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**Microbiological and physicochemical evaluation of Biquinho-type peppers from different markets.** Avaliação microbiológica e físico-química de pimentas do tipo Biquinho de diferentes mercados.

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## Abstract

Given that storage conditions can affect the composition and commercial viability of peppers, this study aimed to evaluate the quality of Biquinho peppers sold in four establishments in Southeastern Brazil. Analyses were performed to assess firmness, color, pH, soluble solids, titratable acidity, moisture, total phenolics, antioxidant activity, water activity, and microbiological quality of the peppers. The levels of the compounds analyzed in the Biquinho peppers varied according to the sampling location. The peppers from market 'A' presented the best antioxidant, phenolics and soluble solids levels. The peppers presented microbiological contamination, emphasizing the importance of care in handling the fruits.

**Keywords:** Storage. Antioxidant activity. *Capsicum chinense*. Quality. Post-harvest.

## Resumo

Tendo em vista que as condições de armazenamento podem afetar a composição e a viabilidade comercial de pimentas, este trabalho teve como objetivo avaliar a qualidade de pimentas Biquinho comercializadas em quatro estabelecimentos do Sudeste do Brasil. Foram realizadas análises quanto a firmeza, cor, pH, sólidos solúveis, acidez titulável, umidade, fenólicos totais, atividade antioxidante, atividade de água e qualidade microbiológica das pimentas. Os teores dos compostos analisados nas pimentas Biquinho variaram de acordo com o local de amostragem. As pimentas do mercado 'A' apresentaram os melhores teores antioxidante, fenólicos e de sólidos

solúveis. As pimentas apresentaram contaminação microbiológica, enfatizando a importância dos cuidados no manuseio dos frutos.

**Palavras-chave:** Armazenamento. Atividade antioxidante. *Capsicum chinense*. Qualidade. Pós-colheita.

## Introduction

*Capsicum chinense* is a species of chili pepper widely cultivated and consumed in Brazil and various parts of the world (FAO, 2020). This species is characterized by a wide range of fruit shapes, sizes, colors, and pungency levels due to the contents of capsaicinoids (JOSHI et al., 2022; HENG et al., 2023). Thus, the wide-ranging diversity of *C. chinense* peppers, as well as their potential health-promoting effects, has attracted interest for fresh consumption and the food industry, owing to their desirable sensory and nutritional properties (GUZMAN et al., 2021; CRUZ et al., 2022). Biquinho-type pepper is one of the varieties of *C. chinense*, and are marketed widely across Brazil, being available both in fresh and canned forms. This cultivar is characterized by its distinctive sweet, aromatic, and flavorful fruits, making it a popular choice among consumers (DANTAS; ARAÚJO, 2015; DANTAS et al., 2017).

The postharvest quality of fresh peppers is influenced by a combination of physiological and pathological factors (SMITH et al., 2006; PORAT et al., 2018). The high perishability of peppers can be primarily attributed to water loss and wilting (BERNARDO et al., 2015; ULLAH et al., 2017; WANG et al., 2023). The rate of these processes is directly related, mainly, to low relative humidity and inappropriate temperature during storage (PORAT et al., 2018; SANATOMBI; RAJKUMARI, 2020). Poor postharvest handling can lead to changes in the fruit's characteristics, making them visually unattractive and unsuitable for consumption (OLIVEIRA et al., 2018).

Some studies have already been carried out to evaluate microbiological contamination (CÁRDENAS et al., 2013; HARADA et al., 2017; LIMA et al., 2019), as well as to characterize the physicochemical attributes (BORGES et al., 2015) of peppers sold in markets. However, few studies have evaluated both microbiological quality and physicochemical characteristics of Biquinho-type peppers sold in their fresh form.

Given these considerations, the present study aimed to evaluate the physicochemical and microbiological attributes of Biquinho-type peppers (*C. chinense*) available in local establishments in a city in the southeastern region of Brazil.

## Materials and methods

### Plant material, location and treatment

Four different markets, identified as 'A', 'B', 'C', and 'D', located in the city of Lavras – MG, Brazil, were selected for sample collection. From each market, two packages of Biquinho peppers were collected. During sample collection, the temperature and storage conditions were verified.

