Changes in Phenolic Compounds During The Development and Cold Storage of Artichoke (Cynara scolymus L.) Heads

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ABSTRACT

The phenolic components of both fresh and stored artichoke heads have been studied. In fresh marketable heads only traces of free apigenin and luteolin were identified, while in badly injured heads measurable amounts of the above flavonoids, as well as of free caffeic acid, were found to occur. In addition to the latter phenolics, vanillic-, syringic-, p-coumaric- and ferulic acids proved to be present in the alkali labile bound state although caffeic acid proved to be the main phenolic component. In addition, the latter phenolic acid increased considerably during storage of the healthy heads for 2 weeks at 20°C or for 1 month at 4°C, the increase at 20°C being the most pronounced. In injured heads (internal blackening) stored for 2 weeks at 20°C, a decrease in caffeic acid, as well as in most other phenolics, has been recorded, while in badly injured heads (external symptoms of injury visible), stored for the same period of time, less than half of the total amount of the caffeic acid present in fresh marketable heads was found. However, in injured heads stored for 1 month at 4°C, the decrease in caffeic acid proceeded less rapidly.

INTRODUCTION

Free and/or bound phenolic compounds are widely distributed in the vegetable kingdom and their amounts vary greatly with the plant part under consideration (Van Buren, 1970; Van Sumere et al., 1975; Harborne, 1980; Lattanzio, 1981, 1984). The metabolism of phenolics is further
compartamentalised and changes in their relative amounts can be expected to occur during the different stages of plant development (Van Buren, 1970; Lattanzio & Morone, 1979; Alibert & Boudet, 1980).

Phenolic compounds have furthermore been implicated in several important biological phenomena such as the germination of seeds and plant growth (Van Sumere et al., 1972; McClure, 1979; Towers & Abeysekera, 1984), the resistance of plants against phytopathogens (Van Sumere, et al., 1975; Swain, 1977; Friend, 1979; Beart et al., 1985), the activation or inhibition of enzymes (Van Sumere et al., 1975; McClure, 1979) and the quality of foods (including colour, flavour and taste) (Van Buren, 1970; Bate-Smith, 1973; Synge, 1975; Van Sumere et al., 1975; Pierpoint, 1983). In addition, phenolics play an important role in the browning response of plant tissues on cutting or injury (Van Sumere et al., 1975; Mayer & Harel, 1981), while their use as markers in chemotaxonomy is quite well known (Van Sumere et al., 1985).

Recently, the importance of phenylpropanoid metabolism in fruit and vegetable tissues under stress (mechanical damage and low temperature) has also been studied (Engelsma, 1970; Rhodes et al., 1981; Blakenship & Richardson, 1985; Siriphanic & Kader, 1985a,b). Such investigations are usually made in order to obtain useful information with regard to the prolongation and improvement of the post-harvest life of fruits and vegetables.

For the same reason artichoke heads, which are economically very important in Mediterranean areas, have also received attention with respect to their phenolic composition (Lattanzio, 1981; Lattanzio, 1984).

So far, in *Cynara scolymus* L., the following phenolic components have been identified: luteolin-7-rutinoside, luteolin-7-glucoside and luteolin-7-rutinoside-4'-glucoside, cyanidin-3-caffeoylglucoside, cyanidin-3-cafeoylsophoroside and cyanidin-3-dicaffeoylsohphoroside, 1-O, 3-O, 4-O and 5-O-cafeoylquinic acids, 1,3-O, 1,4-O, 1,5-O and 3,5-O-dicaffeoylquinic acids. Amongst the latter components chlorogenic acid was further found to be the main substance (Aubert & Foury, 1981; Lattanzio, 1981).

During this study the variation of the phenolic acid and flavone (aglycone) content of artichoke heads has been investigated under different conditions of development, injury and storage. Furthermore, a possible correlation between the phenolic content and the quality of the heads is discussed.

