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Influence of Application of Microwave Energy on Quality Parameters of Mate Tea Leaves (*Ilex paraguariensis* St. Hil.)

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Summary

Polyphenol oxidase and peroxidase are enzymes responsible for browning and quality deterioration in mate tea leaves. The main objective of this work is to investigate the influence of microwave energy on the oxidase activity, moisture content and colour of this raw material. The polyphenol oxidase was inactivated after 30 s of microwave treatment of the samples exposed to high and low light intensity. In samples exposed to low light intensity, the peroxidase activity was reduced for about 60 % after 120 s. The exposure of mate tea leaves to microwave energy for 220 s resulted in the moisture content required by the manufacturing process. The measured colour parameters showed that after microwave treatment, mate tea leaves showed a more intense green colour. In a general sense, the results show that the inhibition of polyphenol oxidase and peroxidase by microwave energy have an anti-browning effect on the colour evolution of mate tea leaves.

Key words: mate tea leaves, microwave energy, oxidase activity

Introduction

Mate (*Ilex paraguariensis* St. Hil.) is an important natural product in the economic and cultural context of Brazil, with many relevant attributed properties, such as anti-inflammatory, therapeutic, anti-rheumatic, stimulating and diuretic (*1–3*).

The three main attributes in the quality control of the mate tea leaves are colour, flavour and texture. Colour is the first criterion applied by the consumer in the acceptability of the product. This attribute in mate tea leaves is determined by the presence of yellow-golden colour pigment and green colour (chlorophyll) (*4*). In this sense, it is important to observe that the drying process used for this raw material should not change the colour of the final product.

Polyphenol oxidase (PPO, EC 1.14.18.1) and peroxidase (POD, EC 1.11.1.7) have been considered as enzymes responsible for browning and quality deterioration in mate tea leaves (*3*). Polyphenol oxidases are copper-containing enzymes that catalyze two types of reactions, both involving molecular oxygen. The first reaction corresponds to hydroxylation of monophenols to *o*-diphenols (cresolase activity) and the second to dehydrogenation of *o*-diphenols to *o*-quinones (catecholase activity) (*5*). Peroxidases are a group of heme proteins having as the main function the oxidation of substrates (monophenols, diphenols, *etc.*) at the expense of H_2O_2 (*6,7*).

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Gomes and Ledward (*8*) related that several methods had been used to inhibit enzymatic browning in plant foods. In the industrial processing of mate tea leaves, a step called sapeco is employed in order to inactivate these enzymes, and consequently inhibit the browning of the product. The sapeco, an industrial process nowadays conducted in a very rudimentary way, consists of a quick passage of the branches with leaves around the flames in the equipment called sapecador (*9*). In a general sense, the quality of the obtained product is highly variable so to obtain a mate of better quality, there is a need to standardize the process conditions. One of the alternatives to achieve this is the application of microwave energy with the main objective of inactivating the oxidases present in mate tea leaves.

Microwave energy transfer causes a rapid evaporation of water from the vegetable tissue, the treatment time is shorter compared to conventional processes and oxidation is limited with a substantial preservation of colour, flavour and sensory qualities of the products (*10*). Microwave has recently been introduced as an alternative for the laboratory and industrial inactivation of oxidases, drying process and blanching of food (*11–14*). In most works it was concluded that the combination of microwave energy with a conventional process increases the final quality of the product, leading to an insignificant mass loss and relatively reduced texture degradation (*15*).

The use of microwave technology for enzyme inactivation and dehydration of green tea enhanced the quality of processing, with greater values of catechins and polyphenols, when compared with a conventional tea dryer (*13*). Huang *et al.* (*16*) observed that microwave-heated green tea had a significantly high vitamin C content, which decomposed more slowly than the oven-heated tea during storage. They also observed that chlorophyll content was higher and more stable compared to conventional drying processes. When microwave was used as the drying method for mate tea leaves, a general tendency of increasing content of the chemical compounds (caffeine, theobromine, phytol, vitamin E and stigmasterol) was observed (*17*).

