applied sciences

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Olive Paste-Enriched Cookies Exert Increased Antioxidant Activities

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Abstract: Functional foods are beneficial to human health and are part of the daily diet of people trying to follow a healthier lifestyle. Olive paste is a good source of functional compounds, mainly phenolic compounds, that have been shown to have health benefits. At the same time, cookies are an ideal snack that can be fortified with additional ingredients to address human dietary needs. The study aimed to enrich cookies with olive paste and extra ingredients for flavor differentiation and evaluate the impact of the enrichment on their antioxidant properties. Enriched cookies were prepared analyzed and tested for sensorial acceptability, total phenolics, and antioxidant activities by DPPH, ABTS, FRAP, and CUPRAC assays. Enriched cookies were sensorially acceptable. Unsaturated fat, total phenolics, and antioxidant activities of enriched cookies were higher compared to control cookies, while among enriched cookies the extra addition of 1% garlic, 0.5% thyme, and 0.5% oregano resulted in higher total phenolics and antioxidant activities compared to cookies that were flavored either with 3% vegetables or 3% orange zest. Antioxidant activity in cookies was strongly correlated with total phenolic content. Cookies enriched with olive paste may be healthy functional food in terms of increased antioxidant activity.

Keywords: cookies; olive paste; antioxidant; dietary added value

Citation: Argyri, E.-A.; Piromalis, S.-P.; Koutelidakis, A.; Kafetzopoulos, D.; Petsas, A.S.; Skalkos, D.; Nasopoulou, C.; Dimou, C.; Karantonis, H.C. Olive Paste-Enriched Cookies Exert Increased Antioxidant Activities. *Appl. Sci.* **2021**, *11*, 5515. https://doi.org/10.3390/app11125515

Academic Editor: Cédric Delattre

Received: 15 May 2021
Accepted: 8 June 2021
Published: 15 June 2021

1. Introduction

The idea of functional foods dates back thousands of years. In 1984, Japan was the first country to promote the idea of functional food [1]. According to one of the most commonly used definitions, functional foods are foods that have been enriched with essential and physiologically useful effects [1].

It is widely acknowledged that functional foods have a positive impact on human health and the lifestyle that people follow has moved toward a healthier one [2], where functional food intake is a new way for consumers to communicate their well-being [3,4]

Cookies are a worldwide popular bakery snack for consumers of all ages. They have high nutritional value, affordable cost, long shelf life, and they are ready to eat in different tastes. The above characteristics make cookies an ideal snack that can be fortified with extra ingredients to suffice health-promoting human dietary demands [5].

As a result of growing interest in functional foods, bioactive components are being used to create innovative cookies with enhanced nutritional value [6]. Antioxidants are a major category of foodstuff molecules through which functional foods have been shown to exert positive effects on human health. Therefore, the enrichment with antioxidants is one of the most interesting concepts in functional food development [7–9].

Olive paste is a product from olives and characteristic of the Mediterranean diet. It contains various phenolic compounds, with hydroxytyrosol being the most abundant that exert health preventive activities associated with various chronic diseases where oxidative stress plays a major role in their onset [10–13].

Therefore, this study aimed to enrich cookies with olive paste and evaluate their antioxidant activities compared to non-enriched cookies to assess the olive paste effectiveness as an ingredient that confers to dietary added value in enriched cookies.

2. Materials and Methods

2.1. Materials

Greek method fermented table olives of Kalamon cultivar and green Chondroelia olives were provided by Amalthia S.A. (Kefalovrison Etoliko, Greece). Stoneless olives were homogenized with a blender (Premier Chef KMC570, Kenwood, UK). Absolute ethanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Trolox and neocuproine were purchased from Acros Organics (Fair Lawn, NJ, USA). Ammonium acetate, sodium chloride, sodium dihydrogen phosphate dehydrate, and copper chloride dihydrate were all purchased from Penta (CZ Ltd., Chrudim, Czech Republic). 2,2′-Azino-bis-(3-ethylbezothiazoline-6-sulphonic acid (ABTS) was purchased from Applichem (Darmstadt, Germany). Potassium persulfate was purchased from Chem-Lab (Zedel-gem, Belgium).

