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Proceeding Paper

Valencian Paella: Synergistic Antioxidant Properties of a Complete Dish versus Its Isolated Ingredients †

Marta Cuenca-Ortolá, Amparo Alegría and Antonio Cilla *

Nutrition and Food Science Area, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain; cuenor@alumni.uv.es (M.C.-O.); amparo.alegria@uv.es (A.A.)

* Correspondence: antonio.cilla@uv.es; Tel: +34-963-544-229

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Abstract: The Mediterranean Diet (MD) is rich in plant-based foods that provide antioxidants associated with a lower risk of chronic degenerative diseases, with Valencia's paella being a representative dish. Antioxidants can be classified into extractable and non-extractable. Extractable antioxidants are easily extracted using aqueous and organic solvents. On the other hand, non-extractable antioxidants are those that are bound to the food's cellular matrix and cannot be easily extracted. This study provides information on these properties in paella, evaluating total polyphenols (TP) (Folin-Ciocalteu method) and total antioxidant capacity (TAC) (TEAC and ORAC). Per 100 g, the total values ranged from 835.65 mg GAE/dry weight (DW) for TP, 1286.80, and 77,803.54 μM Trolox/DW for TEAC and ORAC (TAC), respectively. Approximately 82% of the TAC comes from non-extractable antioxidants. Furthermore, there is an antioxidant synergy in a portion of paella compared to the sum of its ingredients: TP (478.74 vs. 168.37), TEAC (2111.10 vs. 1669.98), and ORAC (127,642.42 vs. 47,023.59), respectively. The results can contribute to expanding the information present in TAC and total polyphenol databases, as there are few studies on complete dishes, and they tend to significantly underestimate the total values due to the lack of determination of non-extractable antioxidants.

Keywords: Mediterranean Diet; antioxidant capacity; total polyphenols; TEAC; ORAC; synergy

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1. Introduction

An increasing number of scientific studies claim the existence of a positive correlation between the consumption of an antioxidant-rich diet and greater protection against chronic degenerative diseases [1]. The Mediterranean Diet (MD) provides a wide variety of plant-based foods, a rich source of antioxidants. Due to the high interest in studying the bioactive components of the MD, Valencian paella was chosen as one of the most representative dishes of Spanish cuisine, with its main ingredients being rice, flat green beans, butter beans, meat (chicken and rabbit), tomato, extra virgin olive oil (EVOO), saffron, water, and salt [2]. Antioxidants are defined as substances capable of delaying, preventing, or eliminating oxidation that damages a molecule by transferring to present free radicals and inhibiting such chemical reactions [3]. Antioxidant extraction in foods is frequently incomplete, resulting in a significant number of phenolic compounds remaining in the extraction residues. Therefore, they are fractionated into extractable antioxidants (aqueous-organic extracts) and non-extractable antioxidants (acid hydrolysis) [4–7]. There are three categories for determining antioxidants and polyphenols (hydrogen atom transfer, single electron transfer, and mixed method). For this study, three widely recognized and commonly used methods from each category were selected: single electron transfer (total polyphenols), hydrogen atom transfer (Oxygen Radical Absorbance Capacity (ORAC)) and mixed mode (Trolox Equivalent Antioxidant Capacity (TEAC)) (for Total

Antioxidant Capacity (TAC)) [8]. It is worth noting that there are few studies evaluating the antioxidant properties of complete dishes, and furthermore, considering both extractable and non-extractable fractions. The objective of this study was to evaluate the antioxidant potential (both extractable and non-extractable fractions) of Valencian paella and its ingredients as a representative dish of the MD. Additionally, bibliographic information was gathered about the TAC and total polyphenols in complete dishes, both Italian and Spanish, to compare them with Valencian paella [4,5,9]. At the same time, the possible synergy of antioxidants in a portion of paella was assessed in comparison to the sum of antioxidants in its ingredients.

2. Materials and Methods

2.1. Sampling

Two samples of paella were collected weekly for three consecutive weeks (January 2023) from the cafeteria of the Faculty of Pharmacy at the University of Valencia. One sample was analyzed as a complete dish, while the other was separated into its individual ingredients (green beans, butter beans, meat, and rice). The samples (complete paella or individual ingredients) were homogenized at maximum speed using a household chopper (Moulinex Robot) and weighed on a precision balance ± 0.001 g (Kern PNS). Subsequently, they were placed in containers (tupperware) and were freeze dryer (Stuart block heater SBH200D). Finally, they were weighed again to determine the moisture content. Using a mortar, they were ground and stored in 50 mL vials in a desiccator.

