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Improving Polyphenol Profile, Antioxidant and Antiglycation Activity of Onion Bulbs

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Abstract: The goal of this research is improved polyphenol profile, antioxidant and antiglycation activity of onion bulbs using Matricariae flos (*Matricaria chamomilla* L.) water extract (12 g L^{-1}). The incubation of 24 h of onion roots was in glass tubes filled with deionized water (control) and in aqueous extract of Matricariae flos (test treatment), in a climate chamber. Total phenols, total hydroxycinnamic acids, total flavonols, antioxidant activity and antiglycation activity were measured in ethanolic (7.5 g L^{-1}) extract before (initial phase) and after *in vitro* digestion (salivary, gastric and intestinal phase). In almost all phases of *in vitro* digestion Matricariae flos treatment statistically improved total phenols, total hydroxycinnamic acids, total flavonols, antioxidant activity (ABTS and FRAP) and antiglycation activity (BSA method). The results presented positive influence of Matricariae flos treatment of onion bulbs. Aqueous chamomile extract increases the proportion of polyphenols and antioxidant and antiglycation activity before and after *in vitro* digestion.

Keywords: *Allium cepa* L., *Matricaria chamomilla* L., antiglycation activity, antioxidant activity, phenolics.

INTRODUCTION

Onion (*Allium cepa* L.) is a biannual plant belonging to the Amaryllidaceae family.[1,2] It consists of a fleshy underground bulb from which broad hollow green leaves emerge above ground. In its second year onion forms a globular inflorescence composed of white or greenish-white small flowers. Onion is a widely cultivated plant, second only to tomato, and is consumed across nearly all cultures.[3] It has been used in cooking since ancient times, but it is also known for its medicinal effects. The most used part of the onion is the bulb, although other parts of the plant are also edible and contain bioactive compounds. Today, onions are processed into many products, such as onion flakes, powders, or dietary supplements.[4] The bulb, which can be white, yellow, or purple, is composed of fleshy leaf bases that store nutrients, enclosed in dry, papery outer scales that provide protection. Onion bulbs are highly regarded for their nutraceutical value, being rich in carbohydrates, proteins, and fats. They also contain essential minerals such as selenium, potassium, phosphorus, and iron, along with vitamins—particularly vitamin C.[5–8] Additionally, onions are a rich source of various bioactive compounds, including polyphenols, fructans, and organosulfur compounds. These contribute to a wide range of health benefits, such as antioxidant, anticarcinogenic, antithrombotic, antidiabetic, antihyperglycemic, antidepressant, anti-inflammatory, neuroprotective, and antiparasitic effects[1,2,9,10] Among the dominant organosulfur compounds found in onion bulbs are alliin and γ-glutamylcysteine. Regarding polyphenols, the yellow and purple onion varieties contain compounds such as gallic acid, 3,4-dihydroxybenzoic acid, catechin, syringin, caffeic acid, rutin trihydrate, *p*-coumaric acid, *trans*-ferulic acid, apigenin-7-glucoside, quercetin, resveratrol, kaempferol, isorhamnetin, naringenin, and *trans*-cinnamic acid.[8] Purple onion varieties contain anthocyanins such as cyanidin 3-glucoside, cyanidin

3-laminaribioside, cyanidin 3-(6”-malonylglucoside), and cyanidin 3-malonyllaminaribioside.[11] Compared to other fruits and vegetables, *A. cepa* contains significantly higher levels of quercetin–ranging from 300 mg kg^{-1}–compared to broccoli (100 mg kg^{-1}), apples (50 mg kg^{-1}), and blueberries (40 mg kg^{-1}).[12] Both polyphenols and organosulfur compounds are recognized as powerful antioxidants.[3]

Chamomile (*Matricaria chamomilla* L.) is a well-known medicinal plant belonging to the Asteraceae family. Chamomile extracts are rich in phenolic compounds, including phenolic acids, flavonoids, and coumarins. The quantity and composition of these compounds can vary depending on several factors, particularly the method of extraction. In our research, we used water extraction, as it is the most commonly used technique and, importantly, poses no harm to the onions we grow. The beneficial properties of chamomile are attributed to its diverse array of bioactive compounds, which can generally be divided into two major classes based on their solubility. The hydrophilic fraction includes numerous polyphenols, primarily flavonoid glycosides and, to a lesser extent, free aglycones. Among these, the flavones apigenin, apigenin-7-O-glucoside, luteolin, and luteolin-7-O-glucoside are particularly abundant. In addition, the flavonols quercetin and quercetin-3-O-rutinoside, as well as the flavanone naringenin, have also been identified.[13]

Interspecific metabolite transfer (ISMT) is a novel approach aimed at enhancing the content of secondary plant metabolites through the uptake of compounds from donor plants.[14,15] With growing awareness of food quality and its impact on human health, the food industry faces increasing pressure to develop sustainable and health-promoting production methods. ISMT offers a natural solution, enabling metabolite enrichment without the use of harsh chemicals or genetic modification—practices that are often poorly received by consumers. Donor extracts are typically applied to the soil and absorbed through the roots of plants, although foliar application is also possible.[14] In the studies conducted by Šola et al.[14] and Davosir & Šola,[15] the transfer of metabolites from donor plant extracts (*Hypericum perforatum*, *Matricaria chamomilla*, *Rosa* spp., *Tamus communis*, *Camellia sinensis*) to crops such as Chinese cabbage, broccoli, cauliflower, and brussels sprouts was explored. These experiments demonstrated the potential of ISMT to increase both the biological activity and phytochemical composition of acceptor plants. The results suggest that treatment with donor extracts of St. John's wort, chamomile, rosehip, yarrow, and green tea can significantly improve the nutritional profile and bioactivity of the recipient vegetables.

