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Improving the Polyphenol Profile, Antioxidant and Antiglycation Activity of Onion Outer Scales with Chamomile Treatment

Running head: Improving Polyphenol Profile and Biological Activity of Onion Outer Scales

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SUMMARY

Research background. Outer scales of common onion (*Allium cepa* L.) are often discarded as biological waste but can be an interesting source of polyphenols with antioxidant and antidiabetic properties that can be used in the food and pharmaceutical industries.

Experimental approach. The study goal is to use dried chamomile flowers (*Matricaria camomilla* L.) containing 6 % flavonoid apigenin in the form of water extract to improve the polyphenol profile, antioxidant and antiglycation activity of onion outer scales. "Red Carmen" onion bulbs with roots were exposed to deionized water (control) and chamomile water extract (test treatment) in a phytotron (23 °C/16 h day/8 h dark). From dried and pulverized outer scales and chamomile, Fourier transform infrared (FTIR) analysis was performed. Also, pure standards of polyphenols (apigenin,

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quercetin and caffeic acid) was used for FTIR spectra comparison.

Results and conclusions. In the initial phase, the salivary, gastric, and intestinal phase; THA (total hydroxycinnamic acids), TFL (total flavonols), antioxidant (ABTS; (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid and FRAP; ferric reducing antioxidant power) activity, and inhibition of BSA (bovine serum albumin) glycation activity of on-ion scales extract was measured. Chamomile treatment improved THA, TFL, antioxidant (FRAP) and antiglycation activity (BSA method) in almost all in vitro digestion phases. Treatment of onions with chamomile water extract is good method of enrichment of biological waste. This is confirmed by FTIR analysis. Enricht onion scales represent good sources of bioaccessible polyphenols with high antioxidant (ABTS>85 % and FRAP>95 %) and moderate antiglycation activity (43–62 %).

Novelty and scientific contribution. The enrichment of the phytochemical composition and biological potential of onions had potential applications of this technique for health and nutritional purposes. Since the method does not require genetic modification but relies on natural absorption mechanisms and stress induction, it could become a valuable tool for enriching the nutritional and therapeutic composition of herbs. These results represent a foundation for future research, which should further elucidate the mechanisms of transmission, long-term effects, and potential industrial applications of this phenomenon.

Keywords: *Allium cepa* L.; *Matricaria chamomilla* L.; inhibition of BSA glycation; antioxidant activity; *in vitro* digestion; polyphenols

INTRODUCTION

In modern society, in which nutrition increasingly goes beyond its basic role of satisfying hunger and is becoming a key component of the prevention of certain diseases and the preservation of health, plants are no longer seen only as a source of nutrients, but also as a wealth of natural healing compounds. The growing interest of consumers in functional foods and nutritionally rich, fresh and minimally processed products thus encourages more and more research aimed at increasing their content with bioactive compounds such as flavonoids, glucosinolates, phenolic acids and vitamin C [*1,2*].

It is known that the composition and concentrations of bioactive substances in plants are subject to changes depending on environmental factors, but also that they can be increased in a targeted manner using agrotechnical procedures, biotechnological methods, or by inducing stressful conditions such as UV radiation and temperature changes [*3,4*]. However, with all the known approaches, in recent years, more and more attention has been paid to a strategy that brings a new

dimension to this issue – the transfer of phytochemical compounds from one plant to another, the so-called interspecific transfer of plant metabolites. This phenomenon, also known as the horizontal natural products transfer (HNPT), involves the movement of specialized (secondary) metabolites from donor plants to recipient plants, most often through the common soil and root system (rhizosphere) [*5*], and it is assumed that compounds from the soil enter by passive diffusion through the plasmalemma of root cells [*6*]. To confirm such a possibility of metabolite transfer, Selmar *et al*. [*7*] conducted a co-cultivation experiment in which they grew plant species *Senecio jacobaea* L., which contains high concentrations of pyrrolizidine alkaloids (PA), and parsley (*Petroselinum crispum* L.) in common pots. After two months of co-cultivation and analysis, significant concentrations of PA were found in parsley plants, averaging more than 200 μg/kg of dry matter. The parsley plant has therefore been able to absorb pyrrolizidine alkaloids from the soil, although it does not have the biosynthetic ability for these compounds itself.

A particularly important group of plant metabolites are flavonoids, phenolic acids, and related polyphenolic compounds, which have numerous beneficial effects on human health [*8,9*]. They are valued for their antioxidant, anti-inflammatory, antiproliferative, and antidiabetic properties, however, their instability in the gastrointestinal tract and low bioavailability limit their full potential. Therefore, ways to increase their concentration in plants are being investigated, and in this context, the model of interspecies transfer of metabolites proves to be a potentially powerful tool for improving the phytochemical profile. In addition to the previously mentioned transfers of metabolites through soil, more recent research includes another strategy – interspecific transfer via plant extracts. This was exactly the focus of the work of Šola *et al*. [*10*], which showed that the treatment of Chinese cabbage seedlings with extracts of plants such as chamomile, St. John's wort, rose and black bryony can significantly change their phytochemical profile and biological activities, including antidiabetic and antioxidant potential. Although the authors cautiously consider whether this can be a real transfer of compounds or an induction of biosynthesis in the acceptor plant, the effect on the concentration of phenolic compounds and antioxidant and antidiabetic activity was measurable and significant.

The greatest wealth of chamomile, as a guardian of traditional medicine, lies in its flower heads. There are flavonoids such as apigenin, quercetin, and luteolin, phenolic acids, including ferulic and caffeic, coumarins, and powerful essential oils with α-bisabolol and camazulene as key components [*11*]. Phenols exhibit antioxidant and sedative properties. It has traditionally been known as the "cure-all" and is officially recognized in 26 pharmacopoeias around the world [*11*]. It is used in the form of teas, extracts, tinctures, creams, and baths. In recent years, its antioxidant and antidiabetic properties have attracted particular attention from science. Apigenin, one of its main flavonoids, together with hydroxycinnamic acids, contributes to the regulation of blood glucose, so these

compounds show exceptional potential in alleviating the symptoms of type 2 diabetes, improving lipid profiles, and preventing metabolic syndrome [*12*].

Among the most common groups of compounds in common onion bulbs and outer onion scales, there is a trio – organosulfur compounds, flavonoids, and fructans. Sulfur compounds, responsible for the tears caused by onion cutting, mostly belong to the group of S-alk(en)yl-L-cysteine sulfoxide, which is dominated by isoalliin. In addition to determining the taste and smell of onions, these compounds have antimicrobial, hypolipidemic, antidiabetic, and anti-inflammatory properties [*13*]. Flavonoids, primarily quercetin, whose concentration in certain cultivars is as much as five to ten times higher than in broccoli, apples, or blueberries [*14*], have antioxidant, anticancer and cardioprotective effects. In addition to quercetin, kaempferol, and, in colored varieties, anthocyanins are also present. Among the phenolic acids, ferulic, caffeic, and gallic acids stand out [*15*]. Fructans, especially inulin, which have a prebiotic effect, and numerous organic acids that contribute to the spiciness and stability of onions, should not be neglected either. Outer scales of common onion are often discarded as biological waste but can be an interesting source of polyphenols with antioxidant and antidiabetic properties that can be used in the food and pharmaceutical industries. According to Ellatar *et al*. [*16*], the diverse phytochemical composition of the outer scales, leaves and roots of the common onion has been proven; the importance of using onion waste parts as a source of valuable biologically active components is emphasized. A total of 103 compounds were reported using the UPLC-ESI-MS/MS. The most abundant were flavonoids. Compounds from the groups of phenolic acids, organic acids, saponins, amino acids, organosulfur compounds and fatty acids were also detected [*16*].

In this study, the extract of chamomile (*Matricaria chamomilla* L.) served as a transfer medium of valuable phytoactive substances to the common onion (*Allium cepa* L.) After treatment with chamomile extract, the onion outer scales were analyzed for changes in the content of flavonols and hydroxycinnamic acids. The biological activity of onions as an acceptor plant was also investigated, with special emphasis on its altered antidiabetic and antioxidant function. All analyses were performed using an *in vitro* model of human digestion that simulates the physiological conditions of the gastrointestinal tract (different pH in different parts of the digestive system, the presence of stomach acid, bile and digestive enzymes, different duration of oral/gastric/intestinal digestion phases, simulation of intestinal peristalsis, etc.). Antidiabetic activity was measured by inhibition of fructose binding to the protein BSA (bovine serum albumin), which mimics our physiological mechanisms associated with the development of diabetic complications, and antioxidant activity by ABTS and FRAP method. FTIR spectra of the powdered onion outer scales of chamomile treatment or control

are recorded and compared with the FTIR spectra of polyphenol standard apigenin, quercetin and caffeic acid and with FTIR spectra of the powdered dried chamomile flowers.

MATERIALS AND METHODS

Chemicals and materials

All chemicals and reagents used were of analytical or HPLC grade. Enzymes required for the *in vitro* digestion protocol (α-amylase, porcine pepsin, pancreatic lipase, and pancreatin), as well as bile extract and α-amylase applied in the antidiabetic assay, were obtained from Merck KGaA Darmstadt, Germany). Commercial polyphenol standards were purchased from Merck KGaA (Darmstadt, Germany) and Extrasynthese (Genay, France). Chemicals and solvents were provided by Merck KGaA (Darmstadt, Germany) or Kemika (Zagreb, Croatia), and deionized water was used throughout all experiments.