2.2. Extraction of Antioxidants

Extractable antioxidants (aqueous-organic extracts) and non-extractable antioxidants (acid hydrolysis) were obtained according to Durazzo et al., (2017, 2019) [4,5] and Hartzfeld et al., (2002) [6], respectively.

2.3. Total Polyphenols

For the determination of total polyphenols, the Folin-Ciocalteu reagent was used, which was obtained by mixing phosphotungstic acid and phosphomolybdic acid. In the presence of an excess of this reagent, polyphenols reduced the acids to blue tungsten and molybdenum oxides. When the sample was treated with an excess of this reagent in the presence of sodium carbonate (Na_2CO_3), the absorbance reading was proportional to the concentration of polyphenols [7]. The method involved centrifuging the non-extractable polyphenol samples for 10 min at 3000 g to eliminate suspended particles formed during freezing. 100 μL of the sample (extractable or non-extractable antioxidants) were mixed with 3 mL of a 2% (*w*/*v*) sodium carbonate solution and 100 μL of 50% (*w*/*v*) Folin-Ciocalteau reagent. The mixture was allowed to stand for one hour at room temperature and in darkness, and the absorbance was measured at 765 nm using a spectrophotometer (Perkin Elmer Lambda 365 UV-VIS). Quantification was done using a calibration curve with a gallic acid standard in a range of 0–300 mg/L. The results were expressed as gallic acid equivalents (GAE)/100 g in both fresh weight (FW) and dry weight (DW) to facilitate comparison with the literature. Each sample was analyzed in triplicate.

2.4. TEAC

The technique for determining antioxidant capacity using the TEAC method was performed according to Re et al. 1999 [10]. This method involved the ability to trap free radicals present in the medium. The greenish-blue cation radical (ABTS+) was obtained from the interaction of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with potassium persulfate ($K_2S_2O_8$). The radical was a stable, water-soluble compound with a characteristic absorption spectrum. The sample was added to the radical dilution, resulting in a decrease in absorbance, and the antioxidant capacity of the sample was measured based on its ability to reduce the concentration of the radical. It was quantified in relation to 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox).

The ABTS+ stock solution was prepared from a 7 mM ABTS and 140 mM $K_2S_2O_8$ solution for 12–16 h in darkness at room temperature (stable for two days). Then, the ABTS was diluted approximately 1/100 with ethanol and adjusted to an absorbance of 0.70 ± 0.02 at 734 nm at 30 °C in the thermostat spectrophotometer (PerkinElmer UV-VIS Lambda 365). A 5 mM Trolox stock solution was prepared for the calibration curve with concentrations ranging from 0 to 1000 μM Trolox in volumetric flasks. Initially, 2 mL of the ABTS radical was added to the cuvette to measure its absorbance, then 100 μL of the samples or standard solutions were added, and the absorbance was measured again after 3 min. The analysis was performed in triplicate, and the results were expressed as Trolox equivalents (TE) μM /100 g FW and DW.

A proper dilution had been previously optimized to obtain an inhibition percentage within the range of 20–80%. For Valencian paella, no dilution was necessary, while for the individual ingredients, a 1:1 dilution was used.

2.5. ORAC

The ORAC method was based on the ability of antioxidant compounds to trap oxygen radicals [11]. This capacity was evaluated by measuring the decrease in fluorescence of fluorescein due to oxidative damage caused by the free radical generated by the thermal decomposition of 2,2′-azinobis(2-amidinopropane) dihydrochloride (AAPH). The antioxidant capacity of the sample was assessed based on time and the percentage of protection against the loss of fluorescence and was quantified using Trolox as the standard antioxidant. The reaction was carried out using a Multilabel Plate Counter VICTOR 1420 (PerkinElmer) with a fluorescence filter for an excitation wavelength of 485 nm and an emission wavelength of 535 nm. The assays were performed at 37 °C using sodium fluorescein and AAPH at concentrations of 0.015 and 120 mg/mL, respectively. The samples were diluted 1/50 with a 75 mM phosphate buffer, pH 7.4 approximately. In a multi-well white plate, 80 μL of fluorescein, 40 μL of AAPH, and 80 μL of diluted sample, Trolox (standard), or phosphate buffer (blank) were introduced. The fluorescence was recorded every 5 min for 90 min until the fluorescence of the assay was less than 5% of the initial value. The results were expressed as ET μM/100 g FW and DW. The analysis was performed in quadruplicate.

2.6. Statistical Analysis

The results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was applied, followed by Tukey's multiple range test for each of the analyzed variables (extractable polyphenols, non-extractable polyphenols, total polyphenols, and TAC) to establish differences between samples. For the comparison between the complete paella and the total sum of ingredients per serving, the Student's *t*-test was used. All statistical tests were applied with a significance level of $p < 0.05$. All analyses were performed using GraphPad Prism 8 software.

