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ASSESSMENT OF PECTINMETHYLESTERASE ACTIVITIES (PME) IN THE JUICE OF TROPICAL FRUITS PURCHASED FROM THE ROMANIAN MARKET

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Abstract

*The purpose of this paper was to evaluate comparatively pectinmethylesterase activities (PME) from the juice of some tropical fruits purchased from the Romanian market. In this respect, a series of parameters, such as: pH, protein content (with Folin-Ciocalteu reagent) and pectinmethylesterase activities (PME) by the titrimetric method, were analyzed from the juice of kiwi, mango, avocado and persimmon fruits. From each type of fruit, three samples were analyzed. The results were statistically evaluated. Descriptive statistical parameters revealed very large variability of PME activity in persimmon juice (CV%=50). The t test highlighted that: pH values differed very significantly between fruits juices, protein content was significantly higher in kiwi juice (0.779 mg%) and persimmon (0.485 mg%), compared to other juices (t varied between 14.246*** - 179.365***; p<0.001). Mango and avocado juices had lower and similar protein content (0.317 mg% and 0.324 mg%, t=0.281 ns). The PME activity (ηEqv carboxyl/gs) had the following values: 29.993 in kiwi, 16.660 in mango, 56.660 in avocado and 106.650 in persimmon juice. The variance analysis (ANOVA) of the PME activity revealed, with a probability of error less than 5%, that there were real differences between at least two of the fruit juices. The post hoc Tukey HSD test revealed that PME activity in persimmon juice was significantly higher than the PME activity in kiwi and mango juices (p=0.035, respectively p=0.015). It can be said that the mango and kiwi fruits, analyzed in our experiment, seemed to be in a more advanced ripening phase, compared to avocado and persimmon fruits.*

Key words**:** *pecti metylesterase activity, statistical assessment, tropical fruits juice*

INTRODUCTION

Pectin methylesterases (PME) are enzymes present in all organisms of the plant kingdom and are involved in adjusting the methylation degree of pectin, which is the main component of cell walls [5]. These enzymes are present in the physiological mechanisms that have as object: vegetative growth, reproductive processes, plant self - defense (plant - pathogen interaction), stress response, or senescent processes. Changes in pectin structure are associated with changes in cellular adhesion, plasticity, pH and ionic status of the cell walls [22]. Pectins are complex polyglucides rich in galacturonic acid, which may contain up to 17 different carbohydrates [32]. Pectins are the major components of the median lamella and of the primary cell wall, reaching up to 30-35% of the cell wall dry matter [29].

In the case of kiwi fruit (*Actinidia sp*.), the strongest scientific researches so far, support evidence of improvement in gastrointestinal tract functions. Preliminary studies have identified positive effects on attenuation of carbon tetrachloride-induced liver damages in rats, modulation of global immune function in mice, stimulation of bone marrow cells proliferation in vitro, reduction of chemotherapy toxic effects, increased resistance to effort [28].

Mango Fruit (*Mangifera indica*) is extensively studied for the polyphenolic content, with magniferin like antioxidant effect (one of the most powerful antioxidants known). The active substances in mango work synergistically and there is hope that they will

be used to reduce the incidence of degenerative diseases such as: cancer, atherosclerosis, diabetes and obesity [19].

The avocado fruit (*Persea american*) is known mainly for the richness of active compounds, present in the lipid fraction (omega fatty acids, phytosterols, tocopherols, squalene), involved in the reduction of cholesterol and of cardiovascular diseases incidence [6].

The persimmon fruit (*Diospyros kaki*) is considered a source of antioxidant substances, mainly from the high molecular weight tannin fraction. A number of in vitro studies have shown that these tannin substances can reduce the cardiovascular diseases risk, hypertension, diabetes and a wide range of cancers. Also, persimmon fruit consumption reduces the rate of alcohol absorption and metabolism, relieving hangover symptoms [8].

