sustainability

MDPI

Article

Trends in Food Innovation: An Interventional Study on the Benefits of Consuming Novel Functional Cookies Enriched with Olive Paste

Olga Papagianni [1], Iraklis Moulas [1], Thomas Loukas [2], Athanasios Magkoutis [2], Dimitrios Skalkos [3], Dimitrios Kafetzopoulos [4], Charalampia Dimou [1], Haralabos C. Karantonis [5] and Antonios E. Koutelidakis [1,*]

1 Laboratory of Nutrition and Public Health, Unit of Human Nutrition, Department of Food Science and Nutrition, University of the Aegean, 81400 Myrina, Greece; olga3_pap@yahoo.gr (O.P.); fns17077@fns.aegean.gr (I.M.); chadim@aegean.gr (C.D.)
2 Outpatient Clinic, 81400 Myrina, Greece; tloukas2002@yahoo.com (T.L.); tmagoutis@gmail.com (A.M.)
3 Laboratory of Food Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece; dskalkos@uoi.gr
4 Department of Business Administration, University of Macedonia, 54636 Thessaloniki, Greece; dimkafe@uom.edu.gr
5 Laboratory of Food Chemistry, Biochemistry and Technology, Department of Food Science and Nutrition, University of the Aegean, 81400 Myrina, Greece; chkarantonis@aegean.gr
* Correspondence: akoutel@aegean.gr

Citation: Papagianni, O.; Moulas, I.; Loukas, T.; Magkoutis, A.; Skalkos, D.; Kafetzopoulos, D.; Dimou, C.; Karantonis, H.C.; Koutelidakis, A.E. Trends in Food Innovation: An Interventional Study on the Benefits of Consuming Novel Functional Cookies Enriched with Olive Paste. *Sustainability* **2021**, *13*, 11472. https://doi.org/10.3390/su132011472

Academic Editor: Mariarosaria Lombardi

Received: 18 July 2021
Accepted: 13 October 2021
Published: 17 October 2021

Abstract: Olive paste may exert bioactivity due to its richness in bioactive components, such as oleic acid and polyphenols. The present interventional human study investigated if the fortification of cookies with olive paste and herbs may affect postprandial lipemia, oxidative stress, and other biomarkers in healthy volunteers. In a cross-over design, 10 healthy volunteers aged 20–30 years, consumed a meal, rich in fat and carbohydrates (50 g cookies). After a washout week, the same volunteers consumed the same cookie meal, enhanced with 20% olive paste. Blood sampling was performed before, 0.5 h, 1.5 h, and 3 h after eating. Total plasma antioxidant capacity according to FRAP, ABTS, and resistance to copper-induced plasma oxidation, serum lipids, glucose, uric acid, and antithrombotic activity in platelet-rich plasma were determined at each timepoint. There was a significant decrease in triglycerides' concentration in the last 1.5 h in the intervention compared to the control group ($p < 0.05$). A tendency for a decrease in glucose levels and an increase in the plasma antioxidant capacity was observed 0.5 h and 1.5 h, respectively, in the intervention compared to the control group. The remaining biomarkers did not show statistically significant differences ($p > 0.05$). More clinical and epidemiological studies in a larger sample are necessary in order to draw safer conclusions regarding the effect of olive paste on metabolic biomarkers, with the aim to enhance the industrial production of innovative functional cookies with possible bioactivity.

Keywords: postprandial bioactivity; bioactive compounds; metabolic biomarkers; functional cookies; olive paste

1. Introduction

COVID-19 pandemic has generated opportunities and challenges for the production of innovative functional foods and nutraceuticals containing bioactive compounds, and highlighted the advances of nutrition to boost consumers' immune system and improve their overall health [1]. Concerning that food companies are seeking new innovative products to cover consumer needs, the production and commercialization of food such as cookies rich with bioactive compounds will increase the interest of health-conscious individuals. Inflammation and oxidative stress are related to diet and play an essential role in the pathogenesis of chronic diseases such as diabetes, cardiovascular disease, neurodegenerative diseases, and cancer. The modern lifestyle associated with COVID-19

pandemic and unhealthy dietary patterns, exposure to a wide range of chemicals and lack of physical activity, seems to play a significant role in the induction of oxidative stress and inflammation [2].

The postprandial state is a normal, metabolic process that takes place throughout the day and involves multiple mechanisms. It refers to the state after meal consumption, when the digestion and absorption of nutrients are completed (duration 6–12 h) [3]. Numerous factors related to dietary intake have been associated with the activation of the endogenous or innate immune system, which is followed by the induction of a mild inflammatory response. Consumption of a meal, rich in fats or carbohydrates or their complex, may lead to the promotion of oxidative stress [3]. Postprandial lipemia and hyperglycemia may cause vascular damage by molecular mechanisms, including endothelial dysfunction, activation of molecules' adhesion, hemostasis activation, oxidative stress, or genetic polymorphisms that affect genes involved in lipoprotein metabolism [3].

After a meal intake and under normal conditions, there is a rapid increase in plasma glucose concentration, while the rate of glucose absorption is higher than the rate of endogenous glucose production. Important factors affecting the postprandial glycemia, are the amount and duration of food intake, carbohydrate content, glucose uptake rate and insulin resistance. Glucose levels 1–2 h after ingestion is an important factor in predicting the risk of cardiovascular disease, as concentrations higher than 7.8 mmol/L mean hyperglycemia [4,5]. In addition, after a meal consumption, plasma is enriched with triglyceride lipoproteins (TRL) of intestinal (chylomicron) or hepatic (very low-density lipoprotein, VLDL) origin [6].

Both postprandial hyperglycemia and hyperlipidemia are involved in inhibiting oxidative phosphorylation of mitochondria, allowing the passage of peroxide anions into the circulation and leading to increased ROS production by leukocytes. The main factor that affects the degree of oxidative stress after eating a meal is the amount of caloric intake [7]. Scientific evidence suggests that low-grade inflammation and endothelial dysfunction, combined with insulin resistance, are associated with increased risk of metabolic syndrome (MS), cardiovascular disease (CVD), and type 2 diabetes. Scientific data show that oxidation, inflammation, and thrombosis are key mechanisms that coexist and underly to the onset of atherogenesis [8].

