Soluções Sustentáveis: Enfrentando os Desafios do Clima e da Alimentação
9º SIMPÓSIO DE SEGURANÇA ALIMENTAR
19 A 21 DE MAIO DE 2025 | BENTO GONÇALVES | RS

# PRODUCTION OF OLIVE POMACE PULP EXTRACT, QUANTIFICATION AND EVALUATION OF STABILITY OF PHENOLIC COMPOUNDS

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ABSTRACT – Extra virgin olive oil production in Brazil has garnered interest over the years generating wastes including olive pomace, which represents approximately 80% of the processed fruits. This byproduct, rich in bioactive compounds such as phenolics, has great biological and innovative potential, but rarely reused due to the increase in the amount of waste. This study sought to develop a concentrated hydroalcoholic extract of olive pomace, with the aim of reducing waste disposal and adding value to the production chain. Hence, the extraction efficiency was evaluated, taking into account the yield of phenolic compounds. In addition, during 90 days of storage at 25 °C, the extract was analyzed for compound stability. The results showed that 70% ethanol provided a good yield in the extraction of polyphenols, with a high content of hydroxytyrosol and low degradation of the compounds over time.

KEYWORDS: Olive pomace; Phenolic compounds; Antioxidants; Waste.

## 1. INTRODUCTION.

Olive production has garnered interest in Brazil in recent years, especially in the state of Rio Grande do Sul (Monteiro *et al*. 2024). In this regard, the increase in olive oil production, and generation of waste from the process grows proportionally. Consequently, resulting in an economic and environmental problem, since olive pomace represents about 80% of the volume of processed fruits (Selim *et al.* 2022). Therefore, the reuse of olive pomace has increasingly been studied by researchers from different areas, considering that this byproduct is rich in nutrients and bioactive compounds, including phenolic compounds that have antioxidant, anti-inflammatory and antimicrobial activity (Monteiro *et al*. 2024).

Olive pomace is composed mainly of pits and pulp, which contain high content of phenolic compounds due to their polar structures, which causes them to migrate to the by-product after oil extraction (De Bruno *et al*. 2018). The production of extracts focusing on phenolic compounds becomes a means of reusing this residue to explore its biological activities as the basis for other

products, such as food supplements (Aronson, 2017). This study aimed to investigate the yield and stability of phenolic extracts produced from OP.

## 2. MATERIAL AND METHODS

### 2.1 Collection of olive pomace, processing and production of olive pulp extracts

The olive pomace (*Olea europaea* L.) was collected in Restinga Seca/RS and stored at -18 °C. For processing, a fruit pulper (model DES-60/1, Brazil) was used, which separated the pulp from the pits, and the pulp was stored and frozen at -18 °C. To produce the extracts, the olive pulp underwent an oven drying process for 6hr at 90 °C.

The dried olive pulp was weighed in 50 mL Falcon tubes (5 g of sample per tube), after which 20 mL of the extracting solvent, etanol:water (70:30 v/v) was added. The tubes were subjected to an ultrasound bath for 30 minutes, followed by shaking for 30 minutes in a rotary shaker (AGROT-BI, IONLAB, PR, Brazil). Thereafter, the sample was centrifuged at 3500 rpm for 10 minutes. The supernatant was collected and stored in amber bottles at 25 °C for 90 days. The extraction method was adapted from Lozano-Sánchez *et al.* (2011).

### 2.2 Stability of phenolic compounds during storage of extracts and determination of total phenolics by colorimetric method

To determine the stability of the phenolic compounds in the extracts during storage, aliquots were removed from the amber bottles at 1, 30, 60 and 90 days and the structures were analyzed. This technique was adapted from Singleton and Rossi (1965). The standard curve consisted of 10 points (10 to 100 $mg.L^{-1}$) of gallic acid, using a stock solution of 100 $mg.L^{-1}$ diluted in ethanol. Then 200 µL were removed from each vial and separated into two tubes with 100 µL in each. Afterwards, each were diluted 50 times and analyzed in triplicate. In each test tube, 400 µL of the solution and 400 µL of the diluted samples were added. Thereafter, 2000 µL of the Folin-Ciocalteu reagent (ratio 1:10) was added, followed by the addition of 1600 µL of 7.5% sodium carbonate. The tubes were shaken and kept in the dark for 2 hours. After this period, absorbance readings were taken using a spectrophotometer (Kasvi, K14-UV-VIS, Paraná, Brazil) at wavelength of 765 nm. The results were expressed in mg of EAG (gallic acid equivalent) per liter of extract (mg $EAG.L^{-1}$).

### 2.3 Quantification of phenolic compounds by HPLC-DAD-RF

Phenolic compounds were quantified using HPLC according to Speroni *et al.* (2019). The system used a C18 reversed-phase column and a mobile phase in a gradient of water with acetic acid

and methanol. The elution followed a 37-minute program with a flow rate of 0.6 mL.min$^{-1}$ at 25 °C. Detection was done using fluorescence for hydroxytyrosol, tyrosol, and oleuropein, with confirmation by diode array at 280 nm. Standard curves were constructed with high-purity compounds, and the analyses was carried out after 90 days of storage, a single time.

