

Extraction and enzymatic modification of dietary fibre from purple aubergine

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Abstract: This research scrutinised the opportunity of upcycling waste from the food industry by extracting dietary fibre from purple aubergine. One of the challenges addressed was enzymatic browning, which negatively influenced the quality of the fibre extracted from fresh aubergine. Various pre-treatment procedures were assessed, including using citric acid, sulphite immersion, peeling and thermal processing, to determine their effects on extraction yield, colour, and the functionality of the resultant dietary fibre. Findings indicated that a pre-treatment method involving aubergine peeling, sulphite solution immersion, and subsequent steaming before extraction produced optimal results, enhancing both whiteness index and water-holding capacity. Experiments were conducted with traditional enzymes supplemented with cellulase, xylanase, and lipase for enzymatic extraction. The addition of lipase notably elevated the extraction yield and water-holding capacity, albeit with an undesired darkening effect on the dietary fibre. In contrast, the application of xylanase emerged as the most effective treatment, delivering the highest overall quality for the derived dietary fibre.

Keywords: browning reaction; cellulase; lipase; sulphite; water holding capacity; xylanase

Aubergine (*Solanum melongena* L.), particularly the purple variety, was a rich source of dietary fibre and bioactive compounds (Raigón et al. 2008). The chemical composition of its insoluble dietary fibre includes 1.60% cellulose, 0.43% hemicellulose, and 0.18% lignin in the raw eggplant. Meanwhile, the soluble dietary

fibre comprises 26.10–33.64% pectin in the peel and 18.36% in the calyx (Zia-ur-Rehman et al. 2003; Karimi et al. 2021). During the production of pickled aubergine, approximately 25% of raw materials are discarded due to unsuitable size, shape, or defects. Previously, these wastes have been managed through

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landfill disposal or used as animal feed, both methods carrying significant transportation and management costs. Upcycling this aubergine waste into dietary fibre offers an innovative and economically viable alternative. This approach could reduce disposal costs and enhance the overall value of aubergine by creating a higher-value product from what would otherwise be considered waste.

The enzymatic browning reaction can occur when the inner part of an aubergine is exposed to oxygen during cutting or size reduction (Mishra et al. 2013). Several methods, including heat treatment, acid application, and using sulphiting agents – individual or combination, have been employed to control this enzymatic browning reaction (Almeida and Nogueira 1995). However, most existing studies have predominantly focused on the efficacy of these antibrowning agents in the context of fruits or vegetables as raw materials. Thus, a gap exists in understanding how these browning agents impact the resulting dietary fibre's visual appearance and functional properties. Further investigations are needed to elucidate these effects and optimise the dietary fibre extraction.

Amylase, protease, and glucoamylase are commonly utilised enzymes in extracting total dietary fibre (TDF), primarily employed to remove non-fibre components (Rodríguez et al. 2006). The application of cellulase or xylanase during extraction has also been reported, with studies showing they can enhance the functional properties of dietary fibre obtained from citrus, rice bran, and potato residue (Wen et al. 2017; Song et al. 2021; Ma et al. 2022). Notwithstanding these developments, the enzymatic modification processes of dietary fibre from purple aubergine remain primarily unexplored, indicating a clear avenue for further investigation.

This article aims to fill this research gap, detailing the pretreatment procedures, extraction process and enzymatic modification of dietary fibre from purple aubergine. The aim of this work is to provide valuable insights into the potential of purple aubergine as a source of functional dietary fibre and contribute to the broader efforts to enhance dietary fibre intake in human diets.

MATERIAL AND METHODS

Materials. Discarded purple aubergines (*Solanum melongena* L. var. Shikon Sendai Naga), specifically those of smaller size (less than 6 cm in length) or irregular shape, were generously provided by Hsu

Chuan Foods Co., Ltd, located in Chiang Rai, Thailand. Upon receipt, intact aubergines were carefully selected and thoroughly washed with tap water to remove any surface debris. The cleaned samples were stored under refrigeration and utilised within 7 days to ensure the freshness and reliability of subsequent analyses.

Alpha-amylase (20 420 U·g⁻¹), cellulase (22 100 U·g⁻¹), and glucoamylase (500 023 U·g⁻¹) were procured from Xi'an Lyphar Biotech, China. Alkaline protease (200 000 U·g⁻¹) was sourced from Shaanxi Jintai Biological Engineering, China. Xylanase (10 000 U·g⁻¹) was obtained from Wellgreen Technology, China. A dietary fibre assay kit was procured from Megazyme International, Ireland. Potassium metabisulphite (KMS) was purchased from Krungthepchemi, Thailand. Citric acid was purchased from RCI Labscan, Thailand.