Epidemiological and clinical studies suggest that the adoption of a diet rich in fruits, vegetables, raw cereals, fish and dairy products, low in saturated fat, such as the Mediterranean diet, reduces the risk of developing cardiovascular disease. However, the achievement of such a diet requires significant modifications to the wider dietary behavior of the population [9]. The development of innovative foods, such as cookies, enriched with components from the Mediterranean diet, could be an alternative way for diet improvement in parallel with the modern lifestyle. Recent scientific evidence suggests that the consumption of functional foods may have a beneficial effect on reducing the risk of chronic diseases such as obesity, cardiovascular disease, and diabetes [10]. Functional foods are defined as natural or processed foods, which have been shown to contribute to the achievement of specific functional goals within the human body, contributing to the possible prevention of diseases and to general health promotion [11–13]. The enrichment of foods in order to produce functional foods is achieved by adding bioactive compounds, such as phytosterols and stanols, antioxidants (carotenoids, polyphenols), dietary fiber (β-glucans), oligosaccharides (fructans), n-3 long chain fatty acids, etc., as they have been shown to play an important role in both inhibiting intestinal cholesterol absorption and in reduced glycemic response and oxidative stress [9,10]. However, limited number of scientific data are available on the potential postprandial bioactivity of innovative foods, such as cookies, enhanced with bioactive compounds from olive products.

Olive products may reduce the pre-inflammatory status as well as the oxidative damage, which are caused by the LDL cholesterol and free radicals' oxidation, respectively. The antioxidant activity possessed by olive phenolic compounds has been supported by an increase in plasma antioxidant capacity, modifying the lipid profile, and preventing

oxidative damage in a group of young and middle-aged healthy volunteers. These results are mainly attributed to the redox properties of phenolic compounds, which allow to act as reducing agents and hydrogen donors [14]. The main bioactive ingredients found in olives are phenolic alcohols, 3,4-DHPEA (hydroxytyrosol) and *p*-HPEA (tyrosol), as well as the secoiridoid derivatives 3,4-DHPEA-EA (an oleuropein aglycon), *p*- HPEA-EA (ligstroside aglycon), 3,4-DHPEA-EDA (oleuropein aglycon di-aldehyde), *p*-HPEA-EDA (ligstroside aglycon de-aldehyde) and oleuropein [15]. Additionally, the oleic acid contained in olive products has been shown to possess a protective effect against insulin resistance, and contribute to the improvement of endothelial dysfunction in response to pro-inflammatory stimuli. Likewise, it has been reported that oleic acid may reduce blood lipids levels, mainly cholesterol, LDL, and triglycerides [15].

The plasma antioxidant levels reflect the general body antioxidant capacity. Total plasma antioxidant capacity is primarily credited to ascorbic acid, α-tocopherol, glutathione, lipoic acid, uric acid and urea, β-carotene, ubiquinone, and bilirubin. Low antioxidants levels in blood have been linked to the pathogenesis of various diseases such as cancer, Alzheimer, Parkinson, diabetes, rheumatoid arthritis, hypertension, heart disease, and aging. In contrast, high concentrations of antioxidants in the blood strengthen the body's defenses against degenerative diseases. The advantages acquired from antioxidants' dietary intake is attributed to the food content of antioxidants with various antioxidant potential that can participate in numerous reactions, neutralizing effectively the free radicals [16]. The nutritional biomarkers' levels, which are determined in biological samples (urine, blood, and other tissues) may reflect either dietary intake levels, the metabolic rate of food components, or nutritional status and could be used as indicators of a person's normal functioning or of a clinical condition [15].

Thus, the development of strong biomarkers is a matter of necessity, as it is presumed that it will contribute to the achievement of an optimal classification of dietary intake or evaluation of the interdependence of nutrition with chronic diseases. Scientific interest in the application of metabolic biomarkers has intensified in recent years, with the aim of discovering further biological elements of dietary intake. Existing findings from dietary interventions in clinical trials suggest that the consumption of refined wheat flour of cookies may cause hyperglycemia, as the source of rapidly digestible starch [17]. Furthermore, it is noteworthy that the lack of scientific data observed, regarding the possible effect of a meal containing cookies enhanced with bioactive compounds, derived from natural food functional sources, on the postprandial state.

The aim of the present interventional study was to investigate if the fortification of cookies with olive paste and herbs may affect postprandial lipemia, oxidative stress, and other biomarkers in healthy volunteers.

2. Materials and Methods

2.1. Subjects

The study protocol was approved by the Ethics Committee at the University of the Aegean and performed in accordance with the Declaration of Helsinki. The study duration was from 1 May to 30 June 2021. All volunteers signed an updated consent form and were informed about the ultimate purpose of the study, the confidentiality of the data obtained, and the voluntary nature of participation. All participants were initially tested using a medical history questionnaire that also included demographic characteristics, level of physical activity, frequency of polyphenol-rich foods, and general habits, as smoking, and alcohol consuming, referring to the last 6 months. Anthropometric measurements, especially measurement of height, weight, and body composition was performed with the use of a suitable body composition analyser (Tanita SC 330).

After an initial screening of 16 potential participants, 11 volunteers were selected according to the inclusion and exclusion criteria and finally 10 healthy volunteers, 4 men and 6 women, aged 20–30 years were recruited to the study. The recruitment of the participants was carried out in a random way via social media and online announcements

at Lemnos Island and Lemnos University Department. The study excluded people over the age of 30, in order to achieve the participation of a homogeneous sample of volunteers, those who had received dietary supplements in the last two months, those with a history of chronic diseases including type I and II diabetes (HbA1c > 5%), those who showed heavy smoking (>10 cigarettes/day), abnormal BMI (>25 kg/m^2), and alcohol overdose (>40 g alcohol/day), factors that could lead to unstable conclusions. Prior to the start of the study, participants underwent biochemical blood tests, in collaboration with external physicians, in order to exclude cases with hematological and biochemical profile beyond normal values (cholesterol > 6.8 mM, triglycerides > 2.8 mM, glucose > 6.11 mM).

Subjects were asked to come after a 12 h fast, as well as to avoid taking medication on the day of the study as well as any dietary supplements. They were also asked to abstain from foods high in antioxidants and alcohol for 24 h before the study.

