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COMPLEX COACERVATION OF CHICKPEA PROTEIN ISOLATE AND PECTIN: EFFECT OF BIOPOLYMER RATIO AND pH

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ABSTRACT
Complex coacervation is an up-and-coming encapsulation technique widely working in the medicinal, food, agriculture, and textile industries. This study investigated the effect of biopolymer ratio and pH on the complexation between chickpea protein isolate (CPI) and pectin (PC) through zeta potential, turbidity measurement, and visual observations. Pectin showed a negative charge profile between pH 2-9. The isoelectric point of the chickpea protein isolate was found as 4.5 (pI). Soluble complexes were formed in the system with pHs below the pI of CPI with positive charges, whereas PC had negative ones. Complex coacervates formed at pH 3.1 with a 4:1(CPI: PC) biopolymer ratio. The turbidity and visual appearance revealed that larger aggregates were formed in CPI-PC coacervates. The findings could help in the development of pH-sensitive biopolymer carriers for use in functional foods and biomaterials.
Keywords: chickpea protein, pectin, complex coacervation, zeta potential

NOHUT PROTEİNİ İZOLATI VE PEKTİNİN KOMPLEKS KOASERVASYONU: BİYOPOLİMER ORANI VE pH'NIN ETKİSİ

ÖZ
Kompleks koaservasyon, farmasötik, gıda, tarım ve tekstil endüstrilerinde yaygın olarak kullanılan, oldukça destekleyici bir kapsülleme tekniğidir. Bu çalışmada, nohut protein izolatı (NPİ) ve pektin (PK) arasındaki kompleksleşme üzerinde biyopolimer oranı ve pH'ın etkisi zeta potansiyeli, bulanıklık ölçümü ve görsel gözlemler kullanılarak araştırılmıştır. Pektin, pH 2-9 arasında negatif yük profili göstermiştir. Nohut protein izolatının izoelektrik noktası 4.5 (pI) olarak bulunmuştur. Çözülebilir kompleksler pH'ları NPİ'nin izoelektirik noktasının pozitif, pektininin de negatif yük taşıdığı sistemde oluşmuştur. Kompleks koaservat oluşumunun 4:1(NPİ:PK) biyopolimer oranı ile pH 3.1'de gerçekleştiği gözlemlenmiştir. Bulanıklık ve görsel görünüm, NPİ-PK koaservatlarında daha büyük agregatların oluştuğunu ortaya koymuştur. Bulunan sonuçlar fonksiyonel gıdalar ve biyomalzemelerde kullanım için pH'ya duyarlı biyopolimer taşıyıcıların geliştirilmesine yardımcı olabilir.
Anahtar kelimeler: nohut proteini izolatı, pektin, kompleks koaservasyon, zeta potansiyeli

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INTRODUCTION

Complex coacervation is the spontaneous associative phase separation of two oppositely charged biopolymers based on electrostatic interactions. One dense phase, coacervate, and another comparatively diluted macromolecular liquid phase, supernatant, are produced as a result (De Kruif et al., 2004). Complex coacervation has gained wide applications in fat substitution, protein separation, cosmetics, enzyme immobilization, sensitive food ingredients (e.g., Omega-rich oils and bioactive compounds), microencapsulation, and pressure-sensitive carbonless paper (Huang et al., 2012; Timilsena et al., 2019). The critical parameters influencing coacervate formation are pH, ionic strength, protein to polysaccharide ratio, and total biopolymer concentration (Kayitmazer, 2017).

Plant proteins have recently gained popularity as a cost-effective and adaptable alternative to animal proteins in human nutrition and as functional components in new products. Animal protein has been associated with climate change, freshwater depletion, biodiversity loss, growing expenses, and health hazards like cardiovascular disease and others due to its scarcity and rising demand (Sá et al., 2020).