Proximate composition. The proximate composition of purple aubergine was determined using the methods described by the Association of Official Analytical Chemists (AOAC 2005). Moisture, protein, ash, fat, and dietary fibre contents were quantified according to AOAC methods 925.10, 2 001.11, 942.05, 920.39, and 991.43, respectively.

Effects of pretreatments on properties of the fibre. Due to the enzymatic browning of fresh purple aubergine, various pretreatments were investigated for reducing brown colour and their impact on the functional properties of fibres (Figure 1).

Extraction of dietary fibre. In the experimental process, 100 g of pretreated aubergines were blended with 200 mL of water, except for the dehydrated aubergine, where a ratio of 7 g to 200 mL of water was utilised to achieve equivalent solid content with the other samples. The pH of the mixture was adjusted to 5.5 to facilitate digestion by amylase at a rate of 150 U·g⁻¹ in a shaking water bath at 90 °C for 30 min. The sample was digested by alkaline protease at 300 U·g⁻¹, pH 9.5, 55 °C for 30 min. The final stage of digestion involved glucoamylase at 50 U·g⁻¹, pH 5.5, 60 °C for 30 min. Upon completion, four volumes of ethanol were added to the mixture and left to stand at room temperature for 18 h to allow dietary fibre precipitation. The sample was centrifuged at 4.173 g for 15 min, after which the residue was lyophilised. The yield of dietary fibre was subsequently calculated. The enzymatic conditions were adapted from Prosky et al. (1988) and modified according to the enzyme supplier's specifications.

Colour measurement. The colour of dietary fibres was determined on the CIE Lab* scale by a CR-400 chro-

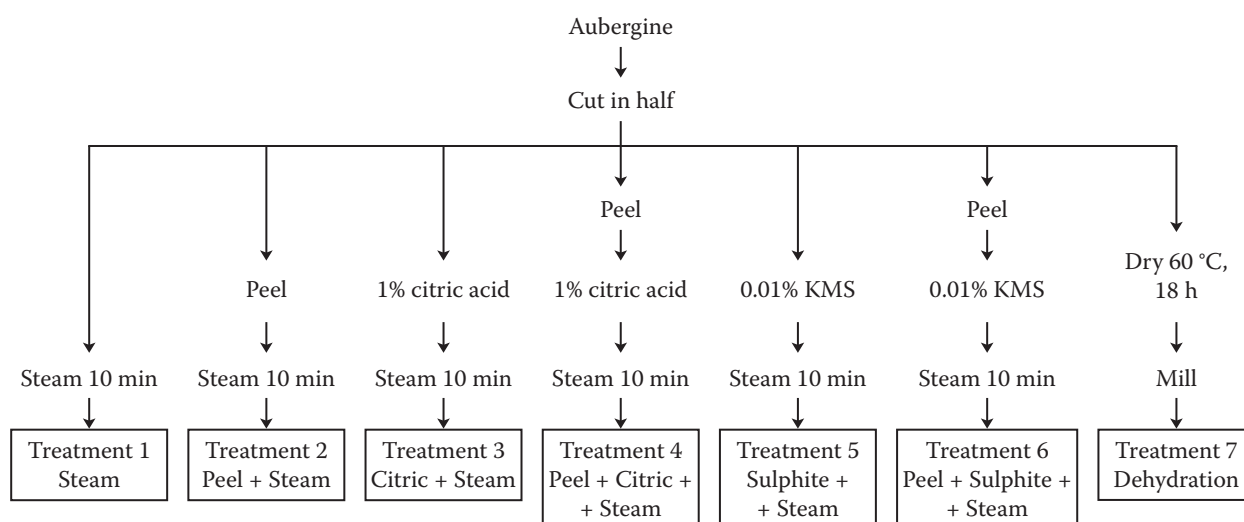


Figure 1. Pretreatments of aubergine before total dietary fibre (TDF) extraction

Peel – aubergines were peeled manually using stainless steel knives; Steam – aubergines were placed approximately 10 cm over boiling water in a steaming pot at ambient pressure for 10 min; KMS – potassium metabisulphite

ma meter (Konica Minolta, Japan). The whiteness index (*WI*) was calculated using Equation 1 (Ullah et al. 2017).