Most literature data show that the kiwi fruit pH is around 3.5 [7; 10]. The pH of mango fruit varies widely within the range of 3.00-4.80, as reported in the literature. The different results reported by the authors are mainly related to the fruit variety and the degree of maturation at which determinations have been made, considering that mango fruit is anyway a fairly perishable tropical fruit [12; 31]. Avocado pH also varies widely, depending on the maturation stage (5.68 - 6.30 on the data reported by Ozdemir et al., 2009) and decreases later on during storage [18, 21]. Certain authors reported for avocado pH values ranging from 6.0 to 6.5 [30]. With regard to the persimmon fruit, the number of storage days and packing are critical factors for the pH value. Khan et al. (2007) reports declines in pH value from 5.7 to 3.66 depending on these factors [16]. Previously, Sargent et al. (1993) reported pH values ranging from 5.29 to 5.86 for a variety of persimmon (*Fuyu* variety) cultivated in California and kept under different temperature conditions over a week [25]. Altunas et al. (2010) reported an average pH value of 5.5 for the same variety grown in the Black Sea region [1]. On the other hand, for varieties cultivated in the eastern Mediterranean part of Turkey, Yýldýz et al. (2007) found an average pH value of 5.0, for the persimmon juice [33].

Fruits protein content is essential for their nutritional value. Data from the literature show that kiwi fruit has a protein content of between 0.81 and 1.52%, depending on several factors, among which the variety and culture conditions seem to be very decisive [27]. For mango fruit, protein content ranges between 0.11-0.42% in juice or nectar and 0.82% in fruit pulp [26]. The average protein content of the avocado fruit is estimated to be about 1.8%, but there is a significant variation between different varieties and between different parts of the fruit. So, in *Fuerte* variety, the protein content is: 1.33±0.12% in peel, 1.20±0. 36% in pulp and 2.22±0.46% in seeds [23]. In the case of persimmon fruit, the protein content may range from 0.58% to 0.8% [4].

Considering that PME activity is correlated with loss of fruit consistency during storage, as a result of pectic substances degradation, the determination of PME activity is essential for overall assessing the degree of maturation and the quality of the fruits. Numerous studies have shown that the dynamics of the process of depolymerization and decrease of the methylation degree of pectic substances is characteristic for each species of fruits, being dependent on the complexity of pectic molecules, pectolytic enzymes, as well as on the intensity of metabolism. Activating or inhibiting factors of the process are: temperature, pH, presence of anions or cations etc. [3]. Practically, PME activity is directly proportional to the level of fruit maturation, going down to overripe fruits, that have passed the stage of consumption [15; 35]. The various methods of determination, the degree of fruits maturation, as well as the units in which the PME activity is expressed, make the results very different from author to author [14]. Also, thermal treatments of fruits significantly diminishes PME activity [9]. Thus, Gonzales (2011) reported the thermal inactivation of PME in mango fruit [11].

The main objective of the research was to highlight the comparative PME activity in the juice of several tropical fruits, provided by supermarkets in Romania.

MATERIALS AND METHODS

There were randomly purchased from a supermarket, imported tropical fruits (3 pieces of each assortment), namely: kiwi, mango, avocado and persimmon, in order to evaluate the pectinmethylestrase activity (PME). The fruits were fresh, ripe, seemingly free from signs of excessive soaking, indicating an advanced maturation.

In order to maintain the variability that we can not control, in low limits, each type of fruit was purchased from a single source or from the same batch, because their ripening stage was similar. Also, the fruits have been selected so, that on the assortments, they can be of comparable size.

The fruits were initially washed with water and dried at ambient temperature. Subsequently, they were peeled and cut into small pieces. These pieces were subjected to a homogenization with a blender, in order to be centrifuged and to obtain the juice. There were three juices replicas of each fruit assortment.

We determined the pH of the juices. Also, the protein content was analyzed by the use of the Folin - Ciocalteu reagent [13; 17; 20]. Extraction of PME was performed according to a protocol initiated by Zainon et al. (2004), which was also used by other authors, such as Avang et al. (2013) [2, 34].

Thus, 10 g of crushed fruit pulp was sampled and diluted with 20 ml of a 0.1 M sodium citrate buffer solution, in which polyvinylpyrrolidone, NaCl, EDTA and β-mercaptoethanol were dissolved according to the recipe for the preparation of the reagent, reported by Awang et al (2013), pH=4.6 [2].

