UNIVERSIDADE FEDERAL DE VIÇOSA

Estudo sensorial e termodinâmico da interação entre a quinina e a proteína mucina: efeito da concentração e força iônica

Gustavo dos Santos Emiliano
Magister Scientiae

VIÇOSA - MINAS GERAIS
2022

GUSTAVO DOS SANTOS EMILIANO

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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de *Magister Scientiae*.

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Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

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E53e
2022

Emiliano, Gustavo dos Santos, 1996-
Estudo sensorial e termodinâmico da interação entre a quinina e a proteína mucina: efeito da concentração e força iônica / Gustavo dos Santos Emiliano. – Viçosa, MG, 2022.
1 dissertação eletrônica (41 f.): il.

Orientador: Márcia Cristina Teixeira Ribeiro Vidigal.
Dissertação (mestrado) - Universidade Federal de Viçosa, Departamento de Tecnologia de Alimentos, 2022.
Referências bibliográficas: f. 37-41.
DOI: https://doi.org/10.47328/ufvbbt.2023.216
Modo de acesso: World Wide Web.

1. Alimentos - Avaliação sensorial. 2. Proteínas e peptídios salivares. 3. Microcalorimetria . I. Vidigal, Márcia Cristina Teixeira Ribeiro, 1981-. II. Universidade Federal de Viçosa. Departamento de Tecnologia de Alimentos. Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos. III. Título.

CDD 22. ed. 664.07

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB-6/2523

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APROVADA: 3 de agosto de 2022.

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Marcia Cristina Teixeira Ribeiro Vidigal
Orientadora

AGRADECIMENTOS

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

RESUMO

EMILIANO, Gustavo dos Santos, M.Sc., Universidade Federal de Viçosa, agosto de 2022. **Estudo sensorial e termodinâmico da interação entre a quinina e a proteína mucina: efeito da concentração e força iônica**. Orientadora: Marcia Cristina Teixeira Ribeiro Vidigal. Coorientadores: Ana Clarissa dos Santos Pires e Luis Henrique Mendes da Silva.

A percepção do gosto amargo é um processo complexo, que pode ser influenciado por vários fatores, dentre os quais se destacam as interações entre as proteínas presentes na saliva (como a mucina) e as moléculas presentes nos alimentos. Além disso, as condições do meio, como presença de sais e pH, influenciam nestas interações, podendo resultar em alterações na sensação sensorial percebida. Neste sentido, a técnica de microcalorimetria de titulação isotérmica foi utilizada para verificar a interação entre a quinina (QN), molécula promotora do gosto amargo, e a proteína mucina (MUC), em diferentes condições de pH (3,0 e 7,4) e de força iônica (0, 10, 50 e 100 mmol L^{-1} de cloreto de potássio - KCl). Além disso, foram determinados os efeitos da concentração da QN (0,04; 0,06; 0,08 e 0,1 mmol L^{-1}) e da força iônica do meio (0, 10 e 50 mmol L^{-1} de KCl) na percepção do gosto amargo pela técnica Tempo-Intensidade. O estudo termodinâmico demonstrou que a QN interage com MUC, formando o complexo QN-MUC em pH 3,0 e 7,4 (?Gº = -45,38 ± 0,51 kJ mol^{-1} e ?Gº = -41,66 ± 0,29 kJ mol^{-1}, respectivamente) apesar da presença de KCl. Em pH 3,0, a estequiometria de formação do complexo foi de 1:3 (QN-MUC), ou seja, cada molécula de QN interage com três de mucina, resultando assim na formação de um agregado proteico, devido à protonação do nitrogênio presente na estrutura química deste alcaloide, que reduz a repulsão eletrostática entre as proteínas. Já em pH 7,4, a estequiometria observada na interação foi de 1:1. A presença de sal não alterou a estequiometria de formação do complexo QN-MUC em pH 3,0. Em relação à avaliação sensorial, houve diferença significativa na intensidade máxima de percepção do gosto amargo das soluções de QN sem adição de KCl, verificando-se uma elevação do amargor com o aumento da concentração de QN. Além disso, para a intensidade máxima de percepção do gosto amargo, os avaliadores detectaram diferenças significativas entre as soluções de QN com adição de 10 mmol L^{-1} de KCl ($p < 0,05$). No entanto, para as soluções de QN com 50 mmol L^{-1} de KCl, este comportamento não foi observado. O efeito do sal na percepção do gosto amargo também foi avaliado para cada uma das quatro concentrações de QN. Para a solução 0,04 mmol L^{-1} de QN, a intensidade máxima do gosto amargo foi significativamente maior nas soluções contendo 50 mmol L^{-1} de KCl em

comparação àquela sem KCl ($p<0,05$). Portanto, as interações QN e MUC são fortemente dependentes da concentração de QN e da presença de sais na solução, influenciando a percepção sensorial do gosto amargo.

Palavras-chave: proteínas salivares; análise tempo-intensidade; microcalorimetria de titulação isotérmica

ABSTRACT

EMILIANO, Gustavo dos Santos, M.Sc., Universidade Federal de Viçosa, August, 2022. **Sensory and thermodynamic study of the interaction between quinine and mucin protein: effect of concentration and ionic strength**. Adviser: Marcia Cristina Teixeira Ribeiro Vidigal. Co-advisers: Ana Clarissa dos Santos Pires and Luis Henrique Mendes da Silva.

The perception of bitter taste is a complex process, which can be influenced by several factors, among which the interactions between proteins present in saliva (such as mucin) and molecules present in food stand out. In addition, environmental conditions, such as the presence of salts and pH, influence these interactions, which may result in changes in the perceived sensory sensation. In this sense, the isothermal titration microcalorimetry technique was used to verify the interaction between quinine (QN), a molecule that promotes the bitter taste, and mucin protein (MUC), under different pH conditions (3.0 and 7.4) and ionic strength (0, 10, 50 and 100 mmol L^{-1} of potassium chloride - KCl). In addition, the effects of QN concentration (0.04, 0.06, 0.08 and 0.1 mmol L^{-1}) and ionic strength of the medium (0, 10 and 50 mmol L^{-1} KCl) on bitter taste perception were determined by the Time-Intensity technique. The thermodynamic study demonstrated that QN interacts with MUC, forming the QN-MUC complex at pH 3.0 and 7.4 (?Gº = -45.38 ± 0.51 kJ mol^{-1} and ?Gº = -41.66 ± 0. 29 kJ mol^{-1}, respectively) despite the presence of KCl. At pH 3.0, the complex formation stoichiometry was 1:3 (QN-MUC), that is, each QN molecule interacts with three mucin molecules, thus resulting in the formation of a protein aggregate, due to nitrogen protonation present in the chemical structure of this alkaloid, which reduces the electrostatic repulsion between proteins. At pH 7.4, the stoichiometry observed in the interaction was 1:1. The presence of salt did not alter the stoichiometry of QN-MUC complex formation at pH 3.0. Regarding the sensory evaluation, there was a significant difference in the maximum intensity of perception of the bitter taste of the QN solutions without the addition of KCl, verifying an increase in bitterness with the increase in the QN concentration. Furthermore, for the maximum intensity of bitter taste perception, the evaluators detected significant differences between the QN solutions with the addition of 10 mmol L^{-1} of KCl ($p < 0.05$). However, for QN solutions with 50 mmol L^{-1} of KCl, this behavior was not observed. The effect of salt on bitter taste perception was also evaluated for each of the four QN concentrations. For the 0.04 mmol L^{-1} QN solution, the maximum intensity of the bitter taste was significantly higher in the solutions containing 50 mmol L^{-1} KCl compared to the one without KCl ($p<0.05$). Therefore, QN and MUC interactions are strongly dependent on QN concentration and the

presence of salts in the solution, influencing the sensory perception of bitter taste.

Keywords: salivary proteins; time-intensity analysis; isothermal titration microcalorimetry

SUMÁRIO

INTRODUÇÃO GERAL

A compreensão do processo sensorial possui elevada importância para a indústria alimentícia, sendo o gosto um dos principais fatores a serem levados em consideração. Dentre os gostos primários, o amargo é conhecido por provocar aversão em algumas pessoas, enquanto para outras é percebido de forma menos desagradável ou até mesmo agradável, o que pode estar relacionado a vários fatores, como por exemplo, a frequência de consumos de alimentos amargos e às alterações nas proteínas presentes na saliva, que podem estar associadas ao modo em que o gosto amargo é percebido [1, 2].

