The purpose of the present study was to investigate the efficiency of refrigeration and 1-methylcyclopropene (1-MCP) on the conservation of fresh-cut watercress. Before processing, the watercress was treated with 0, 500 or 1000 nL L⁻¹ of 1-MCP for 8 hours at 10°C. Detached leaves were packed in rigid polyethylene packages and stored at 5°C and 15°C for 7 days. The plant material was assessed daily for colour, respiration rate, visual yellowing and, every three days, for the soluble protein content. Only the storage temperature influenced the loss of the green colour. After 5 days of storage at 5°C the watercress showed 25% yellowing, while those stored at 15°C were already 100%. Respiration during the storage period was similar for all treatments with 1-MCP. The temperature at 5°C reduced the respiratory rate and protein degradation. 1-MCP had no significant effect on increasing the shelf life of fresh-cut watercress, while low temperature was the main factor increasing conservation. The fresh-cut watercress could be stored for 4 days at 5°C. Some possible reasons for the failure of 1-MCP in increasing the conservation of watercress leaves are discussed.
Fresh cut products are increasingly present on the fresh product market. In spite of the increasing demand, the offer of these products is hindered by their short storage life. Watercress is a highly perishable vegetable, showing pronounced chlorophyll loss during storage as compared to other vegetables such as parsley leaves and spinach (PHILOSOPH-HADAS et al., 1989). The senescence process in watercress leaves is quite rapid and occurs within a few hours after detachment (PHILOSOPH-HADAS et al., 1994). Changes that occur during the senescence of these products are induced and/or enhanced by the physical action of processing (WATADA, 1990). The mechanical injury caused by processing can induce several alterations in metabolic routes, with consequent changes in the whole metabolism. These changes include elevation of the respiration rate, production of secondary metabolites, weight loss and the induction of ethylene synthesis (ROLLE & CHISM, 1987; BRECHT, 1995).

Techniques such as cold storage and control of ethylene can be used to minimize the losses of these products. Temperature control is the most useful and important technique, minimizing the effects caused by processing, increasing the shelf life of fresh-cut products. In general, the optimum temperature for the conservation of minimally processed products ranges from 0°C to 5°C. However, higher temperatures are often encountered during handling and marketing which harm the conservation of fresh-cut products.

Ethylene has been shown to play an important role in the senescence of detached leaves (GEPSTEIN & THIMANN, 1981; PHILOSOPH-HADAS et al., 1994). Detached watercress leaves exhibit an increase in ethylene production in the first few hours after processing, reaching a peak five hours later (PHILOSOPH-HADAS et al., 1994). An alternative technique to avoid the effects of ethylene is the use of 1-MCP, an effective inhibitor of ethylene action in ornamental plants (Serek et al., 1989). The senescence process in watercress leaves is quite rapid and occurs within a few hours after detachment (PHILOSOPH-HADAS et al., 1994). Changes that occur during the senescence of these products are induced and/or enhanced by the physical action of processing (WATADA, 1990). The mechanical injury caused by processing can induce several alterations in metabolic routes, with consequent changes in the whole metabolism. These changes include elevation of the respiration rate, production of secondary metabolites, weight loss and the induction of ethylene synthesis (ROLLE & CHISM, 1987; BRECHT, 1995).

Techniques such as cold storage and control of ethylene can be used to minimize the losses of these products. Temperature control is the most useful and important technique, minimizing the effects caused by processing, increasing the shelf life of fresh-cut products. In general, the optimum temperature for the conservation of minimally processed products ranges from 0°C to 5°C. However, higher temperatures are often encountered during handling and marketing which harm the conservation of fresh-cut products.

Ethylene has been shown to play an important role in the senescence of detached leaves (GEPSTEIN & THIMANN, 1981; PHILOSOPH-HADAS et al., 1994). Detached watercress leaves exhibit an increase in ethylene production in the first few hours after processing, reaching a peak five hours later (PHILOSOPH-HADAS et al., 1994). An alternative technique to avoid the effects of ethylene is the use of 1-MCP, an effective inhibitor of ethylene action in ornamental plants (Serek et al., 1989 and 1995) and fruits (Golding et al., 1998, FAN et al., 1999, TIAN et al., 2000, FENG et al., 2000, IACOMINO et al., 2002). However, there are few reports on the effect of 1-MCP on vegetable leaves (ABLE et al., 2003, JIANG et al., 2002). Besides, the most studied vegetable crop is broccoli (FAN & MATTHEIS, 2000; Ku & Wills, 1999; POGSON & MORRIS, 1997), but there is evidence that 1-MCP treatment is not as effective for leafy tissues as it is for floral organs (ABLE et al., 2003). In addition, the effect of ethylene on excised tissue senescence is enhanced by the wounding effect (PHILOSOPH-HADAS et al., 1991). The efficiency of these inhibitors in retarding senescence of detached leaves should be reviewed.

