Food Chemistry 120 (2010) 591–598

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Application of thermal inactivation of enzymes during vitamin C analysis to study the influence of acidification, crushing and blanching on vitamin C stability in Broccoli (*Brassica oleracea* L *var. italica*)

Ann Wambui Munyaka, Indrawati Oey, Ann Van Loey, Marc Hendrickx *

Laboratory of Food Technology, Leuven Food Science and Nutrition Research Centre (LFoRCe), Department of Microbial and Molecular Systems (M2S), Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, 3001 Leuven, Belgium

ARTICLE INFO

Article history:
Received 1 October 2008
Received in revised form 30 July 2009
Accepted 13 October 2009

Keywords:
L-ascorbic acid
Dehydroascorbic acid
Broccoli
Blanching
Acidification
Crushing
Enzymes

ABSTRACT

The effectiveness of heat inactivation of oxidative enzymes e.g., ascorbic acid oxidase (AAO) to stabilise vitamin C during extraction and analysis was evaluated. The influence of different sequences of performing treatments including acidification (pH 4.3 vs. pH 6.5), crushing, high temperature short time (90 °C/4 min–HTST) and low temperature long time (60 °C/40 min–LTLT)) blanching on vitamin C stability in broccoli florets and stalks was also investigated. Heat inactivation of enzymes prior to matrix disruption resulted in higher vitamin C values mainly in L-ascorbic acid (L-AA) form, while lack of enzyme inactivation resulted in high vitamin C losses resulting from conversion of L-AA to dehydroascorbic acid. Various treatments and their sequence of application influenced vitamin C stability as follows: (i) crushing prior to blanching reduced vitamin C stability and (ii) in the absence of heating, acidification increased vitamin C stability (iii) blanching prior to crushing resulted in higher vitamin C retention, with HTST blanching retaining more vitamin C than LTLT blanching.

1. Introduction

Vitamin C is one of the important vitamins for human health particularly popular for its role in prevention of scurvy. In addition, vitamin C is important for the enhancement of the immune system, absorption of iron, prevention of some cancers and cardiovascular disease through its free radical-scavenging activities (Davey et al., 2000; Naidu, 2003). Due to lack of the enzyme gluconolactone oxidase, humans cannot synthesise vitamin C and must entirely rely on dietary sources. Fruits and vegetables are the main contributors of vitamin C in the diet, with the percent contribution amounting to as high as 90% (Lee & Kader, 2000). In fruits and vegetables, vitamin C mainly occurs in the form of L-ascorbic acid (L-AA). However, dehydroascorbic acid (DHAA), the oxidised form of L-AA could occur but in small quantities (Wills, Wimalasiri, & Greenfield, 1984). Both L-AA and DHAA exhibit vitamin C activity.

L-AA is a very labile molecule that could be easily degraded through chemical and enzymatic oxidation during processing (Lee & Kader, 2000). The extent of vitamin C loss during processing is influenced by temperature, presence of oxygen, pH, and metal ions such as Cu^{2+}, Fe^{2+} and Ag^{+} (Davey et al., 2000; Lee & Kader, 2000). In addition, matrix disruption during processing can bring into contact L-AA and enzymes such as ascorbic acid oxidase (AAO, E.C. 1.10.3.30) and ascorbic acid peroxidases (APx, EC 1.11.1.11) hence facilitating oxidation of L-AA (Nishikawa et al., 2003). AAO catalyses the oxidation of L-AA in the presence of molecular oxygen resulting in DHAA and water, while APx catalyses the reduction of hydrogen peroxide (H_2O_2) by L-AA, leading to the production of water and DHAA. It has been demonstrated that the contribution of DHAA to the total vitamin C content in fresh fruits and vegetables is less than 10%. However, DHAA levels could increase during processing and/or storage (Davey et al., 2000; Wills et al., 1984). During processing it is important to minimise the oxidation of L-AA to DHAA since the latter is highly unstable and can be rapidly and irreversibly hydrolysed to 2,3-diketogulonic acid, a compound devoid of vitamin C activity (Deutsch & Santhosh-Kumar, 1996; Nishikawa, Toyoshima, & Kurata, 2001).