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (1)$$

where: *L* – lightness; *a*, *b* – colour parameters of green to red, blue to yellow, respectively.

Functional properties. Water holding capacity (*WHC*) and oil binding capacity (*OBC*) of dietary fibre were analysed by the method described by Luo et al. (2017).

The optimal pretreatment method was determined using normalised scores of yield, *WI*, *WHC*, and *OBC*, where each response was assigned an equal weight.

Enzymatic modification of dietary fibre. A completely randomised design was utilised to investigate the effect of lipase, cellulase, and xylanase on the functional properties of dietary fibre. As described above, the aubergine was pre-treated using an appropriate pre-treatment method and digested by amylase, alkaline protease, and glucoamylase (control). Then, the samples were digested by lipase (3 U·g⁻¹, pH 7.5, 55 °C, 30 min), cellulase (300 U·g⁻¹, pH 4.5, 60 °C, 60 min), or xylanase (300 U·g⁻¹, pH 3.8, 50 °C, 120 min) (Ma et al. 2022). The required concentrations for extraction were determined by multiplying these values with the activity of the respective enzyme. The digested samples were precipitated by ethanol, centrifuged, and lyophilised. Yield, colour, *WHC*, and *OBC* of dietary fibre were analysed as described above.

Statistical analysis. The analysis of variance and Duncan's multiple range test were performed with R version 4.2.2 (R Core Team 2022). The weighted normalised score was calculated by the 'dplyr' package (Wickham et al. 2022).

RESULTS AND DISCUSSION

Proximate composition of purple aubergines.

The fresh purple aubergines contained 93.00 ± 0.13% moisture content, 1.29 ± 0.01% protein, 0.41 ± 0.01% lipid, and 0.64 ± 0.01% ash. Regarding dietary fibre, the aubergines under study had 0.17 ± 0.04% soluble and 2.96 ± 0.07% insoluble dietary fibres, culminating in a total dietary fibre of 3.13%. These values also echo the fibre content in Nigerian (2.21 to 3.07%) and Ethiopian (2.44 to 2.50%) samples (Ossamulu et al. 2014; Eletta et al. 2017; Bidaramali et al. 2020). However, it is important to note that the variation in these proximate contents could be ascribed to both the genotypic characteristics of the aubergines and the post-harvest handling techniques employed, which have the potential to affect the moisture content and consequently alter the ratio among other components.

Dietary fibre yield. Among pretreatments and extraction using fresh raw materials, Citric + Steam pretreatment yielded more dietary fibre than other methods (Figure 2). However, the values were significantly lower than those obtained using dehydrated aubergines as raw material. This disparity can be attributed to dehydration, which may induce cell shrinkage and foster cross-link-

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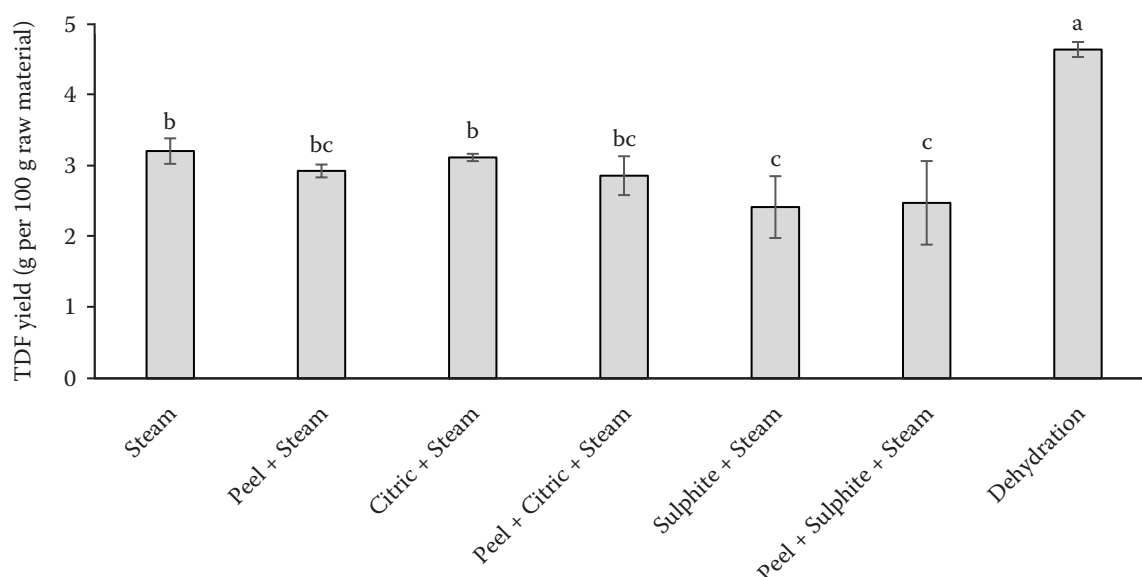


