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Antioxidant properties in Food Composition Database: new update about some Italian popular recipes

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Abstract:

Four popular dishes belonging to Italian cuisine and widely consumed in the country were experimentally prepared in a dedicated lab-kitchen following a validated and standardized protocol. This study provides their antioxidant properties evaluating the contribution of extractable and non-extractable bioactive compounds and identifying the assessment of interactions between their natural active compounds and the food matrix.

FRAP values in aqueous-organic extract ranged from the highest antioxidant activity in *torta di mele* (10.72 μmol/g d.m.) to *besciamella* (2.47 μmol/g d.m.) whereas in residue *pasta alla carbonara* reached the highest value (73.83 μmol/g d.m.) following by *pasta alla amatriciana* (68.64 μmol/g d.m.). Total Polyphenol Content (TPC) ranged between 64.3 mg/100 d.m. and 36.5 mg/100 d.m. (*torta di mele* and *pasta alla carbonara* respectively).

Our findings contribute to the updating of Italian Food Composition Database, by providing for the first time a value on antioxidant properties, in order to encourage the consumption of recipes rich in key nutrients and bioactive molecules useful and important for determining the association between diet and a healthy status.

Keywords: Italian popular recipes; Food Composition Database; antioxidant properties; extractable compounds; non-extractable compounds; FRAP; TPC.

1. Introduction

The study of food bioactivity and epidemiological investigations are increasing, underlining/emphasizing more and more the view/perspective of considering the whole food matrix of interest for the risk of disease. Following the evolution of nutrition science, currently, researchers are trying to identify the concept of "optimal nutrition" also by studying not just the characteristics and functions of the individual foods or food components, but also their combination in composite foods, dishes, meals and diets, in order to understand their global importance and impact on health.

There are very few foods that are consumed raw, mainly vegetables and fruit, while most of them are heat treated using different methods chosen according to the matrix, type of food preparation and recipe and affected by the various cultures and culinary traditions. Cooked foods and composed dishes are in fact the most used/consumed in our daily diet, but there is still little information concerning them, both in terms of their nutritional characteristics and their potential functions, whereas numerous data are available in literature on the single ingredients, without taking into account either the formulation or the effects of technological process [1]. The interactions between single food components and/or between the different ingredients of a composite dish can play an important role, amplifying the importance of the concept of "food synergy" on health [2]; also studies on technological and cooking treatments during the preparation of a recipe highlight how they can influence their total characteristics, reflecting on the state of health and well-being of people. It is well known that the physical, organoleptic and chemical changes produced in food by heat treatments, influence different parameters such as sensorial characteristic, nutrient content, quality and availability, bioactivity and phytochemical composition [3-5].

To accurately estimate the dietary intake of the population and prevent diseases such as cardiovascular disease, cancer, diabetes, etc., in recent years, the focus has increasingly been on studying the nutritional characteristics of foods and traditional recipes, ready for consumption [6-23] also with the purpose of formulating the dietary recommendations [14, 15]. This indicates the need to have comprehensive data sets and databases that include composite and processed foods and food preparations as well as accurately dietary information just to investigate the links between diet and health, but currently are still limited for two reasons: the scarcity of data on the nutritional composition of commonly consumed foods, such as processed foods and composite dishes [16] and the deficit of up-to-date knowledge about dietary habits. Food composition databases (FCDBs) are used as the main tool to assess the dietary intake of individuals and groups of people at the regional, national and international levels [17-20]. In addition to providing dietary information, the survey on their characteristics and overall nutritional role, also wants to preserve important cultural elements, such as the great variety of traditional Italian cuisine that distinguishes the gastronomy of the country reflecting the history and local characteristics [21].

Specific research projects, such as the European Food Information Resource (EuroFIR) network [22] (and the Italian national project Food Quality and Functional (QUALIFU) [23], were born and developed precisely to study, protect and maintain the production of significant cultures and culinary traditions of a country; also Italy, particularly rich and various of traditional foods and dishes, has taken part in these projects by studying several national traditional recipes widely consumed and playing an important role on preserving the local and regional food cultures.

