

ANISE (*PIMPINELLA ANISUM*) AS A FUNCTIONAL INGREDIENT IN MUSTARD: IMPACT ON PHYSICOCHEMICAL PROPERTIES, ANTIOXIDANT CAPACITY AND COLOR PARAMETERS

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RESEARCH ARTICLE

Abstract

This study investigates the fortification of mustard with anise (*Pimpinella anisum*) at concentrations of 0%, 0.5%, 1%, and 1.5%, with the aim of improving its functional properties. Physicochemical parameters (pH, total acidity, moisture), antioxidant capacity (DPPH and FRAP), total phenolic content, total flavonoid content, and color characteristics (CIE Lab*) were evaluated. The results show that pH remained unaffected by anise addition, while total acidity decreased significantly at 1.5% anise. Moisture content declined in all fortified samples, especially at 1% and 1.5%. Total phenolic content increased significantly in the 1% and 1.5% formulations, whereas flavonoid levels showed no significant variation. Antioxidant capacity improved markedly with increasing anise concentration, particularly in the samples containing 1% and 1.5%. Color analysis revealed a decrease in luminosity, yellowness, and chroma with higher levels of anise, although the hue angle remained stable. Overall, anise fortification enhances the antioxidant potential and phenolic profile of mustard while modifying its visual characteristics.

Keywords: mustard; anise (*Pimpinella anisum*); fortification; phenolic compounds; antioxidant activity; color parameters; functional foods

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INTRODUCTION

The concept of functional fortified foods has gained increasing attention in recent decades, driven by greater consumer awareness of the link between diet and health. Traditionally, food fortification aimed to prevent nutrient deficiencies by adding essential vitamins and minerals; however, functional foods go beyond basic nutrition, providing additional physiological benefits due to bioactive compounds, probiotics, and antioxidants (Vignesh et al., 2024; Jendyose, 2024). Functional fortified foods combine both approaches, incorporating essential nutrients and natural bioactives into commonly consumed foods to enhance their nutritional and functional value (Kyazimova et al., 2024; Nagar et al., 2024). This strategy contributes to preventing micronutrient deficiencies and promoting overall health, aligning with current clean label trends that favor natural antioxidants over synthetic additives (Kaur et al., 2022; Grasso et al., 2023).

Mustard (*Brassica* spp.), a member of the Brassicaceae family, is widely cultivated for its seeds, which are used as condiments and sources of edible oil (Rahman et al., 2024). Mustard seeds are rich in oil (28–42%),

proteins (25–40%), carbohydrates, and secondary metabolites such as glucosinolates, phenolic compounds, and tannins (Shahidi, 1990; Rahman et al., 2020; Von Der Haar et al., 2014). These bioactive compounds are associated with antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Hartmann, 2007; Idrees, 2019; Ramirez et al., 2020). Despite their nutritional and therapeutic potential, mustard seeds may also contain antinutritional factors, such as erucic acid and glucosinolate degradation products, which require controlled processing (Lietzow, 2021).

Anise (*Pimpinella anisum*), belonging to the Apiaceae family, is a medicinal and aromatic plant rich in essential oils and phenolic compounds. Its main constituent, trans-anethole, along with flavonoids, coumarins, and vitamins, contributes to its antioxidant, antimicrobial, and anti-inflammatory effects (Picon et al., 2010; Yamini et al., 2008; Moazzami et al., 2020).

This study aimed to evaluate the potential of fortifying a traditional product, mustard, with Anise (*Pimpinella anisum*) to develop a food with enhanced functional value. The objectives were to assess the physicochemical properties,

total phenolic content, antioxidant capacity, and color parameters of mustard samples fortified with three different concentrations of anise extract.

MATERIAL AND METHOD

Reagents and ingredients used

The ingredients used for the preparation of the mustard formulations were sourced from certified suppliers to ensure quality and reproducibility. Mustard seeds were obtained from SIA ELPIS SRL (Latvia), while fermentation vinegar (9°) was provided by SEINEANA PRODUCT SRL (Romania). Native corn starch and guar gum were supplied by BARENTZ SRL (Romania). Additional ingredients included refined salt from SOCIETATEA NAȚIONALĂ A SĂRII SA (Romania), sugar and sunflower oil from PARHAN COM SRL (Romania), and citric acid, turmeric, and ground star anise from SOLINA ROMANIA SRL (Romania). All ingredients were food-grade and used without further purification.

