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Quantificação de flavonoides em folhas trigo-sarraceno (*Fagopyrum esculentum* Moench*)*: uma avaliação comparativa entre o medidor portátil e a espectrofotometria

Quantification of flavonoids in buckwheat leaves (*Fagopyrum esculentum* Moench): a comparative assessment between the portable meter and spectrophotometry

Cuantificación de flavonoides en hojas de trigo sarraceno (*Fagopyrum esculentum* Moench): una evaluación comparativa entre el medidor portátil y la espectrofotometría

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Resumo
Os flavonoides são os principais metabólitos secundários do trigo-sarraceno (*Fagopyrum esculentum* Moench), porém sua quantificação laboratorial é onerosa e demorada,

demandando métodos mais rápidos e baratos. Portanto, este estudo objetivou comparar o teor de flavonoides totais entre o método de espectrofotometria e o medidor portátil MPM-100 em folhas de trigo-sarraceno. O experimento foi conduzido em Montes Claros-MG, com 20 indivíduos da cultivar IPR-91, em delineamento inteiramente casualizado. Após 46 dias, mediu-se o índice de flavonoides (IF) em três folhas por planta e determinou-se o teor de flavonoides totais (TFT) por espectrofotometria ($AlCl_3$) a 430 nm, utilizando curva de calibração com rutina. As maiores médias do IF foram os indivíduos 4 (1,25), 5 (1,14), 7 (1,22), 8 (1,27), 9 (1,35), 10 (1,21), 12 (1,19) e 17 (1,30) e do TFT os indivíduos 6 (37,62 mg g^{-1}), 7 (35,78 mg g^{-1}) e 9 (28,53 mg g^{-1}) pelo teste Scott-Knott ($p \leq 0,05$). A correlação de Pearson ($p \leq 0,01$) foi positiva ($r = 0,46$), porém baixa, indicando correspondência limitada entre os métodos. Conclui-se que o medidor portátil, embora promissor para avaliações rápidas, apresenta baixa correspondência com o método espectrofotométrico e há necessidade de calibração específica para espécie. Os resultados obtidos a partir dele devem ser confirmados com métodos laboratoriais de referência.
Palavras-chave: Cloreto de alumínio; Metabólito secundário; Rutina.

Abstract
Flavonoids are the main secondary metabolites of buckwheat (*Fagopyrum esculentum* Moench); however, their laboratory quantification is costly and time-consuming, requiring faster and more affordable methods. Therefore, this study aimed to compare total flavonoid content between the spectrophotometric method and the portable MPM-100 meter in buckwheat leaves. The experiment was conducted in Montes Claros, Minas Gerais, Brazil, with 20 individuals of the cultivar IPR-91, in a completely randomized design. After 46 days, the flavonoid index (FI) was measured in three leaves per plant, and total flavonoid content (TFC) was determined by spectrophotometry ($AlCl_3$) at 430 nm, using a rutin calibration curve. The highest mean FI values were observed in individuals 4 (1.25), 5 (1.14), 7 (1.22), 8 (1.27), 9 (1.35), 10 (1.21), 12 (1.19), and 17 (1.30), and for TFC in individuals 6 (37.62 mg g^{-1}), 7 (35.78 mg g^{-1}), and 9 (28.53 mg g^{-1}), according to the Scott-Knott test ($p \leq 0,05$). Pearson's correlation ($p \leq 0,05$) was positive ($r = 0.46$), but low, indicating limited correspondence between methods. It is concluded that the portable meter, although promising for rapid assessments, shows low agreement with the spectrophotometric method, and species-specific calibration is required. Results obtained using it should be confirmed with reference laboratory methods.
Keywords: Aluminum chloride; Rutin; Secondary metabolite.

