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NON-THERMAL INACTIVATION OF POLYPHENOLOXIDASE FROM JERUSALEM ARTICHOKE (*HELIANTHUS TUBERUSUS* L.)

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Abstract
Polyphenoloxidase (PPO) is an enzyme that causes brown-colour in fruits and vegetables, and has been the object for the development of anti-enzymatic browning agents for food industries. In this study, PPO was extracted using the method as described by ÖNez, Karaku, & Pekyardimci, (2008) at 4 °C from Jerusalem artichoke (Helianthus Tuberusus L.). The PPO was inactivated using microwave and pulse magnetic field techniques. The kinetics of the non-thermal inactivation process was effectively modelled using Bigelow, Weibull and Hülsheger models. Results of this study revealed that, an increase in Tesla enhanced the inactivation of the PPO. Thus, treatment at 4.0-Tesla with 45-pulses were found to reduce the residual-activity of the enzyme to 32.6%. Also, the lowest PPO residual activity (≤ 30 %) occurred at high power (180 W – 300 W) for 15, 20, 25 and 30 min. when microwave was employed. With regards to the kinetic models, Hülsheger fitted the research data with an $R^2 \geq 0.980$. The application of pulse magnetic field and microwave was able to inactivate PPO from Helianthus tuberusus L., and may have a huge potential in the food industry for the control of enzymatic browning, which occurs during fruit and vegetable processing.

Keywords: Non-thermal inactivation, Polyphenoloxidase, Pulse magnetic field, Jerusalem artichoke

Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.), which belongs to the Asteraceae family, is a root vegetable, sweet in taste due to its high inulin content. The roots have uneven shape and skin-colour, but are moderately large. The recent surge for the cultivation and production of Jerusalem artichoke (JA) is associated with its health benefits. These are linked to the consumption of inulin and polyphenols (Bock *et al,*. 2014, Chen *et al,*. 2013). Inulin is a primary dietary fibre in JA tuber (JAT), and it exists in the form of stored carbohydrate within the tuber cell (Afoakwah *et al,*. 2015; Carpita, Kanabus & Housley,1989). It is also rich in folic-acid, vitamin C, coumarins, unsaturated fatty acids, polyacetylenic derivatives and sesquiterpenes (Chen *et al,*. 2013, Baba, Yuan *et al,*. 2012, Yaoita, & Kikuchi, 2005). Epidemiologic-evidence has recommended that diets rich in vegetables are linked with reduced risk of a number of diseases due to potent antioxidant properties of phytochemicals decreasing oxidative-stress in consumers (Darfour-Oduro *et al*,. 2018; Yaya & Bishwajit, 2018; Goyeneche, Di Scala, & Roura, 2013).

JA has been reported to possess therapeutic properties. This includes aperient, cholagogue, diuretic, spermatogenic, stomachic, and tonic effects. JA has been utilized as a folk-medicine for the treatment of diabetes, rheumatism and wounds (Chen *et al,*. 2013, Yuan *et al.* 2012, Ahmed *et al.* 2005). The extracts of JA have anti-microbial and anti-fungal activities (Chen *et al,*. 2013). The heliangin, a germacrane sesquiterpene lactone, isolated from JA plant, showed activity in vitro against Ehrlich ascites carcinoma cells (Ahmed *et al.* 2005). More so, JA Inulin, a non-digestible dietary fibre, can stimulate the development and activity of preferred bacteria in the colon. Thus enhancing the health status of human beings (Roberfroid, 2005). Inulin in JA has a positive

influence on blood sugar reduction, lipid-homeostasis, and immunomodulation property. Again, it retards colon cancer, assimilates calcium, magnesium, and potassium in the gastrointestinal tract (Coudray, Demigné, & Rayssiguier, 2003). Besides, with the capability of inulin to influence texture and enhanced rheological attributes as well as nutritional qualities of food, it has been considered as a functional food. JA has been recommended to be frequently consumed to ensure normal cholesterol content and perfection of vision. In addition, JA has the potential to prevent liver diseases, obesity, atherosclerosis, gout, arthritis, kidney stones and anemia (Gedrovica & Karklina 2013).