Due to their widespread consumption and existing medicinal value, vegetables such as onions represent an ideal model for ISMT. This research specifically focuses on enhancing the polyphenol content in onions through metabolite transfer using chamomile tea extracts. To test this, we measured the content of total polyphenols (TP), total hydroxycinnamic acids (THA), total flavonols (TFL), before (initial phase) and after *in vitro* digestion (salivary, gastric and intestinal phase) in control samples and in samples chamomile tea treatment. During digestion antioxidant (ABTS and FRAP) and antiglycation (inhibition of BSA glycation) activity were also monitored.

EXPERIMENTAL

Chemicals and Materials

Enzymes (α-amylase, porcine pepsin, pancreatic lipase, and pancreatin) and bile utilized for *in vitro* digestion and antidiabetic activity (α-amylase) were products of Merck KGaA (Germany). Commercial polyphenol standards were produced by Merck KGaA and Extrasynthese (France). All chemicals and reagents were of analytical grade and supplied by Merck KGaA or Kemika (Croatia). Deionized water was used in all experiments, and the solvents and chemicals were of analytical or HPLC grade.

All absorbance and fluorescence measurements related to polyphenol profile antiglycation, and antioxidant potential were performed using a Fluostar Optima microplate reader (BMG Labtech GmbH, Germany).

Plant Material

Matricariae flos (*M. chamomilla* L.) in dry tea form were obtained from Gradska ljekarna Zagreb (Zagreb, Croatia). These flores were selected due to the high content of flavonoid apigenin. Mass of 3 g was poured with 250 mL hot water with and then incubated with stirring for 60 minutes. After incubation tea was filtered, cooled down to room temperature and then used for experimentation.

A. cepa L. var. Red Carmen bulbs (NL-244085226, code 2704) were purchased at a local store in December 2024. Equal-sized (1.5–2.0 cm) healthy bulbs were selected for experiment. Old dried adventive roots were removed. Root growth was started by placing bulbs into glass tubes filed with deionized water for 48 h in a Fito-Clima 600 PLH climate chamber (Aralab, Rio de Mouro, Portugal) at 23 °C / 16 h day / 8 h dark, humidity 65 %. For the treatment experiment with Matricariae flos water extract, only bulbs with normally developed roots were selected. Bulbs where the roots had visible morphological abnormalities were discharged from the experiment. The incubation of 24 h of nine onion bulbs (N = 9) were in glass tubes filed with deionized water (control) and nine onion bulbs (N=9) were in aqueous extract of Matricariae flos (test treatment), in a Fito-Clima 600 PLH climate chamber (Aralab, Rio de Mouro, Portugal) at 23 °C / 16 h day / 8 h dark, humidity 65 %. After

incubation onion bulbs and roots are rinsed with water. Onion bulbs are cut into 5 mm pieces and put to dry in the oven at 90 °C. Dry onion bulbs parts were ground in a mortar. In plastic tubes with a cap, the plant material was weighed and 70 % ethanol was added to obtain an extract concentration of 50 g L^{-1} that we used for *in vitro* digestion experiment.

Extract Preparation

The extracts at the concentration of 50 mg mL^{-1} were prepared from dry and powered onion bulbs using 70 % aq. ethanol (*V* / *V*) at room temperature on a rotary extraction device for 60 min. After extraction the extracts were centrifuged for 5 min at 10,000 rpm, and supernatants were stored at –20 °C until analyses.

Model of Human *in vitro* Digestion

The *in vitro* model of human digestion was based on the method described by Vujčić Bok et al.[16] Firstly, 0.15 mL of extract was combined with equal volume of 20 mmol L^{-1} phosphate buffer (pH 7.0). To initiate the salivary phase of digestion, 5 µL of amylase (0.48 mg mL^{-1} in 20 mmol L^{-1} phosphate buffer, pH 7.0) was added, and the mixture was incubated for 5 minutes at 37 °C in a shaking water bath at 150 rpm. For the gastric digestion phase, 0.2 mL of porcine pepsin solution (3 mg mL^{-1} in 0.1 mol L^{-1} HCl) was added, and acidified with 1 mol L^{-1} HCl (pH 2.0). The samples were then incubated in a shaking water bath at 37 °C for 1 hour at 150 rpm. To simulate the upper intestinal phase, the pH was first adjusted to 5.3 with 5 µL of 1 mol L^{-1} $NaHCO_3$. After the pH adjustment, 0.45 mL of pancreatic juice (containing 2.4 mg mL^{-1} bile acids, 0.2 mg mL^{-1} porcine pancreatic lipase and 0.4 mg mL^{-1} pancreatin, in 20 mmol L^{-1} phosphate buffer, pH 7.0) was added. The total volume of each sample in the intestinal phase was then adjusted to 1 mL using 20 mol L^{-1} phosphate buffer (pH 7.0), and the final pH was brought to 7.0 by adding 1 mol L^{-1} NaOH. These samples were subsequently incubated for 2 hours at 37 °C in a shaking water bath at 150 rpm. After digestion, the final volume of each sample, both pre- (initial phase of *in vitro* digestion) and post-digestion (salivary, gastric and intestinal phase of *in vitro* digestion), was adjusted to 1 mL with 20 mmol L^{-1} phosphate buffer (pH 7.0). The samples were centrifuged at 15,000 rpm for 5 minutes at 4 °C, and the supernatants were stored at –20 °C until further spectrophotometric analyses.

