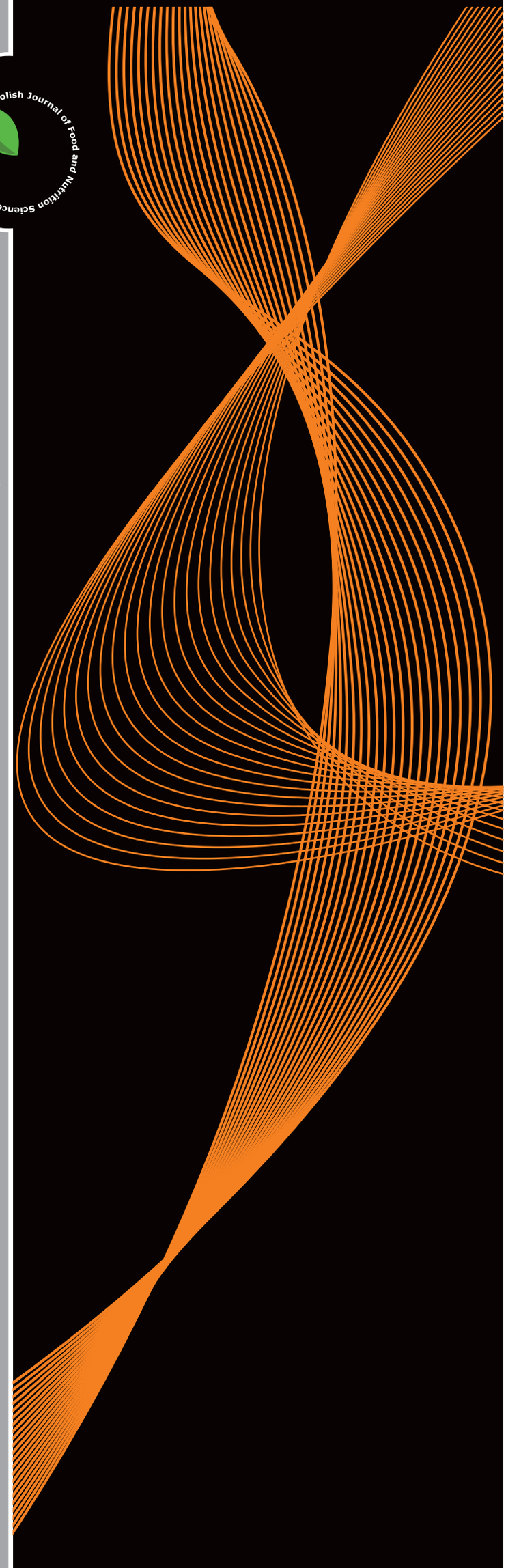
ISSN (1230-0322)
2023, Vol. 73, No. 4

Food

Polish Journal of Food and Nutrition Sciences

formerly Acta Alimentaria Polonica



Published

by Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn

Published since 1957 as
Roczniki Chemii i Technologii Żywności and Acta Alimentaria Polonica (1975–1991)

SCOPE: The Journal covers fundamental and applied research in food area and nutrition sciences with a stress on interdisciplinary studies in the areas of food, nutrition and related subjects.

POLICY: Editors select submitted manuscripts in relation to their relevance to the scope. Reviewers are selected from the Advisory Board and from Polish and international scientific centres. Identity of reviewers is kept confidential.

AUTHORSHIP FORMS referring to Authorship Responsibility, Conflict of Interest and Financial Disclosure, Copyright Transfer and Acknowledgement, and Ethical Approval of Studies are required for all authors.

FREQUENCY: Quarterly – one volume in four issues (March, June, September, December).

COVERED by Web of Science, Current Contents/Agriculture, Biology & Environmental Sciences, Journal Citation Reports and Science Citation Index Expanded, BIOSIS (Biological Abstracts), SCOPUS, FSTA (formerly: Food Science and Technology Abstracts), CAS (Chemical Abstracts), AGRICOLA, AGRO-LIBREX data base, EBSCO, FOODLINE, Leatherhead FOOD RA data base FROSTI, AGRIS and Index Copernicus data bases, Biblioteka Nauki ICM, Biblioteka Narodowa – POLONA, and any www browser; ProQuest: The Summon, Bacteriology Abstracts, Immunology Abstracts.

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Polish Journal of Food and Nutrition Sciences

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W latach 2022–2024 kwartalnik naukowy ***Polish Journal of Food and Nutrition Sciences*** realizuje projekt nr RCN/SP/0520/2021/1 finansowany ze środków budżetu państwa – Ministerstwo Edukacji i Nauki w ramach programu „Rozwój czasopism naukowych". Dofinansowanie wynosi 120 000 zł. W ramach projektu podejmowane są działania zmierzające do podniesienia poziomu praktyk wydawniczych i edytorskich, zwiększenia wpływu czasopism na rozwój nauki oraz utrzymania się czasopism w międzynarodowym obiegu naukowym.

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In the years 2022–2024, the scientific quarterly ***Polish Journal of Food and Nutrition Sciences*** accomplishes a project no. RCN/SP/0520/2021/1 financed from the state budged – Ministry of Education and Science Republic of Poland in the framework of a program "Development of scientific journals". The financing amounts to 120,000 PLN. The program aims at improving the level of publishing and editing practices, increasing the impact of scientific journals on science development, and extending the international range of scientific journals.

Subscription
2023 – One volume, four issues per volume. Annual subscription rates are: Poland 150 PLN, all other countries 80 EUR. Prices are subject to exchange rate fluctuation. Subscription payments should be made by direct bank transfer to Bank Gospodarki Żywnościowej, Olsztyn, Poland, account No 17203000451110000000452110 SWIFT code: GOPZPLWOLA with corresponding banks preferably. Subscription and advertising offices at the Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, ul. J. Tuwima 10, 10-747 Olsztyn, Poland, tel./fax (48 89) 5234670, fax (48 89) 5240124, e-mail: pjfns@pan.olsztyn.pl; http://journal.pan.olsztyn.pl

Zamówienia prenumeraty: Joanna Molga (e-mail: pjfns@pan.olsztyn.pl)

Wersja pierwotna (referencyjna) kwartalnika PJFNS: wersja papierowa (ISSN 1230–0322)
Nakład: 70 egz.; Ark. wyd. 16,5; Ark. druk. 14
Skład i druk: ITEM

ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 301–310
DOI: 10.31883/pjfns/172244
ISSN 1230-0322, e-ISSN 2083-6007

Effects of Mulberry Pomace Addition and Transglutaminase Treatment on the Quality of Pasta Enriched with Antioxidants and Dietary Fiber

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Mulberry pomace powder, a by-product of mulberry juice processing, was added to pasta recipe to make pasta with high dietary fiber and antioxidant contents. The effects of mulberry pomace ratio on the nutritional, textural and cooking properties as well as the sensory overall acceptance of the product were investigated. A significant increment in dietary fiber and total anthocyanin contents as well as decrement in cooking quality, texture and color change were observed. The cooking loss increased with the substitution level of mulberry pomace while the optimal cooking time, swelling index and water absorption index decreased. The quality improvement of 10% mulberry pomace fortified pasta was investigated by adding a transglutaminase preparation with enzyme dosage from 0.25 to 1.00 U/g protein. The fortified pasta treated with transglutaminase at 0.50 U/g protein showed a significant improvement in chewiness, tensile strength and elongation rate but was not significantly affected in terms of the swelling index and water absorption. The use of transglutaminase also improved the overall acceptability of the fortified pasta. Mulberry pomace powder may, therefore, be considered a potential antioxidant-rich and dietary fiber-rich material for incorporation into pasta products.

Key words: antioxidant, dietary fiber, mulberry pomace, pasta, transglutaminase

INTRODUCTION

Pasta is a common staple food over the world with a production rate of 16.9 million tons in 2021 [IPO, 2021]. Durum wheat semolina is a key ingredient in making high-quality pasta. Despite being rich in calories, durum wheat semolina contains very little dietary fiber and bioactive compounds, such as antioxidants. In this regard, pasta made of durum wheat semolina could be supplemented with functional ingredients to satisfy the growing demand of consumers for healthy food products [Bresciani *et al.*, 2022].

Fruit processing by-products are reported to contain many functional components such as dietary fiber, protein, minerals, vitamins, and antioxidants [Martins *et al.*, 2017]. As a sustainable approach, the utilization of fruit by-products to improve the nutritional value of pasta has been attracting attention from researchers [Bianchi *et al.*, 2021]. Using cashew apple pomace from 5 to 20% of the semolina weight increased total dietary fiber of the pasta up to 4.1 times [Nguyen *et al.*, 2023b]. For pasta supplemented with 10% grape pomace, the total phenolic content and antioxidant capacity were 6-fold and 38-fold, respectively,

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Submitted: 17 May 2023
Accepted: 12 September 2023
Published on-line: 10 October 2023

higher than those of the control sample [Tolve *et al.*, 2020]. Fruit peels from mango and banana were also reported as mineral and ascorbic acid-fortified ingredients for pasta preparation [Jalgaonkar *et al.*, 2018; Puraikalan, 2018]. However, a significant consequence of adding fruit by-products to pasta formulation is significant deterioration of the cooking and textural attributes as well as the sensory quality of the fortified pasta [Carpentieri *et al.*, 2022; Padalino *et al.*, 2017, 2018]. In order to improve textural and cooking properties of high-fiber pasta, use of auxiliary ingredients or additional treatment of pasta dough are essential [Padalino *et al.*, 2016].

Transglutaminase (TG, EC 2.3.2.13) has been reported to improve the protein network due to its cross-linking reaction between the protein molecules in food materials [Zhu *et al.*, 2019]. In biochemistry, TG catalyzes the formation of a covalent bond between the γ-carboxamide group of protein or peptide-bound glutamine (acyl donors) and the free amino group of protein or peptide-bound lysine (acyl acceptors). In pasta making, this covalent bond formed by protein intermolecular crosslink could help to considerably decrease the quantities of soluble protein fraction, thereby strengthening the structural integrity of pasta [Gharibzahedi *et al.*, 2019]. The effects of TG on pasta texture have been demonstrated in different studies to improve the overall quality of pasta made with low-protein durum wheat semolina [Aalami & Leelavathi, 2008], semolina-pollard enriched pasta [Sissons *et al.*, 2010], cellulase-treated wheat bran-fortified pasta [Nguyen *et al.*, 2020] or corncob powder-supplemented pasta [Nguyen *et al.*, 2023a]. However, the use of TG in the making of pasta with fruit pomace added has not been reported.

Fruits of mulberry, a tropical *Morus* genus of the Moraceae family, are widely used in the processing of juice, jam, vinegar, and alcoholic beverage [Jan *et al.*, 2021]. The by-product of mulberry juice processing, mulberry pomace, is rich in dietary fiber and phenolic compounds with high antioxidant activity [Zhang *et al.*, 2011]. Mulberry pomace is supplemented to recipes of various food products including chocolate [Hwang *et al.*, 2012], bread [Kim *et al.*, 2013] and cookies [Jeon *et al.*, 2013] to improve their antioxidant capacity. Nevertheless, the addition of mulberry pomace to pasta formulation has not been considered in the literature.

In this study, mulberry pomace powder and transglutaminase preparation were used in pasta recipe to make the product with high-fiber and antioxidant contents. The objective of the study was to investigate the effects of the mulberry pomace ratio on the nutritional, textural and cooking properties as well as the overall acceptability of the product. The effects of transglutaminase dosage in the treatment of pasta dough supplemented with mulberry pomace were also evaluated.