Formulation of Mustard Samples Fortified with Anise

Four mustard formulations were prepared by incorporating different concentrations of ground anise (*Pimpinella anisum*): 0% (control), 0.5%, 1.0% and 1.5%. The base recipe was adapted from an industrial-scale formulation and scaled to 100 kg for laboratory production. The ingredients used in the formulations included water, fermentation vinegar (9°), mustard seeds, native corn starch, salt, sugar, sunflower oil, turmeric, guar gum, citric acid, and ground anise. The quantities corresponding to the standard formulation (0% anise) and the fortified variants (0.5%, 1.0%, 1.5%) were calculated proportionally, replacing part of the dry mix with the appropriate amount of anise powder.

Physicochemical determinations

- *Determination of moisture*

Moisture content was determined by the gravimetric method, since the water level influences the texture, microbiological stability, and shelf life of mustard. An empty weighing vial was first weighed (M_1), after which 5 g of mustard sample was added and the vial was weighed again (M_2). The vial was then placed in a drying oven at 105 °C for 4 hours, cooled in a desiccator for 30 minutes, and weighed (M_3). Drying was repeated in 30-minute intervals until

a constant mass was reached. Moisture content (%) was calculated using the formula:

$$\text{Moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

- *pH-determination*

The pH of the mustard samples was determined using an InoLab pH 7310 pH meter. The sample was first homogenized, after which 5 g of mustard were dispersed in 50 mL of distilled water, stirred, and allowed to equilibrate for 5 minutes. The pH was then measured by immersing the electrode fully into the mixture, and the final value was recorded once the reading stabilized.

- *Total acidity determination*

Total acidity was determined by titration. A 5 g portion of mustard was diluted in 50 mL of distilled water, homogenized, and filtered if necessary. Two to three drops of phenolphthalein were added, and the sample was titrated with 0.1 N NaOH under continuous stirring until a persistent pale-pink endpoint was reached. The volume of NaOH used (V , mL) was recorded. Total acidity, expressed as acetic acid (%), was calculated using the formula:

$$\text{Total acidity (\% acetic acid)} = (V \times N \times 60)/m$$

where V is the NaOH volume (mL), N is the normality of NaOH (0.1 N), m is the mass of the sample (g), and 60 represents the molecular weight of acetic acid.

Biochemicals analysis

- *Total phenols content*

Total phenolic compounds were determined using the Folin-Ciocalteu method (Singleton et al, 1999; Vicaș et al, 2011). A volume of 100 μ L mustard sample was mixed with 1700 μ L distilled water and 200 μ L freshly diluted Folin-Ciocalteu reagent (1:10, v/v). After approximately 3 minutes, 1 mL of 7.5% sodium carbonate solution was added. The mixtures were incubated at room temperature in the dark for 2 hours, after which the absorbance was measured at 765 nm using a Shimadzu miniUV-Vis spectrophotometer. The calibration curve was constructed using gallic acid standards in the range of 0.05–0.25 mg/mL, and results were expressed as mg gallic acid equivalents (GAE) per 100 mL sample.

- *Total Flavonoids content*

Total flavonoid content was determined using an aluminum chloride colorimetric method, with quercetin serving as the standard.

Calibration was performed using quercetin solutions ranging from 0.1 to 0.5 mg/mL, yielding a regression equation of $y = 0.8388x + 0.0003$ and an $R^2 = 0.996$, with all measurements carried out in triplicate. For sample preparation, 1 mL of mustard extract (P1, P2, P3) was transferred into a 10 mL volumetric flask, followed by the addition of 4 mL distilled water and 3 mL of 5% NaNO₂ solution. After 5 minutes, 0.3 mL of 10% AlCl₃ was added, the mixture was homogenized, and after an additional 6 minutes, 2 mL of 1 M NaOH was added and the volume was adjusted to 10 mL with distilled water. After 15 minutes, absorbance was measured at 510 nm using a Shimadzu miniUV-Vis spectrophotometer. Results were expressed as mg quercetin equivalents (QE) per gram of mustard based on the calibration curve.