Figure 2. Yield of aubergine total dietary fibre (TDF) obtained by various pretreatments

a–c – statistically significant difference at $P < 0.05$; data represented as mean with standard deviation

ing between cell components. Such changes potentially hinder enzyme accessibility. As a result, the TDF derived from the dehydrated sample might still contain starch or protein bound to the fibre, leading to a higher yield (Sun and Chen 2008). Extraction of peeled aubergines tends to have a lower result than those obtained from non-peeled aubergines. This may be due to higher amounts of dietary fibre in the peel compared to the peel than in the inner part. A study of Egyptian aubergine found that the aubergine flour obtained from peel had higher soluble and insoluble dietary fibre content than the flour obtained from the pulp (Mohamed et al. 2019).

Colour of dietary fibre. Previous dietary fibre extraction studies typically employed dry matter as raw materials to facilitate size reduction and ensure sample homogeneity. However, the present study used fresh samples as raw materials to minimise processing steps and energy consumption from drying. Notably, polyphenol oxidase in fresh aubergine induced darkening during fibre extraction (Figure 3). Hence, various pretreatments were evaluated for their ability to prevent enzymatic browning and their subsequent impact on the properties of the extracted dietary fibre.

TDF prepared from peeled aubergine had a slight green colour (negative a^*), while those prepared from unpeeled counterparts had a slight red value (positive a^*). All TDFs had colour in the yellow region (positive b^*) (data not shown). Nevertheless, both a^* and b^* values were low compared to the whole range of the colour space, indicating that aubergine TDF had low

chroma and may be suitable for addition in food with white or pale colour.

The whiteness index (WI) was highest for the TDF pretreated with Peel + Sulphur + Steam (70.32 ± 2.24 , $P < 0.05$). This was followed by Sulphite + Steam (64.58 ± 1.87), Peel + Steam (64.44 ± 3.30), and Peel + Citric + Steam (64.36 ± 3.55), respectively (Figure 4A). These findings suggest that a sulphiting agent was the most effective in mitigating enzymatic browning in aubergine TDF. Extractions using peeled aubergine generally resulted in a higher WI than the pretreatment of unpeeled aubergine using the same solution. However, it's worth noting that the WI value of aubergine TDF was still lower than those obtained from bamboo shoots (75.23) via enzymatic extraction and coffee silverskin (77.54) via alkaline hydrogen peroxide extraction (Behrouzian et al. 2016; Wicharaew et al. 2019).

Water holding capacity. WHC refers to the ability of a material to retain moisture when subjected to external forces or gravitational pull. Factors influencing WHC include the chemical characteristics and abundance of hydrophilic sites, surface areas, densities, and structures of dietary fibres. Dietary fibre with high WHC can improve textural properties and prevent syneresis of water in food products (Wang et al. 2021). The highest WHC ($17.66 \pm 1.66 \text{ g} \cdot \text{g}^{-1}$) was observed in TDF acquired through Peel + Sulphite + Steam pretreatment, followed by Dehydration ($15.92 \pm 3.66 \text{ g} \cdot \text{g}^{-1}$). The remaining pretreatment methods had no significant difference (Figure 4B).

