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Functional and conservation value of fruits - a lab approach

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Abstract

Fruits are a relevant source of phenols and ascorbate, biomolecules which scavenge reactive oxygen species. For this reason, they are considered as healthy for the human being. Fruits quality depends on their levels of antioxidants and enzyme activities that ensure their conservation. The aim of this work was to plan and execute a laboratory class of Enzymology, a discipline of Biochemistry degree of University of Évora, Portugal, for determining the functional and conservation value of three different fruits types, sold in the market of Évora, Portugal. The development of this activity allowed that students of a pilot class participate in a laboratory activity which intended to compare the content of phenols, ascorbate, and polyphenol oxidase enzyme activity present in apple, peach and blueberries pulp. At Lab activity, the students successfully determined markers of functional and conservation value of selected fruits. The skills acquired by the students, in terms of obtaining fruit pulp and their composition in antioxidants, stimulated their commitment degree on the application of biochemistry in the everyday, acquiring thereby significant learning, with a high degree of satisfaction.

Keywords: Malus domestica, Prunus persica, Vaccinium myrtillus, antioxidants, conservation, enzymology class.

1. Introduction

Natural antioxidants present in foods have attracted special attention due to their nutritional effects, prophylactic and therapeutic action in various human pathologies (Ratnam *et al.*, 2006). Among the main antioxidants of plant origin are phenolic compounds and L-ascorbate. These can block lipid peroxidation by scavenging free radicals, decomposing peroxides or acting as metal ion chelators (Çam and Durmaz, 2009). L-ascorbate is a metabolic derivative of glucose or galactose that has ubiquitous distribution among plants and animals, with some exceptions including humans, some primates and teleosts (Mann, 2002). The ability to participate in oxidation-reduction reactions is one of the well-known functions for this vitamin which is often used as a food preservative. Humans get this vitamin from their diet, using natural or preserved foods where they are in abundance, so it is imperative to provide consumers with vitamin C-rich products (Pénicaud *et al.*, 2010).

Polyphenoloxidase enzyme (PPO) plays a relevant role in modulating the quality of food products as its presence has been correlated with the loss of fruit or vegetable quality during processing causing browning, loss of taste and nutritional qualities (Liu *et al.*, 2010). From a structural and functional point of view PPO is a metalloenzyme that catalyzes two distinct reactions involving phenolic compounds and dioxigen: (*i*) monophenol monooxygenase (EC 1.14.18.1) and (*ii*) diphenol oxygen oxidoreductase (EC 1.10.3.1) (Mayer, 2006). In the plant cell occurs a physical separation between PPO enzymes that express themselves in the plastides and their phenolic substrates stored in vacuoles which prevents the oxidation of phenols and consequent darkening of tissues. Physical aggression or senescence disrupt this barrier facilitating the contact of the enzyme with phenolic substrates, occurring the hydroxylation of phenols to o-quinones and respective darkening of the fruit (Toivonen and Brummmell, 2008).

This class took place in two three-hour laboratory sessions, using apples, peaches and blueberries, sold at points of sale in the city of Évora, as biological models. The lesson plan, as well as the assessment of the experimental protocols were elaborated in the scope of the discipline Enzymology of 2nd year of the Biochemistry degree of the University of Évora (Gomez *et al.*, 2007). In this experimental activity, it was intended, in a first phase, to compare the content in water-soluble proteins, antioxidants and polyphenol oxidase activity of three different fruit type, applying the teaching of biochemistry, using centrifugation and UV/Vis spectrophotometry, in the determination of the functional and conservation value of wilde-type fruits, a target that deserved particular importance given the progressive importance attached to these food properties by the juice industry.

2. Aims

Estimate the functional and conservation value of *Malus domestica*, *Prunus persica* and *Vaccinium myrtillus*, using centrifugation and UV/Vis spectrophotometry.

3. Skills to be acquired/assessed

Develop the ability to *i*) planning the experimental activity: "Functional and conservation value of fruits - a lab approach"; *ii*) apply centrifugation for fruit juice clarification; *iii*) apply UV/Vis spectrophotometry for antioxidant quantification and enzyme detection; *iv*) outline and construct calibration curves; *v*) outline and construct reaction curves; *vi*) compare functional and conservation value of fruits (Gomez *et al.*, 2007).

4. Strategy

The theme, addressed in the scope of the discipline Enzymology highlights the relevance of analytical biochemistry in determining the functional and conservation value of fruits. It took place in two moments: *i*) short theoretical exposition and planning of experimental procedure; *ii*) lab activity for obtaining juices fruit, for determining its antioxidant properties and a conservation marker.