Absorbance and fluorescence measurements related to hydroxycinnamic acids and flavonols, antioxidant and antiglycation activity were recorded using a Fluostar Optima microplate reader (BMG Labtech GmbH, Ortenberg, Germany).

Plant material

Dried chamomile flowers (*Matricaria chamomilla* L.) with a declared flavonoid content of 6 % apigenin were purchased from City Pharmacies Zagreb (GLJZ), Galenic and Analytical laboratories, code 240245 (Zagreb, Croatia). A portion of 3 g was infused with 250 mL of hot water and stirred for 60 min, after which the extract was filtered, cooled to room temperature, and then used for subsequent experiments. Bulbs of red onion (*Allium cepa* L. var. Red Carmen, Nakt, Roelofarendsveen, Netherlands, NL-244085226, code 2704) were obtained from a local store in December 2024. Bulbs of uniform size (1.5–2.0 cm) and good health were selected, while dried adventitious roots were removed. For root induction, bulbs were placed in glass tubes with deionized water and incubated for 48 h in a Fito-Clima 600 PLH climate chamber (Aralab, Rio de Mouro, Portugal) at 23 °C, 65 % humidity, under a 16 h light/8 h dark regime. Only specimens with normally developed roots were used in experiments; bulbs with visible root malformations were excluded. For treatment, selected bulbs were incubated for 24 h either in deionized water (control) or in the aqueous chamomile extract (test). After incubation, outer scales were rinsed with water, dried, pulverized, and used for ethanolic extraction. Powdered onion scales were extracted with 70 % ethanol to yield a final concentration of 50 mg/mL, which was subsequently applied in the *in vitro* digestion assays.

Extract preparation

Ethanolic extracts were prepared by suspending powdered onion outer scales in 70 % aqueous ethanol (*V*/*V*) to a concentration of 50 mg/mL. Extractions were carried out at room temperature using a rotary extractor (Thermo Fisher Scientific, Shanghai, China) for 60 min. After centrifugation (10,000 rpm, 5 min) in laboratory centrifuge (Hettich MIKRO 220R Centrifuge, Tuttlingen, Germany), the supernatants were collected and stored at –20 °C until analysis.

Model of human in vitro digestion

The *in vitro* digestion of onion outer scales extract was conducted following a modified procedure based on Vujčić Bok *et al*. [*17*]. An aliquot of 0.15 mL extract was combined with 0.15 mL of 20 mmol/L phosphate buffer (pH=7.0). The oral (salivary) phase was initiated by adding 5 µL of α-amylase (0.48 mg/mL in the same buffer) and incubating the mixture for 5 min at 37 °C in a thermal shaker (HP 15A and TH15 Edmund Bühler, Tübingen, Germany) at 150 rpm.

To simulate gastric conditions, 0.2 mL of porcine pepsin solution (3 mg/mL in 0.1 mol/L HCl) was added. The pH was then adjusted to 2.0 with 1 mol/L HCl, and samples were incubated for 1 h at 37 °C with constant shaking.

For the intestinal phase, the pH was first raised to 5.3 by the addition of 5 µL of 1 mol/L $NaHCO_3$. Pancreatic juice (0.45 mL), containing 2.4 mg/mL bile acids, 0.2 mg/mL porcine pancreatic lipase, and 0.4 mg/mL pancreatin in 20 mmol/L phosphate buffer (pH 7.0), was then added. The total sample volume was brought to 1 mL with phosphate buffer, and the final pH was adjusted to 7.0 using 1 mol/L NaOH. Incubation continued for 2 h at 37 °C with shaking at 150 rpm.

After each digestion step, including the initial (pre-digestion) phase, the volume of samples was standardized to 1 mL with 20 mmol/L phosphate buffer (pH=7.0). All samples were centrifuged at 15,000 rpm for 5 min at 4 °C in laboratory centrifuge (Hettich MIKRO 220R Centrifuge, Tuttlingen, Germany), and supernatants were stored at –20 °C until further spectrophotometric fluorescence analysis.

Phytochemical analysis

The total contents of hydroxycinnamic acids (THA) and flavonols (TFL) were quantified according to Howard *et al*. [*18*] using caffeic acid and quercetin as standards. Briefly, 50 µL of extract (7.5 mg/mL) was combined with 50 µL HCl (1 mg/mL in ethanol) and 0.91 mL HCl (2 mg/mL). Absorbance readings at 320 and 360 nm were used for THA and TFL, respectively. Results were expressed as milligrams caffeic acid equivalents per gram of dry mass (mg CAE/g DM) or milligrams quercetin equivalents per gram of dry mass (mg QE/g DM). Bioaccessibility (%) was determined as the ratio of compound concentration in each digestion phase relative to the initial undigested extract.

Antioxidant and antiglycation activity

The antioxidant properties of the extracts were evaluated using ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] and FRAP (ferric ion reducing antioxidant power assays). For the ABTS test [*19*], 2 µL of extract was mixed with 200 µL of ABTS solution and incubated at room temperature for 6 min. The absorbance decrease at 740 nm was measured, and the percentage of ABTS radical inhibition was calculated.

The FRAP assay [*20*] involved combining 10 µL of extract with 190 µL of freshly prepared FRAP reagent. Following 4 min of incubation at room temperature, absorbance at 595 nm was recorded, and the reduction of Fe^{3+}-TPTZ was expressed as a percentage. Trolox served as the positive control for both antioxidant assays.

To determine antiglycation activity, inhibition of BSA glycation was performed according to Rusak *et al*. [*21*]. Samples containing 100 µL BSA solution (10 mg/mL), 100 µL fructose solution (0.5 mol/L), and 40 µL extract were incubated in a shaker incubator (HP 15A and TH15 Edmund Bühler, Tübingen, Germany) at 37 °C for 24 h. After incubation, fluorescence was measured at an excitation wavelength of 405 nm and emission wavelength of 460 nm. Catechin solution was used as a reference, and inhibitory activity was calculated accordingly.

FTIR analysis

Powdered chamomile and onion outer scales (chamomile treatment or control) or polyphenol standard was placed onto the sample holder in a FTIR (Fourier transform infrared) spectrophotometer (Spectrum Two, PerkinElmer, Inc., MA, USA) controlled by software (Spectrum Touch PerkinElmer, Inc., MA, USA). The absorption spectra of each sample were recorded in the range of 4000 to 500 cm^{-1}. A background scan was done prior to analysis of each sample scan, with an empty sample plate. Each treatment was recorded in three replicates. The IR spectra were analysed by observing vibrations of sample atoms when these were exposed to IR region of electromagnetic spectrum.

Statistical analysis

Data were processed with Statistica v. 13.3 software [*22*]. One-way variance analysis together with Duncan's *post hoc* test was used to assess statistically significant differences ($p \leq 0.05$). Principal component analysis (PCA) was applied to visualize grouping patterns among samples. Pearson's correlation coefficients were calculated to evaluate the relationships between total polyphenols and the measured biological activities, including antioxidant and antiglycation potential.

RESULTS AND DISCUSSION

Polyphenols, antioxidant and antiglycation activity

Fig. 1 presents total hydroxycinnamic acids (THA) and total flavonols (TFL) content of control sample (deionized water) and test treatment (chamomile) of onion outer scales before (initial phase) and after (salivary, gastric and intestinal phase) of *in vitro* digestion.

In the initial phase of digestion, the chamomile-treated sample (25.89 mg CAE/g DM) had a statistically significantly higher THA value compared to the untreated control (24.23 mg CAE/g DM) (Fig. 1a). During the oral phase, the highest value of THA was observed in the entire experiment in the treated sample (27.08 mg CAE/g DM), statistically significantly higher compared to all other phases except the treatment in the initial phase and control and treated samples in the intestinal phase. In the gastric phase, THA concentrations generally decrease, especially in the control group (19.33 mg CAE/g DM), where the lowest proportion of THA was recorded, statistically significantly lower compared to other phases. In the treated sample in the gastric phase (21.93 mg CAE/g DM), the proportion is slightly higher, but still lower compared to the previous phases. In the intestinal phase, there is a renewed increase in the content of THA in both the control (25.20 mg CAE/g DM) and treated samples (25.24 mg CAE/g DM), but without a statistically significant difference between them.

The TFL content (Fig. 1b) of the treated sample was significantly higher in the initial phase (44.01 mg QE/g DM) compared to the control sample (34.63 mg QE/g DM). During the oral phase, there was a statistically insignificant decrease in flavonol levels in treatment (42.40 mg QE/g DM), but the difference from control (32.06 mg QE/g DM) was still statistically pronounced. In the gastric phase, a decrease in flavonoid concentration was observed in both groups, with the value of 34.70 mg QE/g DM in treatment, and 24.25 mg QE/g DM in control. The lowest concentrations of flavonols were recorded in the intestinal phase in both samples – treated (15.38 mg QE/g DM) and control (13.60 mg QE/g DM), with no statistically significant difference between them.

Fig. 1.

Bioaccessibility of THA and TFL of onion outer scales extract control and chamomile treatment after salivary, gastric and intestinal phase of *in vitro* digestion is presented in Fig. 2. High (>79.79 %) bioaccessibility of THA (Fig. 2a) was detected for control and chamomile treatment in all phases of digestion. Statistically significant higher percentage of THA bioaccessibility was observed in the treated sample (105.52 %) compared to the control sample (99.56 %) in the salivary phase of digestion. In the gastric phase, test treatment (84.69 %) also had a statistically significant higher percentage than the control onion scales sample (79.79 %). In this phase, we can see a statistically significant decrease in bioaccessibility compared to the oral and intestinal phases.

