

**Protein solubility of jackfruit seed flour: pH and salt concentration influence**  
**Solubilidade de proteínas da farinha do caroço de jaca: influência do pH e da**  
**concentração de sal**

**Solubilidad proteica de la harina de yaca: influencia del pH y la concentración de sal**

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## Abstract

Solubility is a fundamental physicochemical property of proteins because of its importance over other protein properties in foods. Water solubility characteristics determine appropriate protein extraction and fractionation conditions in foods. In this study protein solubility of jackfruit seed flour (*Artocarpus integrifolia* L.) was determined, combined with the effect of pH and sulfate, chloride and trisodium citrate salt concentration variations. Protein solubility was higher in more acidic (pH =2.0) and alkaline ( $8.0 \leq \text{pH} \leq 10.0$ ) mediums, in the absence of salts. It was also verified that the best solubility conditions under pH 2.0 using trisodium citrate was at 0.25 mol/L; and for pH 6.0, it was 1.0 mol/L. For sodium chloride, the best conditions occurred at 0.25 mol/L for pH 2.0, 6.0 and 10.0; and the best solubility values using sodium sulfate under pH 4.0 and 8.0 was 0.25 mol/L; and 0.50 mol/L under pH 6.0 and 10.0. Jackfruit seeds flour can be used as an alternative source of edible protein and as a substitute for products already available in the market.

**Keywords:** *Artocarpus integrifolia* L.; Isoelectric point; Sodium salts; Jackfruit; Solubility.

## Resumo

A solubilidade é uma propriedade físico-química fundamental das proteínas devido à sua importância sobre outras propriedades proteicas nos alimentos. As características de solubilidade em água determinam as condições adequadas de extração e fracionamento de proteínas nos alimentos. Neste estudo, a solubilidade proteica da farinha do caroço de jaca (*Artocarpus integrifolia* L.) foi determinada combinando-se o efeito das variações de pH e concentrações de sulfato, cloreto e citrato trissódico. A solubilidade das proteínas foi maior em meios mais ácidos (pH = 2,0) e alcalinos ( $8,0 \leq \text{pH} \leq 10,0$ ), na ausência de sais. Verificou-se também que as melhores condições de solubilidade em pH 2,0 com citrato trissódico foi 0,25 mol/L; e em pH 6,0 foi 1,0 mol/L. Para o cloreto de sódio, as melhores condições ocorreram em 0,25 mol/L para pH 2,0, 6,0 e 10,0; e os melhores valores de solubilidade usando sulfato de sódio sob pH 4,0 e 8,0 foram 0,25 mol/L; e 0,50 mol/L sob pH 6,0 e 10,0. A farinha do caroço de jaca pode ser utilizada como fonte alternativa de proteína comestível e como substituta de produtos já disponíveis no mercado.

**Palavras-chave:** *Artocarpus integrifolia* L.; Jaca; Ponto isoelétrico; Sais de sódio; Solubilidade.

## Resumen

La solubilidad es una propiedad físico-química fundamental de las proteínas debido a su importancia sobre otras propiedades proteicas en los alimentos. Las características de solubilidad en agua determinan las condiciones adecuadas para la extracción y el fraccionamiento de proteínas en los alimentos. En este estudio, se determinó la solubilidad proteica de la harina de yaca (*Artocarpus integrifolia* L.) combinando el efecto de las variaciones de pH y las concentraciones de sulfato, cloruro y citrato trisódico. La solubilidad de la proteína fue mayor en medios más ácidos (pH = 2.0) y alcalinos ( $8.0 \leq \text{pH} \leq 10.0$ ), en ausencia de sales. También se encontró que las mejores condiciones de solubilidad a pH 2.0 con citrato trisódico fueron 0.25 mol/L; y a pH 6.0 fue de 1.0 mol/L. Para el cloruro de sodio, las mejores condiciones ocurrieron a 0.25 mol/L para pH 2.0, 6.0 y 10.0; y los mejores valores de solubilidad usando sulfato de sodio a pH 4.0 y 8.0 fueron 0.25 mol/L; y 0,50 mol/L a pH 6,0 y 10,0. La harina de yaca puede utilizarse como fuente alternativa de proteína comestible y como sustituto de productos ya disponibles en el mercado.

**Palabras clave:** *Artocarpus integrifolia* L.; Punto isoeléctrico; Sales de sodio; Yaca; Solubilidad.

## 1. Introduction

Proteins, commonly used as food ingredients, are of fundamental importance for human diets. In addition, they attribute sensorial properties and provide adequate functionality conditions (Waghmare et al., 2019; Zang et al., 2019).