- *Antioxidant capacity*

The antioxidant activity was evaluated using the DPPH radical scavenging assay, according to the method described by Vicaş et al (2011). Briefly, 2.9 mL of DPPH solution (80 µM) was transferred into a test tube, followed by the addition of 100 µL mustard extract. The mixture was allowed to react in the dark for exactly 30 minutes, after which the absorbance was measured at 515 nm. The percentage of DPPH inhibition was calculated using the formula:

$$\text{DPPH inhibition (\%)} = [(A_0 - A_s) \times 100] / A_0$$

where A_0 represents the absorbance of the blank and A_s the absorbance of the sample.

The ferric reducing antioxidant power (FRAP) assay was used to evaluate the antioxidant capacity of the mustard samples, based on the ability of antioxidants to reduce Fe³⁺ to Fe²⁺, forming a blue Fe²⁺-TPTZ complex with maximum absorbance at 593 nm. The working FRAP reagent was prepared fresh by mixing 300 mM acetate buffer (pH 3.6), 20 mM FeCl₃·6H₂O, and 10 mM TPTZ (dissolved in 40 mM HCl) in a 10:1:1 (v/v/v) ratio. Trolox was used as the standard, starting from a 2.5 mM stock solution, with calibration dilutions between 0.25 and 2 mM. The calibration curve showed the regression equation $y = 15439x + 0.118$ with an $R^2 = 0.9970$. Absorbance measurements were performed at 593 nm using a Shimadzu miniUV-Vis spectrophotometer.

Determination of color parameters

Color parameters of the mustard samples were measured using a portable colorimeter (3nh TS7030, Shenzhen Threneh Technology Co., Ltd., China), operating in the CIE Lab* color space. The instrument was calibrated before each measurement session using the standard white and black calibration plates provided by the manufacturer. Approximately 5 g of mustard were placed evenly in a plastic Petri dish, and measurements were performed in triplicate under constant ambient lighting conditions. The parameters L* (lightness), a* (green-red component), and b* (blue-yellow component) were recorded, and mean values with standard deviations were calculated for each sample. Based on the obtained a* and b* values, chroma (C*, color saturation) and hue angle (h*, perceived color tone) were also determined.

Statistical analysis

All measurements were performed in triplicate. Statistical comparison between the fortified samples and the control (0% anise) was carried out using the Student's t-test (two-tailed, independent samples). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The effect of anise on physicochemical parameters

Fortifying the mustard with anise did not significantly affect the product's pH (Table 1). In contrast, the total acidity was strongly affected at a concentration of 1.5% anise, which showed a significant reduction ($p < 0.05$) compared to the control and the rest of the formulated variants (Table 1). The moisture content significantly decreased in the fortified samples, especially at concentrations of 1% and 1.5% (Table 1), which can be correlated with both the absorption of water by the phenolic compounds of anise and the effects on the food matrix structure. The results indicate that the addition of anise significantly modifies the physicochemical properties of mustard, particularly in terms of acidity and moisture content.

Table 1

Physicochemical parameters (pH, total acidity and moisture) of mustard fortified with anise

Samples	pH	Total acidity	Moisture (%)
Control	4.24 ± 0.02a	2.19 ± 0.06a	67.14 ± 0.09b
M-0.5	4.19 ± 0.07a	1.77 ± 0.20a	66.51 ± 0.01c
M-1	4.25 ± 0.01a	2.01 ± 0.12a	65.89 ± 0.16a
M-1.5	4.27 ± 0.01a	1.65 ± 0.12b	66.28 ± 0.25a

Values represent mean ± standard deviation (n = 3). Different letters within the same column indicate statistically significant differences between samples (p < 0.05).

The effect of anise on total phenols and flavonoids content

The total phenolic compound content increased significantly (p < 0.05) in all anise-fortified samples compared to the control (M₀) (Figure 1A). The increase in TPC can be attributed to the contribution of bioactive compounds from *Pimpinella anisum*, known for

its high content of flavonoids, phenolic acids, and their derivatives (Leonard et al., 2021). Additionally, the mustard's food matrix structure, rich in water and soluble compounds, can facilitate the release and diffusion of phenolic compounds into the final product.