TFL bioaccessibility (Fig. 2b) of both samples (control and test) in the salivary and gastric phase of *in vitro* digestion was high (>70.01 %). In these two phases, chamomile test treatment (97.15 % for salivary and 78.84 % for gastric phase) had higher percentage of bioaccessibility compared to control samples (92.58 % for salivary and 70.01 % for gastric phase). A statistically significant decrease was observed for both samples in the gastric phase compared to the oral phase. This decline continues in the intestinal phase, where bioaccessibility for both samples was significantly lower compared to the first two phases of *in vitro* digestion.

Hydroxycinnamic acids are phenolic acid derivatives of cinnamic acid, and they include caffeic, ferulic, p-coumaric, and sinapic acids [*3*]. In plants, they help strengthen cell walls, defend against pathogens and oxidative stress, and signaling, while in the human body, they have an antioxidant and hypoglycemic effect, modulating enzymes involved in the breakdown of carbohydrates [23]. In our experiment, in three of the four observed phases, a statistical increase in THL content can be observed, which indicates a positive impact of chamomile treatment. Chamomile is an excellent source of caffeic and ferulic acid derivatives [*24*] and for this reason, chamomile treatment could be a donor of these phenolic acids in the common onion, the acceptor plant. In the oral phase, the treated sample reached the highest proportion of THA in the entire experiment. The increase may indicate that hydroxycinnamic acids, often bound to polysaccharides and cell walls, are rapidly released by the action of amylase at neutral pH. This phase, although short, allowed the enzymes to release a significant part of the bioactive molecules, which is confirmed by the high bioaccessibility (105.52 %) in the oral phase of digestion. This is followed by the gastric phase, whose acidic environment often destabilizes the more sensitive phenolic acids. In this case, this trend came to life – there was a decrease in the concentration of THA in both samples, but in the treated sample, as expected, statistically significantly higher values were maintained, which suggests the relative resistance of the transferred acids to gastric conditions. This resistance could be the result of interactions with onion plant matrices that protect them from degradation, which is also noted in similar papers related to cinnamic acids in plant foods [*25*]. In the intestinal phase, the THA stabilized. Although the values were slightly lower compared to the oral phase, they remained at a level comparable to the initial one. According to Manach *et al*. [*26*], this may mean that the compounds that reached this stage were stable and potentially ready for absorption, or that there was a partial degradation into smaller phenolic fragments that could have the same, and perhaps even more, biological activity. Overall, hydroxycinnamic acids showed relative stability and high bioaccessibility during digestion. In the treated sample, their profile indicates that interspecies transmission can not only increase the initial content of these compounds, but also enable their more efficient release and an increase in stability

that enables the "survival" of digestive conditions. For the development of functional foods with a targeted effect on glucose metabolism, this information is of great importance.

Flavonoids are known for their strong antioxidant activity, the ability to bind free radicals, but also the regulation of key enzymes in glucose metabolism [*27*]. In plants, they act as pigments, defense mechanisms, and signaling molecules, and in the human body as silent allies in preserving homeostasis. Therefore, it is not surprising that flavonoids are the subject of numerous studies when it comes to functional nutrition. In this study, total flavonols, as a subgroup of flavonoids, were analyzed, which showed a significantly higher proportion in the treated onion compared to the control sample, in all digestive phases except the intestinal phase, in which the elevation was not statistically significant. During the oral phase, the concentration of TFL in the treated sample is still higher than the control sample. The latter may indicate that chamomile flavonols are mostly free-form and therefore quickly released and are more stable in a neutral pH environment, while endogenous onion flavonols can be bound to sugar or protein structures that still require degradation. In the gastric phase, where the pH is low (≈2), a statistically significant decrease in TFL in both samples is expected, but the values in the treated sample remained higher. This stage is known for its challenges to the stability of flavonoid compounds because many of them are sensitive to hydrolysis in an acidic medium, especially if they are present in the form of glycosides. However, the fact that they have not completely disappeared speaks in favor of the resistance of the transferred compounds, perhaps thanks to the protection provided by the onion plant matrix. Furthermore, the intestinal phase, a key part for absorption, records the lowest flavonol values throughout the model. The treated and control samples are statistically almost equal in concentration, and the bioaccessibility is only 35 %. This result is not surprising, given the known instability of flavonoids at higher pH values [*28*]. A higher pH in the intestine (≈7), therefore, does not bring complete "relief" to flavonols. On the contrary, this phase represents a second wave of stress for these compounds – oxidation, conjugation or complete degradation, which leads to a significant reduction in their absorption. Similar trend of decrease of bioaccessibility was observed for flavonols in ginkgo casein, ginkgo glucose, ginkgo olive oil and spinach lemon juice formulation [*17,21*]. However, there are also experiments with opposite results. For example, in the literature [*21,29*] was reported an increase in flavonoid bioaccessibility in the intestinal phase in berry, green tea, and ginkgo water extracts, which can be attributed to the difference in the plant matrix and the structure of the flavonoids present [*21*]. These discrepancies, however, confirm that the stability and utilization of flavonols are highly contextual and depend on many factors, including the source of compounds, as well as the physicochemical properties of the digestive environment. The amount of flavonols through the digestive model is progressively reduced. In addition to the chemical delicacy of flavonoids, these data also indicate the importance of design

in the development of functional products to protect flavonols during their journey through the digestive system, either through microencapsulation, the use of synergistic plant matrices or the selection of more resistant molecular forms. In the combination of onion and chamomile, the loss of distinctiveness between treatment and control in the intestinal phase suggests that the transfer of chamomile flavonols has been successful, but their stability in the later stages of digestion remains a challenge for future formulations.

Fig. 2.

ABTS and FRAP methods were used to test the antioxidant activity of the control sample (deionized water) and the test treatment (chamomile) of onion outer scales before (initial phase) and after (salivary, gastric, and intestinal phase) of *in vitro* digestion (Fig. 3). High antioxidant activity was observed in both methods. The percentage of ABTS radical inhibition ranged from 85.14 to 92.98 %, and the reduction percentage for the FRAP method ranged from 91.91 to 97.46 %. In the ABTS method, a statistically significant higher value of the treated sample compared to the control sample was observed only in the intestinal phase of *in vitro* digestion (91.51 *vs* 86.21 %). For FRAP methods, onions treated with chamomile showed statistically significant higher values before (initial phase) and after (salivary, gastric, and intestinal phase) *in vitro* digestion compared to the control onions treated with water.

In our experiment, two antioxidant methods were used to test the control sample (deionized water) and the test treatment (chamomile) of onion outer scales before (initial phase) and after (salivary, gastric, and intestinal phase) of *in vitro* digestion. We combined the ABTS method able to evaluate both hydrophilic and lipophilic antioxidants, with the FRAP method able to evaluate primarily measures hydrophilic antioxidants [*30,31*]. Combining the antioxidant activity methods reduces the limitations of a particular method. The DPPH method was not suitable for the measurement of our samples due to the purple color of the onion outer scales extract, which affected the DPPH measurement. In both methods, onion outer scales samples before and after all stages of digestion showed high antioxidant activity (ABTS>85.14 % and FRAP>91.91 %). The high antioxidant activity of onion samples is in accordance with the literature data [*32–34*]. In the FRAP method statistically significant increase was observed for all treatment samples in comparison to control samples for all phases of *in vitro* digestion, and for the ABTS method only in the intestinal phase of *in vitro* digestion. This could be attributed to the high concentration of hydroxycinnamic acids and their high bioaccessibility for chamomile-treated onion samples. As mentioned before, chamomile is an excellent source of hydroxycinnamic acids, especially caffeic and ferulic acid, with high antioxidant activity [*24,35*].

Fig. 3.

All treated samples (Fig. 4) had moderate antiglycation activity, ranging from 42.86 to 62.24 %. The control samples had weak (26.92 % initial phase) to moderate antiglycation activity (36.35–54.60 %). In the initial phase, the treated sample (43.52 %) showed statistically significantly higher inhibition of BSA glycation compared to the control (26.92 %), indicating that the transfer of specialized metabolites from chamomile to onion had a rather positive effect on antiglycation properties. During the oral phase, activity increases further in control (36.35 %) versus treatment (49.85 %), with the difference in the percentage of inhibition being statistically significant. In the gastric phase, a decrease in inhibitory activity was observed in the treated sample, but the treated sample still maintained higher activity (42.86 %) compared to the control (36.98 %). In the intestinal phase, the highest percentage of inhibition of bovine albumin glycation was recorded in the treated sample (62.24 %), statistically significantly higher compared to all other phases and samples, which suggests that bioactive compounds from treated onions not only remain stable during digestive conditions, but also potentially increase their bioaccessibility and efficacy at this stage.

