The Effect of Cold Storage on Some Quality Characteristics of Minimally Processed Parsley (*Petroselinum crispum*), Dill (*Anethum graveolens*) and Lovage (*Levisticum officinale*)

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Abstract. The aim of the present paper is to evaluate the effect of storage at refrigeration temperature on some quality characteristics of the most important Romanian seasoning herbs, presented as minimally processed products. The herbs, sealed in polyethylene bags, were stored at 4°C for 12 days. The evaluation of quality characteristics: sensory analysis, humidity, chlorophyll and vitamin C was done in the 1st, 5th, 8th and 12th day. Total quality of parsley, dill and lovage assessed by sensory analysis (color, texture and flavor) decreased little after 12 days. The water content of each sample did not statistically differ along the storage period. During storage, total chlorophylls content decreased for all samples as they started yellowing. After 12 days, the decrease of total chlorophylls was of 42% for parsley, 45% for dill and 38% for lovage. The content of vitamin C determined for the three aromatic plants can be estimated as high. During the 12 day of storage, the content of ascorbic acid diminished by 18% for parsley samples, 8% for dill and 3% for lovage. In conclusion, minimally processed parsley, dill and lovage sealed in polyethylene bags and stored at 4°C for 12 days have a shelf life of up to 12 days of storage, with little modifications of quality parameters.

Keywords: parsley, dill, lovage, quality, refrigeration

INTRODUCTION

Consumers are becoming more and more concerned about the quality and safety of their food, but they also take into consideration the nutritional and sensory aspects (Artés and Allende, 2005). Human nutritional research is continually showing that a well-balanced diet, rich in fruit and vegetables, promotes good health and may reduce the risk of certain diseases. As we are in the age of fast food, minimally processed fresh fruit, vegetables and aromatic herbs are a becoming more popular. Minimally processed fresh plant food, also named fresh-cut or ready-to-eat, undergoes light treatments such as washing, cutting, grating, shredding, pulling the leaves off, etc. and packing at refrigeration temperatures (Artés and Allende, 2005). This kind of foodstuffs are generally free from additives and only needs minimal or no further processing prior to consumption. Traditional preservation methods such as freezing, dehydration or salting are never applied and these foods are highly perishable. Therefore, these products are more susceptible to deterioration than the original products as plant tissues were damaged in the processing stages (Artés and Allende, 2005). Moreover, the minimal processing cannot guarantee microbial stability, nor can it slow down the plant metabolism. In conclusion, the limited shelf life of ready-to-eat plant foods is caused by physiological ageing, biochemical changes and microbial spoilage stages (Artés and Allende, 2005). Therefore, the quality losses consist of changes in color (discoloration, yellowing), texture (loss of crispness or juiciness), flavor (off-flavors) and water losses.

Temperature is the most important environmental factor that influences the quality of minimal processed plant foods (Artés and Allende, 2005). In consequence, a good knowledge
of time-temperature relation in the cold chain is critical to evaluate the effect of the actual
cold chain on the quality loss and the shelf life of these products.

As Romania is one of the leading European spice consumer, according to
EUROSTAT and FAOSTAT, the present study was aimed at this category of commodities. The
aromatic herbs most consumed Romania are parsley, dill and lovage. Fresh parsley, dill and
lovage can be stored at 18-20°C and 85–90% humidity for about 3 days. As, minimal
processed parsley, dill and lovage were little studied in Romania, we wanted to asses the
evolution their quality characteristics in relation to shelf-life and temperature. In order to do
this we stored parsley, dill and lovage samples packed in polyethylene bags at 4°C for up to 12
days and tested the samples during storage for sensory characteristics, humidity, chlorophyll,
ascorbic acid and volatile flavor compounds.

MATERIALS AND METHODS

1. Raw material and preparation of samples

Fresh parsley (Petroselinum crispum), dill (Anethum graveolens) and lovage
(Levisticum officinale) were purchased from a local market in Cluj-Napoca. The batches were
homogenized and examined visually. Foreign bodies and plant materials were removed,
together with yellow and withered leaves. The stems were cut to approximately 10 cm. The
leaves were cleaned, washed with tap water, air-dried. The batches of each plant were divided
into 6 groups of 30g and 60 groups of 5g. Each sample of 30 g was termosealed in a 15x20
cm polyethylene bag, and each sample of 5 g in a 10x10 cm polyethylene bag (Krups
Vacupack Plus F380).