Based on its health benefits, JA is extensively used as salad and pickle preparations as well as food additives (Praznik *et al,.* 2002). However, the rapid enzymatic-browning, which occurs during JA processing, reduces its health associated benefits. Therefore, technological-strategies are required to inhibit polyphenoloxidase (PPO) (Taranto *et al.,* 2017), which is well known to be responsible for enzymatic-browning in fruits and vegetables (Reyhan *et al.,* .2017). Therefore, the characterization of JA PPO is vital to identify its biochemical characteristics and mechanism and, as well, recognize how to avert its deteriorative act throughout storage and processing. In addition, the inactivation of PPO is imperative for the preservation of the health properties linked to JA consumption.

Hence, the objectives of this work were to inactivate JA PPO activities using microwave and pulse magnetic field and to study the kinetic inactivation of JA PPO using kinetic inactivation models.

Materials and Methods

Materials

The tubers of JA were collected from Yanchen, Jiangsu province of China. The tubers without bruises were selected and washed. They were packed in polyethylene bags and stored at -20 °C prior to usage. Enzymatic reactions were conducted with Beckman DU800 UV/Vis spectrophotometer.

Methods

Enzyme Extraction

Using the modified method of Önez et al., (2008), JA PPO was obtained at 0 - 4°C. 50 g of JA tubers were weighed. Liquidizer was used for the homogenization of the tubers for 2 min in a 100 mL phosphate buffer (0.2 M, pH=7.3), 10% polyethylene glycol and 50 mM ascorbic acid. Filtration was carried out using filter cloth. Refrigerated centrifuge was then used to centrifuge the homogenate at 30,000 x g for 35 minutes at 4°C. The extraction of crude JA PPO was repeated three times.

Microwave Inactivation Effect on JA PPO

The experiments were carried out using microwave technique (Discover® System S-Class, CEM Coperation, Smith Farm Road, Mathews. USA). In this study, 25 mL of extracted JA PPO was placed into a 50 mL round bottomed flask; magnetic agitator was positioned within the reaction PPO in the round bottomed flask. Microwave powers were selected from 60 to 300 W using the exposure time of 5–30 min. Different microwave power levels were produced as the microwave power selected was attuned from 60–300 W in relation to change in time (5–30 min). After the microwave exposure, the round bottom flask was inserted in an ice bath for 3 min, which was then followed by the determination of the enzymatic activities as described previously.

Pulse Magnetic Field (PMF) Inactivation Effect on JA PPO

Eight (8) mL of extracted JA PPO was placed into a 10 mL test tube tightly capped. The tube was placed into the instrument cavity which contains the pulse treatment chamber. The number of pulses was selected at a linear adjustable level (5.0–45.0), using a magnetic field strength 1.0–4.0 Tesla. Different magnetic field strengths were produced when the number of pulses selected were adjusted from 5–45. After the magnetic field exposure, the 10 mL tubes were placed in an ice bath for 3 min, which was then followed by the determination of the enzymatic activities.

Tristimulus Colorimetry for the Evaluation of Enzymatic Browning

Five (5.0) mL of inactivated JA PPO was mixed with 12 mL of 2 mM caffeic acid. Colour measurements were determined by DC-P3 automatic colour meter using 2 cm cuvette. Before colour measurement, the instrument was calibrated with white and black tiles. Three colour

determinations were made for each sample group. The colour was measured in triplicates. From the values obtained the evaluation of browning was done by calculating the whiteness index (WI) as described by Yu and Zeng (2013) equation (2) and % inhibition value (IV) as described by (Sapers & Douglas 1987) equation (3). JA PPO sample without PMF inactivation was used as a control.

$$WI = \sqrt{(100-L)^2 + a^{*2} + b^{*2}} \quad (2)$$

L= lightness, a*=redness or greenness and b*= yellowness or blueness

$$\%\ \text{Inhibition value (IV)} = \frac{\Delta\ L\ \text{in control} - \Delta\ L\ \text{in PMF treatment}}{\Delta\ L\ \text{in control}} \quad (3)$$

Δ L = Percentage difference between the control and PMF treated sample.

