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The Study of Kinetics of Polyphenol Oxidase Inactivation in Carrot Juice by Ohmic Heating

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ABSTRACT

Carrot juice color changes from orange to brown immediately after production. Blanching is a suitable way to preserve and commercialize this product. In this study, an ohmic device was used as a heating source, and polyphenol oxidase enzyme was selected as an indicator of enzyme inactivation sufficiency to color change of carrot juice. Fresh carrot juice was subjected to thermal processing under 3 temperature levels of 70, 80, and 90 degrees of Celsius and 4 time levels of 0, 20, 40 and 60 seconds with a constant voltage of 100 volts and the enzyme inactivation, Brix, pH and color indexes L* , a* and b* were checked. Constant values of reaction rate, activation energy, D-value and Q_{10} were investigated. The kinetics of inactivation of polyphenol oxidase of carrot juice samples were calculated at 70, 80 and 90 degrees of Celsius. The changes of enzyme inactivation, Brix, pH and a* parameter with increasing temperature and time were significant at 1% probability level by completely random design. The level of enzyme inactivation was also measured by the conventional water bath method and compared with the ohmic method, and the results indicated the greater efficiency of the ohmic method. As the temperature increased, the Brix level increased and the color of carrot juice changed from orange-yellow to orange-red ($p \leq 0.05$).

1. Introduction

The carrot, a pivotal root vegetable globally cultivated for its edible taproots [1], is esteemed for its sweet flavor profile, nutrient density, and myriad health benefits, including antioxidative, anticarcinogenic, antiseptic, and palliative properties. Composed of approximately 90% water and 5% carbohydrates [2], carrots are subject to various preservation techniques like freezing, canning, or dehydration to prolong their shelf life. Prior to these methods, carrots typically undergo blanching in hot water or steam to expel air, solidify color, hydrolyze protopectins, and inactivate pathogens and enzymes [3]. Enzymes, specialized proteins within foods, can induce alterations (some beneficial, others detrimental), indicating the necessity for their deactivation [4]. The enzymatic culprits of browning and discoloration in fruits and vegetables are polyphenol oxidase and peroxidase. Polyphenol oxidase (EC 1.14.18.1), a copper-containing oxidoreductase enzyme ubiquitous in plant tissues, catalyzes the oxidation of phenols to quinones, engendering melanin pigments that manifest as black, brown, or red hues, thereby diminishing the nutritional, functional, and sensory qualities of fresh produce[5, 6, 7]

Given the drawbacks of traditional enzyme inactivation methods like water baths, which can negatively impact food quality [8], researchers are exploring alternative techniques such as ultrasonication, microwave treatment, infrared radiation, high hydrostatic pressure, enzymatic hydrolysis with hydrocolloids, high-pressure carbon dioxide, and the novel ohmic heating [9]. Ohmic heating, predicated on the transmission of alternating electric currents through foodstuffs to induce thermal effects, is lauded for its rapid and homogeneous heating capabilities [10], even in particulate-laden products, thereby enhancing the quality over conventionally processed counterparts [11]. Its applications span heating, enzyme inactivation, pasteurization, and sterilization of food products [12]. Advantages of ohmic heating over conventional thermal methods include the preservation of food color and nutritional value, swift volumetric heating, absence of temperature gradients, eco-friendliness, and superior efficiency [13].

This study investigated the application of ohmic heating for the inactivation of the polyphenol oxidase enzyme in carrot juice, evaluating the impact of this method on enzyme activity.

2-Materials and methods

2.1Materials

The carrot specimen, classified under the family Apiaceae, specifically within the genus Daucus and species Carota, was purchased from a horticultural site in Isfahan and subsequently conveyed to the research lab. Analytical-grade reagents were procured from Merck Co., located in Germany, to ensure the highest standard of experimental integrity.

2.2Methods

2.2.1. Sample preparation

Post-excision of non-essential components, the pristine carrot specimen underwent a thorough cleansing with distilled water to eliminate potential contaminants such as soil and detritus. Subsequent to the preparatory procedures, the sample was meticulously peeled using a culinary knife, and the terminal segment was detached. Thereafter, the carrot was subjected to a juicing apparatus to procure the aqueous extract for analysis [14].

2-2-2- Determination of electrical conductivity

Prior to initiating the experimental protocol, the electrical conductivity (expressed in millisiemens per centimeter) of the freshly prepared carrot juice was quantified utilizing a conductometer (Model 712, Metrohm AG, Switzerland) [15].