The students, supported by the teachers, selected information available in on-line databases that allowed them to design the experience, write the protocol and obtain useful results to discuss fruit quality.

The activity occurred in the Laboratory of Analytical Biochemistry, Department of Chemistry, School of Science and Technology, University of Évora, Évora, Portugal, using as biological models the species *Malus domestica*, *Prunus persica* and *Vaccinium myrtillus*. Assays included **a**) obtaining peach, apple and blueberries pulp homogenates, **b**) determining content in water-soluble protein, phenols and ascorbate as well as **c**) determining PPO enzyme activity present in fruit pulp, using techniques such as centrifugation and UV/Vis spectrophotometry.

The universe covered a 15-member pilot class, enrolled in the discipline of Enzymology of the Biochemistry degree of the University of Évora in the academic year of 2017/2018, with an age distribution of 19 (28%), 20 (60%) and over 20 (12%) years old, where 44% were male and 56% female, attending for the first time in that discipline. The action lasted two 3-hour sessions. The activity assessment focused on skills acquired during and after the action, using the same set of problem questions asked before and after the completion of the three phases of the experimental activity (**a**, **b** and **c**).

5. Methods

Approximately 5 g of pulp of *Malus domestica*, *Prunus persica* and *Vaccinium myrtillus* fruits were homogenized in water (2:1, w/v). Homogenates were clarified by centrifugation at 18000 *g*, 40 min, 4 °C in a Hermle Z 323K centrifuge. Water-soluble protein content (720 nm) (Lowry *et al.*, 1951), ascorbate (534 nm) (Cai and Tang, 1999), total phenols (760 nm) (Singleton and Rossi, 1965) and PPO enzymatic activity (420 nm) were determined at 37 °C (Valero *et al.*, 1991), using a Genesys 10S spectrophotometer.

Students assessment skills survey realised before and after the lab approach had a score from 0 to 100. Significant differences between the two moments were detected by the t-student test for independent samples and the analysis of the highest scores in the phases **a**, **b** and **c** by ANOVA II, post-hoc test HSD of Tuckey, by Software SPSS 24, licensed to the University of Évora. The degree of students' personal satisfaction regarding the lab activity was detected by an opinion survey, and analyzed by the percentage value of the answers obtained in each item (Sokal and Rohlf, 1997, Gomez *et al.*, 2007).

6. Results

6.1. Obtaining fruit pulp homogenates and their clarification by centrifugation

In this sub-theme, students successfully prepare homogenates (5 replicates) of apple, peach and blueberry pulp, having the possibility to acquire laboratory skills in the execution of homogenization and centrifugation techniques. The students obtained a higher volume of peach pulp homogenate and a smaller volume of blueberry pulp homogenates (Figure 1).

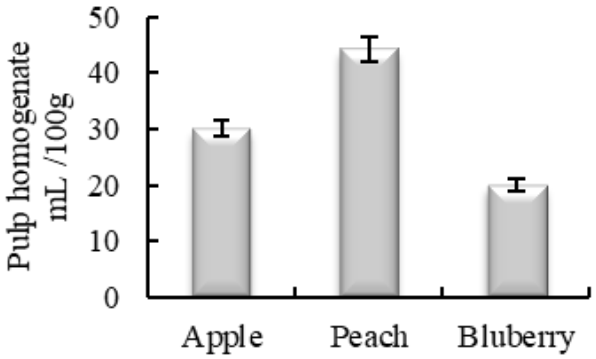

Figure1. Pulp homogenate volume of Malus domestica, Prunus persica and Vaccinium myrtillus (mL/100g fruit). Each bar represents the mean of five replicates, and the error bars represent ±1 SE..