Residual Activity

Residual activities of PMF and microwave treated samples were calculated as:

$$\text{Residual enzyme activity (RA)} = \frac{P}{P_O} X\ 100 \quad (4)$$

P = JA PPO activity after microwave and PMF activity.
PO = Original JA PPO activity.

Kinetics analysis of enzyme inactivation

The inactivation of JA PPO was characterized using Bigelow, Weibull and Hülsheger kinetic models as presented in equation 5, 6 & 7 respectively (Ma, Huang, & Zhu, 2011).

$$\text{Log}(Q) = -\frac{ty}{d} \quad (5)$$

Q = Relative activity, ty = number of pulses used for PMF treatment and d = Decimal reduction value

$$\ln(Q) = -\left(\frac{t}{\alpha}\right)^{\beta} \quad (6)$$

α and ß = Scale and shape factors

$$\ln(Q) = -XY \ln\left(\frac{t}{tp}\right) \quad (7)$$

XY = Kinetic rate constant; tp = Inactivation time

Estimation of Model Parameters

Model parameters of Bigelow, Weibull and Hülsheger models were calculated from the mean experimental data for each specific experimental condition employing non-linear least squares procedures utilizing the function lsqcurvefit of the software Matlab 7.1

Experimental Design and Statistical Analysis

Six durations (5, 10, 15, 20, 25 and 30 min.) while 5 power levels (60, 120, 180, 240, and 300 W) was used for microwave exposure. Also, 5 pulses (0, 5, 15, 25, 35, 45 selected at linear adjustable level) by 4 magnetic field strengths (1, 2, 3, 4 Tesla) factorial design was used for PMF. Three complete replications of these experiments were conducted. Data for this experiment were analysed using General Linear Model (SPSS Version 13, Chicago).

Main effects and interactions were indicated as significant at $P < 0.05$. The means were distinguished using Duncan multiple range test. Accuracy factor (*Af*) of the models was evaluated using Valero *et al,.* (2007) method equation (8).

$$Af = 10^{\frac{\sum \log[(\text{fitted} / \text{observed})]}{n}} \quad (8)$$

n = number of observations.

Results and Discussion

Effect of Microwave and Pulse Magnetic Field on the Activity of JA PPO

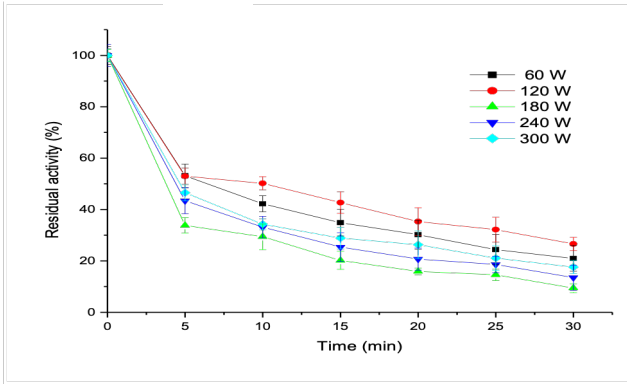
The effect of microwave treatment on PPO activity is presented in **Fig. 4A-1.** Subjecting JA PPO to the lowest power level (60 W), 20.98% PPO residual enzyme activity (RA) was observed after 30 min. However, increasing microwave power to 120 W for 30 min recorded 26.6% RA. JA PPO subjected to 180 W for 30 min reduced RA to 19.4% of its original activity. The lowest PPO RA (≤ 30%) occurred at high power (180 W – 300 W) for 15 min, 20 min, 25 min and 30 min. The RA decreased with an increased of microwave treatment duration. With regards to the effect of power level on inactivation of PPO activity, the following was observed: 180 W > 240 W > 300 W > 60 W > 120 W, this result was in agreement with de Ancos *et al,.* (1999), who reported that microwave at various conditions of power and time produced inactivation of polyphenol oxidase (PPO, EC 1.14.18.1).