3. Results

The results of total polyphenols and TAC (ORAC and TEAC) per sample DW per 100 g in both complete paella and its ingredients are collected in Table 1. In this case total, extractable and no extractable polyphenol contents of complete paella show a significant higher value than each of its ingredients, except for extractable and no extractable values in the butter beans. With the ORAC, the paella still provides a higher value compared to the rest of the ingredients, with green beans and butter beans being the ones with the highest contribution in non-extractable antioxidants. For TEAC, the meat provides a higher contribution of antioxidants, as it contains non-extractable antioxidants, which are non-detectable in the other ingredients.

Table 1. Total antioxidant capacity evaluated through the TEAC and ORAC method and total polyphenols per 100 g dry weight (DW).

	Antioxidant	Complete Paella	Ingredients			
			Green Beans	Butter Beans	Rice	Meat
Total polyphenols (mg GAE/100 g DW)	*Extractable*	80.2 ± 8.1 ^{a}	205.2 ± 34.7 ^{b}	88.0 ± 16.1 ^{a}	107.5 ± 11.7 ^{ac}	n.d ^{d}
	Non-extractable	755.5 ± 202.3 ^{a}	324.1 ± 15.1 ^{b}	681.6 ± 9.2 ^{a}	327.3 ± 10.0 ^{b}	n.d ^{d}
	*Total **	835.7 ± 477.5 ^{a}	529.3 ± 84.1 ^{b}	769.6 ± 419.7 ^{c}	434.8 ± 155.4 ^{b}	n.d ^{d}
ORAC (μM Trolox/100g DW)	*Extractable*	3556.3 ± 183.1 ^{a}	5919.4 ± 513.1 ^{b}	5794.9 ± 547.7 ^{b}	11,262.2 ± 959.5 ^{c}	11,841.5 ± 1108.7 ^{c}
	Non-extractable	74,247.2 ± 11,170.3 ^{a}	40,821.9 +3459.0 ^{b}	37,615.2 ± 3259.2 ^{bc}	24,450.5 ± 2563.1 ^{c}	34,123.6 ± 3841.0 ^{d}
	*Total **	77,803.5 ± 2800.4 ^{a}	46,741.4 ± 24,679.8 ^{b}	43,410.1 ± 22,500.4 ^{b}	35,712.7 ± 9325.5 ^{b}	45,965.1 ± 15,755.9 ^{b}
TEAC (μM Trolox/100g DW)	*Extractable*	268.1 ± 12.8 ^{a}	1171.6 ± 160.5 ^{b}	310.0 ± 52.1 ^{ac}	934.5 ± 112.5 ^{b}	489.1 ± 34.9 ^{c}
	Non-extractable	1018.8 ± 51.9 ^{a}	n.d ^{b}	n.d ^{b}	n.d ^{b}	3669.8 ± 606.7 ^{c}
	*Total **	1286.8 ± 530.8 ^{a}	1171.6 ± 160.5 ^{a}	301.0 ± 52.2 ^{b}	934.5 ± 112.5 ^{a}	4158.8 ± 2249.1 ^{c}

The results are expressed as mean values ± standard deviation. $^{a–d}$: Different letters in the same row indicate that there are significant differences between paella and ingredients (t-student) ($p < 0.05$). n.d: not detectable. * Sum of extractable and non-extractable antioxidants. The average moisture content of the analyzed samples is: complete paella 65.09 g; green beans 83.45 g; butter beans 63.36 g; rice 60.83 g; meat 60.83 g. GAE: Gallic Acid Equivalent; ORAC: Oxygen Radical Absorbance Capacity; TEAC: Trolox Equivalent Antioxidant Capacity.

Total polyphenols, as well as ORAC and TEAC, showed a synergy in the complete paella, attributed to the non-extractable fraction (see Figure 1).