2.2. Olive Paste Cookies Preparation

Cookies were prepared based on the formulations presented in Table 1. Control cookies were prepared without the addition of olive paste. Cookies enriched with olive paste were prepared by replacing fat and water with olive paste at a level of 20% taking into account the moisture and oil content of olive paste. The enriched cookies were prepared based on Greek traditional cuisine in three different flavors. The first with garlic, oregano, and thyme, the second with a powdered mixture of tomato, carrot, zucchini, green pepper, onion, green onion, and celery in equal amounts, and the third with orange zest, aiming at enhancing flavor and nutritional value.

Table 1. Formulation of cookies.

Ingredients (%)	OPC_C	OPC_{GTO}	OPC_{VEG}	OPC_{ORG}
Hard margarine	21.1	17	17	17
Sugar	6	6	6	6
Water	15.9	-	-	-
Olive Paste	-	20	20	20
Wheat Flour	44	44	44	44
Oat flour	13	13	13	13
Soda	0.7	0.7	0.7	0.7
Salt	0.8	-	-	-

OPC_C: Olive Pomace Cookie $_{Control}$; OPC_{GTO}: Olive Pomace Cookie Garlic Thyme Oregano Enriched with 1.0% garlic, 0.5% thyme, and 0.5% oregano; OPC_{VEG}: Olive Pomace CookieVEGetables Enriched with 3.0% powdered mixture of tomato, carrot, zucchini, green pepper, onion, green onion, and celery in equal amounts; OPC_{ORG}: Olive Pomace Cookie $_{ORanGe}$ Enriched with 3.0% orange zest.

For the cookie's formulation, hard margarine was heated to 45 °C and mixed in a mixer (Premier Chef KMC570) with the sugar and either water and salt for control, or olive paste, and ingredients for extra flavor for enriched cookies. Then, flours were combined with the soda and added to the mixture. The resulted dough was placed in the freezer for

10 min. Finally, the dough was rolled and cut into a round shape with 4.0 cm diameter and 0.7 cm height and baked at 170 °C for 30 min.

2.3. Analysis of Olive Paste and Cookies

For moisture content, olive paste samples were placed in an oven at 70 °C for 72 h. Moisture content was calculated as the difference in weight before and after drying and expressed as mean ± standard deviation in g per 100 g.

For the oil content determination, olive paste samples were placed in glass tubes. Then 20 mL of n-hexane was added, and the fat was extracted with the aid of ultrasonic pulses at 37 kHz and 100% power for 5 min. Centrifugation at 2500× *g* for 5 min followed and the supernatant was collected in a pre-weighed ground conical flask. 20 mL of hexane were added to the centrifuge precipitate and the procedure was repeated two more times. The entire supernatant phases of the hexane were evaporated on a rotary evaporator under vacuum. Oil content was calculated by weighing and expressed as mean ± standard deviation in g per 100 g olive paste.

Cookie analyses were carried out with standard protocols (AOAC, 2006). Protein was analyzed by the Kjeldahl method (AOAC 976.05, N × 6.25). Total, saturated and unsaturated fat was estimated by the GC-FID method (AOAC 996.06). Moisture content was determined by drying in a hot air oven (AOAC 934.01). Fiber content was determined by the Ceramic Fiber Filter Method (AOAC 962.09). Total carbohydrate and energy were estimated by calculation [14]. Water activity was determined using aw meter (Novasina Lab Touch-aw meter, Novasina AG, Zurich, Switzerland). Salt was determined based on ISO 1738.1997.