This study was carried out to investigate the efficiency of cold storage and 1-MCP in increasing the conservation of minimally processed watercress.

### 1. INTRODUCTION

Watercress (Nasturtium officinale R. Br.) bunches were acquired from a local wholesale market and immediately transported to the postharvest laboratory. Only bunches without wounds and signs of yellowing were used. Before the processing, the bunches were treated with 0, 500, and 1000 nL L⁻¹ of 1-MCP during 8 hours at 10°C. Only undamaged and totally green leaves were separated from the bunches, being surface-sterilized in 100 µg L⁻¹ sodium hypochlorite for 4 minutes. All minimal processing was done at 10°C. The detached leaves (60g) were packed in rigid polyethylene packages and stored at 5°C and 15°C for 7 days. These temperatures were used because although 5°C is the recommended temperature for minimally processed products, it is common to find these products being commercialised at 15°C. The plant material was assessed daily for visual yellowing, colour, respiration rate and every three days, for the soluble protein content.

Visual yellowing was subjectively assessed using the following scale: 0%, up to 25%, 25-50%, 50-75% and 75-100% of yellowing. When the leaves showed 50% of yellowing, they were considered to have reached the end of their shelf life.

The colour of the leaves was directly evaluated at four points with a colorimeter (Minolta CR-300, Osaka, Japan). The colour was reported as the hue angle (H°) in which a value of 90° represents a totally yellow leaf and 180° a totally green leaf.

For the respiration rate determination, samples consisting of 50g detached leaves were enclosed in 1.8L chambers for 1 hour. The respiration rate was determined by measuring the CO₂ accumulated during one hour using a gas analyser (PBI-Dansensor CheckMate 9900 O₂/CO₂, Ringsted, Denmark).

The soluble protein content was measured in leaf samples (200-300mg) homogenized in 5ml of phosphate buffer, pH 6.8. Following centrifugation at room temperature for 5 minutes at 7000 rpm, the protein content in the supernatant was determined according to Bradford (1976).

The experiment was performed twice and had qualitatively and quantitatively similar results. The experimental design was completely randomised, with four replicates for colour and soluble protein analyses and ten replicates for the respiration rate determination. The data were analysed using the ANOVA procedure and the Tukey test (p<0.05) to compare the means.

### 2. MATERIAL AND METHODS

#### 2.1. INSTRUMENTS AND REAGENTS

The instruments and reagents used were: Minolta CR-300 colorimeter, 1.8L chambers, gas analyser (PBI-Dansensor CheckMate 9900 O₂/CO₂), Bradford (1976) method for protein determination, ANOVA procedure and Tukey test (p<0.05) for data analysis.

#### 2.2. PROCEDURE

The experiment was performed twice and had qualitatively and quantitatively similar results. The experimental design was completely randomised, with four replicates for colour and soluble protein analyses and ten replicates for the respiration rate determination. The data were analysed using the ANOVA procedure and the Tukey test (p<0.05) to compare the means.

No significant differences between untreated and 1-MCP treated leaves were observed for the leaf colour during storage at both temperatures (p<0.05) (Figure 1). The decrease in hue angle of treated and untreated watercress was more accentuated when it was stored at 15°C, showing that only the temperature was effective in delaying yellowing, mainly after 4 days of storage (p<0.05) (Figure 1).

#### 3. RESULTS AND DISCUSSION

No significant differences between untreated and 1-MCP treated leaves were observed for the leaf colour during storage at both temperatures (p<0.05) (Figure 1). The decrease in hue angle of treated and untreated watercress was more accentuated when it was stored at 15°C, showing that only the temperature was effective in delaying yellowing, mainly after 4 days of storage (p<0.05) (Figure 1).
For treatments with 0 and 500 nL. L⁻¹ of 1-MCP stored at 15°C, the hue angle decreased from 122º to 107º. Although there were no significant differences between the 1-MCP treatments [p<0.05], we observed that in leaves treated with 1000 nL. L⁻¹, the decrease was less accentuated at 15°C, from 121º to 112º. At 5°C, all the treatments caused a reduction in hue angle of, at the most, 4º (Figure 1).