Spectrophotometric Phytochemical Analysis of Chamomile Tea and Onion Extract

The total polyphenols (TP) of onion bulbs extract were determined with Folin–Ciocalteau reagent according to Zhishen et al.[17] A volume of 20 µL of the chamomile tea extract and onion bulbs extract were diluted with 1580 µL of deionized water and then 100 µL of Foline–Ciocalteau reagent was added. Subsequently, 300 µL Na_2CO_3 (1.88 M) were added, and the mixture was incubated for 30 min at 45 °C. The absorbance of the mixture was measured at 740 nm. The TP content was calculated from the calibration curve and expressed as gallic acid equivalents (mg GAE g^{-1}).

The total content of hydroxycinnamic acids (THA) and total flavonols (TFL) of the chamomile tea extract and onion bulbs extract were measured according to the method of Howard et al.[18] using caffeic acid and quercetin as standards. A volume of 50 µL of the extract (7.5 mg mL^{-1}) was mixed with 50 µL HCl (1 mg mL^{-1} in ethanol) and 0.91 mL of HCl (2 mg mL^{-1}). The absorbance of the solution was read at 320 and 360 nm, respectively. The THA and TFL contents were calculated from the corresponding calibration curves and expressed as caffeic acid (mg CAE g^{-1}) and quercetin equivalents (mg QE g^{-1}), respectively.

Antioxidant and Antiglycation Activity of Chamomile Tea and Onion Extract

The ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] assay was carried out as described by Vujčić et al.[19] A volume of 2 µL of the tested plant extract was added to 200 µL of ABTS solution and incubated for 6 min at room temperature. The decrease in absorbance of the reaction mixture was read at 740 nm, and the radical scavenging activity was calculated as a percentage of ABTS inhibition.

The ferric reducing antioxidant power (FRAP) assay was carried out as described by Vujčić Bok et al.[20] The tested plant extracts (10 µL) were mixed with 190 µL of freshly prepared FRAP reagent. Absorbance was measured at 595 nm after 4 min of reaction time, and the percentage of ferric tripyridyl triazine (Fe^{3+}-TPTZ) reduction was calculated. Trolox was used as a positive control for all antioxidant activity methods.

Inhibition of BSA glycation was performed as described by Rusak et al.[21] Volume of 100 µL of BSA solution (10 mg mL^{-1}) was mixed with 100 µL of fructose solution (0.5 mol L^{-1}) and 40 µL of the tested extract. Incubation was done in an incubator shaker for 24 h at 37 °C; after incubation, fluorescence was measured (excitation wavelength 405 nm and emission wavelength 460 nm). Catechin solution was used as a positive control, and BSA inhibitory activity was calculated.

Statistical Analysis

Experiment with nine biological replicates per treatment and minimum of three technical replicas was performed in December 2024. All results were processed using Statistica 14.0.1.25. software package (StatSoft Inc., USA). The Shapiro–Wilk test of normality of variance was carried out

to determine normal distribution of data. One-way variance analysis (ANOVA) followed by Duncan's multiple range test was applied for assessment of significant differences between the samples. Principal component analysis (PCA) was employed for the visualization of sample grouping. Pearson's correlation coefficients between total polyphenol compounds, and antioxidant activity, and antiglycation potential were calculated to assess possible correlations between the measured parameters. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Total Polyphenols Content, Antioxidant Activity and Antiglycation Activity

Table 1 presents the total phenolic content, total hydroxycinnamic acids, total flavonols, antioxidant activity (measured by ABTS and FRAP assays), and antiglycation activity (BSA method) of the Matricariae flos (chamomile) water extract used for the treatment of onion plants in this experiment.

Compared to the undigested control onion extract (Figure 1), the chamomile infusion contained (Table 1) higher levels of phenolic compounds (13.04 ± 3.74 mg GAE g^{-1}) than onion extracts in the initial phase (3.40 ± 2.22 mg GAE g^{-1}) (Figure 1). Similarly, the total hydroxycinnamic acid content was higher in chamomile (8.83 ± 1.09 mg CAE g^{-1}) than in onion extracts (3.30 ± 0.22 mg CAE g^{-1}), as was the total flavonol content (8.11 ± 1.60 mg QE g^{-1} in chamomile vs. 3.70 ± 0.35 mg QE g^{-1} in onion).

Regarding antioxidant activity (Table 1, Figure 2), onion control samples in the initial (pre-digestion) phase exhibited lower antioxidant capacity in both assays compared to the chamomile water extract. In the ABTS assay, the chamomile extract demonstrated moderate antioxidant potential (51.59 %), while in the FRAP assay, it exhibited high antioxidant potential (92.57 %) according to antioxidant classification.[16,19–21] These results suggest that the antioxidants present in chamomile are predominantly hydrophilic in nature.[13,22]

Similarly, with respect to antiglycation activity (Table 1, Figure 2), the chamomile extract showed a greater antiglycation potential than the undigested onion control samples, further supporting its functional potential in oxidative and glycation-related processes.