2-2-3- Ohmic device

The experimental setup comprised a benchtop ohmic heating apparatus, inclusive of an electrical power source, an adjustable autotransformer, and a power analysis unit (Arduino Mega 2560). Additionally, the system featured a microcontroller board and several laboratory-grade cells fabricated from polytetrafluoroethylene (PTFE), commonly known as Teflon. The specified cell was cylindrical, with an internal diameter of 0.07 (m), an external diameter of 0.09 (m), and a height of 0.26 (m), equipped with a pair of stainless steel electrodes, each 0.2 (cm) thick. Throughout the ohmic heating process, temperature fluctuations were precisely monitored via a K-type thermocouple, which was meticulously insulated with Teflon tape to obviate electrical field disturbances [16].

2-2-4- Blanching

In this investigation, both water bath and ohmic heating modalities were employed for the inactivation of enzymatic activity. Within the ohmic heating protocol, post-assessment of the carrot juice electrical conductivity, enzymatic inactivation was executed at a sustained voltage of 100 volts. The ohmic chamber was charged with 250 ml of carrot juice, between two electrodes, an electric field was established, and a K-type thermocouple was strategically positioned at the center of the cell to monitor the temperature. The application of 100 volts facilitated the enzymatic inactivation at temperatures of 70°C, 80°C, and 90°C, for durations of 0, 20, 40, and 60 seconds, across triplicate trials. The duration required for the sample to escalate from ambient to the desired enzymatic inactivation temperature varied and is referred to as the 'come up time' in literature [17,18].

Conversely, for the conventional water bath technique, enzymatic inactivation was conducted at 70°C, 80°C, and 90°C, for 1 and 2 minutes in triplicate [19].

2-2-5- Polyphenol oxidase enzyme measurement

The quantification of polyphenol oxidase activity was conducted in the control specimen and samples subjected to the inactivation process utilizing ohmic or water bath methodologies. Following enzymatic inactivation, the carrot juice subjected to the aforementioned treatments was promptly cooled to a temperature range of 20-25°C for a brief period of 1-2 minutes. Owing to the criticality of temporal factors in this study, the sample was expeditiously filtered using a vacuum pump coupled with a Büchner funnel. The extract was centrifuged at13000 rpm for 20 min at 4ºC. Subsequently, 100 microliters of the resultant extract was mixed with 2.9 milliliters of the reaction substrate, comprising a 0.1 M catechol solution and 0.1 M phosphate buffer solution. The absorbance of the mixture was then assayed at a wavelength of 420 nm. For the control enzyme measurement, prior to the inactivation step, all procedures were perforemed except the thermal treatment [20].

6-2-2- Measurement of soluble solids

The concentration of total soluble solids (TSS) was ascertained in the control specimen prior to enzymatic inactivation and subsequently following the ohmic inactivation process. This assessment was conducted at thermal set points of 70°C, 80°C, and 90°C for intervals of 0, 20, 40, and 60 seconds, with each condition being replicated three times. A refractometer was employed for the precise measurement of TSS levels [21].

2-2-7- Color measurement

The chromatic attributes of the analyzed sample were quantified utilizing a photometric chamber, with the CIELAB color space parameters including L* (luminosity), a* (red-green chromaticity), and b* (yellow-blue chromaticity), ascertained through an image analysis technique. The samples were positioned within the photometric chamber at intervals of 0, 20, 40, and 60 seconds post-enzymatic inactivation, ensuring uniformity in experimental conditions—namely, the placement of the illumination source and the spatial arrangement between the imaging device and the specimen—prior to photographic documentation[22].

2-2-8-1- Modeling

For the mathematical modeling aspect, the reaction kinetics were characterized using polynomial reaction rate equations of zero, first, and second order:

$$A = A_0 + K_0 t \quad (1)$$

$$A = A_0 \exp(-K_1 t) \quad (2)$$

$$\frac{1}{A} = K_2 t \frac{1}{A_0} \quad (3)$$

A is the value of the variable measured at time t, A_0 is the initial value of the variable measured at time t_0, and t is the retention time (seconds). K_0 is the rate constant of the zero-order reaction, K_1 is the rate constant of the first-order reaction, and K_2 is the rate constant of the second-order reaction.

The temperature dependence of the deactivation rate constant was calculated by the Arrhenius equation:

$$K_2 = K_1 \exp\left(-\frac{E_a}{RT}\right) \quad (4)$$

T is the temperature (K), E_a is the activation energy of the reaction ($J.mol^{-1}K^{-1}$), R is the gas constant (8.314 $J.mol^{-1}K^{-1}$) and K_1 is the reaction rate constant at the first temperature and K_2 is the reaction rate constant is at the second temperature.