Dentre as várias substâncias que provocam o gosto amargo, pode-se destacar a quinina, que é um alcaloide de baixa solubilidade em água (1,541 mmol L^{-1}) [3] e com um intenso amargor, sendo frequentemente usada como molécula modelo para estudos envolvendo este gosto [4, 5, 6]. A percepção sensorial do gosto amargo também envolve as proteínas salivares, que possuem a capacidade de interagirem com estas moléculas [7, 8], podendo então resultar em alterações na percepção do amargor. A mucina é um exemplo de proteína salivar que pode ser usada para o estudo de interações com compostos amargos e possui várias funcionalidades na cavidade oral [9].

Existe evidência de que a interação entre compostos com características amargas e proteínas podem diminuir a intensidade do gosto amargo. Nunes et al. (2020) relataram que a interação entre naringenina e a proteína lactoferrina foi capaz de atenuar a percepção deste gosto [10]. Logo, a capacidade de tornar o gosto amargo menos intenso é importante para as indústrias de alimentos e farmacêutica, visto que o amargor de alimentos e medicamentos pode ser considerado desagradável, dependendo da concentração. Ademais, algumas condições podem interferir no processo de interação, como a presença de íons e variações de pH [11], e consequentemente afetar diretamente na percepção do amargor pelos indivíduos.

Considerando a importância do estudo de interação entre moléculas amargas e diferentes proteínas, o presente estudo se propôs pesquisar a interação entre a proteína mucina e o alcaloide quinina em diferentes condições do meio, como pH e força iônica, pela técnica de calorimetria de titulação isotérmica, que é um método eficaz e confiável para determinar as propriedades termodinâmicas, como a constante de ligação (K_b), variação de entalpia padrão ($\Delta H°$), variação de energia livre de Gibbs padrão ($\Delta G°$), variação de entropia padrão ($T\Delta S°$) e estequiometria da reação (n).

Além disso, também foi realizado um estudo sensorial a fim de caracterizar a percepção do gosto amargo promovido pela quinina na presença e ausência de cloreto de potássio (KCl) por meio do método sensorial de tempo-intensidade, que avalia a intensidade percebida do atributo ao longo de um tempo pré-determinado.

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ARTIGO

THERMODYNAMIC INTERACTIONS BETWEEN QUININE AND MUCIN PROTEIN INFLUENCE THE PERCEPTION OF BITTER TASTE: EFFECT OF CONCENTRATION AND IONIC STRENGTH

Abstract

Different factors can contribute to the perception of bitter taste, including the interactions that occur between salivary proteins and bitter taste-inducing molecules, as well as environmental conditions, such as the pH and the presence of salts. In this sense, isothermal titration microcalorimetry was used to evaluate the interaction between quinine (QN) (substance representative of bitter taste) and mucin (secreted protein) (MUC) under different conditions of the medium, such as pH and ionic strength. In addition, the effects of the concentration of QN and the ionic strength on the perception of the bitter taste were evaluated using the time-intensity technique. QN interacts with MUC, forming the QN-MUC complex at pH 3.0 and 7.4 (ΔG^o = -45.38 ± 0.51 kJ mol^{-1} and ΔG^o = -41.66 ± 0.29 kJ mol^{-1}, respectively) despite the presence of the salt KCl in the media. The perception of the bitter taste caused by the addition of QN can be influenced by interactions between QN and salivary proteins and by the ionic strength of the medium. In fact, the perception of the bitter taste can be modulated by salivary proteins, in the sense that mucins can interact with QN molecules. The QN and MC interactions are strongly dependent on the concentration of QN and the presence of salts in the solution.

Keywords: Sensory perception, Isothermal titration microcalorimetry, Salivary proteins.

1. Introduction

The understanding of the mechanisms involved in the sensory perception of foods is extremely important for the food industry, as well as for the general population, since these mechanisms can impact the development and improvement of food products. It is known that the bitter taste in foods can strongly impact the perception of the consumers. In this sense, the degree of bitterness, which is determined by the concentration of bitter substances, is essential for the palatability of foods and beverages in the food industry [1], [2]. Moreover, the perception of the bitter taste can be also associated with the detection of harmful compounds, leading to aversive reactions [3].

However, the share of the consumers who has a preference for foods with a bitter taste can have higher rejection thresholds regarding the compounds responsible for the bitterness in foods. Proportionally, there is a relationship between the frequency of consumption of bitter foods with the tolerance to this taste [1]. In addition, there is still the possibility of alterations in salivary proteins (PS), such as the increase in the expression of protein bands, which has already been evidenced in studies involving animals, such alteration may influence the perception of taste [4].

Among the compounds that play an important role regarding the bitterness in foods, quinine is a well known substance (Fig. 1) [5], [6], [7], [8]. Extracted from a tree known as Cinchona *(Rubiaceae)*, quinine belongs to the group of alkaloids and presents a water solubility of 1.541 mmol L^{-1} [9].

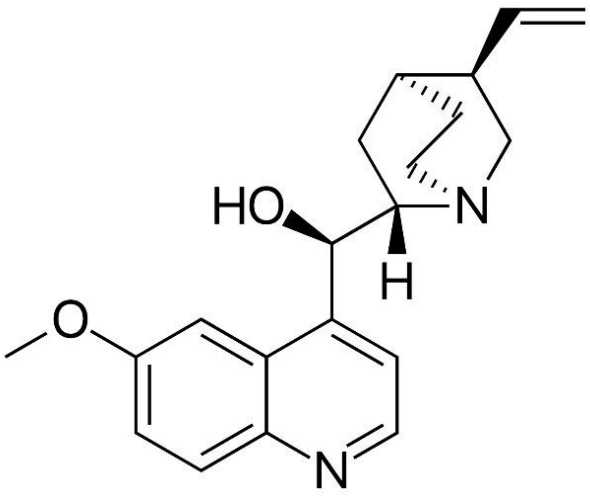

Fig.1. Chemical structure of quinine.

The perception of bitter taste can be influenced by several factors, some of which are related to salivary proteins (PS) [4], [10], [11], [12], [13]. According to Ployon et al. (2018) and

Matsuo, (2000), salivary proteins rich in proline (PRPs) have the ability to interact with bitter substances, mainly tannins that have bitter characteristics, in addition to being astringent. However, other PS may also play a role in the detection of bitter molecules [14], [15].

According to Pushpass et al. (2019), in general, bitter compounds are hydrophobic and can activate taste receptors depending on the interactions with mucins (MUCs) [2], an anionic glycosylated protein of high molar mass (0.5 -20 MDa) [16]. Although saliva is a dispersion with 99% aqueous continuous phase, the presence of MUC contributes to the rheological properties of saliva (viscosity, elasticity), water retention and lubrication [17], [18]. In addition, MUCs can protect the oral cavity against a number of diseases with different defense mechanisms [19].

The molecular mechanisms involved in the perception of bitter taste are not fully elucidated in the literature. The thermodynamic evaluation of QN-MUC complex formation can contribute to the understanding of the perception of this taste. The understanding of the driving forces involved in the interactions between QN and MUC can provide insights into ways to decrease the perceived intensity of such a taste, which would likely result in a greater acceptance of bitter foods. In addition, the effect of some factors, such as the pH and the presence of salt on the formation of the QN-MUC complex can also affect the perception of the bitter taste, since the presence of ions and pH conditions can strongly affect the binding sites present in the protein structure [20].

Nunes et al. (2020) evaluated the thermodynamic interaction between naringenin (NG), a bitter flavonoid, and bovine lactoferrin (LF) through isothermal titration nanocalorimetry (ITC) analysis. Temporal sensory perception of NG bitterness was obtained using time-intensity analysis. The authors confirmed the formation of the NG-LF complex and verified that LF reduced the maximum intensity and the general perception of bitterness of NG [21]. However, no studies were found with the simultaneous application of thermodynamic and sensory techniques on the interaction of bitter compounds and salivary proteins.

Therefore, this study proposes: (1) to study the interaction between mucin protein (MUC) and QN alkaloid through the thermodynamic study of complex formation under different conditions of pH and ionic strength, and (2) to evaluate the intensity of sensory perception of bitter taste over time of solutions with different concentrations of QN, in the absence and presence of a potassium chloride (KCl).