2.2. Treatments

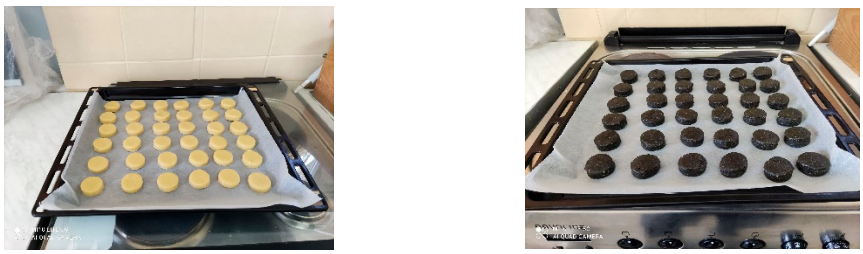
Cookies were supplied by Greek olive company AMALTHIA SA. Each functional cookie weighed a total of 12.5 g, contained 5.4 g of soft wheat flour, 1.6 g of oat flour, 0.08 g of soda, 2.04 g of vegetable margarine, 0.72 g of sugar, 2.5 g of olive paste, as well as 0.12 g garlic, 0.06 g of oregano, and 0.06 g of thyme. The functional meal weighed a total of 50 g, and contained 4 functional cookies, enhanced with 20.0% olive paste, 1.0% garlic, and thyme and oregano at a percentage of 0.5 % for each.

Each control cookie, which weighed a total of 12.5 g, contained 5.4 g of soft wheat flour, 1.6 g of oat flour, 0.08 g of soda, 4.7 g of vegetable margarine, and 0.72 g of sugar. The difference from the functional ones lies in the replacement of the fats, derived from the olive paste from an additional amount of fats, derived from vegetable margarine, while in the control cookies no herbs and spices were added. The control meal weighed a total of 50 g and contained 4 control cookies. Dietary composition of the test meals is shown in Table 1.

Table 1. Nutritional composition of each meal cookies.

Meals' Dietary Composition	Control	Functional
Energy (kcal)	242.32	185.1
Carbohydrates (g)	23.68	24.27
Fat, total (g)	15.04	8.37
Protein (g/kg)	3.2	3.28
Saturated fat (g)	2.84	1.52
Unsaturated fat (g)	6.38	6.52
Cholesterol (mg)	0	0
Dietary fiber, total (g)	1.24	1.59
Sugar, total (g)	2.96	2.96

Figure 1 presents control coolies (a) and functional cookies (b), prior to the baking.



(**a**) (**b**)

Figure 1. Control (**a**) and Functional (**b**) cookies prior to the baking.

In vitro preliminary studies for the tested cookies have been performed in order to test sensorial acceptability, total phenolics, and antioxidant activity by DPPH, ABTS, FRAP, and CUPRAC assays, and the results have been recorded by Argyri et al. [18].

2.3. Study Design

It was an acute cross-over and two-period, interventional study. All participants on enrollment were randomly assigned to group C:F (Control:Functional-Control:Functional) or to group F:C (Functional:Control-Functional:Control). Individuals who joined the C:F group during the first period received the control meal and during the second trial period ate the functional meal, while participants in the F: C group received the functional meal during the first period and the control meal in the second period. Figure 2 shows the crossover design study illustration.

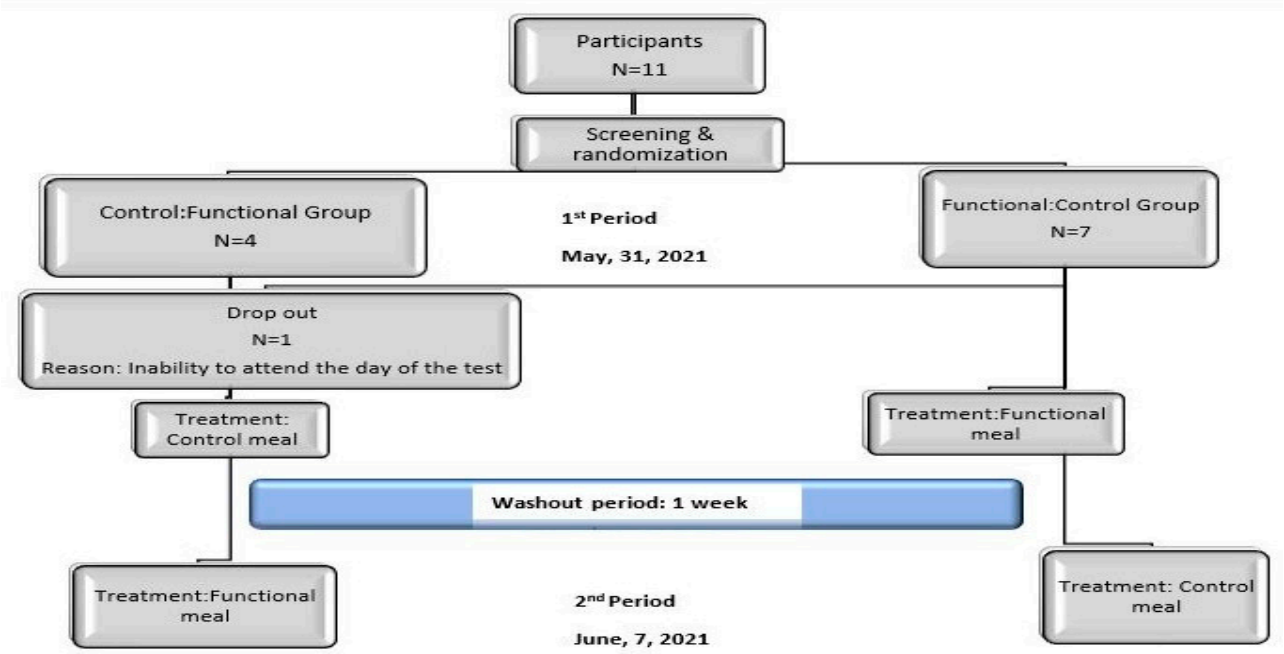


Figure 2. Illustration of study crossover design.

The volunteers arrived at the Human Nutrition Unit at 9 a.m., after a 12 h fast and abstinence from dietary supplements and any medication. The participants were asked to complete a short 24 h recall questionnaire, which recorded all meals eaten in the last 24 h.

A meal consisting of 4 biscuits (50 g) was then offered for consumption, while a glass of water (250 mL) was available for each participant. For the first trial period, volunteers in the C:F group consumed the control cookie meal, while individuals in the F:C group received the functional cookie meal. Respectively, during the second trial period, the participants in the C:F group received the functional cookie meal and those in the F:C group received the control cookie meal.

Ten mL of blood was drawn from all volunteers, shortly before the meal (baseline) and 30 min, 1.5 h and 3 h (Figure 3) after meal consumption. Blood samples were collected in EDTA and citric acid tubes for plasma separation or in heparin tubes for serum separation. Plasma and serum of each volunteer and for each time point were separated by 10-min centrifugation at 20,000× *g* and cooled to −4 °C in a tabletop high speed refrigerated centrifuge immediately after blood collection. Aliquots of plasma or serum were stored at –40 °C until analysis.