Despite the importance of mate tea leaves in the social and economic context of Brazil, the evidence of oxidases as the enzymes responsible for mate tea leaf browning and the fact that the microwave energy could be introduced in technological process without great difficulties, the literature is very scarce regarding the alternative processes that could enhance the quality of the final product. As an example, the literature does not present studies comparing oxidase activities of mate tea leaves under different cultivation conditions, but it has been shown that the high light intensity is responsible for differences in the chemical composition of this raw material. Based on these aspects, the main objective of this work is to investigate the influence of microwave energy on the quality parameters of mate tea leaves (oxidase activity, moisture content and colour) under different conditions of cultivation (low and high light intensity).

Materials and Methods

Sampling and raw material

Samples of mate tea leaves (*Ilex paraguariensis* St. Hil.) were collected in an experiment conducted under agronomic control at Indústria e Comércio de Erva-Mate Barão LTDA (Barão de Cotegipe, RS, Brazil). Details of the experiment can be found in the work of Esmelindro *et al.* (*17*). The samples of trees without the addition of fertilizers were selected. All samplings were conducted in the morning in July (cold month, with plants in their physiological sleep). Half of the samples were covered with a covering device that absorbed 75 % of the incident light (low light intensity). Seven plants were selected to assemble the treatment sample; the top, middle, and bottom of each tree were sampled and homogenized to form the sample of each treatment. The samples with high and low light intensity were dried in a PMV-101 Philco microwave, a domestic microwave (800 W) with frequency of 2400 MHz, for different periods of time, performing a kinetic evaluation (from 15 to 220 s). All samples were submitted to microwave energy on the day of collection. After drying, the samples were stored without light at temperature controlled at (25±1) °C.

Reagents

Pyrocatechol and polyvinylpyrrolidone K90 (PVP K90) were obtained from Fluka (Steinheim, Germany). Guaiacol was obtained from Reagen (Rio de Janeiro, RJ, Brazil). H_2O_2 was purchased from Vetec (Rio de Janeiro, RJ, Brazil), and sodium phosphate from Synth (São Paulo, Brazil). Triton X-100 was obtained from JT Baker (Phillipsburg, USA). All reagents were of high purity grade and used without further treatment.

Oxidase activity

In all assays, 40 g of mate tea leaves (before and after the treatment with microwave energy) were homogenized with 90 mL of 3 % (by mass per volume) PVP K90 in 0.05 M sodium phosphate buffer (pH=7.5). The suspension was filtered through five gauze layers and centrifuged for 30 min at 11 000×*g* and 4 °C. The pellet was discarded, and the supernatant was used as crude enzymatic extract. The enzyme extraction was performed in triplicate, on days 0, 2, 7, 16 and 29 of storage (*7*).

The enzyme activity was determined by monitoring the time course of the change in absorbance with a spectrophotometer UV-Visible (Agilent 5100, USA) upon oxidation of the substrates catalyzed by the enzymes. Absorbance increase was monitored for up to 3 min with the slope of linear portion of the curve used to determine the enzyme activity that was calculated using the molar absorption coefficient for each substrate. One unit of enzymatic activity was defined as the change (0.001) in the absorbance per minute per milliliter of extract.

The polyphenol oxidase activity used pyrocatechol as substrate and the absorbance was monitored at 420 nm. The assay mixture contained 50 μL of crude extract, 2.85 mL of 0.05 M sodium phosphate buffer (pH=9.0) and 100 μL of 0.1 M pyrocatechol solution in 0.1 % Tween 80 (by mass per volume) (*18*).

Peroxidase activity was determined spectrophotometrically at 470 nm. The assay mixture contained 50 μL of crude extract, 2.75 mL of 0.05 M sodium phosphate buffer (pH=4.0), 100 μL of 0.1 M guaiacol solution (used as substrate) in 0.1 % Tween 80 (by mass per volume) and 100 μL of 2 mM hydrogen peroxide (*19*).