Total phenolics in the olive paste and cookies extracts before and after baking were determined according to the method of Singleton and Rossi [15] with minor modifications. Samples in 1.0 g were homogenized for 5 min in 10.0 mL methanol: water 70:30 (*v/v*) plus 2% (*v/v*) acetic acid using a homogenizer (Ultra Turrax, IKA Werke, Staufen, Germany). The suspension was left at room temperature under magnetic agitation for 15 min and was then centrifuged for10 min at 2000× *g* in a Hermle Z 383 centrifuge (Hermle Labortechnik, Wehingen, Germany). The supernatants were collected and the volume of each one was made up to 10.0 mL [16]. Various volumes of extracts in the range of 0.01 to 0.10 mL were dissolved in a final volume of 1.8 mL with distilled water followed by the addition of 0.1 mL Folin-Ciocalteu reagent. Then, samples were vigorously stirred and incubated for 2 min in the dark. After that, 0.3 mL of aqueous Na_2CO_3 20% (*w/v*) was added followed by vigorous stirring and incubation in a water bath at 40 °C for 30 min. Absorbance was measured at 765 nm, using a Spectrophotometer Lambda 25 (Perkin-Elmer, Norwalk, CT, USA). Gallic acid was used for standard curve preparation. Results were expressed as mean ± standard deviation in mg of gallic acid per g of cookies [15]. The rest of the extracts were stored under a nitrogen atmosphere at −40 °C for antioxidant determination.

2.4. Sensory Acceptability of Cookies

Sensory acceptability testing was conducted by 50 consumers. Appearance, flavor, mouthfeel, and general acceptability were the hedonic sensory attributes that consumers evaluated using a 9-point hedonic scale (1 = disliked extremely, 9 = liked extremely). Control and olive paste enriched cookies were served to consumers randomly as coded samples on plates. In-between the assessment of each sample, the panelists were asked to take some water to minimize carry-over effects [17]. In addition, the consumers were allowed to comment on each sample.

2.5. In Vitro Antioxidant Activity of Cookies

The antioxidant activity of cookies before and after baking was evaluated by the DPPH, ABTS, FRAP, and CUPRAC assays. The capacity of methanolic extract of cookies to scavenge the free radical of DPPH was evaluated by the method of Miller et al. [18] with minor modifications. An aliquot of the extract (10 to 100 μL) or appropriate standard

solution of Trolox was diluted with methanol up to 0.9 mL. Then, 0.1 mL of 0.6 mM DPPH reagent in methanol was added, followed by vigorous stirring. After 15.0 min in the dark, the absorbance was measured at 515 nm against a reference sample containing methanol. The results were expressed as Trolox equivalents in μmol per g of cookie.

Determination of ABTS radical scavenging activity of samples was performed by the method of Brand-Williams et al. [19] with minor modifications. ABTS radical cation ($ABTS^{\bullet+}$) was produced by the oxidation of ABTS with potassium persulfate ($K_2S_2O_8$). The $ABTS^{\bullet+}$ was generated by reacting 7 mmol/L stock solution of ABTS with potassium persulphate in a final concentration equal to 2.45 mmol/L. The $ABTS^{\bullet+}$ working solution was prepared by dilution of the stock solution using distilled water to give an absorbance of 0.700 at 734 nm. Aliquots of cookie extracts (10 to 100 μL) or appropriate amounts of Trolox standards were dried under a stream of nitrogen followed by the addition of 1.0 mL working $ABTS^{\bullet+}$ solution and were vigorously stirred. Samples remained for 15.0 min in the dark at ambient temperature and the absorbance was measured at 734 nm. The ability of the extracts to scavenge the $ABTS^{\bullet+}$ was evaluated relative to a reference sample that did not contain any quantity of extract. The results were expressed as Trolox equivalents in μmol per g of cookie.

The reducing potential of the samples was determined using the FRAP assay as described by Benzie and Strain [20]. The method is based on the reduction of the Fe^{3+}-tripyridyltriazine complex to its ferrous-colored form at low pH in the presence of antioxidants. The FRAP reagent was freshly prepared and contained 0.2 mL of a 10 mM TPTZ (2,4,6-tripyridy-s-triazine) solution in 40 mM HCl plus 0.2 mL of 20 mM $FeCl_3 \bullet 6H_2O$ plus 0.2 mL of 3.0 M acetate buffer, pH 3.6. Aliquots of cookie extracts (0.01 to 0.10 mL) were transferred in glass test tubes, dried under a stream of nitrogen, and dissolved in distilled water to a final volume of 900 μL, followed by addition of 300 μL of FRAP solution and vigorous stirring. The samples were incubated for 10 min in a 37 °C water bath and the absorbance was measured at 593 nm. A standard curve was prepared using Trolox. The antioxidant activity of the cookie extracts was expressed as Trolox equivalents in μmol per g of cookie.

