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Effects of maltodextrin-to-collagen ratio on antioxidant activity, anthocyanin retention, and hydroxyproline content in spray-dried mulberry collagen powder

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ABSTRACT This study investigates the effects of different maltodextrin–to-collagen ratios on the chemical properties of spray-dried mulberry powder enriched with fish collagen. Three formulations were prepared with maltodextrin-to-collagen ratios of 26:3 (M1), 23:5 (M2), and 20:7 (M3). The powders were analyzed for hydroxyproline content, antioxidant activities (DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,20-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) and ferric reducing antioxidant power (FRAP) assays), and anthocyanin content. Hydroxyproline analysis confirmed increasing collagen content across formulations, with values of 2.72 ± 0.07% (M1), 3.40 ± 0.02% (M2), and 3.61 ± 0.02% (M3). Among the formulations, M1 exhibited the highest antioxidant activities, with DPPH inhibition at 33.71 ± 0.24%, ABTS at 0.0349 ± 0.0003 mg AEAC/g, and FRAP at 0.120 ± 0.0002 mM/g. Significant differences ($p < 0.05$) were observed for DPPH and ABTS activities across all samples, whereas FRAP values did not differ significantly between M2 and M3. Anthocyanin content also varied significantly ($p < 0.05$), with M1 yielding the highest concentration (3.60 ± 0.003 mg cyd-3-glu/g DW). Overall, the M1 formulation, containing 26% maltodextrin and 3% collagen, demonstrated the most favorable powder characteristics with satisfactory collagen content, antioxidant properties, and anthocyanin retention, highlighting its potential as a functional food ingredient.

KEYWORDS: Mulberry; Collagen; Maltodextrin; Antioxidant activity; Spray-drying.
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Original Article

INTRODUCTION

The growing interest and demand for healthy food products have led to a surge in research regarding functional foods in the food, health, and technology sectors (Baker *et al*., 2022). Mulberry fruit is notable for its richness in bioactive compounds, anthocyanins, vitamin C, minerals, and phenolic substances, making it a prominent ingredient for functional food (Khatri *et al*., 2024). The phenolic compounds contribute to mulberry's diverse biological activities, such as antioxidants, anti-inflammatory, and antibacterial effects, which have promising applications in food and medicine (Huang *et al*., 2022). However, these compounds are highly susceptible to thermal degradation, oxidation, and interactions with the food matrix during processing (Thuy *et al*., 2022).

Spray drying is a widely used technique to convert fruit extracts and protein-rich solutions into stable powders suitable for food and nutraceutical applications. This technique enables rapid dehydration while allowing the incorporation of wall materials that protect labile bioactive compounds (Vargas *et al*., 2024). Commonly used carrier agents such as maltodextrin enhance powder yield, reduce stickiness, and improve storage stability. However, its concentration and interaction with proteinaceous carriers such as hydrolyzed collagen can markedly influence the retention of antioxidant capacity and anthocyanin content after spray drying. Previous studies have demonstrated that the effectiveness of carrier systems during spray drying is highly dependent on the specific fruit matrix and its dominant bioactive compounds, particularly heat-sensitive pigments such as anthocyanins (Díaz-Montes, 2023).

The incorporation of collagen peptides into functional drink formulations improves the physicochemical and microbiological capacity of these products (Hajj *et al*., 2024). Furthermore, collagen-enriched fruit juice drinks offer benefits such as supporting skin and joint health, and also providing antioxidant and immune support due to the synergistic effects of nutrient-rich fruit compounds and collagen. Driven by the growing demand for natural healthy products, mulberry-collagen powder drink shows promising potential for development into a functional drink with extended shelf-life compared to conventional drinks, and it is convenient for on-the-go consumption. In such formulations, collagen functions not only as a nutritional component but also as part of the drying matrix, where its interaction with fruit polyphenols may influence the retention and expression of antioxidant activity.

Studies on wall materials and carrier blends demonstrate that the choice and proportion of carriers affect encapsulation efficiency, antioxidant activity, and powder functionality, highlighting the importance of evaluating carrier systems for fruit-derived pigments (Díaz-Montes, 2023; Vargas-Muñoz & Kurozawa, 2020). While similar carrier-agent systems have been investigated for other fruits, differences in phytochemical composition and thermal sensitivity mean that findings cannot be directly extrapolated to mulberry-based systems, which are particularly rich in anthocyanins and prone to degradation during drying.