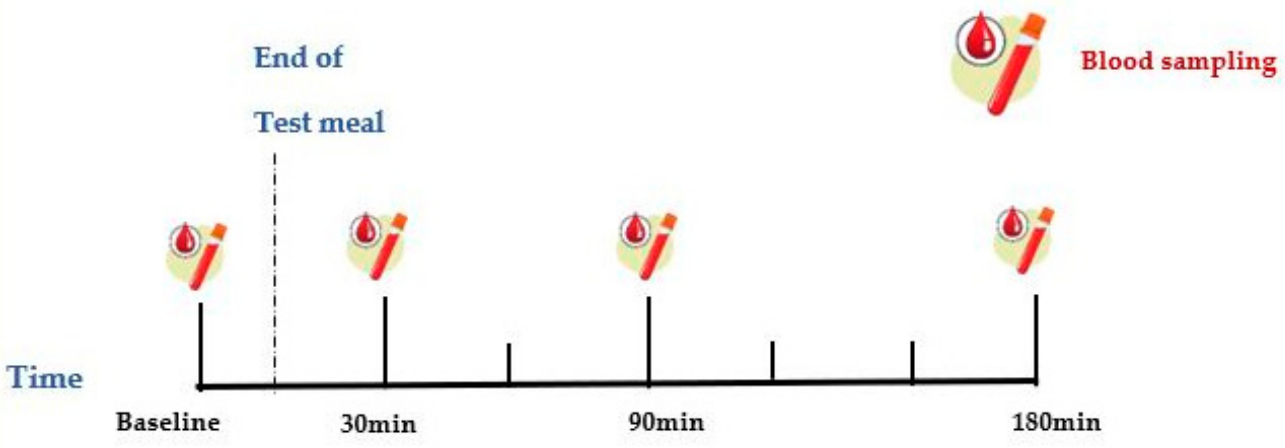


Figure 3. Test visit flow diagram.

2.4. Biochemical Analyses in the Blood Samples

Total Antioxidant Capacity (TAC) was evaluated in plasma by Ferric Reducing Antioxidant Power (FRAP assay), as described by Argyri et al. [18,19]. Antiradical activity was determined by Trolox Equivalent Antioxidant Capacity (TEAC) assay, as previously described in deproteinized plasma according to Prymont-Przyminska et al. [20]. Resistance to copper-induced plasma oxidation was determined according to Sakka and Karantonis [21].

Inhibition of platelet activating factor (PAF)—induced thrombosis in platelet-rich plasma was determined according to Antonopoulou et al. [22].

Total, HDL and LDL cholesterol, triglycerides, glucose and uric acid were measured in serum with an automated analyzer (COBAS c111, Roche, Basel, Switzerland).

2.5. Statistical Analysis

The statistical analysis was performed using SPSS (SPSS V210). The computational power of each sample was calculated for the outcome, as well as the venous plasma antioxidant capacity (TAC) using Statmate version 2.0 (GraphPad Software, Inc., San Diego, CA, USA). Taking $\alpha = 0.01$, the sample of 10–14 individuals allows the detection of a difference of 0.21 mmol TAC/L between the control group and the intervention group, calculated from the expected SD between the differences of the meal group 0.21 mmol/L. The level of statistical significance was at $p < 0.05$. Prior to any statistical analysis, all variables were tested for normal distribution. For the variables that followed normal distribution, repeated post-hoc test ANOVA and Bonferroni test, as well as Wilcoxon sign rank test were performed for the differences between plasma and serum samples at 0.5 h, 1.5 h, and 3 h for each meal group and by change from baseline for venous plasma TAC, TEAC, and copper-induced oxidation, platelet rich plasma thrombosis, and serum biomarkers. Differences between the two treatment groups at any time and time period from the baseline were also examined by the paired *t*-test. For variables that did not follow a normal distribution, Wilcoxon sign rank tests were performed, both for changes in each biomarker and for each treatment group, and for significant differences between the two treatments, at different times, at intervals between blood sampling and at intervals for changes from baseline. The variables LDL and HDL cholesterol, antioxidants, uric acid, ABTS, copper-induced plasma oxidation, and platelet rich plasma thrombosis did not follow a normal distribution. The variables cholesterol, triglycerides, and glucose followed a normal distribution. All data were taken into account for statistical analyses.

3. Results

3.1. Baseline Characteristics

Ten participants completed the study, while one volunteer was unable to attend the study appointments. The volunteers' baseline characteristics during the initial screening

are presented in Table 2. No difference was observed between men and women in all tested parameters. The analysis of food frequency questionnaires showed that the majority of the participants consumed fruits 1–2 times a week and vegetables 1–2 times a week, while they stated that in their diet, they included starchy foods 1–2 times a week (data not shown).

Table 2. Volunteers' characteristics at baseline.

	N
Volunteers	11
Men	4
Women	7
Smokers	8
Dietary Supplementation	1
Physical Activity	
Low	5
Medium	4
High	2
	Mean ± SD
Age (years)	22.8 ± 1.6
Weight (kg)	75.2 ± 10.3
Height (cm)	168 ± 6.7
BMI	25.8 ± 6

3.2. Plasma Total Antioxidant Capacity and Oxidation Resistance

3.2.1. Plasma Total Antioxidant Capacity

Plasma TAC differed between the two groups for value changes from 1.5 to 3 h after the meal consumption. Venous plasma TAC increased from 1.5 h to 3 h after the functional meal (MD = 0.02, 6.5%) compared to the control meal, where TAC decreased significantly (MD = −0.13, 27.5%, p = 0.05) as shown in Table 3. No other statistically significant differences were observed between the control group and the treatment group.

Table 3. Effects of functional cookies on plasma TAC, serum glucose, and triglycerides levels.

	a. Significant Changes of Each Treatment over Time			
				Mean Difference
Biomarker	p Value [a]	Timepoints' Difference	P (Bonferroni Test) [b]	P (Wilcoxon Test) [c]
Antioxidant Capacity (TAC) (mmol/L)				
Control		30 min–Baseline		0.109
		1.5 h–30 min		0.285
		3 h–1.5 h		0.05 *
Functional		30 min–Baseline		0.109
		1.5 h–30 min		0.109
		3 h–1.5 h		0.285
Glucose(mg/dL)				
Control	0.024 *	Δbaseline–Δ30 min		0.012
		Δ30 min–Δ1.5 h		0.05
		Δ1.5 h–Δ3 h		0.035
	0.001 *	Δ1.5 h–30 min	−23	
Functional	0.896			
Triglycerides (mg/dL)				
Control	0.130			
Functional	0.05 *	3 h–1.5 h	−19.8	

[a] p values represents the statistical significance over time for each treatment, and for the variables that follow a normal distribution. [b] p indicates the mean difference obtained from the Bonferroni test for the baseline time periods for each treatment, and for the variables following a normal distribution. [c] p values represent the statistical significance over time for each treatment, and for variables that do not follow a normal distribution. * correspond statistical significant differences (p < or = 0.05).