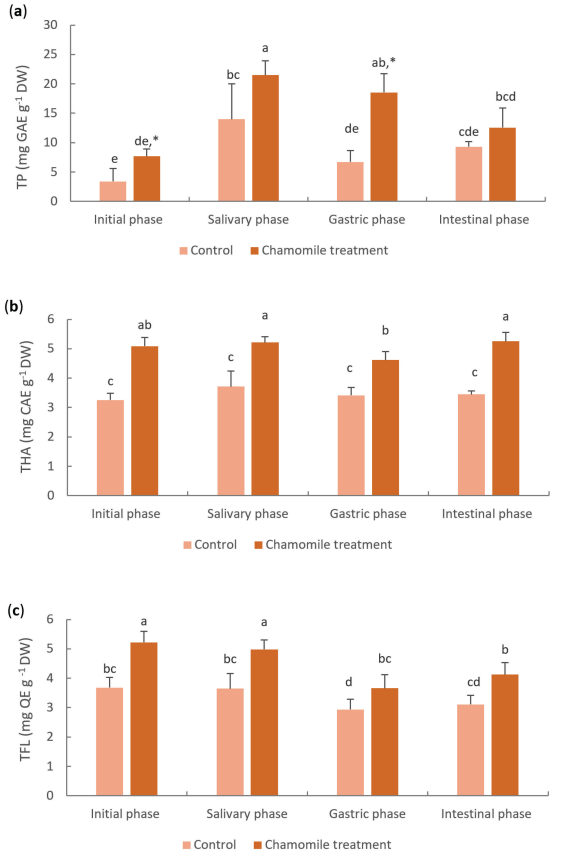

Figure 1. (**a**) Total phenols (TP), (**b**) total hydroxycinnamic acids (THA), (b) total flavonols (TFL) content of onion bulb extract. Onion bulbs were treated with deionized water (control) and aqueous extract of Matricariae flos (test treatment). Data are presented as mean value ± S.D. *N* onion bulbs = 9. Different letters indicate significant differences at $p \leq 0.05$ (ANOVA, Duncan test) between the initial, salivary, gastric and intestinal phase together. Asterisk indicates significant differences at $p \leq 0.05$ for each phase individually (ANOVA, Duncan test). GAE= gallic acid equivalents, CAE = caffeic acid equivalents, QE = quercetin equivalents, DW = dray weight of onion bulbs.

Table 1. Total phenols (TP), total hydroxycinnamic acids (THA), total flavonols (TFL) content and antioxidant activity (ABTS and FRAP) and antiglycation activity (BSA method) of Matricariae flos (*Matricaria recutita* L.) water extract. Data are presented as mean value ± S.D, *N* = 9. GAE= gallic acid equivalents, CAE= caffeic acid equivalents, QE= quercetin equivalents, DW= dray weight of Matricariae flos.

TP / mg GAE g^{-1} DW	THA / mg CAE g^{-1} DW	TFL / mg QE g^{-1} DW	ABTS / inhibition %	FRAP / 0reduction %	BSA / inhibition %
13.04 ± 3.74	8.83 ± 1.09	8.11 ± 1.60	51.59 ± 7.77	92.57 ± 0.70	80.18 ± 3.96

Figure 1 illustrates the total phenolic content (TP), total hydroxycinnamic acids (THA), and total flavonols (TFL) in onion bulbs following a 24-hour treatment with chamomile tea, compared to untreated control samples, across different phases of *in vitro* digestion. Treated samples exhibited statistically higher TP, THA, and TFL levels than controls during almost all digestion phases.

Considering that a 24-hour exposure is generally a short time for plants to synthesize substantial amounts of endogenous phenolic compounds, the observed increase can be attributed not only to synthesis but also to the absorption of low molecular weight, water-soluble phenolics present in chamomile tea—such as gallic acid, ferulic acid, and caffeic acid. Among phenolic compounds, phenolic acids are particularly relevant due to their small molecular size and capacity for root uptake. In plants, these metabolites function as specialized compounds with allelopathic properties, capable of inhibiting the growth of neighboring plants. Upon interaction with root cell membranes, phenolic acids have been shown to cause membrane depolarization, ion efflux, and disruption of water and nutrient uptake.[23] Furthermore, Kobayashi et al.[24] demonstrated that wheat roots treated hydroponically with ferulic acid and *p*-coumaric acid absorbed nearly all of the applied acids within 14 and 18 hours, respectively. These low molecular weight acids are presumed to enter root cells via passive diffusion in their non-ionized forms.[25] Hydroxycinnamic acids were significantly more abundant in treated samples throughout all digestive phases, consistent with the notion that most phenolic compounds absorbed by onion bulbs from chamomile tea are phenolic acids. While concentrations in control samples remained stable throughout digestion, treated samples exhibited a significant decrease in hydroxycinnamic acid content during the gastric phase compared to the oral and intestinal phases, likely due to pH-induced chemical modifications. Similar results were published by Moreno-Ortega et al.[26] Phenolic acids are rarely found in plants in free form; they are generally bound to the plant matrix and released during digestion. In the study by Odriozola-Serrano et al.,[27] some phenolic acids such as ferulic and rosmarinic acid were more abundant in the initial phases, whereas others, like chlorogenic acid, increased after the gastric phase. Additionally, *p*-salicylic acid showed an increase during the gastric phase but decreased during the intestinal phase. Overall, the quantity of phenolic acids is influenced by their chemical structure, the transformations they undergo during digestion, and the specific types of acids initially present in the plant extract. As growth inhibitors that induce stress responses[28], phenolic acids also stimulate endogenous synthesis of phenolic compounds.[29] The biosynthesis of phenolic compounds can commence within a few hours after stress exposure and may continue for several days.[30] Therefore, these results likely indicate that the observed increase in total phenolic content is primarily attributable to the absorption of phenolic acids. However, the endogenous synthesis of more complex phenolic compounds, such as flavonols, may also contribute to this increase, though to a lesser extent, due to the short time frame, which is insufficient for their substantial accumulation.