**MATERIALS AND METHODS**

**Plant material**

Artichoke heads (cv. Catanese) were harvested at different physiological stages and then analysed for their phenolic acid (free and alkali-labile
bound), apigenin and luteolin aglycone content. In addition, heads of marketable quality, i.e. immature flower buds (100–140 g fresh weight), were also harvested at different periods during the plant growing season. Some of the heads were immediately analysed for phenolic compounds (reference values), while others were stored in closed polyethylene bags at 20°C (2 weeks) and 4°C (1 month). In this way most of the artichokes could be kept in good condition. After either 2 weeks (at 20°C) or 1 month (at 4°C) the phenolic acid (free and alkali labile-bound) and flavone aglycone content was, in each case, analysed (see also Lattanzio, 1982).

**Extraction of the plant material and analysis of the free and alkali-labile bound phenolic acids and apigenin and luteolin aglycones**

For the qualitative and quantitative determination of the phenolics the plant material (20 g fresh weight) was first homogenised during 3 min with 100 ml hot MeOH–EtOH (1:1) and then refluxed for 2 h under N₂ (repeat five times) (Vande Casteele, et al., 1981). After centrifugation and pooling of the extracts, the combined solutions were first concentrated under vacuum to ±10 ml and then partitioned between n-BuOH and 6% Na₂CO₃. After removal of Et₂O-soluble substances, the aqueous layer was acidified to pH 3.5 and re-extracted with Et₂O giving fraction A (free phenolics). The acidic aqueous layer was then basified with concentrated NaOH until 2m and refluxed 2 h, acidified and then extracted with Et₂O to give fraction B (carbonate-soluble alkali-labile bound phenolics). The n-butanol layer from the above was refluxed in 2m NaOH for 2 h, the cooled hydrolysate extracted with n-BuOH followed by Et₂O and the aqueous layer then acidified as above and re-extracted with Et₂O to give fraction C (carbonate-insoluble, alkali-labile bound phenolics). Fraction D was obtained from the residue insoluble in MeOH–EtOH after basic hydrolysis, acidification, ether extraction, partitioning the ether solubles between Na₂CO₃ (pH 9) and Et₂O, acidifying the carbonate layer and re-extracting with Et₂O (alcohol-insoluble, alkali labile-bound phenolics). (Full details of this procedure can be found in the supplementary publication* to the paper by Vande Casteele et al., 1981). Finally, all Et₂O-extracts were concentrated and, after addition of MeOH to the concentrates, the latter were qualitatively and quantitatively analysed for phenolic acids by HPLC. For the qualitative and quantitative determination of flavonoid aglycones, the extracts were first hydrolysed under N₂ with 2m HCl. After neutralisation and concentration, MeOH extracts (for HPLC analyses) were prepared as described for the phenolic acids.

* In this supplementary paper (SUP 90049), which has been deposited at the National Lending Library at Boston Spa, Yorkshire LS23 7BQ, Great Britain, from which copies can be obtained, flow sheets are given for the detailed extraction of the phenolic acids.
HPLC

HPLC analysis was performed with a Perkin-Elmer Series 4 liquid chromatograph, which was equipped with a 3600 Data Station, a fluorimetric detector LS-3 and a spectrophotometric photodiode array 1040 (Hewlett Packard), coupled with a HP-85 computer and a HP-9121 disc drive. The 'pilot signal' to the spectrophotometric detector was set at 325 nm and the fluorimetric detector at 265 nm (excitation) and 345 nm (emission).

An analytical Merck (Darmstadt, GFR) column (250 mm × 4 mm) prepacked with LiChrosorb RP-18 (7 µ) was used throughout this work. The employed solvent system consisted of A: MeOH and B: acetic acid-water (5/95; v/v). Two different elution profiles were used:

(i) A linear gradient profile: 0–25 min 15–40% A in B; 25–30 min 40% A in B (isocratic); 30–45 min 40–63% A in B; 45–47 min 63% A in B (isocratic); 47–51 min 63–99% A in B.

(ii) A concave gradient profile: starting with 10% A; 0–25 min 10–40% A in B (curve 3); 25–40 min 40–99% A in B (curve 3).