In this framework, we want to provide a new update of the antioxidant properties of some Italian recipes -respect to a previously work [12], in terms of extractable and non-extractable compounds, to examine any healthy aspects and identify their potential beneficial role.

The total antioxidant properties identify the assessment of interactions between natural active compounds and other food matrix components of foods; each food matrix has its own antioxidant capacity that derives from the combined action of bioactive compounds such as carotenoids, lignans, polyphenols, vitamins C and E, etc.; natural antioxidants can show different physiological properties such as the anti-inflammatory, antimicrobial, anti-allergic, anti-atherogenic, anti-thrombotic, cardioprotective and vasodilatory effects [24-33]. In respect to antioxidant chemicals, it is worth mentioning the current review of Yeung et al. [34] based on scientific literature landscape analysis of works since 1991, concluded that, a transition of the scientific interest has been observed from research focused on antioxidant vitamins and minerals into more research attention dedicated on antioxidant phytochemicals (plant secondary metabolites).

In particular, in the last years, the distinction of extractable and non-extractable antioxidants took consensus in scientific community: a development and assessment of methodologies were achieved [35]. Antioxidants occur in two forms [33, 36]: on the one side, as easily extractable compounds (free forms), made soluble by aqueous-organic solvents; on the other side, as less extractable compounds that are in bound forms because they remain in the residue after aqueous-organic extract. Their

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incidence in foodstuffs as raw, cooked and processed products, were studied because they represent a significant portion in some groups of foods, i.e., cereals, fruits, vegetables, nuts, and legumes [37-48]. As marked previously by Durazzo, [35] related to the presence of multiple aspects and factors, it becomes difficult to carry out a categorization of the main trends of the contribution of extractable and non-extractable compounds to the total antioxidant properties of the major food groups. Generally, researches remarked that the analysis of antioxidants in plant foods that remain in the residues was necessary. Particular attention should be given to high fat food matrices [49] and complex food matrices [12].

The assessment of bioactive compounds to dietary intake is a key issue. Also, a proper assessment of the contribution of extractable and non-extractable compounds to the dietary intake is required. Most of the studies presented daily intakes of extractable polyphenols, whereas few researches have been performed on intake of non-extractable polyphenols [50-54], an important fraction contributing to total polyphenol intake [55]. Saura-Calixto et al. [50] estimate the amount of total polyphenols (as extractable and non-extractable polyphenols) consumed in a whole diet (Spanish Mediterranean diet) and their intestinal bioaccessibility: the amount of non-extractable polyphenols was almost double that of extractable polyphenols. A further study of Pérez-Jiménez, and Saura-Calixto, [51] evaluated Non-extractable polyphenols s for the 24 most consumed fruit and vegetables in four European countries (France, Germany, The Netherlands and Spain): macromolecular antioxidants, made up of hydolysable polyphenols and polymeric proanthocyanidins, are major contributors (mean value 57%) to the total polyphenol content of fruit and vegetables and the intake of non-extractable polyphenols was estimated at about 200 mg; in particular, the authors reported that Spain had the highest daily per capita non-extractable polyphenols intake from fruit, whereas the Netherlands had the highest derived from vegetables [51]. It is worth mentioning the work of Koehnlein et al. [52] on estimation of the total dietary antioxidant capacity –TDAC- in by the Brazilian population: TDAC, evaluated as the ferric-reducing antioxidant power and as the trolox equivalent antioxidant capacity, was 10.3 and 9.4 mmol/d, respectively. In a further works [53], the same authors, compares the phenolic contents and the total antioxidant capacity of the 36 most popular Brazilian foods submitted to aqueous extraction or in vitro digestion: after in vitro digestion, cereals, legumes, vegetables, tuberous vegetables, chocolates and fruits showed higher phenolic contents and higher antioxidant activities than those obtained by aqueous extraction. whereas, the digestion caused a reduction in the phenolic contents and antioxidant activities of beverages (red wine, coffee and yerba mate) [53]. Another work to mention is the research of Faller et al. [54] on chemical and cellular antioxidant activity of feijoada whole meal coupled with an in vitro digestion: bound and residue contributions to total phenolics were 20.9% and 32.2%, respectively, suggesting that phenolics reach the colon after intake.