After collection, the samples were homogenized in a single container, obtaining single samples that were evaluated. Physicochemical and microbiological analyses were performed at the postharvest of fruits and vegetables laboratory and food microbiology laboratory, respectively, at the Department of Food Science, of the Federal University of Lavras (UFLA), Brazil.

### Firmness

The firmness was measured on the equatorial region of the peppers using a manual penetrometer (Instrutherm Ltda., PTR-300, São Paulo, SP, Brazil), with a 5 mm diameter probe. Each measurement was made on a randomly chosen pepper and was expressed in Newton (N). Twenty peppers were selected for analysis.

### Color

Color was measured with a colorimeter (Konica Minolta CR-400, Osaka, Japan) using L\* C\* h° color space: lightness (L\*), chromaticity (C\*) and hue angle (h°). Each pepper surface reading was taken on a randomly chosen fruit. Twenty peppers were selected for analysis.

## **pH, soluble solids and titratable acidity**

Analyses were carried out based on the methodology described by the Instituto Adolfo Lutz (IAL, 2008), following the official physicochemical methods for food analysis (procedures 016/IV, 017/IV, and 315/IV). pH was determined using a pHmeter (Tecnal<sup>®</sup>, R-TEC-7-MP, Piracicaba, SP, Brazil) by dilution of 3 g of homogenized sample into 30 mL of distilled water. The soluble solids content was determined using a digital refractometer (Atago PAL-1, Tokyo, Japan). Results were expressed in percentages. The titratable acidity was performed by titration of 3 g of sample diluted into 30 mL distilled water with 0.1 N sodium hydroxide using phenolphthalein as indicator. Results were expressed as citric acid ( $\text{g } 100 \text{ g}^{-1} \text{ FW}$ ). The ratio between soluble solids and titratable acidity was calculated.

## **Moisture**

The moisture content was determined using the gravimetric method through oven drying (Fanem, 315 SE, São Paulo, Brazil), as described by Instituto Adolfo Lutz (IAL, 2008) (procedure 012/IV). At a temperature of 105°C, 5 g of fresh Biquinho peppers were dried until a constant mass was reached.

## **Extract for total phenolic compounds and antioxidant activity determination**

The extracts were obtained following the procedure described by Waterhouse (2002), using acetone 70% and methanol 50% as solvents.

## **Total phenolics**

The determination was performed according to the method described by Waterhouse (2002), using Folin-Ciocalteu reagents. Absorbance readings of supernatant at 720 nm were taken using a spectrophotometer (Biochron, EZ Read 2000, Cambridge, United Kingdom). A blank prepared with distilled water was used as control. The content of total phenolics for each sample was determined by using a standard curve developed with gallic acid (GAE), expressed in  $\text{mg } 100 \text{ g}^{-1}$ .

## **Total antioxidant activity by the phosphomolybdenum assay**

The determination followed the procedure described by Prieto et al. (1999). Readings were performed in a spectrophotometer at 695 nm. A blank prepared with distilled water was used as control. The results were expressed in ascorbic acid ( $\text{mg } 100 \text{ g}^{-1}$ ).

### **Water activity**

Water activity was determined using the AquaLab equipment (METER, 4TE, Pullman, USA), with approximately 10 g of sample used for the analysis.

### **Microbiological analysis**

The microbiological analysis was conducted according to Silva et al. (2021). For analytical units, 25 g of pepper were added 225 mL of 0.1% peptone water (1:10), then homogenized in a Stomacher-type homogenizer (Metroterm<sup>®</sup>, 1204M, Porto Alegre, Brazil) at 490 strokes  $\text{min}^{-1}$  for 3 min. An aliquot of the solution was then serially diluted in sterile peptone water 0.1% (1:10). For molds and yeast counts, aliquots of 0.1 mL of each dilution were withdrawn and plated onto Dichloran Rose Bengal Chloramphenicol (DRBC) agar, followed by incubation for 5 to 7 days at 25 °C. For enterobacteria and mesophilic aerobes, 1 mL aliquots were inoculated onto sterile and dry plates, and then culture medium was subsequently poured over the plates. Lactose Bile Violet Agar (VRBG) was used with an over layer for enterobacteria, and Soy Tryptone Agar (TSA) for mesophilic aerobes. All plates were incubated in B.O.D (SOLAB científica, SL-200, Piracicaba, Brazil) at 37 °C for 24h to 48 h. Culture media used were Himedia (HiMedia Laboratories, Mumbai, Maharashtra, India).