The flow rate was always 1 ml/min and at the start the column pressure was 15 MPa. Samples of 6 µl were applied to the column by means of a 6 µl loop valve.

Identification of the compounds

The retention times (t_R min) of the unknowns were compared with the t_R's of known standards. Furthermore, the UV spectra obtained with the photodiode array detector, as well as the excitation and emission maxima (fluorimetric detection) of both the test substance and the unknown were compared. In addition, the unknown peaks were also collected and further analysed by TLC.

TLC analysis

The collected HPLC samples were concentrated under vacuum and then further analysed by TLC on silica gel thin layers (Merck; Darmstadt, GFR). For this purpose the following solvents: toluene–HCO_2Et–HCOOH (TEF, 5/4/1) and CHCl_3–HOAc–H_2O (CAW, 4/1/1) were used (Van Sumere et al., 1972; Vande Casteele et al., 1981b). For qualitative identification the plates were first viewed under UV both before and after treatment with 2M NaOH. Thereafter, the chromatograms were treated with diazotised p-nitroaniline and the colours were compared with those of suitable test substances (Van Sumere et al., 1972).
Quantitative HPLC determinations of identified compounds

For this purpose the HPLC-equipment was first calibrated with known amounts of each of the substances under discussion.

Correction of the results obtained

Due to the fact that certain phenolic compounds are unstable in basic (e.g. caffeic acid and flavonoids) or acid (e.g. ferulic acid and flavonoids) solution, the results of the extracts obtained after hydrolysis must be corrected (Van Sumere et al., 1972; Vande Casteele et al., 1981a,b).

The overall correction factors, which have been employed for this purpose, embrace corrections for losses during the extraction, the sodium carbonate treatment (and, where required, also hydrolysis with either 2M NaOH or 2M HCl), etc.

Throughout this work the following correction factors have been employed: caffeic acid = 2.22; ferulic acid = 1.03; p-coumaric acid = 1.02; vanillic acid = 1.02; syringic acid = 1.02; luteolin = 4.58 and apigenin = 4.50.

RESULTS AND DISCUSSION

The flavone aglycones, apigenin and luteolin, as well as a series of phenolic acids—vanillic, syringic, p-coumaric, caffeic and ferulic acid—were identified in artichoke heads. Usually, and as shown in Table 1, the phenolics occur in fresh healthy heads in the bound state both in fraction B and fraction D. As can be seen from the latter Table, fraction B contains, next to the phenolic acids, of which caffeic acid is by far the most abundant component (± 4% of the dry weight), also apigenin and luteolin aglycones. Caffeic acid occurs further in artichoke heads to a large extent as the phenolic 'moiety' of chlorogenic (III) and neochlorogenic acid (I) (Fig. 1) (Lattanzio, 1981). With regard to the presence of other caffeoylquinic acid derivatives, it is so far rather difficult to state whether they really do occur in artichokes or whether they arise from isomerisation reactions during the extraction procedure (see, also, Scarpati & Esposito, 1964). Indeed, when the extraction procedure (see 'Methods') was applied to pure chlorogenic acid (III) or 1,5-O-dicafeoylquinic acid (cynarin), two different series of isomers were found. From pure chlorogenic acid, caffeic acid, the original compound (III) and two of its isomers: neochlorogenic (I) and cryptochlorogenic acid (II) (Fig. 1), were obtained* while, from cynarin, only 5% was isomerised to mainly 1,4-O- and 3,5-O-dicafeoylquinic acid.

* The three quinic acid esters being present in about equivalent amounts, 97% of which were found in fraction B.
### TABLE 1

Amounts (mg/100 g dry weight) of Phenolic Compounds during Development of Artichoke Heads