MATERIALS AND METHODS

Materials

Fresh mulberry (*Morus alba* L.) fruits were harvested from a farm in Da Lat City (Lam Dong province, Vietnam) in July 2022, packed in perforated carton boxes and transported to the laboratory within 8 h after harvesting. At the laboratory, the fruits without physical damage and decay were manually selected and washed with tap water. After 1 h draining, the fruits were pressed in a screw press (AC-130, Kheo Sung World Inc., Seoul, South Korea). The obtained pomace was subsequently dried in a convection oven (CD-20-08, Tung Viet Ltd., Dong Nai, Vietnam) at 60°C for about 6 h. The dried mulberry pomace with moisture content of 10–12 g/100 g was powdered in a hammer mill (HM-20-10, Tung Viet Ltd., Dong Nai, Vietnam), sieved through a 40-mesh sieve and stored in polyethylene bags at −18°C for experimentation.

Durum wheat semolina and table salt were purchased from Vietnam Flour Mills Ltd. (Ba Ria – Vung Tau, Vietnam) and Southern Salt and Trade Joint Stock Company (Ho Chi Minh City, Vietnam), respectively.

Protiact TG-RA, a clean-labelling preparation used to improve the texture of protein-rich food products, was supplied by Rama Production Co. (Bangkok, Thailand). The transglutaminase (TG) activity of Protiact TG-RA was 100 U/g. One unit (U) of TG activity was defined as the quantity of enzyme that catalyzes the formation of 1 µmol of hydroxamate from *N*-carbobenzoxy--L-glutaminylglycine within 1 min under the assay conditions [Ando *et al.*, 1989].

Protease (Alcalase 2.5 L), amyloglucosidase (Dextrozyme GA) and α-amylase (Termamyl SC) used for determination of dietary fiber content were from Novozyme Inc. (Bagsvaerd, Denmark). All chemicals of analytical grade quality used in this study were purchased from Sigma Aldrich Co. (Saint Louis, MO, USA) and Merck Co. (Darmstadt, Germany).

Pasta preparation

Pasta was prepared with 200 g of a blend of durum wheat semolina and mulberry pomace, 1 g of table salt and 95 g of water. The ratio of mulberry pomace was 0, 5, 10, 15, 20% of the blend weight. Semolina, mulberry pomace and table salt were premixed in a KitchenAid 5K5SSWH flour mixer (Whirlpool Co., Guangzhou, China) at about 80 rpm for 5 min. Water was heated to 42°C, added to the flour mixer and mixed at 80 rpm for 2 min. The dough was kneaded for 18 min at 60 rpm. The pasta was prepared using a Philips HR2355 extruder (Philips Co., Guangdong, China) with extrusion force of 7,117 N. The fresh pasta was dried at 50°C until the moisture content achieved 10–12 g/100 g. The product was then stored in 18×26 cm polyethylene bags at −18°C for further analysis. Pasta fortified with 10% mulberry pomace was used for enzymatic treatment. TG was mixed with water before the wet mixing. The enzyme was added with the dosage of 0.25, 0.50, 0.75, and 1.00 U/g protein of the blend weight.

Proximate composition analysis

Moisture content was determined at 105°C using a moisture analyzer (ML-50, A&D Co., Tokyo, Japan). Crude protein content was evaluated by Kjeldahl method with a nitrogen-to-protein conversion factor of 5.8 for wheat. Lipid content was measured using Soxhlet method with diethyl ether. Ash was quantified by incineration at 600°C in a furnace (EF11/8, Lenton Co., Hope Valley, UK). Insoluble dietary fiber (IDF) and soluble dietary fiber

(SDF) contents were estimated according to AOAC International methods no. 991.42 and 993.19, respectively [AOAC, 2000].

Determination of total phenolic and anthocyanin contents and antioxidant capacity

Total phenolic content (TPC) of dried mulberry pomace, durum wheat semolina and dried pasta samples was determined according to the procedure reported by Biney & Beta [2014] with some modifications. About 1 g of dried mulberry pomace, durum wheat semolina or dried pasta was extracted with 10 mL of 60% (*v/v*) acetone for 30 min. The extract was recovered by centrifugation at 1,600×*g* for 20 min (3K30, Sigma Zentrifugen Ltd., Osterodeam Harz, Germany). The obtained supernatant (0.2 mL) was added to 1 mL of the Folin-Ciocalteu reagent followed by vortexing for 30 s. The tubes with the reaction mixture were left at room temperature in dark for 2 h, and the absorbance was measured at 760 nm (UV 2600i spectrophotometer, Shimadzu Co., Kyoto, Japan). TPC was calculated as mg gallic acid equivalent *per* 100 g dry weight of the dried mulberry pomace, durum wheat semolina or dried pasta (mg GAE/100 g dw).

For total anthocyanin content determination, 1 g of dried mulberry pomace, durum wheat semolina or dried pasta was extracted with 10 mL of acidified ethanol (the volume ratio of ethanol to 2% HCl was 1:1, *v/v*) for 2 h [Peng *et al.*, 2021]. The centrifugation was performed at 1,600×*g* for 20 min. The resulting supernatant was then diluted in acetate buffer with pH 1.0 and pH 4.5. Total anthocyanin content was calculated as follows:

$$A = (A_{520}^{pH_{1.0}} - A_{700}^{pH_{1.0}}) - (A_{520}^{pH_{4.5}} - A_{700}^{pH_{4.5}}) \quad (1)$$

$$\alpha = \frac{A \times M_W \times D_f \times 1000}{\varepsilon \times l} \quad (2)$$

in which, α is the total anthocyanin content (mg cyanidin 3-*O*--glucoside equivalent/100 g dw); M_W is molecular weight of cyanidin 3-*O*-glucoside, equal to 449.2 g/mol; D_f is dilution factor; ε is molecular extinction coefficient, equal to 26,900 L/(mol×cm); l is length of the cuvette (cm), $A_{520}^{pH_{1.0}}$ and $A_{700}^{pH_{1.0}}$ are absorbances of the reaction mixture with extract diluted in the buffer with pH 1.0 at 520 and 700 nm, respectively, $A_{520}^{pH_{4.5}}$ and $A_{700}^{pH_{4.5}}$ are absorbances of the reaction mixture with extract diluted in the buffer with pH 4.5 at 520 and 700 nm, respectively.

Antioxidant capacity of dried mulberry pomace, durum wheat semolina and dried pasta was evaluated as 2,2-diphenyl--1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) as described by Nguyen *et al.* [2020]. Briefly, about 1 g of dried mulberry pomace, durum wheat semolina or dried pasta was mixed with 10 mL of a 60% (*v/v*) aqueous acetone solution for 30 min. The supernatant recovered by centrifugation at 1,600×*g* for 20 min (3K30 centrifuge, Sigma Zentrifugen Ltd.) was diluted and used for antioxidant capacity determination. For DPPH assay, 0.1 mL of diluted extract was combined with 3.9 mL of a 60 µM DPPH radical solution in methanol. The reaction mixture was incubated at room temperature and in the dark. Absorbance at 515 nm was measured at zero and after 30 min of incubation using methanol as the blank. The results were given as µmol of Trolox equivalent (TE) *per* 100 g dw of dried mulberry pomace, durum wheat semolina or dried pasta. For FRAP assay, 3.8 mL of the FRAP working solution (25 mL of 0.3 M acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tris(2-pyridyl)-*s*-triazine solution in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3 \times 6H_2O$) was mixed with 0.2 mL of the diluted extract. The reaction mixture was incubated in the dark at 37°C for 5 min, and the absorbance at 593 nm was measured with an acidified methanol as blank. The FRAP was expressed as µmol TE/100 g dw of dried mulberry pomace, durum wheat semolina or dried pasta.

Determination of water holding capacity

Water holding capacity (WHC) of mulberry pomace and semolina was determined as described by Caprez *et al.* [1986] with slight modification. About 1 g of the sample was soaked in 10 mL of distilled water for 2 h and then centrifuged at 1,200×*g* for 10 min at room temperature. The supernatant was discarded, and the residue was weighted. WHC (g water/g) was calculated by the following equation:

$$WHC = (M_1 - M_0)/M_0 \quad (3)$$

in which, M_0 is the initial weight of the sample and M_1 is the weight of the obtained residue.

Determination of cooking properties

Cooking quality of pasta was evaluated through optimal cooking time (OCT), cooking loss (CL), swelling index (SI) and water absorption index (WAI) according to the method described by Nguyen *et al.* [2020]. About 10 g of the pasta sample (~5 cm in length) were cooked in 100 mL of boiling distilled water until the white inner core of pasta strand disappeared for its OCT. The cooked pasta strands were drained for 2 min, then dried at 105°C to constant weight. The total dry matter of cooking water was determined by drying at 105°C. The CL, SI and WAI were calculated as follows:

$$CL = P_1/P_0 \times 100\ (\%) \quad (4)$$

$$SI = (P_2 - P_3)/P_3 \quad (5)$$

$$WAI = (P_2 - P_0)/P_0 \quad (6)$$

in which, P_1 is the total dry matter of cooking water, P_2 is the weight of cooked and drained pasta, P_3 is the weight of cooked pasta after drying at 105°C, and P_0 is the weight of raw pasta.

Instrumental color analysis

Color of uncooked pasta, mulberry pomace powder and semolina was determined using a chromameter CR400 (Konica Minolta Co., Osaka, Japan) with CIELab color space. The total color difference (ΔE) was determined through L^* (lightness), a^* (redness) and b^* (yellowness) values as follows:

$$\Delta E = \sqrt{(L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2} \quad (7)$$

in which, L^*_0, a^*_0 and b^*_0 are the color values of the pasta without mulberry pomace supplementation; L^*, a^*, and b^* are the color values of the mulberry pomace-enriched pasta.

Determination of textural properties

Texture profile analysis (TPA) was employed to assess the textural quality of pasta samples using a TA-XT plusC (Stable Micro Systems Co., Godalming, UK) with a Windows version of Exponent Connect Lite 7.0 software (Texture Technologies Co., Hamilton, MA, USA). The measurements were done on 5 sections from 5 different cooked pasta strands, using a 40 mm diameter acrylic probe with 70% axial compression and compression speed of 1 mm/s. The second compression cycle was set after 1 s. The hardness and chewiness of cooked pasta samples were calculated from the force-time curve. In the tensile strength test, two ends of a 15 cm cooked pasta strand were fixed to a pair of parallel rollers (Pasta Tensile Rig, Stable Micro Systems). The extension speed was 1 mm/s. The tensile strength (TS) and elongation rate of cooked pasta samples were determined by the formulas reported by Nguyen *et al.* [2020].

Estimation of overall acceptability

The overall acceptibility of pasta was evaluated using a consumer test with 60 untrained panelists, including 32 men and 28 women, aged from 18 to 25, recruited from the students and staff at the Ho Chi Minh City University of Technology (Ho Chi Minh City, Vietnam). Pre-screening for potential wheat allergies and pasta consumption (at least once a week) was done on the panelists. Pasta samples were prepared by boiling 100 g of pasta in 1 L of water at OCT. Three-digit codes were used to label samples of pasta. Each panelist received approximately 30 g of cooked pasta at 40°C, one serving at a time and in a random order. Water was provided between samples for mouth cleansing. The overall acceptability was evaluated using a 9-point hedonic scale, ranging from 1 (extremely dislike) to 9 (extremely like).

Statistical analysis

Each type of pasta was prepared in triplicate. The data were subjected to one-way analysis of variance (ANOVA) following a Tukey post-hoc test with $p \leq 0.05$, and correlations between the overall acceptance and the pasta hardness and elasticity were evaluated based on the Pearson correlation coefficient (Statgraphics ver. 18.1.12, Statgraphics Technologies, Inc., The Plains, VA, USA).

