Vol.53, n. 6: pp.1497-1502, November-December 2010
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

The Effect of Mg^{2+}, Cu^{2+} and Zn^{2+} Pre-treatment on the Color of Yerba Maté (*Ilex paraguariensis*) Leaves

Griselda Patricia Scipioni, Beatriz del Valle Argüello and Miguel Eduardo Schmalko[*]
Facultad de Ciencias Exactas, Químicas y Naturales; Universidad Nacional de Misiones Félix de Azara; 1552; 3300; Posadas - Argentina

ABSTRACT

The aim of this work was to study the effect of alkaline blanching followed by an immersion in salt solutions of Mg^{2+}, Cu^{2+} *and* Zn^{2+} *on color preservation of Yerba Maté leaves. The concentration of NaOH in the alkaline solution influenced all of the color parameters. Ion concentration and dipped time influenced only some color parameters. The color parameters of the product obtained with different treatments were different from the control sample (blanched with boiling water instead of alkaline blanching) and from the product obtained via industrial processing. The green color in the pre-treated and control samples was more intense (greater values of –a) and darker (low values of L and b). Ionic and ash content in the leaves increased with the treatments.*

Key words: color, yerba maté, ionic treatment, drying

INTRODUCTION

Yerba Maté (*Ilex paraguarienses* Saint Hilaire) is a plant that grows in the central region of South America. A product that is consumed as "chimarrão", or tea, is derived from the leaves and young branches of this plant. Its industrial processing consists of four stages: heat treatment with gases from burning the wood at high temperature (300 – 350 ºC) in order to inactive the enzymes, a drying step below 100 ºC, grinding and seasoning (Schmalko et al., 1997). The color preferences differ in the countries where yerba maté is consumed. The consumers in Argentina, Paraguay and Uruguay prefer a light olive green color. In this case, the plant's chlorophyll is degraded during the seasoning step. In Brazil, the consumers prefer a bright green color, hence, the product is not seasoned. However, chlorophyll degradation occurs in the packaged product, thus the finished product is only marketable for a short period of time, usually 30 – 45 days.

Chlorophylls are the source of green color in vegetables. Their structure may be modified by the enzymatic or chemical reactions. Generally, heat treatment is employed in order to avoid the enzymatic degradation. However, chemical degradation may take place. Recently, several strategies have been carried out to avoid chlorophyll degradation and to maintain the green color in kiwi fruit (Leunda et al., 2000) and spinach (von Elbe and Schwartz, 1996). The treatments currently in use, are high temperature, short time (HTST) treatments (Maharaj and Sankat, 1996; Negi and Roy, 2000; Rocha et al., 1993); high intensity pulsed electric fields (Yongguang et al., 2007); microwave heating (Olivera et al., 2008); pH control, treatments with alkali (Min et al., 2004); the addition of chloride salts; and osmotic treatments with zinc and copper

---

[*] Author for correspondence: mesh@fceqyn.unam.edu.ar

salts (Guzmán et al., 2002; LaBorde and von Elbe, 1994; Rinaldi, 2000). The last three treatments are only applied to liquid or semi-liquid materials.

A loss of green color in food products is associated with pheophytin formation, in which the Mg^{2+} of the chlorophyll is replaced by H^+. Food preservation processes usually induce this loss. The addition of alkaline agents, such as magnesium carbonate during blanching is one method used to avoid pheophytin formation. In dasheen leaves (Maharaj and Sankat, 1996), alkaline blanching with magnesium carbonate reduced chlorophyll losses. These losses reached 9.6% after drying. Higher losses have occurred with water blanching without pre-treatment. In broccoli, a similar treatment enhanced the color stability. Lioutas (1989) determined that a pH between 7.0 and 9.0 was important for the stability of chlorophyll.

Because they form new complexes of chlorophyll derivatives without Mg^{2+}, salt solutions of Zn^{2+} and Cu^{2+} have been used to preserve the green color of vegetables. The formation of Zn^{2+} complexes in the material depends on the pH, Zn^{2+} and chlorophyll-derivative concentrations (Ngo and Zhao, 2005). The effect of both the cations in avocado puree has been studied by Guzmán et al. (2002). They found that color was best preserved when the material was treated with a salt solution (120 ppm of $ZnCl_2$ and 12 ppm of $CuCl_2$) and micro-waved for 30 s. Better results were obtained with zinc chloride than with copper chloride treatment. Ngo et al. (2005) studied the formation of Zn^{2+}-chlorophyll complexes in thermally processed green peas. They found that zinc-pheophytin complexes were the dominant green compound. In addition, they found that the value for the pigment reached 242% of the control, which was treated with water.