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>32 g fresh weight</th>
<th>69 g fresh weight</th>
<th>133 g fresh weight</th>
<th>156 g fresh weight</th>
<th>205 g fresh weight</th>
<th>419 g fresh weight</th>
<th>506 g fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15·49% dry weight</td>
<td>13·79% dry weight</td>
<td>10·93% dry weight</td>
<td>17·49% dry weight</td>
<td>18·34% dry weight</td>
<td>23·18% dry weight</td>
<td>34·05% dry weight</td>
</tr>
<tr>
<td>Fraction B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>0·9</td>
<td>0·4</td>
<td>2·9</td>
<td>0·2</td>
<td>1·3</td>
<td>1·0</td>
<td>2·5</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>7·1</td>
<td>3·2</td>
<td>11·0</td>
<td>2·3</td>
<td>4·9</td>
<td>4·2</td>
<td>9·1</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>+</td>
<td>3·9</td>
<td>3·5</td>
<td>3·3</td>
<td>7·2</td>
<td>4·8</td>
<td>2·1</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>4926·2</td>
<td>3685·2</td>
<td>2639·6</td>
<td>1964·7</td>
<td>1187·7</td>
<td>779·2</td>
<td>366·3</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>3·2</td>
<td>5·1</td>
<td>9·3</td>
<td>0·8</td>
<td>+</td>
<td>0·7</td>
<td>1·8</td>
</tr>
<tr>
<td>Apigenin</td>
<td>68·4</td>
<td>44·1</td>
<td>9·0</td>
<td>35·6</td>
<td>48·2</td>
<td>21·2</td>
<td>39·2</td>
</tr>
<tr>
<td>Luteolin</td>
<td>114·1</td>
<td>76·0</td>
<td>14·2</td>
<td>18·8</td>
<td>16·9</td>
<td>23·4</td>
<td>38·0</td>
</tr>
<tr>
<td>Fraction D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>0·6</td>
<td>0·2</td>
<td>1·2</td>
<td>0·1</td>
<td>0·3</td>
<td>0·3</td>
<td>1·3</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0·7</td>
<td>0·3</td>
<td>1·9</td>
<td>0·2</td>
<td>0·6</td>
<td>0·5</td>
<td>2·5</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>1·8</td>
<td>6·1</td>
<td>6·3</td>
<td>2·9</td>
<td>5·3</td>
<td>4·1</td>
<td>2·6</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>572·8</td>
<td>606·1</td>
<td>548·3</td>
<td>412·9</td>
<td>124·3</td>
<td>95·5</td>
<td>84·4</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1·1</td>
<td>2·1</td>
</tr>
</tbody>
</table>

Fraction B: Alcohol- and carbonate-soluble but ethylether-insoluble alkali-labile bound phenolics.
Fraction D: Alcohol-insoluble alkali-labile bound phenolics.
The isomers formed occurred mainly in fraction A, while the residual 1,5-
O-dicafeooylquinic acid was recovered from fraction B. For these reasons
no attempts were made to quantise the quinic acid derivatives as such. How
far other esters† of fraction B could, at least in part, also be related to the
natural intermediates of lignin biosynthesis discussed by Neish (Neish,
1968) requires additional investigations. From Table 1 it follows further
that the residue (fraction D), which is free from flavonoids and which also
contains less phenolic acids than the alcohol-soluble fraction B, possesses

† In the case of acid hydrolysis the amounts of substituted cinnamics liberated are strongly
diminshed owing to their decomposition under acid conditions (Van Sumere et al., 1972;
Van Brussel et al., 1978; Newby et al., 1980). It is further reasonable to suppose that the
phenolics occur mainly in ester form, although some hydrolysis of amide linkage could also
have occurred (Newby et al., 1980; K. Vande Casteele and C. F. Van Sumere, unpublished
results). In addition, the cinnamics are normally present in the plant world in the trans form
(Newby et al., 1980; C. F. Van Sumere, unpublished work).
### TABLE 2

Changes in the Levels (mg/100 g dry weight) of Phenolic Compounds in Artichoke Heads, stored at Room Temperatures (20°C) and at 4°C

