Research Article

Consumer Acceptability and Impact of Handling Conditions on Sensory, Microbiological and Nutritional Quality of Beet Leaves

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Abstract

Background and Objective: Beet leaves (BL) constitute a currently wasted but highly nutritious resource. The aims of this study were to assess Argentinians consumer habits towards beet leaves (BL) to determine how information affects consumers' acceptability and to assess the impact of storage conditions on BL quality and shelf-life. Materials and Methods: A web-online survey was conducted and data of 416 respondents were analyzed. Then, quality evolution under different storage conditions (A: 25±1 °C and 60-62% of relative humidity (RH), B: 5±1 °C and 97-99% RH) were followed. Data were analyzed by two-way analysis of variance and Turkey-Kramer test (p<0.05). Results: Most consumers (79%) don't use BL due to the poor state at retailers and/or the lack of knowledge about their nutritional value but when this information was provided, acceptability (p<0.05) increased significantly. BL quality was severely affected by storage conditions. “A” condition introduces a rapid drop in sensory quality, losses of 60-82% in bioactives complete loss of antioxidant activity (in 9 days) and severe injure of microbiological quality. Conversely, at “B” condition significantly lower losses in quality attributes were detected, reaching a shelf-life extension of at least 14 days. Conclusion: Development of a product based on BL requires the improvement of handling conditions as well as increasing efforts to inform consumers about their benefits. Through this recovery, economic, environmental and social profits could be achieved.

Key words: Beet leaves, quality characteristics, consumer acceptance, postharvest, waste recovery, sensory evaluation, bioactive compounds


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Data Availability: All relevant data are within the paper and its supporting information files.
INTRODUCTION

Beet (Beta vulgaris L. var. conditiva) is a commonly consumed vegetable around the world. Although it was originally grown for the consumption of their leaves, nowadays the root is the main product obtained. Beet plants are harvested by pulling the base of the stems to release roots from soil. Beet leaves (BL) are not usually cut and separated at this moment and the whole plant goes through the supply chain without control of temperature or relative humidity since roots do not require refrigeration to maintain its quality. However, these factors are critical for the leaves. In fact, under the current handling conditions, once BL get to the retailers, they usually show severe symptoms of quality loss, determining their cutting and removal and their disposal as waste.

In recent years, several studies have focused on the high nutritional value that this type of resources, which are not fully exploited, have especially before they undergo such degradation. Particularly, it was demonstrated that BL are a rich source of nutrients and present a high antioxidant capacity. Thus, if the initial quality was preserved, this by-product, which represents around 50% of the harvested plant, could be recovered for fresh consumption.

Consumers’ acceptance of new food products depends on sensory, economic, social and environmental factors. Moreover, several studies have demonstrated that information could have a large impact on consumers’ perceptions and liking of food products. When raw materials or the products itself are completely new for consumers, exploration of consumer attitudes towards the product as well as the impact of providing information to consumers on product acceptability is crucial as one of the first steps for development of revalorization strategies.

Another main challenge is related with the stabilization of the raw material in order to retain its quality attributes and to extend its shelf life, providing sufficient time for processing, distribution and marketing. Therefore, the aims of this research were: (a) To assess Argentineans consumer habits and attitudes towards BL consumption and to determine how nutritional information affects consumers’ perceptions and acceptability and (b) To determine the enhancement in sensory, nutritional and microbiological quality and shelf life of BL that is achieved by improving the conditions to which the product is exposed after harvest.

MATERIALS AND METHODS

Survey: A web-online survey was conducted during November, 2016. Respondents were contacted through mobile phone messages, email, professional and social networks. The minimum required sample size was set at 384 (95% confidence level and 5% margin of error). For this survey data, 416 completed questionnaires were analyzed.

The questionnaire (Table 1) started with some mandatory questions (Q1, Q2) that allow only people living in Argentina and aged >18 years old to fully complete the survey. Next, general information (age, gender and education level) were required (Q3-Q5). After that, Q6 and Q7 intended to survey consumers’ habits towards leafy vegetables consumption, while Q8 and Q9 specifically explored beet and BL consumption. Finally, nutritional information about BL was provided to respondents and a last question was performed in order to evaluate how this positive information affects consumer attitude towards BL consumption (Q10).