Chickpeas are the third most widely cultivated variety of legumes in the world and have a high protein content (18–25%), with the globulin fraction constituting the significant protein fraction and legumin-like proteins (11S) being the dominant globulins (Boukid, 2021). Chickpea protein isolate presented techno-functional properties, such as high solubility, emulsifying capacity, foam formation, and gelation. Additionally, it works as a powerful antioxidant by neutralizing free radicals, peroxides, and chelating metals (Xu et al., 2021). The isoelectric point of the chickpea protein isolate is around pH 4.0. The advantages of chickpea protein isolate are its low price, biodegradability, biocompatibility, high digestibility, and non-toxicity. Hence, it is known as GRAS (Generally Recognized As Safe), which can be safely used in food formulations (Sarabi-Aghdam et al., 2021).

Pectin has a negative charge because of the presence of ionized carboxylic groups through its backbone (pKa = 3.5). Pectin is a hydrophilic polysaccharide derived from plant cell walls made up of esterified d-galacturonic acid residues in the α-(1–4) chain. Pectin and its derivatives are primarily used as gelling and thickening agents, food product stabilizers, colon-specific drug delivery, and gastrointestinal digestion agents, among other things (Joshi et al., 2018; Wu and McClements, 2015). Pectin's main constituent is galacturonic acid, which can be partially methoxylated or amidated. Pectin is classified into two types based on the degree of methoxylation (DM): high methoxyl pectin (HMP) with DM greater than 50% and low methoxyl pectin (LMP) with DM less than 50% (Xiong et al., 2018).

The combination of chickpea protein and pectin with different hydrocolloids has been previously used to form complex coacervation. Such as chickpea protein-Persian gum (Mousazadeh et al., 2018), pea protein isolate-pectin (Lan et al., 2020), chickpea protein isolate- Persian gum (Sarabi-Aghdam et al., 2021), whey protein-pectin (Raei et al., 2018), gelatin-pectin (Xiong et al., 2021) and lysozyme-pectin (Souza et al., 2018). Additionally, some studies about using chickpea protein-pectin complexes for encapsulation (Moser et al., 2019; Moser et al., 2020). However, there is no study done on complex coacervation between chickpea protein isolate and pectin.

Since the complex coacervation between chickpea protein isolate and pectin has not been reported yet, this study aimed to explore the coacervation between the two biopolymers. Initially, the changing in zeta potential and turbidity of individual chickpea protein isolate and pectin were assessed as a function of pH. Thereafter, the influences of pH and biopolymer ratio on the formation of coacervates were investigated.

MATERIALS AND METHODS

MATERIALS

Chickpea protein isolate (CPI) (>85% protein) was supplied by AGT Foods (SK, Canada). Pectin (PC) (86.3% galacturonic acid) from citrus fruits, analytical grade sodium hydroxide (NaOH), and

hydrochloric acid (HCl) were obtained from Sigma Aldrich.

METHODS

Preparation of stock solutions

The stock solution of PC (1 wt%) was prepared by dissolving the exact amount of PC powder in Milli-Q water and then stirred for 2 h at room temperature to ensure complete solubilization. As for the CPI stock solution, 1 g of CPI powder was suspended in 100 mL Milli-Q water and stirred on a magnetic stirrer for 2 h at ambient temperature. The solution was exposed to ultrasonic treatment (Branson Sonifier SFX250, Danbury, CT) at 50% amplitude for 3 minutes. The pH of solutions was adjusted to pH 7 using 0.1-1 M NaOH or HCl. Sodium azide (0.02 wt%) was added to both solutions to avoid microbial growth. Both solutions were stored at 4°C overnight for further analysis.

Formation of complexes and coacervation

The mixtures containing PC and CPI were prepared by mixing at appropriate CPI:PC (1:1, 2:1, 4:1, 6:1) ratios of the biopolymers stock solutions, with a final concentration of 1 wt %. Then the suspensions were homogenized on a magnetic stirrer for 30 minutes. 0.1-1 M NaOH or HCl was used to adjust the pH of the solutions after mixing.

Zeta Potential measurement

The zeta potential values of the pure individual and mixed PC and CPI solutions at different pH were measured using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd, Malvern, Worcestershire, UK) and disposable capillary cells (DTS 1060) (Huang et al., 2012).

Turbidimetric measurements

The pH-dependent turbidity of the pure individual and mixed PC and CPI solutions was measured at 600 nm using a spectrophotometer (Jenway 6715, Bibby Scientific Limited, Beacon Road, Stone, Staffordshire, ST15 OSA, UK), which was calibrated with milli-Q water to 100% transmittance.100-T% was used to define turbidity (Souza et al., 2018).