The protein content of the extracts was determined according to the Bradford method, measuring absorbance at 595 nm, using bovine serum albumin (BSA) as a standard (*20*).

Moisture content

Moisture content was measured according to the methodology described in the Analytical Principles of the Institute Adolfo Lutz (*21*). The samples were dried for 8 h at 105 °C, and assayed in triplicate.

Colour evolution assessment

The samples of mate tea leaves were triturated to colour determination assays, using the measurement on the day of the extraction as a standard. Each sample was assayed in triplicate, and the mean and standard error were plotted. The CIELAB coordinates L^* (lightness), a^* (red-green), and b^* (yellow-blue) of mate tea leaves were determined by using a Minolta CR400 Colorimeter (Konica Minolta, Japan). Three readings were obtained for each replicate to obtain uniform colour measurements. Hue (H^*), chroma difference (ΔC^*), and colour difference (ΔE^*) were calculated using the following equations:

$$H^* = \tan\left(\frac{b^*}{a^*}\right) \qquad /1/$$

$$\Delta C^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2} \qquad /2/$$

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad /3/$$

Results and Discussion

Influence of microwave energy on oxidase activity

The inactivation of PPO and POD in mate tea leaves was achieved by treating the samples in microwave oven for different times, at the operational conditions described before. The PPO initial activity in mate tea leaves was similar in samples cultivated at high and low light intensity. In this way, it could be observed that different cultivation conditions did not influence PPO activity. For POD, the initial activity presented the difference among the samples, showing a significant effect of light intensity on POD activity. The literature does not contain studies comparing oxidase activities of mate tea leaves under different cultivation conditions, but it has been shown that the light intensity is responsible for differences in the chemical composition of this raw material. Coelho *et al.* (*22*), for instance, showed that the content of methylxanthines is higher under the conditions of low luminosity. Esmelindro *et al.* (*17*) evaluated the influence of some agronomic variables on the chemical composition of the extracts obtained from high pressure CO_2 extraction of mate tea leaves and observed that the concentrations of caffeine, theobromine, phytol and stigmasterol increased in plants cultivated at low light intensity, except for squalene, concentration of which was reduced. Primo *et al.* (*23*) submitted crude extract of oxidases from mate tea leaves to compressed carbon dioxide and verified that the POD activity was enhanced under some conditions (around 25 %) and inactivated under others, and the PPO activity showed a decrease under all experimental conditions tested.

Fig. 1 shows the activities of polyphenol oxidase of samples exposed to microwave energy at high and low light intensity, and stored for 29 days. The PPO activity presented a continuous decrease with the increase of the exposure time to the microwave energy and storage time. The inactivation of enzyme was achieved after 30 s of exposure to the microwave energy. This result is in agreement with those obtained for the inactivation of PPO in mushrooms by microwave energy (*11*). Devece *et al.* (*11*) compared different heat treatments on PPO activity of mushrooms and observed that using microwave and the effect of combined microwave/conventional heating, the enzyme was completely inactivated in a short time. Rodríguez-López *et al.* (*24*) inactivated PPO of mushrooms after 15 s of microwave irradiation.

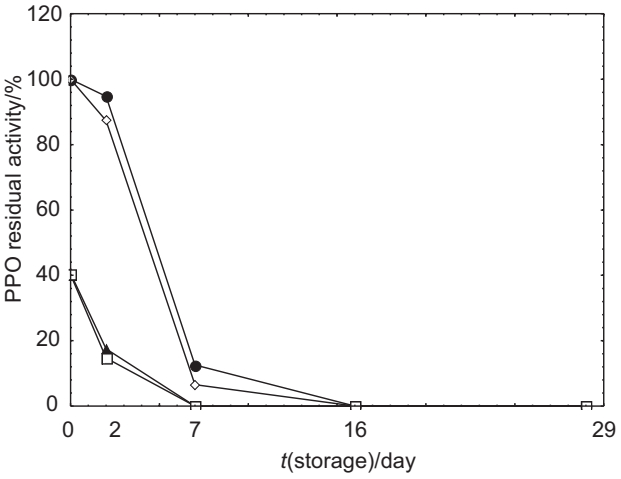

Fig. 1. Effect of the exposure of mate tea leaves to microwave energy on polyphenol oxidase at high light intensity after exposure for 0 (●) and 15 s (▲) and low light intensity after exposure for 0 (◇) and 15 s (□); initial activity of 2466.62 U/mg of protein; overall average absolute deviation of 5.67 %