Despite the regularity of maltodextrin and protein carrier usage, there is limited information on how specific maltodextrin-to-collagen ratios affect the chemical constituents of spray-dried mulberry–collagen powders. Such information could be beneficial for product developers seeking to formulate powders that simultaneously preserve the vibrant color and antioxidant potential of mulberry while delivering the functional and nutritional advantages of collagen peptides. Addressing this gap, the present study provides a matrix-specific evaluation of defined maltodextrin–collagen ratios and examines their effects on antioxidant activity, anthocyanin retention, and hydroxyproline content in spray-dried mulberry collagen powder.

METHODOLOGY

Sample Collection and Materials

Mulberry fruits of the *Morus Alba* species were obtained from a local harvest in Kampung Tudan Kiulu, Tuaran, Sabah. Fish collagen was purchased from Membrahealth Marketing Sdn. Bhd., Kuala Lumpur, Malaysia. The chemicals used in the study were of analytical grade.

Preparation of mulberry collagen powder

Preparation of mulberry fruit puree

Fresh mulberries were sorted to remove damaged fruits, washed thoroughly with tap water to eliminate dirt and impurities, and manually destemmed. The cleaned fruits were then homogenized using a blender to obtain a uniform mulberry puree.

Preparation of collagen-infused mulberry juice

Mulberry puree was mixed with maltodextrin and fish collagen in distilled water to produce feed solutions with maltodextrin-to-collagen ratios of 26:3 (M1), 23:5 (M2), and 20:7 (M3). The mixtures were stirred continuously until the complete dissolution of the carrier materials was achieved. The resulting solutions were filtered through a nylon cloth to remove insoluble residues, transferred into sterile containers, and stored at 4 °C prior to spray drying.

Spray drying process

The mulberry-collagen feed solutions were subjected to spray drying with an inlet temperature of 160 °C and an outlet temperature of 78 °C to produce mulberry collagen powder.

Collagen content

The collagen content was analyzed following the hydroxyproline analysis method as described by Bilek and Bayram (2015).

Antioxidant activity assays

The antioxidant activity of the mulberry juice and mulberry collagen powder was assessed by measuring DPPH radicals scavenging activity, ABTS radicals scavenging activity and ferric reducing antioxidant power (FRAP), according to the methods described by Abu Bakar *et al*. (2016).

Anthocyanin content assay

The anthocyanin content of the mulberry juice and mulberry collagen powder was measured following the method described by Do & Nguyen (2018).

Statistical analysis

All experiments were conducted in triplicate, and data are expressed as means ± standard deviation (SD). The statistical analysis was performed using one-way ANOVA in Statistical Package for the Social Sciences (SPSS) version 25.

RESULT AND DISCUSSION

Hydroxyproline content

The collagen content in the mulberry collagen powder, determined by measuring hydroxyproline content, is shown in Table 1. Hydroxyproline levels increased significantly ($p < 0.05$) with higher collagen inclusion, ranging from 2.72 ± 0.07% in formulation M1 to 3.61 ± 0.02% in M3.

Table 1. Collagen content in the mulberry collagen powder.

Formulation				Hydroxyproline (%)
	Ratio	Maltodextrin	Collagen	
M1	26:3	26%	3%	2.72±0.07[a]
M2	23:5	23%	5%	3.40±0.02[b]
M3	20:7	20%	7%	3.61±0.02[c]

* Mean values with the different superscripts were significantly different at $p < 0.05$.

Since hydroxyproline is an amino acid present almost exclusively in collagen, its concentration is considered a reliable biochemical marker for collagen content and stability in processed foods (Oliveira *et al*., 2021). Increasing collagen from 3% (M1) to 5% (M2) produced a substantial rise in hydroxyproline by 0.68%, whereas the further increase from 5% to 7% resulted in a comparatively smaller increment of 0.21%. This diminishing increment suggests that collagen incorporation efficiency may decrease at higher protein loadings, likely due to changes in feed composition and matrix behavior during spray drying rather than analytical limitations.

According to Anandharamakrishnan & Ishwarya (2015), excessive protein carriers can reduce drying efficiency by lowering the glass transition temperature (Tg), thereby increasing stickiness and wall deposition. Although drying yield and physical properties were not quantified in this study, the observed hydroxyproline trend is consistent with reported effects of high protein content on spray-drying performance and matrix formation.