### 2.4 Statistical analysis

The analyses were performed in triplicate and the results were subjected to one-way analysis of variance (ANOVA), followed by Tukey's test ($p$<0.05), using GraphPad Prism 7.0 software.

## 3. RESULTS AND DISCUSSION

### 3.1 Stability of phenolic compounds during storage

Although the Folin-Ciocalteu method exhibits low specificity for phenolic compounds, the observed losses indicate oxidative degradation of phenols present in the extracts. This degradation is considered in the analysis, as it encompasses bioactive molecules involved in the process.

The stability of the extracted phenolic compounds plays a fundamental role in assessing the quality of the extracts over time. Furthermore, it is desired that there be minimal degradation rates, although to some extent losses are expected. As a result, it was observed that on day 1, the total phenolic content for the extract was 4230 ± 38.34 mg.L$^{-1}$ EAG, evidencing a high extraction efficiency of 70% ethanol as shown in Table 1.

**Table 1** – Analysis of the stability of total phenolic compounds (mg.L$^{-1}$ EAG) in olive pulp extracts for 90 days.

| **Extractor solvent** | **Storage days** | | | |
|---|---|---|---|---|
| | 1 | 30 | 60 | 90 |
| **Ethanol 70%** | 4230±38 a | 4250±23 a | 3939±19 b | 3763±8 c |

Identical lowercase letters indicate no significant differences ($p$>0.05) between the extracts. Different lowercase letters indicate significant differences ($p$>0.05) between the extracts.

The analysis of the stability of phenolic compounds in the first 30 days of storage did not indicate significant differences ($p$<0.05), with the variations being more pronounced only in the last two periods (60 and 90 days). These results reinforce the high stability of phenolic compounds in the first 30 days of conservation. After 90 days of storage, a degradation rate of 11% was observed in relation to day 1. In a shelf stability study carried out by Grigoletto *et al*. (2024), it was found that hydroalcoholic extracts presented a relatively low degradation of phenolic compounds in a period of two months, considering a loss of 15-20% acceptable. In this context, the extract presented stood out for yielding a rate of less than 15% within 90 days of storage.

Considering both extraction efficiency and conservation during storage, the concentration of phenolic compounds in a portion corresponding to one tablespoon (15 mL) was 63.45 mg for the extract. Such content is relevant for potential use as a dietary supplement, as long as the portion is adjusted in accordance with the guidelines of the Agência Nacional de Vigilância Sanitária (ANVISA). According to IN 102/21, for olive pulp extracts to be considered as "sources of phenolic compounds, they must have a minimum content of 16 mg and a maximum of 23.2 mg per portion".

### 3.2 Quantification of phenolic compounds by HPLC-DAD-RF

The quantification of phenolic compounds in the extract was performed after 90 days of storage and is presented in Table 2. The assay was conducted using HPLC-DAD-RF, focusing on the three main compounds: hydroxytyrosol, tyrosol, and oleuropein, with specific standard curves constructed for each of them. As shown in Table 2, hydroxytyrosol was the most abundant compound in the samples, followed by tyrosol, which corroborates the findings of Martins *et al.* (2024), who identified the predominance of these structures using different phenolic compound extraction techniques from olive pomace.

**Table 2** – Hydroxytyrosol, tyrosol and oleuropein content ($mg.L^{-1}$) in olive pulp extracts after 90 days of storage.

| Extractor solvent | Hydroxytyrosol | Tyrosol | Oleuropein | Total |
|---|---|---|---|---|
| Ethanol 70% | 996.4±1.9 a | 47.5 ±0.1 a | 56.4±2.1 a | 1100.3±0.1 a |

Identical lowercase letters indicate no significant differences ($p$>0.05) between the extracts.

The amount of hydroxytyrosol present in the extract is significant, since this compound has important biological activities that are widely discussed in the literature. This content is great relevance considering the requirements of the ANVISA by IN 304/24, which establishes that olive pulp extracts intended to function as a "source of hydroxytyrosol" in dietary supplements must contain between 5 mg and 35 mg per unspecified portion. Thus, in a tablespoon portion (15 mL) of the analyzed extract, a content of 15 mg of hydroxytyrosol was observed, which falls within the parameters established by ANVISA allowing the standardization of the product as long as the portion is defined.

The amount of tyrosol was 47.6 ± 0.1 $mg.L^{-1}$, which may vary over time, since it may originate from metabolic or chemical processes of hydroxytyrosol. This compound is frequently targeted by extractive processes due to its antioxidant activity, as reported by Marković *et al.* (2019). However, the transformation of hydroxytyrosol into tyrosol may affect the antioxidant potential of the extract, given that hydroxytyrosol has a greater free radical scavenging capacity compared to tyrosol, as

observed by Nieto *et al*. (2024), who compared the chemical structures of these compounds. This transformation can be considered an indication of extract degradation.