In the context of a metabolic disorder such as diabetes lies not only elevated blood glucose levels, but also a whole series of accompanying biochemical processes that damage cells and tissues over time. Among them, protein glycation occupies a special place. This process, also known as the Maillard reaction, is a non-enzymatic interaction between reducing sugars, such as glucose, and protein molecules [*36*]. Sugars irreversibly bind to proteins, creating AGEs (advanced glycation end products), molecules that accelerate cell aging, disrupt the elasticity of blood vessels, and activate inflammatory pathways [*37*], and also cause nerve and retinal damage, cataracts, and chronic kidney disease [*36*]. Due to such negative effects of glycation, it is crucial to find compounds that can suppress this process and thus reduce the aforementioned diabetic complications. That is why the ability of plant extracts to inhibit glycation is increasingly used as an indicator of their antidiabetic potential. Throughout this work, glycation suppression was investigated using the BSA method: the ability of the test extract to prevent glucose binding to bovine serum albumin, which is used as an analogue to human albumin due to its structural and functional similarity. Overall, the results show that both control and treated samples of onion outer scales provided protective antiglycation activity. Treated samples had moderate antiglycation activity, while the control samples had weak to moderate antiglycation activity. This activity was statistically significantly higher in the treated samples in comparison to the control samples. The control sample in the initial phase showed the lowest value in relation to all samples. In the initial phase, the inhibition of BSA in the treated sample was moderate compared to the weak inhibition in the control sample. It is already evident here that the compounds transferred from chamomile, known for its richness in flavonoids and phenolic acids, have greatly contributed to the antiglycation effect. Given that apigenin and luteolin have already been confirmed

as inhibitors of AGEs formation, their presence may be a key factor in explaining the results obtained. By entering the oral phase, which is dominated by neutral pH and mild enzymatic activity, the inhibition of glycation further increases. It seems that here most of the compounds are activated or released from the plant matrix, especially in the treated sample, where a statistically significant difference is still maintained compared to the control sample. The gastric phase showed the lowest inhibitory activity in the treated sample, which is expected, given the already mentioned sensitivity of certain phenolic compounds to low pH. Nevertheless, the treated sample retained a statistically significantly higher level of glycation inhibition compared to the control. From this, we can again conclude that, although there is a decline in relation to the oral phase, the transferred compounds from chamomile extract to onions show a certain level of resistance, or are at least partially protected by the onion plant matrix that mitigates their acid degradation. The intestinal phase, as the last and most important stage in the context of absorption and the biological effect of compounds, gave rise to the highest percentage of BSA glycation inhibition, especially in the treated sample. This upward trend may indicate the cumulative effect of the release of compounds throughout the digestive process, but also their stability and potential activation in the more alkaline environment of the small intestine. Likewise, it may be that the compounds that reached this stage were more effective or reactive in the presence of BSA itself, and it is also possible that new metabolites with antiglycation properties appeared during digestion. From the results of the experiment, it was already evident that the bioaccessibility of flavononols in the intestine was relatively low (35 %), so the high level of glycation inhibition at this stage suggests that these compounds are not the exclusive carriers of the antiglycation effect. Given that in the same digestive phase, the bioaccessibility of hydroxycinnamic acid was significantly higher (97 %), it could be the main actor of this biological action. Namely, chlorogenic and ferulic acids have been shown to prevent the formation of AGE products through various mechanisms, including the capture of reactive carbonyl compounds and chelation of metal ions [*38,39*]. It is possible, therefore, that a kind of "phenolic power transition" occurs in this part of digestion, where the dominant role is taken over by more stable and available compounds, and their synergy with residual flavonols further enhances the overall effect. The highest inhibition of BSA glycation at this stage, therefore does not necessarily reflect the amount of one type of compound, but indicates the efficacy and cooperation of several groups of bioactive metabolites.

Fig. 4.

Fourier transform infrared (FTIR) spectroscopy is one of the most important non-destructive analytical techniques used to identify the functional groups of organic compounds and widely used for quality control in the pharmaceutical industries. Also, FTIR has become increasingly useful in the field of evaluating herbal qualities [*40–43*].

The absorption spectra of dried onion outer scales and dried chamomile flowers powder is presented in Fig. 5, FTIR spectra of pure phenolic compounds (apigenin, caffeic acid and quercetin) are presented in Fig. 6. Onion outer scales and chamomile flower powder are composed of polyphenolic compounds with O–H (around 3400–3200 cm^{-1}) bonds, C=O bonds (around 1670–1660 cm^{-1}), and along with other characteristic peaks for C-C, C-H, C-O-C and C-O bonds around 1030-1023 cm^{-1} as is recorded in literature [*40–43*]. The FTIR spectra of the onion outer scales control treatment had a broad peak at 3331 cm^{-1}, for chamomile treatment at 3298 cm^{-1}, and for pure chamomile powder at 3293 cm^{-1}. The region of 3331-3293 cm^{-1} indicates stretching of polymeric hydroxyl group. O-H bonds are mainly derived from the phenols and flavonoids [*44*].

Fig. 5.

Quercetin, the main flavonoid in the onion, had a characteristic broad peak at 3353 cm^{-1} for its hydroxyl groups. O-H groups of quercetin are located at C-3, C-3', C-4', C-5 and C-7, and this peak is in the range of 3600-3100 cm^{-1} [*45*]. Apigenin, the main flavonoid in chamomile, has O-H groups at the C-5, C-7, and C-4′positions [*45*]. This O-H group is located at 3282 cm^{-1} on the apigenin FTIR spectra. As we previously discussed, chamomile is an excellent source of caffeic acid [*24*]. Caffeic acid had two O-H groups located at the C-3 and C-4 positions. In the FTIR spectra of caffeic acid, is visible peak is at 3401 cm^{-1}.

Transmittance of O–H (around 3600-3100 cm^{-1}) bonds of the pure polyphenolic compound's quercetin was 82.99 %, for apigenin was 88.15 %, for caffeic acid was 96.67 % for onion outer scales control treatment was 98.33 %, for chamomile treatment 95.31 % and for pure chamomile powder 95.20 %. A higher number of O-H groups has a positive effect on antioxidant activity [*3*,*44]*. The lowest transmittance was reported for quercetin, the compound with five O-H groups, and the highest transmittance for caffeic acid, the compound with two O-H groups. The increase in THA (control 39.20 CAE/g DM, chamomile treatment 48.80 CAE/g DM) and TFL (control 51.75 QE/g DM, chamomile treatment 78.77 QE/g DM) measured by spectrophotometry (data not shown) was confirmed based on FTIR analysis, which showed that the transmittance of the O–H group, mainly derived from the polyphenols, was lower for chamomile treatment in comparison to control onion scales samples. The above points to the positive effect of chamomile treatment on the polyphenolic composition of the outer onion scales and also on the antioxidant and antiglycation activity. Chamomile treatment could have benefited the entry of apigenin and caffeic acid from chamomile tea, as well this treatment may cause stress in the plant that results in the additional synthesis of quercetin in the onion.

Fig. 6.

Pearson's correlations of polyphenolic content and bioaccessibility, antioxidant and antiglycation activity

Pearson's correlations of the total hydroxycinnamic acids (THA), total flavonols (TFL), bioaccessibility of the total hydroxycinnamic acids (BA THA %), bioaccessibility of the total flavonols (BA TFL %), antioxidant (ABTS and FRAP method) and antiglycation (BSA method) activity in onion scales extracts (control and test treatment with chamomile extract) during simulated *in vitro* gastrointestinal digestion are presented in Table 1. Evans' correlations coefficient from 0.00–0.19 indicates very weak correlation, from 0.20–0.39 weak correlation, from 0.40–0.59 moderate correlation, from 0.60-0.79 strong correlation and from 0.80–1.00 very strong correlation [*46*]. The highest positive correlation was observed between the THA and BA THA % and between the TFL and BA TFL %. This correlation is very strong and its value is 0.95. Very strong positive correlation (0.91) was observed between the antioxidant (FRAP) and antiglycation (BSA) activity. Strong positive correlation was observed between the FRAP and THA (0.79), BSA and THA (0.64), FRAP and BA THA % (0.62). While a moderate positive correlation has been observed between BA THA % and BSA (0.52). According to our results of *in vitro* digestion of onion outer scales extract, hydroxycinnamic acids significantly contribute to the antioxidant and antiglycation activity which is in line with the literature [*10,17,19–21,47–49*].

Table 1.

PCA of polyphenol group and antioxidant and antiglycation activity

Visualization between the similarity and diversity of samples and parameters based on distances on the diagram is possible with PCA (principal component analysis) [*17,21,47,49*]. PCA diagram press the diagram of the measured polyphenols (THA, TFL), bioaccessibility of the THA and TFL, antioxidant (ABTS and FRAP) and antiglycemic (BSA) activity of onion scales extracts (control and test treatment with chamomile during simulated *in vitro* gastrointestinal digestion).

The first (Factor 1) and the second (Factor 2) principal components accounted for 57.20 and 28.78 %, respectively (Fig. 7). Together, the first two factors represented 85.98 % of the total variability. In the upper left quadrant are the test treatment samples in the salivary phase of *in vitro* digestion and the THA, BA THA, and FRAP. On the same side, but in the lower quadrant, both samples (C=control and T=test treatment with chamomile) in the intestinal phase were located with the BSA. Control samples in the salivary phase of digestion are located in the upper right quadrant, and the control samples in the gastric phase of digestion are located in the lower right quadrant. Treatment samples in the gastric phase of digestion are located on the border between the upper and lower quadrants on the right side. From the above, it is evident that the greatest similarity between

control samples in the gastric phase of digestion means that the differences between the treatments are not as noticeable as in the oral and gastric phases. In the test treatment in the salivary phase of *in vitro* digestion, the highest values of THA, BA THA, FRAP, TFL, and BA TFL were measured. The test samples of the intestinal phase of *in vitro* digestion had the highest values of BSA and FRAP, which is clearly noticeable when looking at the distances between the samples and the measured parameters on the PCA diagram.

Fig. 7.