Evolution of quality parameters under different storage conditions

Plant material and storage conditions: Beet (Beta vulgaris L. var. conditiva) was grown and harvested in Escobar, Argentina. Once harvested, plants were immediately transported to the laboratory within the 1st postharvest h. A cut was done 2 cm below the base of each leaf in order to separate leaves from roots and stems. Next, leaves were divided into three lots, one used for initial quality determination and the other two for the evaluation of the impact of environmental conditions on quality attributes and shelf life. Conditions selected for this purpose were: (A): 25±1°C and 60-62% relative humidity (RH), representing an average unfavorable condition that can typically be found in spring and summer in temperate climates and (B): 5±1°C and 97-99% RH, representing the typical commercial refrigeration conditions recommended for leafy vegetables. The extension of the evaluation period was established taking into account the evolution of quality indices. At each sampling time, moisture content, sensorial attributes, microbiological load and nutritional quality was assessed in samples by triplicate.

Moisture content: Moisture content was determined according to the AOAC 934.01 drying oven method. Results were expressed as grams of water per kilogram of fresh tissue (g kg⁻¹).

Sensory quality: Sensory quality of samples was determined by 8 judges, aged 28-60 years, with sensory evaluation experience in leafy vegetables. The coded samples were randomly presented to the judges who evaluated the sensory parameters (color, texture, defects and overall visual quality)
using a 1-9 hedonic scale, where 9: best, excellent, 5: acceptance limit and 1: fully objectionable.

**Microbiological quality:** Fresh leaf tissue samples (10 g) were homogenized with 90 mL of sterile, 0.1% peptone water (Biokar Diagnostics, France) in stomacher (Interscience Laboratories Inc. BagMixer® 400P, France) for 120 sec. Decimal dilutions were prepared with sterile 0.1% peptone water and plated in the growth media for microbial counts. The mesophilic aerobic bacteria count was determined in plate count agar (PCA, Biokar Diagnostics, France) incubated at 37 °C during 24-48 h. *Enterobacteriaceae* were determined in neutral red bile lactose crystal violet (VRBL, Merck) with double layer, incubated at 37 °C during 24 h. Yeast and molds counts were determined in yeast extract glucose chloramphenicol agar (YGC, Biokar Diagnostics, France) incubated at 28°C during 48-72 h and psychrotrophic bacteria count was determined in PCA incubated at 5°C during 5 day. The results were expressed as the logarithm of colony forming units per gram of leaf (log CFU g⁻¹).

**Nutritional quality:** For antioxidant capacity and total phenolic content determination, an extraction was conducted following the methodology proposed by Viacava *et al.*¹¹. Antioxidant capacity was evaluated through the DPPH radical scavenging assay, following the methodology described by Viacava *et al.*² and was expressed as milligrams of ascorbic acid equivalents kg⁻¹ of dry tissue (mg kg⁻¹). Total phenolic content was determined using the Folin-Ciocalteu method and was expressed as milligrams of gallic acid equivalent kg⁻¹ of dry tissue (mg kg⁻¹).
Total chlorophyll and carotenoids contents were determined according to Horwitz and Scott, respectively and results were expressed as milligrams of chlorophyll or carotenoids kg\(^{-1}\) of dry tissue (mg kg\(^{-1}\)).

Betaxanthins and betacyanins were determined following the methodology proposed by MoBhammer et al. and reported as milligrams kg\(^{-1}\) of dry tissue (mg kg\(^{-1}\)).

**Statistical analysis:** Data from the survey were analyzed and qualitative responses from questions 9 and 10 were converted into numbers by coding “Yes” as 1, “No” as 0 and “Perhaps” as 0.5. Next, comparison was assessed by taking into account whether or not difference was greater than the established margin of error (5%) and if so considered significant.

The results of the quality parameters evaluated along storage were expressed as LSMEAN values (means estimators by the method of least squares) together with their standard deviation. Statistical analysis was performed using SAS software version 9.0. (SAS Inst. Inc., Cary, N.C., U.S.A.). Data was subjected to an analysis of variance, using a two-way ANOVA, considering as sources of variation: DAY (storage time), COND (environmental condition during storage) and DAY-COND interaction. Differences between factors were determined by the Tukey-Kramer multiple comparison test (p<0.05)\(^5\).

**RESULTS**

**Survey:** Among respondents, 30.5\% were in the range of 18-25 years old, 39.2\% between 26 and 35, 17\% between 36 and 50 and 13.2\% older than 51 years old. Most of them were females (74\%). In regards to maximum education level achieved, 37\% have secondary degree, 14.2\% a tertiary degree and 48.8\% declared to have a university degree. The results related to leafy vegetables consumption frequency revealed that roughly half of the respondents (45.9\%) consume them 2-3 times/week. Many respondents stated to consume every day (27.6\%), while very few declared to consume them once a week (16.3\%), rarely (8.4\%) or never (1.7\%). In regard to the reason for choosing leafy vegetables (Q7), 80\% of the respondents agreed that “their consumption is good for health”, 57\% considered that “they are tasty” and very few declared to choose them only for their convenient price (0.24\%).