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Fresh marketable head</th>
<th>Stored heads</th>
<th>Healthy marketable quality</th>
<th>Internal blackening</th>
<th>External visible symptoms of injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stored 1 weeks at 20°C</td>
<td>Stored 2 weeks at 20°C</td>
<td>Stored 1 month at 4°C</td>
<td>Stored 2 weeks at 20°C</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>–</td>
<td>0·5</td>
<td>0·5</td>
<td>0·8</td>
<td>1·4</td>
</tr>
<tr>
<td>Apigenin</td>
<td>+</td>
<td>2·8</td>
<td>2·9</td>
<td>1·4</td>
<td>3·3</td>
</tr>
<tr>
<td>Luteolin</td>
<td>+</td>
<td>5·3</td>
<td>14·9</td>
<td>3·6</td>
<td>5·9</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td></td>
<td>5·1</td>
<td>18·3</td>
<td>4·2</td>
<td>3·9</td>
</tr>
<tr>
<td>Syringic acid</td>
<td></td>
<td>31·0</td>
<td>44·6</td>
<td>10·4</td>
<td>13·1</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td></td>
<td>36·2</td>
<td>78·3</td>
<td>12·4</td>
<td>17·9</td>
</tr>
</tbody>
</table>

**Fraction D**

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Fresh marketable head</th>
<th>Stored heads</th>
<th>Healthy marketable quality</th>
<th>Internal blackening</th>
<th>External visible symptoms of injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanillic acid</td>
<td></td>
<td>563</td>
<td>1410</td>
<td>429</td>
<td>276</td>
</tr>
</tbody>
</table>

Fraction A: Free phenolics.
Fraction B: Alcohol- and carbonate-soluble but ethylether-insoluble alkali-labile bound phenolics.
Fraction D: Alcohol-insoluble alkali-labile bound phenolics.
The data in the Table represent the pondered average over six heads.
changes in phenolics during development and storage of artichoke

Caffeic acid 'moieties' in a relatively high proportion (±0.55% of the dry weight).

The alcohol-insoluble phenolic esters of the residue may, on the one hand, and again at least in part, be related to certain of the esters described by Neish (1968) and Majak & Towers (1973), and on the other hand they could also be akin to the ester-bound phenolics which have been found to be linked to cell wall materials and which eventually can be released by alkaline extraction and/or hydrolysis (Rhodes, 1985). In any case, the relatively high amount of alkali-labile caffeic acid in fraction D is rather remarkable. From Table 1 it can further be seen that the quantity of the phenolic compounds present in the artichoke heads varies considerably with the stage of development. In this respect reference must first be made to the alcohol-soluble bound caffeic acid (fraction B), which decreases rapidly in the first stages of development and then reaches a relatively low level in the preflowering buds.

With regard to the evolution of the caffeic acid 'moiety' of the residue (fraction D), a slower decrease in 3,4-dihydroxycinnamic acid was at first noticed but when the preflowering stage was reached the alkali-labile content of the latter acid decreased rapidly. Indeed, in preflowering heads the caffeic acid 'moiety' content was only ±15% of that of heads of marketable quality.

Moreover, the decrease in alkali-labile caffeic acid runs parallel with a more extensive lignification of the outer bracts. It is therefore quite possible that the latter content may, in part, be related to the above-mentioned 'Neish intermediates' of lignin biosynthesis and that a decrease in the 3,4-dihydroxycinnamic acid content of the artichoke heads may be a measure for the progress of their lignification process. In addition, compounds like chlorogenic and caffeic acids can be excellent substrates for the classical polyphenoloxidase (Vande Casteele et al., 1981a) and the latter enzyme may further, to a large extent, explain the brown-blackening response, which can be observed in preharvested heads obtained from lots with a too high field temperature (V. Lattanzio, unpublished results). Indeed, plants respond to various types of injuries and stresses in a generalised metabolic way and, as stated by Rhodes & Wooltorton (1977), these reactions can include a rise in respiration rate, the evolution of ethylene as well as an important change in the phenylpropanoid metabolism, which, in turn, may involve synthesis, oxidation and polymerisation of phenolic compounds (Rhodes & Wooltorton, 1978; Rhodes et al., 1981).