CONCLUSIONS

The hypothesis of improving polyphenolic content and biological (antioxidant and antiglycation) activity of the onion scales, achieved by simple immersion of onion root in chamomile extract, was confirmed. The results of the analysis show an increase in the content of flavonols and hydroxycinnamic acids (initial, oral and gastric digestion phase), as well as the preservation of their bioaccessibility during simulated digestion, with the exception of flavonols in the intestinal phase. Antioxidant activity was also improved in FRAP method before (initial phase) and after (salivary, gastric and intestinal phase) in vitro digestion for onions treated with chamomile and in ABTS method only in intestinal phase of in vitro digestion. In addition, extracts of chamomile-treated onion showed a higher inhibitory activity on BSA glycation, thus proving a potentially stronger antidiabetic effect. Treatment of onions with chamomile water extract is good method of enrichment of biological waste. This is confirmed by FTIR analysis. Onion scales represent good source of bioaccessible polyphenols with high antioxidant and moderate antiglycation activity.

The enrichment of the phytochemical composition and biological potential of onions builds on recent research into the horizontal transfer of natural products and raises new questions about the potential applications of this technique for health and nutritional purposes. Since the method does not require genetic modification but relies on natural absorption mechanisms and stress induction, it could become a valuable tool for enriching the nutritional and therapeutic composition of herbs. These results represent a foundation for future research, which should further elucidate the mechanisms of transmission, long-term effects, and potential industrial applications of this phenomenon.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

V. Vujčić Bok conceived the research, supervised the research, carried out the experiments and statistical analysis, prepare graphic and tables, wrote and edited the manuscript. S. Gagic wrote part of the draft and caried experiments. Student L. Rubinic wrote part of the draft and caried experiments. I. Šola review, edited the manuscript and provide resources. Student P. Kašnjar Perković carried out the part of the experiments. Student N. Celinić carried out the part of the experiments. S. Jurić review and edited the manuscript. Ž. Maleš review, edited the manuscript and provide resources.

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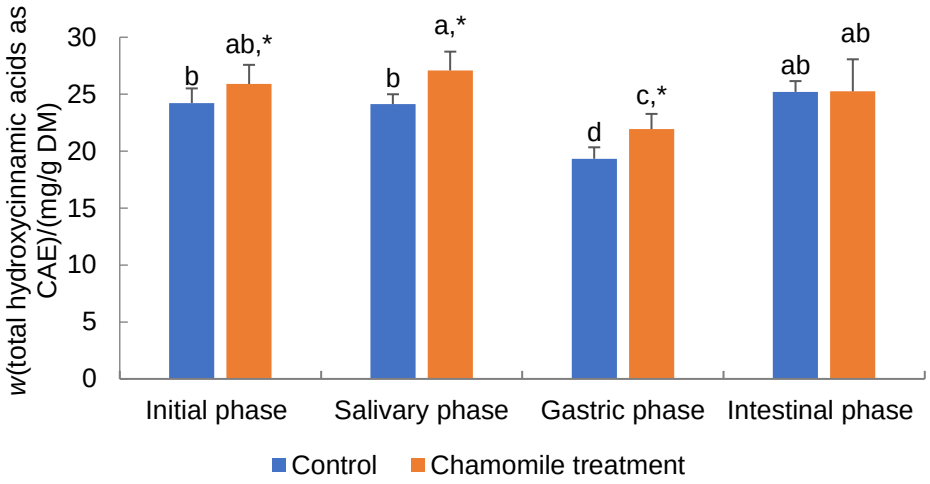
Table 1. Correlations of the total hydroxycinnamic acids: THA, total flavonols: TFL, bioaccessibility of the total hydroxycinnamic acids: BA THA/%, total flavonols: BA TFL/%, antioxidant (ABTS and FRAP method) and antiglycation (BSA method) activity in onion extracts (control and test treatment with chamomile extract) during simulated *in vitro* gastro-intestinal digestion

Variable	THA	TFL	BA THA/%	BA TFL/%	ABTS/%	FRAP/%	BSA/%
THA	1.00						
TFL	0.09	1.00					
BA THA/%	0.95	0.00	1.00				
BA TFL/%	-0.04	0.95	-0.05	1.00			
ABTS/%	-0.55	0.31	-0.62	0.40	1.00		
FRAP/%	0.79	-0.15	0.62	-0.41	-0.51	1.00	
BSA/%	0.64	-0.50	0.52	-0.71	-0.55	0.91	1.00

a)



b)

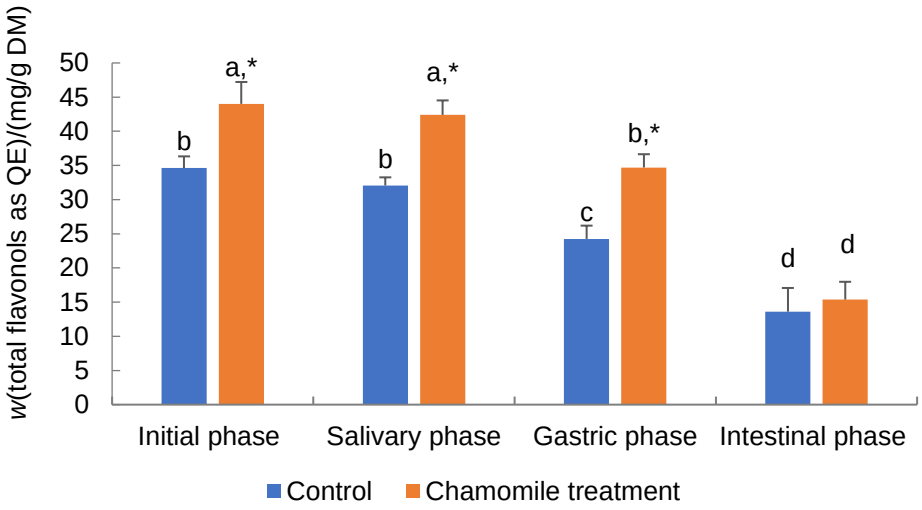
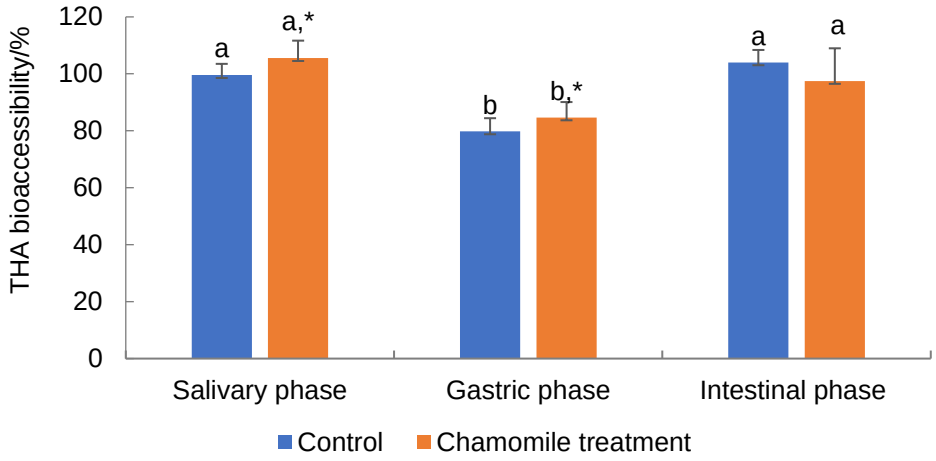


Fig. 1. a) total hydroxycinnamic acids, b) total flavonols of control sample (deionized water) and test treatment (chamomile) of onion outer scales before (initial phase) and after (salivary, gastric and intestinal phase) *in vitro* digestion. Data are presented as mean value±S.D., *N*(onion bulbs)=6. Different letters indicate significant differences at p≤0.05 for all samples and phases together. Asterisk indicates significant differences at p≤0.05 for each phase individually. CAE=caffeic acid equivalents, QAE=quercetin equivalents, DM=dry mass

a)



b)

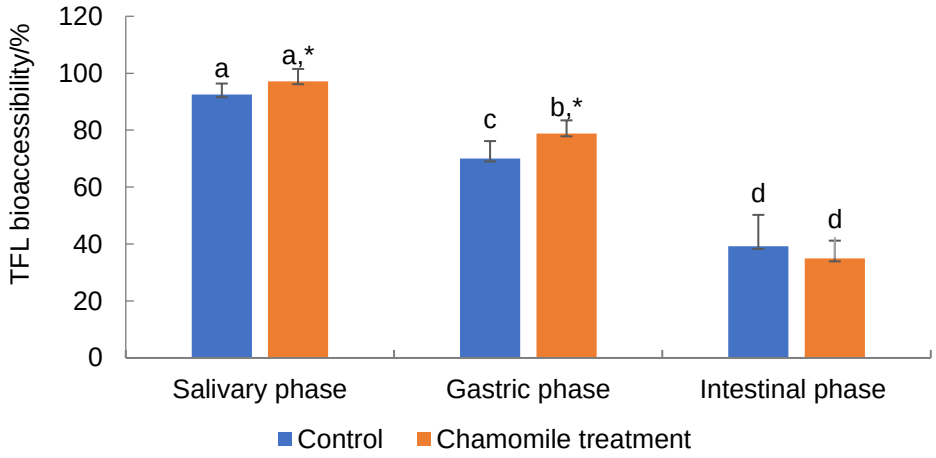
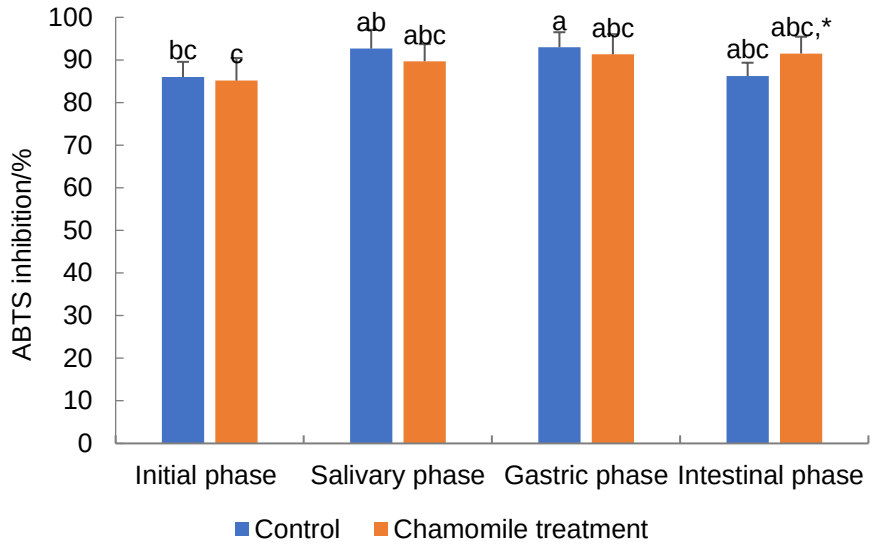


