Effects of pH on the Adsorption and Desorption of Naringin onto Zymosan A in Aqueous Systems

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This study aimed to investigate the interactions between naringin and dietary fiber from yeast (zymosan A) through adsorption and desorption processes. The effects of different solution pH conditions (pH 3.0, pH 7.0, and pure water at pH 5.5), as well as varying initial concentrations of zymosan A and naringin, on their interaction were examined. The adsorption capacity of naringin onto zymosan A was higher at pH 7.0 (12 to 275 mg g^{-1}), lower in water (6 to 83 mg g^{-1}) and lowest at pH 3.0 (0 to 55 mg g^{-1}). The desorption ratio was lowest in pH 3.0 solution, followed by water and pH 7.0 solution. UV-Vis and FTIR spectral analyses confirmed the binding of naringin onto zymosan A. The antiradical activity for naringin was highest at pH 3, followed by pH 7 and pure water. For the naringin-zymosan A complex, antiradical activity was highest at pH 3, with similar but lower values at pH 7 in pure water.

Keywords
adsorption, naringin, zymosan A, pH

Introduction

Polyphenols are a large group of plant metabolites found in various fruits, vegetables, and their derived products. Flavanones, a group of phenolic compounds, constitute the majority of phenolic compounds in citrus fruits. Given the widespread dietary consumption of citrus fruits, the bioactive effects of their flavanone content have been extensively studied[1,2]. Understanding the bioactive effects of flavanones requires investigating various digestive processes that influence the release of these compounds from the food matrix, their bioaccessibility, absorption into tissues and organs, and overall bioactivity.

Interaction with other food components during digestion can affect the release of flavanones, their absorption[3], and ultimately their potential bioactive effects. Dietary fibers, which are resistant to digestion and absorption in the human small intestine and are partially or fully fermented in the large intestine, may serve as carriers for adsorbed flavanones, delivering them to the lower digestive tract, where they can be released and exert their beneficial effects. Previous studies have explored the role of dietary fibers as colon-targeted delivery systems for polyphenols[4].

One of the main citrus flavanone glycosides is naringin, predominantly found in grapefruit, oranges, lemons, and limes[5,6]. Naringin has shown various potential health benefits[7,8], and may interact with dietary fibers. Dietary fibers are classified as soluble or insoluble[9]. Zymosan A or β-(1,3)-glucan, is an insoluble dietary fiber derived from the yeast *Saccharomyces cerevisiae*[10] with diverse biological activities, including roles in inflammatory and immune responses, drug protection during digestion, and toxin adsorption[11–14]. Due to these biological activities, zymosan A can be used as a functional food or an immunity booster[10].

The interactions between naringin and dietary fiber such as zymosan A, can be studied through adsorption processes[15], where naringin acts as the adsorbate and zymosan A is the adsorbent. The desorption process occurs simultaneously with adsorption until the adsorption equilibrium is reached. Investigating these processes can provide insights into the amount of naringin bound to zymosan A and how digestive factors, such as pH, influence this binding. Variations in pH can alter the chemical structures of naringin[16,17] and zymosan A, potentially affecting adsorption. Therefore, the influence of pH on the interactions is also regularly studied.

This study aimed to examine the interactions between zymosan A and naringin, a process relevant to the bioaccessibility and potential bioactive effects of naringin in the digestive tract. Specifically, *in vitro* experiments were conducted to investigate the adsorption and desorption processes, evaluating the influence of pH and component concentrations on the surface binding. Adsorption was performed in pure water (pH 5.5), and in aqueous solutions pH

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3 and 7, representing different sections of the digestion system. FTIR and UV-Vis analyses were conducted to observe naringin binding to zymosan A, while the DPPH˙ assay was used to assess the antiradical activity. To the best of our knowledge, no previous studies have examined the effects of pH on the interactions of naringin with zymosan A.

Materials and methods

Chemicals and reagents

Zymosan A from *Saccharomyces cerevisiae* and 2,2-diphenyl-1-picryl-hydrazyl (DPPH˙) were purchased from Sigma Aldrich (St. Louis, USA), and naringin hydrate (≥ 97 %) from Acros Organics. Sodium carbonate, hydrochloric acid (37 %) were purchased from Avantor (Arnhem, Netherlands), and Folin-Ciocalteu reagent from Merck (Darmstadt, Germany). Sodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate, citric acid monohydrate, and sodium citrate dihydrate were purchased from Kemika (Zagreb, Croatia). Citrate and phosphate buffer solutions (0.1 M) were used for pH 3.0 and pH 7.0, respectively.

Determination of naringin in the adsorption process

Naringin concentration during adsorption and desorption experiments was determined according to the Folin-Ciocalteu method[18], with calibration curves prepared in solutions of pH 3.0, pH 7.0, and pure water. Standard solutions of naringin at concentrations of 1, 10, 50, 100, 200, and 500 mg L^{-1} in water, and pH 3 and pH 7 solutions were prepared from stock solutions (1000 mg L^{-1}). For each measurement, 30 μL of naringin standard solution was mixed with 2370 μL of distilled water, 150 μL of Folin-Ciocalteu reagent, and 450 μL of Na_2CO_3 (200 g L^{-1}) in glass tubes. The mixtures were vortexed (Grant Bio, Cambridgeshire, England), and incubated at 40 °C for 30 min in an incubator (Memert, IN 30, Schwabach, Germany). Absorbance was measured at 765 nm against a blank solution using a UV-Vis spectrophotometer (Shimadzu, UV-1280, Kyoto, Japan). The naringin concentration in samples after adsorption and desorption experiments in pH 3.0 and pH 7.0 solutions, and in pure water, was determined using the same procedure, and quantified against the corresponding calibration curves.