As indicated in **Fig. 1B-1**, the number of pulses and magnetic strength confirmed a significant ($P < 0.05$) influence on JA PPO RA. The RA reduced from 100% to 32.6%, when JA PPO was exposed to 5–45 pulses on 4.0 Tesla, the JA PPO activity reduced as the pulse magnetic field number and pulse increased.

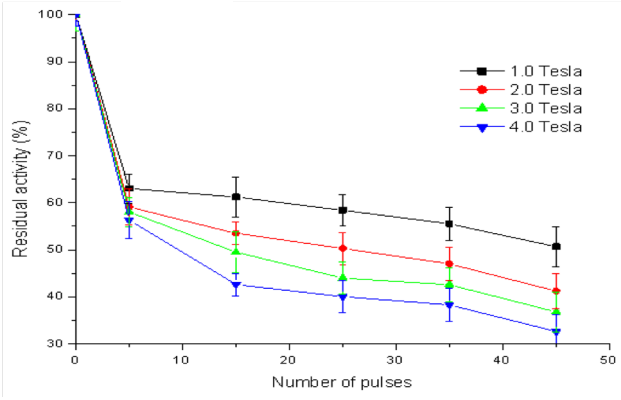
Activity of JA PPO Using Microwaves and Pulse Magnetic Field after Storage

The microwave inactivation on JA PPO was further studied after 48 h of storage at 4 °C. This was carried out to establish if the PPO would continue to be inactivated (**Fig. 1A-2**). However, it was found that the enzyme activity of the treated samples reduced after 48 h of storage as compared to the untreated samples. The results suggested that microwave treatments may inactivate the JA PPO irreversibly. The activity of JA PPO was examined instantly after pulse magnetic field inhibition and after storing for 48 h at 4°C. It was realized that there is no significant difference ($P > 0.05$) of PPO activity detected in samples immediately after PMF and subsequent storage for 48 h (**Fig.1B-2**). This result suggests that pulse magnetic field may cause permanent inactivation of this enzyme.

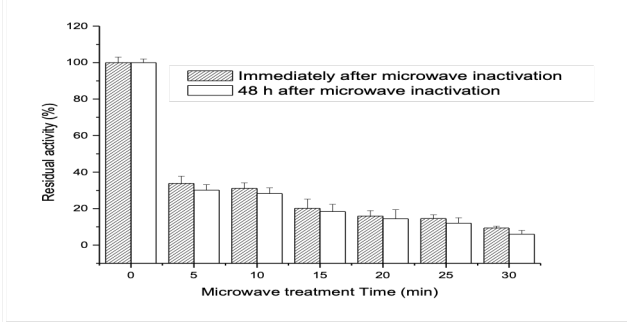
A-1



B-1



A-2



B-2

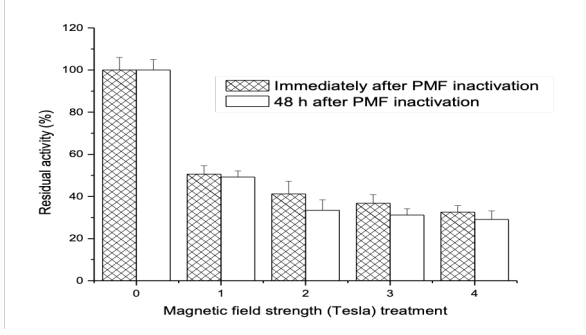


Fig.1: A1: Microwave power (W) and time (min) effect on the activity of JA PPO; A2: Microwave effect on the activity of JA PPO immediately and after 48 h storage; B1: Pulsed field strength and number of pulse effect on the activity of JA PPO; B2: Magnetic field strength treatment effect on the activity of JA PPO immediately and after 48 h storage. Bars represent standard error of the difference