3.2.2. Plasma Antiradical Activity Based on TEAC Assay

A gradual increase in plasma antiradical activity expressed as % scavenging activity of the ABTS radical cation was observed in the functional group, reaching an increase of MD = 35.0 (92.1%), 3 h after consumption in comparison with baseline values, when for the control group a decrease of MD = 6.0 (12.5%) was observed at 0.5 h compared to baseline and an increase of MD = 18 (37.5%) and 8 (16.7%) at 1.5 h and 3 h, respectively. All differences failed to reach statistical significance.

3.2.3. Coper-Induced Plasma Oxidation Resistance

A non-significant, gradual increase in oxidation resistance was observed in the functional group, reaching an increase of MD = 9.06 (65.6%), 3 h after consumption in comparison with baseline values, when for the control group a gradual decrease in oxidation resistance was observed, especially in the last 1.5 h (1.5 h−3 h), reaching a decrease from the baseline of MD = −1.4 (−28.5%). No statistically significant difference was observed between different fractions for oxidation resistance measurements.

3.3. Antithrombotic Activity

PAF-induced platelet rich plasma thrombosis was not affected by the intervention, since no differences were noticed in the intervention compared to the control group.

3.4. Serum Lipids, Glucose, and Uric Acid Concentrations

Serum triglyceride concentrations differed significantly between the two treatments 3 h after each meal intake (paired sample *t*-test, $p = 0.041$ and treatment * time, $p = 0.02$) (Table 4). In addition, a statistically significant difference was found between the two treatments in the different time intervals ($p = 0.003$) and between each timepoint and baseline ($p = 0.004$). Although, triglyceride levels 3 h after the control meal are represented as not significant increase, while triglyceride values were decreased significantly in the last 1.5 h (the period from 1.5 h to 3 h) after functional meal consumption (MD = −19.87, 34.9%, $p = 0.05$) (Table 3).

Serum glucose concentration decreased to a non-significant, greater degree 3 h after the consumption of the functional meal (18.7%, MD = −16.1) compared to the degree of decrease observed 3 h after the control meal consumption (2.1 %, MD = −1.6). A statistically significant difference was observed for changes in glucose values over time in the control group for changes from 30 min to 3 h ($p = 0.001$, $P_{Bonferroni} = -23$), and for all time intervals between blood sampling (Δ30-baseline $p = 0.012$, Δ90–30 $p = 0.051$, Δ180–90 $p = 0.035$) (Σφάλμα! Το αρχείο προέλευσης της αναφοράς δεν βρέθηκε). Serum glucose concentration differed significantly in terms of sampling timepoints ($p = 0.022$) and for all changes observed from baseline ($p = 0.046$), after consuming both meals (Table 4).

No statistically significant differences were observed for any interaction regarding the remaining biomarkers tested (total, HDL and LDL cholesterol and uric acid), as there was a similar response to the levels of these biomarkers after consuming both meals.

Table 4. Significant differences between control and functional cookies consumption, over time and for changes from baseline, regarding plasma TAC concentrations as well as serum glucose and triglycerides.

	a. Significant Difference for Each Time Point			**Treatment Comparison**		
	Treatment	Time	Treatment * Time	Mean Difference	Paired-Samples *t*-Test	Wilcoxon Sign Rank Test
Biomarker	*p* Value [a]	*p* Value [a]	*p* Value [a]	Timepoint	*p* Value [b]	*p* Value [c]
Glucose (mg/dL)	0.114	0.022 *	0.450			
Triglycerides (mg/dL)	0.171	0.297	0.02*	3 h	0.041	
Antioxidant capacity (TAC) (mmol/L)				30 min		0.782
				1.5 h		0.312
				3 h		0.153
	b. Significant difference for baseline changes					
	Treatment	Time	Treatment * Time period		Paired-Samples *t*-test	Wilcoxon sign rank test
Biomarker	*p* value [d]	*p* value [d]	*p* value [d]	Time period	*p* value	*p* value [e]
Glucose (mg/dL)	0.146	0.046 *	0.777			
Triglycerides (mg/dL)	0.004 *	0.182	0.297			
Antioxidant capacity (TAC) (mmol/L)				Δ30 min-baseline		0.875
				Δ1.5 h-baseline		0.381
				Δ3 h-baseline		0.081

* Indicates the statistical significance. [a] *p* values indicate the statistical significance for the effect of treatment, the effect of time, and the effect of treatment x time interaction, obtained by repeated ANOVA tests, for the changes between the time points, and for the variables that follow a normal distribution. [b] *p* value indicates the statistical significance for the effect of treatment on the time points obtained with Paired samples *t*-test for the changes between the time points, and for the variables that follow a normal distribution. [c] *p* value indicates the statistical difference between the two treatments (Control-Functional) in the time points obtained with Wilcoxon sign rank test, and for the variables that do not follow a normal distribution. [d] *p* value indicates the statistical significance for the effect of treatment, the effect of time and the effect of therapy x interaction time, obtained by repeated ANOVA tests for changes up to 3 h from the baseline (fasting, 0 h), and for the variables that follow a normal distribution. [e] *p* value indicates the statistical difference between the two treatments (Control-Functional) for the changes up to 3 h from the baseline (fasting, 0 h), obtained with Wilcoxon sign rank test, and for the variables that do not follow a normal distribution.

4. Discussion

The significance of the findings of the acute effect of this dietary intervention on the metabolic, postprandial biomarkers lies in the scientific data which suggest that postprandial hyperlipidemia, hyperglycemia, and induction of oxidative stress are associated with vascular dysfunction [23]. The main finding of this nutritional intervention study was that serum triglyceride levels gradually decreased after consuming the meal containing cookies enriched with olive paste and herbs, as presented in Table 3. In particular, serum triglyceride concentration was significantly reduced at 3 h after the intake of the functional meal. In contrast, for the control group, it was observed that 3 h after consuming the meal containing the control cookies, serum triglyceride values were significantly higher than the baseline values (Tables 3 and 4). The development of innovative functional foods rich in bioactive compounds from the Mediterranean diet could be an alternative way to enhance nutrients intake, improve dietary habits, and decrease the risk of chronic diseases and infections, which is of high importance in the period after COVID-19 pandemic.