Since most raw vegetables are unpalatable and can undergo progressive quality changes associated with enzymatic reactions, vegetables have to be processed to increase their shelf life and improve their eating quality. During processing, vegetables are often subjected to thermal (blanching) and mechanical (peeling, cutting, mixing, homogenisation, coring, and juicing amongst others) treatments. In addition, vegetables with a high natural pH are often acidified to prevent microbial growth and spore germination.

* Corresponding author. Address: Laboratory of Food Technology, Centre for Food and Microbial Technology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 23, B-3001 Leuven (Heverlee), Belgium. Tel.: +32 16321585; fax: +32 16321960.
E-mail address: marc.hendrickx@biw.kuleuven.be (M. Hendrickx).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.10.029

These treatments and the sequences of performing them could influence the stability of vitamin C during the treatments themselves and/or during subsequent processing, due to occurrence of chemical and enzymatic oxidation reactions.

Therefore, the current study was carried with two main objectives in mind: (i) to study the effect of a heating step prior to vegetable matrix disruption and during extraction on vitamin C quantification and (ii) to investigate the influence of different treatments i.e. mixing, blanching and acidification, and different sequences of executing these treatments on the stability of vitamin C in vegetables. Broccoli was chosen as a model vegetable because it is a rich dietary source of vitamin C.

2. Materials and methods

2.1. Broccoli sample preparation

One batch of broccoli was purchased from a local supplier. Before the treatments, the broccoli was stored at 4 °C for a period not longer than 5 days. Both the florets and the stalks were used. The broccoli heads were obtained by cutting the main stalk at the point slightly below the emergence of the smaller stalks. The florets, together with about 1 cm of stalk, were cut off from the rest of the stalk and these were used as broccoli floret samples. The rest of the stalk was cut into small pieces of about 1 cm and used as broccoli stalk samples. The bottom main stalk was discarded. The sampling was done randomly.

2.2. Study on the influence of treatments on vitamin C stability

2.2.1. Treatments

2.2.1.1. Crushing (with or without acidification) – blanching treatment sequence. In this sequence of treatments, broccoli florets and stalks were first crushed at different pH i.e. the natural pH of broccoli around 6.5 and acidic pH of 4.3 and then blanched. To obtain pH 6.5 and 4.3, 100 g of broccoli florets or stalks were crushed (Buchi mixer, B-400, Switzerland) in the presence of distilled water or acetate buffer (0.2 M, pH 3.7) respectively, at a ratio of 1:1 (w/v) for a standardised time of 20 s. A 30-min period (which included the time for vacuum-packing) after crushing and before blanching was standardised. The crushed samples were vacuum-packed (Multivac A300/16, Wolfertschwenden, Germany) in plastic bags (50 g per bag) and blanched in a water bath (Memmert, WBU 45, Germany). High temperature short time (HTST) and low temperature long time (LTLT) blanching was conducted at 90 °C for 4 min and at 60 °C for 40 min, respectively. After blanching, samples were immediately cooled in ice water for 5 min, frozen in liquid nitrogen and stored at −80 °C until vitamin C extraction. Samples from this treatment sequence were later referred to as crushed (pH 6.5)-unblanched, crushed (pH 4.3)-unblanched, crushed (pH 6.5)-HTST, crushed (pH 6.5)-LTLT, crushed (pH 4.3)-HTST and crushed (pH 4.3)-LTLT.

2.2.1.2. Blanching-crushing (with or without acidification) treatment sequence. Intact broccoli florets and stalks were vacuum-packed (Multivac A300/16, Wolfertschwenden, Germany) in plastic bags (50 g per bag) and HTST- and LTLT-blanched in a water bath (Memmert, WBU 45, Germany). After immediate cooling in ice water for 5 min, the blanched broccoli samples were crushed (Buchi mixer, B-400, Switzerland) in the presence of distilled water or acetate buffer (0.2 M, pH 3.7), respectively, at a ratio of 1:1 (w/v) for 20 s and vacuum-packed. A 15-min period after crushing (which included the time for vacuum-packing) was standardised. The samples were then frozen in liquid nitrogen and stored at −80 °C until vitamin C extraction. Samples from this treatment sequence were later referred to as HTST-crushed (pH 6.5), HTST-crushed (pH 4.3), LTLT-crushed (pH 6.5) and LTLT-crushed (pH 4.3).