The use of Cu^{2+} is not recommended due to its toxicity at high concentrations. In yerba maté leaves, pre-treatment with Cu^{2+}, Mg^{2+} and Ca^{2+} did not influence the color (Scipioni et al., 2004). This was probably due to the low diffusion rate of ions inside the cells. Yerba maté leaves are covered with a lipophylic layer that resists the diffusion of ions from a salt solution. This layer may be hydrolyzed with alkaline solutions. Pre-treatment with an alkaline solution has been used in some fruits (Tulasidas et al., 1996). Such pretreatments may allow ionic diffusion into the cells and neutralization of the acidic compounds, thus delaying chlorophyll degradation.

The aim of this work was to study the effect of alkaline blanching and immersion in a solution of Mg^{2+}, Zn^{2+} and Cu^{2+} on the color of yerba maté leaves during processing.

MATERIAL AND METHODS

Material

Yerba maté branches obtained from a factory in Misiones, Argentina, were used as test materials. They were cut the day before processing and kept in a vase with water to avoid the loss of turgidity. Before processing, the leaves were separated from the branches.

Experimental design

Experiments were carried out using a central composite design with 17 experiments, including three at the central point (Table 1). Different ionic concentrations were used for each experiment. The independent variables were as follows: ionic concentration (x_1), NaOH concentration in the blanching solution (x_2) and dip time (x_3). The color parameters were fit to the following equation:

$$y = a_0+a_1x_1+a_2x_2+a_3x_3+a_{11}x_1^2+a_{22}x_2^2+a_{33}x_3^2 +a_{12}x_1x_2+a_{13}x_1x_3+a_{23}x_2x_3 \qquad (1)$$

Sample treatment

Samples consisting of 50 g of leaves were blanched at 100ºC for 30 s in 1500 mL of NaOH solution. The leaves were washed immediately afterwards with cold water (5ºC) in order to stop the heat treatment and to remove the remaining NaOH. Next, the leaves were dipped in an ionic solution in a flask at room temperature (approximately 25ºC). As a control, one sample was treated with boiling water for 30 s. After treatment, the leaves were washed with water and dried in a cross-flow drier at 60 ± 2ºC for 2 h. In order to simulate the industrial seasoning, the dried samples were grounded and put into a chamber at 50ºC and 60% relative humidity.

Color measurement

The color of each sample was measured using a HunterLab D25-9 colorimeter (Hunter Associates

Laboratory, Reston, Virginia, USA). Response parameters, reported using the HunterLab scale, were L= lightness (or black-white axis), a= green-red axis and b= blue-yellow axis. Measures were carried out in triplicate and the mean values are reported. Time t= 0 corresponds to the beginning of the seasoning. Experiments were carried out until the control sample was no longer green.

pH

The pH was determined by preparing a mixture of 2 g of sample and 100 mL of water. In order to avoid CO_2 absorption, the mixture was heated until boiling. Then it was cooled in a closed flask at 20ºC, and pH was measured with a Cole-Palmer pH meter using a glass combined electrode. The pH meter was calibrated at pH= 4 and pH=7.

Table 1 - Factor levels for Ion treatments.

Ion	Level	Ion Concentration (x_1) (ppm)	NaOH concentration (x_2) (% w/v)	Dip time (x_3) (h)
Mg^{2+}	-1	1000	1	3
	0	1500	1.5	5
	+1	2000	2	7
Cu^{2+}	-1	10	1	5
	0	20	1.5	8.5
	+1	30	2	12
Zn^{2+}	-1	50	1	5
	0	100	1.75	8.5
	+1	150	2.5	12

Spectrophotometric analysis

In order to determine ion concentrations, 6 g of sample was calcined in a furnace at 525ºC ± 25ºC for 5 h (IRAM 20505, 1996). The ash was dissolved in 50 mL of HCl (10% v/v) and distilled water was added to a final volume of 100 mL. A Perkin Elmer Analyst 200 Spectrophotometer of Atomic Absorption was used to measure the ionic concentration. The working conditions for ionic concentration were wavelength= 285.21 (for Mg^{2+}), 324.75 (for Cu^{2+}) and 213.86 (for Zn^{2+}) ; Standard concentration= 0.3-0.6-0.9 ppm (for Mg^{2+}), 0.5-1.0-2.0-4.0 ppm (for Cu^{2+}) and 0.5-1.0-1.5 ppm (for Zn^{2+}). The oxidant mixture was air-acetylene in all cases.