2. Material and methods

2.1. Material

Mucin from porcine stomach (M1778) (MUC), quinine (QN) 90%, potassium phosphate monobasic (KH_2PO_4), sodium hydroxide (NaOH), sodium acetate (CH_3COONa), acetic acid, and potassium chloride (KCl) were purchased from Sigma-Aldrich (St Louis, USA). The solvents used to prepare the solutions were the acetate buffer (0.1 M, pH 3.0) or phosphate buffer (0.1 M, pH 7.4), in the absence or presence of KCl. Deionized water obtained from a Milli-QII system (Millipore, USA) was used to prepare all solutions.

MUC was chosen as the substrate as its structure is very similar to human mucin and it is a well-established source of mucin for the preparation of model saliva [13], [23], [24].

2.2. Isothermal titration calorimetry

Calorimetry analyses were performed in an isothermal titration calorimeter, CSC 4200 model (TA Instruments, New Castle, DE, USA) at 298.15 K, controlled by the ITCRun software. QN solutions (titrant) at concentrations of 0.1 mmol L^{-1} were prepared in each of the buffer solutions, pH 3.0 and pH 7.4. QN solutions added to 0, 10, 50 and 100 mmol L^{-1} of KCl were also prepared in each of the described buffer solutions. MUC solutions 1% (w/w) (titrand) were ultra-centrifuged for 60 min at 10,000 rpm and 4 °C to discard aggregates and ensure a stable heat flow. Then, the sample cell was loaded with 1.8 mL of the MUC solution (interaction experiment) or the buffer solution (dilution experiment). After reaching the thermal equilibrium, the titration experiment was carried out with the injection of 48 aliquots of 10 µL of the QN solution into the sample cell, using a Hamilton microliter syringe. The interval between injections was 300 s and the sample cell content was stirred constantly at 180 rpm throughout the experiment.

2.3. Sensory analysis

2.3.1. Time-intensity analysis

The temporal evaluation of the intensity of bitter taste perception of QN was carried out by using the software SensoMaker [25] developed by the Federal University of Lavras (UFLA). The software has a user-friendly and easy-to-understand interface, which facilitates the performance of procedures in addition to the free access. The analysis consisted of marking the perceived intensity on a scale as a function of a time. For this study, the time of 90 seconds was determined by carrying preliminary tests. The data resulting from the evaluation of each panelist

are automatically stored and can be analyzed in the software itself. All solutions used in sensory analysis were prepared using water as a solvent.

The analyzes were carried out in the laboratory of Applied Molecular Thermodynamics (THERMA) of the Federal University of Viçosa (UFV), individually, being previously approved by the ethics committee in research with human beings under number 4.946.598 (CAAE: 46997821.8.0000.5153).

2.3.2. Recruitment and pre-selection of evaluators

The recruitment of potential participants was carried out through the application of a questionnaire in order to verify the interest and availability of each participant, the presence of any health problems that could affect the analysis, as well as the ability to use scales. A total of 21 participants were recruited for this study, most of them students.

The pre-selection was performed through triangular test sessions, in which the evaluators received samples of solution containing QN in two different concentrations (0.04 and 0.1 mmol L^{-1}), served at room temperature (approximately 25 °C). 3 samples were presented to the panelists, who were instructed to identify on a printed sheet which of the three was different. In this phase, those who were able to correctly identify the different sample in at least three sessions (60%) were approved, being able to perform a maximum total of 5 sessions (n= 14 evaluators, 4 males, 10 females; age 20–30 years old).

2.3.3. Evaluator training

The next step consisted of training the evaluators with the bitter taste patterns and with the SensoMaker software. The participants were familiarized with the bitter taste and trained to use the software in order for them to identify the perceived intensity of bitterness, as well as the duration of the analysis.

The evaluators performed the training by using two patterns of bitter taste: weak (QN 0.02 mmol L^{-1} solution) and strong (QN 0.12 mmol L^{-1} solution), corresponding to the extremes in an unstructured intensity scale of zero to ten.

2.3.4. Final selection of evaluators

The final selection of the tasters consisted of an analysis of two QN solutions at concentrations of 0.04 mmol L^{-1} and 0.1 mmol L^{-1}, performed in the software itself. The samples were coded with three-digit random numbers and presented in a monadic and randomized manner to the panelists, in three repetitions. Fourteen panelists previously approved

in the triangular test and previously trained in using the software SensoMaker participated in this stage.

The data provided by each evaluator was analyzed with the software SensoMaker by using ANOVA. Ten panelists were able to distinguish the differences ($p < 0.05$) between the samples and also obtained repeatability ($p > 0.05$). Therefore, the final panel was defined by the ten selected trained panelists (3 males and 7 females).

2.3.5. Final analysis

For the final analysis, the samples coded with three-digit numbers were presented randomly and individually to the panelists in three repetitions. The scale used for the analysis was ten points, with 0 = no bitter taste and 10= strong bitter taste.

In the first step, the objective was to evaluate the perception of the bitter taste of the solutions containing QN without the addition of salt. For this purpose, four solutions with different concentrations of QN (0.04 mmol L^{-1}; 0.06 mmol L^{-1}; 0.08 mmol L^{-1} and 0.1 mmol L^{-1}) were evaluated.

After that, the influence of the addition of potassium chloride (KCL) on the perception of the bitterness of QN solutions was also evaluated. Solutions containing QN were prepared with the addition of KCL. The concentrations of QN were the same as in the previous step, while those of KCL were 10 (G1) and 50 mmol L^{-1} (G2). Importantly, the KCl concentrations were established based on thermodynamic analyses.

The parameters obtained with SensoMaker were: *Imax* (maximum perceived intensity); *TI 5%* (time in which the intensity is 5% of *Imax* in the increasing part of the curve); *TD 5%* (time in which the intensity is 5% of *Imax* in the decreasing part of the curve); *TI 90%* (time in which the intensity is 90% of *Imax* in the increasing part of the curve); *TD 90%* (time in which the intensity is 90% of *Imax* in the decreasing part of the curve); *Plateau* (time interval where intensity is ≥ 90% of *Imax*) and *Area* (area under the curve).

2.3.6. Statistical analysis

Analysis of variance (ANOVA) was performed for the data obtained from the thermodynamic and sensory parameters. For the sensory test, in ANOVA, sample and panelist were used as sources of variation, as well as the interaction between sample and panelist, for each parameter. Tukey's test was used to evaluate any significant differences between the means of each sample ($\alpha = 0.05$). Statistical analyzes were carried out using the R and Sisvar statistical programs.

3. Results and Discussion

3.1. Thermodynamic analysis of the QN-MUC interaction by ITC

The thermodynamic evaluation is essential to determine the main driven power in a complex formation. It allows us to understand how different molecular processes, e.g., direct intermolecular interactions (electrostatic, van der Waals, or hydrogen bond) and conformational changes, contribute to the stability of the complex formed [26]. Moreover, by performing this analysis under varied conditions, such as different ranges of pH and ionic strength, it is possible to evaluate how the interactions can be affected by the changes in the molecules' charge distribution and chemical structure, as well as in their medium. In fact, this information is very useful to optimize a complex formation for a determined application

ITC is a very well stablished thermodynamic technique to evaluate the binding parameters involved in the association between proteins and ligands. Some binding parameters, such as the binding constant (K_b), the standard enthalpy change ($\Delta H°$), and the stoichiometry can be obtained by using this technique. In addition to allowing the direct measurement of the heat involved in the binding process, the data can be obtained in a single calorimetric experiment without using the van't Hoff approach [27]. Therefore, we used ITC to investigate the effect of pH and ionic strength on the thermodynamics of the QN-MUC interaction. Fig. 2 shows the raw data obtained from the dilution (Fig. 2a) and interaction (Fig. 2b) experiments at pH 3.0, in the absence of KCl. The raw data obtained at pH 7.4 are presented in Fig**.** S1 (Supplementary material).

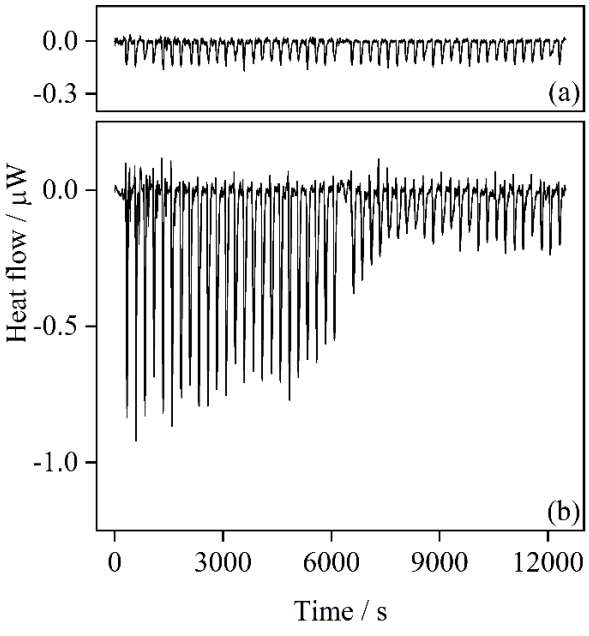

Fig.2. Calorimetric raw data for the titration of a QN solution (4.0 x 10^{-5} mol L^{-1}) in **(a)** a phosphate buffer solution, or **(b)** a PSM solution (1.56 x 10^{-5} mol L^{-1}), at pH 3.0 and 298.15 K.