The overall goal is the development of specified and dedicated databases as well as the inclusion of extractable and non-extractable compounds in current comprehensive and harmonized FCDBs for a better and correct dietary intake assessment. Studies direction [56, 57] on this are carried out in eBASIS BioActive Substances in Food Information System [58-60]:search protocols and data collection systems are developed to enable the expansion of eBASIS with new quality evaluated data on extractable and non-extractable antioxidants, producing a valuable unique resource [56, 57]

This study evaluates the contribution of extractable and non-extractable bioactive compounds on the antioxidant properties of four popular Italian dishes commonly consumed, previously characterized by their nutrient content [13], for a better understanding of their nutritional role and provide additional dietary information to be included in the next update of national Food Composition Database (FCDB) of the CREA Research Centre for Food and Nutrition.

2. Materials and Methods

2.1 Recipes: Identification of standard recipe, sampling and dish preparation

Four recipes (Table 1), all identified from survey Italian National Food Consumption Survey INRAN-SCAI 2005-2006 [61], and selected in the QUALIFU project [23], were experimentally

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prepared [13] in a dedicated lab-kitchen following a validated and standardized protocol developed within the EuroFIR Network [62]; three preparations represented some popular, most commonly consumed Italian dishes: *Pasta alla amatriciana*; *Pasta alla carbonara*, *Torta di mele*; one Souce, the *Besciamelle*, was selected and studied for its great use, allowing you to make other traditional food preparations (lasagna, vegetables au gratin, baked pasta, rustic cakes). The different ingredients included pasta, cured meat, milk, cheeses, eggs, vegetables, fruits, and extra virgin olive oil and butter, by covering a wide range of antioxidant properties. Our previous studies on traditional Italian dishes were focused on determination of proximate composition and the dietary intake evaluation [12, 13], and also aimed at applying an integrated and emerging (analytical) approach to classifying dishes [63, 64].

Table 1. Italian popular dishes: ingredients, preparation procedures, and method and time cooking .

Original Name	Food Name	Ingredients (g/100g)	Cooking	Timing (min.)
Pasta alla amatriciana	*Amatriciana pasta*	Short pasta (37.5), tomato pulp (37.5), Amatrice cheek lard diced (16), Amatrice Pecorino cheese PAT (hard cheese from sheep) (7.5), extra virgin olive oil (1.1), salt (0.3), chili pepper (0.1).	Boiling, pan-fried and simmering	25
Pasta alla carbonara	*Carbonara pasta*	Short pasta (47.3), bacon cubes (20.3), Roman Pecorino cheese PDO (hard cheese from sheep) (6), eggs (16.6), extra virgin olive oil (1.4), salt (0.4), black pepper (0.4).	Boiling and pan-fried	13
Besciamella	*Béchamel sauce*	Milk (83), butter (8), flour (8), salt (0.5)	Simmering gently	33.
Torta di mele	*Apple Pie*	Apples (37.7), sugar (15), wheat flour (18), butter (9.3), eggs (9.2), whole milk (7.5), baking powder (1), vanilla (0.03), grated lemon peel (0.2), lemon juice (2.4).	Baking	30

In detail, at first a document collection was carried out from the most popular and traditional cookbooks in Italy (Il cucchiaio d'argento; La cucina italiana, etc.) for every recipe selected; therefore, for every dish one "standard recipe" was identified and one "preparation protocol" was drafted in detail identifying ingredients, quantities, preparation techniques, type, temperature and time cooking; dishe production had been carried out according to the standard procedures developed within the EuroFIR network.