The reducing capacity of the samples was also determined using the CUPRAC assay according to Özyürek et al. [21]. Amounts of the samples in the range of 10 to 100 μL were transferred in test tubes and were dried under a stream of nitrogen. After that, 10 mM $CuCl_2 \bullet 2H_2O$, 7.5 mM neocuproine, and 1mM CH_3COONH_4 buffer solution with pH = 7.0 were added in 300 μL each, followed by the addition of distilled water up to the volume of 1200 μL. The samples were well stirred and remained at room temperature for 30 min. The absorbance of the samples was then measured at 450 nm. A standard curve was prepared by the same procedure using Trolox. The antioxidant activity of samples was expressed as Trolox equivalents in μmol per g of cookie.

2.6. Statistical Analysis

Normal distribution of the data was confirmed by Shapiro-Wilk's test. One-way analysis of variance (ANOVA) and post hoc Tukey test was used for comparison of the means between samples. Data are presented as mean ± standard deviation (SD) and analyzed using Statistical Package for Social Sciences (SPSS version 20, IBM, Armonk, NY, USA). The significance level for the differences between the sample means was set at $p < 0.05$. Data were also evaluated using Pearson's correlation coefficients for identification of any relationships between total phenolics in cookies and their antioxidant activities as determined by DPPH, ABTS, FRAP and CUPRAC assays.

3. Results

3.1. Analysis of Olive Paste

The moisture content of olive paste samples from olives cultured in different areas showed that OP1, OP2, and OP3 were at the same level but statistically significantly lower

compared to OP4. Similarly olive content was not significantly different among OP1, OP2 and OP3 but it was higher compared to OP4 (Table 2).

Table 2. Analysis of olive paste.

Samples	%Moisture	% Oil Content	GAE (mg/g)
OP1	64.6 ± 1.5^{b}	20.3 ± 0.8^{a}	1.74 ± 0.06^{a}
OP2	66.1 ± 1.5^{b}	19.7 ± 0.8^{a}	1.35 ± 0.08^{b}
OP3	66.8 ± 1.4^{b}	20.9 ± 0.9^{a}	1.45 ± 0.07^{b}
OP4	80.6 ± 0.6^{a}	6.6 ± 0.1^{b}	0.49 ± 0.02^{c}

Results are expresses as mean ± standard deviation of % content (*w/w*) from triplicate measurements. OP1-PO3: Olive paste of Kalamon Cultivar cultured in Greece at the areas of Kefalovriso Mesologiou, Stamnas Mesologiou, and Drimou Etoloakarnanias respectively; OP4: Olive paste of green olives cultured in Greece at the area of Etoloakarnania; Different letters in each column denote significant difference ($p < 0.05$).

The results for the content of total phenolics in olive paste samples showed that the green olive paste (OP4) has the lowest phenolic content compared to the other samples. Among the samples OP1, OP2, and OP3 a significantly higher content was recorded in OP1 (Table 2). For this reason, OP1 was chosen for the formulation of olive paste cookies.

3.2. Analysis of Cookies

The results from the analysis of cookies are presented in Table 3. The fortified cookies did not differ from each other in terms of the results of their analyzes. However, fortified cookies had a lower percentage of saturated fat and higher levels of unsaturated fat, and fiber compared to control cookies.

Table 3. Analysis of cookies.

	OPC_C	OPC_{GTO}	OPC_{VEG}	OPC_{ORG}
Protein (%)	9.2	9.1	9.3	8.9
Fat (%)	20.5	20.5	20.6	20.8
Saturated Fat (%)	18.8^{a}	7.0^{b}	6.9^{b}	7.1^{b}
Unsaturated Fat (%)	1.7^{b}	13.5^{a}	13.7^{a}	13.7^{a}
Carbohydrates (%)	64.0	63.6	62.8	64.0
Dietary fibers (%)	3.3^{b}	4.2^{a}	4.4^{a}	4.1^{a}
Moisture (%)	5.7	5.9	6.0	5.9
Salt (%)	1.0	0.9	1.2	0.9
Energy (Kcal/100g)	478	475	474	479
Water Activity (aw)	0.430	0.413	0.425	0.437

Results are expresses as mean ± standard deviation from triplicate measurements. OPC_C: Olive Pomace $Cookie_{Control}$; OPC_{GTO}: Olive Pomace $Cookie_{Garlic\ Thyme\ Oregano}$; OPC_{VEG}: Olive Pomace $Cookie_{VEGetables}$; OPC_{ORG}: Olive Pomace $Cookie_{ORanGe}$. Different letters in each line denote significant difference ($p < 0.05$).