Table 2 shows the changes of the phenolics during the storage of fresh marketable heads at either 20°C or 4°C. In this respect especially the important increase in caffeic acid content should be noticed. Here again references can again be made to Rhodes (1985) (see also above).
Furthermore, when internal blackening appeared on the inner bracts, the level of the phenolics decreased and this decrease was greater in heads which showed external visible symptoms of injury. In the latter samples caffeic acid, apigenin and luteolin were also found in the free state, presumably owing to hydrolytic phenomena. In addition, in the insoluble residue a higher p-coumaric acid content, coupled with a lack of firmness, was observed (see, also, Shibuya, 1984; Towers & Abeysekera, 1984).

Low temperature effects on phenolic metabolism and/or biosynthesis have further been dealt with in a vast series of papers (Rhodes & Wooltorton, 1977; Rhodes et al., 1981; Graham & Patterson, 1982; Blankenship & Richardson, 1985; Siriphanic & Kader, 1985a). During their studies with potato tubers (containing relatively higher amounts of caffeoyl quinic acids), Rhodes et al. (1981) showed that the phenolic metabolism was enhanced under chill stress and that the behaviour of the same metabolism was further dependent on the storage temperature (Rhodes & Wooltorton, 1978). It is also interesting that certain enzymes, which are important for phenylpropanoid metabolism (e.g. hydroxycinnamoyl CoA: quinate hydroxycinnamoyl transferase (CQT) and phenylalanine ammonia lyase (PAL) can be stimulated at low temperatures.

Moreover, the increase in enzyme activity seems to be a feature of the defence system of many plants and leads to the formation of flavonoids and polymeric phenolics (Rhodes, 1985). The polymeric phenolics may, in turn and according to the latter author, be the so-called 'wound lignin', although doubts on the real nature of this special type of lignin remain. It is possible that the production of polymeric phenolics may, on the one hand, play a role in the defence mechanism of the plant against infections (Van Sumere et al., 1975) and, on the other, may be responsible for a decrease in esterified caffeic acid 'moieties'. This hypothesis would further be in agreement with the decreasing amounts of alkali-labile caffeic acid 'moieties' in heads showing either internal blackening or, even worse, external visible symptoms of injury. Moreover, other enzymes such as cinnamic acid 4-hydroxylase (CAH) and polyphenoloxidase (PPO) could also be activated (Blankenship & Richardson, 1985; Siriphanic & Kader, 1985a,b) and an increase in activity of such enzymes would most likely, in combination with temperature-dependent phase changes in the cellular membranes (postulated as the primary response to chilling temperatures (Lyons, 1973; Rhodes et al., 1981), contribute enormously to the enzymatic browning process. Nevertheless, phenolic browning can also be caused by metal–polyphenol complexing and in this connection iron seems to be the most common metal involved (Mathew & Parpia, 1971). According to Vande Casteele et al. (1981), the brown-black colouring of the cut ends of Medinilla magnifica, an hydrolysable tannin-containing ornamental, is due
to two reactions: (a) an oxidation of hydrolysable tannins mediated by the ferrous ions of the Murashige and Skoog medium used, (b) an oxidation of hydrolysable tannins by means of the hydrolysable tannin polyphenol-oxidase present in the tissues. It is further known that iron chelators like citric acid and ascorbic acid (Mathew & Parpia, 1971; Vande Casteele et al., 1981; Subba Rao & Narasinga Rao, 1985) can affect plant tissue coloration (see also Hughes & Swain, 1962). This is also relevant to artichoke heads which contain high amounts of diphenols (chlorogenic acid, etc.) and iron (Lattanzio, 1981; Magnifico & Lattanzio, 1981). Indeed, in heads treated with 1% citric acid a delayed browning reaction has been noticed. This result is also in good agreement with the findings of Van Sumere et al. (1981), who noticed that the brown coloration of the mixture of Murashige and Skoog medium and 0.17% hydrolysable tannin could be strongly retarded by supplementing ferrous ions in the medium by ferric ions and/or using a combination of EDTA (final concentration, $4 \times 10^{-4}$ M) and citric acid (final concentration, $2 \times 10^{-4}$ M). Finally, it is quite obvious that an inhibition of the browning reaction will greatly improve the shelf life of stored artichoke heads.

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