

ORIGINAL ARTICLE**Effect of Hot-Water Blanching on *Colocasia esculenta* and *Corchorus olitorius* Leaves**Jerry Ampofo-Asiama^{*a} / Francis Amoako-Andoh^b / Bernard Abakah^a / Angela Abla Zebede^a / Bright Quay^a / Rosemond G. Dadzie^c**Authors' Affiliation**^aDepartment of Biochemistry, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana^bCouncil for Scientific and Industrial Research - Crops Research Institute, Fumesua, Ghana^cDepartment of Agricultural Engineering, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana**Corresponding author**

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None

Abstract

The leaves of *Colocasia esculenta* and *Corchorus olitorius* deteriorate quickly after harvest due to the loss of green color caused mainly by the activity of enzymes. Blanching, can be used to inactivate these enzymes to help slow down the loss of green color, although this may affect the nutritional quality of the leaves. In this study, the effect of hot-water blanching on the stability of chlorophyll, ascorbate, and phenols in the leaves of *C. esculenta* and *C. olitorius* was investigated. Blanching was carried out in hot-water at temperatures of 71, 82 and 93 °C for durations of 1, 2, 3 and 5 min. The efficiency of blanching was assessed based on the inactivation of peroxidase. Blanching inactivated peroxidase with more than 90 % activity lost after 3 min of blanching *C. esculenta* leaves at the different temperatures. However, a 90 % inactivation of peroxidase activity in *C. olitorius* leaves was observed after 5 min of blanching. The total phenolic content of both leaves was not affected by blanching, however the retention of ascorbate decreased and the loss of chlorophyll increased with increasing duration of blanching. After 5 min of blanching, about 38, 34 and 15 % of ascorbate levels were retained at 71, 82 and 93 °C respectively, in *C. esculenta* leaves. With the exception of blanching at 82 °C, similar ascorbate levels were retained in the leaves of *C. olitorius*. Less than 15 % of chlorophyll was lost when *C. esculenta* leaves were blanched at 71 °C for 2 min or less. Similar loss in chlorophyll was observed when *C. olitorius* leaves were blanched for 2 min or less.

Practical application

The leaves of *C. esculenta* and *C. olitorius* deteriorate quickly after harvest due to degreening caused mostly by action of enzymes. Blanching can therefore be used to inactivate enzymes with the aim to enhance the quality of the leaves during storage.

Keywords: Ascorbate, Chlorophyll, Hot-water blanching, Peroxidase activity**1. Introduction**

Many people in sub-Saharan Africa consume the leaves of *Colocasia esculenta* and *Corchorus olitorius*. These green leafy vegetables serve as a main component of several dishes and are rich in fibre, minerals such as magnesium and iron, and serve as a good source of provitamin A carotenoids (Lewu *et al.*, 2009). In spite of their vast usage, the consumption of these vegetables

is limited to within a few days after harvest (Oktavianingsih *et al.*, 2017) owing to the short storage life of the leaves. This is mainly linked to the degreening of the leaves by enzymes after harvest. Indeed, the intensity of greenness plays an important role in determining the quality and economic value of these vegetables. The inactivation of the relevant enzymes can

therefore, help enhance the storage life and quality of these vegetables.

Thermal processing methods such as blanching can be used to inactivate enzymes and help stabilize the quality of these vegetables prior to their usage (Halpin & Lee; Hong-wei *et al.*, 2017). Blanching involves exposing vegetables to high temperatures for a short period (Rawson *et al.*, 2011). This process can help sustain the color, flavor and overall quality of these vegetables. Additionally, blanching can help minimize non-enzymatic browning reactions and also destroy contaminating microorganisms (Hong-wei *et al.*, 2017). Blanching, however, can cause a decrease in quality. Especially, heat-labile nutrients such as ascorbate can easily be degraded due to blanching (Xanthakis *et al.*, 2018). Also, blanching can lead to loss of chlorophyll which is responsible for the green color, therefore leading to a reduction in greenness of the leaves. It is, therefore, important to gain a deeper understanding into how blanching can be used to inactivate enzymes and, also, how it will affect the quality of these leaves.