![Figure 1](image)

**FIGURE 1.** Leaf color (H°) of fresh-cut watercress untreated (Control [a]) and treated with 1-MCP [500 nL. L⁻¹ (b); 1000 nL. L⁻¹ (c)] and stored at 5°C (open symbol) or 15°C (closed symbol). Data represent mean ± SE (n=4).

When evaluating the yellowing percentage, the temperature was again the main factor in the retarding of senescence (Table 1). After 3 days of storage at 5°C, all the treatments showed 100% of packages free from yellow leaves, different from the treatments stored at 15°C, which showed a high percentage of packages with 25-50% yellowing. On the fifth day, all the packages stored at 15°C were more than 25% yellow, whereas half of those maintained at 5°C presented no sign of yellowing (Table 1). It is reasonable to assume that the fresh-cut watercress could be stored for 4 days at 5°C.

Since yellowing is associated with chlorophyll degradation and increased xanthophyll content (TIAN et al., 1994; YAMAGUCHI & WATADA, 1991), low temperatures may be useful to delay these processes. In fact, low temperatures during storage can slow the metabolic processes, reduce deterioration, and can therefore minimize the effects of wounding, including chlorophyll loss. Contrarily, treating broccoli with exogenous ethylene promotes yellowing (BOROCHOV & WOODSON, 1989; TIAN et al., 1994).

According to ABLE et al. (2002), 1-MCP had no significant effect on increasing the shelf life or reducing yellowing of pak choy (Brassica rapa var. chinensis) leaves, regardless of the temperature. Temperature had a direct effect on yellowing, with higher temperatures shortening shelf life equally for treated and untreated leaves.

**TABLE 1.** Changes in the visual yellowing of fresh-cut watercress untreated and treated with 1-MCP and stored for 7 days at 5°C and 15°C.

<table>
<thead>
<tr>
<th>%1</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>7th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15°C</td>
<td>5°C</td>
<td>15°C</td>
<td>5°C</td>
<td>15°C</td>
</tr>
<tr>
<td>Untreated</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Up to 25</td>
<td>53</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25-50</td>
<td>37</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>50-75</td>
<td>10</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>75-100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>500 nL. L⁻¹</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>93</td>
<td>0</td>
</tr>
<tr>
<td>Up to 25</td>
<td>58</td>
<td>33</td>
<td>0</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>25-50</td>
<td>26</td>
<td>0</td>
<td>33</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>50-75</td>
<td>16</td>
<td>0</td>
<td>34</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>75-100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>1000 nL. L⁻¹</td>
<td>0</td>
<td>100</td>
<td>94</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>Up to 25</td>
<td>63</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>25-50</td>
<td>37</td>
<td>0</td>
<td>33</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>50-75</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>75-100</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>27</td>
<td>0</td>
</tr>
</tbody>
</table>

1 yellowing percentage

At the beginning of the storage period, the respiration rate was higher in watercress stored at 15°C [p<0.05] (Figure 2). The increase in respiration, as well as in other reactions associated with wounding, is a common feature when some products are stored at high temperatures (BRECHT, 1995). After processing, the respiration rate at 15°C increased in all treatments, reaching a peak on the second day of storage (Figure 2). JIANG et al. (2002) also observed a peak in the respiration of coriander (Coriandrum sativum L.) treated with...
1-MCP and stored at 20°C. From the second day of storage, the values of respiration rate at 15°C decreased until the fifth day, when the respiration increased again, reaching a peak on the sixth day (Figure 2). This peak at advanced stages of senescence is suggested to be intimately related to the progressive increase in ACC content (PHILOSOFP-HADAS et al., 1991).

It has been shown that respiration rates were significantly reduced by 1-MCP in banana, apple and broccoli (FAN et al., 1999, FAN & MATTHEIS, 2000; MACNISH et al., 2000). However, JIANG et al. (2002) observed no significant differences in respiration rate of coriander treated with 1-MCP until the fifth day of storage. From the sixth day, the coriander leaves treated with 1-MCP presented higher respiration rates, in accordance with the results for respiration between the second and fourth days at 5°C, presented herein.

The soluble protein content in the leaves declined during storage at 15°C (Figure 3). Although this decrease was more pronounced in untreated leaves, at the end of the storage period there was no significant difference between the treatments ($p<0.05$). Protein degradation was significantly reduced by temperature ($p<0.05$), with leaves stored at 5°C maintaining their protein content during storage, independent of 1-MCP treatment ($p<0.05$) (Figure 3).