Fig. 2. Bioaccessibility (%) of: a) total hydroxycinnamic acids (THA), and of b) total flavonols (TFL) of control sample (deionized water) and test treatment (chamomile) of onion outer scales after (salivary, gastric and intestinal phase) of *in vitro* digestion. Data are presented as mean value±S.D, *N*(onion bulbs)=6. Different letters indicate significant differences at p≤0.05 for all samples and phases together. Asterisk indicates significant differences at p≤0.05 for each phase individually

a)



b)

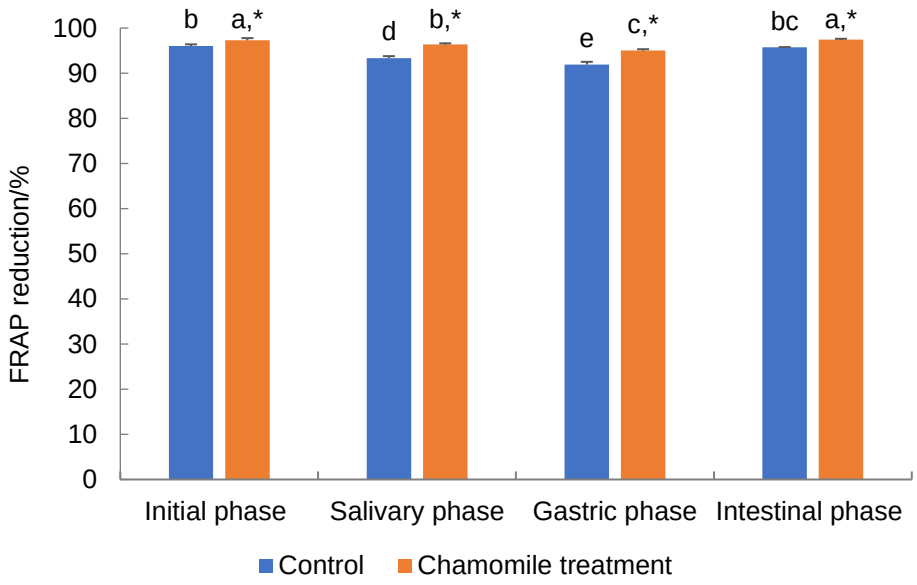


Fig. 3. Antioxidant activity (a) ABTS and (b) FRAP of control sample (deionized water) and test treatment (chamomile) of onion outer scales before (initial phase) and after (salivary, gastric, and intestinal phase) of *in vitro* digestion. Data are presented as mean value±S.D, *N*(onion bulbs)=6. Different letters indicate significant differences at p≤0.05 for all samples and phases together. Asterisk indicates significant differences at p≤0.05 for each phase individually. ABTS=[2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)], FRAP=ferric ion reducing antioxidant power

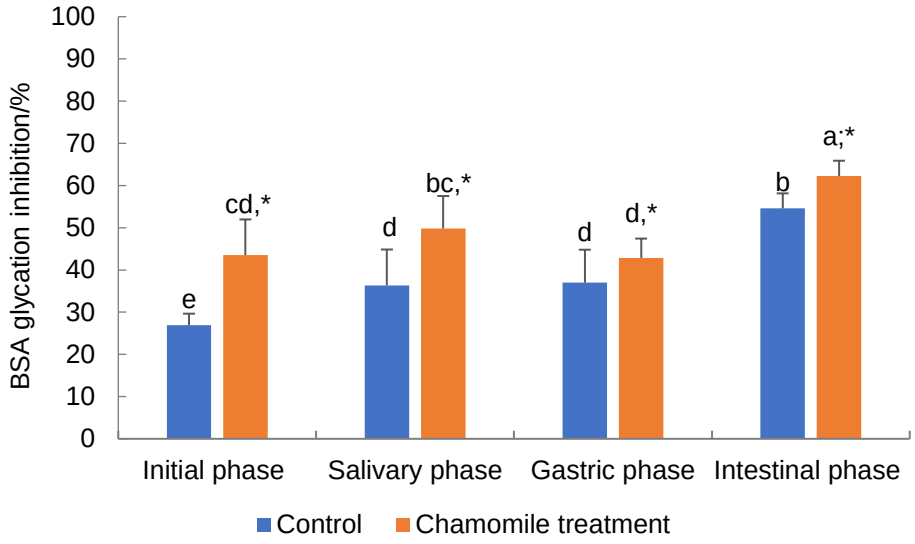
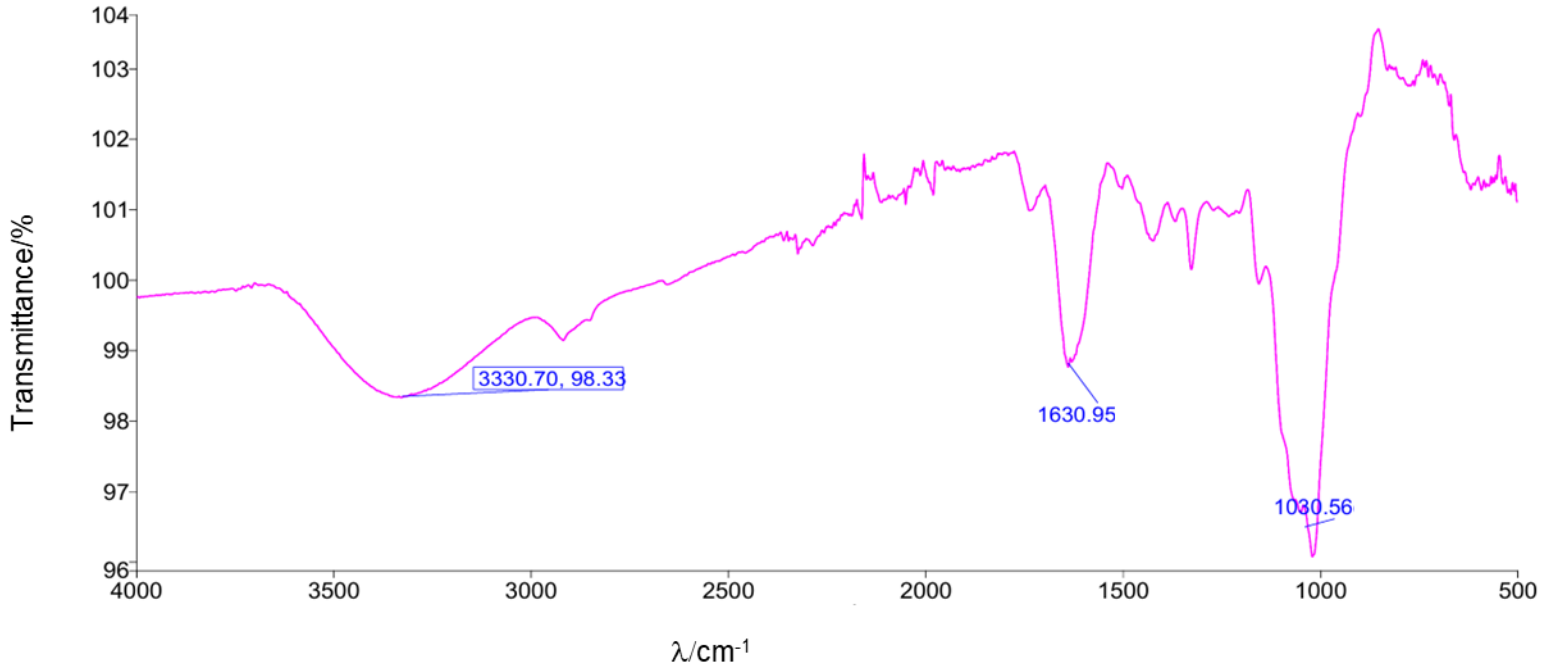


Fig. 4. Antiglycation activity (BSA method) of control sample (deionized water) and test treatment (chamomile) of onion outer scales before (initial phase) and after (salivary, gastric and intestinal phase) of *in vitro* digestion. Data are presented as mean value±S.D, *N*(onion bulbs)=6. Different letters indicate significant differences at $p \leq 0.05$ for all samples and phases together. Asterisk indicates significant differences at $p \leq 0.05$ for each phase individually. BSA=bovine serum albumin

a)