The objective of this work was to investigate the effect of blanching on *C. esculenta* and *C. oleraceus* leaves. Hot-water blanching was carried out on the leaves of both plant species and the efficiency of the process assessed based on peroxidase inactivation. Generally, blanching of most vegetables is usually carried-out at temperatures between 85-95 °C (Dauthy, 1995). This study also sought to determine whether temperatures lower than this range can be employed to successfully blanch the selected vegetables. Therefore, 71 and 82 °C were also tested as possible blanching temperatures for *C. esculenta* and *C. oleraceus* leaves. In addition to peroxidase inactivation, the effect of blanching

on the retention of ascorbate as well as other quality parameters such as chlorophyll and phenolic content were assessed. Finally, a kinetic model was developed to explain the inactivation of peroxidase and the retention of ascorbate in these vegetables due to blanching.

2. Materials and Methods

2.1. Materials and sampling preparation

C. esculenta and *C. oleraceus* leaves were obtained from the School of Biological Sciences Garden and authenticated by the Herbarium of the Department of Conservation Biology and Entomology all within the University of Cape Coast, Ghana. The leaves were picked fresh in the morning and used within 2 h after harvest.

Whole leaves without any physical defects were harvested and used in the study. The freshly harvest leaves were transported to the laboratory under cold storage, and washed to remove dirt and other foreign particles. The leaves were then air-dried and cut into pieces of 1 cm² average size for blanching experiments.

2.2. Blanching of leaves

The cut leaves were blanched in hot-water, immediately cooled on ice and stored frozen at -20 °C until further analysis. Four independent blanching experimental conditions per time duration (1, 2, 3 and 5 min) and temperature (71, 82 and 93 °C) were carried out. Unblanched fresh leaves were used as control.

2.3. Determination of peroxidase activity

Peroxidase activity was determined according to Bania & Mahanta, (2012). Essentially, crude peroxidase extract was obtained by homogenizing 0.5 g leaf sample in 50 mL of 0.1 M sodium phosphate buffer (pH 6.5) pre-chilled on ice. The homogenate was centrifuged at 4400

rpm for 20 min at 4 °C. To 0.2 mL of the enzyme extract, 0.1 mL freshly prepared O-dianisidine solution was added. Hydrogen peroxide (H₂O₂) was also added (0.2 mL of 0.2 M) and the absorbance of the mixture measured immediately at 430 nm every 30 sec for 3 min.

Peroxidase activity was expressed based on the amount of protein which was determined by adding 2 mL Biuret reagent to 5 mL of phosphate extracts and incubated at 37 °C for 10 min. The absorbance of the mixture was measured at 562 nm using a spectrophotometer (Bibby Scientific Ltd, UK, Jenway 6400). Calibration graphs were plotted using bovine serum albumin (BSA), from which the measured absorbance were used to estimate protein levels.

2.4. Determination of chlorophyll, ascorbate and total phenolic content

The chlorophyll content of the leaves was determined after extracting with acetone. To 0.25 g of leaves, 80 % acetone was added, homogenised and centrifuged at 4000 rpm for 10 min. Re-extraction was carried-out to a total volume of 10 mL and the absorbance measured at 646.6 and 663.6 nm. The total chlorophyll content of the leaves was estimated based on the method of Pora *et al.* (1989) using Equation 1.

$$Chls\ a+b = 17.76A^{646.6} + 7.34A^{663.6} \quad \text{Equation 1}$$

Ascorbic acid (ascorbate) was extracted with metaphosphoric-acetic acid solution by homogenising 1 g of leaf sample with a solution containing 3 % metaphosphoric acid in 8 % acetic acid to a final volume of 10 mL. The mixture was centrifuged for 15 min at 4000 rpm and bromine water (90 uL) added to 1.5 mL of the supernatant. Further, thiourea and 2,4-dinitrophenylhydrazine were added and the

mixture incubated for 3 h at 37 °C after which the mixture was placed on ice for 30 min. Prior to the spectrophotometric analysis at 521 nm, H₂SO₄ was added (Ampofo-Asiama & Quaye, 2019a). L-ascorbic acid was used as standard in plotting calibration curves from which the concentration of ascorbate in the leaves was interpolated.

