

# Effects of blanching on microbial adhesion and hydrophobicity in fresh *Brassica oleracea* L.

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**Abstract**—Aiming to increase the collard greens (*Brassica oleracea* L.) shelf life, we assessed the inactivation of the enzyme polyphenol oxidase (PPO) in fresh vegetables subjected to four different times of blanching (2, 4, 6, and 8 minutes) at a constant temperature of 90°C. We estimated the number of microorganisms (aerobic mesophiles, fungi, and yeasts) at the surface of fresh vegetables before and after blanching. Leaves blanched for 2 minutes showed the most significant reduction in microorganism adhesion ( $p < 0.05$ ). The blanching solution is a viable method to conserve fresh-cut collard, and do not affect the health of consumers.

**Keywords**—conservation, vegetables, minimally processed, inactivation, polyphenol oxidase.

## I. INTRODUCTION

The World Health Organization recommends the consumption of 400g of vegetables and fruits per day. Daily consumption of fruits and vegetables, besides other healthy habits, prevents cardiovascular diseases and some sorts of cancers [16]. The anticancer action bears upon the natural presence of antioxidants in the foods [21].

Collard greens (*Brassica oleracea* L.) have a high concentration of vitamins, mineral elements, and fibers, which are essential for a healthy and balanced diet [5]. However, the short shelf life of collards impairs its commercialization and consumption.

The polyphenol oxidase (PPO) enzyme affects physical-chemical stability in vegetables and aids the adhesion of microorganisms at the surface, causing food deterioration and risks to consumers' health [19]. Thus, several studies suggest methods to decrease the action of PPO [24].

Aiming to enhance the conservation of vegetables, this work assessed the inactivation of the PPO enzyme in fresh collard greens using the blanching method. We tested different time durations of blanching and verified its influence on microbial adhesion and hydrophobicity on the vegetable surface.

## II. MATERIAL AND METHODS

### 2.1 Collard green samples

Fresh collard green leaves with 35 to 40cm of length were used to test for enzymatic inactivation, microbial adhesion, and hydrophobicity. We collected collard greens randomly and aseptically in a rural property of Pombal, northeastern Brazil, which commercializes the vegetables in the urban fair and adjacencies.

The samples were packed in individual plastic bags, sterilized, and transported in ice boxes to the Laboratory of Microbiology of the Federal University of Campina Grande, Pombal Campus, where we carried out the physical-chemical and microbiological tests.

### 2.2. Polyphenol oxidase (PPO) inactivation by blanching

The thermal inactivation of PPO enzyme was performed by blanching procedure. Collards were cut into slices of 100 cm<sup>2</sup> and immersed in 500 mL of water at 90°C during for different time durations: 2 (T1), 3 (T2), 4 (T3), and 8 minutes (T4) (Table 1). The control treatment (T0) comprised leaves not immersed in hot water. Afterward, collards were cold down in an ice bath for 1 to 2 minutes. The leaves were placed in test tubes containing 10 mL of deionized water. Then, we poured 2 mL of hydrogen peroxide at 3% (v/v) and 2 mL of guaiacol at 1% (v/v) at each tube. The guaiacol test with hydrogen

peroxide allows checking the inactivation of PPO enzyme. According to this technique, the change of test tube color to a reddish brown hue indicates that the peroxidase remains active while the color maintenance indicates the inactivation of the enzyme.

### 2.3 Microbiological analyses

Microbiological analyses were performed according to American Public Health Association (APHA) [3] and described in the Compendium of Methods for the Microbiological Examination of Foods [13].

A sample of 200 g of collards was homogenized, and 25 g of leaves were weighed and immersed in 225 mL of saline solution at 0.85% (w/v). Then, we proceeded the subsequent deceduous dilutions.

The microorganisms were counted by the pour-plate method. Aerobic mesophiles were grown in Nutrient Agar medium (Himedia) and incubated at  $35 \pm 1$  °C during 24 h  $\pm$  2 h. Filamentous fungi and yeasts were grown in Agar Sabouraud Dextrose medium (Himedia) and incubated at 25 °C for five days. The results were expressed in Colony-Forming Unit per gram (CFU g<sup>-1</sup>) [1].