The use of alternative sources of lower cost for substituting or simulating traditional high protein value food relies on the knowledge of functional properties of its protein and its behavior in certain food systems. Recent studies on human nutrition deal with low cost sources of food protein, due to animal's protein high cost and scarce supply, especially in underdeveloped countries. On the other hand, legumes are abundant and they are a cheaper food protein source (Lawal et al., 2007). New compounds or protein sources such as concentrates and protein isolates require in-depth studies for greater awareness of their behavior on different food systems. Some seeds of high protein content have become research targets, such as *Camelina sativa* (Anderson et al., 2019); cumin (*Cuminum cyminum*) (Chen et al., 2018), jackfruit (Zhang et al., 2019), among others.

Jackfruit (*Artocarpus heterophyllus* Lam.), originally from Asia, has acclimated well in Brazil. It is rich in carbohydrates, protein, vitamins, and minerals (Waghmare et al., 2019;

Zhang et al., 2019). Its berries can be freshly consumed and if processed, it can be consumed as dessert, compote, frozen pulps, beverages, among others. The seeds can be baked or used in different recipes. Jackfruit seed powder is used as alternative flour in bakery and confectionary products by blending it with wheat flour and other low-cost flours. The lack of low cost and nutritive products led researches to seek for alternative protein sources which attend low cost production demands. Jackfruit seeds could be used as an economic alternative protein source to tackle the malnutrition (Waghmare et al., 2019).

In literature there is a lack of data on studies concerning protein properties of jackfruit seeds for technological applications, an obstacle for its utilization as a protein source on food production. Characterization of protein properties is necessary for underlying basic and applied studies about seed proteins. Such studies aim material suitability and applicability in new products in food industries and even as a protein supplement in food systems (Furtado et al., 2001).

According to Ajibola et al. (2016), solubility is an important requirement for proteins to be useful as functional ingredients in food. Solubility is affected by environmental conditions which influence its physico-chemical characteristics, such as pH, ions nature, concentration and strength, temperature, and the presence of organic solvents (Zayas, 1997), which reduce the medium dielectric constant and protein molecules hydration, causing denaturation and, consequently, reducing protein solubility (Ribeiro & Seravalli, 2007).

Concerning the importance of new products development, the search for alternative protein sources for food and beverage application, and the scarce utilization of jackfruit seeds, the objectives of this study are: to determine protein solubility of jackfruit (*Artocarpus integrifolia* L) seed flour; to evaluate the effect of pH and sulfate, chloride and trisodium citrate salts concentration on protein solubility.

## **2. Methodology**

The present work was conducted in the Laboratory of Process Engineering at the Southwest Bahia State University (UESB). Jackfruits were obtained at the local market, washed, peeled, and pulp was removed. Seeds were washed in water, grinded in an industrial blender, sieved using a n° 10 sieve, and washed in running water. After filtration the residual part was oven dried in 20 °C for 24 h and grinded in order to obtain typical flour texture. In sequence, the product was stored in plastic containers under room temperature.

## 2.1 Effect of pH and salt concentration in jackfruit seed flour solubility

Solubility of jackfruit seed flour was determined using the methodology proposed by Machado et al. (2007), modifying pH values. 0.5 g of jackfruit seed flour of known protein content was weighed and 100 ml of buffer solution was added. Buffer solution of pH 4.0, 6.0 and 8.0 were adjusted with disodium phosphate (0.05M) and citric acid (0.05 M), hydrochloric acid (0.05 mol/L) and disodium phosphate (0.05M) in pH 2.0 and with disodium phosphate (0.05 mol/L) and sodium carbonate (0.05 mol/L) in pH 10.0. The material was magnetically stirred for 30 minutes at constant speed and room temperature ( $25 \pm 3$  °C). In sequence, the material was centrifuged at 1200xG for 30 min. Supernatant aliquots were collected for protein analysis according to the method used by Bradford (1976).

Salts of sodium chloride, sodium sulfate and trisodium citrate were used in concentrations of 0.25mol/L, 0.50 mol/L, 0.75 mol/L and 1.0 mol/L.

Solubility (S, g protein/ 100 g solution) was defined as the protein amount in the supernatant, according to Equation 1:

$$\%S = \frac{MP}{MS} \times 100 \quad (1)$$

Where: MP is the protein in the supernatant (g) and MS is the supernatant (g), calculated from the density found using the pycnometer method.