The sampling plan had taken into account the collection of simple ingredients at various retail stores, and supermarkets in Rome; to represent the variability/ heterogeneity of the ingredients, , the main brands and cultivars of the same product were considered and purchased: 8 brands of pasta and tomato pulp, 7 brands of eggs, 6 brands of bacon, 4 samples of Amatrice cheek lard and Pecorino cheese, 4 brands of extra virgin olive oil, 2 brands of wheat flour, butter and milk. Each brand (primary sample) for every ingredient (secondary sample) was properly prepared, weighed, and

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then combined to make a composite sample (pool) before using it, for the preparation of the final dish (laboratory sample). This was weighed, assembled and cooked in a laboratory and dedicated kitchen in CREA-Research Centre for Food and Nutrition by trained persons according to preparation protocol of the standard recipe, applying methods and utensils commonly used in households. The recipes, once completed and cooked, were homogenized, frozen at -30 °C and lyophilized, and freeze-dried before being analyzed in triplicate.

2.2 Evaluation of antioxidant properties by Ferric Reducing Antioxidant Power (FRAP) and Total Polyphenol Content (TPC)

2.2.1 Extraction procedure

Extractable and Non extractable polyphenols were extracted as described by Durazzo et al. [12]. Aqueous-organic extracts (extractable polyphenols) and their residues (non-extractable polyphenols) were isolated and studied.

In particular, among non-extractable antioxidants, we focused our attention on hydrolysable polyphenols, that were isolated and determined following a specific and suitable acid hydrolysis as reported below.

Aqueous-organic extract. On the basis of dish ingredients and literature data, about 3-5.5 g of each sample were placed in a test tube and 20 ml of acid methanol/water (50:50 v/v, pH 2) were added. The tubes were vortexed at room temperature for 3 min, then mildly shaken for 1 h in a water bath at room temperature. The tubes were then centrifuged at 2500 g for 10 min and the supernatants were recovered. Twenty millilitres of acetone/water (70:30 v/v) were added to residues, then all operations (vortexing, shaking, centrifugation) were repeated. Methanolic and acetonic extracts were combined and centrifuged at 2800 g for 15 min. The resulting supernatant was transferred into tubes and directly used for the determination of FRAP and TPC.

Residue. The residue remained after the extraction previously described, were left in a ventilated and heated apparatus (max temperature 25 °C), until dryness. Briefly, about 200-450 mg of the residue, respectively were mixed with 20 ml of methanol and 2 ml of concentrated sulphuric acid (18 M). The samples were gently stirred for 1 min and were shaken in a water bath at 85 °C for 20 h; samples were then centrifuged (2500 g for 10 min) and the supernatant was recovered. After two washings with minimum volumes of distilled water and recentrifuging as necessary, the final volume was taken up to 50 ml. The tube was centrifuged at 2800 g for 20 min and the resultant supernatant directly used for the determination of FRAP and TPC

2.2.2. Antioxidant Assays

Several methods have been proposed for evaluating the antioxidant properties of single compounds and foods [65]. The common are: Folin-Ciocalteu assay used widely to determine the total phenolics, the Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP), ferric-reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay [66-68]. All these methods are based on measurements of the capacity of food component or a food to scavenge specific free radicals or reduce a target molecule.

These assays differ in their principles, mechanisms, and experimental conditions as well as in how end points are measured, so that different methods to estimate and/or determine the antioxidant activity of the compounds should be carried out. Three are the main mechanisms throughout the antioxidants acts, encompassing the direct reaction with radicals and the chelation of free metals (involved in reaction finally generating free radicals): -the H atom transfer, the single electron transfer and the metals chelation [69]. Literature data reported that the use of at least two or three assays is strongly recommended for assessing antioxidant properties [70]. Prior et al. [71] proposed , proposed that procedures and applications for three assays be considered for standardization: the

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oxygen radical absorbance capacity (ORAC) assay, the Folin-Ciocalteu method, and possibly the Trolox equivalent antioxidant capacity (TEAC) assay [71].