The time required to inactivate 90% of polyphenol oxidase enzyme activity compared to its initial value is called the decimal reduction time (D-value) and is calculated as follows:

$$D = \frac{Ln10}{K} \quad (5)$$

K is the reaction rate constant of polyphenol oxidase enzyme inactivation.

The Q_{10} coefficient is a dimensionless parameter that quantifies the sensitivity of biological reaction rates to a temperature increment of 10°C. It reflects the factor by which the reaction rate amplifies in response to this temperature change. Commonly, for biochemical processes such as enzymatic discoloration, flavor transformation, degradation of inherent pigments, non-enzymatic browning, and microbial proliferation, the Q_{10} value approximates to 2. This implies that the rate of these reactions typically doubles with every 10°C rise in temperature. The calculation of Q_{10} is articulated as follows [23]:

$$K_2 = K_1 [Q_{10}]^{\frac{(T_2 - T_1)}{10}} \quad (6)$$

T is the temperature (K), K_1 is the reaction rate constant at the first temperature, and K_2 is the reaction rate constant at the second temperature.

2-2-8-2- Validation of the model

The model validation was achieved through empirical data derived from triplicate laboratory assays. This involved meticulously documenting the kinetics of the enzymatic degradation process. Subsequently, the mean values from these assays were compared to the model prediction to ensure congruence.

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(EA_{experimental} - EA_{simulation})^2} \quad (7)$$

where ($EA_{experimental}$) is the enzyme activity of the laboratory data, ($EA_{simulation}$) is the enzyme activity of the data obtained from the model and N is the number of data [11].

2-2-8-3- Statistical analysis of data

Associated production facilities conducted quantitative assessments of the polyphenol oxidase (PPO) activity, solid matrix constituents, and chromatic parameters (L*, a*, b*) prior and subsequent to enzymatic degradation via ohmic heating, employing a completely randomized design (CRD) in triplicate experiments. Furthermore, comparative analyses of PPO activity alterations were executed between ohmic heating and conventional water bath treatments, adhering to a CRD protocol. Key kinetic parameters, including activation energy (E_a), rate constant (k), decimal reduction time (D-value), and the (Q_{10}), were meticulously determined. The enzymatic degradation kinetics were elucidated through a kinetic model. Statistical comparisons were conducted utilizing the Least Significant Difference (LSD) test at a 5% significance with SPSS software.

3- Result and discussion

3-1- Polyphenol oxidase enzyme changes

The thermal inactivation kinetics of the polyphenol oxidase (PPO) enzyme were described at three distinct temperatures, with the findings delineated through appropriate kinetic models. The models demonstrating the highest fitness to the empirical data, as evidenced by their regression coefficients R^2, were as follows: at 70°C (R^2= 0.970), at 80°C (R^2= 0.943), and at 90°C (R^2= 0.934), detailed in Table 1.

Table 1. Kinetic model of inactivation of polyphenol oxidase enzyme by ohmic method

R^2	Second order reaction equation	R^2	First order reaction equation	R^2	Zero order reaction equation	Temperature
0.970	y=- 0.0312 x -0.0799	0.966	y=- 0.0031 x -0.0104	0.959	y=- 0.0003 x -0.0013	70
0.934	y=- 0.0175 x -0.0861	0.938	y=- 0.001 x -0.0081	0.933	y=- 0.0001 x-0.0008	80
0.930	y=- 0.032 x -0.214	0.934	y=- 0.0026 x -0.0167	0.926	y=- 0.0002 x-0.0014	90

Enzymatic activity was characterized by kinetic models that exhibited the highest R^2. Validation performed through both R^2 and Root Mean Square Error (RMSE), with these results presented in Table 2. The thermodynamic parameters—activation energy (E_a), rate constant (k), decimal reduction time (D-value), and temperature coefficient (Q_{10})—were quantified across the temperature spectrum of 70°C to 90°C and are elucidated in Table 3. The D-value, indicative of the duration required for the parameter of interest to diminish to 10% of its original magnitude, typically inversely correlates with temperature. However, in this study, an anomalous escalation in D-value was observed with rising temperatures, attributable to the diminished coloration resultant from PPO inactivation. Consequently, the residual color intensity necessitated protracted measurement intervals at elevated temperatures, thereby manifesting an upward trend in D-value.