### **Statistical analysis**

A completely randomized design (CRD) was used, with 20 replicates for color and firmness analysis and five replicates for the other analyses. The microbiological analyses were performed with three replicates, in duplicate. Data were analyzed using a one-way analysis of variance – ANOVA. Means of the treatments ('A', 'B', 'C', and 'D'), the Tukey test was applied at the 0.05 level of significance. The data were analyzed using SAS university version. The graphs were prepared using the program OriginLab version 2021.

## Results and discussion

The optimum storage temperature for chili ranges from 7 °C – 7.5 °C, and the use of packaging contributes to the maintenance of their postharvest quality by reducing wilting and water loss (KADER, 2002). In market 'A', the samples were packaged within polyethylene terephthalate (PET) trays with lids and maintained at a temperature of approximately 22 °C. In market 'B' and 'D', the fruits were packed within expanded polystyrene trays (EPS) covered with polyvinyl chloride (PVC) and maintained at a temperature of 4 °C. The peppers from market 'C' were packed in EPS and wrapped with PVC, maintained at a temperature of approximately 25 °C.

In all four markets, it was noticed that the optimal storage temperature for chili peppers was not used, which can directly impact the overall quality of the fruits. If the fruits are stored for a prolonged period, this could result in water loss for fruits from markets 'A' and 'C', as well as chilling injury and decay for fruits from markets 'B' and 'D' (CHITARRA; CHITARRA, 2005; SIDDIQUI, 2015; YAHIA, 2019).

Visually, the fruits showed variations in terms of shape (width and length) and color (Figure 1). The presence of greenish-colored fruits suggests that they might have been harvested before reaching the optimal stage of ripeness. Also, the difference in red color among the fruits could potentially signify their classification into different cultivars. Notably, only the peppers from market 'B' were accompanied by traceability label, providing detailed information about their origin and characteristics.

The fruits from the four markets did not differ significantly from each other for water activity, with the observed values (0.89) within the range considered suitable for microbiological growth (PARK et al., 2014; LIMA et al., 2023). It is important to note that bacteria generally require higher water activity values compared to fungi for optimal development. In particular, only a few spoilage bacteria tend to thrive in environments with water activity below 0.91, while fungi tend to exhibit better growth in environments with water activity around 0.85 (JAY et al., 2005; HOLBAN; GRUMEZESCU, 2018).



Figure 1 – Biquinho peppers collected from four different markets ('A', 'B', 'C' and 'D').

The Biquinho pepper samples obtained from market 'A' showed a mold and yeast count of  $4.02 \log \text{CFU g}^{-1}$ . Samples from market 'B' showed  $5.86 \log \text{CFU g}^{-1}$ , while those from market 'C' reached  $5.17 \log \text{CFU g}^{-1}$ , and in market 'D', the count was  $3.96 \log \text{CFU g}^{-1}$ . These high incidences contribute to a decline in product quality and shelf life. Consequently, it can lead to substantial postharvest losses with a potential economic impact on the value chain, reducing availability, increasing prices, and potentially lower quality peppers reaching consumers (JAY, 2005; MADIGAN et al., 2016; HOLBAN; GRUMEZESCU, 2018). The fruits from market 'B' exhibited large and visible mold colonies during sampling.

Mesophilic aerobes and enterobacteria are commonly used as microbiological quality indicators for fresh vegetables and in the food industry (JAY, 2005; HOLBAN; GRUMEZESCU, 2018; GUO et al., 2022;). The elevated counts of mesophilic aerobes in the Biquinho pepper samples from the market's 'A', 'C' and 'D' can indicate improper handling hygiene. The adoption of Good Agricultural Practices (GAP) (EMBRAPA, 2004) is fundamental to guarantee safety fruits to consumers.