Statistical Analysis

All measurements were done in triplicates. The SPSS statistical packet (Version 22, Polar Engineering and Consulting, Nikiski, USA) was used to perform one-way analysis of variance. Means of the obtained data were compared using Duncan's multiple range test at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Effect of biopolymer ratio on the formation of complex coacervates

The ratio of protein to polysaccharide in the mixture will affect the charge balance of complexes and, thus, their behavior. Maximum complexation in a mixture occurs at a specific protein-to-polysaccharide ratio under certain conditions, e.g., pH and ionic strength. Because of the presence of non-neutralized charges, soluble complexes can form when one of the components (protein or polysaccharide) in the mixture is in excess. No complexation occurs when the polysaccharide or protein in the solution is in excess at high biopolymer concentrations (Elmer et al., 2011; Ye, 2008). The turbidity values at pH 3 for the systems with different proportions of chickpea protein isolate: pectin (CPI:PC) are shown in Figure 1. The values were 78.99±0.11, 87.00±0.11, 90.06±0.06, 90.11±0.01 for 1:1, 2:1, 4:1, 6:1 mixing ratio, respectively. It is obvious from the results that increasing the concentration of CPI led to an increase in turbidity. Increasing ratios > 4 did not show a significant increase in turbidity value. Since the increase in the protein ratio will cause aggregation among the proteins, the 4:1 ratio was chosen as the optimum ratio (Flanagan et al., 2015). Gulão et al. (2014) showed similar behavior in forming complex coacervates between lactoferrin and pectin and stated that increasing protein concentration led to decreased coacervates formation.

Zeta Potential

As evidence of electrostatic or hydrophobic interactions between biopolymers, measuring the net charge of biopolymers or complexes is often helpful in evaluating the effectiveness of various mechanisms governing the stability of complexes (Mousazadeh et al., 2018). Therefore, the zeta

potential of individual CPI, PC, and their complexes and/or coacervates was measured to see the electrical charge profile at different pHs (Figure 2). At pH 4.5, the zeta potential of CPI was zero, which corresponds to its isoelectric point (pI). The CPI acquires a positive charge below its pI due to protonation of the amino groups (NH_3^+), while it acquires negative charges above pI due to deprotonation of the carboxyl groups caused by the presence of hydroxyl (OH^-) groups. Boukid (2021) mentioned that the pI of chickpea protein is between 4-6, and the value of 4.5 is found in the present work in the limit of this range. Ladjal-Ettoumi et al. (2016) reported that the charge of CPI was also zero at pH 4.5. According to Figure 2, zeta potential of PC was negative at pHs 2 up to 9, and its magnitude increased by increasing the pH, which was already expected because pectin is an anionic polysaccharide containing carboxyl groups. These results are in accordance with those reported by Joshi et al. (2018) and Souza et al. (2018).