Table 2. Validation of kinetic models

RMSE	R^2	Reaction equation	Kinetic model	temperature
0.0011	0.970	y=- 0.0312 x -0.0799	second order	70
0.001	0.943	y=- 0.0175 x -0.0861	second order	80
0.0022	0.934	y=- 0.032 x -0.214	First order	90

Table 3. Effects of temperature on reaction rate constant, activation energy, D-value and Q_{10}

Q_{10}	E_a (KJ/mol^{-1})	D-value(s)	K (s^{-1})	T (°C)
-	-	73.814	0.0312	70
0.560	60.068	131.6	0.0175	80
0.288	129.126	885.76	0.0026	90

The Q_{10} coefficient elucidated that a 10°C augmentation in temperature engendered a proportional acceleration in the reaction rate. This was particularly pertinent to the color formation reaction, which, upon initial temperature elevation, decelerated to a rate of 0.5 and, upon a subsequent 10°C increment, reverted to 0.288 of its baseline rate. The rate constant (k), representing the velocity of color development, inversely varied with temperature, underscoring the temperature-mediated enzyme inactivation. The E_a, denoting the energy threshold requisite for reaction progression, escalated concomitantly with temperature increments, aligning with the findings presented by Icier [24].

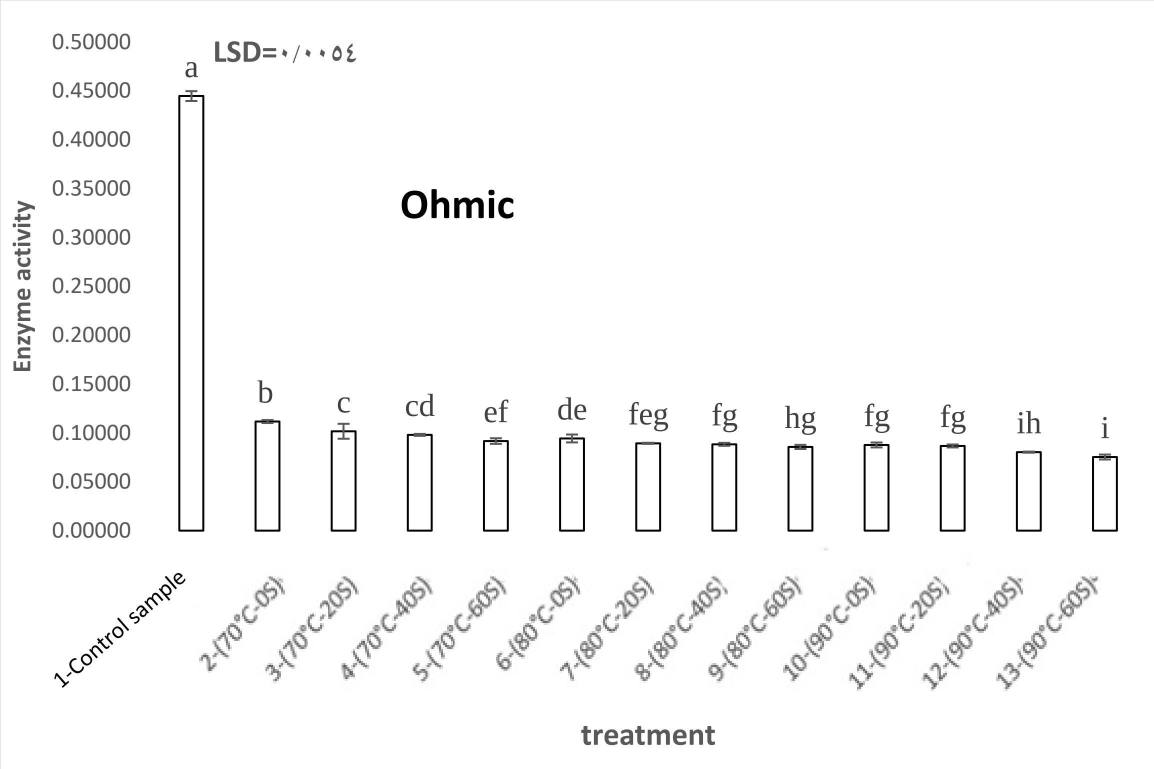

Figure 1. Mean comparisons of enzyme activity in ohmic method

Figure 1 delineates the differential impact of ohmic treatments on average polyphenol oxidase (PPO) activity, where Treatment 1 (control) exhibited a statistically significant deviation from subsequent enzyme-depleted treatments at 1% level of probability. As the thermal threshold escalated to 70°C, a diminution in PPO activity was observed, with Treatments 3 and 4 displaying non-significant reductions. However, relative to Treatments 2 and 5, the decrement was statistically significant at the 1% level. At an elevated temperature of 80°C, the decrement in PPO activity within Treatments 6 and 7 was not statistically significant, nor was there a discernible difference when compared to Treatments 7, 8, and 9. Advancing the temperature to 90°C, the reduction in PPO activity for Treatments 10 and 11 was statistically non-significant, paralleling