In market 'A', the count of mesophilic aerobes at  $4.22 \log \text{CFU g}^{-1}$  was found in chili peppers. The fruits from market 'C' showed a count of  $4.83 \log \text{CFU g}^{-1}$ , while the fruits from market 'D' showed a count of  $4.69 \log \text{CFU g}^{-1}$ . In market 'B', the counts were approximately one logarithmic cycle less, at  $3.49 \log \text{CFU g}^{-1}$ .

Regarding the presence of enterobacteria in fruits, they consistently showed a low count despite the markets of study. In market 'A', a count of  $3.90 \log \text{UFC g}^{-1}$  was observed, while in market 'B', the fruits showed a count of  $3.18 \log \text{UFC g}^{-1}$ . In markets 'C' and 'D', the counts reached  $3.88 \log \text{UFC g}^{-1}$  and  $3.95 \log \text{UFC g}^{-1}$ , respectively.

The presence of a high count of molds and yeasts and aerobic mesophilic microorganisms on the pepper samples can indicate poor hygiene practices during production, processing, or storage. It may also suggest the presence of other microorganisms with the potential to cause foodborne illness.

Therefore, it is essential to ensure proper sanitation and hygiene practices throughout the production and handling of Biquinho peppers to minimize the risk of microbial contamination. This includes implementing good agricultural practices, maintaining clean handling facilities, and appropriate temperatures for these fruits during storage and transportation. Implementing these preventive measures is essential for maintaining the safety and quality of chili peppers.

The exocarp color, firmness, soluble solids and acid content have been used as indicators to determine the ideal harvest time of peppers, so that their quality attributes can be maintained for a longer period during the storage and commercialization (TADESSE et al., 2002; ABUD et al., 2018). Color is an important quality characteristic for chili peppers as it indicates the level of maturity, visual appeal, and adherence to market standards (CHITARRA; CHITARRA, 2005; BRÜCKNER; WYLLIE, 2008; TEIXEIRA, 2009).

The fruits from market 'D' showed the highest  $L^*$  and  $h^\circ$  (Table 1), which can be attributed to the high number of green-colored Biquinho peppers in the package, indicating harvest before the complete ripening process. The fruits showed no significant difference in relation to the  $C^*$ . The values ranged from 61.56 to 64.97, indicating homogeneity in the color saturation of the fruits.

Comparing the samples from the four markets, an association between firmness and color was observed as expected. Samples from market 'A' exhibited the lowest firmness and an intense red color, visually appearing to have been harvested at adequate stage of maturation for consumption (Table 1). Generally, the ripening of fruit is also associated to breakdown of cell wall compounds that leads to its softening (YAHIA, 2019).

Table 1 – Physical and physicochemical characterization of Biquinho peppers from different markets

Quality characterization	Markets			
	A	B	C	D
<b>Firmness (N)</b>	7.38±1.12 <sup>c</sup>	9.16±1.55 <sup>b</sup>	9.96±1.55 <sup>b</sup>	11.45±1.54 <sup>a</sup>
<b>L*</b>	52.62±3.28 <sup>bc</sup>	49.17±5.12 <sup>c</sup>	54.40±4.23 <sup>b</sup>	59.91±6.52 <sup>a</sup>
<b>h°</b>	39.29±3.07 <sup>b</sup>	41.35±5.28 <sup>b</sup>	42.73±5.28 <sup>b</sup>	51.94±5.99 <sup>a</sup>
<b>Moisture (%)</b>	88.19±0.24 <sup>c</sup>	90.02±0.20 <sup>ab</sup>	90.34±0.23 <sup>a</sup>	89.79±0.18 <sup>b</sup>
<b>pH</b>	4.91±0.03 <sup>b</sup>	5.04±0,07 <sup>a</sup>	4.88±0,07 <sup>b</sup>	4.77±0,04 <sup>c</sup>

<b>Soluble solids (%)</b>	7.26±0,60 <sup>a</sup>	5.94±0,60 <sup>b</sup>	6.16±0,60 <sup>b</sup>	6.60±0,00 <sup>ab</sup>
<b>Soluble solids/titratable acidity ratio</b>	12.89±1,07 <sup>a</sup>	11.10±0,80 <sup>b</sup>	11.83±0,92 <sup>ab</sup>	11.71±0,00 <sup>ab</sup>

Means followed by the same letter in the row do not differ by Tukey's test at 5% probability. Source: Elaborated by the authors. Lavras, Minas Gerais, Brazil, 2024.