For the data analysis, the integration of the raw data provides the heat involved in each injection. The heat generated by the dilution was subtracted from the heat released or absorbed during the interaction experiment ($q_i = q_{\int -q_{dil}}$). The accumulated heat ($Q_T = \sum_{i=1}^{N} q_i$) for the binding process, after N injections, can be related to K_b, $\Delta H°$, and the number of QN molecules per MUC in the complex formed (n), through the single set of binding sites (SSIS) model [28] as shown in Eq. 1.

$$Q_T = \frac{V_c \Delta H°}{2K_b}\left[1 + K_b[QN]_T + nK_b[MUC]_T - \sqrt{(1 + K_b[QN]_T + nK_b[MUC]_T)^2 - 4n{K_b}^2[MUC]_T[QN]_T}\right] \quad (1)$$

where V_c represents the effective volume of the sample cell; $[MUC]_T$ and $[QN]_T$ are the total concentrations of MUC and QN species in the system, respectively, after N injections. By determining Q_T, the heat involved in each injection can be given by Eq. 2.

$$q_i = \Delta Q_T = Q_T(i) - Q_T(i-1) + \frac{V_{inj}}{V_c}\left(\frac{Q_T(i) + Q_T(i-1)}{2}\right) \quad (2)$$

where $Q_T(i)$ and $Q_T(i-1)$ are the total heat accumulated after the ith and $(i-1)$th injections, and V_{inj} corresponds to the volume injected in the sample cell. For a better visualization of the obtained data, q_i values were divided by the number of moles of QN of each injection, resulting in enthalpy change values (ΔH_i), which were plotted against the molar ratio ([QN]:[MUC]), as shown in Fig. 3.

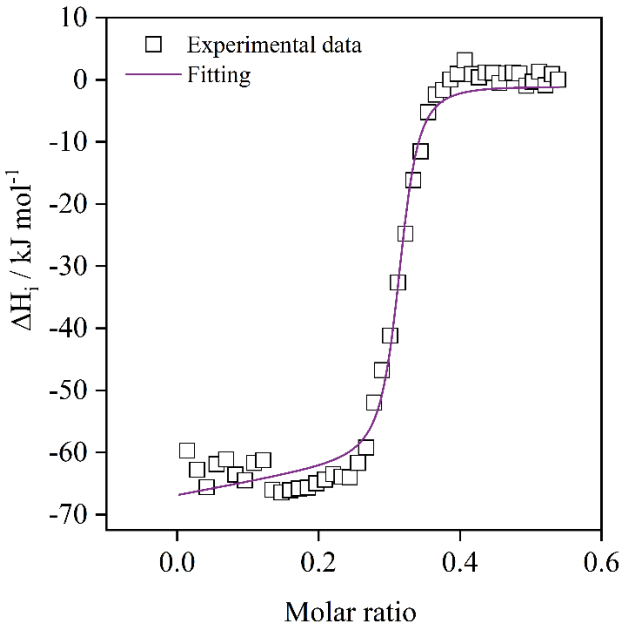

Fig. 3. Plot of ΔH_i versus the molar ratio for the interaction of QN with MUC, at pH 3.0 and 298.15 K, and the best fitting obtained from the SSIS model.

The sigmoidal profile of the curve obtained carries important information about the binding process. For molar ratios < 0.24, there is an excess of MUC molecules in the sample cell, causing approximately all QN molecules injected to form complexes. With the further injection of the QN solution, the molar ratio between the interacting partners reaches the stoichiometry of the complex, which is represented by the inflection point at 0.28. Finally, for molar ratios > 0.37, nearly all protein molecules are bounded and the new QN molecules added remain free in solution. A similar profile of the ΔH_i versus molar ratio curve was obtained for the QN-MUC interaction at pH 7.4 is shown in Fig S2.

By fitting the experimental data shown in Fig. 3 to Eq. 2, the values of K_b, n and $\Delta H°$ were obtained, which allowed the calculation of the standard free energy change ($\Delta G°$) and standard entropy change ($\Delta S°$), through Eqs. 3 and 4, respectively.

$$\Delta G° = -RTlnK_b \tag{3}$$

$$T\Delta S° = \Delta H° - \Delta G° \tag{4}$$

where R is the universal gas constant, and T is the absolute temperature of the system. All the thermodynamic parameters obtained at pH 3.0 and 7.4 are shown in Table S1 (Supplementary material).

3.1.1. pH effect on the thermodynamics of QN-MUC interaction

Regardless of the pH, the K_b values were in the order of 10^7 L mol^{-1}, which resulted in a negative $\Delta G°$, which indicates that the formation of complexes between MUC and QN is favored in the equilibrium. Data regarding the binding parameters between QN and MUC are nonexistent in the literature. These findings show that the affinity of QN to MUC is much higher compared to other proteins. For instance, Liu et. al. (2015) investigated the formation of complexes between QN and bovine serum albumin, reporting K_b values of 10^4 M^{-1} [29]. In the same sense, Wu et. al. (2021) determined values even lower for the interaction of this alkaloid with human serum albumin [30].

The complex formation of QN-MUC was more favored at pH 3.0, as indicated by the lower $\Delta G°$ value (-45.38 ± 0.51 kJ mol^{-1}), in comparison to the interaction at pH 7.4 (-41.66 ± 0.29 kJ mol^{-1}), which can be explained by different charges on both molecules at the pH values studied. The isoelectric point of MUC is around pH 2-3, indicating that at pH 3.0 the protein has almost no net charge due to the balance between the negative sulfate groups ($pK_a < 1.0$) in the glycosylated "naked" region and sialic acid groups ($pK_a \approx 2.6$) in the oligosaccharide side chains, and the positive amino acid residues (e.g., glutamic and aspartic acids, $pK_a \approx 4.0$) in the polypeptide backbone [31]. However, at pH 7.4 there is an increase of the negatively charged groups in the protein, resulting in a negative net charge. As for QN, the pK_a values are 4.25 and 8.72 [32], indicating that it contains a positive charge distribution at pH 3.0 and neutral at pH 7.4.

Therefore, while attractive electrostatic interactions between QN and MUC can occur at pH 3.0, other forces are responsible for the formation of the complex at pH 7.4. Moreover, when protonated, QN appears to induce the aggregation of MUC when the complex is formed, as indicated by the stoichiometry obtained of ~1:3. This behavior has been reported for the interaction between mucin and cations, in which the presence of Ca^{2+} ions reduce the intra- and inter-electrostatic repulsions between negative oligosaccharide side chains of MUC, causing them to aggregate. This hypothesis is supported by the 1:1 stoichiometry obtained at pH 7.4. Since QN is no longer ionized at this pH, there is no charge neutralization on the proteins' side chain, preventing their aggregation.

Although important, the direct intermolecular interaction between QN and MUC is not the solely molecular event contributing to the complex formation since. The desolvation of the molecules, release of counterions from the electric double layer, and conformational changes must occur simultaneously. To understand the contribution of each one of these processes and

determine the complexes' driving forces, $\Delta H°$ and $T\Delta S°$ were analyzed. At pH 3.0 the formation of the QN-MUC complex was enthalpically driven ($\Delta H° =$ -66.88 ± 0.70 kJ mol^{-1} and $T\Delta S° =$ -21.50 ± 0.19 kJ mol^{-1}). According to Ross and Subramanian (1981), ionic interactions slightly contribute to $\Delta H°$ values, whereas other intermolecular interactions such as hydrogen bonds and van der Waals forces negatively contribute the system's standard enthalpy change of complex formation [33]. Therefore, the negative $\Delta H°$ values obtained indicate that, at pH 3.0, the positively charged QN molecules, besides contributing to attractive electrostatic interactions with MUC, play an important role in decreasing the electrostatic repulsion between the negative oligosaccharide side chains of MUC. Once the repulsion is reduced, the more flexible polypeptide regions of the proteins can approach to perform intra- and intermolecular interactions, contributing to a decrease in $\Delta H°$ values. As a result, when the MUC's structure changes from a more rigid and linear conformation to a folded one upon binding, the protein's rotational degrees of freedom increase. Besides this conformational change, another process that can contribute to an increase in the standard entropy change in the system is the release of counterions from the MUC's electric double layer to the bulk [34] when the interactions occur. However, the entropy increase caused by these processes is overcome by the greater loss of both molecules' translational degrees of freedom upon binding, resulting in negative $T\Delta S°$ values.