RESULTS AND DISCUSSION

Characterization of mulberry pomace powder

The results of nutritional quality and physical characteristics of mulberry pomace powder and durum wheat semolina used in the present study are presented in Table 1. The mulberry pomace powder contained slightly less protein than the durum wheat semolina but had a greater content of lipid (9.8-fold) and ash (2.8-fold) than the semolina. In addition, starch was also present at low level in the mulberry pomace while it was the main compound in the durum wheat semolina. It can be noted that the total dietary fiber (TDF) content of mulberry pomace powder was 13.9-fold higher than that of the semolina. Nevertheless, the mulberry pomace powder used in this study contained less TDF than the pomace powder of blueberry (62.3 g/100 g dw), cranberry (62.2 g/100 g dw) [Wang *et al.*, 2019] and strawberry (67.6 g/100 g dw) [Juśkiewicz *et al.*, 2015]. The IDF:SDF ratio of mulberry pomace powder (6.8:1.0, *w/w*) was also greater than that of durum wheat semolina (1.4:1.0, *w/w*) (Table 1). Both soluble and insoluble dietary fibers provide positive effects to human health; SDF is mainly associated with lowering blood cholesterol and reducing the absorption of glucose in the small intestine while IDF is in charge of improving fecal bulk, and water absorption that improves laxative effects [Esteban *et al.*, 2017; Yangilar, 2013].

The mulberry pomace contained a considerable amount of anthocyanins (Table 1). This group of mulberry pomace phenolics is known for its high antioxidant activity [Du *et al.*, 2021; Zhang *et al.*, 2011]. The total phenolic content of mulberry pomace powder was 7.7-fold greater than that of the semolina (Table 1). Different non-anthocyanin monomeric phenolics, including resveratrol, catechin, rutin, quercitrin and quercetin, were identified in mulberry pomace and these compounds were also reported to exhibit high antioxidant activity [Du *et al.*, 2022]. As a result, the ferric reducing antioxidant power and DPPH radical scavenging activity of mulberry pomace powder were 38.9- fold and 15.1-fold, respectively, greater than those of durum wheat semolina (Table 1). Mulberry pomace can be considered as an antioxidant source for incorporation into different food products.

In terms of water holding capacity, the mulberry pomace exhibited a threefold increase relative to the semolina (Table 1). Fiber is reported to have good water holding capacity due to a high number of hydroxyl groups in the chemical structure of its components [Tejada-Ortigoza *et al.*, 2017]. Supplementation of mulberry pomace to pasta recipe could change cooking quality of the product since fiber can compete for water with starch granules during pasta cooking, affecting starch gelatinization [Qiu *et al.*, 2016].

The mulberry pomace had different color compared to the wheat semolina. The lightness (L^*) of mulberry pomace was 2.3-fold lower than that of the semolina counterpart (Table 1), meaning it was much darker. The redness (a^*) of mulberry pomace was also 5.9-fold greater while its yellowness (b^*) was 7.7-fold lower than those of the semolina. The color of pasta supplemented with mulberry pomace could be changed as compared to that of the conventional pasta.

Effects of mulberry pomace ratio in the pasta formulation on nutritional quality and antioxidant capacity of the product

The nutritional quality and antioxidant capacity of pasta samples incorporated with different mulberry pomace powder ratios are shown in Table 2. The use of mulberry pomace was found to have no impact on the protein content of pasta probably due

Table 1. Nutritional quality, antioxidant capacity and physical characteristics of mulberry pomace and durum wheat semolina.

Component/characteristic		Mulberry pomace	Durum wheat semolina
Proximate composition	Crude protein (g/100 g dw)	12.88±0.41^b	13.89±0.56^a
	Lipid (g/100 g dw)	10.82±0.42^a	1.05±0.18^b
	Ash (g/100 g dw)	2.81±0.05^a	0.95±0.03^b
	Starch (g/100 g dw)	3.33±0.20^b	81.72±1.10^a
Fiber composition	TDF (g/100 g dw)	45.80±0.89^a	3.32±0.47^b
	IDF (g/100 g dw)	39.89±1.54^a	1.92±0.18^b
	SDF (g/100 g dw)	5.91±0.69^a	1.39±0.30^b
	IDF:SDF ratio (*w/w*)	6.84±1.09^a	1.40±0.18^b
Phenolic content	Total anthocyanin content (mg C3GE/100 g dw)	508.9 ± 1.9	ND
	Total phenolic content (mg GAE/100 g dw)	1,095±43^a	141.6±4.7^b
Antioxidant capacity	Ferric reducing antioxidant power (µmol TE/100 g dw)	8,558±429^a	220±23^b
	DPPH radical scavenging activity (µmol TE/100 g dw)	2,529±82^a	167±21^b
Physical characteristics	Water holding capacity (g water/g dw)	2.35±0.13^a	1.02±0.08^b
	*L**	39.58±0.03^b	91.19±0.01^a
	*a**	5.25±0.05^a	0.89±0.01^b
	*b**	1.30±0.02^b	9.97±0.01^a

Data are expressed as mean ± standard deviation (n=3). Values that do not share a lowercase letter within a row are significantly different (p≤0.05). TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; C3GE, cyanidin 3-*O*-glucoside equivalent; GAE, gallic acid equivalent; TE, Trolox equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; dw, dry weight; *L**, lightness; *a**, redness; *b**, yellowness; Δ*E*, total color difference; ND, not determined.

to the little difference in protein content between mulberry pomace powder and wheat semolina. However, the mulberry pomace addition to the pasta recipe increased its lipid and ash contents but reduced the starch content. Mulberry pomace was reported to be rich in essential fatty acids, including omega-3 and omega-6 fatty acids [Yılmaz & Durmaz, 2015]. In addition, various minerals including macro-minerals (K, Ca, Na, Mg) and micro-minerals (Fe, Zn, Ni) were identified in mulberry [Imran *et al.*, 2010]. As a result, the partial replacement of semolina with mulberry pomace in pasta formulation significantly improved nutritional value of the product.

When the amount of mulberry pomace in the recipe increased from 0% to 20%, the TDF, IDF, and SDF contents of pasta increased by 232%, 406%, and 46%, respectively. As the IDF:SDF ratio of mulberry pomace was 4.9-fold greater than that of durum wheat semolina, the enhanced amount of mulberry pomace in the recipe led to an augmented IDF:SDF ratio of the pasta ranging from 1.23 to 4.16. The total fiber content of pasta sample with 10% mulberry pomace powder was greater than 6 g/100 g pasta, and this product could be considered as food "high" in fiber [Bröring & Khedkar, 2018]. It was reported that the IDF:SDF ratio of pasta fortified with 10% orange pomace was 1.9 while that of pasta supplemented with 10% cucumber pomace was 5.2 [Kaur *et al.*, 2021]. Thus, the IDF:SDF ratio of pasta added with 10% mulberry pomace met the recommended value of 3:1 (*w/w*) suggested by the Dietetic Association to enhance the nutraceutical functionality of dietary fiber [Borderías *et al.*, 2005]. Foods with proper ratio of IDF:SDF improve physiological effects of both dietary fiber fractions on human health [He *et al.*, 2022].

The higher the mulberry pomace level in pasta formulation, the greater the total phenolic and total anthocyanin contents, as well as the antioxidant capacity of pasta. Results collated in Table 2 reveal that the pasta sample with 20% of mulberry pomace powder (M20) showed the greatest TPC being 2.6-fold higher than in the control sample (M0); in addition, its antioxidant capacity assessed by FRAP and DPPH assays was 11.8-fold and 36.1-fold, respectively, higher than that of the M0 sample. When orange pomace was added to pasta recipe at the level of 20% of the flour blend, a 4.8-fold increase in TPC and a 3-fold increase in DPPH radical scavenging activity were recently recorded [Kaur *et al.*, 2021]. Increment in TPC and antioxidant capacity in the final product may vary depending on the fruit by-product composition and pasta preparation conditions. It can be confirmed that the incorporation of mulberry pomace into pasta recipe highly improved antioxidant capacity of the product.

Effects of mulberry pomace ratio in the pasta formulation on textural and cooking quality of the product

Textural properties

The textural properties of pasta samples incorporated with different mulberry pomace powder ratios are shown in Table 3. Increase in mulberry pomace ratio in the pasta recipe resulted in an increased hardness of the product. At 20% mulberry pomace level (M20), the hardness of pasta was about 22% higher than

Table 2. Nutritional quality and antioxidant capacity of pasta incorporated with mulberry pomace at different levels.

Component/characteristic		M0	M5	M10	M15	M20
Proximate composition	Crude protein (g/100 g dw)	13.92±0.50^{a}	13.75±0.77^{a}	13.54±0.93^{a}	13.43±0.75^{a}	13.08±0.47^{a}
	Lipid (g/100 g dw)	1.65±0.14^{b}	1.53±0.04^{ab}	1.99±0.15^{ab}	2.07±0.08^{ab}	2.57±0.19^{a}
	Ash (g/100 g dw)	1.00±0.03^{e}	1.09±0.03^{d}	1.22±0.08^{c}	1.52±0.03^{b}	1.65±0.02^{a}
	Starch (g/100 g dw)	81.72±1.10^{a}	77.80±1.05^{b}	73.88±1.01^{c}	69.96±0.96^{d}	66.04±0.92^{e}
Fiber composition	TDF (g/100 g dw)	3.35±0.08^{e}	5.35±0.65^{d}	7.27±0.77^{c}	9.32±0.99^{b}	11.33±0.97^{a}
	IDF (g/100 g dw)	1.85±0.05^{d}	3.73±0.70^{cd}	5.56±0.89^{bc}	7.32±1.03^{b}	9.14±0.90^{a}
	SDF (g/100 g dw)	1.51±0.03^{c}	1.62± 0.14^{b}	1.71±0.14^{b}	2.00±0.10^{a}	2.19±0.08^{a}
	IDF:SDF ratio (*w/w*)	1.23±0.02^{c}	2.33±0.57^{bc}	3.29±0.76^{ab}	3.68±0.64^{a}	4.16±0.28^{a}
Phenolic content	Total anthocyanin content (mg C3GE/100 g dw)	0.0±0.0^{e}	22.1±3.0^{d}	33.5±1.9^{c}	62.4±1.5^{b}	83.4±2.8^{a}
	Total phenolic content (mg GAE/100 g dw)	134±9^{e}	190±8^{d}	224±10^{c}	297±6^{b}	348±11^{a}
Antioxidant capacity	Ferric reducing antioxidant power (μmol TE/100 g dw)	60±4^{e}	448±14^{d}	869±31^{c}	1,521±92^{b}	2,168±133^{a}
	DPPH radical scavenging activity (μmol TE/100 g dw)	129±15^{e}	491±92^{d}	855±94^{c}	1,259±201^{b}	1,526±249^{a}

Data are expressed as mean ± standard deviation (n=3). Values that do not share a lowercase letter within a row are significantly different ($p \leq 0.05$). M0, M5, M10, M15, M20, pasta incorporated with 0% (control), 5%, 10%, 15%, and 20% mulberry pomace powder, respectively; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; C3GE, cyanidin 3-*O*-glucoside equivalent; GAE, gallic acid equivalent; TE, Trolox equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; dw, dry weight.

that of the control sample (M0). Similar observation was reported when tomato peel or gac fruit powders were supplemented to the pasta recipe [Chusak *et al.*, 2020; Padalino *et al.*, 2017]. Fibers were reported to have high water absorption capacity [Dhingra *et al.*, 2012]; the amount of water for starch gelatinization during the cooking of enriched fiber pasta was therefore reduced [Nguyen *et al.*, 2020], resulting in an enhanced hardness of the product [Gallo *et al.*, 2020]. Various interaction types between phenolics and starch are reported. Tannins might interact with amylose through hydrogen bonding and hydrophobic interaction, resulting in microstructure alteration of starch granules [Amoako & Awika, 2016]; in addition, polyphenols might adsorb onto surface of the starch granules and interact with amylose to form an unordered inclusion complex [Liu *et al.*, 2011], which is suggested to interact with free water. This phenomenon might lead to less hydrated starch granules during the pasta cooking and decreased starch gelatinization. Otherwise, anthocyanins might limit the gluten development by forming disulfide linkages with glutenin and gliadin, resulting in increased starch crystallinity as well as deformed and disrupted microstructure of the starch-gluten network [Ou *et al.*, 2022]. Such disruption of the starch-protein matrix and decrement in starch gelatinization may affect the textural properties of the incorporated pasta. The chewiness of pasta samples supplemented with mulberry pomace was always lower than that of the control pasta sample (Table 3). Similar observation was also recorded by Kultys & Moczkowska-Wyrwisz [2022] when carrot and beetroot-apple pomace were incorporated into pasta.