Resumen
Los flavonoides son los principales metabolitos secundarios del trigo sarraceno (Fagopyrum esculentum Moench); sin embargo, su cuantificación en laboratorio es costosa y requiere mucho tiempo, lo que demanda métodos más rápidos y económicos. Por lo tanto, este estudio tuvo como objetivo comparar el contenido de flavonoides totales entre el método de espectrofotometría y el medidor portátil MPM-100 en hojas de trigo sarraceno. El experimento se llevó a cabo en Montes Claros, Minas Gerais, Brasil, con 20 individuos del cultivar IPR-91, en un diseño completamente al azar. Después de 46 días, se midió el índice de flavonoides (IF) en tres hojas por planta y se determinó el contenido de flavonoides totales (TFT) mediante espectrofotometría ($AlCl_3$) a 430 nm, utilizando una curva de calibración con rutina. Los mayores promedios de IF se observaron en los individuos 4 (1,25), 5 (1,14), 7 (1,22), 8 (1,27), 9 (1,35), 10 (1,21), 12 (1,19) y 17 (1,30), y para TFT en los individuos 6 (37,62 mg g^{-1}), 7 (35,78 mg g^{-1}) y 9 (28,53 mg g^{-1}), según la prueba de Scott-Knott ($p \leq 0,05$). La correlación de Pearson ($p \leq 0,01$) fue positiva ($r = 0,46$), pero baja,

indicando una correspondencia limitada entre los métodos. Se concluye que el medidor portátil, aunque prometedor para evaluaciones rápidas, presenta baja correspondencia con el método espectrofotométrico y existe la necesidad de una calibración específica para la especie. Los resultados obtenidos a partir de este deben confirmarse mediante métodos de laboratorio de referencia.
Palabras clave: Cloruro de aluminio; Metabolito secundario; Rutina.

1. Introduction

Buckwheat, also known as common buckwheat (*Fagopyrum esculentum* Moench), is a pseudocereal with a rich composition of nutrients and flavonoids, producing gluten-free flour as a by-product (FIGUEIRÓ *et al*., 2023; SILVA *et al*., 2002). It can be used in human and animal nutrition, in the pharmaceutical industry, and in agriculture (SILVA *et al*., 2002), for example, as a cover crop and for weed and erosion control. Additionally, buckwheat grows well in dystrophic soils and mountainous regions, requiring fewer inputs (JOSHI *et al*., 2019; ZHOU *et al*., 2016), which facilitates its cultivation. Among the flavonoids present in buckwheat, rutin stands out as a compound that contributes to plant resistance against pests and diseases. Rutin is extracted from the plant for medicinal use (MOMENTÉ *et al*., 2025), exhibiting antioxidant, antidiabetic, and anti-inflammatory activities (BECHO *et al*., 2009), and is one of the main compounds of pharmaceutical interest in buckwheat, being a focus of research on this species.

However, the quantification of total flavonoid content in plants is commonly performed using spectrophotometry (MARQUES *et al*., 2012), which requires high reagent consumption, skilled personnel, infrastructure, and robust and expensive equipment, in addition to being time-consuming. In both research and commercial production, the high cost and slow pace of laboratory analyses are limiting factors.

Therefore, studies aimed at identifying accurate, faster, and more accessible methods are needed to reduce financial and time costs, such as the use of portable meters that can be applied in the field. The method should be practical, low-cost, and non-destructive to plant material, similar to that used in grape studies (THAIPONG *et al*., 2006), in which the authors describe it as a complementary screening tool, upon prior calibration, for continuous crop monitoring. Thus, the objective of this study was to compare total flavonoid content obtained by the spectrophotometric method and a portable meter (MPM-100) in buckwheat leaves.

2. Methodology

The study was conducted at the Institute of Agricultural Sciences of the Federal University of Minas Gerais (ICA/UFMG), located in Montes Claros, Minas Gerais, Brazil. The experimental field is situated at 16°40′50″ S and 43°50′22″ W, at an altitude of 628.2 m above sea level. The mean annual temperature and precipitation in the region are 23.5 °C and 998.6 mm, respectively (INSTITUTO NACIONAL DE METEOROLOGIA, 2024), where the Cerrado biome predominates (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2024). The experiment was carried out from January to February 2024, totaling 46 days, coinciding with the peak of the reproductive period, at full flowering, prior to fruit maturation. A completely randomized design was used, with 20 buckwheat plants of the cultivar IPR-91 (Baili), developed and provided by the Instituto de Desenvolvimento Rural do Paraná (IAPAR-EMATER), with three replicates, each replicate consisting of one

