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The effect of different lycopene dyeing solutions on rice colour stability

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Lycopene is a bright red substance that belongs to a group of naturally occurring pigments known as carotenoids, being the most efficient antioxidant of this group. Tomatoes are the major source of natural lycopene in the human diet, but synthetic lycopene is commonly used as a food ingredient. In this experiment, three varieties of Portuguese “Carolino” rice (*Oryza sativa* L., subsp. *japonica*) were coloured by saturated solutions of lycopene powder (10% of purity, with starch as excipient), with vinegar (56,5% w/w), ethanol (99,0% w/w), rice bran oil (92% w/w) and water (100% w/w). Colour was measured after colouring and after 21, 42, 63 days of storage. The antioxidant properties of dyed rice were also evaluated at 0 and 63 storage days. The antioxidant activity of most of dyed rice samples did not significantly changed over time. Results showed that vinegar and water conferred a redder colour to the rice kernels and ethanol led to the lower red colour values immediately after dyeing. Red colour intensity of all samples steadily decreased over time. Samples that used water and vinegar still presented at day 63 an evident orange colour, that was considered an appealing sensory attribute being an alternative to conventional white rice.

1. Introduction

Rice (*Oryza sativa*) is one of the most important foods in world supplying as much as half of the daily calories of the world population (Abbas et al., 2011). In many parts of the world, but specially in the East, South and South East Asia, it is a staple food and the second-most consumed cereal grain. Though in smaller amounts, it contains all the essential amino acids required for a good health (Patil & Khan, 2011). Lycopene belongs to the group of natural carotenoids that is found in many fruits, vegetables, and other green plants, being predominantly found in tomatoes and tomato-based products. It is a natural pigment synthesized by plants and microorganisms to absorb light during photosynthesis and to protect them against photosensitization (Rao, Ray, & Rao, 2006). Lycopene is the most prevalent carotenoid in the Western diet and normally the most abundant in human serum, with a very long history of safe use by dietary consumption on a worldwide basis (McClain & Bausch, 2003), and has attracted considerable interest in recent years as an important phytochemical with a beneficial role in human health due to its antioxidant properties (Rao et al., 2006). Synthetic lycopene is a red crystalline fat-soluble powder that is synthetised by the Wittig reaction, with raw materials commonly used in the production of other carotenoids (McClain & Bausch, 2003). Hoppe, Kramer, van den Berg, Steenge, & van Vliet, (2003), determined the relative bioavailabilities of synthetic and tomato-based lycopene and concluded that synthetic and natural lycopene are equivalent sources of lycopene with no interaction with other circulating carotenoids. Appearance is an all-inclusive term involving size, shape, texture, mass, gloss, colour and others, and one of the most important sensory quality attributes of fresh and processed food, products and their marketing (Pathare, Opara, & Al-Said, 2013). The challenge to the food industry is to provide visually appealing foods that taste good and meet the consumers demands on quality and price (Downham & Collins, 2000). Colour is considered a fundamental physical property of agriculture products and foods, correlating with other physical, chemical and sensorial indicators of product quality (Mendoza, Dejmek, & Aguilera, 2006), that could influence flavour perception (Solymosi, Latruffe, Morant-Manceau, & Schoefs, 2015), and appetite is stimulated or dampened in almost direct relation to the observer's reaction to colour (Downham & Collins, 2000). The colour of an object can be described by several colour coordinate systems developed for colorimeter measurement, where all are mathematically convertible (Martins & Silva, 2002), and one of the most used in the food industry

is the CIELAB coordinates (L*, a*, b*), where parameter a* takes positive values for red colour and negative values for the green, whereas b* takes positive values for yellow colour and negative values for blue. L* is an approximate measurement of luminosity, which is the property according to which each colour can be considered as equivalent to a member of the greyscale, between black and white (Granato & Masson, 2010). Colour changes can be measured as the modulus of the distance vector between the initial colour values and the actual colour coordinates. This value, called the total colour difference (ΔE), indicates the magnitude of colour difference between samples (Pathare et al., 2013). The objective of this work is to determine the effectiveness of the solvents used for the incorporation of lycopene in different Portuguese Carolino rice kernel varieties, respecting to colour stability and antioxidant properties overtime.