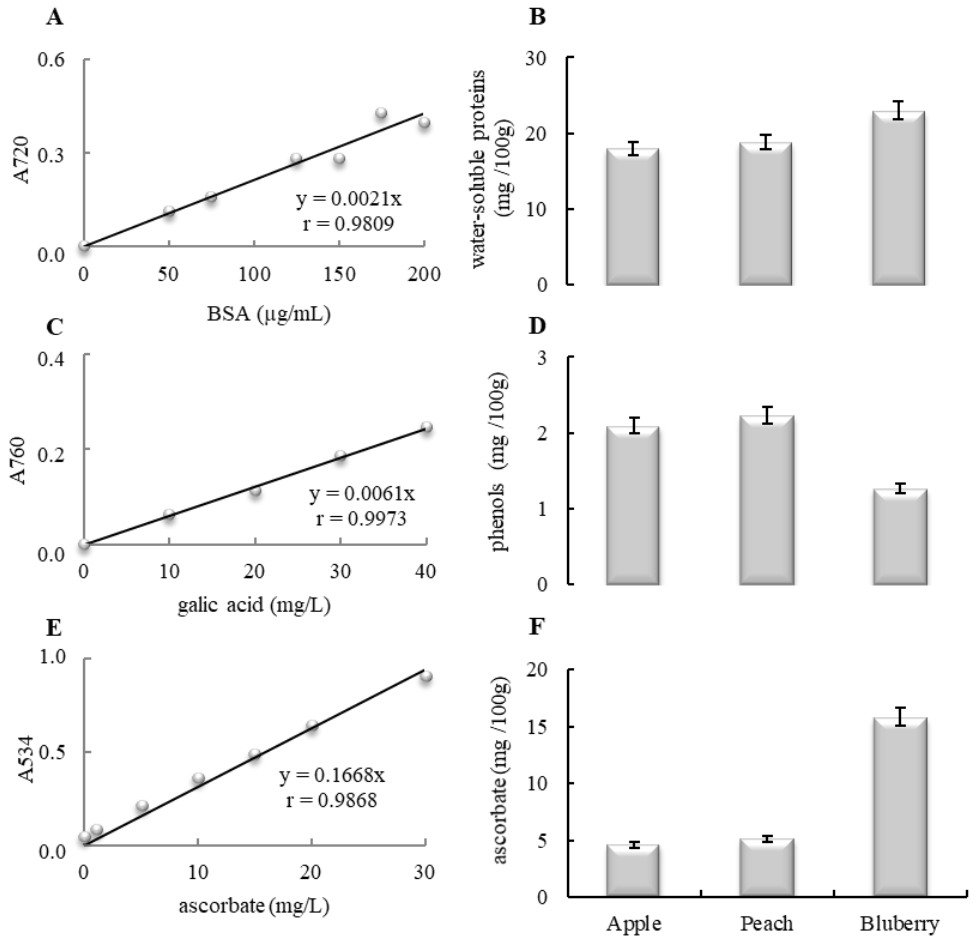

Figure 2. Calibration curves of proteins (A), phenols (C) and ascorbate (E). Content of water-soluble proteins (B), phenols (D) and ascorbate (F) from samples of Malus domestica, Prunus persica and Vaccinium myrtillus. Each bar represents the mean of five replicates, and the error bars represent ±1 SE.

6.2. Determination of the composition in water-soluble proteins, ascorbate and phenols by spectrophotometry.

In this sub-theme students had the opportunity to use UV/Vis spectrophotometry for prepare calibration curves for quantification of protein, ascorbate and phenol using BSA, gallic acid and ascorbate as standard. The critical analysis of the results allowed them to evaluate the quality of the obtained curves in terms of slope (positive), origin (0.0) and correlation coefficient (0.976 <r <0.997), confirming its agreement with Beer's law (Figure 2A, 2C and 2E). The students were also able to determine the content of water-soluble proteins, ascorbate and phenols, by graphically interpolation the readings of the samples in the obtained curves,

managing, for example, to identify the fruits richest in water-soluble proteins (blueberries), ascorbate (blueberries) and phenols (peaches and apples) (Figure 2B, 2D and 2F).

6.3. Determination of PPO enzyme activity by UV/Vis spectrophotometry

Regarding this sub-theme, the students were able to assess the linearity of the reaction catalyzed by PPO enzyme, using reaction curves such as the one illustrated in Figure 3A, which exhibited correlation coefficients that varied between 0.9887 and 0.9734. Subsequently, they also had the opportunity to determine the enzyme activity from the slope of reaction curves as well as to calculate the specific activity using the protein content estimated for each sample in the sub-theme **a**. Thus enabling students to acquire skills in determining enzymatic activities by UV/vis spectrophotometry. Figure 3B show that peach and apple pulp exhibited PPO values higher than those detected in the blueberry pulp, a marker of the greater tendency of these fruits to darken by mechanical action. The discussion of these results highlighted the contribution of this methodology in the definition of fruit preservation strategies.

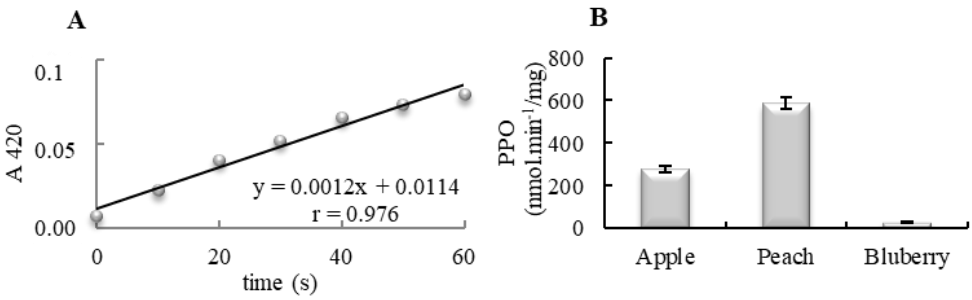

Figure 3. PPO (A) enzymatic reaction curve and PPO specific activity of samples from M. domestica, P. persica and V. myrtillus (B). Each bar represents the mean of five replicates, and the error bars represent ±1 SE).