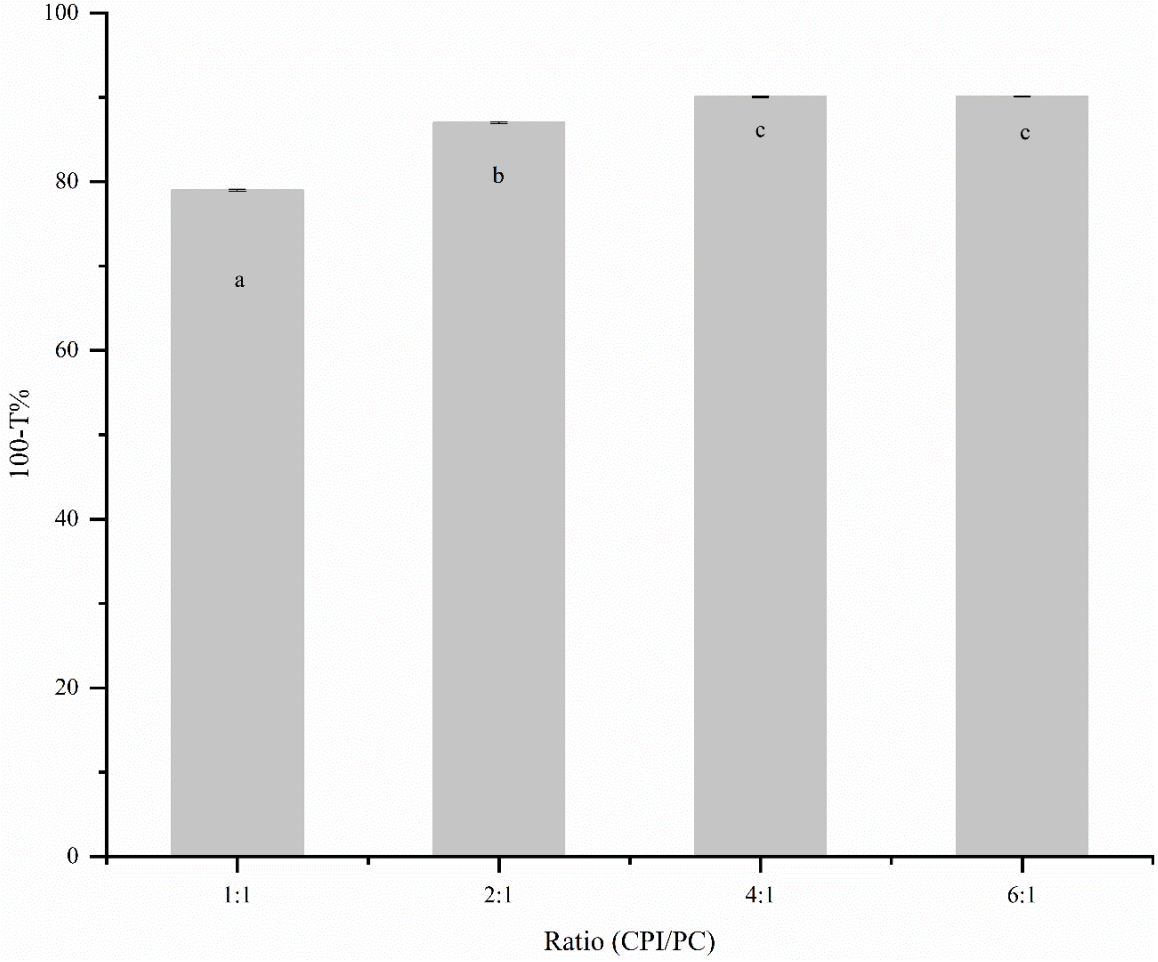

Figure 1. Turbidity values of the sample prepared different CPI/PC ratios at pH 3. Error bars represent standard deviations, and small letters indicate differences between samples by Duncan's multiple range test ($p<0.05$) (CPI: chickpea protein isolate, PC: pectin).