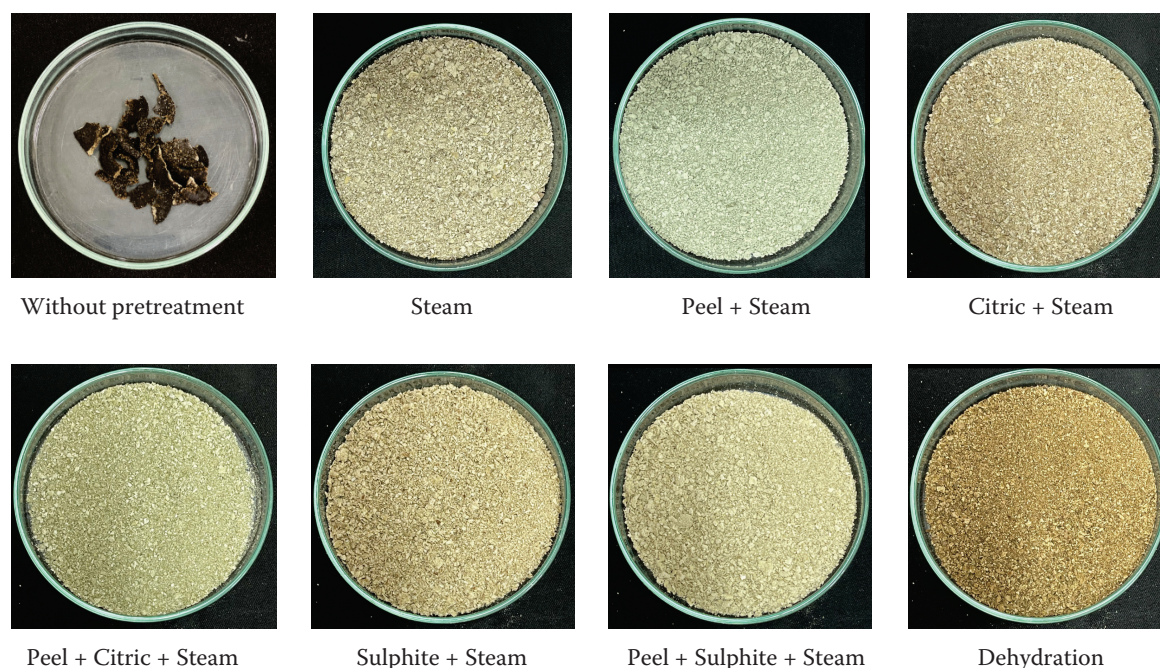


Figure 3. Appearance of aubergine total dietary fibre (TDF) obtained by various pretreatments

WHC of aubergine TDF was higher than TDF from potato residue of $6.06\text{--}10.29\text{ g}\cdot\text{g}^{-1}$ and $9.03\text{ g}\cdot\text{g}^{-1}$ (Waliullah et al. 2021; Ma et al. 2022), pomelo fruitless $9.74\text{ g}\cdot\text{g}^{-1}$ (Liu et al. 2021). The obtained value was also higher than insoluble and soluble dietary fibre from kiwifruit ($6.38 \pm 0.15\text{ g}\cdot\text{g}^{-1}$ and $13.34 \pm 0.11\text{ g}\cdot\text{g}^{-1}$, respectively) (Wang et al. 2021).

Oil binding capacity. *OBC* plays a pivotal role in minimising oil loss during food processing and reducing oil absorption in the human digestive system (Ma et al. 2022). In this research, the highest *OBC* was achieved using TDF pretreated with Sulfur + Steam ($18.35 \pm 3.03\text{ g}\cdot\text{g}^{-1}$), a value not significantly different from that of TDF treated with Peel + Sulfur + Steam ($17.38 \pm 3.48\text{ g}\cdot\text{g}^{-1}$) (Figure 4C).

The results from this study demonstrated a higher oil holding capacity (*OHC*) for TDF compared to potato residue ($2.95 \pm 0.22\text{ g}\cdot\text{g}^{-1}$) and pomelo fruitlets ($1.17 \pm 0.32\text{ g}\cdot\text{g}^{-1}$) (Liu et al. 2021; Ma et al. 2022). However, the *OHC* was lower than that of soluble dietary fibre (SDF) from kiwifruit ($23.00 \pm 0.08\text{ g}\cdot\text{g}^{-1}$) (Wang et al. 2021), indicating that the type of dietary fibre (soluble and insoluble) may also influence *OHC* (Dhingra et al. 2012). Factors affecting *OHC* encompass the surface properties of hydrocolloids, the overall electric charge density, and the hydrophobic attributes of dietary fibre (Wang et al. 2021). These factors can vary based on the source, processing methods, and pretreatments employed.

Based on the previous data, TDF obtained from dehydration pre-treatment exhibited the highest yield, while Sulfite + Steam provided the highest *OBC*. Peel + Sulfur + Steam had the highest values for both *WI* and *WHC*. The weighted normalised scores indicated that Peel + Sulfur + Steam had the highest overall score (0.72), followed by Sulfur + Steam (0.49) and dehydration (0.39). Therefore, the Peel + Sulfur + Steam pretreatment was selected for further experiments.