When the mulberry pomace ratio increased from 0 to 20%, the tensile strength and elongation rate of pasta decreased by 45 and 86%, respectively (Table 3), probably mainly due to the decrement in gluten content. Replacement of gluten by components with high water absorption capacity, like fibers from fruit by-products, was reported to reduce firmness of pasta due to a weak gluten network [Gull *et al.*, 2015]. A sub-incorporation of gluten is reported to compensate for these properties of fiber-enriched pasta [Nguyen *et al.*, 2020].

Cooking properties

As seen from Table 3, when the replacement level of semolina by mulberry pomace was increased from 0 to 20%, the optimal cooking time (OCT) of pasta reduced by about 36%, while the cooking loss (CL) increased 2.1-fold. This could be explained by the higher total dietary fiber content of sample M20 (11.33 g/100 g dw), in comparison to that of sample M0 (3.35 g/100 g dw) which could interfere with the starch-gluten network and result in "capillary texture" of fiber-enriched pasta [Gallo *et al.*, 2020]. During the pasta cooking, water diffusion into the central core of the pasta strands could be more rapid, facilitating the gelatinization of starch granules [Tolve *et al.*, 2020]. As a result, the cooking time of pasta supplemented with mulberry pomace was shorter. Moreover, the weakening of the gluten matrix of fiber-enriched pasta could cause higher leaching of starch and other components from the pasta strands into the cooking water, significantly increasing the cooking loss of pasta. Increase in CL and reduction in OCT were previously reported for tomato peel-fortified spaghetti [Padalino *et al.*, 2017].

The presence of 20% mulberry pomace in pasta formula also decreased the swelling index (SI), and water absorption index (WAI) compared to those of the control pasta (Table 3), probably due

Table 3. Texture and cooking quality of pasta incorporated with mulberry pomace at different levels.

Characteristic		M0	M5	M10	M15	M20
Textural properties	Hardness (g)	2,438±13^{e}	2,600±44^{d}	2,715±44^{e}	2,825±21^{b}	2,977±57^{a}
	Chewiness (g)	1,613±26^{a}	1,537±39^{b}	1,520±10^{b}	1,557±26^{b}	1,542±18^{b}
	Tensile strength (kPa)	31.31±0.82^{a}	21.91±0.93^{b}	19.59±1.05^{c}	18.18±0.64^{cd}	17.06±0.91^{d}
	Elongation rate (%)	365±35^{a}	309±48^{b}	159±12^{c}	130±22^{c}	50±9^{d}
Cooking quality	Optimal cooking time (min)	14.00±0.50^{a}	13.00±0.50^{b}	12.50±0.17^{b}	10.50±0.20^{c}	8.88±0.33^{d}
	Cooking loss (%)	3.76±0.19^{e}	5.12±0.17^{d}	5.94±0.24^{c}	6.58±0.37^{b}	8.05±0.45^{a}
	Swelling index	1.97±0.11^{a}	1.79±0.01^{b}	1.69±0.07^{bc}	1.65±0.06^{c}	1.59±0.07^{c}
	Water absorption index	1.52±0.03^{a}	1.39±0.02^{b}	1.28±0.06^{c}	1.26±0.01^{c}	1.13±0.04^{d}

Data are expressed as mean ± standard deviation (n=3). Values that do not share a lowercase letter within a row are significantly different (p≤0.05). M0, M5, M10, M15, M20, pasta incorporated with 0% (control), 5%, 10%, 15%, and 20% mulberry pomace powder, respectively.

to the reduction in starch content of the fiber-enriched pasta [Rakhesh *et al.*, 2015]. Similar reduction in SI and WAI was recorded for pasta enriched with tomato peel when the addition level increased from 0 to 15% [Padalino *et al.*, 2017]. However, the opposite results were observed by Simonato *et al.* [2019] when the olive pomace was added to durum wheat semolina pasta. The quantity of water absorbed by pasta cooked at OCT is reported to be associated with starch swelling and gelatinization as well as with physical properties of material flours, such as water-binding capacity and particle size distribution [Bustos *et al.*, 2015; Steglich, 2013]. Further study on fiber-protein-starch-phenolic interaction of high-fiber pasta dough is therefore essential to justify the impacts of mulberry pomace on textural and cooking properties of the product.

■ Effects of mulberry pomace ratio in the pasta formulation on instrumental color and overall acceptability of the product

Table 4 reveals that the lightness (L*) and yellowness (b*) of pasta samples notably decreased while the redness (a*) increased with the increasing amounts of mulberry pomace incorporated into the product recipe. It can be explained that color of mulberry pomace had a higher redness value (5.9 folds) but lower yellowness (7.7 folds) and lightness values (2.3 folds) than the durum wheat semolina. The color of pasta supplemented with mulberry pomace could be changed as compared to that of the conventional pasta. Addition of fruit and vegetable pomace was reported to enhance the pasta darkness [Kultys & Moczkowska-Wyrwisz, 2022]. High redness value of berry fruits is due to high anthocyanin content. A similar increment in redness was also observed in berry-enriched pasta [Bustos *et al.*, 2019].

The overall acceptability of the pasta sample fortified with 5% mulberry pomace and the control sample was statistically (p>0.05) similar (Table 4). As the incorporation level of mulberry pomace increased from 5 to 20%, the overall acceptance of pasta decreased by 22% due to changes in its textural properties. Strong correlations between the overall acceptance and the pasta hardness and elasticity were recorded. The Pearson correlation coefficient between the overall acceptance and the hardness was –0.96 (p=0.009) while the coefficient between the overall acceptance and the tensile strength was 0.95 (p=0.015). However, all the pasta samples with mulberry pomace had acceptable scores, ranging from 5.13 to 6.58.

■ Effects of transglutaminase treatment on quality of the mulberry pomace-fortified pasta

Following the recommendation of Dietetic Associations on dietary fiber requirement [Borderías *et al.*, 2005], the fortified pasta

Table 4. Color parameters and overall acceptability of pasta incorporated with mulberry pomace at different levels.

Characteristic	M0	M5	M10	M15	M20
L*	89.32±0.04^{a}	66.71±0.12^{b}	60.11±0.23^{c}	57.37±0.11^{d}	55.40±0.15^{e}
a*	1.07±0.01^{e}	4.55±0.07^{d}	6.52±0.03^{c}	8.07±0.06^{b}	8.58±0.06^{a}
b*	8.74±0.02^{a}	3.63±0.04^{b}	2.68±0.04^{c}	2.18±0.02^{d}	1.52±0.03^{e}
ΔE	0.00±0.00^{e}	23.44±0.08^{d}	30.33±0.22^{c}	33.36±0.13^{b}	35.48±0.13^{a}
Overall acceptability	6.67±1.46^{a}	6.58±1.22^{a}	6.00±1.13^{b}	5.19±1.32^{c}	5.13±1.37^{c}

Data are expressed as mean ± standard deviation (n=3). Values that do not share a lowercase letter within a row are significantly different (p≤0.05). M0, M5, M10, M15, M20, pasta incorporated with 0% (control), 5%, 10%, 15%, and 20% mulberry pomace powder, respectively. L*, lightness; a*, redness; b*, yellowness; ΔE, total color difference.

with 10% mulberry pomace was chosen in the TG treatment for improvement in its textural and cooking quality.

The effects of TG dosage on textural properties, cooking quality, color values as well as overall acceptability of the high-fiber pasta are shown in Table 5. The pasta hardness enhanced with the increased TG dosage from 0.00 to 0.50 U/g protein; however, the chewiness of the fortified pasta increased only 4.2% when increasing the TG dosage from 0.50 to 1.00 U/g protein. Similar observation was recently reported when TG was used to improve the quality of corncob powder-enriched pasta [Nguyen *et al.*, 2023a]. In addition, increase in TG dosage from 0 to 0.50 U/g protein improved the tensile strength and elongation rate of mulberry pomace-supplemented pasta by 26 and 10%, respectively (Table 5). It is reported that the TG treatment of pasta dough induces the formation of crosslinks between gliadin and glutenin in wheat proteins, strengthening the protein network and enhancing the dough elasticity [Gharibzahedi *et al.*, 2019]. Increase in tensile strength and elongation rate of pasta fortified with 20% wheat bran was recently reported when the pasta dough was treated with TG dosage of 0.75 U/g [Nguyen *et al.*, 2020]. Otherwise, as the TG dosage continued to increase from 0.50 to 1.00 U/g protein, both tensile strength and elongation rate began to decrease (Table 5). High level of TG treatment may lead to high degree of cross-linking and reduce the flexibility of the protein network [Aalami & Leelavathi, 2008]. The tensile strength and elongation rate of corncob-incorporated pasta were also decreased by 5–8% and 16–28%, respectively when the excessive TG concentration (1.00–1.25 U/g) was used [Nguyen *et al.*, 2023a].

Increase in TG dosage from 0 to 0.75 U/g protein slightly enhanced the OCT of high-fiber pasta while reduced its cooking loss (Table 5) probably due to the strengthened gluten network. Similar results were recently reported when TG treatment was applied to pasta dough supplemented with wheat bran [Nguyen *et al.*, 2020] and corncob powder [Nguyen *et al.*, 2023a]. However, further increase in TG level from 0.75 to 1.00 U/g protein decreased the OCT while enhanced the CL of mulberry pomace-added pasta (Table 5). According to Aalami & Leelavathi [2008], high degree of gluten crosslinking at high TG dosage might decrease protein-starch interaction, resulting in an increased leaching of starch component into the cooking water. This effect was observed in both non-fortified pasta [Sissons *et al.*, 2010] and pasta enriched with fiber ingredients, such as wheat bran and corncob powder [Nguyen *et al.*, 2020, 2023a]. In addition, the TG treatment of pasta dough had no effect on SI ($p>0.05$) while the WAI of high-fiber pasta treated with TG at 0.50 U/g protein was slightly greater than that of the counterpart without TG treatment (Table 5).

Table 5 also reveals that the pasta samples treated with TG showed an increased lightness. This could be due to limitation of Maillard reaction during pasta drying as TG crosslink effect could reduce available lysine content [Aalami & Leelavathi, 2008]. Moreover, change in yellowness and redness of the pasta samples was very little; both *a** and *b** values varied in narrow ranges (Table 5).

All pasta samples were considered acceptable since their overall acceptability score was higher than 5 points (Table 5). It should be noted that the high-fiber pasta samples treated with TG at 0.50 and 0.75 U/g protein had similar overall acceptance which was significantly ($p\leq0.05$) higher than that of the other pasta samples. These results were consistent with the tensile strength and elongation rate of the product. The appropriate

Table 5. Texture profile, cooking quality, color parameters and overall acceptability of pasta supplemented with 10% mulberry pomace and treated with transglutaminase (TG) at different dosages.