Regarding beet consumption (Fig. 1), 34.1\% of respondents were not beet consumers, while 44.9\% declared...
to consume only the roots and only 21.0% of respondents affirmed that they consume not only the roots but also the leaves (the vast majority, 81.6% after cooking them and the remaining 18.4% consume them raw). Among beet root but not BL, consumers about a quarter of them declared to buy beet roots without the leaves while a higher percentage (60.8%) manifested that they cut and discard leaves in their own home.

After receiving the information about nutritional value of BL, roughly half of respondents showed a positive attitude towards BL consumption (Fig. 1) and only a few refused to consider the consumption. An important feature is that some people responded ambiguously to this question but without dismissing the possibility to consume BL. Furthermore, when comparing converted qualitative responses from Q9 and Q10, significant differences were found with rating’s means of 0.21 and 0.67, respectively.

**Evolution of quality parameters under different storage conditions**

**Sensory quality:** Results of sensory quality evaluation of BL exposed to different conditions during storage are presented in Table 2. The statistical analysis of data obtained for each sensorial parameter yielded significant interactions among factors under study (COND and DAY, p<0.0001), indicating that changes registered along storage were different depending on the environmental condition applied after harvest. In this way, samples exposed to “A” condition, exhibited a rapid drop in the scores of all evaluated sensory attributes that drastically limited the sensory shelf life of samples, which were rejected at day 6 as they presented extended wilting, total texture loss, darkening and visible molds. On the other hand, samples exposed to “B” condition did not show evident signs of dehydration and all assessed sensory attributes had scores higher than the acceptability limit (5 points) even until 21 days of storage. Panelists rejected these samples at 28 days (Table 2) when the scores were below 5 in all evaluated attributes.

Moreover, results of moisture content determination indicated that samples exposed to “A” condition showed high dehydration (Table 3) during storage, while samples exposed to “B” condition did not show significant changes in water content over the storage time.

**Microbiological quality:** Results of microbiological quality assessment are presented in Fig. 2. Initial microbiological counts obtained in fresh material were in the range of 3.8-5.7 log CFU g⁻¹. For all the analyzed microorganisms, ANOVA showed significant interactions among factors under study (COND and DAY, p<0.0001) indicating that changes in microbial counts were different depending on the environmental condition to which samples were exposed to. Indeed, an abrupt increase in all assessed microbial groups was observed in samples exposed to “A” condition (Fig. 2) since the beginning of storage, with increases of 2.7, 3.5, 2.7 and 2.2 log cycles for mesophilic aerobic bacteria (MAB), Enterobacteriaceae (EB), yeasts and molds (Y and M) and psychrotrophic bacteria (PB) at day 6, respectively. On the other hand, when samples were exposed to “B” condition, MAB, EB and PS presented a lag phase of 5 day followed
Fig. 2(a-d): Microbial counts of beet leaves exposed to different environmental conditions (A: 25±1°C 60-62% RH and B: 5±1°C 97-99% RH): (a) Mesophilic aerobic bacteria, (b) Enterobacteriaceae, (c) Yeast and molds and (d) Psychrotrophic bacteria

Error bars represent the standard deviation of the mean. Different letters indicate significant differences by Tukey-Kramer test (α = 0.05)

by a MAB, EB and PS presented a lag phase of 5 day followed by a slow and steady growth reaching final values of 7.62±0.62, 6.47±0.59 and 7.88±0.87 log CFU g⁻¹, respectively, at day 28. Y and M, meanwhile, presented an increase from the beginning of storage, reaching values around 6 log cycles at the end of the evaluated period.

**Nutritional quality:** Results obtained for all nutritional parameters of BL exposed to different conditions during storage presented in Fig. 3. The statistical analysis of data yielded significant interactions among factors COND and DAY (p = 0.0002, p<0.0001, p = 0.0353, p<0.0001, p = 0.0011 and p = 0.0155 for antioxidant capacity, total phenolic content, total chlorophyll, carotenoids, betacyanins and betaxanthins, respectively) indicating that changes were different depending on the environmental condition applied after harvest.