## 2.2 Experimental Design and Statistical Analysis

The experiment was conducted according to a completely random experimental design in a factorial block of five pH values, five salt concentrations and two repetitions. Experimental data was analyzed using the procedure PROC GLM from SAS software (SAS version 9.1). Some were tested by Analysis of Variance, and once appropriate, through Turkey test for average comparisons. The level of significance was equal or lower than 5%.

## 3. Results and Discussion

Solubility was related to pH (2.0, 4.0, 6.0, 8.0, 10.0) and trisodium citrate, sodium chloride and sodium sulfate concentrations (0.25 mol/L, 0.50 mol/L, 0.75 mol/L and 1.0 mol/L).

In Pictures 1, 2 and 3 it is possible to observe that protein solubility was higher in more acidic (pH=2.0) and alkaline ( $8.0 \leq \text{pH} \leq 10.0$ ) mediums, in the absence of salts, in accordance to values found in literature for plant protein, such as protein in rice flour (Cao et al., 2009), chickpeas, lentils and beans (Boye et al., 2010), and potato protein (Koningsveld et al., 2001).

Some neutral salts, among them NaCl and KCl, promote salting in effect, in another words, the increase of solubility even in high molar concentrations. The effect of salting in occurs due to low concentration of ions and their interaction with charged groups, such as proteins, progressively increasing their electronegativity and therefore, intensifying intermolecular electrostatic repulsion (Arogundade et al., 2004).

Studies concerning characterization of additives and salts effect on protein solubility have been often conducted in order to understand solubility profiles for its application in food industries. In the present study, the influence of three salts on jackfruit seed flour solubility was analyzed: trisodium citrate, sodium chloride and sodium sulfate.

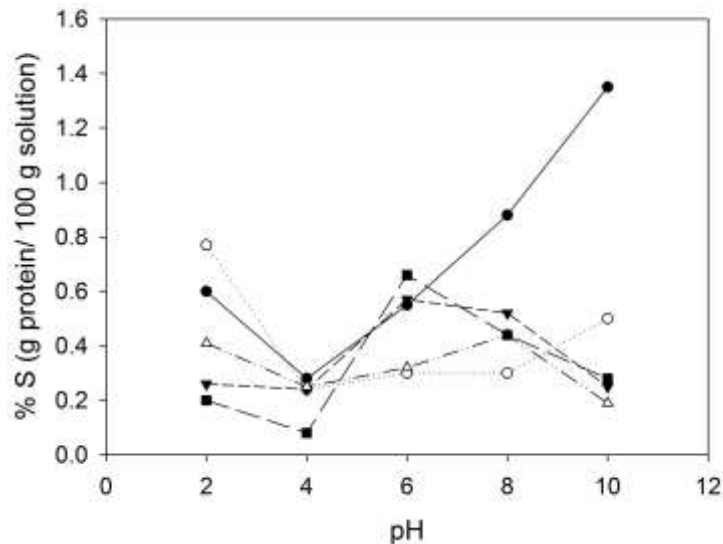
### 3.1 Trisodium Citrate

The effect of pH and trisodium citrate concentration on protein solubility of jackfruit seed flour is presented in Figure 1. For all concentrations under analysis jackfruit seed flour protein presented the lowest solubility in pH 4.0. This result suggests that the isoelectric point of the protein is around pH 4.0. This result was also observant by Zhang et al. (2019) and Ulloa et al. (2017) where the minimum solubility of jackfruit seed was also found to be at pH 4.0. At the isoelectric point, the reduced interaction between water and protein enhances protein-protein interactions, contributing to protein aggregation and precipitation, which disfavors solubility (Zhang et al., 2019).

In Figure 1 it is possible to observe that protein solubility slightly increases as salt concentration decreases, phenomenon which is not observed for most leguminous seeds, especially around the isoelectric region in which solubility is higher in the presence of salts, such as lentils (*Lens Culinaris Medik*) (Neves et al., 1998).

Solubility peak occurs in pH 6.0 and 1.0 mol/L and the lowest solubility is found in pH 8.0 and 0.25 mol/L, which promotes pI displacement. The behavior can be explained by the interaction between added salts and other substances used in pH correction solution.

**Figure 1.** Solubility curves of jackfruit seed flour protein as a function of pH (2.0 and 10.0) and trisodium citrate concentration varying from 0 to 1.0 mol/L, T = 25°C. ● 0.0 mol/L; ○ 0.25 mol/L; ▼ 0.50 mol/L; △ 0.75 mol/L; ■ 1.0 mol/L



Source: Research data.