![Figure 2](image2.png)

**FIGURE 2.** Respiration rate of fresh-cut watercress untreated (Control (a)) and treated with 1-MCP (500 nL. L$^{-1}$ (b); 1000 nL. L$^{-1}$ (c)) and stored at 5°C (open symbol) or 15°C (closed symbol). Data represent mean ± SE (n=4).

![Figure 3](image3.png)

**FIGURE 3.** Soluble protein of fresh-cut watercress untreated (Control (a)) and treated with 1-MCP (500 nL. L$^{-1}$ (b); 1000 nL. L$^{-1}$ (c)) and stored at 5°C (open symbol) or 15°C (closed symbol). Data represent mean ± SE (n=4).
Chlorophyll degradation was accompanied by a loss of protein content, a process also related to senescence. In addition, senescence processes are related to the accumulation of free amino acids and products of lipid catabolism, which are often used as reference parameters to follow the progress of senescence (PHILOSOPH-HADAS et al., 1989). In general, protein hydrolysis precedes chlorophyll degradation, resulting in yellowing before chlorophyll degradation becomes evident. In leaves with accelerated senescence, those two processes happen at the same time (PHILosoph-HADAS et al., 1991).

The senescence process was similar among the concentrations of 1-MCP studied (Figures 1, 2 & 3 and Table 1). These results might indicate that ethylene by itself does not play a fundamental role in the regulation of senescence in fresh-cut watercress, as attested by PHILOSOPH-HADAS et al. (1991). The stress caused by wounding has a pronounced effect on senescence, as reported for spinach leaves (PHILOSOPH-HADAS et al., 1991). 1-MCP appears to have no effect during the later stages of senescence, usually correlated with higher levels of ethylene production. Besides, according to HALL et al. (2000), new binding sites are synthesized and 1-MCP is not permanently attached or it binds to other receptors, decreasing the efficiency of the 1-MCP. SISLER et al. (1995) reported that 1-MCP might not be as active in leaves due to the possibility of post-translational receptor modification. In stress situations the competition with ethylene for binding sites may affect the efficiency of 1-MCP. The wounding effect seems to induce senescence not only through the increased ethylene production but also increasing tissue sensitivity to ethylene (KAO & YANG, 1983) and triggering other metabolic processes (THOMAS, 1986).

ABLE et al. (2002) suggest that endogenous wound ethylene binds to receptors during harvest in an extremely rapid process, so the binding sites were occupied before the 1-MCP treatment. Once engaged in autocatalytic ethylene production, the senescence process became partially independent of further ethylene action (GOLDING et al., 1998).

The efficiency of 1-MCP also depends on the plant organ tissue. A leafy tissue was expected to respond less to 1-MCP than a florot tissue, possibly due to different ages of the leaves and inherent differences between flowers and leaves (ABLE et al., 2002). Florot vegetables produce significant quantities of ethylene in comparison to leafy vegetables (POGSON & MORRIS, 1997), suggesting a greater role for ethylene in the senescence of floral parts.

1-MCP is able to reach and attach to the ethylene receptor sites when it is applied at high temperatures (SISLER & SEREK, 1997). Thus, the efficacy of 1-MCP has been reported to reduce when applied to banana and to some cut flowers at low temperatures. In these situations, 1-MCP binding to the receptor is incomplete, resulting in low efficacy. Application of 1-MCP at 2°C was not effective for penstemon, although application at 20°C resulted in complete protection from exogenous ethylene (SEREK et al., 1995). ABLE et al. (2002) and KU & WILLS (1999) also found that 20°C was best for broccoli treatment. Maybe the low temperature during 1-MCP application might be one of the reasons for the reduced effect of 1-MCP in fresh-cut watercress studied herein. JIANG et al. (2002) observed low sensibility to ethylene of coriander at low temperatures, which might be one of the reasons for the reduced effect of 1-MCP on cold stored leaves.

The application of 1-MCP had little effect on pak choy shelf life in the absence of exogenously applied ethylene (ABLE et al., 2002). According to these authors, 1-MCP may be useful during retailing with ethylene-producing commodities. In a study conducted by ABLE et al. (2003), the application of 1-MCP caused responses in only two of six studied species of leafy asian vegetables, when stored in the absence of ethylene.

4. CONCLUSION

Under the conditions studied, this study indicated that 1-MCP has no effect on the conservation of fresh-cut watercress leaves, while the low temperature was the main factor increasing conservation.

REFERENCES

Influence of Low Temperature Storage and 1-Methylcyclopropene on the Conservation of Fresh-Cut Watercress

Braz. J. Food Technol., v.8, n.2, p. 121-126, abr./jun. 2005