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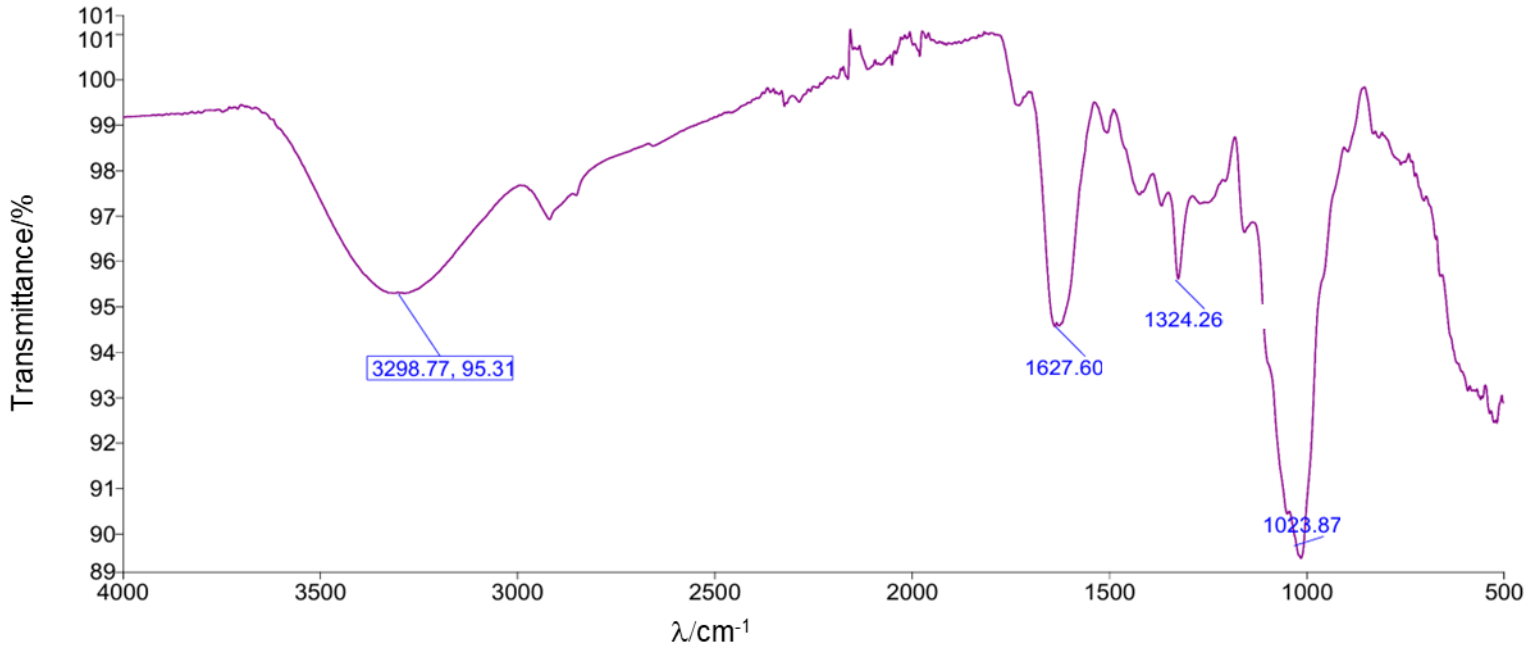


c)

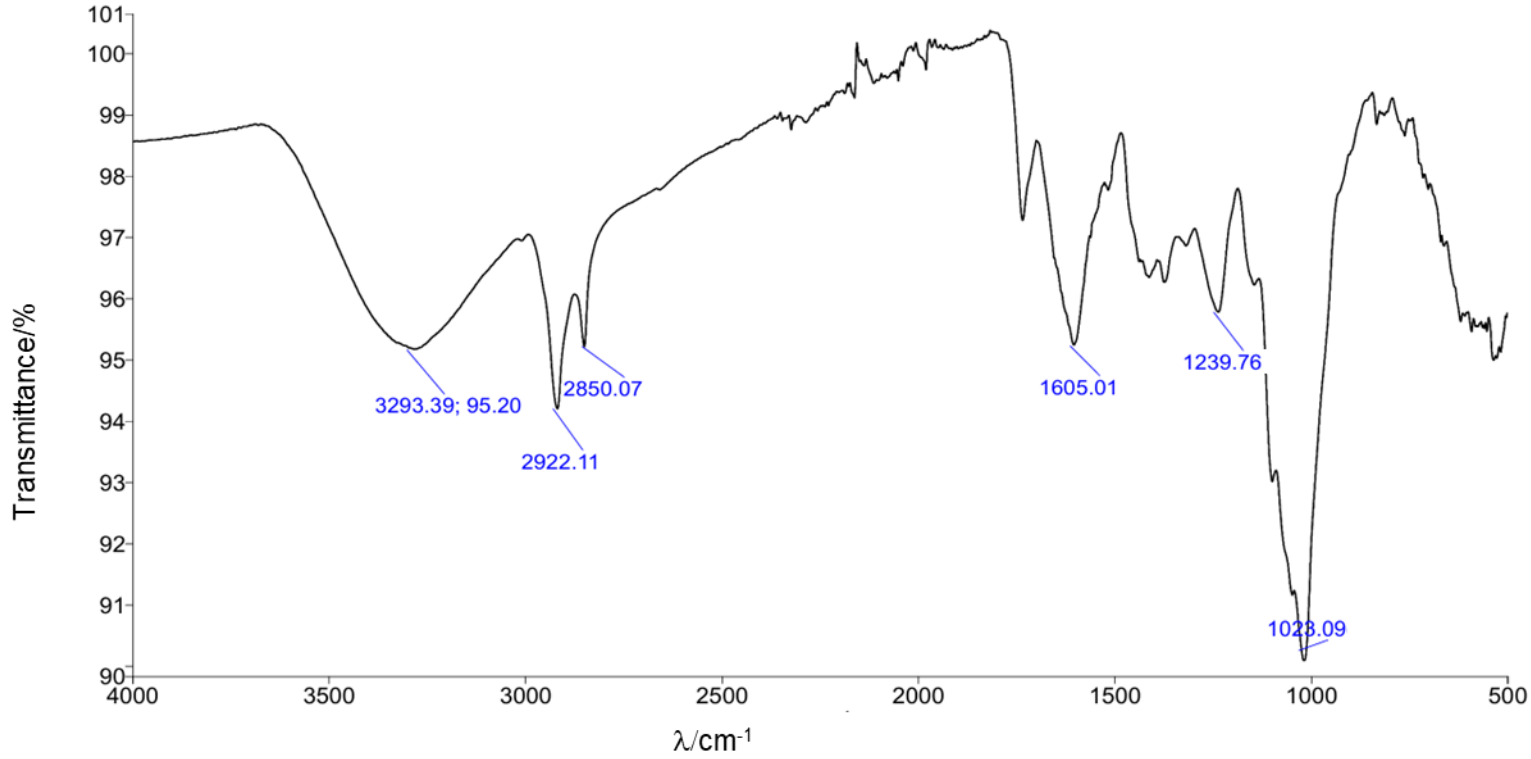
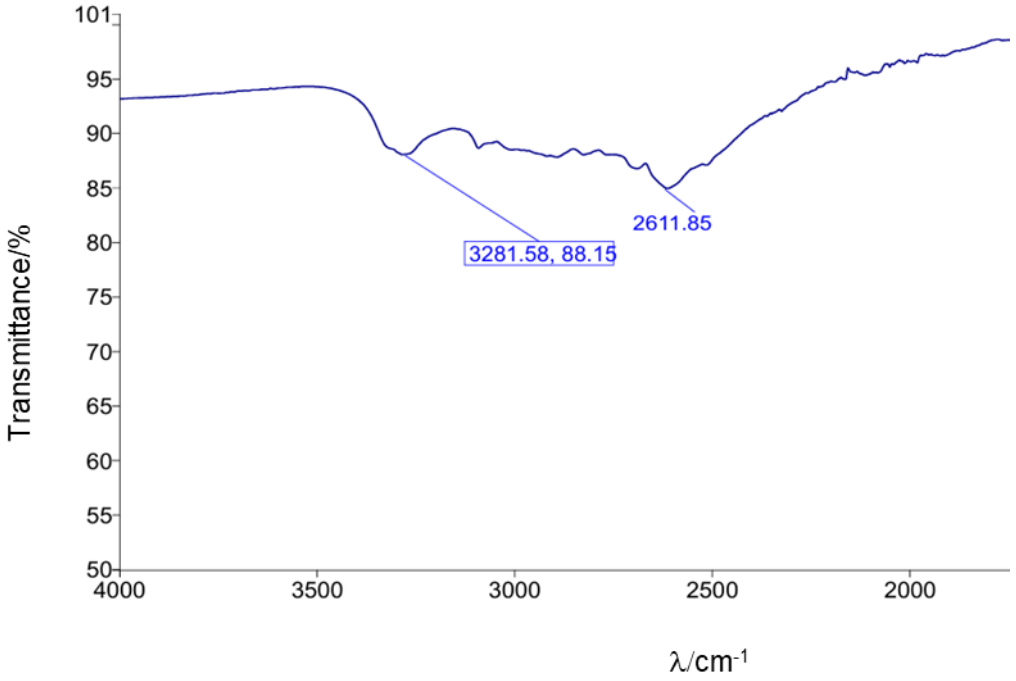
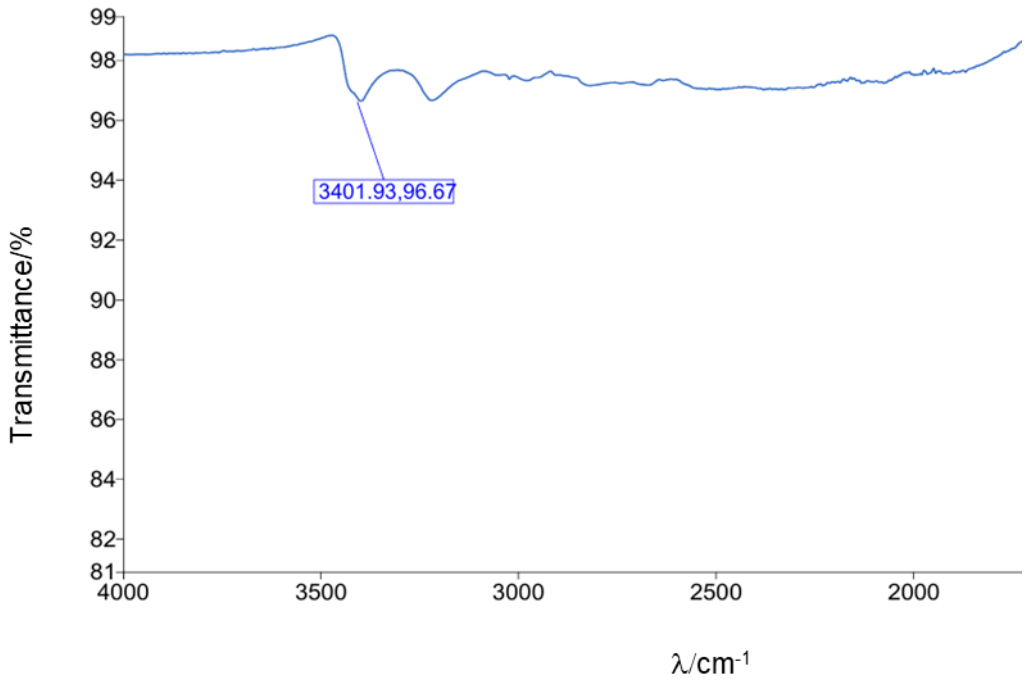