the lack of significant difference in Treatments 12 and 13. Increase in both parameters of temperature and time leaded to an augmentation in enzyme inactivation. The zenith of enzyme elimination was achieved at 90°C with a duration of 60 seconds. Contrastingly, Treatment 2, conducted at 70°C for an instantaneous duration (time zero), facilitated an 80% reduction in PPO activity. This effect is attributed to the temporal duration required for the sample to attain the target temperature, which inherently contributed to enzyme inactivation. The temporal intervals (come up time) necessary for the ohmic method to reach the target temperatures of 70°C, 80°C, and 90°C were 65, 75, and 85 seconds, respectively, each exerting a lethal effect on the enzyme.

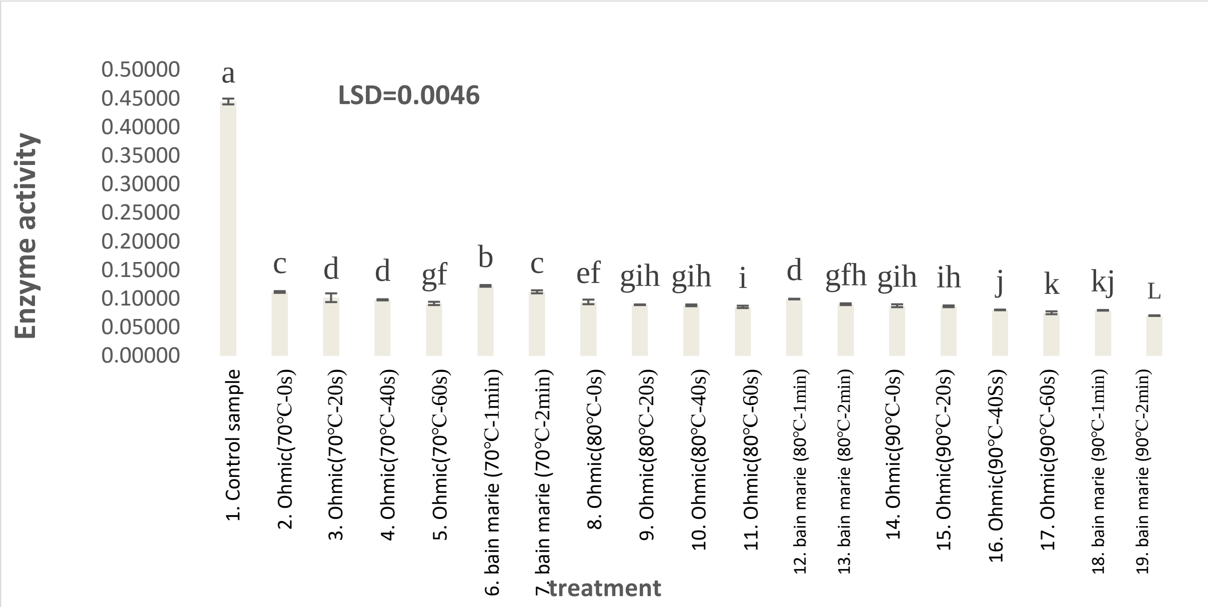

Figure 2. Mean comparisons of enzyme activity by ohmic and water bath methods

Interpreting the mean comparison in Figure 2, Treatment 1, serving as the control, exhibited a statistically significant divergence from the remaining treatments, indicating a pronounced reduction in polyphenol oxidase (PPO) activity after heating. The experimental conditions encompassed three temperature settings, employed both ohmic, and water bath modalities. At 70°C, no significant statistical disparity was discerned between Treatments 2 and 7, as well as between Treatments 3 and 4. However, Treatments 5 and 6 were significantly distinct from other treatments at 1% probability threshold. The ohmic treatments at 70°C consistently resulted in lower PPO activity compared to the water bath at equivalent durations, underscoring the superior efficacy of the ohmic approach.

Elevating the temperature to 80°C, Treatments 8 and 13 did not significantly differ, nor did Treatments 9, 10, and 13. Similarly, no significant statistical difference was noted between Treatments 9, 10, and 11, yet Treatment 12 stood out significantly from the others. The ohmic treatments at 80°C again demonstrated reduced enzyme activity relative to the water bath at one-minute interval, further affirming higher efficiency of ohmic method.