3.3. Sensory Acceptability

The results for the sensory acceptability of cookies are presented in Table 4. All sensory attributes were acceptable based on the 9-point hedonic scale. Mouthfeel was comparable to all cookies. The appearance of control cookies was slightly more acceptable compared to the olive paste enriched cookies. Concerning flavor and general acceptability, olive paste cookies with garlic thyme and oregano were the most acceptable followed by the one enriched with vegetables, then control, and finally, the one enriched with orange zest.

Table 4. Sensory acceptability of cookies.

	OPC_C	OPC_{GTO}	OPC_{VEG}	OPC_{ORG}
Appearance	6.45 ± 0.92^{a}	6.08 ± 0.83^{b}	5.98 ± 0.75^{b}	6.06 ± 0.74^{b}
Flavor	7.04 ± 0.66^{c}	7.96 ± 0.79^{a}	7.52 ± 0.96^{b}	5.86 ± 0.86^{d}
Mouthfeel	7.69 ± 0.88	7.43 ± 0.89	7.39 ± 0.82	7.41 ± 0.69
General acceptability	6.86 ± 0.74^{c}	7.55 ± 0.80^{a}	7.20 ± 0.88^{b}	5.76 ± 0.62^{d}

Results are expresses as mean ± standard deviation. OPC_C: Olive Pomace $Cookie_{Control}$; OPC_{GTO}: Olive Pomace $Cookie_{Garlic\ Thyme\ Oregano}$; OPC_{VEG}: Olive Pomace $Cookie_{VEGetables}$; OPC_{ORG}: Olive Pomace $Cookie_{ORanGe}$. Different letters in each line denote significant difference ($p < 0.05$). a: higher acceptability of OPC_C compared to the enriched ones; b: higher acceptability of OPC_C compared to OPC_{ORG}; c: higher acceptability of OPC_{GTO} compared to the rest of the cookies; d: higher acceptability of OPC_{VEG} compared to OPC_C and OPC_{ORG}.

3.4. In Vitro Antioxidant Activity of Cookies

The results for total phenolics and antioxidant activity of cookie extracts are presented in Table 5. All enriched cookies before and after baking showed higher total phenolics and antioxidant activities compared to control cookies. Among enriched cookies samples enriched with garlic, thyme and oregano (OPC_{GTO}) showed higher total phenolics and antioxidant activities compared to cookies enriched with vegetable (OPC_{VEG}) or orange zest (OPC_{ORG}) in both before and after baking samples.

Table 5. Total phenolics and antioxidant activities of cookies before (B) and after (A) baking.

	OPC_C	OPC_{GTO}	OPC_{VEG}	OPC_{ORG}
Total phenolics (B)	0.79 ± 0.04^{c}	2.20 ± 0.07^{a}	1.83 ± 0.06^{b}	1.76 ± 0.07^{b}
Total phenolics (A)	0.77 ± 0.03^{c}	2.26 ± 0.04^{a}	1.89 ± 0.04^{b}	1.79 ± 0.05^{b}
DPPH (B)	1.10 ± 0.26^{c}	3.23 ± 0.29^{a}	2.70 ± 0.26^{b}	2.64 ± 0.19^{b}
DPPH (A)	1.04 ± 0.18^{c}	3.47 ± 0.2^{a}	2.85 ± 0.27^{b}	2.67 ± 0.05^{b}
ABTS (B)	0.73 ± 0.07^{c}	2.05 ± 0.13^{a}	1.78 ± 0.09^{b}	1.62 ± 0.09^{b}
ABT (A)	0.69 ± 0.09^{c}	2.16 ± 0.02^{a}	1.84 ± 0.11^{b}	1.67 ± 0.08^{b}
FRAP (B)	23.39 ± 0.71	80.35 ± 1.87	65.57 ± 1.57	61.05 ± 1.74
FRAP (A)	22.71 ± 0.54^{c}	82.73 ± 1.55^{a}	68.30 ± 3.08^{b}	62.83 ± 2.23^{b}
CUPRAC (B)	33.48 ± 3.36^{c}	134.28 ± 6.23^{a}	107.59 ± 6.0^{b}	95.28 ± 6.79^{b}
CUPRAC (A)	32.52 ± 3.10^{c}	138.56 ± 7.62^{a}	110.44 ± 6.74^{b}	97.04 ± 6.08^{b}