Fig. 5. The FTIR spectra of: a) the onion outer scales control treatment, b) of the onion outer scales chamomile treatment and c) of the chamomile powder

a)



b)



c)

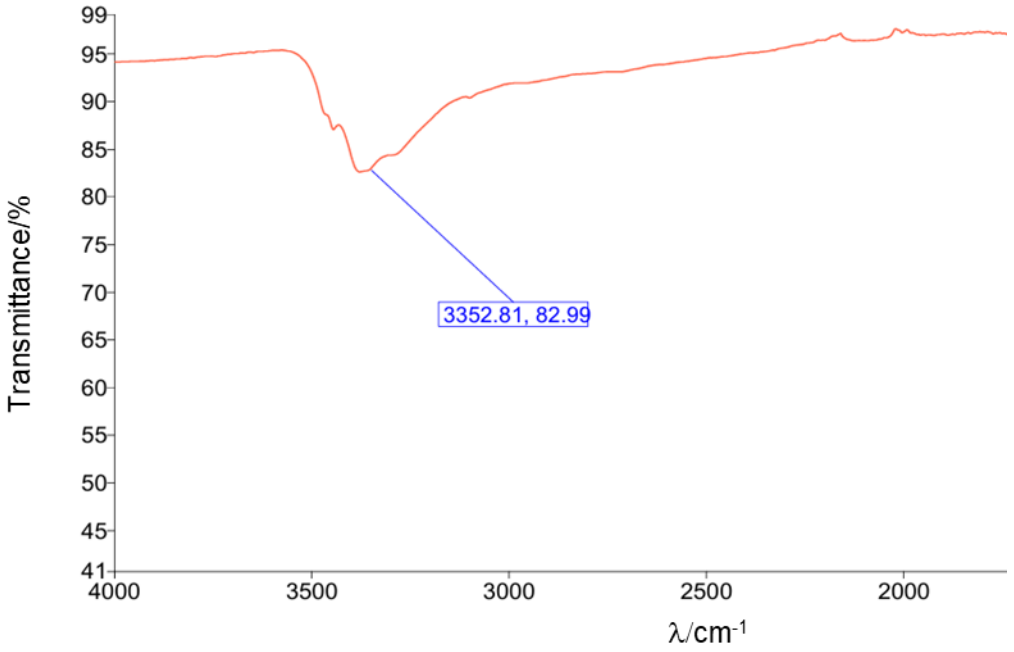
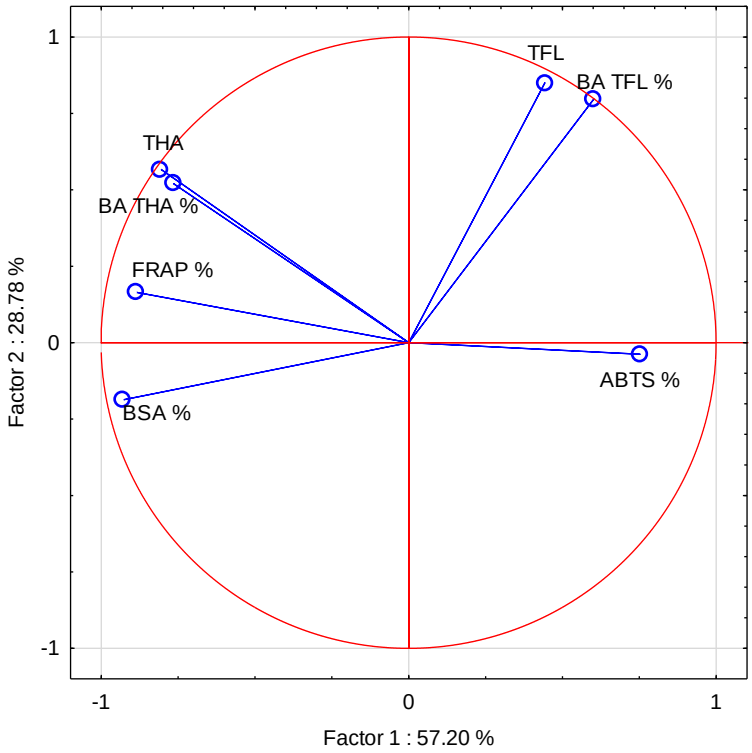


Fig. 6. The FTIR spectra of: a) apigenin, b) caffeic acid and c) quercetin

a)



b)

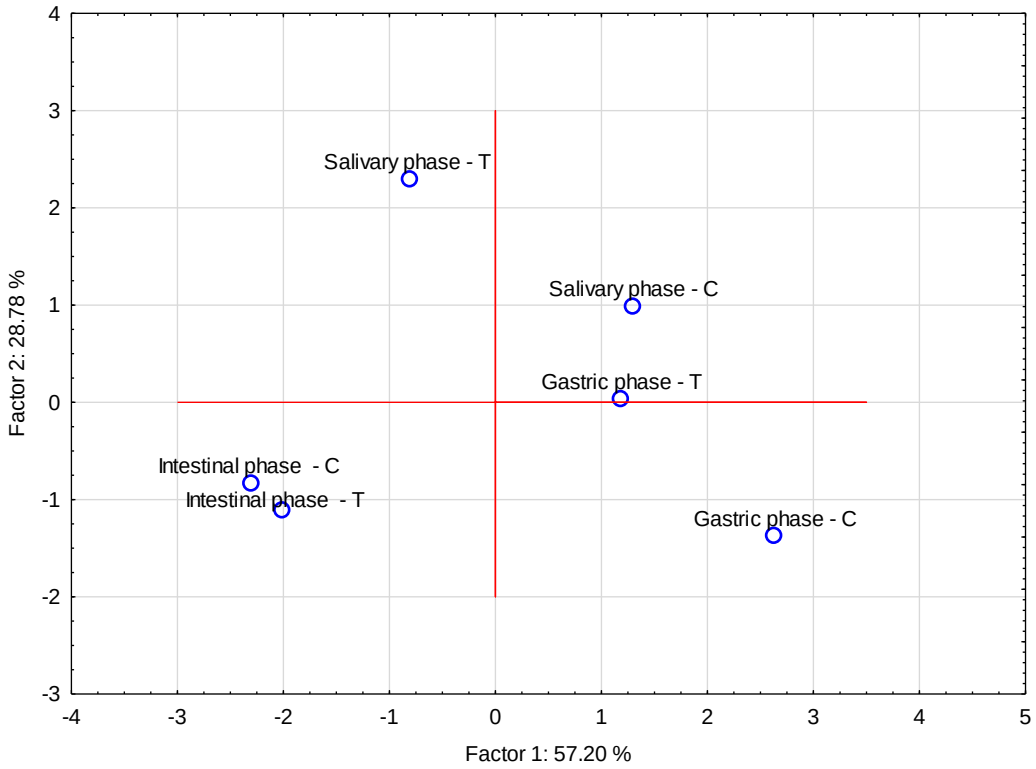


Fig. 7. Principal component analysis (PCA) diagram of the measured polyphenols (total hydroxycinnamic acids: THA, total flavonols: TFL, bioaccessibility of the total hydroxycinnamic acids: BA THA %, bioaccessibility of the total flavonols: BA TFL %), antioxidant (ABTS and FRAP) and antiglycemic (BSA method) activity of onion scales extracts (C=control and T=test treatment with chamomile during simulated *in vitro* gastrointestinal digestion). a) grouping of analyzed parameters, b) grouping of samples