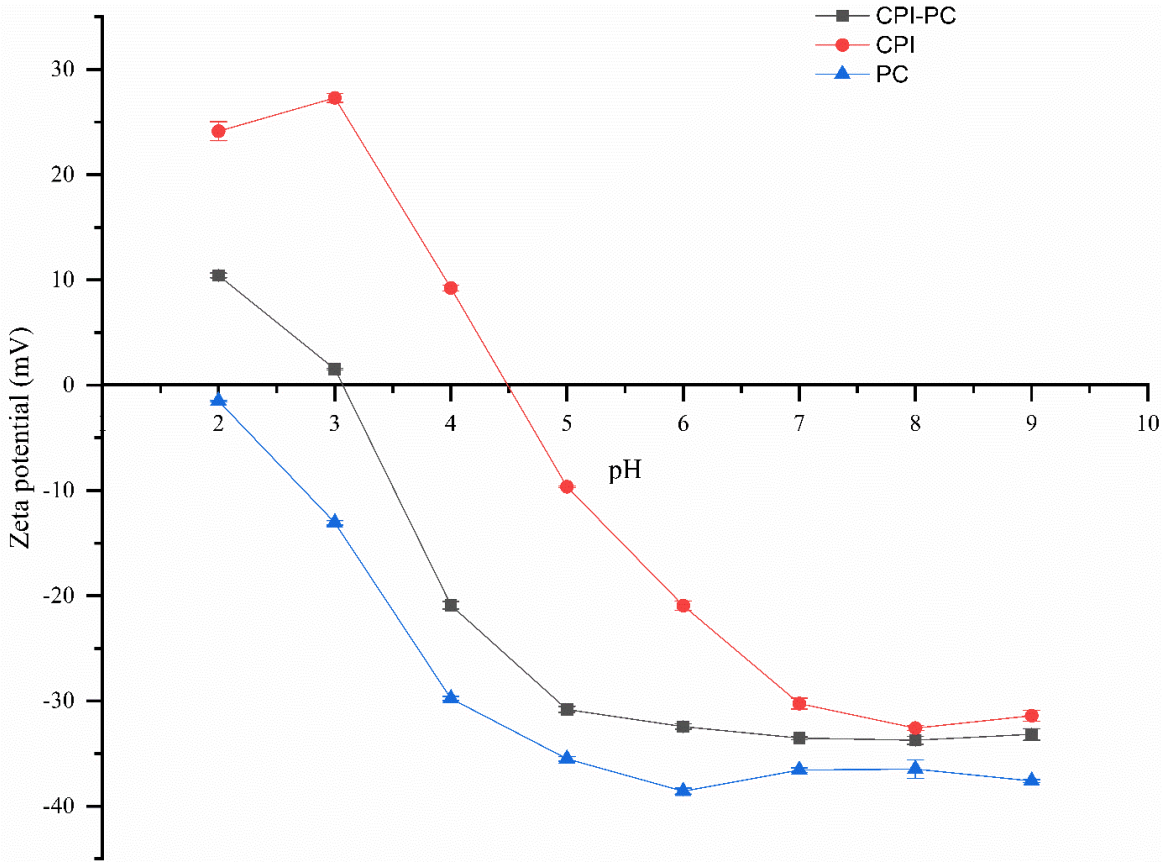

Figure 2. Effect of pH on zeta potential of CPI, PC and CPI-PC mixture (4:1 ratio) as a function of pH (CPI: chickpea protein isolate, PC: pectin). Error bars represent standard deviation.

When the CPI and PC were mixed in the 4:1 ratio, the zeta potential changed from +10.43±0.22 to -33.17±0.56 as a function of pH, with values nearing zero in the pH range from 3-4. The zeta potential of the mixed CPI and PC solutions was in between the zeta potential values of individual CPI and PC solutions due to the neutralization of positive and negative charges. Below pH 3, CPI molecules were cationic, whereas PC was anionic, which indicates the presence of soluble complexes. At pH 3.1, the zeta potential reached zero (data not shown), where mixed solutions showed coacervate formation owing to the electrostatic complexation. As the biopolymer mixture's net charge approaches zero, coacervates begin to form (Lan et al., 2020). Xiong et al. (2021) reported that gelatine and pectin could form complex coacervates mainly through electrostatic interaction at pH 3.5. Moser et al. (2019) reported that the pH 3 was suitable to produce chickpea protein-high methoxyl pectin emulsion, favoring the formation of a bilayer around the droplets due to electrostatic complexation induced by oppositely charged macromolecules.

As seen in Figure 3, PC showed a transparent appearance in all working pH ranges. CPI showed phase separation between pH 4-6 due to precipitation of protein molecules near the pI. Below and above this pH range, there was a distinct cloudy appearance in the CPI solutions. When we compared CPI and CPI-PC mixture, the presence of phase separation at pH 3 only in the CPI-PC mixture was proof of the formation of complex coacervation (insoluble complexes). This is in line with the zeta potential result. Similar visual observations were reported between pea protein isolate and pectin by Lan et al. (2020).