Methanolic extracts of the leaves (prepared by homogenising 1 g of leaf samples with 10 mL of 80 % methanol) were used to determine total phenolic content as described by Ampofo-Asiama & Quaye (2019b). The homogenate was centrifuged for 10 min at 4000 rpm and Folin-Ciocalteu reagent (1.25 mL) was mixed with 0.25 mL of supernatant, followed by the addition of 1 mL sodium carbonate (Na₂CO₃) solution. The mixture was incubated in the dark for 30 min and the absorbance measured at 765 nm. Gallic acid was used as the standard and the phenolic content of the leaves expressed as gallic acid equivalent (GAE)

2.5. Kinetic modelling of changes in peroxidase activity, ascorbate and chlorophyll levels

Models to explain the effect of blanching on peroxidase inactivation and retention of ascorbate were developed by fitting first-order kinetic models (Equation 2) to the obtained experimental data as explained by Ampofo-Asiama & Quaye (2019a).

$$\frac{d[C]}{dt} = k \cdot [C] \quad \text{Equation 2}$$

In these models, [C] represents the activity of peroxidase or concentration of ascorbate and *k* is a first-order rate constant for the inactivation of peroxidase or degradation of ascorbate.

The temperature dependence of the rate constant was modeled with the Arrhenius equation (Equation 3):

$$k_c = k_{ref} \cdot e^{\frac{E_a}{R} \left(\frac{1}{T_{ref}} - \frac{1}{T} \right)}$$

Equation 3

Where k_{ref} is the reference first-order rate constant at a chosen reference temperature of 100 °C, E_a is the activation energy in J/mol and R is the universal gas constant.

2.5. Statistical analysis

Statistical analysis to determine the effect of hot-water blanching on peroxidase activity, chlorophyll, ascorbate and total phenolic content was carried out using analysis of variance (ANOVA) in SPSS (IBM, SPSS Statistics 20). When a significant effect was observed, the Tukey test was performed to determine which means were different. The difference among means was identified at a significance level of 0.05. All presented results are the means of four independent replicates with the error bars being standard deviations.

3. Results and Discussion

3.1. Peroxidase inactivation

The efficiency of blanching of *C. esculenta* and *C. olitorius* leaves was assessed based on peroxidase inactivation. Generally, peroxidase is a relatively stable enzyme so some residual activity may still exist over a short duration of blanching. Indeed, its inactivation, whether partial or absolute, may also lead to the inactivation of other relevant enzymes responsible for quality deterioration (Hong-wei *et al.*, 2017). Figure 1 shows the residual activity

of peroxidase as expressed as a function of the activity in the fresh leaves. The activity of peroxidase in the fresh leaves of *C. esculenta* and *C. olitorius* was 0.085 and 0.11 µmol/min/mg protein respectively. After blanching for 1 min at 71, 82 and 93 °C, peroxidase activity reduced by 73, 86 and 93 % respectively, in *C. esculenta* leaves. Similarly, peroxidase activity of in *C. olitorius* leaves reduced by 47, 54 and 68 % respectively, after 1 min of blanching at 71, 82 and 93 °C. In both plant species, peroxidase activity in the fresh leaves was significantly higher than in the blanched leaves irrespective of the temperature and duration of blanching.

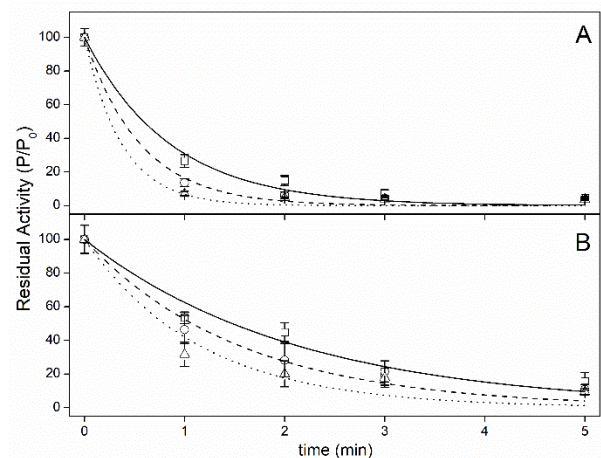


Figure 1: Residual activity of peroxidase following hot-water blanching of *C. esculenta* (A) and *C. olitorius* (B) leaves. The modeled changes in the residual activity of peroxidase (71 °C, solid line; 82 °C, dashed line; 93 °C, dotted line) are plotted along with the experimentally determined values [71 (□), 82 (○), and 93 °C (Δ)].

Enzyme inactivation was correspondingly more effective at higher temperatures. This was demonstrated after 1 min of blanching at 93 °C, with recorded peroxidase activity being lower compared to the two lower blanching temperatures. However, after blanching for 2 min and beyond, no significant differences in peroxidase activity were observed between the

two species for temperatures at 82 and 93 °C. Generally, about 95 % reduction in peroxidase activity was observed after 5 min of blanching at the different temperatures.