Antioxidant capacity (AC) of samples exposed to “A” condition presented a rapid loss since the beginning of storage, with an almost total loss at day 9 (Fig. 3a). On the contrary, samples exposed to “B” condition, showed smaller losses of AC, retaining the 61.0% of the initial value after 9 day of storage and a 35.8% at the end of storage (28 day). Total phenolic content (TPC) followed a similar pattern (Fig. 3b) than AC, but the last one was lost faster.

Regarding pigments, samples exposed to “A” condition presented a rapid loss of chlorophyll (TC) and carotenoids (C) contents since the beginning of storage (Fig. 3c and d), with a decrease in the range of 82.1 and 70.7%, respectively, at the end of storage (9 day). On the other hand, samples exposed to “B” condition showed a smaller loss in both components, losing only around 33.7 and 24.0%, respectively, at 9 day and 63.6 and 39.6%, respectively, at the end of storage (28 days). Results obtained for betalainic pigments of samples exposed to “A” condition showed a rapid decrease in betacyanins (Bc) and betaxanthins (Bx) concentrations, reaching losses around 50% at the end of storage (Fig. 3e and f). On the contrary, when samples were exposed to “B” condition, no significant changes were observed for both pigments, showing retentions around 90% at 28 days.
Fig. 3(a-f): Nutritional quality of beet leaves exposed to different environmental conditions (A: 25 ± 1 °C 60-62% RH and B: 5 ± 1 °C 97-99% RH): (a) Antioxidant capacity, (b) Total polyphenols, (c) Total chlorophyll, (d) Carotenoids, (e) Betacyanins and (f) Betaxanthins  
Different letters indicate significant differences by Tukey-Kramer test (α = 0.05). Error bars represent the standard deviation of the mean.

**DISCUSSION**

It is known that, worldwide the amount of people who choose more natural, fresh and healthy food is rapidly increasing\(^1^\) since they have become more aware of the importance of nutrition to maintain good health. Indeed, in the survey, when respondents were encouraged to select the reason for choosing leafy vegetables (Q7), the great majority (80%) agreed that “their consumption is good for health”, demonstrating the importance of this factor for consumers.

Results regarding beet consumption revealed that the great majority of beetroot consumers did not use the leaves and this could be associated with the poor conditions that leaves suffered after harvest determining that many retailers or consumers cut and discard this portion. Also, it is probable that in many cases in which leaves are well preserved, the lack
of knowledge about their properties leads to their discard. Moreover, after receiving the information about nutritional value of BL, a significant change in consumer attitude towards beet leaf consumption was observed indicating that consumers show a good predisposition to receive information and follow recommendations about consumption of healthy foods and also that their of acceptability could be easily increased if further information were given to consumers. In fact, it was demonstrated that influencing the construction of consumer perceptions of food results in a predictable behavior change towards the consumption of the product. When considering the use of BL for the development of a product for human consumption, sensory aspects are critical as they impact directly on purchase and consumption intentions. As previously presented, the samples exposed to different environmental conditions showed significant differences in their sensory quality evolution. This may be associated with the water vapor pressure deficit (WVPD) generated as a result of temperature and relative humidity of each condition. This parameter that represents the driving force for water loss was calculated resulting in values of 1235.14 ± 44.79 and 17.45 ± 12.34 Pa for “A” and “B” conditions, respectively. When vegetables are exposed to environments with high WVPD, water and weight losses occur affecting product quality through wilting and other deleterious effects. Hence, it is not surprising that samples exposed to “A” condition showed high water loss accompanied by a rapid loss of sensory quality. Moreover, it is important to highlight that leafy vegetables are usually sold by weight and water is their major component. So, water loss not only affects their appearance but also results in weight losses that lead to enormous economic harm for producers.

Initial microbiological counts obtained in fresh material were in the range of those usually informed in others leafy vegetables. It is important to note that, at the 2nd day of exposure to “A” condition, MAB counts exceeded the usually accepted limit of 7 log CFU g⁻¹ for fresh consumption of leafy vegetables, shortening the shelf life even more than when the sensory criteria is taken into account. Additionally, on day 6, Y and M reached values of 6.47 ± 0.25 log CFU g⁻¹, which are consistent with the observations arising from the sensory evaluation, associated with the appearance of visible mold at that moment. On the other hand, microbiological criteria for BL exposed to “B” condition sets the end of shelf life (for fresh consumption) between day 16 and 21, even when the sensory characteristics are still acceptable. Thus, taking into account this criteria, BL exposed to conditions typically recommended for leafy vegetables (“B”) presented a shelf life at least 14 days longer than when they were exposed to those experimented in “A” situation. Indeed, adequate environmental conditions are crucial for the maintenance of the microbiological quality as it was demonstrated previously in numerous studies with leafy vegetables. Additionally, it is important to note that, in this research, BL were not subjected to any disinfection process, which together with adequate environmental conditions, could improve its microbiological quality.