Regarding flavonol content, chamomile-treated samples exhibited significantly higher flavonol levels compared to controls from the initial phase onward, and this difference was maintained throughout all phases of digestion. Flavonols are typically synthesized endogenously in plants in response to stress; however, some studies

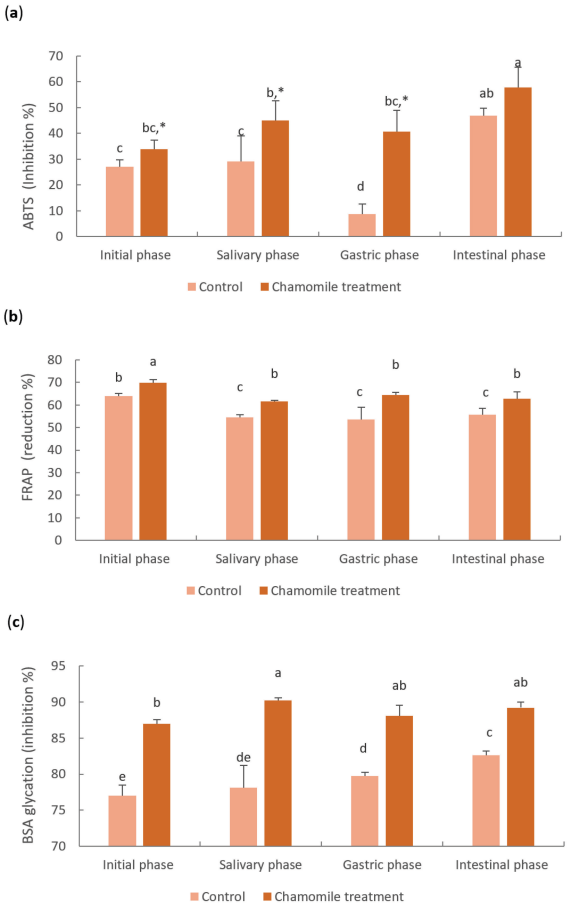

Figure 2. Antioxidant **activity (a) ABTS and (b) FRAP); and (c) antiglycation activity (BSA** method) of onion bulb extract. Onion bulbs were treated with deionized water (control) and aqueous extract of Matricariae flos (test treatment). Data are presented as mean value ± S.D., *N* onion bulbs = 9. Different letters indicate significant differences at $p \leq 0.05$ (ANOVA, Duncan test) between the initial, salivary, gastric and intestinal phase together. Asterisk indicates significant differences at $p \leq 0.05$ for each phase individually (ANOVA, Duncan test).

indicate that these compounds can also be absorbed from the environment, such as from the growth medium. Buer et al.[31] demonstrated that flavonoids - including naringenin, dihydrokaempferol, dihydroquercetin, kaempferol, and quercetin—can be taken up by plant roots, particularly flavonoid biosynthesis precursors such as naringenin, dihydrokaempferol, and dihydroquercetin. In untreated samples, flavonol content progressively declined during digestion, with the highest concentrations observed in the initial and salivary phases, followed by a significant reduction during the gastric phase. Treated samples showed a similar decreasing trend but maintained comparatively higher flavonol concentrations during the initial and gastric phases. Nevertheless, in both treated and control groups, the gastric phase ultimately corresponded to the lowest flavonol content. Similar results regarding the *in vitro* digestion of onion flavonols were reported by Herranz et al.[32] and Fernandes-Jalao et al.[33] The observed decrease in total flavonol (TFL) content during the gastric phase is likely attributable to acid-induced degradation under low pH conditions. In contrast, the reduction observed in the intestinal phase may result from the formation of complexes between flavonols and other dietary constituents such as dietary fiber, proteins, or iron.[33]

Regarding total phenolic content, control samples exhibited the highest levels during the salivary phase, which significantly exceeded those observed in the initial phase and in the subsequent stages of *in vitro* digestion. A similar pattern was observed in treated samples, where the salivary phase also showed the greatest concentration of phenolic compounds, significantly higher than both the initial and intestinal phases. Phenolic content peaks during the salivary and gastric phases of *in vitro* digestion for treated onion samples. These findings are consistent with previous studies conducted on other model plants[16,34] as well as on onion,[26] which attribute the observed increases in phenolic content to enzymatic activity and acidic hydrolysis occurring during the early phases of digestion. During these early digestive stages, high molecular weight polyphenols may be depolymerized into more quantifiable monomers, or their solubility may be enhanced, resulting in increased measurable concentrations. Thus, the observed rise in phenolic content through phases is often linked to the chemical transformation of phenolic compounds under enzymatic or acidic conditions.[35,36] The discrepancies observed between the results for hydroxycinnamic acids and flavonols compared to total phenolic content are likely due to the presence of additional phenolic compounds in onion and chamomile extracts, which were captured in the total phenolic measurements but not individually quantified.

Collectively, these findings indicate that a 24-hour treatment with chamomile tea enhances the phenolic profile of onion bulbs by promoting the uptake of exogenous phenolic acids from the tea, as well as stimulating the endogenous biosynthesis of more complex phenolic compounds. The observed increase in total phenolic content during the early phases of digestion is likely attributable to both the improved bioaccessibility of absorbed phenolics and the structural transformation of complex polyphenols into more readily detectable forms.

The antioxidant activity of onion samples was evaluated using ABTS and FRAP assays. Previously determined phenolic compounds are potent antioxidants. Treated samples exhibited a significantly higher percentage of free radical inhibition compared to control samples with an exception for the ABTS method in the intestinal phase of *in vitro* digestion which corresponded with them being richer in phenolic compounds, as discussed above. The ABTS assay demonstrated the highest inhibition percentage during the intestinal phase for both treated and control samples. Similar results were published by Hur et al.[37] As previously discussed, variations in pH and other parameters during digestion can result in the degradation of certain antioxidants, while simultaneously promoting the release of others from the plant matrix, thereby potentially enhancing the overall antioxidant capacity, particularly in the intestinal phase. In treated samples, no significant differences were observed among the other digestion phases (initial, salivary and gastric phase), suggesting that the antioxidant content remains relatively stable throughout *in vitro* digestion, except for the intestinal phase, where a notable increase was recorded. In contrast, the control samples exhibited no significant difference between the initial and salivary phases, but a marked decrease in antioxidant activity was observed during the gastric phase. This decline was not evident in the treated group. This discrepancy may be attributed to the antioxidant profile of the samples: while control samples contained only native onion phenolics, the treated samples were enriched with phenolic compounds derived from chamomile, which may possess greater stability or distinct reactivity in acidic environments such as the gastric phase. As noted, the profile of hydroxycinnamic acids changes throughout the digestive phases for test treatment, which may account for the pronounced variations in antioxidant potential. It is plausible that the uptake of hydroxycinnamic acids from the chamomile tea solution contributed to the altered phenolic profile observed in the treated samples. Chamomile is known to be particularly rich in hydroxycinnamic acids, as evidenced both by the data presented in Table 1. and by previous studies,[38] whereas onion contains only relatively low levels of these compounds.[39] This may result in a greater release, rather than degradation, of hydroxycinnamic acids during the gastric phase. Following this phase, a marked increase in ABTS radical scavenging activity is observed, while in the