Adsorption of naringin onto zymosan A

Naringin and zymosan A were weighed directly into 10 mL of solutions (water, pH 3, and 7). The final concentrations of both compounds were 200, 300, and 400 mg L^{-1}. The solutions were incubated for 3 hours at 37 °C. After adsorption, the solutions were filtered through a vacuum filtration unit equipped with LLG membrane filters, MCE, 0.22 μm. The concentration of non-adsorbed naringin was determined using the Folin-Ciocalteu method with appropriate calibration curves. The adsorption capacity (q_e) (mg of naringin adsorbed per gram of zymosan A) was calculated using the following equation:

$$q_e = \frac{(\gamma_0 - \gamma_e) \cdot V_m}{m_a} \quad (1)$$

where γ_0 is the initial mass concentration of naringin (mg L^{-1}), γ_e is non-adsorbed mass concentration after adsorption (mg L^{-1}), V_m is the total volume of solution (L), m_a is the mass of zymosan A in solution (g).

Desorption of naringin from zymosan A

After vacuum filtration, the retained naringin-zymosan complex remained on the filter, while the non-adsorbed naringin passed through. The naringin-zymosan A complex was then transferred to 10 mL of solution (water, pH 3 and 7), and incubated at 37 °C for 3 hours to allow desorption. The solutions were subsequently filtered, and the desorbed naringin concentration was determined using the Folin-Ciocalteu method. Desorption yield (eq. 2), desorption ratio D (%) (eq. 3), and recovery R (%) (eq. 4) were calculated using the following equations:

$$q_d = \frac{\gamma_d \cdot V_m}{m_a} \quad (2)$$

$$D = \frac{(\gamma_d \cdot V_m)}{(\gamma_0 \cdot V_m) - (\gamma_e \cdot V_m)} \cdot 100\ (\%) \quad (3)$$

$$R = \frac{(\gamma_d \cdot V_m)}{(\gamma_0 \cdot V_m)} \cdot 100\ (\%) \quad (4)$$

where q_d is the desorption yield (mg g^{-1}), γ_d is the concentration of desorbed naringin in the desorption solution (mg L^{-1}), m_a is the zymosan A mass in the solution (g), V_m is the total volume of solution (L), γ_0 is the initial concentration of naringin in the solution before adsorption (mg L^{-1}), γ_e is the concentration of non-adsorbed naringin in the solution after adsorption (mg L^{-1}).

The naringin-to-zymosan experimental mass ratio was calculated (eq. 5) as the overall mass of naringin present in the adsorption solution divided by the overall mass of zymosan.

$$q_t = \frac{m_{naringin}}{m_{zymosan}} \quad (5)$$

where q_t is the naringin-to-zymosan experimental mass ratio (mg g^{-1}), $m_{naringin}$ is the initial mass of naringin in adsorption solution (mg), and $m_{zymosan}$ is the mass of zymosan A in the adsorption solution (g).

UV-Vis spectra

The UV-Vis spectra were recorded for pure naringin, pure zymosan A, model solution of naringin and zymosan A, and naringin-zymosan complex in different solutions (water, pH 3, pH 7). Pure naringin and pure zymosan A solutions were prepared in concentrations of 100 mg L^{-1} in 3 mL of pure water, and pH 3 and pH 7 solutions. The UV-Vis spectra were recorded in the range of 200–1000 nm with a UV-Vis spectrophotometer (Shimadzu, UV-1280, Kyoto, Japan). For obtaining model solutions of the naringin-zymosan complex, adsorption experiments were performed with concentrations of 100 mg L^{-1} naringin and zymosan A in 10 mL of pure water, and pH 3 and pH 7 solutions at 37 °C for 3 h. The solution was then vacuum filtered, the naringin-zymosan complex from the filter was transferred to 3 mL of the corresponding solution (water, and pH 3 and 7 solutions), and the UV-Vis spectrum was recorded.

FTIR analysis

The FTIR spectra of pure naringin and pure zymosan A solids, as well as of naringin-zymosan complex were recorded in the range of 450–4000 cm^{-1} with a scanning resolution of 4 cm^{-1} using a FTIR spectrometer (PerkinElmer UATR, Massachusetts, U.S.). The naringin-zymosan complex was prepared in solutions containing 400 mg L^{-1} of both naringin and zymosan A in 10 mL of water, and pH 3 and pH 7 solutions. The samples were then incubated at 37 °C for 3 h, and filtered through the vacuum filtration unit with LLG membrane filters (MCE, 0.22 μm). The naringin-zymosan complex remaining on the filters was then put on the FTIR sample holder, dried out with a fan, and scanned by the FTIR.