It was also possible to make an association between the color of the peppers peel and the pH value. Market 'B' had the highest pH, while 'D' had the lowest (Table 1). It is known that the acidity of fruits tends to decrease as ripening progress (SIDDIQUI, 2015). Market 'D' presented the highest quantities of green peppers, evidencing their incomplete ripe. Consequently, the higher acid contents present in these peppers contributed to the observed lower pH value.

Soluble solids represent the number of solids dissolved in the juice or pulp of fruits and vegetables (CHITARRA; CHITARRA, 2005). The peppers from market 'A' and 'D' had the highest content of soluble solids (Table 1). Peppers from market 'A' appeared to have been harvested at an optimal stage of ripeness compared to the others, as evidenced by their less consistent firmness. The lower levels of soluble solids observed in peppers from markets 'B' and 'C', may be attributed to the timing of their harvest, as some of them were visibly harvested earlier due to their greenish coloration (Figure 1).

There were no significant differences for titratable acidity. The acidity levels found for the peppers from the four markets ranged on average from 0.52 g 100 g<sup>-1</sup> to 0.56 g 100 g<sup>-1</sup> citric acid. Other authors have reported similar levels of titratable acidity as the ones found in this study. Martinez et al. (2021) found citric acid values ranging from 0.36% to 0.66% in their study of *C. chinense* hybrids, while (BORGES et al., 2015) found values ranging from 0.15% to 0.56% citric acid. Titratable acidity is an important maturity attribute of chili peppers as it contributes to their flavor (CHITARRA; CHITARRA, 2005). Adequate acidity levels enhance taste and ensure quality and safety during storage and conservation (AROUCHA et al., 2010). The ratio between soluble solids and titratable acidity is often used as an important indicator of fruit maturity and flavor, as it relates the sugar content to the acid content (CHITARRA; CHITARRA, 2005; SIDDIQUI, 2015). There were no differences in titratable acidity; then, the lower ratio for peppers from market B can be explained by their low soluble solids content (Table 1).

Fruits from market 'A' had the lowest moisture content, while those from market 'C' had the highest. The lower moisture content in peppers from market 'A' may also have contributed to their decreased firmness (Table 1), as the turgor of fruits and vegetables is influenced by their water content (TAIZ et al., 2017). Malakar et al. (2019) and Segura-Campos et al. (2016) reported moisture content for *C. chinense* chilis comparable to those observed in this study.

Phenolic compounds play an antioxidative role in plants, protecting them from the harmful effects of sunlight, insect predators, and microbial pathogens (BUCHANAN et al., 2015; TAIZ et al., 2017). These compounds have been extensively studied in fruit and vegetables for their antioxidant activity, which contributes to their beneficial impact on health (SHAHIDI; YEO, 2016; GULSUNOGLU et al., 2019).

The market 'A' sample had the highest total phenolics content and antioxidant activity, while the market 'D' samples had the lowest (Figures 2A and 2B). The loss of green color in vegetables is related to the degradation of chlorophyll, which is responsible for the green pigment in plants. Conversely, the development of a red color, as observed in peppers and certain fruits, is associated with the presence of compounds such as carotenoids and flavonoids (BAE et al., 2012; TAIZ et al., 2017).