The kinetic Inactivation Model of JA PPO

With regards to the principles of inactivation of JA PPO, kinetic models of equation 5, 6, & 7 were calculated as shown in **Table 2**. Coefficient of determination (R^2) and model accuracy (*Af*) were used as statistical measures for comparing the experimental model simulated values. The statistical test parameters indicated that the assayed model explains the relationship between JA PPO activity and the number of pulses well enough. The Value *d,* of the Bigelow kinetic model reduced from 500.00 to 200.00 with an increase in Tesla (1.0 - 4.0 T). The model fitted the experimental data at 4.0 and 2.0 Tesla. With regards to the Weibull kinetic model, it was clear that α and β are influenced noticeably by PMF strengths. In the study, the scale indicator α reduced when the field strength of PMF was higher. However, β was between the range of 0.305 and 0.517. These figures show that, the endurance curvature of the JA PPO was curved inward. Besides, it has a less probability of being inactivated. Also, **Table 2** shows *t*p and *XY* of the Hülsheger kinetic model. The high value of *XY* shows the enhanced inactivation impact. It improved starting from 0.239 to 0.517 with an increase in the PMF field strength. It can be deduced that kinetic model of the Hülsheger better fitted the outcome of this present investigation with $R^2 \geq 0.980$.

Table 2: Kinetic inactivation models

Bigelow model				Weibull model					Hülsheger model				
Tesla	D	R^2	*Af*	Tesla	*B*	α	R^2	*Af*	Tesla	Tp	XY	R^2	*Af*
1.0	500.00±233.91	0.9572	1.02	1.0	0.305±0.032	221.08±8.12	0.9895	1.00	1.0	0.239±0.09	0.159±0.02	0.9987	0.98
2.0	333.30±112.31	0.9876	1.10	2.0	0.423±0.021	193.41±5.03	0.9998	1.02	2.0	0.316±0.01	0.210±0.08	0.9802	1.02
3.0	250.00±103.03	0.9644	1.12	3.0	0.495±0.010	178.67±4.50	0.9897	1.00	3.0	0.363±0.04	0.241±0.04	0.9891	1.06
4.0	200.00±189.56	0.9895	1.00	4.0	0.517±0.011	162.87±7.84	0.9875	1.00	4.0	0.414±0.05	0.275±0.009	0.9994	1.00

D: decimal reduction time (Min), R^2; **c**oefficient of determination, *Af*: accuracy factor, ß: shape factor, α: scale factor, Tp: inactivation time (Min) and XY: kinetic rate constant

Evaluation of enzymatic browning after JA PPO exposure to PMF

Significant fall of whiteness index (WI) for the treated samples as compared to the control was observed in this study. It was clear that the WI of JA PPO was influenced by the magnetic-strengths and number of pulses. The number of pulses and magnetic-strengths did not have effects on the WI at $P < 0.05$. As presented in **Table 3**, WI reduced, as the pulse number (0-45) and magnetic strengths increased from 1.0 to 4.0 Tesla. This research had provided that JA PPO might be inactivated by PMF and may maintain the WI of JA tubers. An inhibitory-value (IV) of JA PPO is also presented in **Table 3**. The % IV for each pulse number was calculated using ΔL values. This was obtained at different magnetic-field exposure. The results showed that the % IV were all positive. The highest % IV of 70.9 % was obtained after 45 pulses at 4.0 Tesla. This result suggested that, the magnetic field strength of 4.0 Tesla had a higher efficiency to inactivate JA PPO. In addition, it was realized that the magnetic field exposure of 1.0 Tesla using 5 pulses recorded the minimum inactivation of JA PPO.