Results are expresses as mean ± standard deviation from triplicate measurements in mg of gallic acid equivalents per g of cookies (mg GAE/g) for total phenolics or μmol of Trolox equivalents per g of cookies (μmol Trolox/g) for DPPH, ABTS, FRAP and CUPRAC. OPC_C: Olive Pomace $Cookie_{Control}$; OPC_{GTO}: Olive Pomace $Cookie_{Garlic\ Thyme\ Oregano}$; OPC_{VEG}: Olive Pomace $Cookie_{VEGetables}$; OPC_{ORG}: Olive Pomace $Cookie_{ORanGe}$. Different letters in each line denote significant difference ($p < 0.05$).

The Pearson correlation coefficients between total phenolics in baked cookies and the antioxidant activities of their extracts as determined by DPPH, ABTS, FRAP, and CUPRAC are presented in Table 6.

Table 6. Correlation of total phenolics and antioxidant activities in baked cookies.

	Total Phenolics
	Pearson Correlation Coefficients
DPPH	0.993
ABTS	0.990
FRAP	0.999
CUPRAC	0.994

Total phenolics were expressed as gallic acid equivalents in mg per gram of cookies while antioxidnt activities in all assays were expessed as Trolox equivalents in μmol per gram of cookies.

In order to understand the origin of total phenolic content in OPC_{GTO} that exerted the higher phenolics content and antioxidant activities, cookies enriched with olive paste (OPC), olive paste and garlic (OPC_G), olive paste and thyme (OPC_T), and olive paste and

oregano (OPC_O) were prepared and total phenolics were determined before and after baking. The results are summarized in Table 7.

Table 7. Total phenolics content in OPC enriched with garlic or thyme or oregano.

Total Phenolics	OPC	OPC_G	OPC_T	OPC_O
Before baking	1.33 ± 0.09^{c}	1.48 ± 0.08^{b}	1.50 ± 0.08^{b}	1.87 ± 0.06^{a}
After baking	1.37 ± 0.08^{c}	1.51 ± 0.10^{b}	1.54 ± 0.07^{b}	1.92 ± 0.1^{a}

Results are expresses as mean ± standard deviation from triplicate measurements in mg of gallic acid equivalents per g of cookies (mg GAE/g) for total phenolics. OPC: Olive Pomace Cookie; OPC_G: Olive Pomace $Cookie_{Garlic}$; OPC_T: Olive Pomace $Cookie_{Thyme}$; OPC_O: Olive Pomace $Cookie_{Oregano}$. Different letters in each line denote significant difference ($p < 0.05$).

4. Discussion

4.1. Analysis of Olive Paste

Moisture content determined in the olive paste samples of this study ranges from 64.6% to 80.6% (*w/w*) (Table 1). This result is in accordance with previous studies that show values in the range of 60% to 81% (*w/w*). Similarly, oil content that ranges in our study from 6.6% to 20.9%(*w/w*) is in accordance with the range 6 to 30% (*w/w*) that has been referred elsewhere [22,23]. Lipids in table olives are the predominant nutrients as protein does not exceed 2.2% (*w/w*) and carbohydrates are found in traces [22].

Total phenolic content in table olives has been previously measured in ranges from 0.37 to 2.51 mg per gram of olives. The results for total phenolics of olive pastes in the present study ranged between 0.49 and 1.74 mg per gram of olive paste (Table 1) and are in line with the published data [12,24–26].

4.2. Analysis of Cookies

Fortified cookies had a lower percentage of saturated fat and higher levels of unsaturated fat, and fiber compared to control cookies due to the replacement of a percentage of 4.1% of hard margarine with olive paste (OP1) based on its oil content.