The samples thus obtained (3 replicas / fruit x 4 fruits = 12 samples) were shaken for 30 minutes in containers immersed in ice water, after which they were centrifuged for 20 minutes with ultracentrifuge at 10,000 rpm at 4° C. The crude extracts (supernatants) were stored in the freezer to avoid alteration and destruction of the enzymatic activity, until analysis of PME activity. Enzymatic analysis was made by the titrimetric method proposed by Rouse and Atkins (1954) and adapted by Awang et al. (2013) [2, 24]. 0.5 ml of crude extract was added to 25 ml of 0.1M acetate buffer, pH 4.5 (containing pectin and NaCl) according to the reagent preparation recipe reported by Awang et al., (2013) [2]. The mixture was titrated with a 0.01N NaOH solution, until the pH had been stabilized at 7.3. The titration was done at 30^0C. The PME activity was expressed in ηEqv carboxil/gs groups.

Descriptive statistics of all analyzed parameters (pH, protein content, PME activity) were calculated and one way variance analysis (ANOVA) was performed for PME activities. Subsequently, the differences in PME activities among the tropical fruits assortments, were revealed by the post hoc Tukey test.

The experimental results have been subjected to computer-assisted statistical calculation, using StatSoft Statistica 7.

RESULTS AND DISCUSSIONS

Descriptive statistical parameters for kiwi, mango, avocado and persimmon pH are shown in Table 1 (n=3)

Table 1. Estimators of tropical fruits pH variability

Fruit	Mean (X)	Stand. dev. (s_x)	Variab. coeff. (CV%)
Kiwi	4.133	0.040	0.960
Mango	4.553	0.028	0.614
Avocado	5.866	0.115	1.960
Persimmon	5.250	0.034	0.647

Source: Own calculation based on the experimental results.

The pH values for kiwi and mango are similar or close to those in the literature (3.1-3.96, respectively 3.40-4.63), in avocado there were slightly lower values of pH (5.866) than those in the literature (6.27 - 6.58). The persimmon fruit had a higher pH (5.25) compared to that reported by other authors (4.42-4.70) [36]. Avocado and persimmon showed more alkaline pH values than kiwi and mango. The variation coefficients have been reduced to all tropical fruits varieties. We observed equal variances, because the variances ratio F between fruits was not significant. Although the tropical fruits pH values are placed in the weak acid range, the test t reveals very significant differences in the pH of tropical fruits (t varies between 8,897*** and

t=36,335***; knowing that: *p<0.05 significant; **p<0.01 very significant; ***p<0.001 extremely significant).

Determination of the protein content of fruit juices, with the Folin-Ciocalteu reagent, was made on the basis of the regression equation of the standard curve:

D.O. 660 nm = 0.0482 + 0.0034x, with a correlation coefficient of r=0.9965 (Table 2).

Table 2. Estimators of protein variability in tropical fruits juice(n=3)

Fruit	Mean(X) %	Stand. dev. (s_x)	Variab. coeff. (CV%)
Kiwi	0,779	0,003	0.385
Mango	0,317	0,018	5.678
Avocado	0,324	0,003	0.925
Persimmon	0,485	0,006	1.237

Source: Own calculation based on the experimental results.

Protein values vary greatly in juices of exotic fruits, taking into account the literature data. Some authors believe that juices contain only protein traces or protein content is zero [37; 38]. The values of the protein content in the fruits juices obtained by us are largely consistent with the data obtained by other authors.

In kiwi juice the protein content was 0.779%, insignificantly low compared to 0.81-1.52% range obtained by other authors [27]. The value of protein content in mango juice (0.317%) was similar to that in the literature (0.11-0.42%) [26]. In avocado and persimon juices, the found protein concentrations were lower than those reported by some authors, namely 0.324% and 0.485%, compared to 1.2% for avocado and 0.58-0.8% for persimmon [4; 26]. Variation coefficients of protein content were kept down, as we planned at the beginning of the experiment, the highest being in mango (5.678%).