In the leaves of *C. olerius*, peroxidase activity was significantly lower at 93 °C compared to the other temperatures for 1 and 2 min of blanching. After 3 min of blanching, however, no significant differences in peroxidase activity were observed between the different blanching temperatures. Comparing the two plant species it was observed that the residual leaf peroxidase activity of *C. olerius* was generally higher, irrespective of the temperature or duration of blanching (Figure 1).

Reduction in the activity of peroxidase has been observed following blanching of several vegetables (Gonçalves *et al.*, 2009; Müftügil, 1985; Murcia *et al.*, 2000). In cabbage and spinach leaves, 47 and 89 % reduction in peroxidase activity was respectively observed after 1 min of blanching at 75 °C. At 85 and 95 °C, the reduction peroxidase activity in cabbage was 88 and 96 % respectively, while no activity was measured in spinach leaves (Müftügil, 1985). During blanching of broccoli leaves, about 90 % of peroxidase activity was lost within 1.5 min (Severini *et al.*, 2016).

The inactivation of peroxidase in vegetables has been explained using both simple first-order (Gonçalves *et al.*, 2009) and biphasic first-order models (Cruz *et al.*, 2006). In this work, a simple first-order model (Table 1) was able to explain peroxidase inactivation in both leaves (R^2 of 0.81 and 0.86 for *C. esculenta* and *C. olerius* leaves respectively). The low rate-constant recorded for *C. olerius* indicates that peroxidase inactivation will occur at a faster rate compared to *C. esculenta*. Varying ranges of kinetic parameters

have been reported for peroxidase inactivation in vegetables (Gonçalves *et al.*, 2009; Thongsook & Barrett, 2005). However, Williams *et al.* (1986) proposed that for optimum quality retention, 90 % peroxidase inactivation is required. This suggests that blanching *C. esculenta* leaves for 3 min at any of the studied temperatures could be effective in deactivating peroxidase, while blanching for 5 min may be effective for peroxidase inactivation in *C. olerius* leaves.

3.2. Ascorbate retention

The effect of blanching on the retention of ascorbate in *C. esculenta* and *C. olerius* leaves is shown in Figure 2. The level of ascorbate in the freshly harvested leaves was 37.07 and 19.57 mg/100 g for *C. esculenta* and *C. olerius* respectively. General reduction in ascorbate levels was observed in the leaves of both vegetable species following blanching. After 1 min of blanching at 71, 82 and 93 °C, about 55, 49 and 29 % of ascorbate respectively, were retained in the leaves of *C. esculenta*. These reductions were all significantly lower compared to the fresh leaves. Ascorbate levels of *C. esculenta* leaves blanched at 71 and 82 °C were not significantly different, irrespective of blanching duration; about 38 and 34 % respectively, of the initial levels were retained. At 93 °C, less than 15 % of the initial ascorbate levels were retained after 5 min of blanching. At this temperature, no significant differences in ascorbate levels were observed after 3, 4 and 5 min of blanching.

In *C. olerius* leaves, about 65 % of ascorbate was retained after 1 min of blanching at 71 and 82 °C. At 93 °C, however, less than half of the initial amount was retained after blanching for 1 min. The residual ascorbate levels after 3 and 5

min of blanching at 93 °C were significantly lower than blanching for 1 and 2 min. A similar observation was made during blanching at 82 °C. Notably, ascorbate retention after 1 min of blanching was always higher in *C. olerius* compared to *C. esculenta* for all the blanching temperatures considered. However, except for blanching at 82 °C, similar ascorbate levels were retained in both vegetable species after 5 min of blanching.

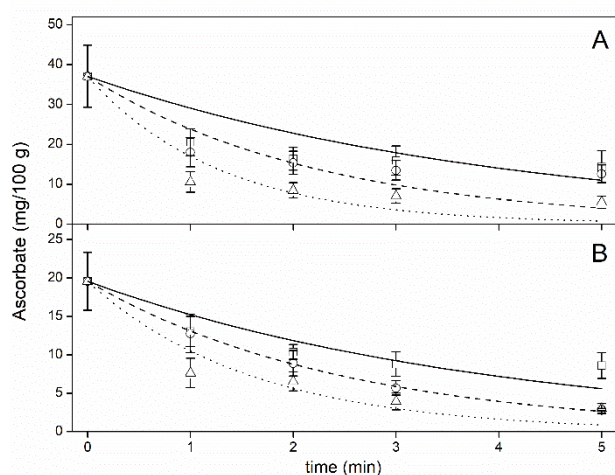


Figure 2: Retention of ascorbate following hot-water blanching of *C. esculenta* (A) and *C. olerius* (B) leaves. The modeled changes in ascorbate retention (71 °C, solid line; 82 °C, dashed line; 93 °C, dotted line) are plotted along with the experimentally determined values [71 (□), 82 (○), and 93 °C (Δ)].