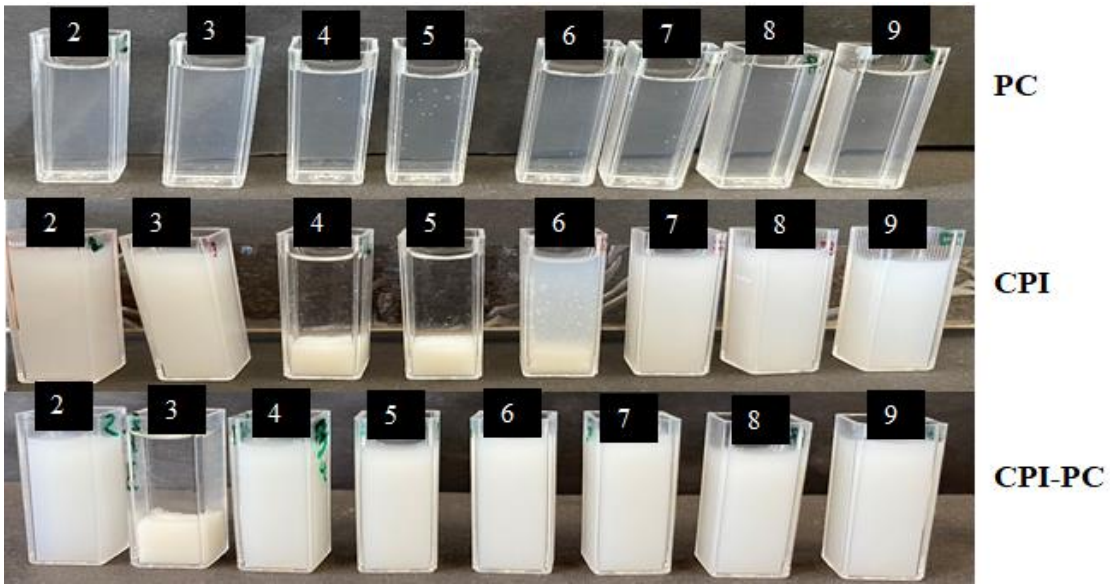

Figure 3. Photograph of CPI, PC and CPI-PC mixture (4:1 ratio) as a function of pH (CPI: chickpea protein isolate, PC: pectin).

Turbidity

Because turbidity is caused primarily by changes in the mass and size of aggregates in solution, changes in turbidity are thought to be caused by the formation and dissociation of protein/polysaccharide coacervates, as in other protein/polymer systems. The turbidity values at different pHs for the CPI, PC, and CPI-PC mixture are shown in Figure 4. The PC solution presented low and constant turbidity values for all studied pHs. There was no phase separation or precipitation because its solubility was not pH-dependent (Moser et al., 2020). The CPI solution's turbidity was low between pH 4-6, as expected from zeta potential measurements and visual observations (Figures 2 and 3). Notably, at pH 5, the turbidity had reached maximum value with the presence of larger aggregates (close to pI of CPI). In the case of the CPI-PC mixture, the turbidity values showed an increase in pH 3-4, highlighting the area of coacervate formation. Looking in the pH range between 3-3.5, the mixture reached the highest turbidity value as 91.14±0.03 (data not shown) at pH 3.1, indicating coacervates formation. This is in line with zeta potential measurement and visual appearance as well. Freitas et al. (2017) studied soy protein isolate-pectin complex characterization based on solubility, zeta potential, and turbidity measurements and observed a similar trend in turbidity values as a function of pH. Souza et al. (2018) reported a similar turbidity change for complex coacervation between lysozyme and pectin.

CONCLUSION

The current study evaluated the effect of pH and biopolymer mixing ratio on zeta potential, turbidity and visual observation of complexes between chickpea protein isolate (CPI) and pectin (PC). Biopolymer ratio and pH were found to significantly affect the formation of soluble and insoluble (coacervate) complexes. An increase in the protein concentration led to an increase turbidity value. When the CPI:PC ratio was increased from 4:1 to 6:1, turbidity was no longer changed. The maximum complex coacervation was observed when the CPI:PC ratio and pH were 4:1 and 3.1, respectively. This optimum condition resulted in coacervates with sharp phase separation and maximum turbidity. Because, under these circumstances, the zeta potential of coacervates was close to zero, promoting their aggregation and precipitation. These CPI-PC complex coacervates may be preferred as new, cost-effective, and nutritionally valuable delivery vehicles for unstable and active food components. Further research is required to

investigate the practical applications of this delivery matrix and its capacity to microencapsulate and protect sensitive components. These results will shed light on future studies.