Antiradical activity of naringin and naringin-zymosan complex

The antiradical activity of pure naringin and naringin-zymosan complex in different solutions (pure water, and pH 3 and pH 7 solutions) was determined using a DPPH$^{\cdot}$ assay. The DPPH$^{\cdot}$ stock solution was prepared in methanol (1 mmol L^{-1}). The reaction solutions were prepared with 300 μL DPPH$^{\cdot}$ solution and 2700 μL of pure naringin dissolved in pure water, and pH 3 and pH 7 solutions (final naringin concentration 400 mg L^{-1}). To obtain the naringin-zymosan complex solution, naringin and zymosan A were dissolved in the concentration of 400 mg L^{-1} in 10 mL in different solutions and the adsorption experiments were performed for 3 h at 37 °C. The samples were vacuum filtered and the naringin-zymosan complex from the filter membranes were transferred into 2700 μL of corresponding solutions. Subsequently, the reaction solutions were prepared by mixing 300 μL of DPPH$^{\cdot}$ solution and 2700 μL of naringin-zymosan complex solution, and rotated (Grant-bio PTR-35, Oxon, England) for 30 min in the dark. The absorbance was measured against the blank solution (which contained 2700 μL of naringin-zymosan complex dissolved in all solutions with the same final concentrations as in reaction solutions and 300 μL methanol instead of DPPH$^{\cdot}$ solution) at 517 nm using a spectrophotometer. DPPH$^{\cdot}$ inhibition (%) was determined by:

$$\text{DPPH}^{\cdot}\text{ inhibition} = \frac{[A_{DPPH} - (A_1 - A_2)]}{A_{DPPH}} \cdot 100\ (\%) \quad (6)$$

where A_{DPPH} is the absorbance of DPPH$^{\cdot}$ radical in t = 0 min, A_1 is the absorbance of the naringin-zymosan complex with DPPH$^{\cdot}$ radical in t = 30 min, and A_2 is the absorbance of blank solution (the naringin-zymosan complex dissolved in different solutions (water, pH 3, and pH 7) and methanol instead of DPPH$^{\cdot}$ radical).

Statistical analysis

MS Excel (Redmond, USA) software was used for the data analysis. All adsorption and desorption experiments were conducted in two parallel experiments, and each concentration was measured three times (n_{TOTAL} = 6) using the Folin-Ciocalteu method. The antiradical activity experiment with DPPH$^{\cdot}$ was performed in two parallels and each value was measured twice. For statistical comparisons of adsorption capacity and DPPH$^{\cdot}$ radical inhibition, a one-way analysis of variance (with post hoc Tukey's honest significant difference test with homogeneous groups) was performed in Statistica (Hamburg, Germany).

Results and discussion

Method for determining naringin in the adsorption and desorption experiments

Previous studies have measured naringin concentrations in orange peel extracts using HPLC and the Folin-Ciocalteu method[19]. In the adsorption studies, the flavanone content from grapefruit peel adsorbed onto dietary fibers was monitored using

Table 1 – *Parameters of Folin–Ciocalteu method validation for naringin as a standard in solutions of pH 3.0, pH 7.0, and water*

Validation parameter[1]	Ultrapure water	pH 3.0	pH 7.0
Calibration curve:			
Range/mg L^{-1}	1–1000	1–1000	1–1000
Calibration equation	y=0.0002x+0.008	y=0.0002x+0.0102	y=0.0002x+0.0120
R^2	0.9946	0.9934	0.9926
LOD/mg L^{-1}	3.5	2.3	1.2
LOQ/mg L^{-1}	10.6	7.1	3.5
Accuracy[2]:			
Slope	1.0126	1.0187	0.9833
95 % confidence interval	0.9697 – 1.0555	0.9709 – 1.0665	0.9353 – 1.0313
Intercept	0.1445	–0.0429	10.1065
95 % confidence interval	–17.7950 – 18.0839	–20.0280 – 19.9417	–11.3479 – 31.5609

[1]results are based on two replicate samples of each standard concentration, each measured twice

[2]accuracy was determined by performing a regression analysis with a confidence interval of 95 %

the same spectroscopic method[20]. Given its simplicity and efficiency, we adopted this validated method for our study. Our earlier research[21] revealed that the calibration curve needed to be prepared in the same solutions used for adsorption to ensure better results, as the solvent used affects absorption.

Thus, calibration curves for naringin were prepared in all investigated solvents (pure water, and pH 3, and pH 7 solutions). Table 1 shows the Folin-Ciocalteu method validation parameters. The calibration curves varied in equations and slopes, confirming the previous findings. Naringin exhibited linear calibration curves in all solvents (R^2 from 0.9926–0.9946), with reasonably low limits of detection (LOD) and quantification (LOQ). The method was accurate, as confidence intervals for the slope and intercepts of the calibration curves included values of 1 and 0, respectively, rejecting the existence of a systematic error.

Effect of pH on adsorption

Naringin (Fig. 1) is found in various citrus fruits, including grapefruit (17.45 mg g^{-1} in fresh fruit)[22] and sour oranges (18.83 mg per 100 g excluding rind, pith, and seeds)[23]. During digestion, naringin from citrus fruits and juices may interact with dietary fibers. Interactions between polyphenols and dietary fibers depend on polyphenol solubility or non-solubility in water, where insoluble dietary fiber will prefer to interact with insoluble polyphenols and vice versa[24]. This was the reason for studying the interactions between water-insoluble naringin and water-insoluble zymosan A as a dietary fiber. Given their shared insolubility in water, naringin and zymosan A may aggregate, promoting hydrophobic interactions. The adsorption experiment was designed to examine the interactions between naringin and dietary fibers. It has been shown that surface binding is influenced by various parameters such as pH[23]. To assess the influence of pH, we investigated naringin adsorption onto zymosan A in pure water (pH 5.5), and in solutions at pH 3 and pH 7, representing conditions in the stomach and small intestine, respectively.