Vegetables and fruits are the most important source for human ascorbate (vitamin C), and together they are responsible for over 90 % of the dietary supply (Lee and Kader, 2000). However, since ascorbate is heat labile, it is essential to always assess their retention during processing. For this purpose, ascorbate retention in vegetables during hot-water blanching has been studied extensively. Low ascorbate retentions of between 8-59 % has been observed in several vegetables after blanching (Gupta *et*

al., 2008). A general decrease in ascorbate retention at increasing blanching temperatures was observed in both *C. esculenta* and *C. olerius* leaves. A similar observation was made in *A. sessilis*, where ascorbate retention of 10.5, 16.9 and 26.8 % was observed when the leaves were blanched at 100, 90 and 80 °C respectively (Ranganathan *et al.*, 2017). Also, at the same blanching temperature, a decreased retention of ascorbate was observed with an increasing duration of blanching. A similar effect was observed in broccoli leaves (Severini *et al.*, 2016).

The kinetic parameters of ascorbate retention in both vegetables is shown in Table 1. First-order models had been confirmed to be adequate to explain ascorbate loss following blanching of vegetables (Ariahu *et al.*, 2011). In this study, the adequacy of the model is confirmed in the high R^2 values obtained (0.98 and 0.96 for *C. esculenta* and *C. olerius* leaves respectively). The rate constant for the loss of ascorbate in *C. olerius* was about twice that of *C. esculenta*, even though the activation energies were not significantly different from each other. Different rate constants and activation energies for ascorbate loss in other vegetables have been reported. In a study of moringa and hibiscus leaves (Musa *et al.*, 2017) and fluted pumpkin leaves (Ariahu *et al.*, 2011), low first-order rate constants of between 0.022-0.19 min^{-1} were reported, while a high rate constant of 3.2 min^{-1} was reported for white sorrel leaves (Dauda *et al.*, 2016). Similarly, varying activation energies for ascorbate loss have been reported (Ariahu *et al.*, 2011). The observed activation energies are in agreement with that observed in other studies (Mauri *et al.*, 1989).

Table 1: Modelled first-order kinetic parameters of peroxidase and ascorbate following hot-water blanching of *C. esculenta* and *C. oleritorius* leaves. The results are the means along with their standard deviations.

	<i>C. esculenta</i>		<i>C. oleritorius</i>	
	K_{ref} (min ⁻¹)	E_a (kJ/mol)	K_{ref} (min ⁻¹)	E_a (kJ/mol)
Peroxidase	3.45 ± 0.40	39.63 ± 5.10	1.02 ± 0.12	29.28 ± 6.35
Ascorbate	0.46 ± 0.04	55.62 ± 9.68	0.81 ± 0.11	43.46 ± 6.74

3.3. Changes in phenolic content

The influence of blanching on the phenolic content of the leaves of *C. esculenta* and *C. oleritorius* is shown in Figure 3. The phenolic content of fresh leaves of *C. esculenta* and *C. oleritorius* was 325.75 and 469.66 mg GAE/100 g respectively. Contrasting data on the effect of blanching on phenolic content are available in the literature. [Severini *et al.* \(2016\)](#), for example, observed no significant changes in phenolic content of blanched broccoli leaves, although

[Gonçalves *et al.* \(2009\)](#) pointed to a significant decrease in phenolic content. In this study, blanching did not significantly influence the phenolic content of the leaves of *C. esculenta* and *C. oleritorius*.