Antioxidant activities

Figure 1 illustrates the antioxidant properties of mulberry collagen powders formulated with different maltodextrin-to-collagen ratios (M1–M3), compared to fresh mulberry juice. Across all three assays, DPPH (A), ABTS (B), and FRAP (C), a consistent trend shows that antioxidant activity decreases with increasing collagen content in the powder formulations, with M1 (lowest collagen) exhibiting the highest activity. The juice control consistently exhibited higher antioxidant activity than all powder formulations, indicating a notable loss of antioxidant capacity following spray drying.

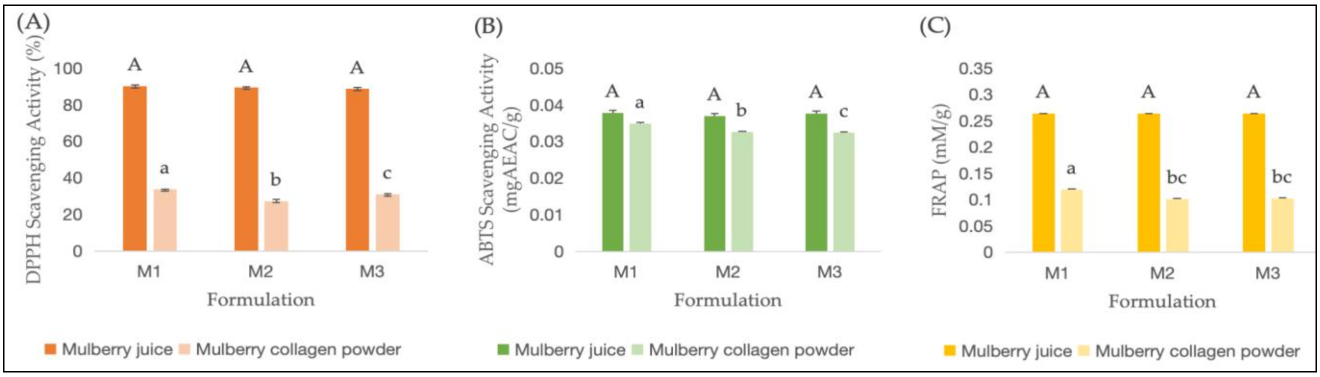

Figure 1. Antioxidants properties. (A) DPPH, (B) ABTS, and (C) FRAP of mulberry juices and the mulberry collagen powders (maltodextrin:collagen ratios M1 (26:3), M2 (23:5) and M3 (20:7)). Bars represent the SD (n=3)[A,B,C] Significant differences in values between juices (p<0.05), [a,b,c] Significant differences in values between powders (p<0.05).

The substantial reduction in antioxidant activity following spray drying reflects the combined effects of thermal degradation of phenolic compounds and dilution by carrier materials, which is commonly reported for anthocyanin-rich fruit matrices (Enaru *et al*., 2021; Zhang *et al*., 2024). This pattern suggests that increasing substitution of mulberry juice with collagen peptides and maltodextrin results in dilution and potential loss of bioactive compounds, particularly anthocyanins and polyphenols, which are primarily responsible for antioxidant activity in mulberries. Additionally, collagen peptides may contribute to reduced antioxidant effectiveness through polyphenol–protein interactions, which can lead to complex formation that hinders the accessibility of reactive phenolic groups (Buljeta *et al*., 2022). Such interactions do not necessarily indicate degradation of phenolics but may reduce their measurable radical-scavenging capacity in chemical assays.

Although maltodextrin enhances powder stability and drying efficiency, it can reduce antioxidant activity by diluting bioactive compounds or entrapping them within the matrix (Kuck & Noreña, 2016). Several studies, such as Pham *et al*. (2022), found that higher maltodextrin levels improve antioxidant retention by protecting hydrophobic compounds during spray drying. However, this protective effect is concentration-dependent, and beyond an optimal level, maltodextrin may lower apparent antioxidant activity due to matrix dilution rather than compound loss (Demircan *et al*., 2023). This explains why M1, with the highest maltodextrin and lowest collagen content, exhibited the most favorable antioxidant profile among the powder formulations.

