant than the ones whose starch contents ranged from 0.60 to 1.20%.FW.

**Figure 1.** Comparison of total soluble sugar content in different varieties of green and purple lettuces (bars, Standard Error; the difference is not significant when the miniscule is same, whereas different minuscules imply significant difference (P<0.01)).
3.3. Comparison of cellulose content of different varieties of leaf lettuces
As shown in Figure 3, the variety G-S9 had the highest cellulose content at 0.46±0.01%.FW. On the other hand, the variety G-S70 had the lowest cellulose content of 0.02±0.01%.FW. There was a difference of 0.44%.FW between the maximum and minimum values of cellulose content. In the purple leaf lettuces, the highest cellulose content was P-S30, 0.43±0.02%.FW, and the lowest cellulose content was P-S3, 0.04±0.01%.FW.

Among the 74 lettuce varieties, cellulose content of 18 varieties (24.32%) was in the range 0–0.10%.FW, these include 5 purple leaf lettuce varieties. Cellulose content of 25 varieties (33.78%) was in the range 0.10–0.20%.FW, these include 5 purple leaf lettuce varieties. Cellulose content of 22 varieties (29.73%) was in the range 0.20–0.30%.FW, these include 3 purple leaf lettuce varieties. Cellulose content of seven varieties (9.46%) was in the range 0.30–0.40%.FW, these include 3 purple leaf lettuce varieties. The varieties G-S9 and G-S30 represented 2.70% of total samples: cellulose content was in the range 0.40–0.50%.FW for the varieties G–S9 and G-S30. Thus, most varieties had cellulose content in the range 0–0.30%.FW; these varieties were more abundant than the ones whose cellulose contents were in the range 0.30–0.50%.FW.

3.4. Comparison of protein content of different varieties of leaf lettuces
As shown in Figure 4, variety G-S42 had the highest protein content at 27.62±0.34 mg/g.FW. On the other hand, variety G-S10 had the lowest protein content of 8.42±0.01 mg/g.FW. There was a difference of 19.20 mg/g.FW between the maximum and minimum values of protein content. In the purple leaf lettuces, the protein content was P-S5, 22.12±019mg/g.FW, and the lowest protein content was P-S6, 12.14±0.11mg/g.FW.

Among the various lettuce varieties, only G-S10 had protein content in the range 0–10 mg/g.FW; the variety G-S10 represented 1.35% of all the samples. Protein content of 62 varieties (83.78%) was
in the range 10–20 mg/g.FW, these include 16 purple leaf lettuce varieties. Protein content of ten varieties (13.51%) was in the range 20–25 mg/g.FW, these include 3 purple leaf lettuce varieties. Protein content of variety G-S42 was 27.62±0.34 mg/g.FW; only this variety had protein content greater than 25 mg/g.FW. Thus, a majority of varieties had protein content in the range 10–20 mg/g.FW; these varieties were more abundant and evenly distributed than the varieties with protein contents in the ranges 0–10 mg/g.FW and 25–30 mg/g.FW.

![Figure 4. A comparison of protein content in different varieties of green and purple lettuces (bars, Standard Error; the same minuscules indicate that the difference is not significant, and different minuscules mean significant difference (P<0.01)).](image)

![Figure 5. A comparison of Vc content in different varieties of green and purple lettuces (bars, Standard Error; the difference is not significant when the miniscule is same, whereas different minuscules imply significant difference (P<0.01)).](image)
3.5. Comparison of \( V_c \) content of different varieties of leaf lettuces

As shown in Figure 5, variety P-S29 had the highest \( V_c \) content of 60.99±0.01 mg/100 g.FW. In contrast, variety G-S23 showed the lowest \( V_c \) content of 3.35±0.51 mg/100 g.FW. There was a difference of 57.64 mg/100 g.FW between the maximum and minimum values of \( V_c \). In the purple leaf lettuces, the lowest \( V_c \) content is P-S34, 11.56±0.07 mg/100 g.FW. In the green leaf lettuces, the highest \( V_c \) content is G-S21, 27.91±0.24 mg/100 g.FW. Among the 74 lettuce varieties, \( V_c \) content of 17 varieties (22.97%) was in the range 0–10.00 mg/100 g.FW. Moreover, \( V_c \) content of 30 varieties (40.54%) was in the range 10.00–20.00 mg/100 g.FW. Furthermore, \( V_c \) content of 18 varieties (24.32%) was in the range 20.00–40.00 mg/100 g.FW, these include 9 purple leaf lettuce varieties. Finally, \( V_c \) content of nine varieties (12.20%) was greater than 40.00 mg/100 g.FW. Vitamin C content of purple leaf lettuces was generally greater than that of green leaf lettuces.