The possible beneficial effect of the meal contained olive paste enriched cookies, in the concentrations of serum triglycerides, could be attributed to oleic acid, the basic monounsaturated fatty acid contained in olive paste. Oleic acid consumption has been associated with an improved or unchanged lipid profile, mainly due to a reduction in total cholesterol and LDL cholesterol levels. Data on the effect of postprandial consumption of oleic acid-rich foods on triglyceride levels are conflicting, when some interventional studies showed that their values decrease, and in other studies, HDL cholesterol concentrations appear an increase. Replacing trans fats with oleic acid has been suggested to increase HDL cholesterol and lower triglyceride levels [24].

The fatty acid composition of a meal greatly affect the subsequent responses, as the first chylomicrons enriched with triacylglycerol, appearing in the post-meal circulation contain the triacylglycerols from the previous meal. In a study examining the responses of a control meal, a butter-fortified meal, and an olive oil-fortified meal, it was found that butter tended to cause significantly higher triacylglycerol concentrations during the 8-h test period than olive oil. In addition, a meal fortified with butter led to a reduction in HDL-cholesterol levels, which was not observed after eating a meal fortified with olive oil. These results are probably attributed to the fact that the administration of triglyceride emulsions leads to increased concentrations of fatty acids, which lies in the activity of the enzyme lipoprotein lipase [25]. These findings are in consistence with the findings of the presented study, where after consuming the meal containing the enriched with olive paste cookies, there was a decrease in the concentration of volunteers' plasma triacylglycerols, in contrast to the control meal. However, no significant difference was found in the response of HDL cholesterol between the control meal and the functional meal. In an intervention study, in which 10 volunteers with high fasting glucose levels consumed a meal with olive oil or not, it was found that the meal containing olive oil led to a statistically significant reduction in triglyceride and Apo B-48 levels and glucose levels, compared to the meal that did not contain olive oil. This beneficial postprandial effect of olive oil was probably attributed to the regulation of incretin secretion [26]. In addition, Gilmore et al. observed that a 5-week diet intervention, with a high content of monounsaturated fats, led to an increase in the concentration of HDL cholesterol and a decrease in the ratio of LDL:HDL cholesterol [27].

The increase in plasma TAC observed 3 h after the consumption of the functional cookie meal could be attributed to increased concentrations of polyphenolic metabolites, such as tyrosol, hydroxy–tyrosol, and oleuropein. The combination of these phenolics has been shown to be potent against free radical oxidation, inhibiting the oxidation of low-density lipoproteins. It is speculated that the metabolites of the aforementioned polyphenols may be transported into the blood plasma [26]. However, research shows that this change can also be attributed to the presence of uric acid, which can contribute up to 60% of antioxidant activity as an endogenous, postprandially increasing antioxidant [28]. Numerous studies confirm the above finding, regarding the antioxidant effect of a meal containing olive paste-enriched cookies, as it has been observed that consuming a meal, high in monounsaturated fatty acid may lead to increased plasma concentrations of antioxidants, when compared with meals, rich in polyunsaturated or saturated fats. This could be explained by the fact that the TRL particles provided by the monounsaturated fatty acid-rich meal could have a greater affinity for the hepatic receptor involved in metabolism, leading to faster and more efficient neutralization of TRLs compared to other fat types [26]. The tendency for an increase of plasma antiradical activity 3 h after consumption of functional meal is in accordance with the increase in plasma TAC (Tables 3 and 4). This tendency failed to reach statistical significance possibly due to the limited number of volunteers, which is a limitation of the study.

The fact that PAF-induced platelet rich plasma thrombosis was not affected in the intervention compared to the control group shows that after digestion of olive paste, garlic, thyme, and oregano constituents do not exert antithrombotic effects at the levels relevant to the present study. To the best of our knowledge, nutritional interventions with olive paste

or olives have not been performed until now. A previous study has been referring an effect on PAF-induced platelet thrombosis in human volunteers who consumed 15 g of olive oil (1 g capsules of olive oil three times per day with meals). Taking into account that olive paste in cookies contained 20.3 ± 0.8 olive oil [18], volunteers in this study consuming 50 g of cookies received about 2 mL olive oil, which is much lower than the study with olive oil to have an effect [29].

The latest finding of this study was the trend toward a more pronounced reduction in volunteer blood glucose levels, 30 min after the consumption of the meal, which included olive paste-enhanced cookies (Tables 3 and 4). This result is confirmed by a clinical study in healthy volunteers, which found that oleuropein, which is included in the composition of olive oil, is believed to reduce postprandial lipemia. This beneficial effect is probably due to the increased activity of the incretins GLP1 and GIP, inhibiting the activity of the enzyme DPP-4 (dipeptidyl peptidase). Incretins are secreted by the peripheral small intestine in response to its stimulation by binding to receptors in the endocrine pancreas and causing insulin secretion and a decrease in postprandial blood glucose [26].

Finally, it is worth mentioning that oregano and thyme added to the cookies probably contribute to a lesser extent to the beneficial effect that the enrichment of the cookies with a mixture of olive paste may possess. The possible bioactivity of these aromatic plants in postprandial lipemia, glycemia, and oxidative stress has been previously investigated, and lies mainly in the bioactive ingredients that they contain such as thymol, carvacrol etc. [30].

During the COVID-19 pandemic, the need for development by the food industry of innovative functional food with increased nutritional value and possible health effects is constantly increasing. Consumers' demands are also focused on novel foods based on both traditional and nutritious raw materials, with a parallel positive environmental impact. The present study indicates that the development of functional cookies enriched with olive bioactive compounds and herbs may contribute to biomarkers which link to possible bioactivity and potential health effects. The use of second sort olives, as well as future use of olive leaves and olive pomace extracts, as olive oil by-products, may help solve a huge environmental problem, by valorization of industrial pollutant wastes, and in parallel contribute to the isolation of valuable bioactive compounds to create innovative food products. Furthermore, the use of traditional natural functional foods, such as olives, olive oil, and herbs could enhance the trend on food industry innovation, via development of processed novel biofunctional foods with nutritional claims and possible bioactivity, such as cookies, cheeses, yogurts, etc. [1,2,10,31,32].