2.3. Study on the influence of enzyme inactivation on vitamin C quantification

To investigate the influence of enzyme activity on vitamin C quantification, some intact broccoli florets and stalks samples prepared as described in the sample preparation section were vacuum-packed (Multivac A300/16, Wolfertschwenden, Germany) in plastic bags (50 per bag). One portion of the vacuum-packed samples was subjected to a heat shock treatment by immersion in boiling water for 10 min in order to inactivate all enzymes (including vitamin C degrading enzymes). After the heat shock treatment, the samples were immediately cooled in ice water. A portion of the heat shocked samples was used to evaluate whether AAO was completely inactivated. The remaining heat shocked sample was frozen in liquid nitrogen and ground (into a frozen powder). The frozen powder was packed into plastic containers and stored at −80 °C until vitamin C analysis. Other vacuum-packed samples that did not receive a heat shock treatment were directly frozen in liquid nitrogen and ground (Grindomix GM 200, Retsch, Germany) into a frozen powder which was packed in plastic bottles and stored at −80 °C until vitamin C analysis.

To evaluate the influence of residual enzyme activity in the treated (crushed, acidified and blanched) broccoli samples on vitamin C quantification, some treated samples were subjected to a heat shock treatment in boiling water for 10 min immediately after the treatments. Other treated samples were not subjected to the heat shock. Afterwards, all samples ware frozen in liquid nitrogen and stored at −80 °C until vitamin C extraction.

2.3.1. Determination of AAO activity in heat shocked broccoli

AAO was extracted from heat shocked broccoli according to a modified method based on Oberbacher and Vines (1963). Five gram broccoli was homogenised together with 10 ml extraction buffer (phosphate buffer (0.1 M, pH 5.6, containing 0.5% Triton X-100 and 1 mM EDTA) using a kitchen blender (J26, Amsterdam, Holland) for 30 s. The homogenate was transferred to Eppendorf tubes and centrifuged (Eppendorf 5417R, Germany) at 17,900*g* and 4 °C for 30 min. The supernatant was filtered using 0.45 μm cellulose filter (Duren, Germany) and stored in ice water prior to AAO activity measurements (within 30 min after extraction).

AAO activity was measured using a spectrophotometer (Ultrospec 2100 Pro UV–Vis, Sweden) at 25 °C and 265 nm. To determine the enzyme activity, 3.0 ml phosphate buffer (0.1 M pH 5.6, 1 mM EDTA), 0.1 ml sample extract and 0.1 ml substrate solution (5 mM L-AA dissolved in double distilled water) were added in a 10 mm cuvette in that order. The blank consisted of 3.1 ml phosphate buffer (0.1 M pH 5.6, 1 mM EDTA) and 0.1 ml extraction buffer. The decrease in absorbance at 265 nm and 25 °C was followed for 3 min. One unit of enzyme was defined as the amount of enzyme catalysing the oxidation of 1 micromole of ascorbic acid per minute at 25 °C.

2.3.2. Vitamin C extraction

Vitamin C was extracted from broccoli using the method described by Galgano, Favati, Caruso, Pietrafesa, and Natella (2007) in accordance to the following three categories:

2.3.2.1. Samples heat shocked prior to storage. Ten grams of frozen broccoli powder from untreated but heat shocked samples was homogenised together with 50 ml cold extraction solution (20 mM NaH_2PO_4, pH 2.1, 1 mM EDTA) using an ultra high speed homogenisation mixer (Ultra Turrax) for 1 min. To extract vitamin C from treated and heat shocked samples, 20 g sample was homog-