Adsorption experiments lasted 3 hours, as equilibrium was previously shown to be reached within this timeframe[25]. Following intragastric administration, naringin is rapidly absorbed rapidly in the duodenum and jejunum within a maximum of 2 h, while most of its metabolites are absorbed in the colon. Some degradation may occur in the small intestine, but overall, naringin and its metabolites are efficiently absorbed in the intestinal tissue[8]. In all three solutions, the adsorption capacities ranged from 0 to 275 mg g^{-1} (Fig. 2). It is difficult to compare these values with the literature, as adsorption capacity depends on multiple properties of adsorbate and adsorbents, such as their concentration, conditions in the solution (pH), and temperature[23].

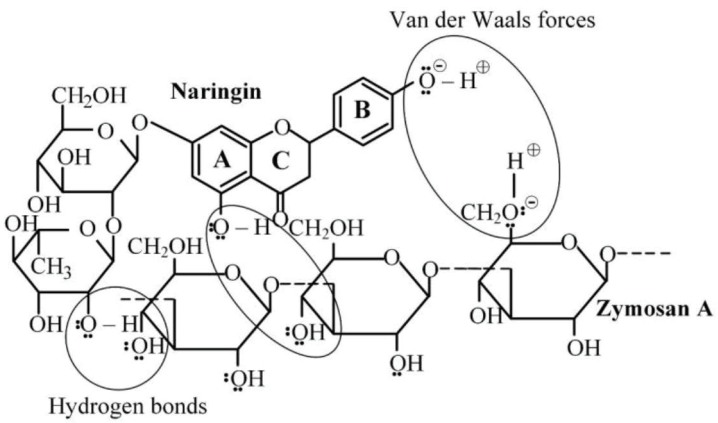

Fig. 1 – *Chemical structure of naringin (4',5,7-trihydroxyflavanone-7-rhamnoglucoside) and zymosan A (β-(1-3)-glucan), and possible interactions between naringin and zymosan A*