At 90°C, no statistical distinction was observed between Treatments 14 and 15, nor between Treatments 16 and 18, and Treatments 17 and 18. Treatment 19, however, marked a two-minute water bath duration, differed significantly from the rest at 1% probability level. Notably, at 90°C, the ohmic treatment effectuated enzyme deactivation instantaneously upon sample collection, achieving completion of the enzyme removal process. The ohmic method, utilizing a constant voltage of 100 volts, achieved 80% enzyme inactivation at 70°C and zero seconds, and over 90% inactivation at both 80°C and 90°C at zero seconds. This rapid thermal transfer and consequent enzyme inactivation align with the findings of Kashani and Fattahi [15, 16], attesting to the superior heat transfer capabilities of ohmic method.

2-3- Changes in soluble solids

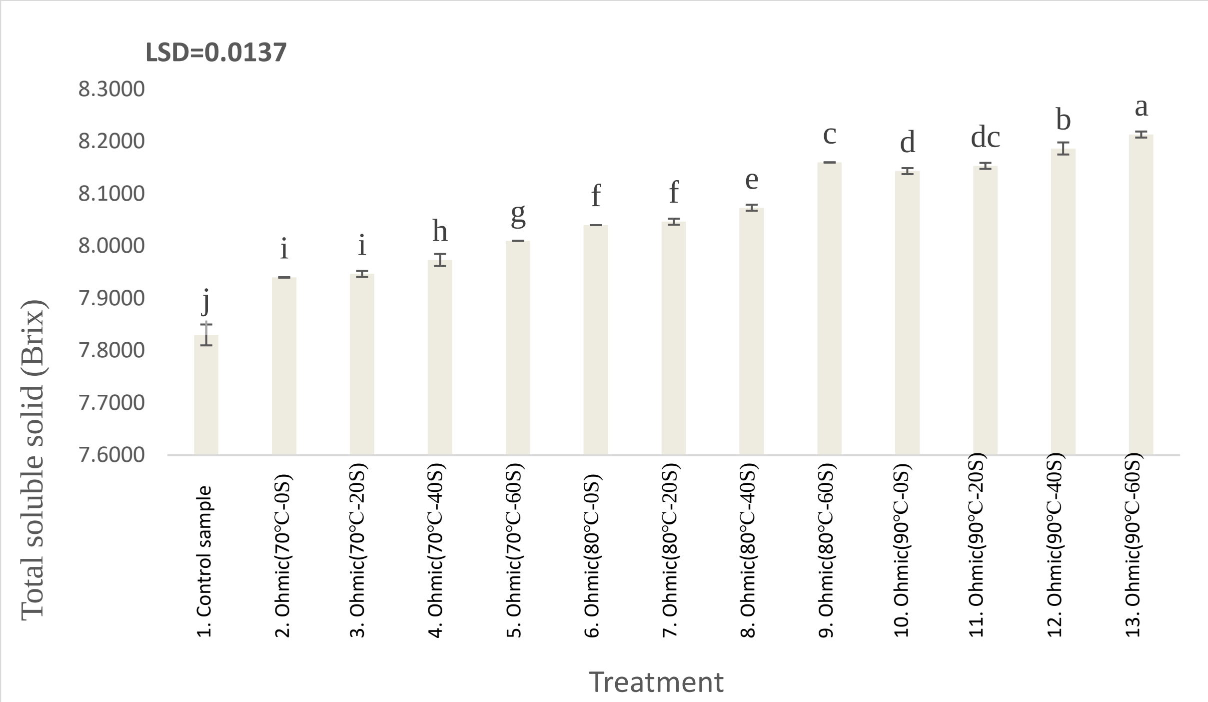

Figure 3. The results of mean comparisons of Brix value

Figure 3 presents a comparative analysis of the mean total soluble solids (TSS) levels, with Treatment 1 (control) not subjected to thermal processing, demonstrating a statistically significant difference when exposed to the subsequent treatments. An escalation in TSS was observed concomitant with the rise in temperature and duration of exposure. Treatment 13 manifested the apex concentration of TSS. At 70°C, Treatments 2 and 3 did not exhibit a significant statistical difference, whereas a marked statistical distinction was noted between Treatments 4 and 5. Progressing to an 80°C regime, Treatments 6 and 7 were statistically indistinguishable, in contrast to Treatments 8 and 9, which displayed a significant difference at the 1% probability level. At the 90°C juncture, Treatments 10 and 11 were statistically analogous, whereas Treatments 12 and 13 were significantly disparate.