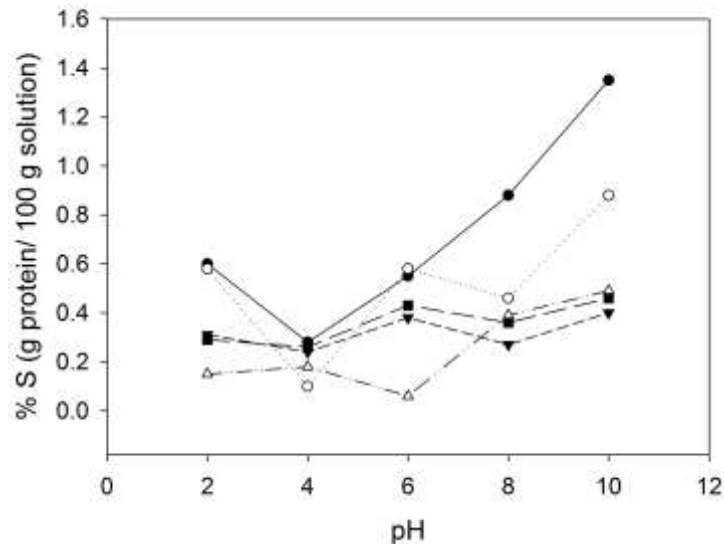
From Figure 1 it is possible to infer that the best solubility conditions using trisodium citrate was pH 2.0 and 0.25 mol/L and pH 6.0 and 1.0 mol/L. Intermediary concentrations of 0.50 mol/L also presented reasonable solubility in the basic regions.

### 3.2 Sodium Chloride

The effect of pH and sodium chloride concentration on protein solubility of jackfruit seed flour is presented in Figure 2.

For almost all concentrations under analysis jackfruit seed flour protein presented the lowest solubility in pH 4.0, except for 0.75 mol/L, in which the lowest solubility occurred in pH 6.0. However, according to Zhang et al. (2019) the isoelectric point of the protein is around pH 4.

**Figure 2.** Solubility curves of jackfruit seed flour protein as a function of pH (2.0 and 10.0) and sodium chloride concentration varying from 0.025 to 1.0 mol/L, T= 25 °C. ● 0.0 mol/L; ○ 0.25 mol/L; ▼ 0.50 mol/L; △ 0.75 mol/L; ■ 1.0 mol/L.



Source: Research data.

Solubility decreased with salt concentration increase in the system. Consequently, NaCl, which is the salt used more often for increasing protein solubility in most leguminous seed, did not present satisfactory results. Chickpea flour (*Cicer arietnum* L.) solubility was higher in the presence of 1.0 M NaCl (Silva et al., 2001).

Other proteins, such as raw beans (*Phaseolus vulgaris*) (Carbonaro et al., 1993), pea (Chavan et al., 2001), sweet and sour lupine flour (El-Adawy et al., 2001), potato protein (Koningsveld et al., 2001), wheat gluten (Mejri et al., 2005), lentil flour (*Lens culinaris Medik*) (Neves et al., 1998), soy flour (McWatters & Holmes, 1979) and sesame flour (Inyang & Iduh, 1996), also became more soluble in the presence of NaCl, especially in the isoelectric region, behaving as proteins classified as globuline, proteins which increase their solubility in the presence of salts (Sgarbieri, 1996).

From Figure 2 one can observe that in 0.75 mol/L concentration, the isoelectric point of jackfruit seed flour protein changed from pH 4.0 to pH 6.0, in accordance to Sgarbieri (1996). In this study the best solubility condition using sodium chloride occurred in concentration of 0.25 mol/L, under pH 2.0, 6.0 and 10.0.