The samples were stored at 4°C up to 12 days. Samples from the 1st, 5th, 8th and 12th
day of storage were taken for analyses.

2. Sensory analysis

A panel of 15 evaluators assessed the sensory quality. Color, texture (with 2
parameters firmness and succulence) and flavor (with 2 parameters taste and odor) were
scored on a scale of 5, 5 being the highest score. The total score for each sample was obtained
by adding the five parameters listed above.

3. Chlorophylls

In order to determine the chlorophyll content, first the humidity of the samples was
assessed.

3.1 Humidity

Humidity of samples was determined gravimetrically, using AOAC 934.01 method
with the following modifications: 5 g of parsley leaves were dried in a drying oven (Memmer
Thermoreglable drying oven) to constant weight at 105° C under pressure <100 mm Hg (ca 3
hours). Weight loss was estimated as water content:

\[ LOD(\%) = \frac{W - DW}{FW} \times 100 \times 100 \quad , (1) \]

where: LOD – loss on drying

W – moisture

FW – fresh weight

DW – dried weight

3.2 Extraction of chlorophyll from tissue

For the extraction of chlorophyll from parsley samples, AOAC 940.03 extraction
procedure was followed with some modifications. 0.5 g of fresh parsley was weight and
grounded in a mortar and pestle using 10 ml of 80% acetone (80:20 v/v, (CH₃)₂CO:H₂O)
(Chem-Lab NV, reagent grade acetone). The extract was transferred to a stoppered
Erlenmeyer wrapped in aluminum foil. The residue was re-extracted with 5 ml of 80% acetone until tissue was devoid of any green and washing solvent remained colorless. The total extract was clarified by centrifugation (EBA 20 Hettich Zentrifugen) at 5000 g for 5 min.

3.3 Total chlorophyll, chlorophyll a and b determination

Total chlorophyll, chlorophyll a and b was determined spectrophotometrically (UV-Vis 1700 PharmaSpec Shimadzu) using AOAC 942.04 method, at 660 and 642.5 nm. The components were calculated as follows:

$$Total \cdot chlorophyll = \frac{(7.12 \cdot A_{650.0} + 16.8 \cdot A_{642.5}) \cdot E}{10 \cdot DWS}$$, mg/100g sample (2)

$$Chlorophyll \cdot a = \frac{(9.93 \cdot A_{660.0} - 0.777 \cdot A_{642.5}) \cdot E}{10 \cdot DWS}$$, mg/100g sample (3)

$$Chlorophyll \cdot b = \frac{(7.6 \cdot A_{642.5} - 2.81 \cdot A_{660.0}) \cdot E}{10 \cdot DWS}$$, mg/100g sample (4)

where: E – total volume of extract
DWS – dried weight of sample

4. Ascorbic acid

Ascorbic acid determination was done on methanolic extracts of samples.

4.1 Extraction of ascorbic acid from tissue

The extracts were obtained following Rodriguez-Saona and Wrolstad (2001) modified protocol: 1 gram of parsley was extracted with 10 ml of acidified methanol (99.09:0.01 v/v, MeOH:HCl) (reagent grade pure methanol Chempur) using a ceramic mortar and a pestle. The extract was filtered through a Whatman no. 1 filter paper. The residue was re-extracted until the extraction solvents remained colorless (the total solvent volume was between 50-80 ml). The filtrates were combined in a total extract. The extraction was done rapidly, at shade and all collectors were wrapped in aluminum foil. The total extract was dried in a vacuum rotary evaporator at 37°C. The dry residues were redisolved in 5 ml of methanol. The extracts were kept at -20°C until further analysis.

4.2 Ascorbic acid separation, identification and dosage

The ascorbic acid in the samples was separated, identified and dosed in a HPLC Agilent 1200 system coupled with UV–VIS detector (DAD). Eclipse XDB-C18 column (5µm, 150x4,6) was used. The column was eluted in isocratic system with a mobile phase consisted of water/acetonitrile/formic acid (94/5/1, v/v/v) at a flow rate of 0,5 ml/min. The chromatograms were registered at 240 nm.

Prior to HPLC injection the methanolic extracts were filtered through syringe 0,45 µm nylon filters (Teknokroma Syringe Filters Nylon 0,45 µm 13 mm diameter).