At pH 7.4, the formation of the complexes with MUC became less enthalpically driven ($\Delta H° =$ -18.65 ± 0.13 kJ mol^{-1}) with an increase of entropy ($T\Delta S° =$ 23.01 ± 0.16 kJ mol^{-1}), due to the deprotonation of QN. Based on the hypothesis presented, the decrease in the positive charge distribution in the QN structure prevents the MUC molecules to fold, due to electrostatic repulsions. Therefore, compared to the binding process at pH 3.0, the formation of the complex is no longer accompanied by an enthalpy decrease coming from the MUC's intra- and intermolecular interactions. Since only 1:1 complex is formed at pH 7.4, the loss of translational degrees of freedom is reduced. Instead, only the intermolecular interactions between MUC and QN (mainly ion-induced dipole forces) contributes to the negative $\Delta H°$ values. Even though interactions in the QN-MUC complex formation can be observed, the counterions from the protein's electric double layer also need to be released, and at pH 7.4, this release is less intense when compared to the same process at pH 3.0. Another process contributes to the positive $T\Delta S°$ value: in its neutral form, QN is a poorly soluble molecule [9], thus, when dispersed in an aqueous solution, the water molecules surrounding this alkaloid form a highly structured 3D network, in which the hydrogen bond interactions are maximized. Hence, the desolvation of

QN molecules occurs with the increase of the water molecules' enthalpy, alongside with a significant increase in their entropy since they are released to the bulk, in which the hydrogen bonds are less effective, and the water molecules are less structured. Therefore, the hydrophobic effect also contributes to the increase in $\Delta H°$ and $T\Delta S°$ values for the formation of the QN-MUC complex at pH 7.4.

3.1.2. Ionic strength effect on the thermodynamics of QN-MUC interaction

Besides the pH, another factor that can modulate the QN-MUC complex formation is the ionic strength of the medium. The ion content in a mucin solution is known to affect the glycoprotein's structure since the cationic species can shield the protein's negatively charged groups, thus reducing the intra and intermolecular electrostatic repulsion [20]. Therefore, to verify how the changes in the ionic strength affect the binding of QN to MUC, the thermodynamic parameters were determined in the presence of different concentrations of KCl, at pH 3.0 and 7.4. The results obtained are presented in Table S1 and Fig. 4.

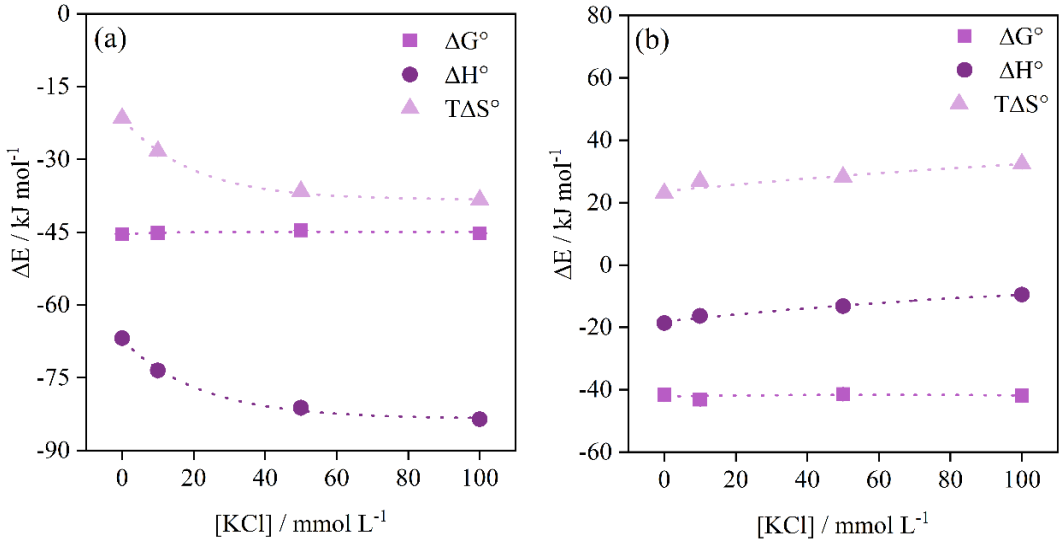

Fig. 4. Dependence of the thermodynamic parameters with the KCl concentration at (a) pH 3.0 or (b) pH 7.4, and 298.15 K. Dotted lines are shown for visual guidance only.

At pH 3.0, the QN-MUC complex presented constant stability (Fig. 4a) with the increase of KCl concentration, as indicated by $\Delta G°$ and n values (Table S1), which were kept constant. Nonetheless, $\Delta H°$ and $T\Delta S°$ showed an exponential decrease with the increasing salt concentration. The addition of salt to a protein solution affects its electric double layer by changing the number of counterions in the internal and diffuse layers [35]. For instance, the increase of KCl concentration in the MUC solution causes the increase of K^+ cations on the

internal layer directly in contact with the proteins, shielding the charges on their surface and, consequently, causing a decrease in the number of ions in the diffuse layers. These changes in the proteins' electric double layer can affect their binding to QN in two different ways. First, the increase of counterions attached to the MUC's surface leads to the decrease of the electrostatic repulsion between the negatively charged groups in the side chains. Therefore, when the complex is formed, MUC molecules are more prone to inter and intramolecular interactions with the presence of KCl, leading to a decrease in $\Delta H°$ values. Secondly, since fewer ions are present in the diffusive layers, the entropy of the system increases as a result from their release to the bulk. On the other hand, the complex formation contributes negatively to $T\Delta S°$. However, the decrease in these thermodynamic parameters with the increase of ionic strength is limited, as shown by the small variation in $\Delta H°$ and $T\Delta S°$ for [KCl] > 50 mmol L^{-1}. This behavior can be explained by the saturation of the internal layer with K^+, i.e., the number of cations on the internal layer remains constant even with the addition of more salt to the system.

At pH 7.4, even though the $\Delta G°$ also remained approximately constant with the increase of the ionic strength in the medium, the other thermodynamic parameters presented a different behavior in comparison to the binding process at pH 3.0. First, the stoichiometry (n) values decreased with the increase of salt concentration. At [KCl] ≤ 10 mmol L^{-1}, the stoichiometry remained approximately constant at 1:1, which can be explained by the fact that the electrostatic shielding promoted by the amount of salt added was not sufficient to cause aggregation of MUC molecules, probably due to the increase in the amount of negatively charged groups in the protein at pH 7.4. However, the increase in the concentration of salt caused the electrostatic shielding to become more effective, which contributes to MUC aggregation when the complex is formed, as indicated by the stoichiometries of 1:2 and 1:4 at [KCl] = 50 mmol L^{-1} and [KCl] = 100 mmol L^{-1}, respectively. On the other hand, $\Delta H°$ and $T\Delta S°$ increased almost linearly with the increase of salt concentration, even with the aggregation of the MUC at [KCl] ≥ 50 mmol L^{-1}. These results indicate that this salt, at pH 7.4, influences the binding of QN to MUC by acting as a kosmotropic agent [36], meaning that the presence of K^+ ions in the solvation shell of the MUC and, especially, QN molecules strengthen the hydrogen bonds between the water molecules. Consequently, the desolvation of the molecules upon binding occurs with a significant increase in the enthalpy and entropy of the system.

3.2. Time-intensity analysis

3.2.1. Evaluation of the bitterness of quinine solutions

Considering the thermodynamic parameters obtained for QN-MUC complex formation, a relationship regarding the perception of the bitter taste caused by the presence of QN can be an interesting approach. The molecules responsible for the taste are perceived interacting with the taste receptors located in the taste buds [3]. QN is known to provoke the perception of bitter taste and, as presented in section 3.1, it interacts with MUC, a salivary protein, forming a thermodynamically stable complex at physiological pH (pH=7.4). In order to verify the effect of the concentration of this alkaloid on the intensity of the bitter taste, solutions containing 0.04 mmol L^{-1}; 0.06 mmol L^{-1}; 0.08 mmol L^{-1} and 0.1 mmol L^{-1} of QN were evaluated sensorially. Figure 5 presents the time-intensity curves obtained for each solution.