The peroxidase activities of the samples of mate tea leaves exposed to microwave energy and storage for different times showed a similar tendency in both samples. The exposure to microwave energy was not sufficient for achieving complete inactivation of POD of the mate tea leaves cultivated at high light intensity (Fig. 2a), while the inactivation of the enzyme from mate tea leaves cultivated at low light intensity was achieved after exposure to microwave energy for 220 s (Fig. 2b). A regeneration of the enzyme activity was observed from the 7th day of storage. Peroxidases are thermostable enzymes, which can regenerate their activity after the thermal treatment (*7*).

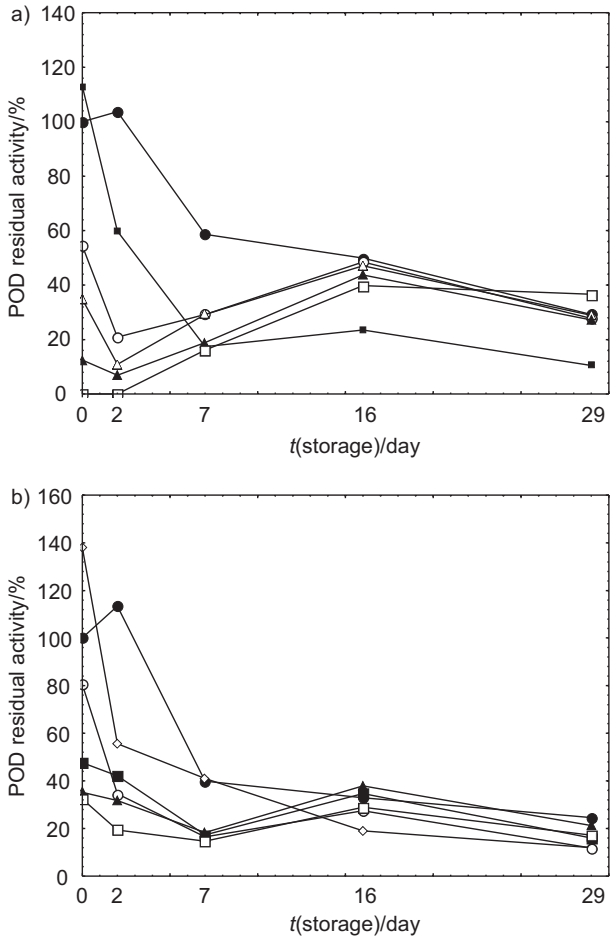

Fig. 2. Effect of exposure of mate tea leaves to microwave energy on peroxidase at (a) high and (b) low light intensity after exposure for 0 (●), 15 (○), 30 (■), 60 (◊), 120 (▲) and 220 s (□); initial activity of 19.39 U/mg of protein; overall average absolute deviation of 3.83 %