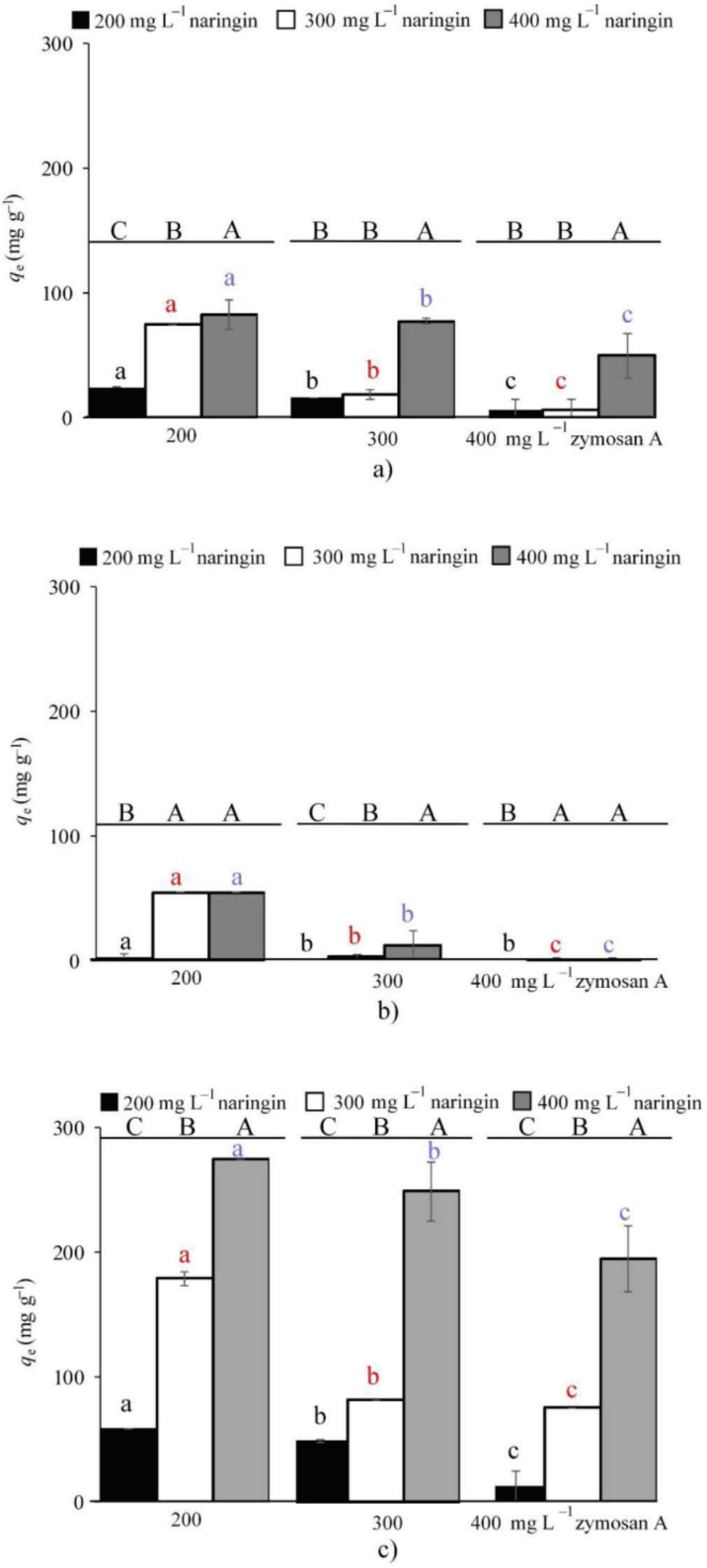

Fig. 2 – *Adsorption capacities (q_e, mg g^{-1}) of zymosan A for naringin at different concentration ranges in (a) water, (b) solution of pH 3, and (c) pH 7. Different lower-case letters correspond to differences between adsorption capacities obtained with the same concentrations of naringin and different concentration of zymosan A at the same pH value. Different upper-case letters correspond to differences between adsorption capacities obtained with the different concentrations of naringin and the same concentration of zymosan A at the same pH value.*

However, earlier studies[22] have reported the adsorption capacity of naringin onto a macroporous resin of 282 to 338 mg g^{-1} aligning with our findings. Furthermore, the adsorption capacity of naringin on zymosan A varied with pH values (Fig. 2). In pH 7 solution the adsorption capacity of naringin onto zymosan A was the highest (from 12 to 275 mg g^{-1}) compared to water (from 6 to 83 mg g^{-1}), and pH 3 solution (from 0 to 55 mg g^{-1}) (Fig. 2). Different ranges of pH could affect the chemical structure of naringin[16,17] and therefore the observed different adsorption capacities. The chemical structure of naringin molecule has a sugar (neohesperidose) moiety attached to the C-7 position of the naringenin aglycon[26], six sugar-based hydroxyl groups and two phenol hydroxyls (Fig. 1). Hydroxyls can be in dissociated/undissociated forms at different pH values[16]. At lower pH, phenol hydroxyls are in undissociated forms. They are acidic and dissociate by increasing pH. The pK_a value and distribution diagram[17] can be helpful in identifying the chemical form of naringin at different pH values. According to the observed pK_a value for naringin (pK_{a1} is 9.00, pK_{a2} is 9.91)[27], it appears that naringin exists in undissociated form at pH values 3 and 7. Some studies point to the possibility that naringin can be in dissociated form at neutral and alkaline conditions[16]. Since adsorption was performed at neutral pH 7, it was possible that a part of the naringin was in dissociated form. Furthermore, with the increasing zymosan A concentration, the adsorption capacity decreased (Fig. 2) in all solutions, and all the results were statistically different. With the increasing concentration of naringin in all three solutions, the adsorption capacity increased, and all results were statistically different at pH 7, and at some concentrations in pure water and pH 3.0 solution (Fig. 2). This agrees with literature where adsorption capacity also increased with concentration of polyphenols from sweet potato leaves on macroporous resins[28], or kiwifruit juice on a resin[29].

Desorption studies

Following adsorption, desorption experiments were conducted to examine how much naringin could be desorbed from the naringin-zymosan complex. Desorption was performed in solutions with different pH (pure water, pH 3, pH 7) (Fig. 3). The desorption ratio, representing the percentage of desorbed naringin relative to total adsorbed naringin, was lowest at pH 3 (from 0 to 38 %), higher in pure water (from 0 to 50 %), and highest at pH 7 (from 0 to 100 %) (Table 2). Recovery, defined as the percentage of desorbed naringin relative to the initial naringin amount in the experiment, was 0 to 3 % in pure water and pH 3 solution, and from 0 to 18 % in pH 7 solution (Table 2). Overall, it appears that