intestinal phase, antioxidant capacity does not differ significantly between treated and control samples. Overall, the ABTS assay indicated a moderate radical scavenging capacity in the intestinal phase of treated samples, with an inhibition percentage of 57.7 %.

It is important to note that the ABTS radical is soluble in both hydrophilic and lipophilic media, enabling interaction with a broad spectrum of antioxidants. In contrast, the FRAP assay specifically measures the reducing power of hydrophilic antioxidants, which likely accounts for the observed differences between the two methods. The FRAP results showed significantly higher antioxidant potential in treated samples. As in treated samples as well in the control ones antioxidant potential peaked in the initial phase prior to digestion and subsequently declined. In treated samples, the antioxidant potential in the initial phase was significantly higher than in all subsequent digestion phases, with no significant differences among the latter. In the research of Hur et al.[37] and Fernández-Jalao et al.[33] of *in vitro* digestion of onion plants antioxidant potential peaked in intestinal phase, but they didn't take into the account the initial phase. Our research didn't record significant differences between oral, gastric and intestinal phases in control and treated samples. Researches on other plant models also showed peaks in initial phase compared to other phases.[16,21] Overall FRAP test showed that treated samples have high antioxidant potential of 69.9 %. Overall, the reducing power remained moderate to high across all phases of digestion in both sample groups. These findings suggest that both untreated and treated onion bulbs are valuable sources of water-soluble antioxidants. However, the treated samples exhibited significantly higher antioxidant capacity, likely due to the presence of additional hydrophilic compounds derived from chamomile. This is further supported by the stronger responses observed with the FRAP assay, which is particularly sensitive to hydrophilic antioxidants.

Antiglycation activity was evaluated using the bovine serum albumin (BSA) assay. Phenolic compounds are well-documented as potent inhibitors of key enzymes involved in the glycation process.[40] Samples treated with the phenolic extract exhibited a significantly higher percentage of glycation inhibition compared to control samples. In control samples, the percentage of inhibition increased progressively across the simulated digestion phases, with the intestinal phase showing a statistically significantly higher level of inhibition than the initial, salivary and gastric phases. This increase is likely attributable to the gradual release of phenolic inhibitors from the onion matrix during digestion. In contrast, treated samples demonstrated the highest antiglycation activity during the salivary phase, with this value being statistically significantly higher only in comparison to the initial phase. The high initial activity and its sustained presence throughout all digestion phases suggest that the phenolic compounds in treated samples are more readily available and remain stable under varying digestive conditions. These findings imply that the absorption and incorporation of tea-derived phenolic compounds into the onion matrix may have modified its phenolic profile. The enhanced antiglycation activity in treated samples likely results from the successful absorption and possible metabolic transformation of phenolic constituents from the tea medium, leading to altered biochemical properties in the plant material.

To date, there are no published studies specifically examining how the antidiabetic activity of onions changes during digestion. However, studies conducted on other plant models provide relevant insights. For instance, Spinola et al.[41] and Šola et al.[34] reported increased enzyme inhibitory activity following simulated digestion in comparison to non-digested plant extracts, highlighting the potential of digestion to enhance the bioactivity of phytochemicals.

Pearson's Correlations of Total Polyphenolic Content and Antioxidant and Antiglycation Activity

In Table 2 is presented Pearson's correlation coefficients between polyphenolic content and antioxidant and antiglycation activity in chamomile and onion extracts (control and test treatment with chamomile extract during simulated *in vitro* gastrointestinal digestion). According Evans' [42] interpretation of correlations (correlation value 0.00–0.19 is very weak correlation, 0.20–0.39 is weak correlation, 0.40–0.59 is moderate correlation, 0.60–0.79 is strong correlation and 0.80–1-00 is very strong correlation), a very strong positive correlation was observed between THA and TFL (0.96), THA and FRAP (0.92), between TFL and FRAP (0.94). Strong positive correlation was observed between the TP and BSA (0.62) and moderate positive correlation was observed between the ABTS and the TP (0.49), THA (0.58) and TFL (0.47). Our results suggest that in chamomile and onion extracts (control and test treatment with chamomile extract during simulated *in vitro* gastrointestinal digestion) THA and TFL significantly contributes to the antioxidant activity and TP significantly contributes to the antiglycation activity.

In Table 3 is presented Pearson's correlation coefficients between polyphenolic content and antioxidant and antiglycation activity in onion extracts (control and test treatment with chamomile extract) during simulated *in vitro* gastrointestinal digestion). Very strong positive correlation was observed between THA and TFL (0.81), THA and BSA (0.93). Strong positive correlation was observed between the TP and BSA (0.66), TP and THA (0.60), THA and ABTS (0.62), THA and FRAP (0.63), TFL and FRAP (0.75), TFL

and BSA (0.63), ABTS and BSA (0.70). Moderate positive correlation was observed between TP and ABTS (0.49) and between BSA and FRAP (0.52). Our results suggest that in onion extracts (control and test treatment with chamomile extract) during initial phase, salivary phase, gastric and intestinal phase of *in vitro* gastrointestinal digestion, THA and TFL significantly contributes to the antioxidant activity and THA significantly contributes to the antiglycation activity.