2. Materials and methods

2.1 Materials

Three varieties of Portuguese Carolino rice (*Oryza sativa* L., subsp. *japonica*) were used: Ariete, Teti and Luna. Each variety used in this experiment corresponds to a different region/river of Portugal (Mondego, Tejo and Sado respectively). Methanol HPLC gradient HPLC grade and ethanol 70% (v/v) from Fisher Scientific, DPPH, ABTS and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) from Sigma Aldrich. Commercial wine vinegar (6.5% acidity) and 100% pure rice bran oil from Alfa One brand. Synthetic lycopene 10% (DC/AF) in a matrix of starch from Divis Nutraceuticals.

2.2 Dyeing method

The three varieties of rice were coloured by saturated solutions of powdered lycopene (10% purity, with starch as excipient). Four dyeing solutions were prepared with different incorporation solvents: vinegar (56.5% w/w), ethanol (99,0% w/w), rice bran oil (92,0% w/w) and water (100% w/w), mixing and agitated manually inside a plastic container. All samples were dried in a vacuum oven (40 ºC, 15 minutes for ethanol, vinegar and water; 60 minutes for oil), vacuum packed and stored at room temperature in the absence of light.

2.3 Colour determination and total colour difference

Rice colour measurements were performed in accordance to the CIE L*, a*, and b* colour system using a Minolta colourimeter model CR-300 (Konica Minolta Tokyo, Japan). The total colour change is given by the colour difference (ΔE) as shown in Eq (1). The instrument was calibrated against a standard white colour Plate (Y=93.9, x=0.313, y=0.321). Samples of rice were inserted in a Petri plate and colour was measured by placing the colorimeter head directly on the rice kernels. Sampling was performed just after drying and after 21, 42 and 63 days of storage. Ten replicates per sample were measured in all sampling moments.

$$\Delta E = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}} \quad (1)$$

2.4 Antioxidant capacity determination

Extraction method was adapted from Shao, Zhang, & Bao (2011). Samples of coloured and white rice were milled. Sampling was performed just after drying and after 63 days of storage. All coloured samples were analysed in triplicate in both sampling moments. White rice samples were analysed just after drying. Extraction was made by mixing 20 mL of methanol (80% v/v) with 5 grams of milled rice using Falcon conical centrifuge tubes. After 24 hours of contact at room temperature, each mix was centrifuged at 4000 g for 15 minutes and the supernatants were pooled and stored at 4°C. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method was based on Brand-Williams, Cuvelier, & Berset (1995). A volume of 0.1 mL of diluted extract stock solution (in methanol) was mixed with 3.9 mL of DPPH in methanol, standing at room temperature in absence of light for 30 minutes prior to measuring the solution absorbance at 517 nm. The control was a DPPH solution containing absolute methanol instead of the sample. The results were obtained as mg of Trolox equivalent per mL of extract. The standard curve was prepared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at 0, 40, 80, 100, 200, 400 and 800 µM. ABTS (2,2´-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging method was based on Porter (2012) and Re et al., (1999). A volume of 30 µL of diluted extract stock solution (in methanol) was mixed with 3.0 mL of ABTS in methanol, standing in the dark at room temperature for 6 minutes prior to measuring the solution absorbance at 734 nm. Control was a ABTS solution containing absolute methanol instead of the sample. The results were obtained as mg of Trolox equivalent per mL of extract. The standard curve was prepared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at 0, 40, 80, 100, 200, 400 and 800 µM.

2.5 Statistical analysis

Statistical analysis was performed using Statistica for Windows software package, version 7 (Stat Soft Inc., Tulsa, USA). Antioxidant activity (DPPH and ABTS) variation over time was tested with variance analysis (ANOVA), followed by post hoc Tukey's test. Differences were considered significant at the significance level of 0.05 ($p < 0.05$).

3. Results and discussion

3.1 Colour determination

Total colour difference (ΔE) was calculated and represented in the graphics of Figures 1, 2, 3 and 4. The value of ΔE indicates the absolute difference between the day when rice was coloured (day 0) with the following sampling times (21, 42 and 63 days). In Figure 1 is represented the graph of total colour difference using vinegar.