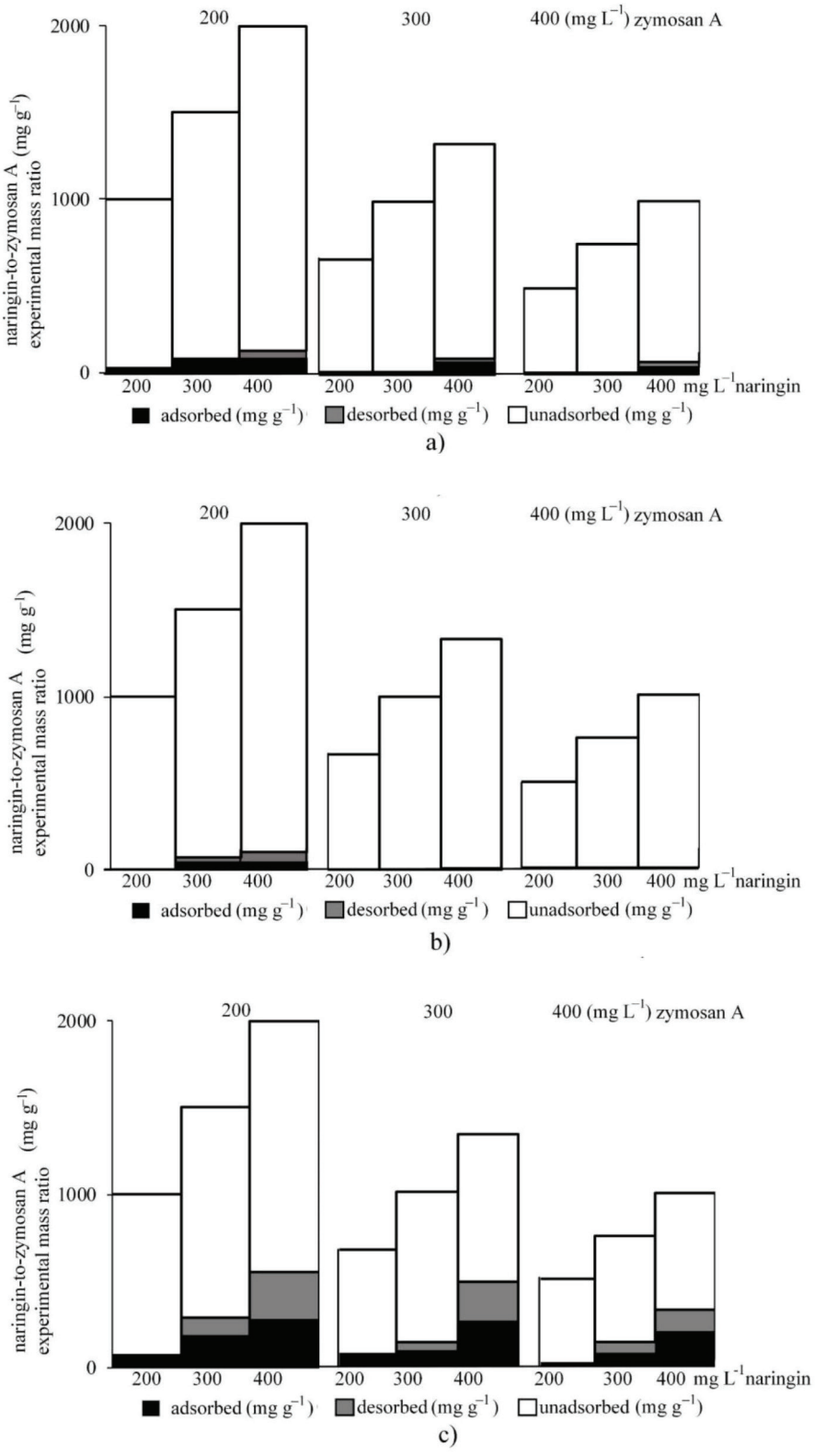

Fig. 3 – *Adsorption capacity, naringin to zymosan A experimental mass ratio and desorption yield of naringin at different concentrations of naringin and zymosan A in water (a), at pH 3 (b) and pH 7 (c)*

Table 2 – *Desorption ratio and recovery of naringin at different concentrations of naringin and zymosan A in water, at pH 3, and pH 7*

γ (mg L^{-1}) naringin	Desorption ratio (%)		
	water	pH 3	pH 7
	200 mg L^{-1} zymosan A		
200	0	0	21
300	0	27	63
400	50	38	100
	300 mg L^{-1} zymosan A		
200	0	0	15
300	0	0	60
400	21	0	93
	400 mg L^{-1} zymosan A		
200	0	0	0
300	0	0	82
400	50	0	69
γ (mg L^{-1}) naringin	Recovery (%)		
	water	pH 3	pH 7
	200 mg L^{-1} zymosan A		
200	0	0	1
300	0	1	7
400	2	3	14
	300 mg L^{-1} zymosan A		
200	0	0	1
300	0	0	5
400	1	0	18
	400 mg L^{-1} zymosan A		
200	0	0	0
300	0	0	8
400	3	0	14

the desorption was affected by pH value. The highest naringin desorption occurred at pH 7, corresponding to the highest adsorption. Desorption was less pronounced in water and pH 3, i.e., in solutions in which the adsorption was weaker as well.

Adsorption and desorption occur simultaneously, reaching dynamic equilibrium when their rates equalize. To our knowledge, the desorption of naringin from zymosan A has not studied been previously studies. Desorption is commonly used to monitor the purification of polyphenols from fruits using various adsorbents[30]. Given that desorption coincides with adsorption, desorption should be taken into account during the study of polyphenols and dietary fiber interactions in the digestive tract.

UV-Vis spectra analysis

The interactions between naringin and zymosan A were studied in different solutions (pure water, pH 3, and pH 7 solution) using UV-Vis spectroscopy. The UV-Vis spectra of pure naringin, the model solution (containing both naringin and zymosan A), and the naringin-zymosan complex are shown in Fig. 4. Zymosan A did not exhibit absorption in UV-Vis region. Naringin showed two maxima in all solutions, one at 285 nm (absorption of the A ring) and another at 330 nm (absorption of the B ring)[31] (Fig. 1). These peaks were also identified in all model solutions (Fig. 4). In comparing the UV-Vis spectra of pure naringin, model solution and naringin-zymosan complex, the band of 330 nm totally disappeared in naringin-zymosan complex solutions (Fig. 4). Also, the peak at 285 nm was very small which could be explained by the small