These results are in agreement with our previous results[14,16,19–22,34,43] in which we monitored the correlations between polyphenolic compounds, antioxidant and antiglycation activity in the original samples as well as in samples during *in vitro* digestion.

PCA (Principal Component Analysis) of Total Polyphenolic Content and Antioxidant and Antiglycation Activity

Principal component analysis or PCA allows us to visualize the similarity and diversity of samples (original samples and samples during *in vitro* digestion) and analyzed parameters such as polyphenols, antioxidant and antiglycation activity parameters based on distances on the diagram. Samples and methods that have a smaller distance on the diagram are more similar, and a greater distance indicates difference.[16,21,22,34]

In the Figure 3. Principal component analysis (PCA) diagram of the measured polyphenols (total phenols: TP, total hydroxycinnamic acids: THA, total flavonols: TFL), antioxidant (ABTS and FRAP) and antiglycemic (BSA method) activity in chamomile and onion extracts (control and test treatment with chamomile extract during simulated *in vitro* gastrointestinal digestion). (a) grouping of samples, (b) grouping of analyzed parameters.

The first (Factor 1) and the second (Factor 2) principal component (PC) accounted for 57.61 % and 28.35 % (Figure 3). Together, the first two PCs represented 85.96 % of the total variability. The smallest distance was observed between the all onion control samples. These control onion samples are found in the upper and lower left quadrant. Chamomile samples and onion samples treatment with chamomile are on the upper and lower right quadrant as well as TP, THA, TFL, antioxidant (ABTS and FRAP) and antiglycemic (BSA method) activity. From the above, the separation of control bulbs from treated bulbs is clearly visible. Similarities between the treated onion samples and chamomile samples contribute to the fact of the positive effect of chamomile treatment of onions by improvement of phytochemical profile and antioxidant and antiglycation activity. When comparing samples, chamomile tea samples have the highest values of all measured methods (TP, THA, TFL, antioxidant (ABTS and FRAP) and antiglycemic (BSA) activity methods), followed by onion samples treated with chamomile tea, and the lowest values of all methods have control onion samples. This is very well confirmed with the help of PCA.

In the Figure 4. Principal component analysis (PCA) diagram of the measured polyphenols (total phenols: TP, total hydroxycinnamic acids: THA, total flavonols: TFL), antioxidant (ABTS and FRAP) and antiglycemic (BSA method) activity in onion extracts (control and test treatment with chamomile extract) during simulated *in vitro* gastrointestinal digestion. (a) grouping of samples, (b) grouping of analyzed parameters.

The first (Factor 1) and the second (Factor 2) principal component (PC) accounted for 64.86 % and 19.39 % (Figure 3). Together, the first two PCs represented 84.25 % of the total variability. In this picture, as in the previous one, we can see the division between the control bulbs samples and the bulbs samples that were exposed to chamomile tea. Onion samples treatment with chamomile are on the

Table 2. Pearson's correlations of the measured polyphenols (total phenols: TP, total hydroxycinnamic acids: THA, total flavonols: TFL), antioxidant (ABTS and FRAP method) and antiglycemic (BSA method) activity in chamomile and onion extracts (control and test treatment with chamomile extract during simulated in vitro gastrointestinal digestion). Significant differences at $p \leq 0.05$.

Variable	TP	THA	TFL	ABTS	FRAP	BSA
TP	1.00					
THA	0.35	1.00				
TFL	0.23	0.96	1.00			
ABTS	0.49	0.58	0.47	1.00		
FRAP	0.08	0.92	0.94	0.45	1.00	
BSA	0.62	0.20	0.07	0.55	0.00	1.00

Table 3. Pearson's correlations of the measured polyphenols (total phenols: TP, total hydroxycinnamic acids: THA, total flavonols: TFL), antioxidant (ABTS and FRAP method) and antiglycemic (BSA method) activity in onion extracts (control and test treatment with chamomile extract) during simulated in vitro gastrointestinal digestion. Significant differences at $p \leq 0.05$.

Variable	TP	THA	TFL	ABTS	FRAP	BSA
TP	1.00					
THA	0.60	1.00				
TFL	0.33	0.81	1.00			
ABTS	0.49	0.62	0.38	1.00		
FRAP	0.02	0.63	0.75	0.34	1.00	
BSA	0.66	0.93	0.63	0.70	0.52	1.00

upper and lower left quadrant as well as TP, THA, TFL, antioxidant (ABTS and FRAP) and antiglycemic (BSA method) activity. Onion bulb control samples are found in the upper and lower right quadrant. From the PCA we can observed that onions treated with chamomile tea initial phase samples had high loadings with TFL and FRAP, onions treated with chamomile tea salivary phase samples had high loadings with TP, THA, ABTS and BSA, and onions treated with chamomile tea gastric and intestinal phase samples had high loadings with THA, BSA and ABTS. This means that in the initial phase of *in vitro* digestion, total flavonols contribute to the antioxidant activity measured by the FRAP method. In salivary phase of *in vitro* digestion, total phenols, total hydroxycinnamic acids significantly contribute to antyglication and antioxidant (ABTS method) activity. For the gastric and intestinal phase of *in vitro* digestion, total hydroxycinnamic acids, contribute to antyglication and antioxidant (ABTS) activity.