For ascorbic acid identification standard L-ascorbic acid (Sigma 99% standard L-ascorbic acid) was used. For dosage of ascorbic acid in the samples, a calibration curve was constructed using dilutions of standard L-ascorbic acid in bidistilled water.

6. Statistics

All results were obtained in triplicate, except for sensory analysis, where results were expressed as means of 15 scores.

Statistical analyses were performed using XLSTAT (Version 2012.4.03) statistical software. Means and standard deviations for each analysis were calculated. ANOVAs computed against the means was employed to determine differences (p<0,05) . To determine whether the differences were significant, Fisher (LSD) analysis of the differences between the different categories was applied with a confidence interval of 95%.
1. Sensory analysis
The test panel made up of 15 members evaluated the samples. Visual quality (color), texture (firmness and succulence) and flavor (taste and odor) were scored on a scale of 5, 5 being the highest score. The total score for each sample was obtained by adding the five parameters listed above. The limit of acceptance was considered a score of 3, for each component, and a total score of 15.

The total score means are presented in fig. 1. It can be observed that the total scores means of each plant taken into study did not statistically differ along the storage period. Although the scores obtained in the first day were higher and there could be observed a decreasing tendency, this tendency could not be statistically confirmed. This suggests that the total quality of parsley, dill and lovage assessed by sensory analysis did not decrease in a twelve days storage period.

In contrast, there were differences between the total scores means of dill and lovage, on one hand and parsley, on the other in each storage day. That is, dill and lovage scored higher, but with a more evident decrease: 2.02 points for dill and 1.97 for lovage. While parsley scored lower in the first day, the overall decrease was slighter, of only 0.47.

![Graph showing total scores for sensory analysis along storage](image)

Note: Different letters for each storage day represent significant differences (Fisher (LSD), p < 0.5) among scores.

2. Humidity
The water content was determined for both leaves and petioles, as they are both used in culinary and medicinal industry.

In the first day, dill had the highest water content 89.2. This value is in agreement with other results: 92.1- 85.1% (Michalik and Dobrzanski, 1987; Witkowska, et al. 1996 quoted by Slupski, 2005) for the whole plant, 88.3-82.1% in leaves, 91%-83.8% in leaves with petioles (Kmiecik, 2002; Slupski, 2005). Parsley had the second water content, 88.7, similar to that cited by current literature: 79–89 % (Azeez, Shamina and V.A. Parthasarathy 2008). Lovage had the smallest water content, of 87.3, close to 89.57% found by Śledź and Witrowa-Rajchert (2012).

The water content of each sample (tab. 1) did not statistically differ along the storage period. There were no differences because the samples were sealed in impermeable polyethylene bags. However, statistically significant differences were found among the three aromatic herbs in each storage day, up to day 8 of storage. In the last day of storage, the water content was similar in all samples.
Tab. 1

<table>
<thead>
<tr>
<th>Plant</th>
<th>Water content, [%]</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 1</td>
<td>day 5</td>
<td>day 8</td>
<td>day 12</td>
</tr>
<tr>
<td>parsley</td>
<td>88,665 ± 0,005% ab</td>
<td>88,988 ± 0,005% ab</td>
<td>89,795 ± 0,001% b</td>
<td>88,396 ± 0,004% a</td>
</tr>
<tr>
<td>Dill</td>
<td>89,225 ± 0,003% a</td>
<td>89,946 ± 0,005% a</td>
<td>91,309 ± 0,000% a</td>
<td>87,987 ± 0,017% a</td>
</tr>
<tr>
<td>lovage</td>
<td>87,307 ± 0,002% b</td>
<td>87,295 ± 0,003 b</td>
<td>87,998 ± 0,004% c</td>
<td>86,133 ± 0,007% a</td>
</tr>
</tbody>
</table>

Note: Different letters for each storage day represent significant differences (Fisher (LSD), p < 0.5) among water content values.

3. Chlorophylls

Chlorophyll content was determined along the storage period in order to assess both the visual quality of the samples (green index) and its nutritional quality. On one hand, chlorophyll is considered crucial to final product acceptance, because green color is associated with fresh vegetables quality (Ferruzzi and Schwartz 2001). On the other hand, chlorophyll may prove to have disease-protective effects attributed to diets rich in green vegetables because its content in these plants is much higher concentrations than the widely studied phytochemicals (Fahey 2005).