Although it is more abundant in olive leaves, oleuropein exhibited a concentration of 56.4 ± 2.1 $mg.L^{-1}$ in the analyzed extracts. Its complex molecular structure confers versatility, enabling multiple biological activities, particularly antioxidant and anti-inflammatory effects, as discussed by Vogel *et al.* (2014). As a result, it has been the focus of several studies aimed at its characterization and isolation.

## 4. CONCLUSION

The 70% ethanol resulted in a good and stable yield of phenolic compounds over 90 days of storage, with a significantly high concentration of hydroxytyrosol. This highlights the extract's potential as a basis for the development of new products, if dose standardization is established, and further studies are conducted to ensure product quality and safety. These should include long-term stability assessments, as well as microbiological and proportion-related evaluations.

## REFERENCES

ARONSON, J. K. Defining 'nutraceuticals': neither nutritious nor pharmaceutical. **British Journal of Clinical Pharmacology**, v. 83, n. 1, p. 8–19, 2017.

BRASIL. Instrução Normativa n°102 de 15 de outubro de 2021. **Diário Oficial da República Federativa do Brasil**, poder executivo, Brasília, DF, 15 out. 2021.

BRASIL. Instrução Normativa n°304 de 26 de junho de 2024. **Diário Oficial da República Federativa do Brasil**, poder executivo, Brasília, DF, 26 jun. 2024.

DE BRUNO, A., ROMEO, R., FEDELE, F. L., SICARI, A., PISCOPO, A., POIANA, M. Antioxidant activity shown by olive pomace extracts. **Journal of Environmental Science and Health**, Part. B, v. 53, n. 8, p. 526–533, 2018.

GRIGOLETTO, L., SALAS, P. G., VALLI, E., BENDINI, A., FERIOLI, F., PASINI, F., VILLASCLARAS, S. S., GARCIA-RUIZ, R., TOSCHI, T. G. HPLC-MS/MS phenolic characterization of olive pomace extracts obtained using an innovative mechanical approach. **Foods (Basel, Switzerland)**, v. 13, n. 2, p. 285, 2024.

LOZANO-SÁNCHEZ, J., GIAMBANELLI, E., QUIRANTES-PINÉ, R., CERRETANI, L. Wastes generated during the storage of extra virgin Olive oil as a natural source of phenolic compounds. **Journal of Agricultural and Food Chemistry**, v. 59, n. 21, p. 11491–11500, 2011.

MARTINS, V, F. R., RIBEIRO, T. B., LOPES, A. I., PINTADO, M. E., MORAIS, R, M, S. C., MORAIS, A, M, M. B. Comparison among different green extraction methods of polyphenolic compounds from exhausted olive oil pomace and the bioactivity of the extracts. **Molecules (Basel, Switzerland)**, v. 29, n. 9, 2024.

MARKOVIC, A. K., TORIC, J., BARBARIC, M., BRALA, C. J. Hydroxytyrosol, Tyrosol and Derivatives and Their Potential Effects on Human Health. **Molecules**. 2019.

MONTEIRO, C. S., ADEDARA, I. A., FAROMBI, E. O., EMANUELLI, T. Nutraceutical potential of olive pomace: insights from cell- based and clinical studies. **Journal of the Science of Food and Agriculture**, v. 104, n. 7, p. 3807–3815, 2024.

NIETO, S., LOZANO, I., RUIZ, F. J., COSTA, J. F., VILLA, R., LOZANO, P. Sustainable synthesis of new antioxidants from hydroxytyrosol by direct biocatalytic esterification in ionic liquids. **Molecules (Basel, Switzerland)**, v. 29, n. 21, 2024.

SELIM, S., ALBQMI, M., AL-SANEA, M. M., ALNUSAIRE, T. S., ALMUHAYAWI, M. S., ABDELGAWAD, H., AL JAOUNI, S. K., ELKELISH, A., HUSSEIN, S., WARRAD, M., EL-SAADONY, M. T. Valorizing the usage of olive leaves, bioactive compounds, biological activities, and food applications: A comprehensive review. **Frontiers in Nutrition**, v. 9, 2022.

SINGLETON, V. L., ROSSI, J. A., Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **American Journal of Enology and Viticulture**, v. 16, n. 3, p. 144–158, 1965.

SPERONI, C. S., STIEBE, J., GUERRA, D. R., BENDER, A, A. B., BALLUS, C. A., SANTOS, D. R., MORISSO, F, D. P., SILVA, L. P., EMANUELLI, T. Micronization and granulometric fractionation improve polyphenol content and antioxidant capacity of olive pomace. **Industrial Crops and Products**, v. 137, p. 347–355, 2019.

VOGEL, P., MACHADO, I. K., GARAVAGLIA, J., ZANI, V. T., DE SOUZA, D., DAL BOSCO, S. M. Polyphenols benefits of olive leaf (*Olea europaea* L.) to human health. **Nutr Hosp**. 2014.