Selected fruits juices contained relatively low amounts of protein, but very different among tropical fruits. In our experiment, the t test reveals an extremely significant higher protein content in kiwi and persimmon juices, compared to other juices (ranged from 14.246*** to 179.365*** p<0.001). Mango and avocado juices had a similar protein content (t=0.281 ns).

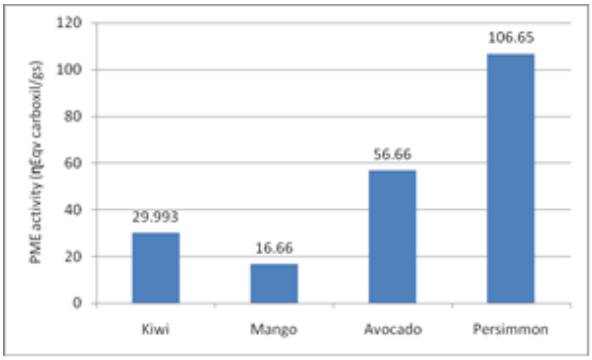
The PME activity titrimetric analysis highlighted the following values for the selected fruits juice (Table 3).

Table 3. Variability estimators of tropical fruits juice PME activity (ηEqv carboxil/gs)

Fruit	Mean (X)	Stand. dev. (s_x)	Variab. coeff. (CV%)
Kiwi	29.993	3.335	11.110
Mango	16.660	3.330	19.980
Avocado	56.660	10.000	17.640
Persimmon	106.650	53.330	50.000

Source: Own calculation based on the experimental results.

The PME activity variability coefficients are increased in selected tropical fruits juice, especially in the juice of persimmon (50%), where the standard deviation is extremely high. Substantial differences are observed between PME activities in the analyzed fruits juice (Figure 1).



Source: Design based on the experimental results.
Figure 1. PME activity of tropical fruits

Analysis of the variance (ANOVA) highlighted the existence of significant differences in PME activity between tropical fruits juice (Table 4).

Table 4. Analysis of PME activity variance in tropical fruits juice

Effect	SS (mean sum of squares)	df (degree of freed.	MS (mean square for errors)	F distri-bution	p
Intercept	33063.45	1	33063.45		
Fruit	14221.75	3	4740.58	**6.392** (4.07)	**0.016**
Error	5932.60	8	741.58		

Source: Own calculation based on the experimental results.

Following the table above, it is noted that the calculated F value of 6.392 was higher than the theoretical value of 4.07 for p<0.05.

It can be argued with a probability of less than 5% to be wrong, that there were real differences in the PME activity, between at least two of the fruits juices. We applied a post hoc single-step multiple comparison analysis, namely Tukey's HSD (honestly significant difference) test, to clarify which of the analyzed fruits had different PME activities from others (Table 5).

Table 5. Tukey HSD test for PME activity

Fruit (mean)		Kiwi	Mango	Persi-mmon	Avo-cado
		29.933	16.660	106.650	56.660
P	Kiwi				
	Mango	0.929			
	Persimmon	**0.035**	**0.015**		
	Avocado	0.644	0.340	0.190	

Source: Own calculation based on the experimental results.

It is noticed that the PME activity at persimmon was significantly higher than the PME activity in kiwi and mango ($p=0.035$ and $p=0.015$ respectively; $p<0.05$). There were no statistical differences between PME activities in persimmon and avocado.

PME activity in juices is variable, so the values found by different authors are very different. Generally, if the PME activity of a fruit is increased, the ripening and soaking process of the fruit follows. As the fruit is ripe, the PME activity goes down, due to the fact that the enzyme substrate, namely the content of pectinmethylesters decreases as well.

CONCLUSIONS

Avocado and persimmon juices had significantly higher pHs than kiwi and mango juices. The protein content was highest in kiwi juice, followed by persimmon juice, but PME activity was significantly higher in persimmon juice, followed by avocado juice. It can be said that the mango and kiwi fruits, analyzed in our experiment, seemed to be in a more advanced ripening phase, compared to avocado and persimmon fruits.

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