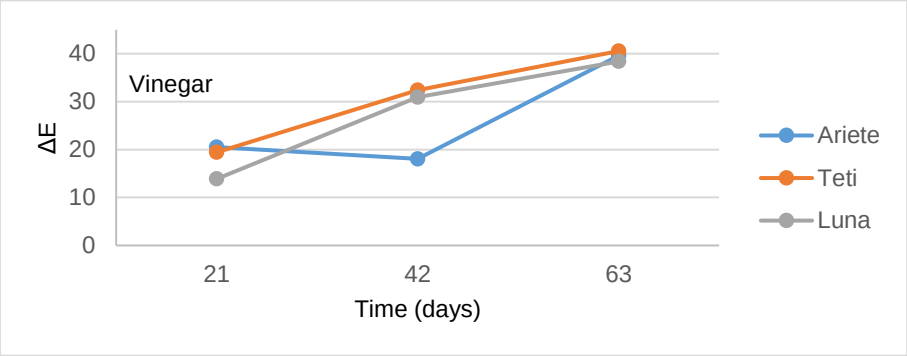

Figure 1: Graphical representation of ΔE values of colouration in rice kernels varieties using vinegar as solvent.

It can be noticed an increase of the total colour difference in samples using vinegar as solvent. ΔE of the day 63 practically doubled in relation to day 21, indicating a substantial change in colour, as also shown in Figure 2. The colour difference trend along days appears to be the same for all varieties, excluding Ariete in day 42, which could be an outlier.

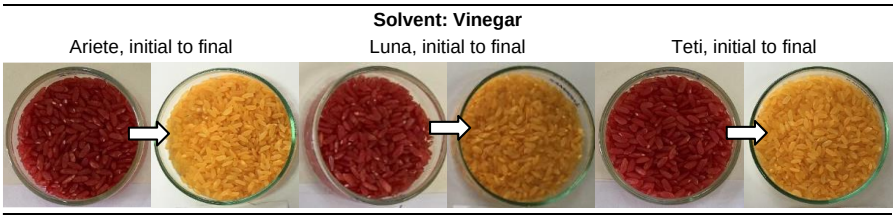

Figure 2: Photos of the colour variations in rice kernels varieties (day 0 to day 63) using vinegar as solvent.

In Figure 3 is represented the graph of total colour difference using ethanol.

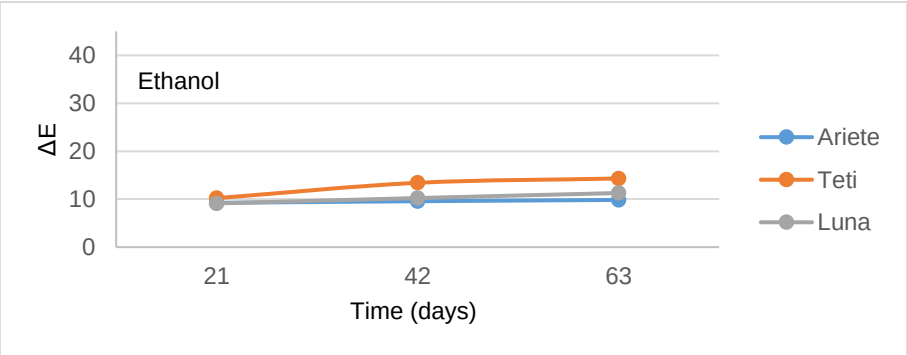

Figure 3: Graphical representation of ΔE values of colouration in rice kernels varieties using ethanol as solvent.

When using ethanol as solvent, values of total colour difference do not vary much, meaning that the measured difference in relation to day 0 was almost the same. It can be explained by the lack colour intensity conferred by ethanol to the rice, as represented in Figure 4.

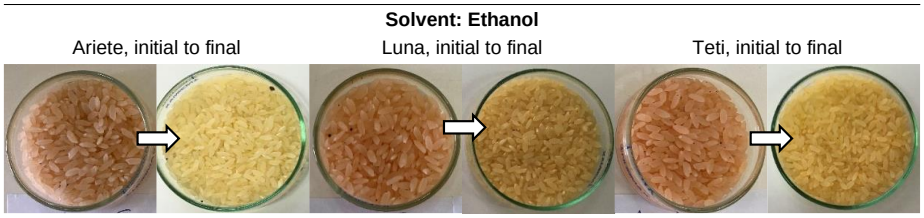

Figure 4: Photos of the colour variations in rice kernels varieties (day 0 to day 63) using ethanol as solvent.