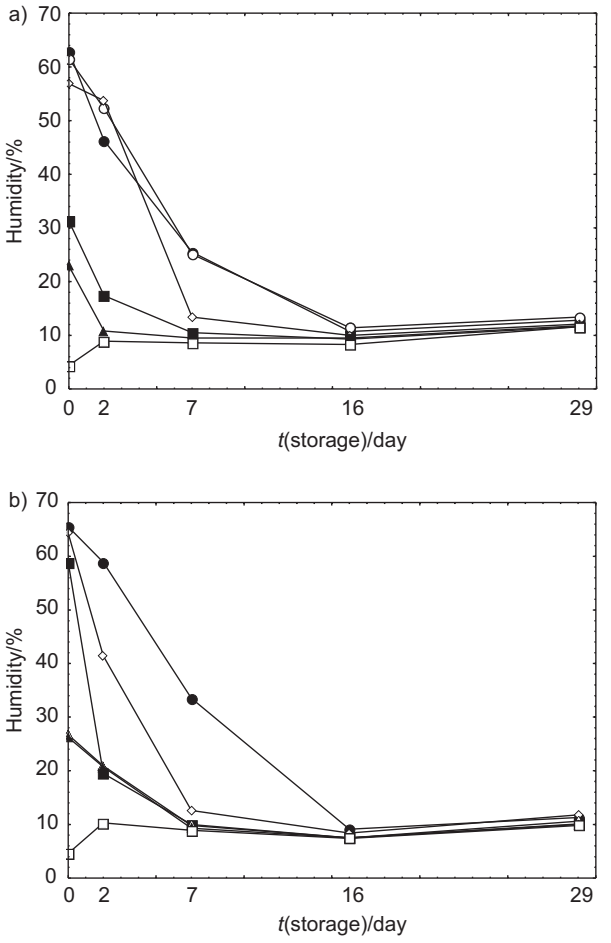

Fig. 3. Moisture content of mate tea leaves after microwave energy treatment in samples with (a) high and (b) low light intensity after exposure for 0 (●), 15 (○), 30 (■), 60 (◊), 120 (▲) and 220 s (□); overall average absolute deviation of 3.77 %

Moisture content of mate tea leaves submitted to microwave energy

Fig. 3 shows the effect of microwave energy on the moisture content of the samples cultivated at high and low light intensity. The 220-second exposure to microwave energy resulted in the moisture content (around 5 %) that is required for manufacturing of mate tea leaves after drying process. The moisture content of all samples, independent of the exposure time to microwave energy, presented similar results, tending towards the equilibrium (around 10 %) during storage.

Colour of mate tea leaves treated with microwave energy

The change of microwave energy exposure and storage duration with time on the L^*, a^* and b^* coordinates was measured in triturated mate tea leaves. The total chroma difference (ΔC^*) and hue (H^*) were calculated. The results obtained in this step are presented in Table 1. The results of L^* showed that the samples exposed to high light intensity during cultivation presented more white colour. With the increase of exposure time to the microwave energy, the intensity of coordinates in the samples increased. These results clearly show that after microwave energy treatment the mate tea leaves presented more green colour. Schmalko and Alzamora (*25*) evaluated the colour parameters (L^*, a^* and b^*) of the mate during processing. The samples were evaluated under natural conditions (fresh), after sapeco and after drying, and the results indicated that greater changes in the colour of mate occur during the sapeco process.

The total colour difference (ΔE^*) was calculated between the coordinates of the samples and standard (on the day of the sampling) (Fig. 4). For both samples, an increase with storage time was observed; however, the samples exposed to microwave irradiation for a longer time presented minor increase of colour difference (minor ΔE^*). In samples treated under natural conditions (fresh) obtained at high light intensity (Fig. 4a), ΔE^* reached a value of 21 in 16 days of storage, whereas when exposed to microwave energy for 60 s for samples of high intensity and 30 s for samples of low light intensity (Fig. 4b), the values of ΔE^* were significantly lower. Núñez-Delicado *et al.* (*26*) observed similar results for colour of Dominga grape evaluated in the absence and presence of the inhibitor of PPO. The results obtained

Table 1. Changes of L^*, a^*, b^*, C^* and H^* coordinates of mate tea leaves with and without microwave treatment on day 0