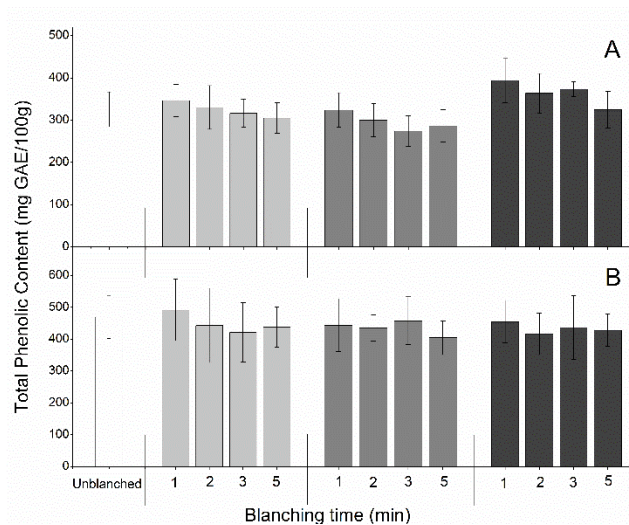


Figure 3: Changes in total phenolic content following hot-water blanching of *C. esculenta* (A) and *C. oleritorius* (B) leaves. The light gray, deep gray and black bars represents the leaves blanched at 71, 82 and 93 °C respectively, while the white bars represent the unblanched leaves.

3.4. Stability of chlorophyll

Figure 4 shows the effect of blanching on the percent loss of chlorophyll. Chlorophyll content of 2.01 and 2.04 mg/g respectively, were observed in the freshly harvested leaves of *C. esculenta* and *C. oleritorius*. Blanching *C. esculenta* leaves for 5 min at 71, 82 and 93 °C resulted in corresponding chlorophyll loss of 29, 31 and 43 %. Under similar conditions, chlorophyll loss of 23, 27 and 40 % were observed in *C. oleritorius* leaves. This shows that for the same duration of blanching, higher loss of chlorophyll occurred with increased temperature of blanching. A similar observation was made during blanching of several green leafy vegetables including *C. oleritorius* ([Faboya, 1985](#)).

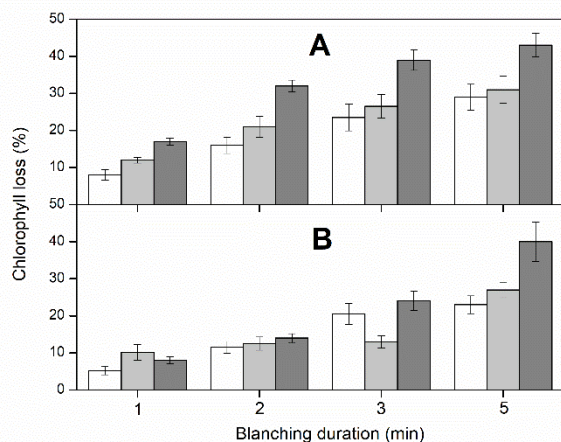


Figure 4: Percent loss of chlorophyll following hot-water blanching of *C. esculenta* (A) and *C. oleraceus* (B) leaves. The cumulative chlorophyll loss after blanching at 71, 82 and 93 °C are represented by the white, gray and black bars respectively.

In *C. esculenta* leaves, chlorophyll loss of 17, 32, 39 and 43 % were observed after blanching for 1, 2, 3 and 5 min respectively, at 93 °C. Similar blanching conditions in *C. oleraceus* leaves resulted in chlorophyll loss of 8, 14, 24 and 40 %. It may be concluded from these observations that there is a general increase in chlorophyll loss with increasing duration of blanching. Other reports corroborate chlorophyll loss in other green vegetables during blanching. Negi & Roy (2000) recorded chlorophyll loss of 8.6 and 42 % respectively, in savoy beet and amaranth/fenugreek leaves after hot-water blanching at 95 °C for 3 min. Also, boiling of broccoli reduced the chlorophyll content by 27 % (Yuan *et al.*, 2009).

The green color of vegetables is an important factor that influences their economic value. Considering that this is due to the presence of chlorophyll, blanching processes should be designed to minimize the loss of this pigment. Studies on other green vegetables have demonstrated that changes in the color intensity

become noticeable when about 15 % of the total chlorophyll is lost (Faboya, 1985). This means that blanching at 71 °C for 2 min or less can be adequate to preserve the green color of *C. esculenta* leaves, while a similar duration can be employed in the blanching of *C. oleraceus* leaves at all the studied temperatures.

4. Conclusion

Blanching *C. esculenta* leaves for 3 min at any of the studied temperatures was effective in inactivating peroxidase while blanching for 5 min was effective in inactivating peroxidase in *C. oleraceus* leaves. The inactivation of peroxidase is, however, accompanied by loss of ascorbate and chlorophyll. Generally, for the same duration of blanching, an increase in ascorbate and chlorophyll loss with increasing temperature of blanching was observed. A similar observation was also made when the duration of blanching was increased.

Conflict of interest

The authors declare that there are no conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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