A lycopene water solution to colour rice was tested and colour difference was calculated, as shown in Figure 5.

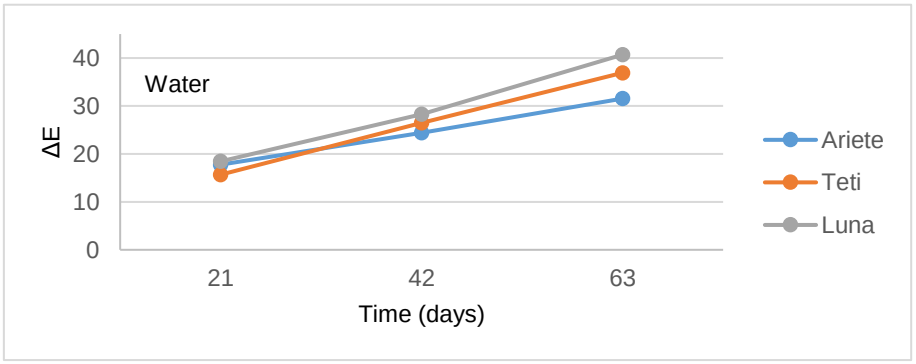

Figure 5: Graphical representation of ΔE values of in rice kernels varieties colouration using water as solvent.

Observing the graph results it is noticeable that total colour difference steadily raised during sampling days, indicating that the initial colour was fading, as seen in Figure 6.

Figure 6: Photos of colour variations in rice kernels varieties (day 0 to day 63) using water as solvent.

In Figure 7 it is represented the total colour difference when using rice bran oil as a solvent.

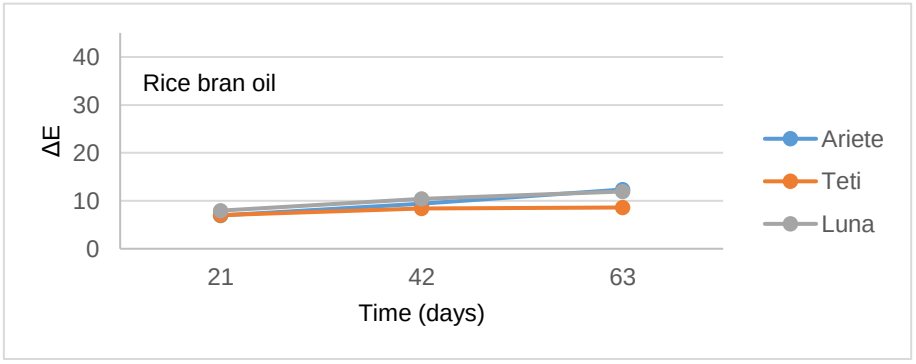

Figure 7: Graphical representation of ΔE values of colouration in rice kernels varieties using rice bran oil as solvent.

Rice bran oil had a similar effect to ethanol in the colouring of rice. As observed in Figure 7, the measured difference in relation to day 0 was very low, also explained by the initial low colour intensity as seen in Figure 8.

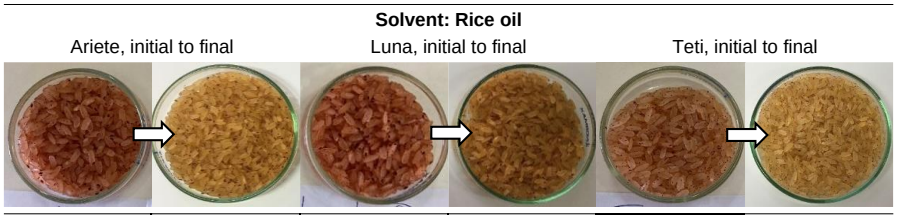

Figure 8: Photos of the colour variations in rice kernels varieties (day 0 to day 63) using rice bran oil as solvent.