2.2.1.2.FRAP

The determination of FRAP assay was carried out according to the methods of Benzie & Strain [72] and Pulido et al. [73] through the use of a Tecan Sunrise® plate reader spectrophotometer. The method is based on the reduction of Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex to ferrous at low pH.

2.2.2. *Total Polyphenol Content (TPC)*

The TPC was determined using the Folin-Ciocalteau procedure [74]. Briefly, appropriate dilutions of extracts were oxidised with Folin-Ciocalteau reagent and the reaction was neutralised with sodium carbonate. The absorbance of the resulting blue colour was measured at 760 nm against an appropriate blank after 2 h of reaction at room temperature. Gallic acid was used as standard.

2.3 Statistical Analysis

All analyses were performed in triplicate. Data are presented as mean ± standard deviation (s.d.) of the analysis carried out on two preparations of every dish. Statistica for Windows (Statistical package; release 4.5; StatSoft Inc., Vigonza PD, Italy) was used to perform One-way Analysis of Variance (ANOVA), post-hoc Test: Tukey honest significant difference (HSD) test.

3. Results and Discussion

In our study, we decided to study the antioxidant properties of the whole matrix as consumed due to the complexity of matrices. The changes in antioxidants and in interactions between components are correlated to phytochemical structure and concentration, typology of food matrixes well as preparation procedure, typology and time of cooking, [75-78].

In Table 2 FRAP values (μmol/g d.m.) and TPC (mg/100 g d.m.) were reported for selected popular Italian dishes.

Table 2. FRAP and TPC of popular Italian dishes*

	FRAP (μmol/g d.m.)		TPC (mg/100g d.m.)	
	Aqueous-organic extract	**Residue**	**Aqueous-organic extract**	**Residue**
Pasta alla Amatriciana	4.01±0.67^{b}	68.64±4.43^{c}	60.87±5.48^{c}	1447.59±70.33^{c}
Pasta alla Carbonara	2.62±0.53^{a}	73.83±3.52^{d}	36.50±6.31^{a}	1747.35±72.91^{d}
Besciamella	2.47±0.17^{a}	52.98±1.22^{b}	51.90±3.38^{b}	1173.44±73.07^{b}
Torta di mele	10.72±0.80^{c}	18.21±5.09^{a}	64.28±2.39^{c}	425.84±63.86^{a}

*Mean ± S.D.; Anova, Tukey HSD Test: by column, means followed by different letters are significantly different ($p < 0.05$)

FRAP values in aqueous-organic extract decreased in the following order: *torta di mele* > *pasta alla amatriciana* > *pasta alla carbonara* = *besciamella*, whereas in residue *pasta alla carbonara* reached the highest value following by *pasta alla amatriciana*. The FRAP values of pasta-based dishes confirmed the value reported in our previous work [11] for *spaghetti alle vongole* 4.20 μmol/g d.m. and 64.22 μmol/g d.m., in aqueous-organic extract and residue, respectively. Generally the antioxidant properties of cereals and derivatives thereof are well documented along years [43, 79, 80]; regards antioxidant in pasta, one of most popular staple foods, the effect of cooking [81, 82] as well as new formulations and functional products are studied [83-86]. In this regard it is worth mention the work of Ioannou et al. [77]the mark how the addition of ingredients with high antioxidant activity to a

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complex preparation can contribute to increase in the total antioxidant capacity, but not in a proportional way.

Besciamella belongs to category of white sauces and is an example of milk-based dishes. In recent years emerging studies on antioxidant properties of milk [87, 88] as well as dairy products [89, 90] are carrying out. As instance Manzi and Durazzo, [76], by evaluating the antioxidant properties of industrial heat treated milk -UHT, Microfiltered and High Quality Pasteurized-, showed that UHT milk has highest values in Total Polyphenol Content, DPPH and FRAP values; the authors [88] explained that the behaviour of UHT is probably related to the development of antioxidant compounds, formed during the Maillard reaction occurring when milk treatment is performed at high temperatures (≥135°C for at least 1 sec),that occur during this severe heat-treatment, in according to previous authors [91-93].