Indeed olives have a large proportion of unsaturated fats that ranges from 66.8% to 82.1% for monounsaturated fatty acids where oleic acid predominates, 4.9% to 14.2% for polyunsaturated fatty acids such as linoleic acid, and a low proportion of saturated fats [22]. Olives are also a good source of dietary fiber, particularly pectin, hemicelluloses, cellulose, and lignin with a total content of around 3% (*w/w*) [22,27].

4.3. Sensory Acceptability

All sensory attributes were acceptable (higher than 5) based on the 9-point hedonic scale. Mouthfeel was not affected by the replacement of 4.1 % of hard margarine and 15.9 of water by olive paste in terms of acceptability ($p > 0.05$). The addition of olive paste resulted in a darker color rendering enriched cookies less acceptable than control ones ($p < 0.05$). Similar effects have been previously referred for cookies high in okara and residues of enzyme-assisted aqueous extraction of soybeans [28,29]. On the other hand, cookies enriched with olive paste and either garlic, thyme, and oregano or powered vegetables received the highest hedonic ratings for flavor (7.96 ± 0.79 and 7.52 ± 0.96, respectively, $p < 0.05$) while the version with orange zest received flavor rating lower than control cookies (5.86 ± 0.86 and 7.04 ± 0.66, respectively, $p < 0.05$). Similar to flavor results were noticed in general acceptability. The results show that the combination of olive paste with herbs such as garlic, thyme, and oregano is more acceptable from Greek consumers than with vegetables ($p < 0.05$) and even more than with orange zest ($p < 0.05$) for which sixteen consumers wrote down that OPC_{ORG} had a sense of bitter.

4.4. In Vitro Antioxidant Activity of Cookies

All enriched cookies before baking showed higher antioxidant activities compared to control cookies ($p < 0.05$). The increases of antioxidant activities as determined with DPPH,

ABTS, FRAP, and CUPRAC assays in OPC_{GTO} compared to OPC_C were 200.8 ± 46.0%, 181.4 ± 9.2%, 240.4 ± 3.5%, and 302.5 ± 21.9% respectively. Similarly, for the four antioxidant determinations the increases of OPC_{VEG} compared to OPC_C were 151.19 ± 36.8%, 144.5 ± 11.2%, 177.8 ± 3.0% and 222.4 ± 14.5% respectively and those for OPC_{ORG} compared to OPC_C were 146.6 ± 42.2%, 122.5 ± 9.0%, 158.6 ± 3.7% and 185.1 ± 8.4% respectively. The same profile was observed after baking, where the increases of antioxidant activities as determined with DPPH, ABTS, FRAP, and CUPRAC assays in baked OPC_{GTO} compared to baked OPC_C were 218.1 ± 16.5%, 214.1 ± 12.1%, 264.3 ± 1.8%, and 327.2 ± 17.4% respectively. Similarly, for the four antioxidant determinations the increases of baked OPC_{VEG} compared to baked OPC_C were 160.7 ± 11.6%, 168.3 ± 19.2%, 200.6 ± 6.4% and 240.4 ± 11.8% respectively and those for baked OPC_{ORG} compared to baked OPC_C were 145.3 ± 19.4%, 143.8 ± 20.4%, 176.9 ± 2.8% and 199.0 ± 9.9% respectively.

At the same time, before baking total phenolics in OPC_{GTO}, OPC_{VEG}, and OPC_{ORG} were higher ($p < 0.05$) compared to OPC_C in percentages equal to 178.8 ± 9.0%, 131.8 ± 5.4%, and 122.9 ± 3.7% respectively. After baking total phenolics in OPC_{GTO}, OPC_{VEG}, and OPC_{ORG} were also higher ($p < 0.05$) compared to OPC_C in similar percentages equal to 193.7 ± 6.3%, 145.6 ± 4.4%, and 132.5 ± 2.6% respectively.