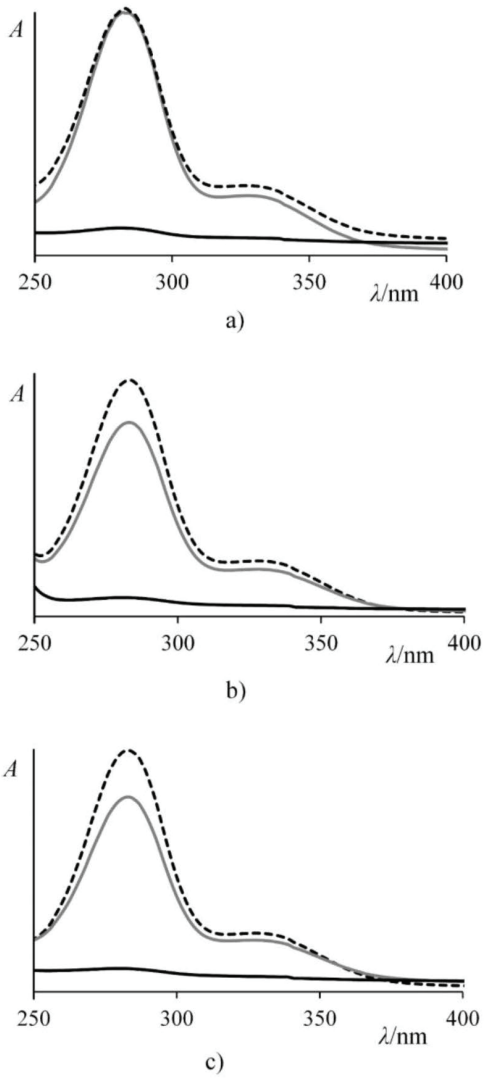

Fig. 4 – *Ultraviolet-visible spectra (UV-Vis) of naringin and naringin-zymosan complex in (a) water, (b) pH 3, and (c) pH 7 (----- pure narigin, —— model solution, ——— naringin-zymosan complex)*

amount of naringin adsorbed onto zymosan A, reducing the observable height of the peaks. In order to investigate which groups of naringin and zymosan A were involved in binding, the FTIR spectra were recorded.

FTIR spectra analysis

FTIR spectra were used to confirm the possible binding of naringin onto zymosan A. FTIR spectra of pure naringin, of naringin-zymosan complexes formed in pure water, pH 3, and pH 7 solutions, and of pure zymosan A are shown in Fig. 5. The FTIR spectrum of naringin was compared to that reported by Wang *et al*.[32] Naringin exhibited characteristic peaks at 1582, 1518, and 1437 cm^{-1} which were assigned to aromatic bending and stretching, and those at 1200–1400 cm^{-1}, 1642 cm^{-1} and 3351 cm^{-1} were attributed to –OH phenolic bending, –C=O stretching and broad phenolic –OH groups stretching of the naringin molecule, respectively[32]. The FTIR spectrum of zymosan A was compared with that of zymosan A found in the literature[10]. Pure zymosan A showed characteristic peaks at 3293 cm^{-1} (OH stretching groups), 2919 cm^{-1} (C–H stretching), 1369 cm^{-1} (C–H bending), 1250 cm^{-1} (CH_2OH stretching), and 1041 cm^{-1} (C–O–C stretching). In the naringin-zymosan complex, the peaks similar to those in the naringin molecule (1642 cm^{-1}), and zymosan A molecule (1041, 576 cm^{-1}) were observed. These peaks indicate the presence of naringin and zymosan A in the naringin-zymosan complex. All peaks corresponding to groups that might be involved in forming the naringin-zymosan complex (Fig. 1) showed low intensity (1200–1400 cm^{-1}). The decrease in intensity can result from reduced –OH bending mobility, which may be due to adsorption via phenol hydroxyls involved in inter-substance hydrogen bonding. For zymosan A, the peaks present in the same wavenumber range include C–H bending, and CH_2OH stretching. Only the latter can be involved in inter-substance hydrogen bonding. OH-stretching is visible in pure naringin at 3351 cm^{-1}, in pure zymosan at 3293 cm^{-1}, while in mixtures it is very pronounced and observable at the intermediate wavenumbers of 3338 or 3326 cm^{-1}.

Antiradical activity

Antiradical activity measurements were performed for naringin and naringin-zymosan complex in different solutions. The highest antiradical activity of naringin was observed in pH 3 solution, followed by pH 7 solution and in pure water, and all the results were statistically different (Fig. 6). For the naringin-zymosan complex, the antiradical activity was statistically higher in pH 3 solution than in pH 7 solution and pure water, and there was no