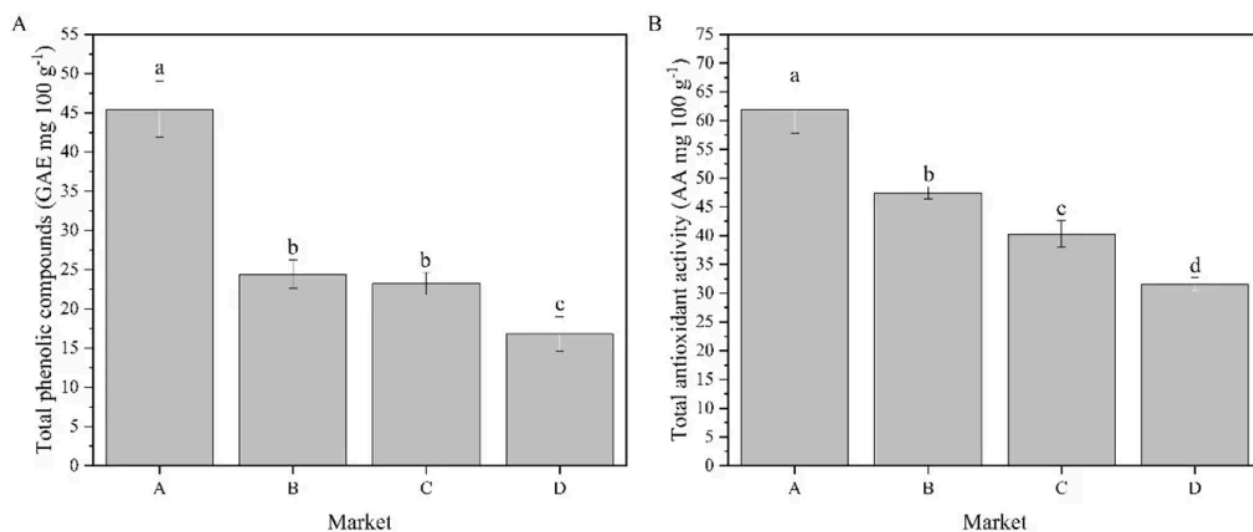


Figure 2 – (A) Total phenolic compounds (GAE mg 100 g<sup>-1</sup>) and (B) Total antioxidant activity (AA mg 100 g<sup>-1</sup>) of Biquinho-type peppers from markets A, B, C and D. \*Data represent the mean ( $\pm$  standard deviation) of 5 replicates by Tukey test at 5%. Means followed by the same letter do not differ from each other.

Ripe peppers are known to contain significant concentrations of compounds beneficial to human health, including flavonoids, carotenoids, vitamin C and capsaicinoids (BAE et al., 2012). These compounds also exhibit antioxidant properties (BUCHANAN et al., 2015). It can be inferred that as peppers advance in ripening stages, the concentration of these compounds is likely to increase, potentially enhancing their antioxidant function and nutritional value.

Therefore, the elevated content of phenolic compounds and antioxidant activity observed in the fruits from the market 'A' can be attributed to their ripening stage at the time of harvest, as these fruits exhibited a more intense red color than fruits from market 'D' (Table 1 and Figures 2A and 2B).

## Conclusion

The Biquinho pepper fruits from the sampled markets exhibited microbiological contamination, emphasizing the need for increased care in handling throughout the entire production chain to preserve produce safety. Furthermore, the color of these commercially available fruits, particularly, showed noticeable heterogeneity, indicating harvesting at different ripening stages and resulting in a lack of product standardization for consumers.

## Conflicts of interest

The authors had no conflicts of interest.

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## Authors' contributions

Carvalho, E. E. N.; Mendes, M. H. A.; Venâncio, A. H.; Honorato, D. R. S.; Souza, B. L. – conceptualization; Pereira, E. P. – data curation; Pereira, E. P.; Venâncio, A. H. – formal analysis; Carvalho, E. E. N.; Boas, E. V. B. V. – funding acquisition; Pereira, E. P.; Venâncio, A. H.; Honorato, D. R. S.; Souza, B. L.; Santos, I. A. – investigation; Mendes, M. H. A.; Pereira, E. P. – methodology; Carvalho, E. E. N.; Boas, E. V. B. V. – project administration and resources; Carvalho, E. E. N.; Boas, E. V. B. V.; Piccoli, R. H.; Pilon, L. – supervision; Carvalho, E. E. N.; Boas, E. V. B. V.; Piccoli, R. H.; Pilon, L.;

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