A trend for lower total phenolics and antioxidant activities was noted in OPCc after baking. On the other hand in OPC_{GTO}, OPC_{VEG} and OPC_{ORG} a trend for higher total phenolics and antioxidant activities was observed. In both cases these differences were not statistical significant ($p > 0.05$). Increased antioxidant activities have been related to the production of reductones during Maillard reaction, while increased phenolics have been noticed due to the release of phenolic compounds from precursor molecules upon heating. These phenomena depend on time, temperature and food ingredients [16,30,31]. In the present study the time and temperature on the studied cookies did not cause significant changes.

According to Tables 5 and 7 total phenolics before baking in OPC_{GTO} are increased due to the presence of olive paste, garlic, thyme and oregano by percentages equal to 68.3 ± 2.9%, 19.1 ± 2.2%, 21.6 ± 2.4% and 68.6 ± 7.3%, respectively. Similarly, after baking the increased percentages of the total phenolic content due to olive pomace, garlic, thyme and oregano are equal to 77.8 ± 3.5%, 18.1 ± 1.9%, 25.3 ± 7.4% and 71.4 ± 0.2%, respectively. The results show that olive paste and garlic contribute to the greatest extentd in total phenolic content of the cookies, followed by thyme and garlic. These observations are in accordance with the findings of Zheng and Wang showing that oregano is one ot the most potent antioxidant herb and that tend to exert higher antioxidant activities compared to fruits and vegetables [32].

The Pearson correlation coefficients between total phenolics in baked cookies and antioxidant activities as determined by DPPH, ABTS, FRAP, and CUPRAC were very strong (Table 6) showing the significant role of total phenolics in the antioxidant activities of the cookies and the high contribution of olive paste and oregano to their antioxidant activites.

The main phenolics that have been referred to exist in olives are the phenolic alcohols of hydroxytyrosol and tyrosol, the flavones of luteolin-7-O-glucoside, luteolin, apigenin-7-O-glucoside, and apigenin, the flavonol of rutin, the anthocyanin of cyanidin-3-O-glucoside, the phenolic acid of 5-O-caffeoylquinic acid and the hydroxycinnamic acid derivative of verbascoside, with hydroxytyrosol being the most abundant one [12,24,26]. The existence of the 3,4-dihydroxy moiety that exists in hydroxytyrosol has been referred to as primarily responsible for the antioxidant capacity in olives and olive oil [33].

Chronic oxidative stress is due to the occurrence of oxidative modifications in main biomolecules and is closely linked to the initiation and progression of chronic diseases in which reactive oxygenated species play a major role [34]. In the scientific literature, it seems that there are no clinical or epidemiologic studies concerning the health effects of the consumption of either olive paste or table olives. On the other hand, oleic acid and hydroxytyrosol that exist in olives are two ingredients that exist also in extra virgin olive oil, the main source of lipids in the Mediterranean diet, and they have been shown to exert

health protective effects in humans. Due to health beneficial effects of hydroxytyrosol on humans that are linked to its antioxidant activity, it has been recommended by the European Food Safety Authority for regular consumption [35–41].

5. Conclusions

Olive paste in an amount equal to 20% (*w/w*) can replace 4.1% of the hard margarine in cookie formulation without compromising sensory quality. This level of replacement significantly increases the unsaturated fat and antioxidant activity of cookies. Olive paste cookies seem an interesting functional snack that may exert health beneficial effects on humans. Future studies could investigate other in vitro bioactivities with a nutritional interest as well as the health effects of the consumption of olive paste cookies through dietary interventions in humans.

Author Contributions: Conceptualization, H.C.K.; A.K.; D.K. and D.S.; methodology, C.N.; software, C.N.; investigation, E.-A.A.; S.-P.P.; D.K.; A.S.P.; C.N. and C.D.; resources, H.C.K.; writing—original draft preparation, H.C.K. and C.N. writing—review and editing, H.C.K.; C.N.; A.K. and D.S. supervision, H.C.K.; project administration, H.C.K.; funding acquisition, H.C.K. and D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the ERDF research regional program of the Western Greece entitled "Production of innovative olive based biscuits with dietary added value" code number: DEP6-0022676, granted to the Greek food—olive company AMALTHIA S.A.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available within this article.

Acknowledgments: Our thanks to the AMALTHIA S.A. company for the supply of the olive paste used in the study and for their cooperation in the cookie production process.

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

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