leaf, totaling three leaves per plant, evaluated independently. The evaluated leaves were the third and fourth from the apex, as they were more expanded and not shaded by the canopy. Sowing was performed in seedling trays with approximately 14 mL per cell, filled with a commercial substrate (composition: pine bark, vermiculite, potassium nitrate, single superphosphate, and peat). After 15 days, the seedlings were transplanted to the field covered with plastic mulch, arranged in four rows, with spacing of 25 cm between plants and 20 cm between rows. Irrigation was performed by drip irrigation up to field capacity, with occasional manual irrigation.

At harvest, between 4:00 and 6:00 p.m., under no precipitation, the flavonoid index (FI) was measured using a portable meter (MPM-100), taking the average of three readings on each of the three evaluated leaves per plant. Subsequently, the leaf samples were placed in a forced-air circulation oven at 60 °C until constant weight was reached for dry matter determination, then ground and stored at 5 °C until total flavonoid content (TFC) analysis.

To obtain the extract for TFC determination, 2 mL of 80% methanol were added to 20 mg of ground sample. The mixture was vortexed for 30 seconds at approximately 25 °C (room temperature), in the dark. It was then centrifuged at 4000 rpm for 10 minutes. After centrifugation, the supernatant was filtered through filter paper (11 cm diameter, particle retention size 4–12 µm) and stored, following the method adapted from FERNANDES *et al*. (2022).

The solution for TFC determination was prepared by adding 5 mL of 1% aqueous $AlCl_3$ solution and 7 mL of distilled water to 1 mL of the previously obtained extract, followed by vortexing for 20 seconds and resting for 10 minutes in the dark at approximately 25 °C. Immediately afterward, absorbance readings were taken using a spectrophotometer at λ = 430 nm, using the aqueous aluminum chloride solution as the blank, with one reading per replicate, adapted from FERNANDES *et al*. (2022). TFC in the extract was determined using the equation obtained from the rutin standard curve: $y = 2.005x + 0.064$ ($r^2 = 0.991$) at λ = 430 nm, where “y” is absorbance and “x” is rutin concentration (mg mL^{-1}). No blank correction was applied, as it showed no absorbance. Subsequently, TFC was expressed as mg of rutin equivalents per gram of leaf dry matter, using the following equation:

$$\frac{\text{Total flavonoid content in the extract} * 1\ \text{g}}{0{,}01\ \text{g (leaf dry matter per mL of extract)}}$$

Data were subjected to analysis of variance using the F-test ($p \leq 0.05$). When significant, means were compared using the Scott-Knott test ($p \leq 0.05$). Additionally, Pearson correlation ($p \leq 0.01$) analysis was performed. All analyses were conducted using R software (R CORE TEAM, 2024).

3. Results and discussion

The mean FI values differed statistically; the highest means were observed in individuals 4 (1.25), 5 (1.14), 7 (1.22), 8 (1.27), 9 (1.35), 10 (1.21), 12 (1.19), and 17 (1.30), all of which showed higher phenotypic values under the evaluated conditions (Table 1). The ability to group individuals with higher and lower flavonoid content using the portable meter method is advantageous, as it allows this method to be used as an auxiliary and preliminary tool for relative screening, after species-specific calibration, of superior genotypes. This contributes to genetic improvement of the crop, as well as to the monitoring of commercial production and other research. Flavonoids have medicinal functions for humans and animals (MOMENTÉ *et al*., 2025; BECHO *et al*., 2009), in addition to protecting plants against solar

radiation, herbivory, and pathogenic agents, and also contributing to the brown coloration of grains (associated with higher commercial value) (CASSA *et al*., 2022; D'ALMEIDA, 2020).