Characteristic		TG0	TG25	TG50	TG75	TG100
Textural properties	Hardness (g)	2,715±44^{d}	2,865±53^{c}	3,044±73^{b}	3,208±114^{a}	3,157±106^{ab}
	Chewiness (g)	1,520±10^{bc}	1,461±15^{c}	1,506±22^{bc}	1,536±52^{ab}	1,584±51^{a}
	Tensile strength (kPa)	19.6±1.1^{c}	22.2±1.5^{b}	24.6±0.9^{a}	22.2±0.5^{b}	20.9±0.9^{bc}
	Elongation rate (%)	159.3±12.0^{b}	163.8±9.6^{ab}	175.1±6.0^{a}	158.4±5.8^{b}	121.7±7.0^{c}
Cooking quality	Optimal cooking time (min)	12.50±0.07^{c}	12.52±0.08^{bc}	12.69±0.08^{ab}	12.85±0.08^{a}	12.03±0.16^{d}
	Cooking loss (%)	5.94±0.24^{a}	5.46±0.25^{bc}	5.49±0.21^{bc}	5.37±0.17^{c}	5.82±0.12^{ab}
	Swelling index	1.69±0.07^{a}	1.68±0.03^{a}	1.67±0.03^{a}	1.66±0.04^{a}	1.60±0.03^{a}
	Water absorption index	1.28±0.06^{bc}	1.32±0.03^{ab}	1.33±0.01^{a}	1.30±0.02^{abc}	1.25±0.03^{c}
Color and overall acceptability	*L**	60.11±0.23^{d}	62.77±0.18^{c}	63.67±0.38^{b}	64.11±0.71^{ab}	64.44±0.50^{a}
	*a**	6.52±0.03^{b}	6.52±0.04^{b}	6.57±0.08^{b}	6.73±0.14^{a}	6.81±0.06^{a}
	*b**	2.68±0.04^{c}	2.77±0.03^{c}	2.77±0.10^{c}	3.12±0.04^{b}	3.27±0.10^{a}
	Δ*E*	0.00±0.00^{c}	2.66±0.30^{b}	3.56±0.46^{ab}	4.02±0.76^{a}	4.37±0.58^{a}
	Overall acceptability	6.04±1.52^{cd}	6.25±1.3^{bcd}	6.88±1.15^{a}	6.48±1.08^{abc}	5.92±1.12^{d}

Data are expressed as mean ± standard deviation ($n=3$). Values that do not share a lowercase letter within a row are significantly different ($p\leq0.05$). TG0, TG25, TG50, TG75 and TG100, pasta treated with TG at dosages of 0, 0.25, 0.50, 0.75 and 1.00 U/g protein of the pasta dough, respectively. *L**, lightness; *a**, redness; *b**, yellowness; Δ*E*, total color difference.

dosage of TG for pasta dough enriched with mulberry pomace powder was therefore 0.50 U/g protein.

CONCLUSIONS

The incorporation of mulberry pomace powder into pasta recipe improved dietary fiber content and antioxidant capacity of the product. The pasta supplemented with 10% mulberry pomace was a high-fiber food with a reasonable ratio of IDF:SDF (3.3:1.0, *w/w*). However, the pasta fortified with mulberry pomace had a decreased optimal cooking time, swelling index, water absorption index and increased cooking loss. The textural properties and overall acceptability of high-fiber pasta were also decreased when the supplementation level of mulberry pomace was 10% or higher. TG treatment slightly reduced the cooking loss of high-fiber pasta while enhanced its tensile strength, elongation rate and sensory score at the enzyme dosage of 0.50 U/g protein. Further studies on fiber-protein-starch-phenolic interaction of pasta dough supplemented with mulberry pomace powder are essential to clarify the effects of this potential material on textural and cooking attributes of high-fiber pasta.

ACKNOWLEDGEMENTS

This research is funded by Vietnam National University Ho Chi Minh City (VNU-HCM) under grant number NCM2020-20-01. We acknowledge the support of time and facilities from Ho Chi Minh City University of Technology (HCMUT), VNU-HCM for this study.

RESEARCH FUNDING

Vietnam National University Ho Chi Minh City (VNU-HCM), grant number: NCM2020-20-01.

CONFLICT OF INTERESTS

The authors have declared no conflicts of interest for this article.

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ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 311–321
DOI: 10.31883/pjfns/173218
ISSN 1230-0322, e-ISSN 2083-6007

Techno-Functional and Bioactive Properties and Chemical Composition of Guava, Mamey Sapote, and Passion Fruit Peels

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Due to their nutritional and sensorial characteristics, tropical fruits like guava, mamey sapote, and passion fruit are regularly incorporated into daily diets. Their by-products, especially peels, are approximately 10 to 35% of their weight and possess an important content of bioactive compounds, such as dietary fiber and phenolics. The nutritional, technological, physio-functional properties and phenolic compound compositions of guava, mamey sapote, and passion fruit peels were studied. Peels had promising techno- and physio-functional characteristics, good dietary fiber contents (45.18-61.42 g/100 g), and phenolic profiles with ferulic acid, gallic acid, *p*-coumaric, and catechin as the main compounds. Peel powders also showed excellent DPPH radical scavenging activity (125.3–252.4 µmol TE/100 g) and Trolox equivalent antioxidant capacity, TEAC (369.2–656.8 µmol TE/100 g). The α-amylase and lipase inhibitory activity varied from 28.15 to 51.4% and 30.89 to 57.15%, respectively. Higher values of α-glucosidase inhibition capacity were found, ranging from 51.64 to 70.32%. The chemical composition and properties reported in the present work suggest that peel powders of these guava, mamey sapote, and passion fruit could be used as constituents in different foods, such as bakery and meat goods, with beneficial health effects like control of hyperglycemia, improved intestinal function, and control of overweight; however, more studies are necessary for animal models and humans to confirm these bioactivities conclusively.

Key words: tropical fruits, phenolic profile, antioxidant potential, enzyme inhibition, glucose adsorption capacity, fat/oil binding capacity, sodium cholate binding capacity

ABBREVIATIONS

GUPP, peel powder of guava; MYPP, peel powder of mamey sapote; PFPP, peel powder of passion fruit; AOAC, Association of Official Analytical Chemists; WHC, water-holding capacity; OHC, oil-holding capacity; GAC, glucose adsorption capacity; DNS, 3,5-dinitrosalicylic acid; FOB, fat/oil binding; SCBC, sodium cholate binding capacity; CAC, cholesterol adsorption capacity, DPPH$^{\bullet}$, 2,2-diphenyl-1-picryl-hydrazyl radical; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TSP, total soluble phenolics; TEAC, Trolox equivalent antioxidant capacity.

INTRODUCTION

Guava, mamey sapote, and passion fruit are tropical fruits accessible in international markets; they are consumed fresh, canning, frozen, or as marmalades, nectars, among others.

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Submitted: 10 February 2023
Accepted: 3 October 2023
Published on-line: 2 November 2023

The processing operations of guava, mamey sapote, and passion fruit generate by-products, such as seeds, peels, and unusable pulp, that may reach up to 52% of the fruit weight [Sagar *et al*., 2018]. These by-products can be reused due to their content of bioactive compounds; among them, dietary fiber which has interesting characteristics like water and oil holding capacity and fat acid adsorption, which increases their excretion and favorably impacts the consumer's lipid profile [Savran *et al*., 2016], while the phenolic compounds have shown antioxidant activity besides diverse health-promoting effects, such as acting in the control of postprandial hyperglycemia and overweight [Marquez-Molina *et al*., 2023]. Obesity is a metabolic disorder resulting from excessive accumulation of body fat, associated with numerous comorbidities like heart disease, stroke, hypertension, and type 2 diabetes mellitus. The complications of people living with diabetes may be partially mitigated by inhibiting α-amylase and α-glucosidase, two enzymes closely related to the digestion of carbohydrates. This strategy counters postprandial hyperglycemia and maintains a stable glycemia [Gutiérrez-Grijalva *et al*., 2018]. Thus, consuming food products that contain compounds efficient in slowing α-amylase and α-glucosidase activity may benefit consumer health. Various efforts have been made to explore plant-based, effective, inexpensive, and non-harmful inhibitors of α-amylase, α-glucosidase, and pancreatic lipase for this purpose. Plant-based foodstuffs, like by-products of tropical fruits, are known to exhibit minimal side consequences in comparison to synthetic drugs [Azizan *et al*., 2020]. Previous findings have shown that phenolic compounds from guava leaves and passion fruit by-products exerted an *in vitro* α-amylase and α-glucosidase inhibition capacity, besides important antioxidant activity [Cao *et al*., 2021; Liu *et al*., 2014]. Guava, mamey sapote, and passion fruit extracts have diverse bioactive compounds, suggesting they may exert health-promoting bioactivities.

Therefore, this study aimed to characterize tropical fruit peels from guava, mamey sapote, and passion fruit regarding their *in vitro* physicochemical, technological, antioxidant, antidiabetic, and antiobesogenic activities. This characterization will allow the proposal of possible applications for the peels instead of discarding them unused, thereby generating additional value for producers, industries, and the consumer.

MATERIALS AND METHODS

Vegetable materials

Guava (*Psidium guajava* L.), mamey sapote (*Pouteria sapota* (Jacq.) H.E. Moore & Stearn) and passion fruit (*Passiflora edulis* f. *flavicarpa*) in commercial ripeness were acquired in the Central de abasto in Hermosillo, Mexico. Guava, mamey sapote, and passion fruit without apparent damage to the skin and free of molds were chosen, then washed in tap water and disinfected with 0.3 mL/L sodium hypochlorite for 3 min. Clean guavas and passion fruits were cut in half using a sanitized stainless-steel knife, pulp and seeds were removed entirely, and peels of mamey sapote were manually eliminated. The peels were dried in an air circulation oven at 40°C for 24 h. Dry peels were ground in a coffee grinder to obtain flour. Dry peel powders of guava (GUPP), mamey sapote (MYPP), and passion fruit (PFPP) were stored in amber vials at 4°C in the dark until subsequent analysis.

Determination of proximate composition and physicochemical properties

Moisture, proteins, lipids, and ash content determinations were performed according to Association of Official Analytical Chemists (AOAC) methods [Helrich, 1990]. Contents of total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) were established using Asp's method [Asp *et al*., 1983]. The peel powders were mixed with distilled water (1:10, *w/v*), and the pH of the mixture was measured with a pH meter (Fisher Scientific AB150, Ottawa, Canada). Water activity (A_w) was measured at 25°C, utilizing an Aqualab CX 2T apparatus (Decagon Devices Inc., Pullman, WA, USA). Tristimulus color parameters, *i.e*., lightness (*L**), red-green chromaticity coordinate (*a**), and yellow-blue chromaticity coordinate (*b**), were measured using a CR-400HS colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan). All parameters were analyzed in triplicate.

Determination of techno-functional properties

The water-holding capacity (WHC) and oil-holding capacity (OHC) of fruit peel powders were evaluated according to the procedure proposed by Wu *et al*. [2010]. In brief, 1 g of peel powder was mixed with 10 mL of water or 10 g of oil in a tube. The mixture was stirred for 1 min, then left to rest for 30 min, and centrifuged at 6,706×*g* (Allegra 64R centrifuge, Beckman Coulter, La Brea, CA, USA) for 30 min. After this time, the supernatant was carefully poured out, and the pellet with retained water or oil was weighed. The results were recorded as the amount of water adsorbed *per* g of peel powder (mL water/g) or oil adsorbed *per* g of peel powder (g oil/g).

Determination of physio-functional characteristics

Glucose adsorption capacity

Glucose adsorption capacity (GAC) of fruit peel powders was evaluated using the procedure described by Ou *et al*. [2001]. In brief, 0.5 g of peel powders were mixed with 25 mL of a glucose solution at different concentrations (10–200 mM), then incubated at 37°C for 6 h with shaking at 120 rpm. The mixtures were centrifuged at 6,706×*g* at 25°C for 20 min. After this, 1 mL of the supernatant was carefully poured and then mixed with 0.75 mL of the reagent with 3,5-dinitro salicylic acid (DNS) (0.63% DNS, 0.5% sodium sulfite, 18.5% sodium potassium tartrate, 2.1% sodium hydroxide, and 0.5% phenol) in order to determine the glucose concentration [Fu *et al*., 2010]. The reaction mixture was incubated at 97°C for 5 min; then left to stand until it reached 25°C; next, 10 mL of distilled water were added. The absorbance of the mixture was determined at 540 nm in a photodiode array spectrophotometer (Model 8452A; Hewlett–Packard Co., Waldbronn, Germany). GAC was reported as mmol of glucose adsorbed *per* g of peel powder (mmol/g).