Table 3: Effects of pulsed magnetic field inactivation on whiteness index (WI) and inhibitory value (IV) of JA PPO

lse Number*	1.0		2.0		3.0		4.0	
	WI	% IV	WI	% IV	WI	% IV	WI	% IV
0	$69.4\pm1.3^{a,\alpha}$	-	$69.4\pm1.3^{a,\alpha}$	-	$69.4\pm1.3^{a,\alpha}$	-	$69.4\pm1.3^{a,\alpha}$	-
5	$58.2\pm19.7^{b,\alpha}$	$22.0\pm14.8^{e,\gamma,\beta}$	$55.1\pm20.7^{b,\alpha}$	$29.9\pm2.3^{e,\alpha}$	$42.2\pm19.8^{b,\alpha}$	$42.5\pm0.6^{e,\beta}$	$40.4\pm19.4^{b,\alpha}$	$61.5\pm2.3^{e\,\gamma,\beta}$
15	$54.6\pm3.9^{c,\alpha}$	$31.3\pm4.1^{d,\gamma,\beta}$	$48.2\pm6.0^{b,\alpha}$	$29.6\pm2.4^{d,\alpha}$	$40.1\pm2.1^{c,\alpha}$	$43.8\pm1.7^{d,\beta}$	$39.7\pm3.7^{c,\alpha}$	$63.5\pm2.9^{d\,\gamma\,\beta,}$
25	$46.6\pm4.6^{d,\alpha}$	$36.0\pm2.8^{c,\gamma\,\beta}$	$43.5\pm2.4^{d,\alpha}$	$31.7\pm2.2^{c,\alpha}$	$39.5\pm12.5^{d,\alpha}$	$46.5\pm3.4^{c\,,\beta}$	$35.0\pm2.9^{d,\alpha}$	$64.5\pm3.0^{c\,\gamma\,\beta,}$
35	$44.8\pm7.1^{e,\alpha}$	$42.7\pm19.6^{b\,\gamma,\beta}$	$38.8\pm6.1^{e,\alpha}$	$32.9\pm7.7^{\,b,\alpha}$	$36.9\pm0.9^{e,\alpha}$	$51.7\pm0.4^{b,\beta}$	$32.8\pm8.7^{e,\alpha}$	$66.3\pm0.5^{b\,\gamma\,,\beta}$
45	$42.2\pm3.4^{f,\alpha}$	$45.7\pm2.4^{a,\gamma\,,\beta}$	$37.5\pm12.2^{f,\alpha}$	$34.9\pm1.9^{a\,,\alpha}$	$33.8\pm0.7^{f,\alpha}$	$53.8\pm0.7^{a,\beta}$	$28.2\pm1.1^{f,\alpha}$	$70.9\pm6.2^{a,\,\gamma\,\beta}$

Different alphabets in each column indicate that number of pulse showed no significant difference at Duncan multiple range test ($p < 0.05$). Same Greek symbols in each column indicates that the magnetic field strength did not show a significant difference at Duncan multiple range test ($p < 0.05$)

Conclusion

The findings of this study have established that, a significant decrease in JA PPO activity was lost at 280 W, 30 min using microwave while the residual activity of JA PPO reduced to 32.6 %, at 4.0 Tesla and 45 pulses. It was evident that the microwave and PMF caused the inactivation of JA PPO. These may suggest the conformational transformation of the JA PPO structure because of PMF and microwave exposure, which may be the cause of its inactivation. The kinetic inactivation studies also confirmed that, PMF could be able to inactivate JA PPO. The kinetic models of Weibull and Hülsheger fitted the research data. It can be deduced that, the presence of PPO in Jerusalem artichoke is the main contributing factor causing brown colour during processing and storage of Jerusalem artichoke tubers. Therefore, JA PPO can be inactivated, and its shelf life extended. It can be established that microwave and PMF may significantly inhibit JA PPO and can be useful for food processors.