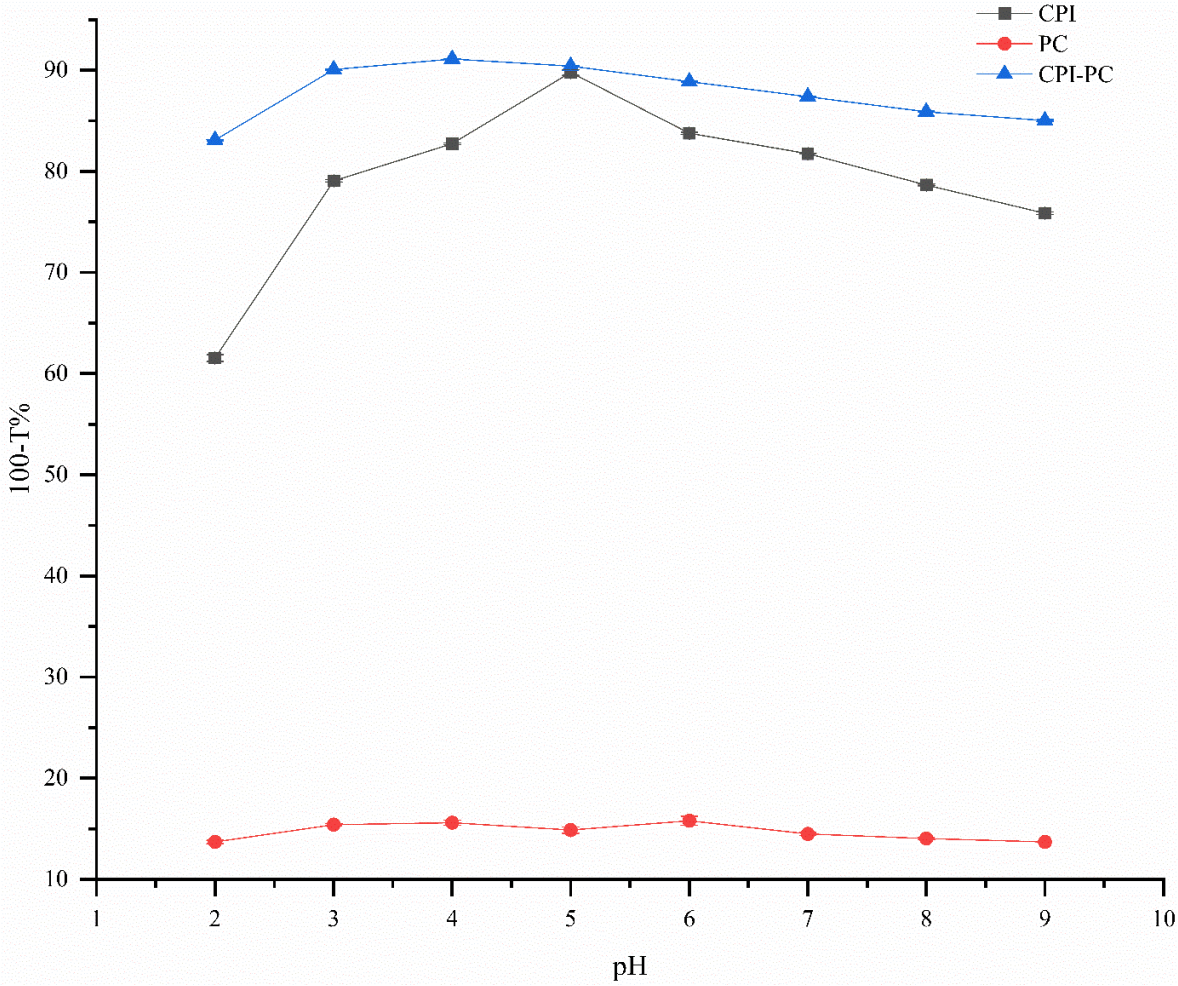

Figure. 4. Effect of pH on turbidity of CPI, PC, and CPI-PC mixture (4:1 ratio) as a function of pH (CPI: chickpea protein isolate, PC: pectin). Error bars represent standard deviation.

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CONFLICT OF INTEREST

The author has declared no conflict of interest.

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