Table 1. Evaluation of parameters in leaves of *Fagopyrum esculentum* (Moench) individuals grown in Minas Gerais, Brazil.

Individuals	Flavonoid Index	Total Flavonoid Content (mg g^{-1})
1	0.99 c	25.97 c
2	0.94 c	25.25 c
3	0.92 c	26.05 c
4	1.25 a	31.74 b
5	1.14 a	32.24 b
6	1.04 b	37.62 a
7	1.22 a	35.78 a
8	1.27 a	27.45 b
9	1.35 a	35.81 a
10	1.21 a	28.53 b
11	1.11 b	28.31 b
12	1.19 a	21.21 d
13	1.07 b	16.94 d
14	0.99 c	16.29 d
15	1.09 b	17.32 d
16	1.02 c	20.58 d
17	1.3 a	32.09 b
18	1.09 b	20.73 d
19	0.99 c	21.78 d
20	1.12 b	23.86 c
Coefficient of variation (%)	7	15.17
Mean	1.12	26.28

Means followed by the same letter in the columns do not differ from each other according to the Scott-Knott test ($p \leq 0.05$).
Source: Authors (2026).

When analyzing TFC results, significant differences among means were also observed; the highest values were found in individuals 6 (37.62 mg g^{-1}), 7 (35.78 mg g^{-1}), and 9 (28.53 mg g^{-1}), with the latter two coinciding with the highest means obtained using the portable meter method (Table 1). The practical implication of this overlap is that, with proper species-specific calibration, it will be possible to perform a preliminary, non-destructive field screening of individuals with higher TFC.

The TFC values were higher than those reported by Fernandes *et al*. (2024) (8.75 mg g^{-1}) in a study using the whole plant of the same buckwheat cultivar with the same extraction method. Because the entire plant was used, lower TFC values are expected due to other plant parts having lower flavonoid accumulation, such as the stem (KIM *et al*., 2023).

Leaves of buckwheat varieties from breeding programs cultivated in Poland, evaluated between 41 and 62 days after sowing (early flowering and flowering/seed formation, respectively), showed 51.17 and 82.37 mg g^{-1} of total flavonoids (ZIELIŃSKA *et al*., 2012). In another study with the species cultivated in Slovenia, approximately 35 to 105 mg g^{-1} of TFC were obtained at different phenological stages (TAVČAR *et al*., 2026). All these values are higher than those obtained in the present study, most likely due to more

advanced breeding for TFC and differences in environmental and experimental conditions, even when using $AlCl_3$ and spectrophotometry.

Figure 1 presents the grouping of means for FI and TFC obtained by the Scott-Knott test ($p \leq 0.05$) for better visualization and comparison. FI was grouped as follows: A (4, 5, 7, 8, 9, 10, 12, and 17), with a mean of 1.24; B (6, 11, 13, 15, 18, and 20), with a mean of 1.09; and C (1, 2, 3, 14, 16, and 19), with a mean of 0.98. TFC was grouped as follows: A (6, 7, and 9), with a mean of 36.40 mg g^{-1}; B (4, 5, 8, 10, 11, and 17), with a mean of 30.06 mg g^{-1}; C (1, 2, 3, and 20), with a mean of 25.28 mg g^{-1}; and D (12, 13, 14, 15, 16, 18, and 19), with a mean of 19.26 mg g^{-1}.