### 2.4 Analyses of hydrophobicity and free energy of adhesion ( $\Delta G_{\text{adhesion}}$ )

The surfaces hydrophobicity of the collard greens, aerobic mesophiles, fungi, and yeasts were evaluated by the contact angle method with liquids of different polarities. We used the standard equipment for measurement

coupled with an image analyzer (Kruss-GmbH) [30], [22]. The hydrophobicity measures the hydrophobic or hydrophilic feature of a surface based on the value of the contact angle of the surface with water ( $\theta_W$ ). Thus, at angles greater than 50° the surface is considered hydrophobic. Otherwise, angles smaller than 50° indicates a hydrophilic surface [6], [11], [15]. The adhesion requires a reduction of global free energy ( $\Delta G_{\text{adhesion}} < 0$ ). Global free energies larger than 0 ( $\Delta G_{\text{adhesion}} > 0$ ) are thermodynamically unfavorable [8], [18].

### 2.5 Experimental design and statistical analyses

The experiment comprised a completely randomized design with three replicates. The analyses of enzymatic inactivation and texture were qualitative. Analyses of variance were used to test the effects of duration of blanching procedure on the number of aerobic mesophiles, filamentous fungi, and yeasts. The Tukey test was used to perform paired comparisons among treatments. The analyses were carried out at the Software Sistema para Análises Estatísticas - SAEG (2007), assuming 5% of probability level.

## III. RESULTS AND DISCUSSIONS

### 3.1. Inactivation of polyphenol oxidase (PPO) by the blanching technique

Blanching for 2 or more minutes (T1 to T4) completely inactivated the peroxidase. The enzyme remained active only in control treatment (To) (Figure 1).

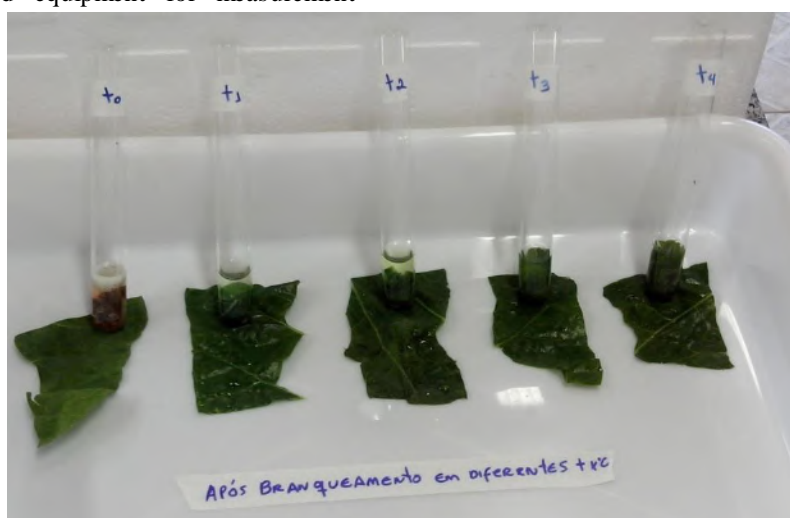


Fig.1: Assessment of enzymatic inhibition of polyphenol oxidase (PPO) showing the color and texture analysis after blanching the collard greens under different time durations.

The fast cooking at low temperatures, besides to promote enzymatic inhibition of PPO, preserves the antioxidant content of vegetables. Heating to low temperatures (< 50°C) keeps the phenolic compounds and antioxidant activity of spinach and cabbage (80 to 100%) as measured by the DPPH free radical scavenging method

[26]. The blanching of apple slices at 100°C for 2 minutes yielded ideal enzymatic inactivation without affecting the surface tissue of the food and increasing its storage time [12]. At a domestic level, cooking in water without pressure comprises the best method to prevent losses of  $\beta$ -carotene in cooked carrots [25].

The above studies suggest that the inactivation of OPP with bleaching over time leads to a low loss of healthy nutritional compounds such as antioxidants and vitamins that reduce the risks of free radicals in the human body and protect foods from undesirable enzyme reactions and oxidative dimming.

### 3.2. Microbiological assessment of fresh kale under different blanching times.

Table.2: Averages ( $\pm$  standard deviation) counts of mesophiles, fungi, and yeasts ( $\log$  CFU  $g^{-1}$ ) in collard greens submitted to different blanching times ( $T_0$  to  $T_4$ ) and a constant temperature ( $90^\circ C$ ).