Fat/oil binding capacity

Fat/oil binding (FOB) capacity of guava, mamey sapote, and passion fruit peel powders was measured according to the method used previously by López-Marcos *et al*. [2015]. In brief, 0.2 g of peel powders were weighed in a flask, and 20 mL of 0.16 M HCl was added and shaken at 120 rpm for 1 h. Then, 20 g of oil was added to the flask. The mixture was stirred in a vortex for 1 min before neutralizing it with 0.01 M NaOH. The flask was then kept at 25°C for 24 h, the unbound oil was carefully decanted, and the pellet was recovered by filtration. FOB was reported as the weight of oil adsorbed *per* g of peel powder.

Sodium cholate binding capacity

Sodium cholate binding capacity (SCBC) of fruit peel powders was measured according to the method described by Xu *et al*. [2015]. In brief, 100 mL of a solution of sodium cholate (150 mM) in phosphate buffer (pH 7.0) was mixed in a flask with 200 mg of each peel powder and stirred at 120 rpm and at 37°C for 2 h. After this time, the mixture was centrifuged at 760×*g* for 20 min. The supernatant (0.5 mL) was mixed with H_2SO_4 (240 µL, 45%, *v/v*), vortexed, incubated at 65°C for 30 min, and cooled at room temperature, then the absorbance was measured at 620 nm. The concentration of unbound sodium cholate was determined based on a standard curve using a standard of sodium cholate. Results were expressed as mg of bound cholesterol *per* g of peel powder.

Cholesterol adsorption capacity

Cholesterol adsorption capacity (CAC) was determined according to the method described by Luo *et al.* [2017]. A solution of fresh egg yolk was prepared in distilled water (1:9, *v/v*) and stirred at 120 rpm for 5 min. Peel powders (1 g) were mixed with 25 mL of the yolk solution and adjusted to pH 2.0 with 6 M HCl to simulate the pH of the stomach, and the second mixture was prepared under the same conditions described above but adjusted to pH 7.0 with 2 M NaOH to simulate the pH of the intestine. The mixtures were set at 37°C for 2 h with shaking at 120 rpm; after this, they were centrifuged at 4,006×*g* and 25°C for 20 min. The supernatants (2 mL) were diluted with 10 mL of an acetic acid solution (90%, *v/v*), adding 0.1 mL of *o*-phtalaldehyde. The absorbance was measured at 550 nm after 10 min of reaction. CAC values were evaluated based on a standard curve for cholesterol. CAC was shown as mg of cholesterol retained *per* g of peel powder and was calculated according to the following Equation (1):

$$CAC = [(C_b - C_d) - (C_y - C_b)] \times V/M \quad (1)$$

where: C_y, concentration of the yolk solution (mg/mL), C_d, cholesterol concentration of the yolk solution after being absorbed by peel powders (mg/mL), C_b, cholesterol concentration of the yolk solution containing no peel powders, V, volume of the yolk solution (mL) and M, weight of peel powder (g).

Crude extract preparation

Briefly, 1 g of peel powder was homogenized in 20 mL of a methanol:water (80:20, *v/v*) mixture and sonicated for 30 min. Samples were then centrifuged at 4,006×*g* and 4°C for 15 min. Supernatants were collected, and the residues were re-extracted twice with 10 mL of a methanol:water (80:20, *v/v*) mixture. Supernatants were combined and filtered through Whatman® no. 1 filter paper, and the filtrate was utilized to determine the total soluble phenolic compound content, phenolic composition and antioxidant capacity of fruit peels. For the determination of enzyme inhibition by extracts, the filtrate was subjected to rotary evaporation at 40°C to remove methanol, the residual water was freeze-dried, and the dried extract was redissolved in sodium phosphate buffer (0.1 M, pH 6.9) at the analysis time.

Determination of the content of total soluble phenolics

The content of total soluble phenolics (TSP) was determined by the method of Singleton *et al*. [1965] with some changes. The extract of peel powder (15 µL) was mixed with 15 µL of 2 M Folin-Ciocalteu reagent and 240 µL of distilled water in a 96 well-microplate and mixed slightly for 3 min, then 30 µL of 2 M Na_2CO_3 were added; microplates were then allowed to stand without light for 90 min. The absorbance was measured at 765 nm in a microplate reader (FLUOstar Omega, B.M.G. Labtech, Durham, NC, USA). TPS content was expressed as mg of gallic acid equivalent *per* 100 g of peel powder (mg GAE/100 g).

Determination of individual phenolic compounds

The phenolic compounds of guava, mamey sapote, and passion fruit peel extracts were evaluated by the method of Velderrain-Rodríguez *et al*. [2018] using an UPLC system (Acquity, Waters Co., Milford, MA, USA) with a photodiode array detector. Separation was performed in an UPLC BEH C18 column (1.7 µm, 3.0×100 mm). The phase solvent was A (0.1%, *v/v*, acetic acid in water) and B (0.1%, *v/v*, acetic acid in methanol) delivered at a 0.7 mL/min flow rate. The solvent gradient was initially 91% of A and 9% of B (0–11 min), 9% to 14% of B (11–15 min), and 15% of B (5 min). The phenolic compounds were identified by matching their retention times and absorption spectra with their respective standards and their contents were estimated based on calibration curves. The results were expressed as µg/g of peel powder.

Antioxidant capacity determination

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of peel powders was evaluated using the method of Brand-Williams *et al*. [1995]. Peel powder extract (20 µL) and 280 µL of 200 µM $DPPH^{\bullet}$ solution were transferred to a microplate, and the mixture was left in the absence of light at 25°C for 30 min. After this time, the decrease in absorbance was determined at 540 nm in a microplate reader. The Trolox curve from 0.05–1 mmol TE/g was used, and the results were expressed as µmol of Trolox equivalent *per* 100 g of peel powder (µmol TE/ 100 g).

Trolox equivalent antioxidant capacity

Trolox equivalent antioxidant capacity (TEAC) was determined by the method of Re *et al*. [1999]. The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was dissolved in a 2.45 mM potassium persulfate solution to obtain a 7 mM solution. The mixture was kept in the absence of light for 18 h to generate $ABTS^{\bullet+}$. The $ABTS^{\bullet+}$ solution was diluted to reach an absorbance of 0.70±0.02 at 734 nm. The extracts of peel powder or a Trolox standard solution (20 µL) were mixed with 255 µL of the $ABTS^{\bullet+}$ solution and left at 30°C for 7 min in a microplate. The absorbance was measured at 734 nm in a microplate reader.

TEAC was expressed as µmol of Trolox equivalents *per* 100 g of peel powder (µmol TE/100 g).

Determination of inhibition of enzymes

α-Amylase inhibition assay

The α-amylase inhibitory activity of peel powder extracts was determined according to the procedure described in Worthington Enzyme Manual [Worthington, 1993]. The substrate (50 µL of a 1% solution of starch in sodium phosphate buffer 0.1 M at pH 6.9), α-amylase (13 U/mL, Sigma-Aldrich, St. Louis, MO, USA) dissolved in the same buffer, and 50 µL of fruit peel extracts (0.25 to 2.50 mg/mL) or acarbose (1 mM) as a control, were incubated at 37°C for 10 min. Then, 1 mL of DNS reagent was added, and the mixture was incubated at 85°C for 15 min. Afterwards, the mixture was moved to an ice bath to cool down at 25°C, and 1 mL of distilled water was added. Finally, 250 µL from the mixture was set in a microplate, and the absorbance was read at 540 nm. The α-amylase inhibitory activity of peel powder extracts was reported in percentage and calculated in the following manner using Equation (2):

$$\%\text{Inhibition} = [(A_{blank} - A_{extract})/A_{blank}] \times 100 \quad (2)$$

where: A_{blank} is the absorbance of the blank (mixture without peel powder extract) and $A_{extract}$ is the absorbance of the mixture with peel powder extract.

In addition, IC_{50} defined as the concentration of extract required to inhibit 50% of the enzyme activity was established. The IC_{50} values were obtained from the regression of the logarithm of the extract concentrations *versus* α-amylase inhibitory activity (%).

α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was performed as established by Cuevas-Juárez *et al.* [2014]. Briefly, 50 µL of peel powder extracts (0.25 to 2.00 mg/mL) and 100 µL of α-glucosidase (0.6 U/mL, Sigma-Aldrich) or acarbose (1 mM) as control were left at 37°C for 10 min in a microplate. Afterwards, 50 µL of *p*-nitrophenyl-α-glucopyranoside (3 mM) was added, and the mixture was left another time in the conditions described above. Enzyme activity was determined at 405 nm in a microplate reader. The α-glucosidase inhibitory activity of peel powder extracts was reported as inhibition percentage and as IC_{50}. Both parameters were defined and calculated analogously to those for α-amylase inhibitory activity as described above.

Pancreatic lipase inhibition assay

Pancreatic lipase inhibitory activity of peel powder extracts was evaluated by the method of Worsztynowicz *et al*. [2014]. In brief, 20 µL of peel powder extracts (0.25 to 3.50 mg/mL) and 20 µL of a pancreatic lipase solution (1 mg/mL) (100–500 U/mg, Megazyme International, Wicklow, Ireland) were incubated at 37°C for 10 min, then 1.8 mL of a cholate solution in 0.1 M sodium phosphate buffer (1.15 mg/mL) containing gum Arabic (0.55 mg/mL) and 20 µL of *p*-nitrophenyl palmitate (0.01 M) in isopropanol were added, and the mixture was incubated at 37°C for 10 min. The released *p*-nitrophenyl was determined at 410 nm using a microplate reader after 10-min incubation at 37°C. The pancreatic lipase inhibitory activity of peel powder extract was calculated according to Equation (2), and the IC_{50} was determined from these values (as described for α-amylase inhibition assay).

Statistical analysis

Experiments were carried out in triplicate, and results were expressed as mean ± standard deviation (SD). An analysis of variance (ANOVA) and Tukey's test were performed ($p<0.05$) to determine statistical differences. Data were analyzed using NCSS 2012 statistical analysis software (NCSS LLC, Kaysville, UT, USA).

RESULTS AND DISCUSSION

Proximate composition and physicochemical properties

The proximate composition of the three peel powders is shown in Table 1. Peel powders had moisture contents from 6.06 to 6.55 g/100 g, which is within the range to prevent the proliferation of microorganisms during storage. GUPP moisture content (6.22 g/100 g) was higher than the values reported by Casarotti *et al*. [2018] (3.97 g/100 g) for this type of guava by-products. MYPP also showed a higher moisture content (6.06 g/100 g) than the one stated by Solís-Fuentes *et al.* [2015] (4.42 g/100 g), and PFPP showed moisture content of 6.55 g/100 g which was higher than that reported for passion fruit peels by Selani *et al*. [2016] (5.85 g/100 g) but lower than that found by Dias *et al*. [2020] (8.29 g/100 g). The protein content of the tested peel powders ranged from 2.07 to 5.02 g/100 g, while their lipid content varied from 1.20 to 4.25 g/100 g (Table 1). The ash content of GUPP and PFPP in this study (0.83 and 6.62 g/100 g, respectively) was similar to that determined by Casarotti *et al*. [2018] and Selani *et al*. [2016] for by-products of guava and passion fruit. Ash content in MYPP (4.11 g/100 g) was higher than the value reported by Solis-Fuentes *et al*. [2015] in mamey sapote peel (3.89 g/100 g).

Table 1 depicts the peel powders TDF, SDF, and IDF content. The researched peel powders had a TDF content from 45.18 to 61.42 g/100 g, and a higher content of IDF than SDF in all samples. Such high IDF content suggests the possibility of important applications of the studied peels in food products due to their various physiological impacts on health. For example,

Table 1. Proximate composition, dietary fiber content, physicochemical and techno-functional characteristics of guava (GUPP), mamey sapote (MYPP), and passion fruit (PFPP) peel powders.