Exposure time/s	Hunter values				
	L^*	a^*	b^*	C^*	H^*
			High light intensity		
0	17.35±1.1	–(3.91±0.5)	14.17±1.7	14.70	74.57
15	16.22±1.2	–(3.62±0.6)	13.46 ±1.5	13.94	74.94
30	30.44±2.2	–(13.8±0.2)	26.32±0.8	29.74	62.33
60	37.27±1.4	–(12.3±0.5)	26.20±0.6	28.93	64.85
220	39.57±1.1	–(13.7±0.4)	25.01±0.8	28.50	62.41
			Low light intensity		
	L^*	a^*	b^*	C^*	H^*
15	32.12±3.7	–(12.54±1.2)	(23.23±1.1)	26.40	61.64
30	39.53±2.6	–(14.22±0.3)	(24.78±0.4)	28.57	60.15
60	38.32±2.6	–(14.23±0.4)	(25.37±1.0)	29.09	60.71
120	39.31±0.7	–(14.91±0.3)	(25.27±0.4)	29.34	59.46
220	40.70±0.8	–(14.71±0.5)	(25.14±0.8)	29.13	59.67

The coordinates L^*, a^* and b^* are expressed as mean values±standard deviation

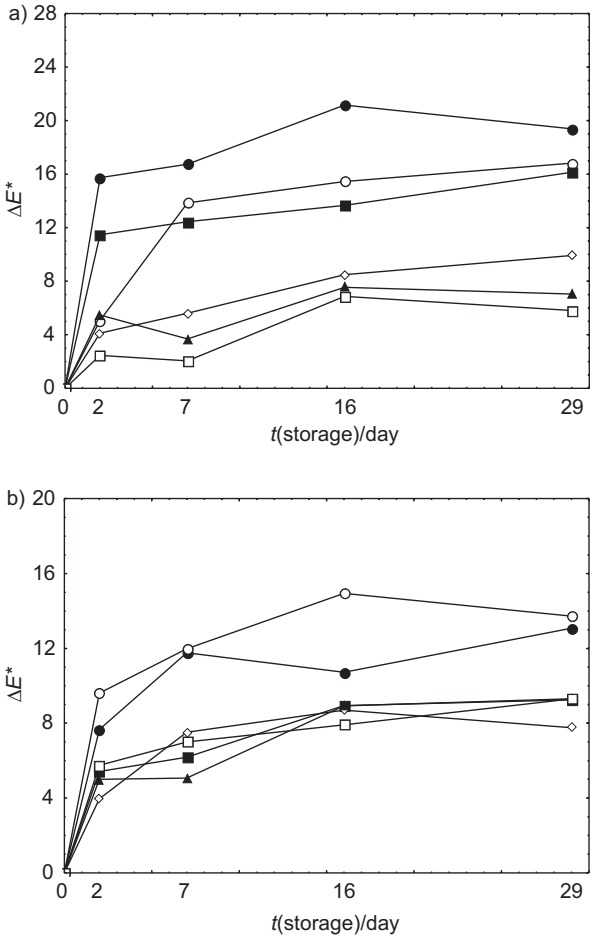

Fig. 4. Evolution of colour difference (ΔE^*) in mate tea leaves after exposure to microwave energy at (a) high and (b) low light intensity after exposure of 0 (●), 15 (○), 30 (■), 60 (◊), 120 (▲) and 220 s (□); overall average absolute deviation of 0.54 %

for mate tea leaves indicate that the oxidative enzymes are responsible for browning of leaves and that the loss of enzyme activity due to microwave energy has an anti-browning effect on the colour evolution.

Conclusions

The use of microwave techniques in the industry has shown promising results for improving product quality and, particularly for shortening the processing times currently used. The results obtained in this work show that the inhibition of the polyphenol oxidase and peroxidase has an anti-browning effect on the colour evolution of mate tea leaves. The studies with mate tea leaves were conducted at laboratory scale with a domestic microwave oven; studies on large sample sizes may be required for use of microwave energy as an alternative to the stage of sapeco at commercial scale. In this work, the exposure of mate tea leaves to the microwave energy for 60 s led to better results, inactivating PPO, reducing significantly the POD activity and leading to a more acceptable colour of the product after the treatment.

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