Treatments	Mesophiles ( $\log$ CFU $g^{-1}$ )	Fungi and Yeasts ( $\log$ CFU $g^{-1}$ )
$T_0$	$6.45 \pm 0.33^a$	$4.23 \pm 0.45^a$
$T_1$	$4.21 \pm 0.11^b$	$3.23 \pm 0.65^b$
$T_2$	$3.98 \pm 0.22^b$	$2.96 \pm 0.41^b$
$T_3$	$3.01 \pm 0.33^d$	$2.01 \pm 0.62^{*d}$
$T_4$	$2.17 \pm 0.44^{*e}$	$< 2.00^{*e}$

$T_0$ : control;  $T_1$ : 2 minutes,  $T_2$ : 3 minutes,  $T_3$ : 5 minutes;  $T_4$ : 8 minutes; \* Estimated; Same letters in the column do not differ from each other by the Tukey test at 5% probability.

Vegetables have an inherent microbiota on their surface, in a survey conducted with American lettuce collected in Viçosa restaurants, mesophilic counts ranged from 5.0 to 6.1  $\log$  CFU  $g^{-1}$ , while fungal and yeast counts ranged from 3.1 to 4.7  $\log$  CFU  $g^{-1}$  [2]. Therefore, it is important to develop and evaluate strategies that contribute to the microbial safety of consumer products. These strategies should consider alternatives that do not result in a decrease in nutrients in the food, one of these strategies is the use of bleaching, that can minimize the presence of these biological contaminants.

Immersion of microorganisms in hot water damages their RNA, DNA, and the permeability of the cytoplasmic membrane, leading to cell lysis. This

Blanching reduced the contamination of collard greens by mesophiles, fungi, and yeasts ( $p < 0.05$ ) (Table 2). The treatment that provided the best inactivation of PPO with the maintenance of texture and color,  $90^\circ C$  for 2 minutes ( $T_1$ ), decreased almost 2.5  $\log$  CFU  $g^{-1}$  of aerobic mesophiles and 1.0  $\log$  CFU  $g^{-1}$  of fungi and yeasts concerning the control, showing excellent food safety.

antimicrobial effect decreases the number of aerobic mesophiles, fungi, and yeasts [1]. The Brazilian legislation lacks a reference value for the count of mesophiles, fungi, and yeasts in vegetables intended for fresh consumption, except for mushrooms [9]. Japanese industries discard vegetables presenting mesophiles counts greater than 5  $\log$  CFU  $g^{-1}$ . They recognize that the number of microbial populations correlates directly with the probability of infection in the individuals [17].

### 3.4 Analysis of contact angles

The surfaces of collard greens without bleaching ( $T_0$ ), and submitted up to 3 minutes of blanching ( $T_1$  and  $T_2$ ) were hydrophobic ( $\theta_A > 50$ ), Table 3.

Table.3: Average values ( $\pm$  standard deviation) of the contact angle of cells aerobic mesophiles, fungi and yeasts, and collard greens surfaces with the water ( $\theta_W$ ), formamide ( $\theta_F$ ) and  $\alpha$ -bromonaphthalene ( $\theta_B$ ) under different treatments of branching,

Surface	Contact angle		
	$\theta_W$	$\theta_F$	$\theta_B$
Collard greens $T_0$	$95.45 \pm 3.80$	$62.67 \pm 4.20$	$21.22 \pm 3.21$
Collard greens $T_1$	$54.66 \pm 4.72$	$52.2 \pm 11.64$	$28.56 \pm 9.60$
Collard greens $T_2$	$53.34 \pm 7.10$	$51.7 \pm 7.53$	$29.76 \pm 7.84$
Collard greens $T_3$	$44.67 \pm 3.38$	$48.4 \pm 3.36$	$33.78 \pm 3.99$
Collard greens $T_4$	$36.65 \pm 8.54$	$33.65 \pm 6.33$	$39.87 \pm 11.82$
Mesophiles	$31.33 \pm 4.74$	$18.56 \pm 4.43$	$43.77 \pm 11.71$
Fungi and Yeasts	$53.65 \pm 5.67$	$51.11 \pm 7.91$	$24.5 \pm 4.62$

$T_0$ : control;  $T_1$ : 2 minutes,  $T_2$ : 3 minutes,  $T_3$ : 5 minutes;  $T_4$ : 8 minutes.

The different values of hydrophobicity found are due, among other reasons, to the presence of natural compounds, such as cutin, suberin, and mainly waxes. Waxes are a complex mixture of long-chain glycolipids extremely hydrophobic. The waxes of the cuticle are synthesized by the epidermal cells and released into droplets, which pass through the pores of the cell wall by a mechanism still unknown. In the leaves, this mechanism builds a layer of cuticle and epicuticular waxes leading to the hydrophobic features of the surface [14]; [32].