Enzymatic modification of TDF. After conventional enzymatic treatments for extraction of TDF using amylase, protease and glucoamylase, the samples were further treated with either cellulase, xylanase or lipase before purification and drying. It was found that lipase and xylanase treatments significantly improved yield [4.52 ± 0.16 and $4.42 \pm 0.19\text{ g}\cdot(100\text{ g})^{-1}$, respectively] compared to control [$3.79 \pm 0.2\text{ g}\cdot(100\text{ g})^{-1}$, $P < 0.05$], as shown in Figure 5. Lipase treatment also improved *WHC* ($15.61 \pm 1.4\text{ g}\cdot\text{g}^{-1}$), although the value was not significantly different from control ($14.57 \pm 1.24\text{ g}\cdot\text{g}^{-1}$). However, the *OBC* of control ($11.76 \pm 1.1\text{ g}\cdot\text{g}^{-1}$) was higher than additional enzymatic treatments (8.43 ± 0.91 to $10.96 \pm 1.41\text{ g}\cdot\text{g}^{-1}$). The whiteness index was not significantly different between control, cellulase and xylanase treatments, whilst lipase treatment caused darkening of the TDF compared to control. The yield might appear higher due to the loss of water from pretreatments such as peeling, heat treatment, and the freeze-thaw cycle, which subsequently increases

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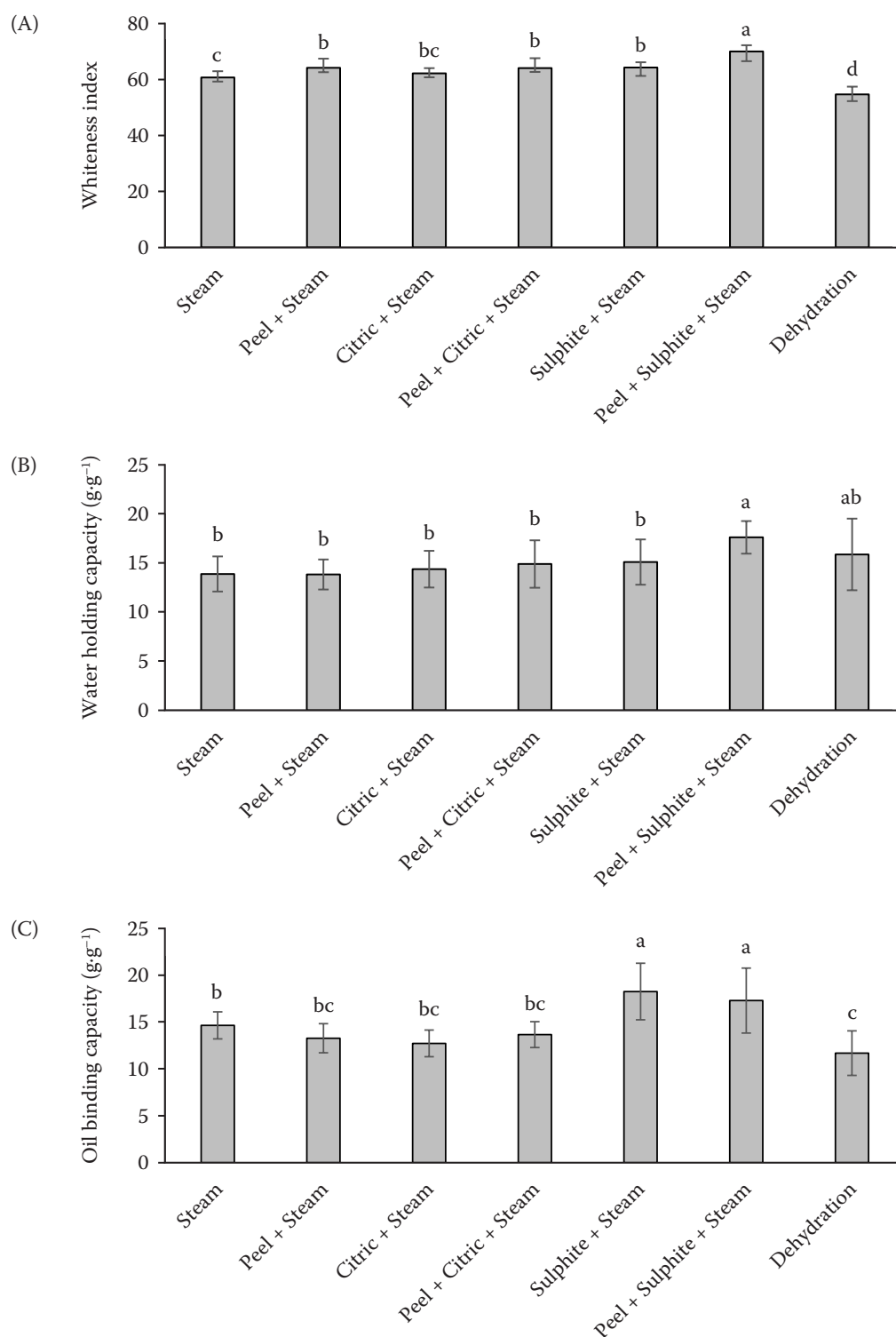