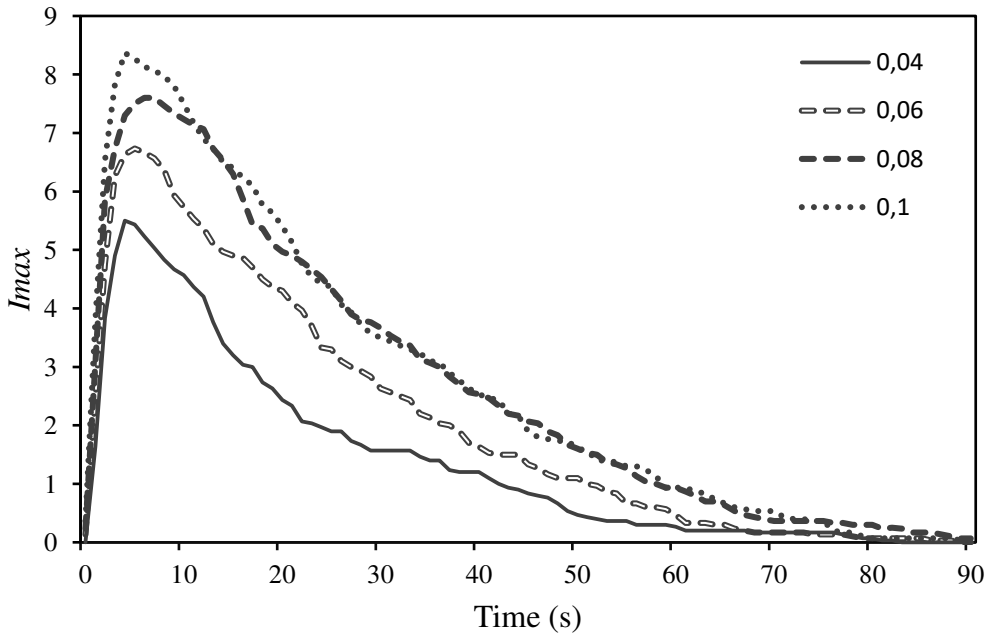

Fig. 5. Time-intensity curves obtained for each quinine solution. Concentrations in mmol L^{-1}.

QN solutions present a marked perception of bitter taste soon after being ingested, however, the bitterness decreases considerably in a small-time interval, meaning that despite the intensity of the bitter taste, especially at high concentrations, it is not perceived for a long period of time. This rapid decline in QN bitterness was also reported by Higgins, Gipple & Hayes (2021). In this study, for QN, there was a rapid decrease in bitterness when compared to some other components, such as narigin and hexalone. The differences observed between the compounds can be related to several factors, such as: chemical characteristics of the compounds, such as whether they are hydrophobic or not; interactions that can occur between

the bitter components and the proteins present in the saliva; characteristics of taste buds, among others [7].

The solutions with different concentrations of QN showed some similarities in the time-intensity profile, however, there were significant differences ($p < 0.05$) in several parameters, as shown in Table 1. The maximum intensity (Imax) increases with the increase in QN concentration, with significant difference between the concentrations evaluated ($p < 0.05$). The solutions containing 0.04 and 0.06 mmol L^{-1} did not differ from each other in terms of time-intensity profile ($p > 0.05$). However, both solutions are significantly different compared to the concentrations of 0.08 and 0.1 mmol L^{-1} of QN. The temporal perception o1f bitter taste presented an interesting behavior regarding the Imax, since the panelists only noticed the differences between the samples with a variation of QN equal or greater than 0.04 mmol L^{-1}. Furthermore, quantitative variations equivalent to 0.02 mmol L^{-1} of QN in the solutions did not result in proportional differences in Imax between samples, meaning that Imax does not increase proportionally to the concentration of QN. This behavior can be justified taking into account the complexity of the bitter taste perception process, which is influenced by several factors, such as the interactions of the molecules with salivary proteins. Due to the ability to form complexes, as observed in the thermodynamic study, there is the possibility of MUC-QN interaction reduction in QN bitterness, suggesting that these interactions decreased the availability of QN to bind to sensory receptors. Similar results were obtained by Torregrossa et al. (2014), who evaluated how PS influence the tannic acid acceptance in an animal model, corroborating the findings of this study. These authors reported that the decrease in the perceived bitter taste is due to the interactions between the tannic acid and salivary proteins rich in proline. Moreover, when using high concentrations of this tannin, the PS would not be able to interact with all the molecules, resulting in less efficiency in reducing bitterness [11]. Proline-rich proteins (PRPs) are known to interact with bitter substances, mainly tannins [14], [15]. In the same sense, there is the possibility that the formation of the QN-MUC complex contributes to the reduction of the perceived bitter taste at certain concentrations of QN, however, at higher concentrations, more QN may be available, thus intensifying the bitterness.

Table 1: Averages obtained for each parameter of the time-intensity curve for the solutions with different concentrations of quinine.

Parameters	Quinine concentrations (mmol L^{-1})			
	0.04	**0.06**	**0.08**	**0.1**
*Imax**	6.08^{c}	7.30^{bc}	8.57^{ba}	9.30^{a}
TI 5%	0.47^{a}	0,43^{a}	0,57^{a}	0,38^{a}
TD 5%	60.89^{a}	59.34^{a}	68.25^{a}	65.75^{a}
TI 90%	2.97^{a}	2.77^{a}	2.82^{a}	2.62^{a}
*TD 90%**	9.41^{b}	13.79^{a}	13.15^{a}	11.89^{ab}
*Plateau**	6.43^{b}	11.01^{a}	10.32^{a}	9.27^{ab}
*Area**	164.54^{b}	218.61^{b}	289.31^{a}	297.90^{a}

** indicate parameters that differed significantly (p <0.05) analyzed by ANOVA and Tukey test. Averages with equal letters in the same row are statistically equal.*

No significant differences were observed between the QN solutions for the TI 5% and TD5% parameters, indicating that, when the intensity represents 5% both in the increasing and decreasing region of the curve, this intensity is not influenced by the amount of quinine. The same behavior was observed when the intensity is 90% of Imax in the increasing region of the curve, which is represented by TI 90%.

There was observed a significant difference between the solutions with different concentrations of QN in the time-interval in the region of the curve where the intensity is 90% of Imax in the decreasing part of the curve (TD 90%), and also in the time interval in which the intensity is ≥ 90% of Imax (plateau) ($p < 0.05$). Regarding these parameters, the solution with the lowest concentration of QN (0.04 mmol L^{-1}) presented the lowest mean values ($p < 0.05$).

The TD_90% values ranged from 9.41 to 13.79 seconds between the QN solutions, meaning that the perception of the bitterness was only perceived in a relative short amount of time. Higgins & Hayes (2019) evaluated differences in the perception of bitterness with QN, sucrose octaacetate (SOA) and tetralone in five different regions of the oral cavity. The authors observed that QN showed a less intense bitter residual taste compared to the other samples in some regions of the oral cavity. In general, after 15 seconds, there was observed a reduction in the perceived bitterness while the sample was still present in the oral cavity. However, for SOA

and tetralone, the intensity of the bitterness did not decrease in the same proportion as observed for QN [37]. The area under the curve (AUC) were significantly different for the solutions containing different concentrations of QN. The AUC for the solutions with lower concentrations of QN (0.04 and 0.06 mmol L^{-1}) were significantly smaller area compared to the solutions containing higher concentrations of QN (0.08 and 0.1 mmol L^{-1}) ($p < 0.05$). These results indicate that the AUC is not linearly proportional to the concentration of QN in the solutions. It is possible to observe that the interactions between QN and salivary proteins can play a role in decreasing the number of the available binding sites in bitter compounds.

Studies regarding the investigation of thermodynamic and sensory properties to clarify the mechanisms of perception of bitter tastes are scarce in the literature. Nunes et al. (2020) performed a thermodynamic study of complex formation between lactoferrin (LF) and naringenin (NG) protein to evaluate the sensory perception of bitterness in a solution of NG, in the absence and presence of LF by using the time-intensity analysis. The solution added with LF presented a significant less bitterness compared to the solution in the absence of the protein ($p < 0.05$). ITC analysis showed that naringenin interacts with lactoferrin, forming NG-LF complexes [21]. NG-LF complex formation may prevent the bitter stimuli from NG from reaching the taste receptors in the mouth, consequently decreasing the perception of the bitter taste [11], [21].