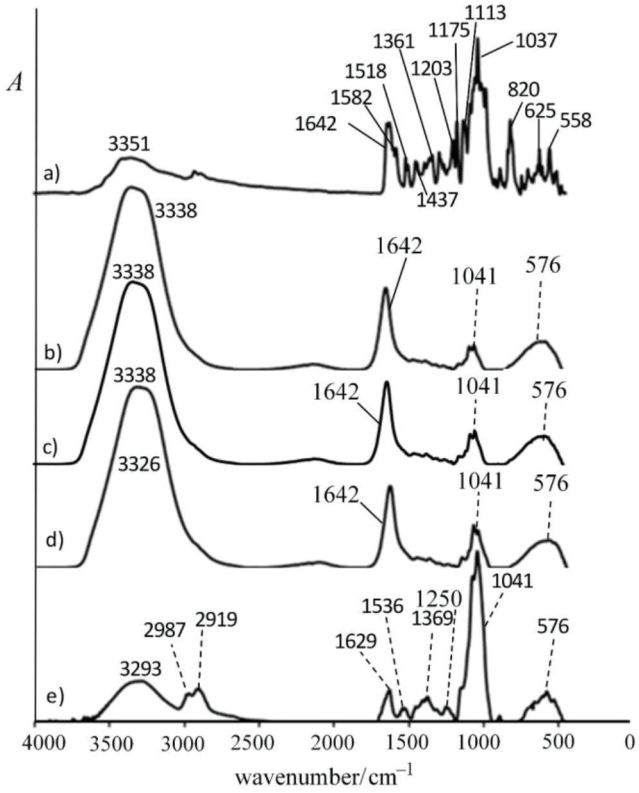

Fig. 5 – *FTIR spectra of (a) pure naringin, (b) naringin-zymosan complex in water, (c) naringin-zymosan complex at pH 3.0, (d) naringin-zymosan complex at pH 7.0, and (e) pure zymosan A*

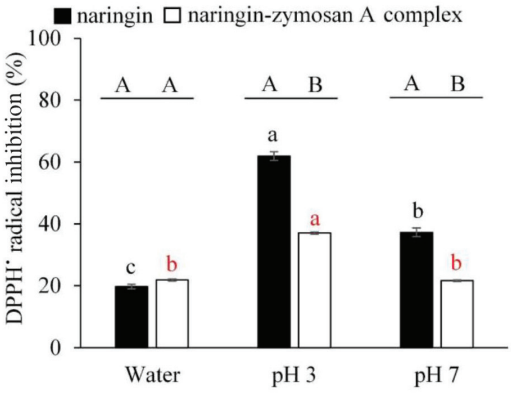

Fig. 6 – *DPPH˙ radical inhibition (%) of naringin and naringin-zymosan complex in water, at pH 3, and pH 7. Different lower-case letters correspond to differences between DPPH˙ radical inhibition obtained for naringin and naringin-zymosan complex at the different pH value. Different upper-case letters correspond to differences between DPPH˙ radical inhibition between naringin and naringin-zymosan complex at the same pH value.*

statistical difference between pH 7 solution and pure water. Antioxidant activity may be affected by steric hindrance, but the relationship between them has not been fully investigated[33]. An earlier study showed that sugar moiety caused steric hindrance of the scavenging groups in naringin molecule[34]. It is possible that reduced antioxidant activity of nar-

ingin-zymosan complex is caused by steric effects. Comparing the antiradical activity of naringin-zymosan complex and naringin, antiradical activity was lower for naringin-zymosan complex and higher for naringin in pH 3 and pH 7 solutions, but statistically not different in water. The tea polyphenols also showed higher antiradical activity than the bound tea polyphenols-β-glucan complexes at lower tea polyphenols concentrations[35].

Zymosan A as a possible carrier for naringin

To the best of our knowledge, there are no reports about naringin and zymosan A interactions so far. The nature of binding of naringin onto zymosan A obviously depends on pH, and different binding can affect their fate in the digestive tract. Although we did not investigate digestive processes, we may recognize that the pH value is different in different parts of the digestive system (stomach pH 1–2.5, small intestine pH 6.6–7.5, colon pH 6.4–7)[25]. Considerable research has been conducted on carriers that facilitate the transport of polyphenols through parts of the digestive tract as it is known that polyphenols absorb in the small intestine, but their chemical stability can be low. However, desorption is also possible and should be taken into account. In an earlier study, naringin was adsorbed onto inulin, pectin, konjac, and chitosan[36]. Zimosan A was previously studied as a carrier in drug delivery systems[37–39]. Therefore, zymosan A could be a potential carrier for naringin through the gastrointestinal tract, but the pH values should also be taken into account during the study of these interactions. This work provides an insight into the interactions of naringin with zymosan A, which is already frequently used, and this is the first work that indicates the binding of naringin to zymosan A. Since the increasing concentration promotes the desorption of naringin from zymosan A, especially at pH 7 which corresponds to pH in the colon, in further studies the encapsulation technique can be applied to prevent undesired desorption. In our study, zymosan A and naringin standards were used to study binding between the two molecules present naturally in yeast and citruses. An earlier study showed that polyphenols can bind to insoluble and soluble dietary fibers and that those fibers can carry polyhenols during the digestion and adsorption processes[40]. Further studies are needed to completely understand the interactions between insoluble zymosan A and naringin.

Conclusion

The spectrophotometric Folin-Ciocalteu method was adjusted for the proper investigation of the adsorption of naringin onto zymosan A in the solutions of pH 3.0, pH 7.0, and pure water, which enabled obtaining accurate data of naringin concentrations. The adsorption of naringin and zymosan A and their interactions could be affected by pH values of the solution as well as by the initial amounts of zymosan A and naringin. The adsorption capacity of zymosan A for naringin was highest in pH 7 solution, and lower in water and pH 3 solution. Increasing the amount of zymosan A decreased the adsorption capacity, while increasing the amount of naringin increased the adsorption capacity. The desorption ratio was lowest in pH 3 solution, followed by pure water, and pH 7 solution. The UV-Vis and FTIR spectra showed that naringin could bind to zymosan A. The antiradical activity of naringin and naringin-zymosan complex was highest at pH 3. The results of this study can find different applications such as in the design of functional food, or as a way to increase naringin bioaccessibility in lower parts of the gastrointestinal tract with a naringin-zymosan A delivery system.

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