Nevertheless, the study has some limitations. Initially, although the adequacy of the sample size used was statistically calculated and is similar to other studies [32], the small sample size may have influenced the lack of statistical significance for most of the biomarkers tested. Studies with a higher number of participants should be performed to determine if a novel cookie with olive paste has more pronounced effects in the postprandial state. Moreover, the study assessed the total antioxidant capacity and oxidation resistance of plasma, but individual polyphenols detection was not performed. Furthermore, the possible functional effect of the novel cookie was investigated in healthy volunteers; more interventional studies should be performed in order to evaluate its postprandial effect in patients with cardiovascular disease or metabolic syndrome. Finally, the present study did not detect differences between men and women in each biomarker tested, possibly due to the small sample size.

5. Conclusions

The findings of this nutritional intervention study indicate that the enhancement of cookies with mainly olive paste, but also with medicinal herbs, is likely to be beneficial, as there was a trend of bioactivity, which is mainly focused on reducing serum triglyceride levels, increasing antioxidant activity, and greater reduction in serum glucose levels, postprandially. Monounsaturated fatty acids, especially oleic acid, and polyphenols contained in olive paste, may have a beneficial effect on metabolic, postprandial biomarkers, such

as blood lipids and glucose. These findings enhance the need for the development of novel industrial functional foods, with the aim to cover the increasing consumers' need for innovative food items, rich in nutrients and bioactive compounds. However, it is necessary to continue and expand clinical trials in a larger sample of the population, in order to draw safer conclusions about the effect of olive paste on postprandial and other biomarkers of lipemia, glycemia, and also oxidative stress, factors that significantly affect the risk of cardiovascular disease.

In addition, epidemiological studies examining consumers' views and acceptance of functional foods suggest that organoleptic characteristics play an important role in their purchasing decision. Thus, a next step could be the design of sensory analysis and organoleptic tests of the produced, functional cookies, fortified with olive paste and aromatic herbs, in order to investigate consumer attitudes and perform possible improvements of the final product.

Author Contributions: Conceptualization, A.E.K.; methodology, A.E.K., O.P. and H.C.K.; software, O.P.; validation, A.E.K. and H.C.K.; formal analysis, O.P. and C.D.; investigation, O.P., I.M., A.E.K., H.C.K. and C.D.; resources, O.P., I.M., C.D., D.S., D.K., T.L. and A.M.; data curation, O.P., A.E.K. and H.C.K.; writing—original draft preparation, O.P., A.E.K. and H.C.K.; writing—review and editing, A.E.K.; visualization, A.E.K. and H.C.K.; supervision, A.E.K.; project administration, A.E.K.; funding acquisition, A.E.K., H.C.K., D.S. and D.K. 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 Western Greece entitled "Production of innovative olive based biscuits with dietary added value" code number: DEP6-0022676, granted to the Greek olive company AMALTHIA S.A.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of University of the Aegean.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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

Acknowledgments: Our thanks to AMALTHIA S.A. for supply of the cookies used in the study.

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

References

1. Galanakis, C.M.; Rizou, M.; Aldawoud, T.M.S.; Ucak, I.; Rowan, N.J. Innovations and Technology Disruptions in the Food Sector within the COVID-19 Pandemic and Post-Lockdown Era. *Trends Food Sci. Technol.* **2021**, *110*, 193–200. [CrossRef]
2. Sharifi-Rad, M.; Anil Kumar, N.V.; Zucca, P.; Varoni, E.M.; Dini, L.; Panzarini, E.; Rajkovic, J.; Tsouh Fokou, P.V.; Azzini, E.; Peluso, I.; et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front. Physiol.* **2020**, *11*, 694. [CrossRef] [PubMed]
3. Moreno-Luna, R.; Villar, J.; Costa-Martins, A.F.; Vallejo-Vaz, A.; Stiefel, P. The Postprandial State and Its Influence on the Development of Atherosclerosis. *Immunol. Endocr. Metab. Agents Med. Chem.* **2011**, *11*, 1–9. [CrossRef]
4. Meessen, E.C.E.; Warmbrunn, M.V.; Nieuwdorp, M.; Soeters, M.R. Human Postprandial Nutrient Metabolism and Low-Grade Inflammation: A Narrative Review. *Nutrients* **2019**, *11*, 3000. [CrossRef]
5. Hiyoshi, T.; Fujiwara, M.; Yao, Z. Postprandial Hyperglycemia and Postprandial Hypertriglyceridemia in Type 2 Diabetes. *J. Biomed. Res.* **2019**, *33*, 1–16. [CrossRef]
6. Bozzetto, L.; Della Pepa, G.; Vetrani, C.; Rivellese, A.A. Dietary Impact on Postprandial Lipemia. *Front. Endocrinol. (Lausanne)* **2020**, *11*, 337. [CrossRef]
7. Diamanti-Kandarakis, E.; Papalou, O.; Kandaraki, E.A.; Kassi, G. Nutrition as a Mediator of Oxidative Stress in Metabolic and Reproductive Disorders in Women. *Eur. J. Endocrinol.* **2017**, *176*, R79–R99. [CrossRef]
8. Demopoulos, C.A.; Karantonis, H.C.; Antonopoulou, S. Platelet Activating Factor—A Molecular Link between Atherosclerosis Theories. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 705–716. [CrossRef]
9. Sirtori, C.R.; Galli, C.; Anderson, J.W.; Sirtori, E.; Arnoldi, A. Functional Foods for Dyslipidaemia and Cardiovascular Risk Prevention. *Nutr. Res. Rev.* **2009**, *22*, 244–261. [CrossRef] [PubMed]
10. Koutelidakis, A.; Dimou, C. The Effects of Functional Food and Bioactive Compounds on Biomarkers of Cardiovascular Diseases. In *Functional Foods Text Book*, 1st ed.; Martirosyan, D., Ed.; Functional Food Center: Dallas, TX, USA, 2017; pp. 89–117.
11. Martirosyan, D.; Miller, E. Bioactive Compounds: The Key to Functional Foods. *Bioact. Compd. Health Dis.* **2018**, *1*, 36. [CrossRef]