Chlorophylls content per fresh weight of samples along storage is presented in tab. 2.

Tab. 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage period</th>
<th>Chlorophyll a (mg/100g)</th>
<th>Chlorophyll b (mg/100g)</th>
<th>Total chlorophylls (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 1</td>
<td>93,554 ± 0,258 d</td>
<td>38,513 ± 0,965 b</td>
<td>131,981 ± 1,169 d</td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td>86,616 ± 0,200 e</td>
<td>34,196 ± 0,383 c</td>
<td>120,734 ± 0,582 g</td>
</tr>
<tr>
<td></td>
<td>day 8</td>
<td>98,545 ± 0,121 c</td>
<td>22,934 ± 0,435 e</td>
<td>121,412 ± 0,550 g</td>
</tr>
<tr>
<td></td>
<td>day 12</td>
<td>54,370 ± 0,189 h</td>
<td>22,523 ± 0,320 e</td>
<td>76,573 ± 0,418 i</td>
</tr>
<tr>
<td></td>
<td>day 1</td>
<td>110,804 ± 0,211 a</td>
<td>41,913 ± 0,760 a</td>
<td>152,620 ± 0,906 a</td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td>109,287 ± 0,176 a</td>
<td>40,208 ± 0,389 ab</td>
<td>149,401 ± 0,331 b</td>
</tr>
<tr>
<td></td>
<td>day 8</td>
<td>109,538 ± 3,473 a</td>
<td>33,891 ± 2,451 c</td>
<td>143,343 ± 1,326 c</td>
</tr>
<tr>
<td></td>
<td>day 12</td>
<td>61,067 ± 0,193 f</td>
<td>22,402 ± 0,451 e</td>
<td>83,417 ± 0,311 h</td>
</tr>
<tr>
<td>dill</td>
<td>day 1</td>
<td>94,398 ± 0,471 d</td>
<td>31,254 ± 0,446 d</td>
<td>125,575 ± 0,065 e</td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td>92,864 ± 0,209 d</td>
<td>30,684 ± 0,214 d</td>
<td>123,472 ± 0,314 f</td>
</tr>
<tr>
<td></td>
<td>day 8</td>
<td>104,871 ± 0,048 b</td>
<td>16,523 ± 0,607 f</td>
<td>121,416 ± 0,579 g</td>
</tr>
<tr>
<td></td>
<td>day 12</td>
<td>56,912 ± 0,316 i</td>
<td>21,175 ± 0,396 e</td>
<td>78,038 ± 0,476 i</td>
</tr>
</tbody>
</table>

Note: Different letters represent significant differences (Fisher (LSD), p < 0.5) among chlorophyll content values.

Chlorophylls content per fresh weight of samples along storage is presented in tab. 2.

In the first day of storage, parsley had a slightly different content of total chlorophylls content than those recorded in literature. Lisiewska and Kmiecik (1997) reported 203
mg/100g FW of total chlorophylls for fresh leaves of Hamburg parsley and 68.5mg/100g FW for the leafy type. Yamauchi, N. and A. E. Watada (1993) studied pigment changes in parsley leaves during storage. They determined a higher content of total chlorophylls: 223 mg/100g FW in the first storage day. But it must be stressed out that chlorophyll content is dependent on biological variability and differences among varieties (Ferruzzi and Schwartz 2001).

Lovage samples had lower total chlorophylls content than dill and parsley. The ratio between chlorophyll a and b content was 3.02.

During the storage period total chlorophylls content decreases for all samples as they start yellowing. This occurs because chlorophylls are extremely sensitive to physical and chemical changes encountered through food processing and storage (Ferruzzi and Schwartz 2001). This decrease leads to perceivable discoloration of vegetable tissue from green to olive brown and yellow. The color modifications are primarily a result of replacement of the centrally chelated magnesium atom by two atoms of hydrogen, producing metal-free pheophytin derivatives (Schwartz and Lorenzo,1990, cited by Ferruzzi and Schwartz 2001).