Figure 4. (A) *WI*, (B) *WHC*, and (C) *OBC* of aubergine total dietary fibre (TDF) obtained by various pretreatments a–d – statistically significant difference at $P < 0.05$; data represented as mean with standard deviation; *WI* – whiteness index; *WHC* – water holding capacity; *OBC* – oil binding capacity

the percentage of fibre in the raw material (Zhang et al. 2022). Lipase increased the yield by removing lipids, making the thread more accessible. On the other

hand, xylanase working by breaking down the cell wall components can further enhance the accessibility for other enzymes during fibre extraction (Ma et al. 2022).

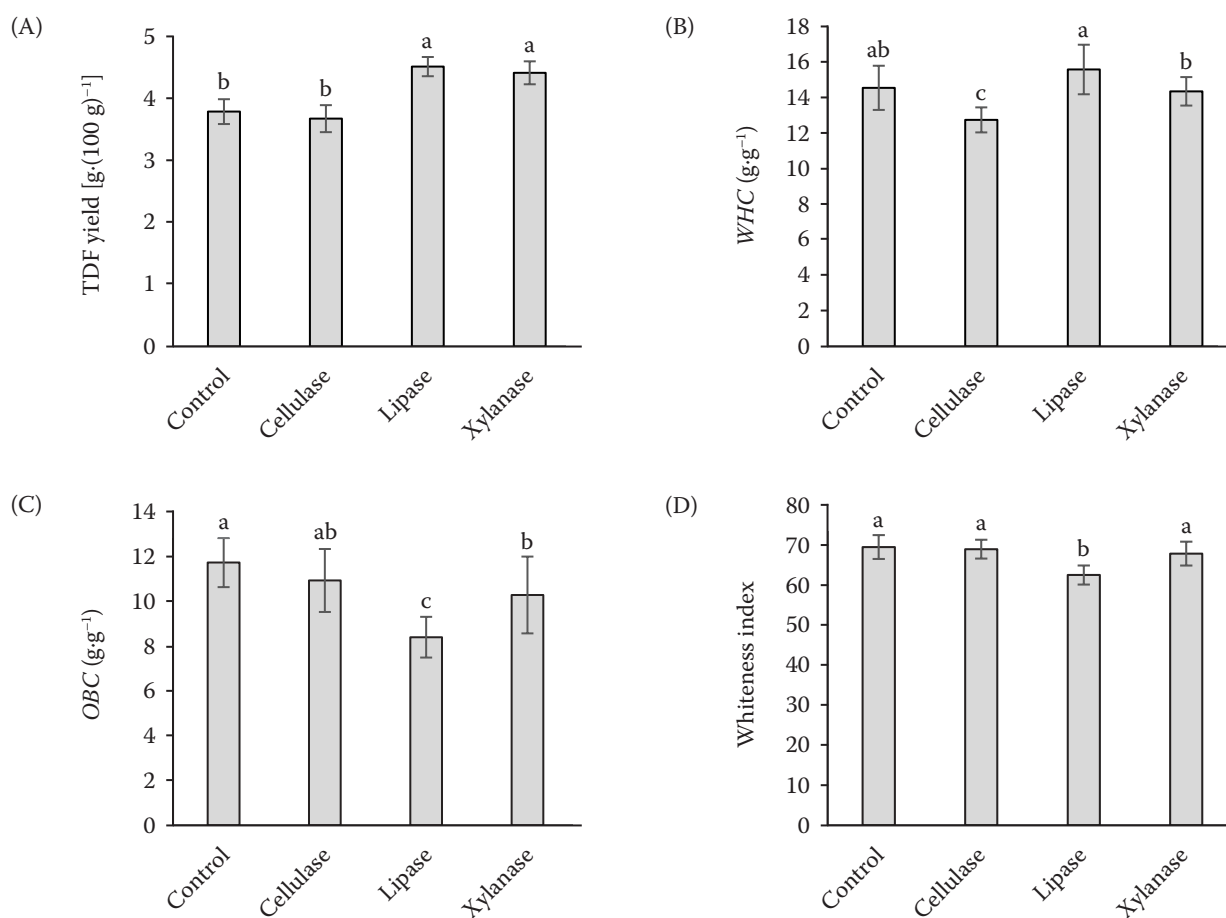


Figure 5. (A) Yield, (B) WHC, (C) OBC, and (D) WI of total dietary fibre (TDF) obtained from different enzymatic modifications

a–c – statistically significant difference at $P < 0.05$; data represented as mean with standard deviation; WI – whiteness index; WHC – water holding capacity; OBC – oil binding capacity

It was previously reported that applications of cellulase and xylanase improved cholesterol absorption capacity (CAC) whilst reducing WHC and OBC of rice bran dietary fibre (Wen et al. 2017). In contrast, applying xylanase enhanced WHC, OBC, and CAC of citrus fibre (Song et al. 2021).

Although cellulase and xylanase hydrolyse cellulose and hemicellulose, components of dietary fibre, limited hydrolysis by these enzymes can enhance the functional properties of the fibre. Cellulase and xylanase cleave bonds within and between cell wall components, transforming water-insoluble elements into water-soluble ones. Simple sugars and smaller carbohydrate molecules could be quantified after applying cellulase and xylanase. Moreover, cellulase and xylanase disrupted the dense lamellar structure of cell wall components, resulting in a loose and porous structure of dietary fibre, which can attach to additional water and oil molecules (Song et al. 2021; Hui et al. 2022; Ma et al. 2022).

Due to the low lipid content of aubergine ($0.41 \pm 0.01\%$ wet basis), a lipid extraction step was not performed before fibre extraction. It was suggested that solvent extraction was required when the lipid content of raw material was more than 10%. However, the presence of lipids may cause incomplete extraction of carbohydrates (BeMiller 2017). The data obtained in this study demonstrates that lipid removal by lipase, as an alternative to solvent extraction, can improve the