3.6. Grey fuzzy relational analysis of the nutritional quality of different varieties of leaf lettuces

When relational degree was between 0 and 1, we observed the following trends: higher the relational degree, better would be the quality of the lettuce. As shown in Table 1, grey relations were highest in the following varieties: P-S23, 6, 5, 32, 3, and 4 and G-S60, 55, and 75; grey relation degree was greater than 0.7 for all these varieties. Grey relations were lowest for varieties G-S68 and 10; the relational degree was less than 0.5 for both these varieties. The mean of grey relations in purple lettuces is 0.67±0.05, the mean of grey relations in green lettuces is 0.62±0.05. The mean of grey relations in purple lettuces was higher than that of green lettuces.

| Variety | Relational degree | Variety | Relational degree |
|---------|------------------|---------|------------------|
| P-S23   | 0.7948           | G-S19   | 0.6285           |
| P-S6    | 0.7339           | G-S71   | 0.628            |
| P-S5    | 0.7281           | G-S50   | 0.6251           |
| G-S60   | 0.7251           | P-S20   | 0.6215           |
| G-S55   | 0.7198           | P-S9    | 0.6211           |
| P-S32   | 0.7197           | G-S56   | 0.6204           |
| P-S3    | 0.7197           | G-S7    | 0.6203           |
| P-S4    | 0.7023           | G-S47   | 0.6189           |
| G-S75   | 0.7008           | G-S26   | 0.6187           |
| P-S33   | 0.6935           | G-S30   | 0.6179           |
| G-S67   | 0.689            | G-S3    | 0.6136           |
| P-S22   | 0.6821           | G-S73   | 0.6134           |
| P-S28   | 0.682            | G-S1    | 0.6127           |
| G-S2    | 0.6727           | P-S30   | 0.6119           |
4. Discussion

Among modern consumers, leaf lettuce has become very popular because it is a refreshing green leafy vegetable with tremendous health benefits. However, the quality of domestic variety is poor. Although many foreign varieties are available in China, their growth conditions are quite inconsistent. Therefore, scientists need to urgently select and cultivate varieties, which suite the local climate and growth conditions in China. Leaf lettuce varieties were judged in terms of their nutritional quality and adaptability to the following environmental conditions: lighting, temperature, humidity, soil, etc. Based on the results, we selected the varieties suitable for cultivation on a commercial scale.

Soluble sugar is an important parameter for determining crop quality[17]. The levels of soluble sugar, protein, and cellulose were high in all the included varieties, with maximum values being

|   |   |   |   |
|---|---|---|---|
| G-S66 | 0.6696 | G-S43 | 0.6045 |
| G-S70 | 0.6678 | G-S54 | 0.6034 |
| P-S29 | 0.6667 | P-S12 | 0.603 |
| P-S34 | 0.6582 | G-S53 | 0.6011 |
| P-S2 | 0.6557 | G-S65 | 0.5994 |
| G-S72 | 0.6487 | G-S33 | 0.5991 |
| G-S6 | 0.646 | G-S20 | 0.598 |
| G-S57 | 0.6456 | G-S48 | 0.5974 |
| G-S25 | 0.6419 | P-S21 | 0.5951 |
| G-S63 | 0.6417 | G-S45 | 0.593 |
| G-S42 | 0.6415 | G-S31 | 0.5925 |
| G-S37 | 0.6395 | G-S12 | 0.5853 |
| G-S22 | 0.6393 | G-S59 | 0.5843 |
| G-S8 | 0.6392 | G-S5 | 0.577 |
| P-S16 | 0.6337 | G-S9 | 0.5757 |
| G-S13 | 0.6331 | G-S21 | 0.564 |
| G-S24 | 0.6308 | G-S58 | 0.5639 |
| G-S41 | 0.6306 | G-S51 | 0.5621 |
| P-S1 | 0.6303 | G-S39 | 0.5574 |
| G-S18 | 0.6301 | G-S23 | 0.5322 |
| G-S34 | 0.629 | G-S49 | 0.5252 |
| G-S52 | 0.6287 | G-S68 | 0.497 |
| G-S64 | 0.6286 | G-S10 | 0.4842 |
0.97±0.03% (G-S66 variety), 27.62±0.34 mg/g (G-S42 variety), and 0.46±0.01% (G-S9 variety), respectively. In a study conducted by Yuan et al., the content of soluble sugar, crude protein, and crude fiber were much lower at 1.23%, 11.26 mg/g, and 0.64%, respectively [18]. Compared to our current study, Yuan et al. obtained different results in their experimental study as they investigated different varieties of leaf lettuces.