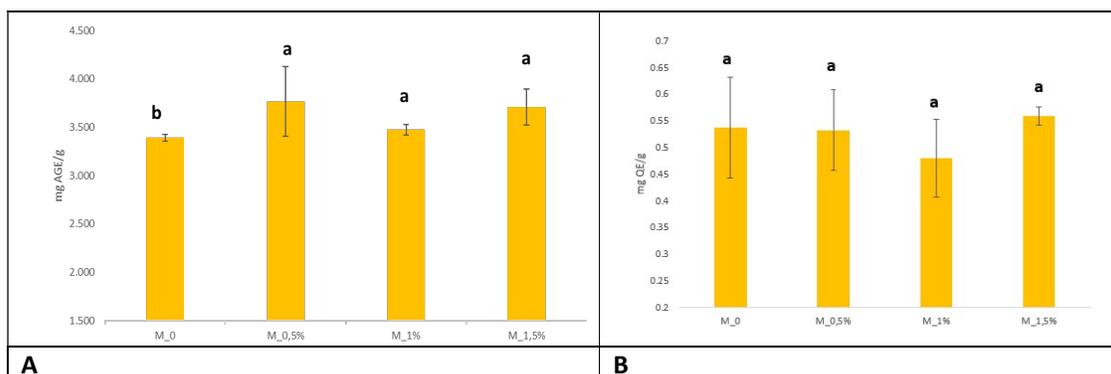


Figure 1. A. Total phenolic content (mg GAE/g) of mustard samples fortified with different concentrations of anise. B. Total flavonoid content (mg GAE/g) of mustard samples fortified with different concentrations of anise. Bars represent mean ± SD (n = 3). Different letters indicate statistically significant differences (p < 0.05).

Although the total flavonoid values vary slightly between samples (Figure 1B), the differences are not statistically significant. The similar levels suggest that flavonoids in anise may be present in moderate amounts, their extractability into the mustard matrix is limited, and the compounds responsible for the increase in TPC are more likely other polyphenols (e.g., phenolic acids, tannins).

The effect on antioxidant capacity

The antioxidant capacity evaluated by the DPPH method showed marked differences between the mustard samples fortified with anise (Figure 2A). The M 0.5% sample showed

the lowest DPPH radical scavenging capacity, being significantly inferior to the control (p < 0.05). In contrast, the M 1% and M 1.5% samples showed superior DPPH inhibition values, both being significantly higher than the control, indicating a cumulative antioxidant effect at higher concentrations of anise. This behavior can be attributed to the phenolic compounds and other secondary metabolites characterized in *Pimpinella anisum*, which contribute to the neutralization of free radicals in the food matrix (Picon et al., 2019).

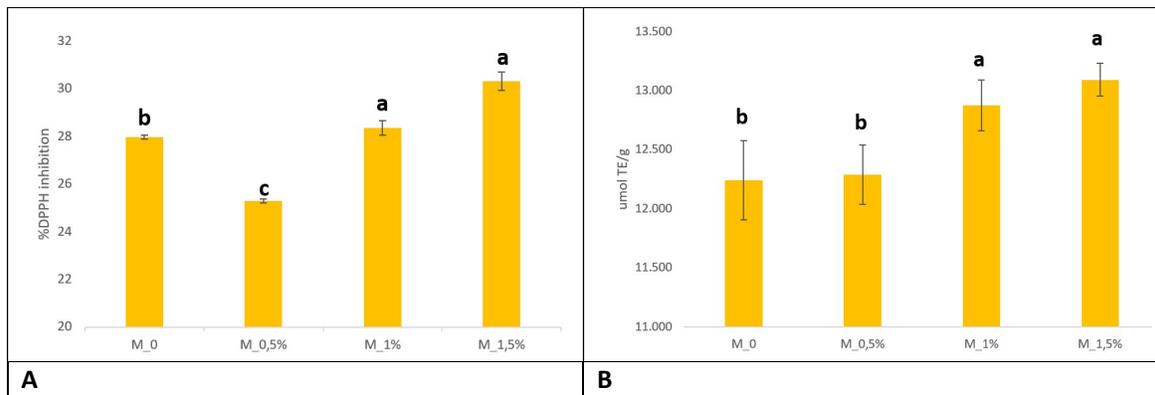


Figure 2. **A. Antioxidant capacity (DPPH) of mustard samples fortified with different concentrations of anise.** **B. Antioxidant capacity (FRAP) of mustard samples fortified with different concentrations of anise.** Bars represent mean \pm SD ($n = 3$). Different letters indicate statistically significant differences ($p < 0.05$).