6.4. Students evaluation

The average score obtained before and after the experimental activity 28.65% and 49.72%, respectively, revealed a positive overall evolution of the students' skills in terms of manipulation of centrifuges and spectrophotometers to obtain fruit extracts and to quantify its antioxidants level and conservation marker (Figure 4A). The t-student test revealed that occurred an increase of 21.08 percentage points, in the average score obtained after the development of the lab activity ($p < 0.05$) (Figures 4A and 4B). Students also demonstrated an increase in the skils to discuss the validity of results, formulating and discussing hypotheses for comparison of the functional and conservation value of the fruits. Figure 4B shows in detail a positive ($p < 0.05$) increment in the experimental development phases: (**a**) obtaining fruit pulp homogenates and clarifying them by centrifugation; (**b**) determining the

composition of water-soluble proteins; ascorbate and phenols by UV/Vis spectrophotometry; (**c**) determination of PPO activity by spectrophotometry on the score previously obtained.

The results of the opinion survey on the teaching activity, where students evaluated the performance of teachers in theoretical and experimental sessions and the impact of biochemistry on the characterization of the functional value of fruits, revealed that most respondents considered the size of the lab activity to be good (87.5%) and suitable the degree of difficulty (93%) as well as the means of work (90%).). Regarding the degree of overall satisfaction, 75% of students were very pleased to be able to apply centrifugation and spectrophotometric techniques to detect biological properties of fruits, relevant to human health and with consequent socioeconomic importance (69%). Among the aspects they liked least, they mentioned only waiting times (10%). Most students (75%) considered that they learned in a very pleasant way. Most students (82%) suggest that this activity be made available to students of the 2nd year of the degree in Biochemistry in order to provide more direct contact between the research environment and its application to everyday problems, such as food quality evaluation, since this activity well-illustrated some aspects taught in the theoretical plenary sessions of Enzymology discipline (98%).

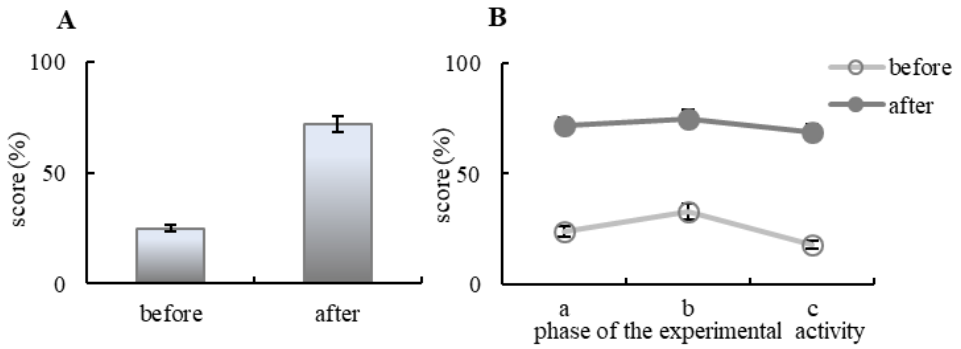

Figure 4 - Overall average score (A) obtained by students, before and after the development of the action and in each phase of the experimental activity (B). The values are presented as the mean of five replicates ± SE and the differences observed between the means of the two groups are statistically significant (p <0.05).

7. Conclusions

Students in the Enzymology Pilot Class, from the 2nd year of the Biochemistry degree, successfully participated in the planning of the activity: "Functional and conservation value of fruits - a lab approach". The preparation of the lab activity allowed them to extend valences such as: *i*) preparing and clarifying fruit pulp homogenates by centrifugation; *ii*) quantifying, water-soluble proteins, ascorbate, phenols and the PPO enzyme activity by UV/Vis spectrophotometry. The obtained results, such as calibration curves for proteins, phenols and ascorbate as well as reaction curves for PPO activity, stimulated in students a great desire

and curiosity to evaluate the antioxidant composition of the analyzed samples, expanding their knowledge regarding the functional value of fruits and their implication in its commercial value. The evaluation of skills developed by the students, confronted with the same set of problem questions posed before and after the different phases of the experimental activity, revealed that they improved their level of achievement, thus acquiring significant learning.

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