Energy is produced from starch in the human body; therefore, starch content is one of the criteria for evaluating the quality of vegetables. Variety G-S68 had the highest starch content at 0.98%; however, this value did not agree with the results of a previous study conducted by Garmendia and Idoia [19]. This is because Garmendia and Idoia used romaine lettuce for investigation, while other varieties of leaf lettuce were included in our experiment. In this study, variety P-S29 had the highest vitamin C content of 60.99 ±0.01mg/100 g, while variety G-S23 had the lowest level of Vitamin C of 3.35 mg/100 g. Vitamin C content of 30 varieties was in the range 10.00–20.00 mg/100 g; these varieties represented 40.54% of the total samples. Ahmet Turhan [20] found that vitamin C content of lettuce cultivar ‘Funuly’ was 16.75 mg/100 g, which agreed with our results.

Grey correlation analysis was first proposed by Ju long Deng [14]. Thereafter, it has been widely used in the fields of agriculture and social economics [21][22]. By performing grey correlation analysis, we determined the quality of optimal varieties and the weight value of each quality in the market; moreover, we determined the correlation degree between different varieties of leaf lettuce and the ideal variety. Larger the correlation degree, closer would be the ideal situation for identification: a variety that is suitable for commercialization as it completely adapts with the market and production conditions. In this study, highest grey relations were observed between the following varieties: P-S3, 4, 5, 6, 23, and 32 and G-S55, 60, and 75. This indicates that the quality of content in these varieties was good enough to be considered as a final choice or reference variety. On the other hand, varieties G-S68 and 10 had the lowest grey relations. This implies that the quality of these varieties was poor.

In summary, different varieties showed differences in their quality: total soluble sugar, starch, cellulose, protein, and vitamin C content were highest in the varieties G-S66, G-S68, G-S9, G-S42, and P-S29, respectively. But, P-S23 had the highest grey relations. Four varieties of green leaf lettuces had the best quality; however, five varieties of green leaf lettuces were of worst quality. At the same time, the mean of grey relations in purple lettuces was higher.

Nutritional properties of plants are affected by phenolic compounds [23]. Phenolic acids and flavonoids are the most common phenolic compounds in lettuce [24]. In purple lettuce, the main phenolic compounds are phenolic acids and flavonoids, whereas green lettuce usually contains phenolic acids in significant amounts. In purple lettuce, the content of phenolic compounds is higher than that in green lettuce [24]. Interestingly, several studies have shown that phenolic compounds interact with proteins and polysaccharides. The functional properties of proteins are affected when they interact with polyphenols. Chung et al. found that tannins react with proteins to form compounds that reduce the nutritional value of food [26]. In some polysaccharides (including starch), biological macromolecules exhibit specific biological activity towards polyphenol; these reactions can be combined with static electricity, hydrogen bonding, and hydrophobic forces. On the other hand, polysaccharides can be combined with phenolics through the following chemical pathways: methylation, acyl interaction with sweets, esterification, etc [26]. In this study, the overall quality of purple leaf lettuce was higher than that of green leaf lettuce. The difference in quality was probably due to the difference in the phenolic content in the different varieties of purple lettuce and green lettuce.

In this study, we included only 74 varieties of leaf lettuce. For germplasm improvement and quality breeding of lettuce, future studies have to include more varieties and determine other nutritional indicators, such as anthocyanin and calcium.

5. Conclusion
In this study, five nutritional indicators (total soluble sugar, starch, cellulose, proteins, and vitamin C) of 74 lettuce varieties were examined. Grey correlation analysis was performed to appraise the quality
of leaf lettuces. The relational degree of P-S23 was 0.7948, which was the highest among all varieties. Therefore, purple leaf lettuce P-S23 is the preferred variety for commercial production. The overall quality of purple leaf lettuces was better than that of green leaf lettuces.

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