Torregrossa et al. (2014) reported that interactions between bitter compounds (tannins) and PS, in particular PRPs, influence the perception of bitter taste, implying that the same approach can be applied to other molecules [11]. Khan et al. (2022) synthesized a hydrogel-based bioelectronic tongue to study bitterness and astringency using MUC as a salivary protein. Quinine sulfate and tannic acid were used as bitter and astringent compounds, respectively. According to the authors, the complex QN-MUC is formed through hydrogen bonds, more specifically between the hydroxyl group of quinine sulfate and the amide groups of the mucin or hydrogel network that makes up the bioelectronic tongue. The synthesized tongue showed superior detection selectivity for the bitter taste compared to other basic tastes and astringency [38]. In fact, the formation of QN-MUC complex was stated according to our thermodynamic study.

3.2.2. Influence of KCl on the perception of the bitter taste of quinine

The ionic strength can cause changes in protein structures [20]. The presence of ions in food systems can affect the interactions between salivary proteins and molecules present in food, which possibly influence the mechanism of taste perception, such as bitterness. Taking this fact into consideration, the behavior of time-intensity parameters was evaluated in the presence of KCl. For this purpose, the same concentrations of quinine (0.04, 0.06, 0.08 and 0.1 mmol L^{-1}) used for the thermodynamic study and time-intensity analysis were evaluated in this experiment. Concentrations of 10 and 50 mmol L^{-1} of KCl were added to each quinine solution. The results obtained for each parameter of the time-intensity curve are presented in Table 2.

Table 2: Parameters of the time-intensity curve for the solutions with different concentrations of quinine added to KCL.

Parameters	10 mmol L^{-1} (KCL) Group 1 (G1)				50 mmol L^{-1} (KCL) Group 2 (G2)			
	QN Concentration (mmol L^{-1})				**QN Concentration (mmol L^{-1})**			
	0.04	**0.06**	**0.08**	**0.1**	**0.04**	**0.06**	**0.08**	**0.1**
Imax(G1)*	6.60[b]	7.82[b]	9.26[a]	9.12[a]	8.51[a]	7.44[a]	8.67[a]	8.57[a]
TI 5%	0.67[a]	0,42[a]	0,90[a]	0,75[a]	0.15[a]	0.31[a]	0.26[a]	0.63[a]
TD 5%	49.26[a]	58.07[a]	66.63[a]	67.31[a]	65.34[a]	53.49[a]	62.79[a]	55.31[a]
TI 90%	2.71[a]	2.29[a]	3.46[a]	2.99[a]	2.29[a]	2.58[a]	2.22[a]	2.78[a]
TD 90%	12.32[a]	11.43[a]	11.90[a]	11.60[a]	14.93[a]	13.66[a]	19.43[a]	14.36[a]
Plateau	9.61[a]	9.14[a]	8.75[a]	8.61[a]	12.64[a]	11.08[a]	17.21[a]	11.60[a]
*Area**	161.07[b]	219.60[ab]	257.18[a]	269.14[a]	266.18[ab]	210.03[b]	310.82[a]	276.20[ab]

** indicate parameters that differed significantly ($p < 0.05$) analyzed by ANOVA and Tukey test. Averages with equal letters in thesame row do not differ within each quinine solution, analyzing each KCL group separately.*

According to Table 2, it is possible to observe that, for concentrations of 10 mmol L^{-1} of KCl, the quinine solutions differ from each other ($p < 0.05$) regarding the Imax and Area. The maximum intensity perceived in the concentration of 0.04 mmol L^{-1} of QN in the presence of KCl did not show any significant different when compared to the solution with a concentration of 0.06 mmol L^{-1} of QN. When compared to the solutions with a concentration of 0.08 and 0.1 mmol L^{-1} of QN, the solutions containing 0.04 and 0.06 mmol L^{-1} were significant different. Moreover, it is possible to observe a difference in the behavior of the parameters when comparing to the quinine solution without the presence of KCl. The other parameters of the curve (TI 5%, TD 5%, TI90% TD90% and Plateau) were statistically equal for all the solutions evaluated. The time-intensity profile of the bitter taste perception of the solutions containing 0 and 10 mmol L^{-1} KCl were similar to the solution containing a concentration of 0.04 mmol L^{-1} of QN, as shown in Figure 6.

On the other hand, the addition of 50 mmol L^{-1} of KCl to the QN solutions affected the perception of the bitter taste for all the solutions evaluated, in the sense that no differences were observed between the samples ($p > 0.05$). This result was in contrast to that observed for both pure QN solutions and those containing 10 mmol L^{-1} of KCl. This behavior can be explained by the fact that possibly the addition of high concentrations of KCl affected the behavior of the salivary proteins. In the thermodynamic study, the addition of 50 mmol L^{-1} of KCl in the quinine solution at pH close to neutrality affected some thermodynamic parameters of the complex formation, such as stoichiometry, which increased from 1:1 to 1:2. This behavior indicates the formation of a protein aggregate. Another interesting alternative to be taken into consideration is the possibility that the high concentrations of KCl directly influenced the sensory perception of bitterness. KCl naturally has a bitter taste, in the sense that the addition of 50 mmol L^{-1} of this salt may have made it difficult to detect different variations regarding the intensity of the bitterness [39].

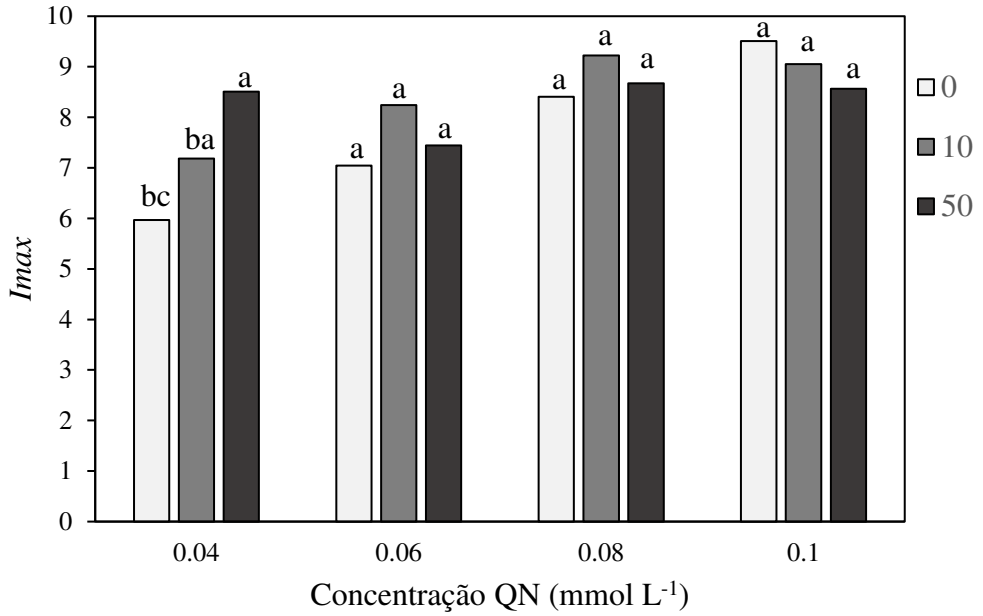

Fig.6. Comparison of the effect of KCL within each QN concentration. Equal letters in the same QN concentration do not differ (p >0.05).

The effect of different concentrations of KCl (0, 10 and 50 mmol L^{-1}) on the perception of bitter taste in each quinine solution was also evaluated. The difference between the mean values of Imax was detected only in the solution containing 0.04 mmol L^{-1} of quinine, in which the solution without the addition of KCl was significantly less bitter compared to the solution added with 50 mmol L^{-1} ($p<0.05$) (Figure 6 and 7).