extraction yield of TDF. Lipase is an enzyme that catalyses the hydrolysis of triacylglycerols into free fatty acids, diacylglycerols, monoacylglycerols and glycerol, which have more hydrophilicity and can be removed along with the extracting solution (Aravindan et al. 2007). Lipase was previously applied to remove lipids to extract dietary fibre from potato residue with the optimal condition of 0.21% lipase at pH 7, 50 °C for 90 min (Huang et al. 2018). However, it was also extensively reported that dietary fibre itself could inhibit pancreatic lipase

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activity and reduce lipid absorption in the small intestine (Tsujita et al. 2007). Therefore, further research is needed to apply lipase as a green alternative to conventional solvent extraction methods. Lipase source, condition and its effectiveness should be investigated.

Considering all the experimental outcomes, the xylanase treatment emerged as the most balanced approach, with the highest overall score of 0.69, based on the weighted normalised score. Interestingly, although this treatment didn't achieve the top values in any individual category, it consistently ranked second or third across all response variables. In contrast, lipase treatment, which excelled in the highest yield and *WHC*, fell to the third overall rank with a score of 0.50 due to its lower performance in *OBC* and *WI*. The enzyme cost of extraction using xylanase and lipase treatment was approximately 0.18 and 0.04 EUR per g TDF, respectively. Nevertheless, it's crucial to note that selecting a suitable treatment will depend on the intended application and the cost implication of the TDF in specific food systems.

CONCLUSION

The optimal preparation method for fresh aubergine is a pre-treatment process involving peeling, immersion in a sulphiting solution, and subsequent steaming before extraction via an enzymatic process. This approach provides exceptional results in terms of *WI* and *WHC*, even though it may result in a yield and *OBC* that are lower compared to certain other treatments. Lipase application boosts the extraction yield and *WHC* when considering enzymatic modifications, but it does come at the cost of darkening the TDF. In contrast, xylanase treatment provides a well-rounded method for enhancing the overall quality of TDF without sacrificing one attribute to improve another.

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