3.2 Antioxidant capacity

The values of antioxidant activity were determined for each coloured rice sample and for white rice (as a control sample) by the DPPH and ABTS methods in all sampling moments. Antioxidant activity of white rice was only determined in day 0. As shown in Table 1, almost all coloured samples had higher values of DPPH and ABTS than control (white rice), indicating that the addition of lycopene increased the antioxidant activity of coloured rice.

Table 1: Antioxidant activity (µmol eq trolox/g) of Ariete, Luna and Teti varieties. Different superscripts within rows (0 days and 63 days) indicate significant differences (p<0.05).

	Ariete			
	DPPH		ABTS	
White rice	0.24 ± 0.02		0.23 ± 0.01	
	0 days	63 days	0 days	63 days
Water	0.25 ± 0.00	0.25 ± 0.01	0.29 ± 0.05	0.28 ± 0.01
Vinegar	0.28 ± 0.01	0.26 ± 0.02	0.36 ± 0.01[a]	0.29 ± 0.03[b]
Ethanol	0.27 ± 0.01	0.25 ± 0.01	0.23 ± 0.01	0.22 ± 0.01
Rice bran oil	0.40 ± 0.01	0.39 ± 0.01	0.42 ± 0.08	0.40 ± 0.04
	Luna			
White rice	0.23 ± 0.01		0.23 ± 0.01	
	0 days	63 days	0 days	63 days
Water	0.25 ± 0.02	0.24 ± 0.04	0.26 ± 0.01	0.25 ± 0.01
Vinegar	0.34 ± 0.01	0.33 ± 0.05	0.29 ± 0.01	0.29 ± 0.01
Ethanol	0.22 ± 0.01	0.21 ± 0.02	0.22 ± 0.01	0.22 ± 0.01
Rice bran oil	0.44 ± 0.01	0.42 ± 0.02	0.49 ± 0.02[a]	0.40 ± 0.01[b]
	Teti			
White rice	0.24 ± 0.01		0.25 ± 0.03	
	0 days	63 days	0 days	63 days
Water	0.27 ± 0.01	0.26 ± 0.01	0.25 ± 0.00	0.24 ± 0.02
Vinegar	0.24 ± 0.01	0.23 ± 0.01	0.36 ± 0.00	0.36 ± 0.03
Ethanol	0.30 ± 0.01	0.28 ± 0.01	0.36 ± 0.01	0.36 ± 0.03
Rice bran oil	0.36 ± 0.00	0.30 ± 0.01	0.42 ± 0.01	0.40 ± 0.01

Results evidence that rice bran oil coloured samples always present the highest antioxidant properties. However, the values of Ariete/Ethanol for ABTS, Luna/Ethanol for DPPH and ABTS, Teti/Vinegar for DPPH and Teti/Water for ABTS were the same as for white rice just after dyeing. This fact could be explained by oxidation of the lycopene by light or solvents during the sample preparation. Antioxidant activity values of day 63 were expected to be lower than day 0, assuming diverse oxidation factors such as light or oxygen. However, just in two samples (Ariete/Vinegar and Luna/Rice bran oil) values of ABTS a statistically significant difference ($p<0.05$) between initial and final time were found, indicating a loss of antioxidant activity. Probably the methods used might not be sufficient sensitive for screening of antioxidant properties of both lipophilic and hydrophilic samples and they might lead to underestimation or overestimation of antioxidant properties (Sadeer et al, 2020). Lycopene presence in the tested samples absorbing in the same wavelength region as the DPPH radicals, might interfere with absorbance readings (Yeo & Shahidi, 2019; Celiz, Renfige & Finetti, 2020).

4. Conclusions

Research studies on this specific subject, rice dyeing and rice dyeing solutions, are scarce and as far as we know this is the first time the effect of different lycopene dyeing solutions on rice colour stability was studied. Results revealed that the best solvents for colouring the tested rice varieties with lycopene, regarding colour stability, were water and vinegar, samples keeping an appealing orange colour after 63 days of storage. In most samples, the addition of lycopene slightly increased antioxidant activity of all rice samples, rice bran oil coloured samples always presenting the highest antioxidant properties. However, as colour stability was the main purpose of this study it can be concluded that kernels of the three rice varieties tested, coloured with lycopene, and using water or vinegar as solvents, can be considered good alternatives to conventional white rice. Further studies of colour stability over a longer storage period are ongoing.

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