Anthocyanin content analysis

Spray-drying significantly reduced the anthocyanin content in mulberry juice, as shown in Figure 2. All powder formulations (M1–M3) exhibited over an 85% loss compared to their respective juice counterparts.

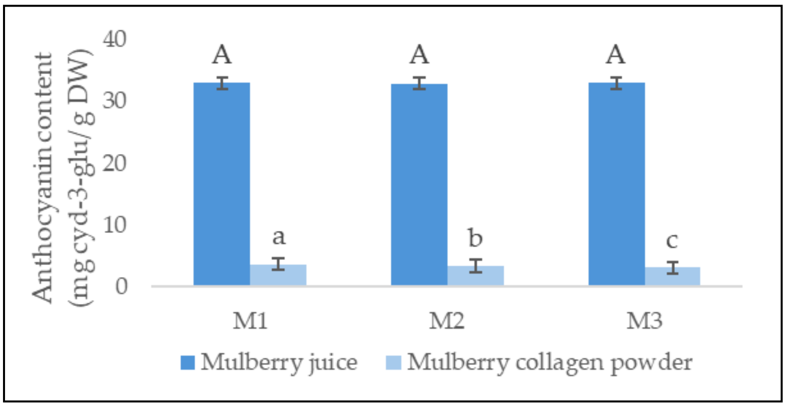

Figure 2. The anthocyanin contents of mulberry juices and the mulberry collagen powders (maltodextrin:collagen ratios M1 (26:3), M2 (23:5) and M3 (20:7)). Bars represent the SD (n=3)[A,B,C] Significant differences in values between juices ($p<0.05$), [a,b,c] Significant differences in values between powders ($p<0.05$).

Such extensive losses are consistent with the high thermal sensitivity of anthocyanins, which readily undergo degradation, polymerization, and structural transformation under elevated temperatures and oxidative conditions during spray drying (Enaru *et al.*, 2021). Anthocyanins are unstable and easily affected by processing conditions such as heat, oxygen exposure, and pH changes (Zhang *et al.*, 2024). Among the powders, M1, containing the highest maltodextrin (26%), showed the greatest anthocyanin retention (3.60 mg/g DW), followed by M2 and M3. This trend indicates that maltodextrin played a protective role by forming a continuous encapsulating matrix that reduced direct exposure of anthocyanins to thermal and oxidative stress during drying. Supporting studies confirm that increased carrier content improves particle structure and stability (Chuwattanakul *et al.*, 2023; Ozcelik *et al.*, 2023; Yudiono, 2024). However, as observed in this study, increasing collagen content while reducing maltodextrin proportion may weaken this protective matrix, resulting in lower anthocyanin retention despite higher protein content. These findings highlight the importance of optimizing carrier ratios to balance encapsulation protection and bioactive concentration, particularly for anthocyanin-rich fruit systems such as mulberries.

Beyond thermal degradation, differences in anthocyanin retention among formulations also reflect the functional roles of maltodextrin and collagen within the spray-drying matrix. Maltodextrin acts as a glass-forming carrier that immobilizes anthocyanins and limits degradation reactions during drying, whereas collagen peptides may exhibit limited encapsulating capacity for water-soluble pigments due to polyphenol–protein interactions (Buljeta *et al.*, 2022). Such interactions can reduce effective physical protection during atomization and drying. In addition, increasing collagen content may disrupt matrix continuity, potentially increasing exposure of anthocyanins to localized thermal and oxidative stress. Similar observations have been reported in spray-dried fruit systems, where adequate polysaccharide carrier content is critical for stabilizing anthocyanins and maintaining particle integrity (Chuwattanakul *et al.*, 2023; Ozcelik *et al.*, 2023; Yudiono, 2024). These findings reinforce the importance of optimizing maltodextrin-to-collagen ratios to balance encapsulation efficiency and bioactive retention in anthocyanin-rich matrices.

CONCLUSION

The study demonstrates that the right balance of maltodextrin and collagen is important to maintain the quality of spray-dried mulberry collagen powder. The M1 formulation (26:3 ratio) gave the most favorable results, exhibiting strong antioxidant activity, good anthocyanin retention, and sufficient collagen content. Although the M3 formulation yielded the highest hydroxyproline content, it showed reduced antioxidant retention and potential processing challenges. These findings highlight the importance of balancing carriers and active ingredient concentrations to ensure both stability and functionality in spray-dried functional food products.

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