RESULTS AND DISCUSSION

Dried product

First, the effects of the pre-treatments on yerba maté without seasoning were studied. Yerba maté without seasoning is consumed in some countries, such as Brazil. The pre-treatment with NaOH solution at 100ºC and then dipping in a Mg^{2+} solution showed that the color parameters *L*, *a* and *b* were dependent on the NaOH concentration, but not on the other factors (Mg^{2+} concentration and dipping time). In the treatments with Zn^{2+}, only a dependence of *L* on dip time was observed. Different behavior was observed in the treatments with Cu^{2+}. For this case, the parameter *a* depended on all the three factors, while *L* and *b* depended on NaOH concentration and dip time, but not on Cu^{2+} concentration. The resulting equations can be observed in Table 2.

When these treatments were compared with the control sample (blanched in hot water), the color parameters in the control sample were slightly higher. However, all these values were higher than those found after the industrial processing (Schmalko and Alzamora, 2001). The mean values of –*a* were 10.46 for the control sample, 9.95 for the treatments with NaOH and ions and 4.78 for product obtained from the industrial. The *L* values were 36.74, 34.74 and 47.39, respectively and *b* values were 15.65, 14.84 and 21.95, respectively. The values of the color parameters obtained from the different treatments were very different from those in the product that underwent industrial processing. Specifically, the products obtained from the treatments were greener and darker than the industrial product.

Seasoned product

In the seasoned product (60ºC for 30 days), color changes were described using a response surface equation. Different behavior was found with each of the different ions. However, in all the cases, the green color (related to the *a* parameter) was better maintained with the ionic treatment than with the blanched water treatment (control) and the industrially processed leaves. For the samples pre-treated with the NaOH solution and for those treated with Mg^{+2}, the three color parameters were influenced by NaOH concentration. Parameter *a* depended on the interaction between Mg^{2+} concentration and dipping time, but it did not depend on either single factor (Table 2). Minor losses of green color occurred at higher Mg^{2+} concentrations and dip time. Figure 1 shows the surface response obtained with Mg^{2+} treatment. The influence of Mg^{2+} concentration at longer dipping time was higher than the treatments at shorter dipping time.

Parameters *L* and *b* depended only on NaOH concentration. An increase in NaOH concentration produced a decrease in *L* and *b* for the seasoned product (Table 2). In the industrially processed product, a decrease of *–a*, an increase of *b* and no variation in *L* were observed (Schmalko and Alzamora, 2001).

Table 2 – Color parameter equations obtained from eq. 1 including parameters that have a significant influence ($P<0.05$) and their determination coefficient (R^2)

Ion		Equation	R^2
Mg^{2+}	Before seasoning	$-a= 13.10 -1.80x_2$	86.12
		$b= 14.10 -1.61x_2 -2.01x_2^2$	85.32
		$L= 36.86 -0.40x_2 -2.88x_2^2$	81.85
	After seasoning	$-a= 7.77 -0.4x_2+ 0.00022x_1x_3+ 0.15x_2x_3$	85.51
		$b= 15.54 +1.02x_2 -2.02x_2^2$	87.04
		$L= 37.88 -1.42x_2 -2.13x_2^2$	85.36
Cu^{2+}	Before seasoning	$-a= 6.40+0.46x_2+0.03x_3 -0.015x_1x_3$	88.83
		$b= 11.94 +1.01x_2-0.32x_3+0.06x_3^2$	90.70
		$L= 32.58+-1.37x_2-0.79x_3+0.10x_3^2$	87.24
	After seasoning	$-a= 4.41 -0.005x_1x_3 +0.003x_3^2$	63.55
		$b= 44.33 -18.48x_2-2.56x_3$	58.17
		$L= 28.37 +4.72x_2-0.50x_3$	84.28
Zn^{2+}	Before seasoning	$-a= 14.94 -5.33x_2$	80.96
		$b= 19.96 -5.95x_2$	87.29
		$L= 41.45 -8.59x_2+0.19x_3+ 0.23x_2x_3$	92.89
	After seasoning	$b=20.07 -4.63x_2$	87.61
		$L= 43.05 -7.37x_2+0.14x_3$	88.30

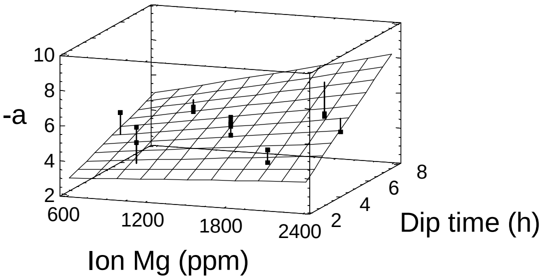

Figure 1 - Color parameter *-a* variation with Mg^{2+} concentration and dip time of the seasoned sample.