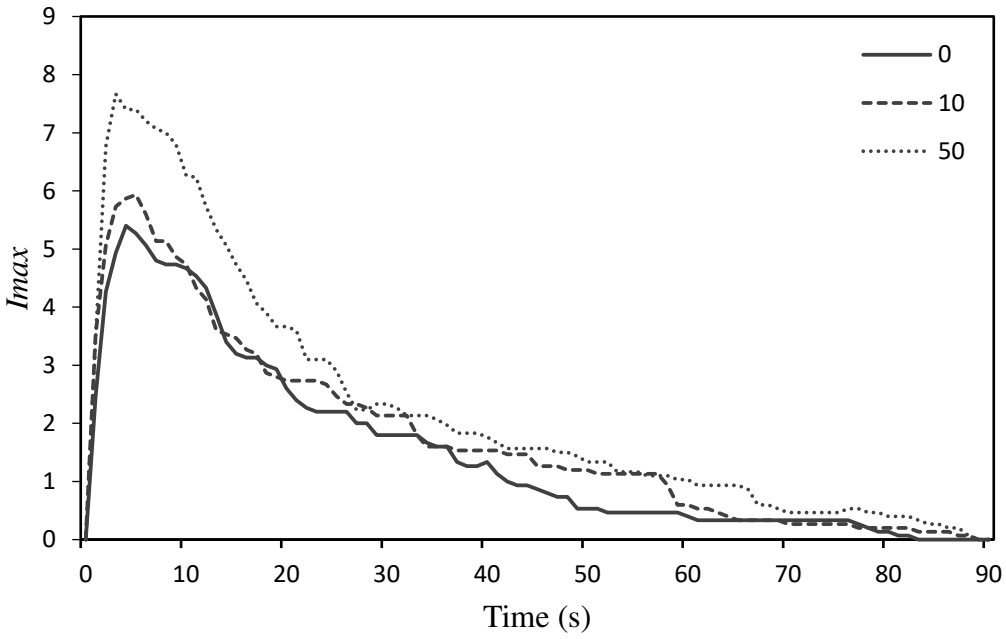

Fig.7. Time-intensity curves obtained for 0.04 mmol L-1 solution of quinine added to KCL. Concentrations of KCL in mmol L^{-1}.

In the thermodynamic evaluation, it was possible to observe the formation of QN-MUC complex, as well as the formation of protein aggregates, which can be favored by the addition of KCl. The presence of ions affects the QN-MUC interaction, which means that the addition of KCl in the solutions can influence the perception of the bitter taste. However, this behavior was only observed for the solutions containing 0.04 mmol L^{-1} of QN, meaning that the intensity of bitter taste perception increased with increasing KCl concentration (Figure 6 and 7). This behavior can be explained by the susceptibility to the addition of 50 mmol L^{-1} for the solution containing 0.04 mmol L^{-1} of QN, since it presents less bitterness compared to the others solutions.

4. Conclusion

The thermodynamic evaluation showed the complex formation between QN and MUC is strongly influenced by the pH and the ionic strength of the medium. Moreover, at pH 3.0, it is more noticeable the formation a protein aggregate compared to the complex formation at pH 7.4. In general, hydrophobic interactions were predominant in the formation of QN-MUC complex. Moreover, the ionic strength of the medium strongly contributed to the formation of this complex at pH 7.4, especially at higher concentration of KCl. These results are extremely important for better understanding the interaction between bitter compounds, especially quinine and salivary proteins.

The sensory perception of the bitter taste was significantly influenced by the concentration of QN. However, the increase in the concentration of QN did not promote a linear proportional increase in the maximum intensity of the bitter taste perception. In addition, ionic strength had a major impact on the temporal perception of bitterness. The addition of 50 mmol L^{-1} of KCL made it impossible to distinguish between solutions with different concentrations of QN, probably due to the salty taste imparted by the salt or the formation of protein aggregates. The sensory perception of bitter taste involves complex mechanisms. In fact, the interaction between quinine and salivary proteins, such as mucin, and the addition of salt are factors that influence the ability to identify bitter compounds.

Supplementary Material

Table S1. Binding constant (K_b), standard Gibbs free energy change ($\Delta G°$), standard enthalpy change ($\Delta H°$) and standard entropy change ($T\Delta S°$) of the [Mucin-Quinine] thermodynamic stable complex formation with different concentrations of KCl and at pH 3 and 7.4.

[KCl]	K_b	$\Delta G°$	$\Delta H°$	$T\Delta S°$	n
mmol L^{-1}	10^7 L mol^{-1}	kJ mol^{-1}			
pH 3.0					
0	9.11 ± 0.49	-45.38 ± 0.51	-66.88 ± 0.70	-21.50 ± 0.19	0.28 ± 0.01
10	8.29 ± 0.58	-45.18 ± 0.33	-73.51 ± 0.51	-28.33 ± 0.18	0.29 ± 0.01
50	6.66 ± 0.99	-44.63 ± 0.33	-81.21 ± 0.58	-36.58 ± 0.25	0.33 ± 0.01
100	8.44 ± 0.89	-45.22 ± 0.27	-83.58 ± 0.46	-38.36 ± 0.18	0.34 ± 0.01
pH 7.4					
0	2.01 ± 0.49	-41.66 ± 0.29	-18.65 ± 0.13	23.01 ± 0.16	0.78 ± 0.01
10	3.82 ± 0.58	-43.24 ± 0.39	-16.32 ± 0.13	26.92 ± 0.26	0.75 ± 0.01
50	1.85 ± 0.99	-41.45 ± 0.29	-13.22 ± 0.11	28.23 ± 0.18	0.50 ± 0.01
100	2.24 ± 0.89	-41.92 ± 0.33	-9.52 ± 0.11	32.40 ± 0.22	0.23 ± 0.01

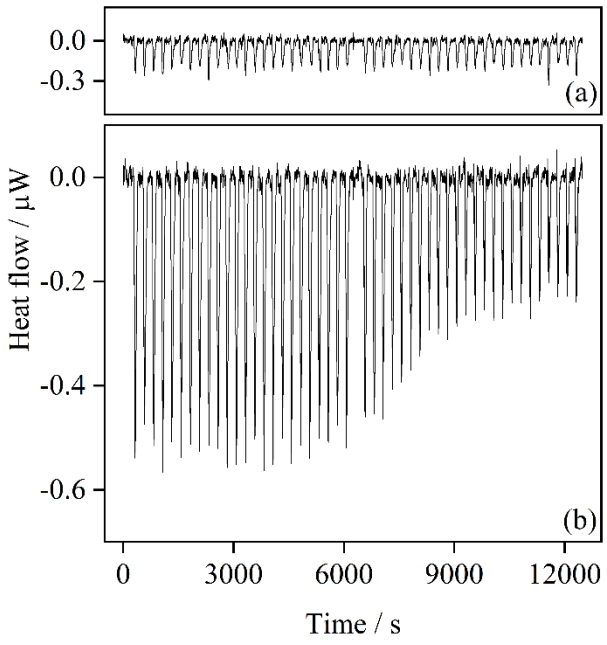

Fig. S1. Calorimetric raw data for the titration of a QN solution (1.0 x 10^{-4} mol L^{-1}) in **(a)** an acetate buffer solution, or **(b)** a MUC solution (1.56 x 10^{-5} mol L^{-1}), at pH 7.4 and 298.15 K.

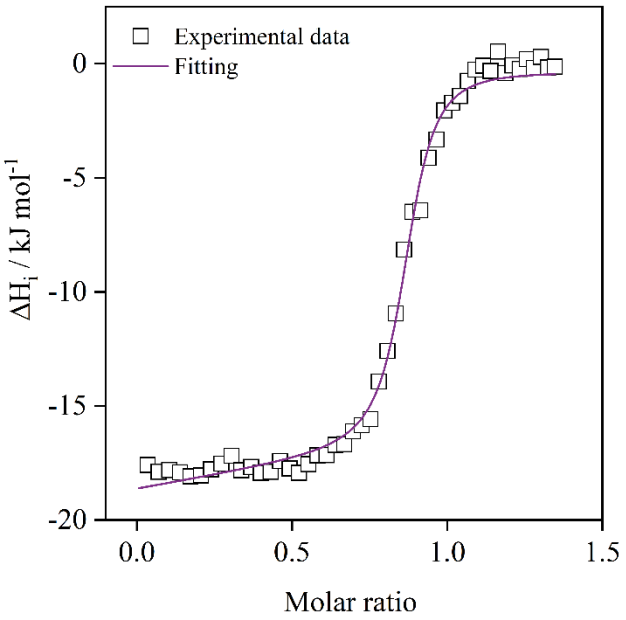

Fig. S2. Plot of 〖ΔH〗_iversus the molar ratio for the interaction of QN with MUC, at pH 7.4 and 298.15 K, and the best fitting obtained from the SSIS model.

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