Characteristic	GUPP	MYPP	PFPP
Moisture (g/100 g)	6.22±0.04^{b}	6.06±0.04^{c}	6.55±0.04^{a}
Proteins (g/100 g)	2.07±0.34^{c}	5.02±0.23^{a}	3.14±0.31^{b}
Lipids (g/100 g)	1.20±0.13^{b}	4.25±0.10^{a}	4.20±0.02^{a}
Ash (g/100 g)	0.83±0.18^{c}	4.11±0.25^{b}	6.62±0.17^{a}
TDF (g/100 g)	48.55±0.05^{b}	61.42±2.08^{a}	45.18±0.83^{c}
IDF (g/100 g)	46.72±0.15^{b}	50.22±0.10^{a}	31.36±1.33^{c}
SDF (g/100 g)	1.83±0.95^{c}	11.12±0.26^{b}	13.71±0.62^{a}
pH	4.00±0.23^{b}	4.04±0.15^{b}	4.13±0.15^{a}
A_w	0.22±0.01^{c}	0.23±0.01^{b}	0.26±0.01^{a}
*L**	86.01±0.30^{b}	68.26±0.05^{c}	90.37±0.11^{a}
*a**	–3.33±0.01^{b}	10.44±0.02^{a}	–5.45±0.15^{c}
*b**	27.48±0.08^{a}	22.43±0.05^{b}	28.48±0.11^{a}
WHC (mL water/g)	4.55±0.23^{b}	4.63±0.04^{b}	8.56±0.07^{a}
OHC (g oil/g)	4.30±0.23^{a}	2.49±0.04^{c}	3.11±0.12^{b}

Results are expressed as mean ± standard deviation (n=3). Means with different letters in rows show significant differences (p<0.05). TDF, total dietary fiber; IDF, insoluble dietary fiber, SDF, soluble dietary fiber; WHC, water-holding capacity; OHC, oil-holding capacity; A_w, water activity; *L**, lightness; *a**, red-green chromaticity coordinate; *b**, yellow-blue chromaticity coordinate.

high levels of IDF in the diet have been shown to induce satiety and increase fecal volume, thereby contributing to maintaining or improving digestive health while preventing disorders like constipation and colon cancer [Luciano, 2018]. The observed differences in the proximate composition of peels from tropical fruits could be primarily due to the origin of the fruit, then to the specific cultivar used, fruit maturity, post-harvested practices, as well as the treatment to obtain powders. Other important factors affecting these variables are the agricultural practices and the environmental conditions when they were grown [Kim *et al*., 2019].

Values of pH showed no significant differences between the samples (Table 1). pH is a significant variable because it can define the type of food matrix where the peels can be added without modifying their technological performance. The present study determined that the pH was acidic (4.00–4.13), implying that the samples could be appropriate for their use in similarly acidic food, such as yogurt [López-Marcos *et al*., 2015]. Regarding A_w, peel powders showed values of 0.22–0.26 (Table 1), comparable to those previously described for powders of by-products from tropical fruits [Casarotti *et al*., 2018; Selani *et al*., 2016]. The low values of pH and A_w of the examined peel powders suggest a low possibility of the growth of microorganisms and the development of non-enzymatic or enzymatic damage, further suggesting the viability of using them as potential food ingredients.

Peel powder color depends on the type of fruit, plant cultivar, and maturity stage when by-products were elaborated into powders. During drying, samples are subjected to high temperatures that promote non-enzymatic and/or enzymatic browning reactions [Deng *et al*., 2018], causing sample darkening. One of the most significant quality attributes in food is the color; thus, the possible changes due to adding peel powders to a food product should limit their potential application to a maximum value. All peel powders showed high *L** values (68.26–90.37), with the highest value in PFPP (Table 1). *L** is highly related to the type and concentration of pigments, surface water availability, and water content. For *a**, the values were different among the peel powders (Table 1). MYPP had the greatest *a**, possibly due to its content of red carotenes [Alia-Tejacal *et al*., 2005]. The highest *b** was measured in PPFP, which was statistically similar (p≥0.05) to GUPP, indicating that these peels have a more notable yellow color because of the color of the passion fruit examined.

Techno-functional properties

WHC is a significant hydration characteristic of dietary fiber, both from a technological and physiological viewpoint, and reflects the capability of a moist material to retain water when it is subjected to compression or centrifugation. It includes water bound, water held, and water trapped within cell-wall associated with the fibers [Nelson, 2001]. WHC of PFPP had the highest (p<0.05) value, followed by MYPP and GUPP, with no significant (p≥0,05) differences (Table 1). WHC of peel powder directly correlates with its SDF content, while samples with a high IDF fraction have a lower WHC [López-Marcos *et al*., 2015].

WHC data suggests that the peel powders may potentially be used in products requiring hydration, such as baked foods,

and viscosity, like yogurts. Comparing our results with literature data, PFPP showed a higher WHC (8.56 mL water/g) than rice bran of two varieties (2.76–3.81 mL water/g), while MYPP and GUPP had a higher WHC than durum wheat of two varieties (2.9–3.76 mL water/g) [Jribi *et al*., 2019].

OHC reflects the capability of a sample to hold oil; it is an important techno-functional characteristic, particularly for cooked meat products, because holding fat stabilizes the emulsion and preserves the flavor of the product [Sánchez-Zapata *et al*., 2011]. Peel powder OHC values varied from 2.49 to 4.30 g oil/g (Table 1), which were higher than those previously reported in guava and passion fruit by-products (2.03 and 0.7 g oil/g, respectively) [Martínez *et al*., 2012]. The variation in the results could be related to the porosity of the peels, which can be modified for drying methods and processing time [Elleuch *et al*., 2011]. To date, no studies describe the techno-functional properties of mamey sapote peel, suggesting that this by-product has been minimally studied and, therefore, its potential use in edible products has not been yet recognized.

Physio-functional properties

Results in Table 2 show that all powder peel samples adsorbed glucose in a concentration-dependent behavior. The GAC of the peel powders may be partly ascribed to dietary fiber content, which promotes glucose molecule adsorption [Qin *et al*., 2020]. Marquez-Molina *et al.* [2023] described that fruit by-products could retain glucose in different concentrations, and the quantity of glucose adsorbed may increase as the concentration in the solution increases. It is also remarkable that GAC of MYPP was higher than that of GUPP and PFPP at all tested glucose concentrations (Table 2), probably due to the higher dietary fiber content and lower moisture content, which contributes to their capacity to adsorb glucose.

Fat/oil binding (FOB) capacity estimates the peel powders' ability to retain or adsorb fats into its matrix, mimicking food digestion conditions. The FOB capacity of PFPP was 10.2 g/g, which was higher than that of MYPP (4.22 g/g) and GUPP (1.33 g/g) (Table 3). This physio-functional property seems to be related to the SDF/IDF ratio, where higher ratios were associated with higher FOB capacity. FOB capacity of the peel powders analyzed was lower than that reported by López-Marcos *et al.* [2015] for other fruit by-products like these of grapefruit (35.43 g/g) and pomegranate (14.00 g/g).

Dietary fiber can bind bile acids in the small intestine and cholesterol in both the stomach and small intestine. Therefore, the sodium cholate and cholesterol binding capacities of dietary fiber-rich materials are important parameters to evaluate [Mudgil & Barak, 2013]. The highest binding capacities was determined in MYPP (2.39 mg/g), followed by GUPP (1.94 mg/g), and PFPP (1.77 mg/g) . These different bile-acid-binding values may result from the different TDF content of peel powders.

The cholesterol adsorption capacity of PFPP was significantly higher than those of GUPP and MYPP. The pH value significantly ($p<0.05$) affected the cholesterol adsorption abilities of all samples, where the CAC values at pH 2.0 were lower than those at pH 7.0. These results indicate that PFPPs have the potential to reduce cholesterol concentration, which could be related to their SDF content [Zhang *et al*., 2023]. Dietary fiber could disturb the absorption of cholesterol and bile acids in the digestive system, promoting bile acid synthesis from cholesterol, which could decrease the total cholesterol concentration [Niu *et al*., 2013].

Total soluble phenolic content and phenolic compound profile

The TSP content of fruit peel powders ranged from 185.2 to 365.7 mg GAE/100 g and decreased in the following order:

Table 2. The glucose adsorption capacity (mmol/g) of peel powders of guava (GUPP), mamey sapote (MYPP), and passion fruit (PFPP) in different concentrations of glucose.

Peel powder	10 mM	25 mM	50 mM	100 mM	200 mM
GUPP	1.10 ± 0.06^{b}	1.98 ± 0.02^{c}	4.41 ± 0.06^{b}	8.61 ± 0.11^{b}	18.67 ± 0.13^{b}
MYPP	1.48 ± 0.18^{a}	2.63 ± 0.06^{a}	4.98 ± 0.07^{a}	9.46 ± 0.09^{a}	20.89 ± 0.11^{a}
PFPP	0.98 ± 0.05^{c}	2.13 ± 0.02^{b}	4.18 ± 0.11^{c}	8.17 ± 0.07^{c}	16.45 ± 0.47^{c}

Values are presented as mean ± standard deviation (n=3). Means with different letters in columns show significant differences ($p<0.05$).

Table 3. *In vitro* fat/oil binding (FOB) capacity, sodium cholate binding capacity and cholesterol adsorption capacity of peel powders of guava (GUPP), mamey sapote (MYPP), and passion fruit (PFPP).

Peel powder	FOB capacity (g/g)	Sodium cholate binding capacity(mg/g)	Cholesterol adsorption capacity (mg/g)	
			pH 2.0	pH 7.0
GUPP	1.33 ± 0.06^{c}	1.94 ± 0.06^{b}	3.53 ± 0.02^{bB}	4.93 ± 0.06^{bA}
MYPP	4.22 ± 0.06^{b}	2.39 ± 0.18^{a}	2.40 ± 0.06^{cB}	4.12 ± 0.07^{cA}
PFPP	10.2 ± 0.06^{a}	1.77 ± 0.05^{c}	9.13 ± 0.02^{aB}	10.48 ± 0.11^{aA}

Values are presented as mean ± standard deviation (n=3). Means with different lowercase letters in the same column show significant differences ($p<0.05$). Different capital letters in rows indicate significant differences ($p<0.05$) between cholesterol adsorption capacity in different pHs.

MYPP > GUPP > PFPP (Table 4). TSP values were higher than those described by Can-Cauich *et al*. [2017] for mamey sapote peel (210 mg GAE/100 g) and guava and passion fruit by-products (254.7 and 175.6 mg GAE/100 g, respectively) [Selani *et al*., 2016]. The variations among studies can be related to the varieties analyzed, fruit ripening state, drying method, and extraction methods used to process the samples.

The individual phenolics determined in the fruit peel powders are listed in Table 4. Gallic, chlorogenic, ferulic, *p*-coumaric, sinapic, and cinnamic acids, besides catechin and quercetin 3-β-D-glucoside were identified. The identification reported herein agrees with these reported by Cao *et al.* [2021], who describe the presence of 31 phenolic compounds, including gallic and ferulic acid, catechin and quercetin in passion fruit peel. Liu *et al.* [2018] determined 69 phenolics in guava peels, among them gallic acid, ferulic acid, catechin and quercetin which were reported in this study, while Yahia *et al*. [2011] detected gallic and *p*-coumaric acids, shown in this study, besides syringic and *p*-hydroxybenzoic acids, hydroxybenzoic acid derivative, *p*-hydroxybenzoic acid dimer and epicatechin dimer. In turn, Torres-Rodríguez *et al*. [2011] found gallic acid, gallocatechin-3-gallate and epicatechin in extracts of mamey sapote pulp. According to our literature review, the profile of phenolic compounds in mamey sapote peels has not been reported so far.