CONCLUSIONS

Based on the presented results, it can be concluded that treating onions with chamomile tea increases their polyphenol levels, as well as their antioxidant and antiglycation activities. Total phenol content was consistently higher in treated samples across initial, salivary and gastric digestion phases of

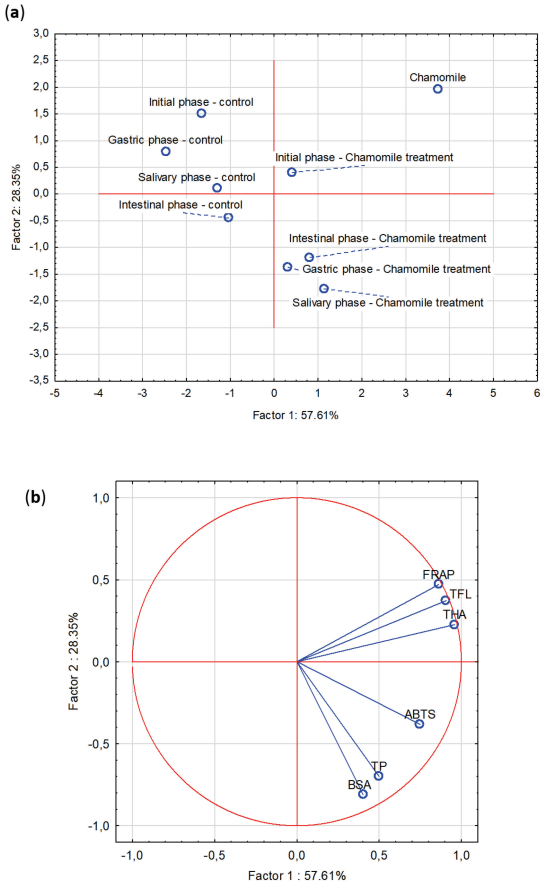

Figure 3. Principal component analysis (PCA) diagram of the measured polyphenols (total phenols: TP, total hydroxycinnamic acids: THA, total flavonols: TFL), antioxidant (ABTS and FRAP) and antiglycemic (BSA method) activity in chamomile and onion extracts (control and test treatment with chamomile extract during simulated in vitro gastrointestinal digestion). (a) grouping of samples, (b) grouping of analyzed parameters. First principal component (PC1) = Factor 1, second principal component (PC2) = Factor 2.

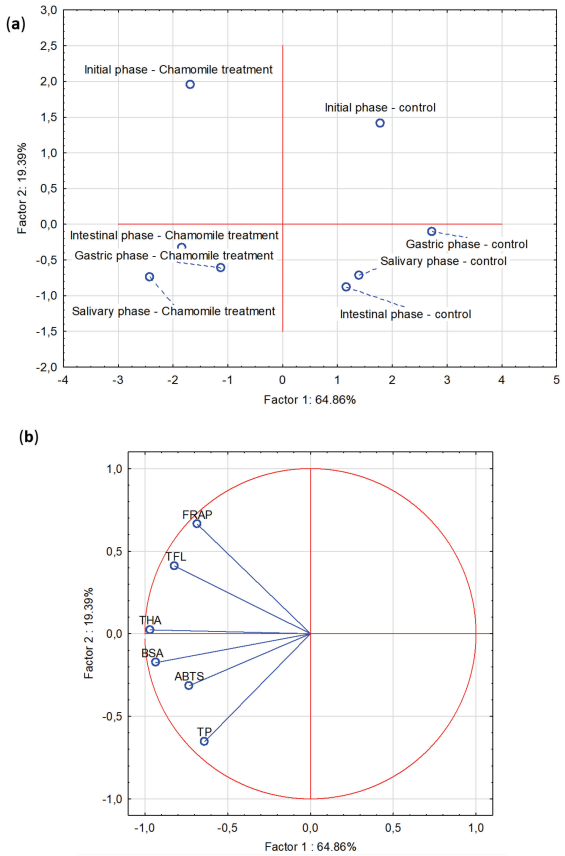

Figure 4. Principal component analysis (PCA) diagram of the measured polyphenols (total phenols: TP, total hydroxycinnamic acids: THA, total flavonols: TFL), antioxidant (ABTS and FRAP) and antiglycemic (BSA method) activity in onion extracts (control and test treatment with chamomile extract) during simulated in vitro gastrointestinal digestion. (a) grouping of samples, (b) grouping of analyzed parameters. First principal component (PC1) = Factor 1, second principal component (PC2) = Factor 2.

in vitro digestion, with the highest levels observed in the salivary phase for treated samples. The most pronounced difference between treated and control samples was noted in the gastric phase. Similarly, total hydroxycinnamic acid content was elevated in all digestion phases in treated samples, although the lowest overall levels were recorded in the gastric phase. Total flavonol content was also higher in treated samples compared to controls throughout the digestion process, peaking in the initial and salivary phases, followed by a gradual decrease in later phases.

Antioxidant activity, measured by the ABTS assay, was higher in treated samples, with the highest activity observed in the intestinal phase. These results suggest that chamomile-treated onion samples possess moderate antioxidant potential according to ABTS. The FRAP assay confirmed a generally higher antioxidant potential in both control and treated samples, placing treated samples in the high antioxidant category. The highest FRAP activity in treated samples was observed in the initial digestion phase. Regarding antiglycation activity, treated samples again demonstrated superior performance, with peak activity occurring during the salivary phase.

Principal Component Analysis (PCA) further substantiated these findings by revealing phase-specific correlations between polyphenolic compounds and bioactivities in onion samples. In the initial digestion phase, treated samples were primarily associated with elevated total flavonol content and FRAP antioxidant activity. During the salivary phase, total phenols, hydroxycinnamic acids, antioxidant activity (as measured by the ABTS method), and antiglycation activity (as measured by the BSA assay) emerged as the most influential variables. In the gastric and intestinal phases, hydroxycinnamic acids and ABTS-based antioxidant activity were the predominant contributors. These results suggest that different classes of polyphenols differentially influence antioxidant and antiglycation responses depending on the stage of digestion.

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