Overall, the TSS levels were significantly amplified with the increment in both temperature and duration of ohmic heating. This phenomenon is partly attributed to the ohmic cell and the consequent evaporation from the sample's surface due to thermal effects, as mentioned in other in references [25].

3-3- Color changes

Within the three colorimetric indices (L*, a*, b*), the comparative analysis of the mean value of the a* parameter (which quantifies the redness intensity of the sample) is of paramount significance in this investigation.

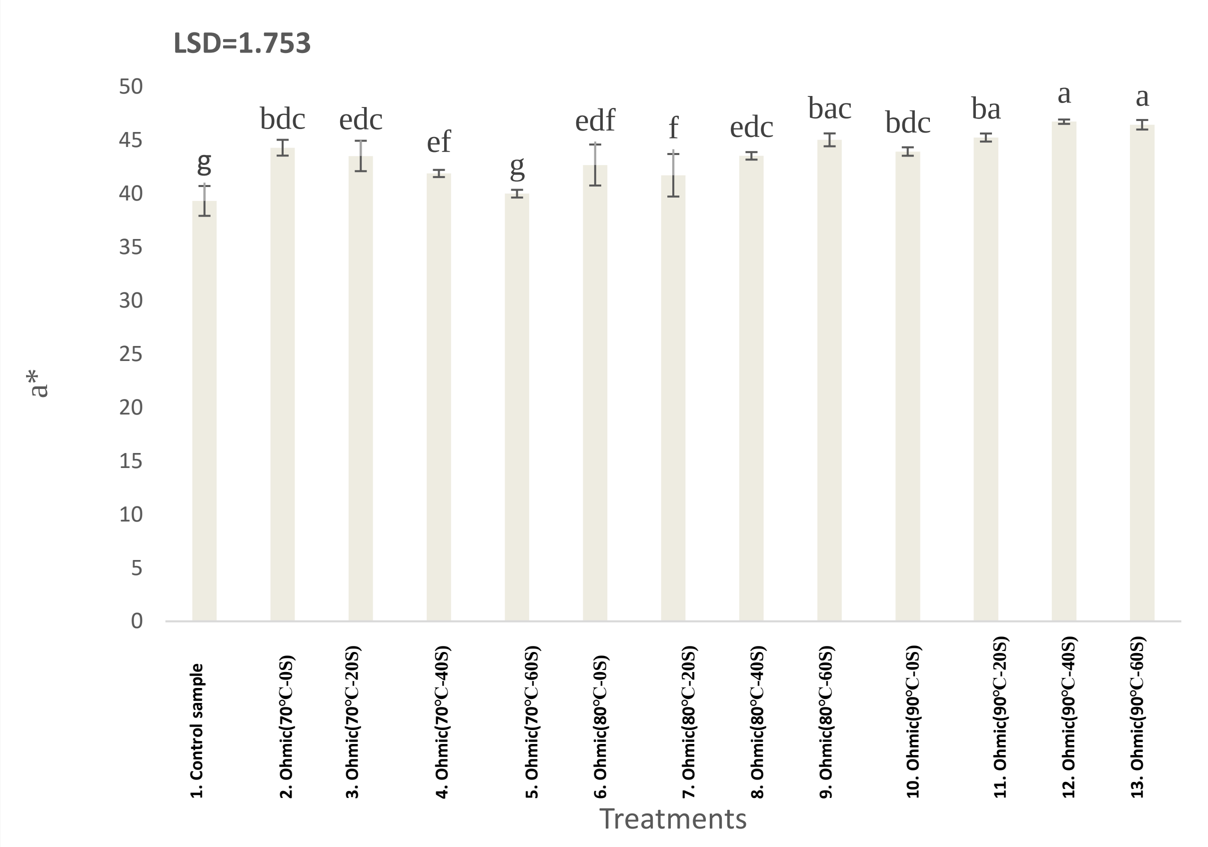

Figure 4. The results of mean comparisons of the a* index

The comparative analysis of the mean parameter of a* (indicative of redness intensity) revealed that Treatment 1, the non-thermally processed control, was statistically indistinguishable solely from Treatment 5. Conversely, it exhibited significant differences when compared to all other treatments. The a* value exhibited an increase concomitant with the escalation in temperature and duration.