Torta di mele belong to subcategory of fruit cake among desserts, present the highest FRAP value in aqueous-organic extract and the lowest in residue respect to other items. This reflect the high content of organic acids in apple [94, 95]. As instance for apple, in Phenol Explorer Databases [96], the total polyphenol content of apple was 131.80 mg/100 g FW, as mean of 53 original content values extracted from 8 publications.

Table 3 shows and summarizes the contribution of extractable and non-extractable compounds to antioxidant properties of all Italian dishes grouped by category, and investigated by us, in this work and in previous ones [12].

Table 3. Contribution % of extractable and non-extractable compounds to antioxidant properties of popular Italian dishes *

Italian Dishes	Aqueous-organic Extract	Residue
Sauces		
Besciamella	4	96
First courses		
*Spaghetti alle vongole**	6	94
Pasta alla amatriciana	6	94
Pasta alla carbonara	3	97
One dish meals		
*Pomodori al riso**	15	85
*Gâteau di patate**	11	89
Side courses		
*Carciofi alla romana**	58	42
Desserts		
*Pan di Spagna**	5	95
Torta di mele	37	63

*Data derived by Durazzo et al. [11]

For all new four dishes, extractable antioxidants (aqueous-organic extracts) were minor contributors to the total antioxidant activity and consequently the hydrolysable polyphenols (residues) contributed significantly more: for *pasta alla amatriciana*, *pasta alla carbonara* and *besciamella* extractable antioxidants contributes less than 6% and non-extractable compounds in a range of 94-97%, whereas for *torta di mele* the percentage of contributions were 37% and 63% respectively for extractable polyphenols and hydrolysable polyphenols.

Regarding total polyphenol content evaluation, as reported in Table 1, TPC values ranged in aqueous-organic extracts between 36.50 mg/100 g d.m. and 64.28 mg/100 g d.m., whereas in residue from 425.85 mg/100 g d.m. to 1747.35 mg/100 g d.m. So hydrolysable polyphenols represent a significant fraction, by accounting between 87% (*torta di mele*) and 98% (*pasta alla carbonara*) of total polyphenols. A good Pearson correlation between TPC values with FRAP ones was found in aqueous-organic extract (r=0.6798) and, in particular, in residues (r=0.9909). As instance, *pasta alla*

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carbonara showed reached the lowest values in aqueous-organic extract and the highest in residue both in FRAP and TPC, whereas *torta di mele* showed the opposite behaviour.

4. Conclusions

In this study antioxidant properties, of 4 commonly consumed popular Italian dishes have been provided for the first time. Our findings contribute to the updating of Italian Food Composition Database, by providing a value of antioxidant properties, useful and important for study the association between diet and a healthy status [97, 98].

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The innovative character of this research is by the fact that the four dishes were experimentally prepared in a dedicated lab-kitchen following a validated and standardized protocol based on harmonized guidelines. The other key aspect is the study of antioxidant properties, in term of extractable and non-extractable antioxidant, applied to complex matrixes, i.e. food preparations and food composite dishes.

Our study highlighted the importance to evaluate the real nutritional information on foods as are taken, often mixed and heat treated to formulate dishes; the availability of these new and appropriate food composition data is needed in order to correctly evaluate the dietary intake of recipes rich in key nutrients and bioactive molecules, facilitating further nutrition-related studies, and can be used to encourage the consumption of recipes. Further studies in this direction to provide a detailed nutritional overview of popular and traditional Italian dishes are carrying out.

Author Contributions: A.D., M.L., A.S. and L.M. have conceived the work and wrote the manuscript. All authors have carried out the experimental study and analyzed the data. All authors have made a substantial contribution to revise the work, and approved it for publication.

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Conflicts of Interest: The authors declare no conflict of interest.

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