Yamauchi, N. and A. E. Watada (1993) studied chlorophyll changes in parsley leaves during storage and obtained a 40% decrease of total chlorophylls after 3 days of storage at 20°C and 64% after 5. In this study, total chlorophylls decreased at a much slower pace. For the three plants under study, this decrease was of 8% for parsley, 6% for dill an 3% for lovage after an 8 day storage period. The reduction in the 12th day of storage was of 42% for parsley, 45% for dill and 38% for lovage, similar to what Yamauchi, N. and A. E. Watada (1993) found after 3 days of storage at 20°C.

4. Ascorbic acid

Ajayi et al. (1980) studied the content of vitamin C and ascorbic acid of six species of leafy vegetables. They concluded that ascorbic acid constituted almost 100% of the vitamin C. Ascorbic acid content (fig. 2) was determined in day 1 and day 12 of storage. Ascorbic acid content was statistically distinct for the three herbs, both in the first and last day of storage.

Dill had the highest ascorbic acid content of 204.55±0.24 mg/100g FW, higher than 116 mg and 159-186 mg/100 g fresh matter reported for vitamin C by Lisiewska et al. (2003, 2006). Galoburda et al. (2012) reported 581 mg/100 g dried weight of vitamin C in fresh dill, a content three times smaller.

Parsley had 179.69±0.26 mg ascorbic acid /100g FW. Azeez (2008) reported for parsley a content of 110–200 mg vitamin C /100 g edible material. Farrel (1990) cited by Shylaja (2004) determined a content of vitamin C in parsley of 122 mg/100 g edible material. On
the other hand, Lisiewska et. al (2003) found a much greater content of vitamin C in fresh parsley leaves: 310 mg/100g for Hamburg type parsley and 257 mg for the leafy type.

Lovage samples had the lowest ascorbic acid content.

According to Agte et al. (2000) who studied 24 species of leafy vegetables, the content of vitamin C in this group of foods considerably exceeds the level of this vitamin in any other groups of crops. So, the content of vitamin C determined for the three aromatic plants can be estimated as high.

During the 12 day of storage, the content of ascorbic acid diminished by 18% for parsley samples, 8% for dill and 3% for lovage. Selman (1994) showed that the main causes for vitamins losses during handling and storage are their solubility in water, thermic degradation and enzymatic oxidation. Howard et al. (1999) confirmed that vitamin C in vegetables decreases linearly during refrigerated storage. Lisiewska et. al (2003) also observed a gradual decrease of vitamin C during the refrigerated storage of dill.

CONCLUSION

Minimally processed parsley, dill and lovage were analyzed during a 12 day storage period for the main quality parameters.

Total scores obtained in sensory analysis did not statistically differ along the storage period, for each plant taken into study. This suggests that the total quality of parsley, dill and lovage assessed by sensory analysis did not decrease in a twelve days storage period. So, from the consumer’s point of view, minimally processed parsley, dill and lovage could have a shelf life of up to 12 days.

The water content of each sample did not statistically differ along the storage period, as it was expected as the samples were sealed in impermeable polyethylene bags. However, statistically significant differences were found among the three aromatic herbs in each storage day, up to day 8 of storage. Dill had a higher water content than lovage in the first 8 days of storage. In the last day of storage, the water content was similar in all samples.

In the first day of storage, dill was found to have the greatest total chlorophylls content. During the storage period total chlorophylls content decreases for all samples as they start yellowing. For the three plants under study, this decrease of total chlorophylls was of 42% for parsley, 45% for dill and 38% for lovage, similar to what Yamauchi, N. and A. E. Watada (1993) found after 3 days of storage at 20°C.

According to Agte et al. (2000) who studied 24 species of leafy vegetables, the content of vitamin C determined for the three aromatic plants can be estimated as high. Ascorbic acid content was statistically distinct for the three herbs, both in the first and last day of storage. During the 12 day of storage, the content of ascorbic acid diminished by 18% for parsley samples, 8% for dill and 3% for lovage. Howard et al. (1999) and Lisiewska et. al (2003) confirmed that vitamin C in vegetables decreases linearly during refrigerated storage.

In conclusion, minimally processed parsley, dill and lovage sealed in polyethylene bags and stored at 4°C for 12 days have a shelf life of up to 12 days of storage, with little modifications of sensory parameters. In what total chlorophyll content in concerned, these samples are comparable to products after 3 days of storage at 20°C. Vitamin C decreases linearly during the storage, but similar to other vegetables stored at refrigerated temperatures.
ACKNOWLEDGEMENTS

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