At 70°C, no significant statistical difference was noted between Treatments 2 and 3, nor between Treatments 3 and 4. However, Treatment 5 was significantly distinct from the other treatments at this temperature. Advancing the temperature to 80°C, Treatments 6 and 8, as well as Treatments 8 and 9, and Treatments 6 and 7, were statistically analogous. At 90°C, Treatments 10 and 11, as well as Treatments 11, 12, and 13, did not demonstrate significant statistical differences.

As depicted in Figure 4, the ohmic treatment at 80°C for 60 seconds, along with all treatment durations at 90°C, resulted in the highest recorded levels of a*, surpassing even the control sample. This is attributed to the rapid inactivation of the enzyme, which consequently preserved the product's color more effectively. In contrast, the control sample exhibited ongoing enzymatic activity during the colorimetric assessment, leading to potential color degradation, whereas the aforementioned treatments prevented enzymatic interference with color integrity.

4- Conclusion

In the enzymatic degradation protocol, thermal elevation is regulated to achieve an 80% reduction in the product's enzyme content. This threshold is optimal as it prevents further detrimental alterations within the product without incurring the excessive energy and temperature demands of complete enzyme inactivation, which is economically impractical. The comparative analysis between ohmic and traditional thermal methods revealed that optimal enzymatic removal via ohmic heating is attained at a temperature of 90°C immediately upon sample introduction, thereby preserving the sample's chromaticity and integrity. Conversely, the water bath method necessitated a 1-minute exposure at 90°C to observe a comparable level of enzymatic degradation. The ohmic approach demonstrated superior efficiency, achieving target temperatures more rapidly than the water bath method. In the context of total soluble solids (TSS), a temperature and time-dependent increase was noted, likely due to augmented evaporation rates. The most pronounced elevation in the a* value, denoting enhanced red color, was recorded at 90°C for durations of 40 and 60 seconds, surpassing the control and other desirable samples. Enzymatic activity was characterized by kinetic models with high regression coefficients. The inactivation of the polyphenol oxidase (PPO) enzyme, responsible for color development, resulted in diminished color formation from the enzymatic reaction. The residual color intensity was quantified over time, revealing that with rising temperatures, the D-value increased, indicating a longer duration for the formation of color, which was the product of an enzymatic reaction. Concurrently, the rate constant (k) for color development decreased with temperature elevation, consistent with the temperature-induced enzyme inactivation.

Conflict of interest

All authors declare that there is no conflict of interest

5-References

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بررسی کینتیک غیر فعال کردن آنزیم پلی فنول اکسیدازآب هویج در اثر حرارت دهی اهمیک

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چکیده

هویج بلافاصله پس از آب‌گیری تغییر رنگ داده و از نارنجی به قهوه‌ای تغییررنگ می‌دهد. آنزیم‌بری راهی مناسب برای حفظ و تجاری‌سازی این محصول است. در این مطالعه از دستگاه اهمیک به عنوان منبع گرمایش استفاده شد و آنزیم پلی‌فنول‌اکسیداز به عنوان شاخص کفایت غیرفعال سازی آنزیم برای تغییر رنگ آب‌هویج انتخاب شد. آب‌هویج تازه تحت ۳ سطح دمای ۷۰ و ۸۰ و ۹۰ درجه سانتی‌گراد و ٤ سطح زمان ۰-۲۰-٤۰ و ٦۰ ثانیه با ولتاژ ثابت ۱۰۰ ولت تحت فرآیند حرارتی قرار گرفت و میزان غیرفعال‌سازی آنزیم، مواد جامد محلول ، pH و متغیرهای رنگی *L، *a و *b بررسی شدند. مقادیر ثابت سرعت، انرژی فعال‌سازی، D-value و Q_{10} مورد بررسی قرار گرفت. کینتیک غیرفعال سازی پلی‌فنول‌اکسیداز نمونه‌های آب‌هویج در دمای ۷۰ و ۸۰ و ۹۰ محاسبه شد. تغییرات غیرفعال‌سازی آنزیم، مواد جامد محلول ، pH و پارامتر*a با افزایش دما و زمان به وسیله‌ی طرح کاملا تصادفی در سطح احتمال ۱٪ معنی‌دار شدند. میزان غیرفعال‌سازی آنزیم به روش متعارف حمام آب نیز اندازه‌گیری شد و با روش اهمیک مقایسه شد که نتیجه‌ی آن کارایی بیشتر روش اهمیک را نشانگر شد. با افزایش دما میزان مواد جامد محلول افزایش پیدا کرد و رنگ آب هویج از نارنجی مایل به زرد به سمت نارنجی مایل به قرمزی پیش رفت($p\leq ۰.۰۵$).