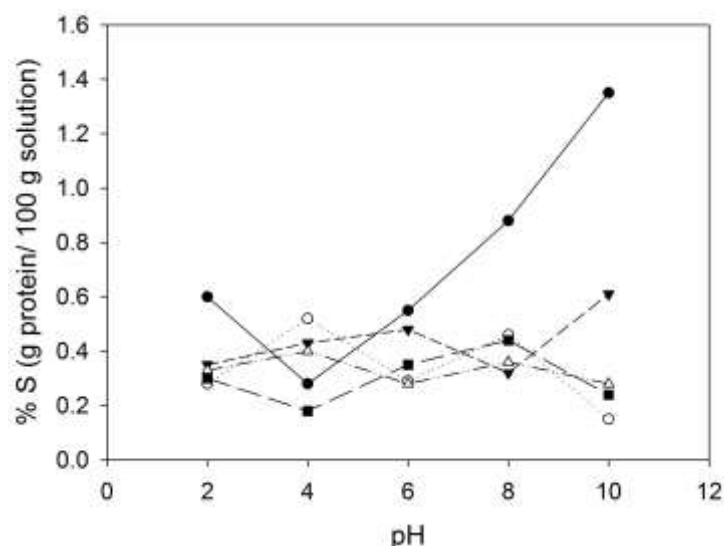
### 3.3 Sodium Sulfate

The effect of pH and sodium sulfate concentration on protein solubility of jackfruit seed flour is shown in Figure 3. Within the concentrations under analysis there was a solubility increase in the isoelectric point, except for 1.0 mol/L, differing from the effects of trisodium citrate. This effect is observed for most of leguminous seeds, especially in the isoelectric region where solubility is higher in salt presence, such as sweet and sour lupine flour (El-Adawy et al., 2001), soy flour (McWatters & Holmes, 1979), sesame protein concentrates (Inyang & Iduh, 1996) and wheat gluten (Mejri et al., 2005).

It was also observed that the lowest solubility occurred in extreme pH values (basic or acid) under all concentrations. Sousa (2007) explained that this can occur because in extreme pH values protein can denature and expose more hydrophobic groups due to weakening of electrostatic bonds' forces. Therefore, protein molecules aggregate and precipitate.

The highest solubility using sodium sulfate occurred under pH 4.0 and 8.0 in 0.25 mol/L and under pH 6.0 and 10.0 in 0.50 mol/L.

**Figure 3.** Solubility curve of jackfruit seed flour protein as a function of pH (2.0 and 10.0) and sodium sulfate varying from 0.0 to 1.0 mol/L, T= 25°C. ● 0.0 mol/L; ○ 0.25 mol/L; ▼ 0.50 mol/L; △ 0.75 mol/L; ■ 1.0 mol/L.



Source: Research data.

Fontanari (2007) reported a decrease in solubility of guava seed protein isolate as sodium chloride concentration increased. According to León (2008), trisodium citrate

presented higher salting-out effect than sodium sulfate. The author stated that citrate ions decrease protein solubility due to an increase of hydrophobic interactions and molecule aggregation caused by ions competition for the water molecule. In this study the opposite occurred: the salt which caused higher solubility was trisodium citrate.

Some salts concentration decreased protein solubility of jackfruit seed flour. The fact can be explained by the salting out effect due to high concentration of some salts in protein solutions or due to the presence of other components in flour, such as carbohydrates and water soluble fibers.

A polynomial model was used for adjusting data related to the emulsifying capacity of jackfruit seed flour compared to sulfate (Equation 2), citrate (Equation 3), and trisodium chloride (Equation 4):

$$y = a - bCs - cCS^2 - dpH + e pH^2 \quad (2)$$

$$y = a - bCs - cCS^2 - dpH + eCs * pH \quad (3)$$

$$y = a + bCs - cCs^2 \quad (4)$$

Where: Cs is NaCl concentration (mol/L), a- represents the constants of the estimated equation.

The models proposed by the Equations were significative (P<0.05) for explaining the emulsifying capacity variation as a function of pH and salt concentration, presenting R<sup>2</sup>=0.82 for sulfate and citrate and R<sup>2</sup>=0.99 for chloride.

Coefficients estimations for Equations 1, 2 and 3 are presented in Table 1.

**Table 1.** Coefficient estimation and Coefficient of determination for adjusted equations.

A	b	C	D	E	R <sup>2</sup>
1162.30	53.73	0.44	146.08	13.00	0.82
304.96	50.34	0.22	72.67	1.69	0.82
869.58	33.78	0.21			0.99

Source: Research data.

#### 4. Final Considerations

Protein content of jackfruit seed flour is 12%, which is characterized as protein source for human diet. Solubility of jackfruit seed flour protein is influenced by pH and salt

concentrations variation. In this study the best system was the one using trisodium citrate 0.25 mol/L. Based on these results, it is believed that this work will encourage the development of new research regarding the properties involved in protein solubility. In addition, it is concluded that jackfruit seed flour can be used as an alternative source of food protein for developing new products and as a substitute of products already available in the market.

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