12. Bidinger, M. Nutraceutical and Functional Food Regulations in the United States and around the World. *J. Chem. Inf. Model.* **2019**, *53*, 1689–1699.
13. Birch, C.S.; Bonwick, G.A. Ensuring the Future of Functional Foods. *Int. J. Food Sci. Technol.* **2019**, *54*, 1467–1485. [CrossRef]
14. Gambino, C.M.; Accardi, G.; Aiello, A.; Candore, G.; Dara-Guccione, G.; Mirisola, M.; Procopio, A.; Taormina, G.; Caruso, C. Effect of Extra Virgin Olive Oil and Table Olives on the ImmuneInflammatory Responses: Potential Clinical Applications. *Endocr. Metab. Immune Disord.-Drug Targets* **2017**, *18*, 14–22. [CrossRef]
15. Flori, L.; Donnini, S.; Calderone, V.; Zinnai, A.; Taglieri, I.; Venturi, F.; Testai, L. The Nutraceutical Value of Olive Oil and Its Bioactive Constituents on the Cardiovascular System. Focusing on Main Strategies to Slow Down Its Quality Decay during Production and Storage. *Nutrients* **2019**, *11*, 1962. [CrossRef]
16. Drouin-Chartier, J.P.; Côté, J.A.; Labonté, M.É.; Brassard, D.; Tessier-Grenier, M.; Desroches, S.; Couture, P.; Lamarche, B. Comprehensive Review of the Impact of Dairy Foods and Dairy Fat on Cardiometabolic Risk. *Adv. Nutr.* **2016**, *7*, 1041–1051. [CrossRef] [PubMed]
17. Ahmad, J.; Khan, I.; Johnson, S.K.; Alam, I.; Din, Z. Effect of Incorporating Stevia and Moringa in Cookies on Postprandial Glycemia, Appetite, Palatability, and Gastrointestinal Well-Being. *J. Am. Coll. Nutr.* **2018**, *37*, 133–139. [CrossRef]
18. Argyri, E.; Piromalis, S.; 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. [CrossRef]
19. Xiao, F.; Xu, T.; Lu, B.; Liu, R. Guidelines for Antioxidant Assays for Food Components. *Food Front.* **2020**, *1*, 60–69. [CrossRef]
20. Przyminska, A.P.; Zwolinska, A.; Sarniak, A.; Wlodarczyk, A.; Krol, M.; Nowak, M.; Johnson, J.D.G.; Padula, G.; Bialasiewicz, P.; Markowski, J.; et al. Isoflavone Intake Inhibits the Development of 7,12 Dimethylbenz(a)Anthracene(DMBA) Induced Mammary Tumors in Normal Andovariectomized Rats. *J. Clin. Biochem. Nutr.* **2014**, *54*, 31–38. [CrossRef]
21. Sakka, D.; Karantonis, H.C. In Vitro Health Beneficial Activities of Pumpkin Seeds from Cucurbita Moschata Cultivated in Lemnos. *Int. J. Food Stud.* **2015**, *4*, 221–237. [CrossRef]
22. Antonopoulou, S.; Fragopoulou, E.; Karantonis, H.C.; Mitsou, E.; Sitara, M.; Rementzis, J.; Mourelatos, A.; Ginis, A.; Phenekos, C. Effect of Traditional Greek Mediterranean Meals on Platelet Aggregation in Normal Subjects and in Patients with Type 2 Diabetes Mellitus. *J. Med. Food* **2006**, *9*, 356–362. [CrossRef]
23. Dimina, L.; Mariotti, F. The Postprandial Appearance of Features of Cardiometabolic Risk: Acute Induction and Prevention by Nutrients and Other Dietary Substances. *Nutrients* **2019**, *11*, 1963. [CrossRef] [PubMed]
24. Pastor, R.; Bouzas, C.; Tur, J.A. Beneficial Effects of Dietary Supplementation with Olive Oil, Oleic Acid, or Hydroxytyrosol in Metabolic Syndrome: Systematic Review and Meta-Analysis. *Free Radic. Biol. Med.* **2021**, *172*, 372–385. [CrossRef] [PubMed]
25. Thomsen, C.; Storm, H.; Holst, J.J.; Hermansen, K. Differential Effects of Saturated and Monounsaturated Fats on Postprandial Lipemia and Glucagon-like Peptide 1 Responses in Patients with Type 2 Diabetes. *Am. J. Clin. Nutr.* **2003**, *77*, 605–611. [CrossRef] [PubMed]
26. Carnevale, R.; Loffredo, L.; Del Ben, M.; Angelico, F.; Nocella, C.; Petruccioli, A.; Bartimoccia, S.; Monticolo, R.; Cava, E.; Violi, F. Extra Virgin Olive Oil Improves Post-Prandial Glycemic and Lipid Profile in Patients with Impaired Fasting Glucose. *Clin. Nutr.* **2017**, *36*, 782–787. [CrossRef]
27. Gilmore, L.A.; Walzem, R.L.; Crouse, S.F.; Smith, D.R.; Adams, T.H.; Vaidyanathan, V.; Cao, X.; Smith, S.B. Consumption of High-Oleic Acid Ground Beef Increases HDL-Cholesterol Concentration but Both High- and Low-Oleic Acid Ground Beef Decrease HDL Particle Diameter in Normocholesterolemic Men. *J. Nutr.* **2011**, *141*, 1188–1194. [CrossRef] [PubMed]
28. Tasaki, E.; Sakurai, H.; Nitao, M.; Matsuura, K.; Iuchi, Y. Uric Acid, an Important Antioxidant Contributing to Survival in Termites. *PLoS ONE* **2017**, *12*, e0179426. [CrossRef] [PubMed]
29. Mori, T.A.; Vandongen, R.; Mahanian, F.; Douglas, A. Plasma Lipid Levels and Platelet and Neutrophil Function in Patients with Vascular Disease Following Fish Oil and Olive Oil Supplementation. *Metabolism* **1992**, *41*, 1059–1067. [CrossRef]
30. Sekhon-Loodu, S.; Rupasinghe, H.P.V. Evaluation of Antioxidant, Antidiabetic and Antiobesity Potential of Selected Traditional Medicinal Plants. *Front. Nutr.* **2019**, *6*, 53. [CrossRef]
31. Dimou, C.; Karantonis, C.; Skalkos, D.; Koutelidakis, A.E. Valorization of fruits by-products to unconventional sources of additives, oil, biomolecules and innovative functional foods. *Cur. Pharm. Biotechnol.* **2019**, *20*, 776–786. [CrossRef]
32. Papagianni, O.; Argiri, K.; Loukas, T.; Magkoutis, A.; Biagki, T.; Dimou, C.; Karantonis, H.; Koutelidakis, A. Postprandial Bioactivity of Spread Cheese, Enhanced with Mountain Tea and Orange Peel Extract, in Healthy Volunteers. An interventional study. *Biomolecules* **2021**, *11*, 1241. [CrossRef]