After the drying, the superior epidermis was not shiny and had many different shades of green, mainly darker ones, and sometimes showed dark spots. The degradation of the wax layer (resulting in the loss of shine) was probably a consequence of a chemical reaction between the ester groups in the waxes and the NaOH. The dark spots in yerba maté are typically evidence of a low quality product because they are usually related to enzymatic browning. However, in this case, they were probably due to an excess of NaOH in some places, and penetrating deeper into the leaves. The high concentration of NaOH may produce oxidation reactions between the Mg^{2+} and metabolites and/or other ions in the leaves. Neutralization of the H^+ in the oxhydrile group of phenolic compounds erases the conjugation of the aromatic ring and produces darker or different colors.

After the seasoning, the color of the leaves varied between a yellow-greenish, indicating that the chlorophyll had degraded, and a green-grayish, with minor chlorophyll degradation. Both were different from the green color of yerba mate leaves (i.e., there were increases in L and b).

In the leaves pre-treated with the NaOH solution and treated with Cu^{2+}, the three color parameters were influenced by the dip time. The parameters *L* and *b* were influenced by the NaOH concentration, while parameter *a* was influenced by Cu^{2+} concentration through its interaction term with the dip time (Fig. 2 and Table 2). This interaction underlined the increased influence of Cu^{2+} concentration at high dip time compared with the treatment at low dip time, where the influence of Cu^{2+} concentration was negligible. In some experiments, the blue shades were observed. The light bluish color was probably due to the formation of complexes between the chlorophyll derivates and Cu^{2+}, while the intense bluish color was probably due to the interaction between the O or N electrons of the organic compounds and Cu^{2+}, which affected the orbital energy. This influence was similar to that exerted by H_2O or NH_3 on the Cu^{2+} (Skoog and Leary, 1996). The values in the color parameters *a*, *b* and *L* obtained with this treatment were similar to that obtained with the Mg^{+2} treatment.

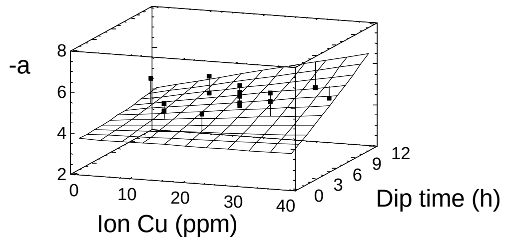

Figure 2 - Color parameter -*a* variation with Cu^{2+} concentration and dipped time of the seasoned sample.

In the leaves pre-treated with the NaOH solution and treated with Zn^{2+}, the NaOH concentration influenced the color parameters *L* and *b*. *L* was also influenced by the dip time. The parameter *a* was not influenced by the three factors. In this treatment, the leaves did not exhibit the bluish color. The values of the color parameters *a*, *b* and *L* obtained with this treatment were similar to those obtained with the Mg^{2+} treatment.

The pH of the different samples was higher than that found in the control sample (Table 3). Few differences were found between the different treatments. This pH range (between 7 and 9) was in the range established by Lioutas (1989), where the chlorophyll was stable because pheophytin formation was minimized.

Table 3 - pH, total ashes and ionic concentration in the seasoned leaves (mean values ± standard deviation of 3 replicates).

Treatment	pH	Total ashes (g/100 g dry solid)	Ion concentration (treated sample) (mg/100 g dry solid)	Ion concentration (control sample) (mg/100 g dry solid)
Mg^{2+}	7.33 ±0.03	5.45 ±0.17	1223.96 ± 15.35	846.36 ± 3.45
Cu^{2+}	7.14 ±0.04	4.27 ±0.02	5.68 ± 0.04	0.99 ± 0.01
Zn^{2+}	7.23 ±0.04	4.40 ±0.02	67.51 ± 1.10	3.35 ± 0.16
Control	5.66 ±0.03	5.13 ±0.02	Not determined	Not determined

The ionic penetration in the three treatments was higher than in the control sample (Table 3). The degree of penetration was dependent on the ionic concentrations in the solution and in the leaves. The ions in the leaves were free to diffuse to the solution, thus increasing or decreasing the total amount of ash produced, depending on the final concentrations. For these experiments, an increase in the total amount of ash was found after the treatment with Mg^{2+} and a decrease was observed with the other two ions (Table 3).