References

Afoakwah, N. A., Dong, Y., Zhao, Y., Xiong, Z., Owusu, J., Wang, Y. & Zhang, J. (2015). Characterization of Jerusalem artichoke (*Helianthus tuberosus* L.) powder and its application in emulsion-type sausage. ***LWT Food Science and Technology***, 64: 74–81.

References

Afoakwah, N. A., Dong, Y., Zhao, Y., Xiong, Z., Owusu, J., Wang, Y. & Zhang, J. (2015). Characterization of Jerusalem artichoke (*Helianthus tuberosus* L.) powder and its application in emulsion-type sausage. ***LWT Food Science and Technology***, 64: 74–81.

Ahmed, M., El-Sakhawy, F., Soliman, S. & Abou-Hussein, D. (2005). Phytochemical and biological study of *Helianthus tuberosus* L. ***Egyptian Journal of Biomedical Science****,* 18: 134-147

Baba, H., Yaoita, Y. & Kikuchi, M. (2005). Sesquiterpenoids from the leaves of *Helianthus tuberosus* L. ***Journal of Tohoku Pharmaceutical University****,* 52**:** 21-25.

Bock, D. G., Kane, N. C., Ebert, D. P. & Rieseberg, L. H. (2014). Genome skimming reveals the origin of the Jerusalem Artichoke tuber crop species: neither from Jerusalem nor an artichoke. ***New Phytologist****,* 201: 1021-1030.

Chen, F., Long, X., Yu, M., Liu, Z., Liu, L. & Shao, H. (2013). Phenolics and antifungal activities analysis in industrial crop Jerusalem artichoke (*Helianthus tuberosus* L.) leaves. ***Industrial Crops and Products****,* **47:** 339-345.

Darfour-Oduro, S. A., Buchner, D. M., Andrade, J. E. & Grigsby-Toussaint, D. S. (2018). A comparative study of fruit and vegetable consumption and physical activity among adolescents in 49 low-and-middle-income countries. *Scientific Report*, 8: 1623.

de Ancos, B., Cano, M. P., Hernandez, A. & Monreal, M. (1999). Effects of microwave heating on pigment composition and colour of fruit purees. ***Journal of the Science of Food and Agriculture*** 663-670.

ÖNez, Z., Karaku, Ş. E. & Pekyardimci, Ş. (2008). Izmir grape polyphenol oxidase (*Vitis vinifera* L.): partial purification and some kinetic properties. ***Journal of Food Biochemistry****,* 32**:** 396-414.

Reyhan, G. G., Kemal, G., Fatma, M. B., Omer, A., Hüseyin, A. & Mehmet, D. (2017). Purification and characterization of polyphenol oxidase from purslane. ***Food Science and Technology****, Campinas,* 37: 356-362**.**

Roberfroid, M. B. (2005). Introducing inulin-type fructans. ***British Journal of Nutrition****,* **93:** 13-26.

Sapers, G. M. & Douglas, F. W. (1987). Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. ***Journal of Food Science****,* 52**:** 1258-1285.

Taranto, F., Pasqualone, A., Mangini, G., Tripodi, P., Miazzi, M. M., Pavan, S. & Montemurro, C. (2017)**.** Polyphenoloxidases in crops: Biochemical, physiological and genetic aspects. ***International. Journal of Molecular Science***, 18: 377

Valero, A., Hervás, C., García-Gimeno, R. & Zurera, G. (2007). Searching for new mathematical growth model approaches for Listeria monocytogenes. ***Journal of Food Science****,* 72**:** M016-M025.

Yaya, S., & Bishwajit, G. (2018). Fruit and vegetable consumption among adults in Namibia: analysis of a nationally representative population. ***Health Promotion Perspectives,*** 8: 283-289.

Yuan, X., Gao, M., Xiao, H., Tan, C. & Du, Y. (2012). Free radical scavenging activities and bioactive substances of Jerusalem artichoke (*Helianthus tuberosus* L.) leaves. ***Food Chemistry,*** **133:** 10-14.