The FRAP method confirmed the trends observed with DPPH, highlighting a superior antioxidant capacity in samples with anise at higher concentrations (Figure 2B). Although M 0.5% did not differ significantly from the control, the M 1% and M 1.5% samples showed significantly higher FRAP values ($p < 0.05$), indicating a superior ability to reduce ferric ions ($\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$). This increase can be explained by the intake of phenolic compounds with redox properties present in anise. The 1.5% concentration generated the highest FRAP antioxidant capacity, suggesting a direct

proportional relationship between the anise content and the samples' ability to act as reducing agents.

Determining the color parameters (L^* , a^* , b^* , C^* , h^*) revealed significant changes in the chromatic profile of mustard as a result of fortification with anise (Table 2). Statistical analysis shows that all samples differ significantly from each other for most parameters, indicating a strong impact of anise concentration on the product's visual appearance.

Table 2

The effect of anise on color parameters of mustard

Samples	L^*	a^*	b^*	C^*	h^*	ΔE
M_0	65.44 \pm 0.34 c	5.08 \pm 0.19 c	62.02 \pm 0.43c	62.23 \pm 0.42c	85.31 \pm 0.19a	0
M-0.5	63.88 \pm 0.12 a	5.49 \pm 0.07a	62.99 \pm 0.13a	63.22 \pm 0.13a	85.02 \pm 0.06a	2.26 \pm 0.14
M-1	63.56 \pm 0.11 b	4.71 \pm 0.07b	58.12 \pm 0.15b	58.31 \pm 0.15b	85.37 \pm 0.08b	4.20 \pm 0.18
M-1.5	62.51 \pm 0.53 d	4.28 \pm 0.27d	53.89 \pm 0.35d	54.06 \pm 0.33d	85.46 \pm 0.30a	8.51 \pm 0.50

Values represent mean \pm standard deviation ($n = 3$). Different letters within the same column indicate statistically significant differences between samples ($p < 0.05$).

The L^* value decreased progressively with increasing anise concentration, the highest lightness being observed in the control sample, and the lowest in sample M_1.5%. The significant decrease in brightness ($p < 0.05$) suggests that anise contributes to the darkening of the mustard, likely thru its composition rich in natural pigments and oxidized phenolic compounds, which can give the final product a deeper hue (Table 2). The a^* parameter showed significant differences between the samples. The highest value was recorded for M_0.5%, indicating a slight tendency toward reddish tones at this concentration. In contrast, the values decreased significantly for M_1% and M_1.5%, indicating a shift in color toward the greenish area. These changes can be explained by the

interaction of the compounds in anise with the natural pigments in mustard, affecting the balance between warm and cool tones. The b^* parameter recorded high values for the control and M_0.5%, indicating the intense yellow color specific to traditional mustard. At higher concentrations of anise (1% and 1.5%), the b^* values decreased significantly ($p < 0.05$), suggesting a reduction in yellow components.

This can be associated with the diluting effect of anise on curcumin and other natural yellow pigments, as well as with possible non-enzymatic browning reactions. The C^* values followed the same trend as the b^* parameter, indicating a significant reduction in color saturation in the samples with 1% and 1.5% anise. The decrease in chromaticity reflects the fact that the product becomes visually "duller"

and less intense in color, an effect explainable by the dispersion of light within the mustard's pigmented matrix in the presence of fine anise particles. The h^* values remained very close between samples, indicating that the overall tone of the mustard (the predominant yellow hue) was not strongly influenced by the addition of anise. Only the M_1% sample showed a statistically significant difference compared to the others, but the variation is small and does not noticeably affect the product's chromatic character. Thus, anise modifies the intensity and brightness, but doesn't fundamentally change the characteristic hue of mustard.

CONCLUSIONS

Fortifying mustard with anise significantly influenced the product's physicochemical, antioxidant, and chromatic profile. Although the pH remained unchanged, the total acidity decreased significantly at a concentration of 1.5%, and the moisture content was lower in the fortified samples, especially at 1% and 1.5%. The addition of anise caused an increase in the total phenolic compound content, especially at concentrations of 1% and 1.5%, while total flavonoids did not show significant differences. The antioxidant activity of mustard was improved, with evident increases in both the DPPH and FRAP tests, especially in the samples with 1% and 1.5% anise. The color parameters were strongly influenced by the addition of anise: brightness, yellowness intensity, and chromaticity significantly decreased with increasing concentration, while the overall hue (h^*) remained relatively stable. Overall, anise proves to be a valuable functional ingredient, capable of enhancing the antioxidant and phenolic properties of mustard while simultaneously generating visible color changes that may be relevant to consumer perception.

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