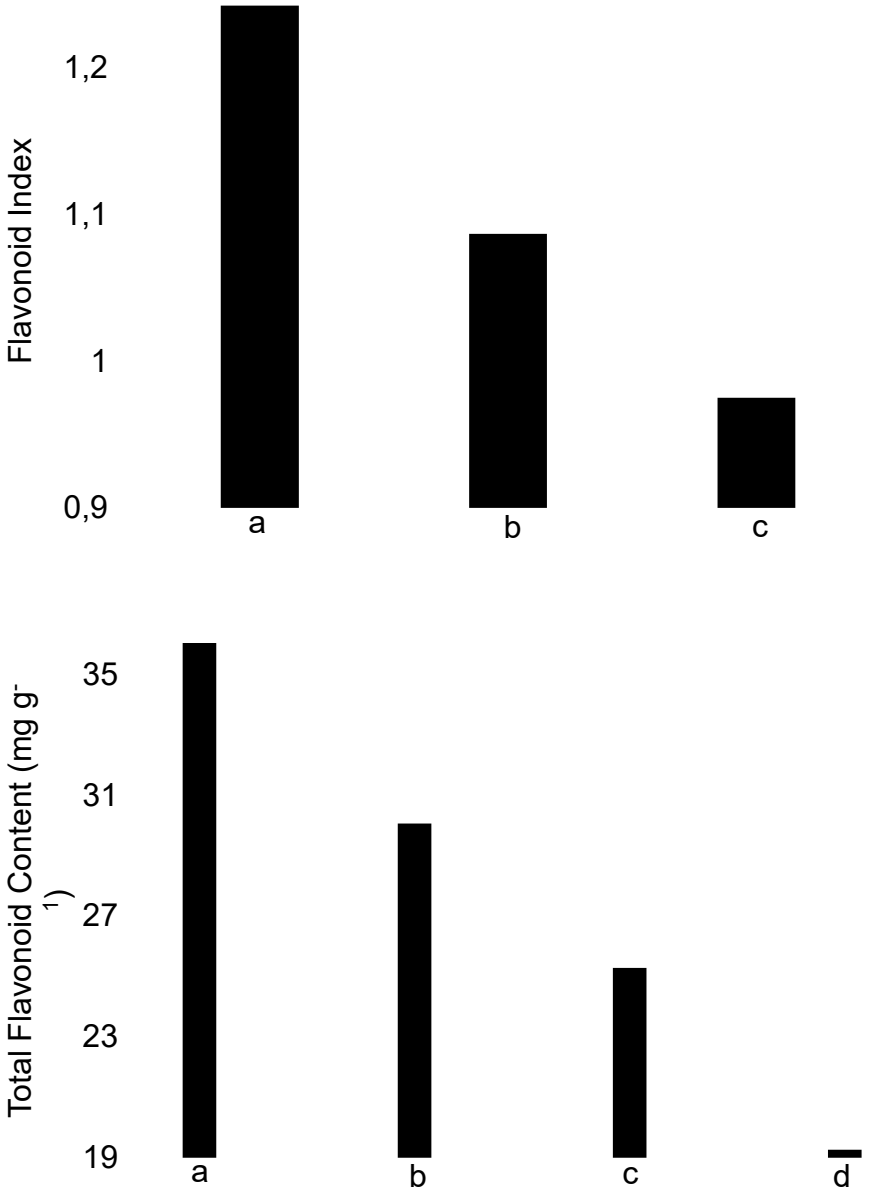


Figure 1. Grouping of means obtained by the Scott-Knott test ($p \leq 0.05$) for the flavonoid index (MPM-100) and total flavonoid content (spectrophotometry) in leaves of buckwheat individuals grown in Minas Gerais, Brazil.
Source: Authors (2026).

A significant positive correlation (r = 0.46) was observed between FI and TFC; however, it was low. Regression analysis between the parameters did not show a significant relationship (Figure 2). This indicates that there is only a linear association, but no agreement, precision, accuracy, or predictive ability between FI and TFC.