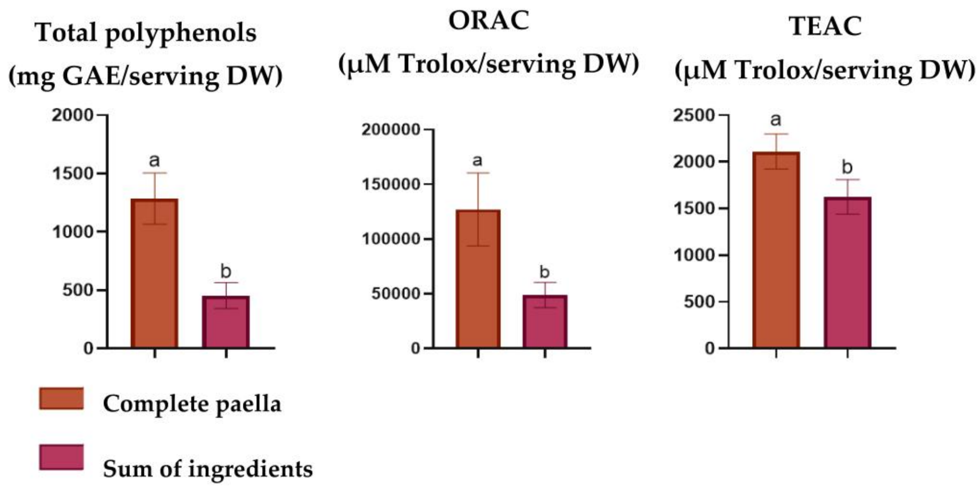

Figure 1. Sinergy in Total polyphenols and Total Antioxidant Capacity (ORAC and TEAC). a,b: Different letters in the same graph indicate that there are significant differences between paella and the sum of ingredients (*t*-test) ($p < 0.05$). GAE: Gallic Acid Equivalent; DW: Dry Weight; ORAC: Oxygen Radical Absorbance Capacity; TEAC: Trolox Equivalent Antioxidant Capacity.

4. Discussion

There is very limited information on the TAC of complete dishes compared to studies that collect information on antioxidants by ingredients. There are studies related to some Italian and Spanish dishes [4,5,9,12].

Regarding total polyphenols, Valencian paella shows values of 292.43 mg GAE/100 g FW, slightly lower than similar Spanish dishes like rice with vegetables and cod with 355.43 mg GAE/100 g FW [9]. Italian dishes have higher values than paella, such as *Carciofi alla romana* with 6643.39 vs. 835.65 mg GAE/100 g FW [4,5]. This is due to a higher presence of plant-based ingredients, which favor a higher content of polyphenols. Non-extractable polyphenols play a fundamental role in the total content, despite being undervalued in other studies [13]. Valencian paella presents non-extractable polyphenols relative percentages ranging from 90% in the complete paella to 61% in green beans. The same happens with Italian dishes [4,5], contributing around 95%, while in Spanish MD, despite being a lower value, it still provides more than half of the TAC [13].

Regarding the TAC, among the Italian dishes, *Carciofi alla romana* stands out with a higher antioxidant potential, measuring 826.68 μM/100 g DW using the FRAP method [4,5], which is lower than Valencian paella with 2111.10 μM Trolox/100 g DW using the TEAC method. In this case, non-extractable antioxidants represent 79% of the TAC. For the Spanish dishes, only extractable values are available, and the rice with vegetables and cod stands out as the dish with the highest TAC, measuring 4455.12 μM Trolox/100 g DW using the ORAC method [9], compared to 77,803.54 μM Trolox/100 g DW in paella. However, another study [12] quantifies the TAC of a dish similar to Valencian paella: vegetable paella. It presents a value of 24,470 μM Trolox/100 g FW, slightly lower than Valencian paella with 27,471.18 μM Trolox/100 g FW. This indicates that paella is a healthy and antioxidant-rich option within the MD. On the other hand, the contribution of the non-extractable fraction ranges from 68% in rice to 95% in paella.

5. Conclusions

The total polyphenol content in the complete paella is moderate compared to that indicated for other traditional dishes. The presence of vegetable ingredients significantly influences its content. Regarding the results obtained in TAC for both methods (TEAC and ORAC), they are higher than the values of the referenced dishes, as both extractable and non-extractable antioxidants were considered for Valencian paella. Therefore, it is important to determine both fractions of antioxidants to have a more comprehensive evaluation of TAC in complete dishes and avoid significant undervaluation of total values. Furthermore, a synergistic effect is confirmed in the determination of total polyphenols and TAC in Valencian paella (both in TEAC and ORAC methods) compared to the sum of its ingredients. Non-extractable antioxidants play a significant role in TAC, contributing 82% to it.

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Abbreviations

The following abbreviations are used in this manuscript:

AAPH	2,2′-azinobis(2-amidinopropane) dihydrochloride
ABTS	2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
DW	Dry Weight
EVOO	Extra Virgin Olive Oil
FW	Fresh Weight
GAE	Gallic Acid Equivalents
MD	Mediterranean Diet
ORAC	Oxygen Radical Absorbance Capacity
TAC	Total Antioxidant Capacity
TE	Trolox Equivalents
TEAC	Trolox Equivalent Antioxidant Capacity

Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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