Heating for a long time reduces the hydrophobicity that, among other factors, is caused by denaturation of the lipids responsible for forming the surface wax, modifying the physical-chemical characteristic from hydrophobic to hydrophilic, as observed in treatments with 5 and 8 minutes of blanching (Table 3).

The bacteria of the aerobic mesophilic group were hydrophilic ( $\theta_A < 50^\circ$ ) (Table 3). Other studies also report the hydrophilic characteristics of mesophilic bacteria, such as *Salmonella* sp., agreeing with our results [20], [23], .

Fungi and yeasts have hydrophobic surfaces (Table 3). Different glycoproteins and hydrophobic

glycolipids, mainly the chitin (a polysaccharide comprised by a long chain polymer of N-acetylglucosamine, highly insoluble in water), give the hydrophobic character of the cells [32].

The correlations between hydrophobicity and the roughness of stainless steel have been studied, however, there have been few reports on the effect of the surface hydrophobicity of fruits and vegetables on bacterial adhesion. Moreover, the relationship between the surface hydrophobicity and surface roughness of fresh produce is largely unknown. Among surfaces' constitutional characteristics, cuticles are typically embedded with cuticular wax, which not only influences plant surface hydration but also alters the interaction between a plant and microorganisms [7].

### 3.5 Free energy of adhesion ( $\Delta G_{\text{adhesion}}$ ) between collard greens and aerobic mesophiles, fungi and yeast in an aqueous medium

Table 4 shows the free energy of adhesion between collard greens and aerobic mesophiles, fungi and yeast in an aqueous medium.

Table 4: Free energy of adhesion values ( $\Delta G_{\text{adhesion}}$ ) between collard greens and aerobic mesophiles, fungi and yeast in an aqueous medium and its apolar ( $\Delta G_{\text{bls}}^{\text{LW}}$ ) and polar ( $\Delta G_{\text{bls}}^{\text{AB}}$ ) components.

Surface of adhesion	Global energy of adhesion ( $\text{mJ}\cdot\text{m}^{-2} \text{ } ^{-1}$ )		
	$\Delta G_{\text{bls}}^{\text{LW}}$	$\Delta G_{\text{bls}}^{\text{AB}}$	$\Delta G_{\text{adesão}}$
Aerobic mesophiles	- 4.3487	6.2357	1.887
Fungi and yeasts	- 7.8975	- 3.8745	- 11.772

The adhesion between mesophilic bacteria and the collard greens is a thermodynamically unfavorable process ( $\Delta G_{\text{adhesion}} > 0$ ), while the adhesion with fungi and yeasts was thermodynamically favorable ( $\Delta G_{\text{adhesion}} < 0$ ) (Table 4). The thermodynamic parameters to predict the adherence of aerobic mesophylls in fresh collard greens were contrary to that observed (Table 4). The thermodynamic theory does not take into account same microbiological factors, such as the presence of flagella, pili, adhesins, and other biological structures that may overcome the physicochemical factors during the adhesion process [30].

Although research studies on adhesion to vegetable surfaces are scarce, it is known that the secretion of mucilage, which is composed of several chemical compounds such as sugars and proteins, helps bacteria grow on vegetable surfaces [10].

The adherence of fungi and yeasts to collard greens was thermodynamically favorable ( $\Delta G_{\text{adhesion}} < 0$ ), (Table 4). Like any system, the microorganism and

surface interaction tends to decrease the free energy of the system, as observed (Table 4). The thermodynamic favoring of the adhesion process between fungi and yeasts and the collards can also be explained by the hydrophobic characteristic of both surfaces (Table 3).

Thermodynamically, adhesion between two hydrophobic surfaces is favored over hydrophilic surfaces in an aqueous medium, since the energy required to remove the water film is smaller between hydrophobic surfaces, which expel the water between them [28], [30]. However, the adhesion between a hydrophobic and a hydrophilic surface or two hydrophilic surfaces still may occur [28].

## IV. CONCLUSIONS

Our results demonstrated that bleaching for 2 minutes at  $90^\circ\text{C}$  presented the best results of stability and microbiota reduction (aerobic mesophiles, fungi, and yeasts), besides the enzymatic inactivation (PPO). The characteristics of adhesion process were

thermodynamically favorable for fungi and yeasts and unfavorable for aerobic mesophylls. Microbiological factors of aerobic mesophiles overcome thermodynamic factors, concerning the hydrophobicity. An appropriate bleaching process and good producing practices may result in a product with higher shelf life and greater microbiological safety for consumers of collard greens and other vegetables.

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