In regard to nutritional quality, again, results indicated that changes were different depending on the environmental condition applied after harvest. The fact that TPC were lost faster than AC may indicate that other antioxidant compounds present in the BL besides TPC would degrade during storage, negatively impacting on the AC of the tissue. The behavior of these indices during storage is quite variable and dependent on factors such as product type, variety and storage conditions, among others. In this way, while Keverst al reported certain stability or only a slight decrease for 15 different vegetables such as spinach, leek and broccoli during refrigerated storage. Rinaldi et al found an increase in TPC and AC during the first 3 days of storage of parsley leaves stored at 0, 5 and 10°C, followed by a significant decrease with a more pronounced loss for samples stored at the highest temperature. Although each product has certain optimum storage conditions, it is accepted for leafy vegetables that low temperatures help to retain the quality attributes, while high temperatures could mean a stress to plant tissues, which could respond by increasing the level of phytochemical compounds. In this study, “A” conditions generated an extreme stress for BL, with high vapor pressure deficit (70 times higher than recommended condition), causing a significant decrease in these indices from the beginning of storage.

The changes observed in pigments concentration were in accordance with the changes in color registered by the sensory panel. Stability of pigments, specially chlorophyll and carotenoids’ ones, during storage has been extensively studied for a wide range of commonly consumed leafy vegetables. In this way, Ferrante and Maggiore found that chlorophyll content decreased by 22 and 35% after 8 days of storage of Valeriana lettuce at 4 or 10°C, respectively, with carotenoids following a similar behavior. Chlorophyll loss usually occurs mainly during the 1st days of storage and their behavior is usually highly affected by storage temperature. The lower metabolic activity induced by the low temperature may contribute to preserve the pigments content of the product. In spite of this, carotenoids generally show a more variable behavior, highly dependent on the plant product. In relation to betalinic pigments, these compounds are not usually assessed in leafy vegetables as they are not rich in
them. Regarding the stability of these pigments, the findings agree with those reported by Gonzalez-Sanchez et al.,
who worked with extracts of beets root stored at different temperatures. They observed a decline of only 2.8% respect its
initial value at 90 day when extracts were exposed to 4°C, whereas losses roughly reaches 50% at 68°C.

An alternative recovery option for BL could be the
extraction of phytochemical compounds for a subsequent application. In this sense, it is important to note that if the goal
were the extraction of these compounds, even after overcoming the limits of sensory and microbiological
acceptability for fresh consume, considerable high retentions could be obtained under recommended conditions, even at
28 days (75.11, 36.41, 60.44, 90.76 and 87.96% for TPC, TC, C,
Bc and Bx, respectively). The phytochemical compounds found in BL have great potential for application in the food
industry and others, as they could be extracted and used in various applications such as ingredients for food fortification,
dietary supplements, etc.

CONCLUSION

The results obtained in the survey exposed that a very high percentage of people in Argentina do not consume beet
leaves (BL) and was related to the poor state in which the leaves reach retailers and also to the lack of knowledge about
nutritional facts of this vegetable. Indeed, after supplying information about nutritional aspects of BL, respondents were
positively influenced and a significant increase in acceptance was achieved. Furthermore, it was proved that by controlling
the temperature and relative humidity conditions to which the product is exposed after harvest it is possible to maintain their
high initial quality and extend the shelf life of this raw material.

Hence, the improvement of handling conditions as well as increasing efforts to inform consumers about BL attributes
are needed to enable their recovery. This study provides a good basis for the development of alternative uses for this
wasted resource, providing useful information both for their use for fresh consumption as well as for the extraction of
bioactive compounds such as antioxidants, phenolic compounds and natural pigments.

SIGNIFICANCE STATEMENTS

This study will help the researchers to uncover the critical areas of horticultural by product recovery, specifically of beet
leaves which have not yet been explored, proposing alternative uses and providing a good foundation for further investigations. The recovery of underutilized resources allows

the achievement of multiple benefits with high impact on society such as reduction of postharvest losses, improvement
of producer’s profitability, reduction of environmental impact, enhancement of sustain ability of production systems, allowance of novel uses of crops, offering novel and healthy
products to consumers, among others.

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