REFERENCES

Guzmán, G. R.; Dorantes, A. L.; Hernández, U. H.; Hernández, S. H.; Ortiz, A., and Mora, E. R. (2002), Effect of zinc and copper chloride on the color of avocado puree heated with microwaves. *Innov Food Sci Emerg*, **3**, 47 - 53.

IRAM 20505. (1996). Instituto de Racionalización de Materiales. Yerba Mate: Determinación del contenido de cenizas a 525 +/- 25 ºC.

Laborde, L. F. and Von Elbe, J. H. (1994), Chlorophyll degradation and zinc complex formation with chlorophyll derivatives in heated green vegetables. *J Agric Food Chem*., **42**, 1100 – 1103.

Leunda, M. A.; Guerrero, S. N. and Alzamora, S. M. (2000), Color and chlorophyll content changes of minimally processed kiwifruit. *J Food Process Pres*, **24**, 17 - 38.

Lioutas, T. S. (1989), Dried feed vegetable and method of preperation. US Patent Nº 4, 832 - 969.

Maharaj, V. and Sankat, C.K. (1996), Quality changes in dehydrated dasheen leaves: effects of blanching pre-treatments and drying conditions. *Food Res Int*, **29**, 563-568.

Min, Z.; Chunli, L. and Ping, C. (2004), Effects of processing conditions of the green-leafy vegetable juice enriched with selenium on its quality stability. *J Food Eng*, **62**, 393-398.

Negi, P.S. and Roy, S.K. (2000), Effect of blanching and drying methods on β-carotene, ascorbic acid and chlorophyll retention of leafy vegetables. *Lebens,Wiss. U.-Technol*, **33**, 295-298.

Ngo, T. and Zhao, Y. (2005), Retaining green pigments on thermally processed peels-on green pears. *J Food Sci*, **70**(9), 568 -574.

Olivera, D.F.; Viña, S.Z.; Marani, C.M.; Ferreyra, R.M.; Mugridge, A.; Chaves, A.R. and Mascheroni, R.H. (2008), Effect of blanching on the quality of Brussels sprouts (*Brassica olearacea L. gemmifera DC*) after frozen storage. *J Food Eng*, **84**, 148-155.

Rinaldi, A. C. (2000), Meeting report - Copper research at the top. *BioMetals*, **13**, 9 - 13.

Rocha, T.; Lebert, A. and Marty-Audouin, C. (1993), Effect of pretreatments and drying condictions on drying rate and color retention of basil (*Ocimum basilicum*). *Lebens,Wiss. U.-Technol*, **26**, 456-463.

Schmalko, M.E.; Morawicki, R.O. and Ramallo, L.A. (1997), Simultaneous determination of specific heat and thermal conductivity using the finite-difference method. *J Food Eng*, **31**, 531-540.

Schmalko, M.E. and Alzamora, S.M. (2001), Color, chlorophyll, caffeine, and water content variation during yerba maté processing. *Dry technol*, **19** (3and4), 599-610.

Scipioni, G.P.; Benitez, J.; Riveros, C.; Antonini, F. and Argüello, B. (2004), Efecto del pretratamiento con sales de Cu, Mg y Ca sobre el color de la yerba mate. Paper presented in the “Congreso Internacional de Ciencia y Tecnología de los Alimentos”, 24-26 November, Córdoba, Argentina.

Skoog, D.A. and Learly, J.J. (1996), Aplicación de la espectroscopía molecular de absorción ultravioleta/visible. In-*Análisis instrumental*. 4th ed. Mc Graw Hill. Interamericana de España S.A, pp. 180-183.

Tulasidas, T.N., Raghavan, G.S.V. and Norris, E.R. (1996), Effects of dipping and washing pre-treatments on microwave drying of grapes. *J Food Process Eng*, **19**, 15-24

Von Elbe, J. H. and Schwartz, S. J. (1996), Colorants. In-*Food Chemistry*, 2nd ed. New York: Marcel Dekker, pp. 651-722.

Yongguang, Y.; Yong, H. and Jingbo, L. (2007), A novel protecting method for visual green color in spinach puree treated by high intensity pulsed electric fields. *J Food Process Eng*, **79**, 125-1260.

Received: January 05, 2009;
Revised: July 03, 2009;
Accepted: May 13, 2010