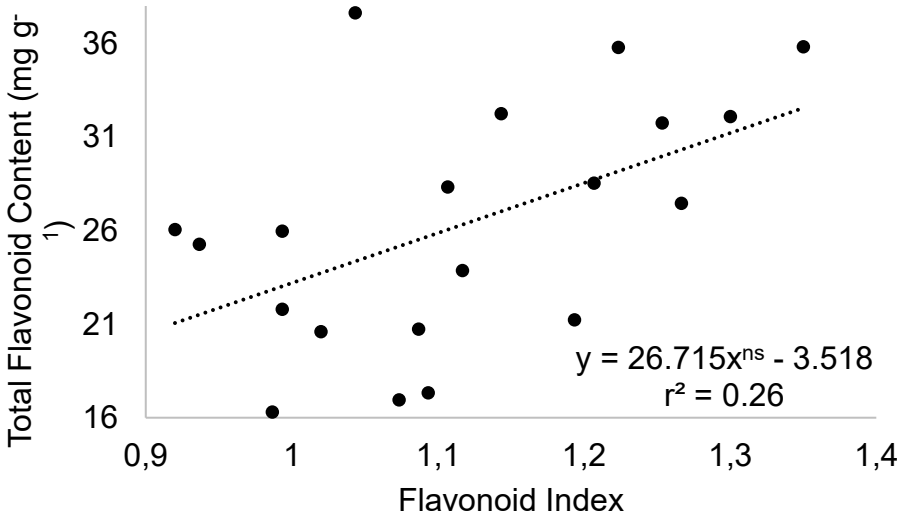


Figure 2. Regression analysis to evaluate the correspondence between the flavonoid index (MPM-100) and total flavonoid content (spectrophotometry) in leaves of buckwheat individuals grown in Minas Gerais, Brazil.
ns: not significant by the F-test ($p \leq 0.05$).
Source: Authors (2026).

Although there is a trend between the measurements, the low correlation and regression analysis suggest that the portable meter, when used alone, does not provide sufficient reliability for accurate estimation of TFC. Its use should be limited to preliminary relative screening, and further studies should be conducted considering species-specific calibration, evaluation of multiple genotypes, and diverse production environments. This inconsistency between methods may be attributed to factors such as limitations of the portable device and environmental influences affecting the biosynthesis and distribution of total flavonoids (KOŁTON *et al*., 2022).

Measurements obtained with both the portable meter and the spectrophotometer are influenced by the plant part evaluated, leaf age, and leaf position within the canopy, due to solar radiation and light incidence (BOROVAYA and KLYKOV, 2020; YATSIV *et al*., 2025; ZIELIŃSKA *et al*., 2012), which may lead to differences in results. In addition, the portable meter may capture optical signals associated with leaf surface properties, pigments, thickness, leaf position, water status, other phenolic compounds (non-flavonoids), and anatomical traits that do not directly correspond to total flavonoids extracted and measured by spectrophotometry.

Spectrophotometry using $AlCl_3$ is an indirect method for total flavonoid analysis, widely employed for this purpose. It is a linear, accurate, precise, and robust technique for the quantitative analysis of total flavonoids in plant leaves; however, it has limitations and may yield overestimated or underestimated results depending on flavonoid class, plant matrix, solvent, interfering substances, and the standard used (LUNA *et al*., 2022; SHRAIM *et al*., 2021). Nevertheless, studies have reported high correlation (approximately 80%)

between total flavonoid content determined by spectrophotometry and rutin quantified by high-performance liquid chromatography (HPLC) in plants of the same buckwheat cultivar (FERNANDES *et al*., 2022). Even so, chromatographic methods such as HPLC are recommended in future studies for the specific quantification of rutin and other relevant flavonoids in buckwheat.

Some limitations may have influenced the results, including the use of a single environment, point-in-time evaluation, and the absence of species-specific calibration of the MPM-100. Given the importance of rapid quantification of secondary metabolites and the need for more efficient screening methods than reference techniques, the current scientific literature lacks validation studies on the effectiveness of portable meters for assessing flavonoid content in buckwheat. Therefore, further studies are required to calibrate and adapt the device to the characteristics of the species. As described by Lacchine *et al*. (2023), the device is practical due to its compactness, facilitating field use, while preserving the leaf for additional relevant measurements.

4. Conclusions

The study demonstrated that the portable meter has low predictive capacity under the evaluated conditions for the relative screening of total flavonoids in buckwheat leaves, not meeting the reliability requirements of conventional laboratory methods such as spectrophotometry. Therefore, its use does not replace laboratory analyses. Continuous calibration and combination with reference methods such as chromatography are essential to obtain reliable results for preliminary relative screening of total flavonoids in buckwheat.

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