Antioxidant capacity

The antioxidant systems chosen for this study were capable of evaluating the activity of hydrophilic compounds (TEAC) and hydrophilic and lipophilic compounds (DPPH assay) [Rufino *et al*., 2010]. Figure 1 illustrates the antioxidant capacity of peel powders, which varied from 125.3 to 252.4 µmol TE/100 g for DPPH$^{\bullet}$ scavenging activity in the order of MYPP > PFPP > GUPP. Other authors have reported different values of the antioxidant activity of tropical fruit by-products; for example, Casarotti *et al*. [2018] reported higher DPPH$^{\bullet}$ scavenging activity than those of the present study, at 1,737.11 and 1,340.23 µmol TE/100 g for guava and passion fruit by-products, respectively. However, Martínez *et al*. [2012] found values like those reported here, with

Table 4. Contents of total soluble phenolics (mg GAE/100 g) and individual phenolic compounds (µg/g) of guava (GUPP), mamey sapote (MYPP), and passion fruit (PFPP) peel powders.

Phenolic compound	GUPP	MYPP	PFPP
Total soluble phenolics	250.3±7.3^{b}	365.7±8.3^{a}	185.2±7.6^{c}
Gallic acid	281.0±8.3^{a}	151.4±9.6^{b}	5.1±0.2^{c}
Chlorogenic acid	ND	60.4±6.2^{a}	ND
Ferulic acid	9.6±0.8^{c}	81.0±2.5^{b}	144.2±2.2^{a}
Sinapic acid	ND	180.6±8.6^{a}	ND
p-Coumaric acid	10.3±0.2^{c}	46.1±3.3^{b}	95.3±6.1^{a}
Cinammic acid	ND	8.4±0.1^{a}	ND
Catechin	330.7±4.7^{b}	ND	11.7±1.5^{a}
Quercetin 3-β-D-glucoside	244.0±2.5^{a}	ND	ND

Values are presented as mean ± standard deviation (n=3). Means with different lowercase letters in the same row show significant differences (p<0.05). GAE, gallic acid equivalent; ND, not detected.

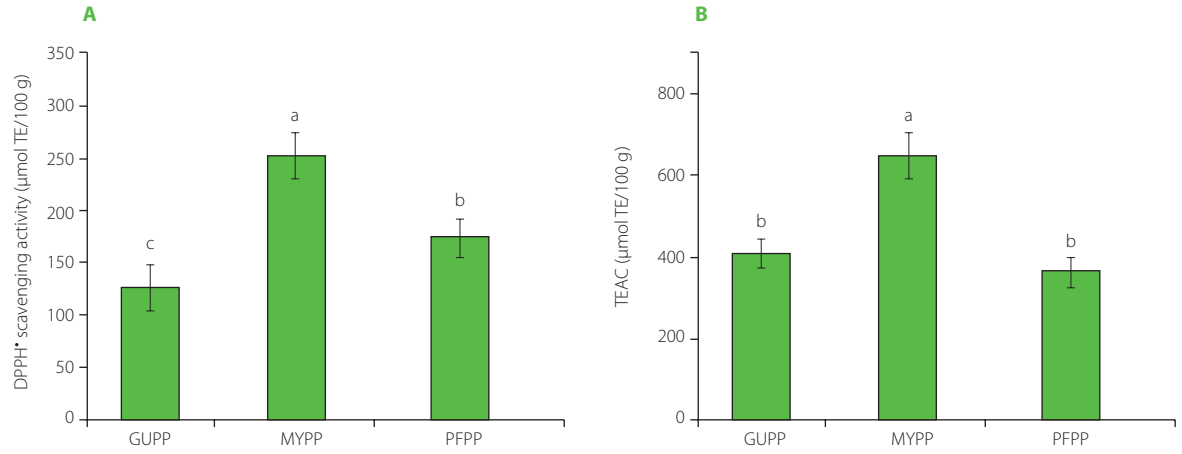

Figure 1. DPPH radical scavenging activity (**A**) and Trolox equivalent antioxidant capacity, TEAC (**B**) of guava (GUPP), mamey sapote (MYPP), and passion fruit (PFPP) peel powders. Values are presented as mean and standard deviation (n=3). Different letters above bars indicate significant differences (p<0.05).

150 and 330 µmol TE/100 g for guava and passion fruit by-products, respectively. Can-Cauich *et al.* [2017] reported antiradical activity against DPPH$^{\bullet}$ of 600 µmol TE/100 g of mamey sapote peels, which was higher than those reported herein. Regarding the TEAC, MYPP showed the highest activity (656.8 µmol TE/100 g) (Figure 1). GUPP and PFPP did not differ significantly in this respect ($p \geq 0.05$), Martínez *et al.* [2012] described TEAC of 190 and 210 µmol TE/100 g for guava and passion fruit by-products, respectively, while Can-Cauich *et al.* [2017] obtained 463 µmol TE/100 g for mamey sapote peel. Our results indicate that mamey sapote peel powder had the highest antioxidant capacity, possibly due to the highest phenolic compound content. Differences in radical scavenging activities are also related to the phenolic compound specific composition of peel powders [Velderrain-Rodríguez *et al.*, 2018].

Inhibition of enzymes

The inhibition of α-amylase and α-glucosidase by peel extracts showed an extract concentration-dependent response (Figure 2A, 2B). Both enzyme inhibitory activity of extracts decreased in the order of MYPP > PFPP > GUPP, with a stronger effect against α-glucosidase than α-amylase. The α-amylase and α-glucosidase inhibitory activities of MYPP extract were 42.46 and 64.15%, respectively, at the maximum concentration tested. Suleria *et al.* [2020] suggest that the presence of ferulic and gallic acid identified in by-products of fruits can inhibit these enzymes. Other studies have confirmed the inhibitory activity of isolated ferulic acid against α-amylase and α-glucosidase and chlorogenic acid against α-amylase [Zheng *et al.*, 2020]. All of these phenolic acids were detected in MYPP, GUPP, and PFPP (Table 4) and may be responsible, at least in part, for the documented effects.

IC_{50} values against α-amylase were >2.50 mg/mL for all extracts. These data were inferior to the data reported by Liu *et al.* [2014] for guava leaves (50.5 µg/mL) and to passion fruit by-products (1.8 mg/mL) as reported by Cao *et al.* [2021]. On the other hand, IC_{50} values against α-glucosidase were 1.11, 1.24, and 1.57 mg/mL for MYPP, GUPP, and PFPP extracts, respectively, which were inferior to the values reported by Cao *et al.* [2021] for passion fruit by-products (0.1 mg/mL) and by Liu *et al.* [2014] for guava leaves (34.6 µg/mL). To date, studies on the inhibitory activity of mamey sapote or its peel against α-amylase or α-glucosidase have not been described.

Even though the inhibitory effects of extracts were lower compared to acarbose (78.22% for α-amylase and 86.42% for

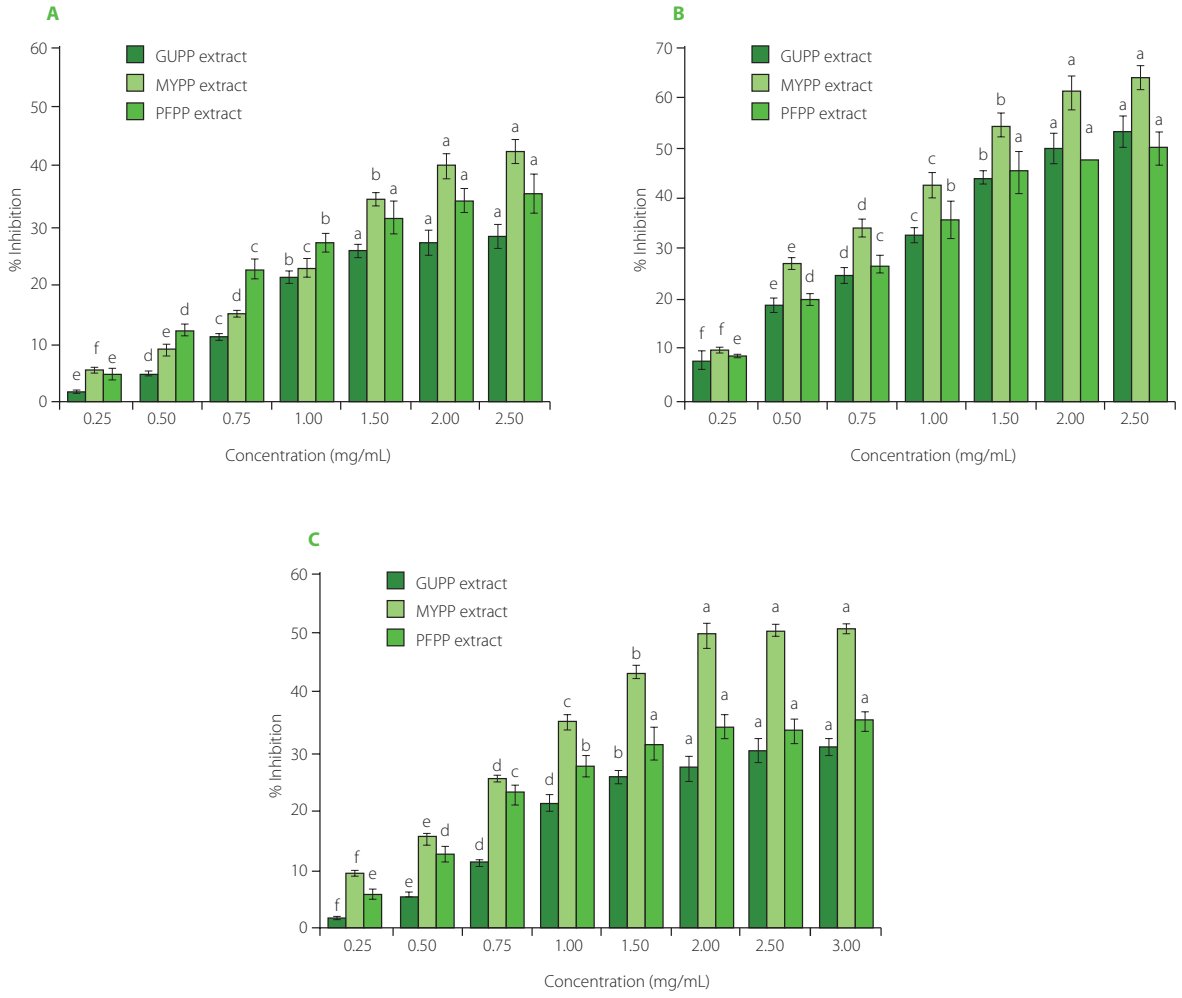

Figure 2. α-Amylase (**A**) α-glucosidase (**B**) and pancreatic lipase (**C**) inhibitory activity of extracts of guava (GUPP), mamey sapote (MYPP), and passion fruit (PFPP) peel powders. Values are presented as mean and standard deviation (n=3). Different letters above bars separately for each extract indicate significant differences (p<0.05).

α-glucosidase), the data suggest that the peel powders could be good inhibitors. Previous studies have established that fruit by-products can inhibit α-amylase and α-glucosidase with minimum effects [Agada *et al*., 2020]. Differences in enzyme--inhibiting effects between the peel fruit extracts may be due to the variety of fruits used, their specific phenolic profile, and the enzymes' origin.

Pancreatic lipase is the enzyme in control of the hydrolysis of fats into monoacylglycerides and free fatty acids in the intestinal lumen [Zdunić *et al*., 2020]; its inhibition may have beneficial effects on weight loss in diabetic patients. The studied peel powder extracts displayed a dose-dependent lipase inhibitory capacity, presenting an IC_{50} of 2.61mg/mL for MYPP extract, and >3.50 mg/mL for PFPP and GUPP extracts. The pancreatic lipase inhibitory activity at the highest concentration of extracts (3.5 mg/mL) was 52.93, 34.92, and 30.15% for MYPP, PFPP, and GUPP, respectively (Figure 2c). Gallic and ferulic acid identified in the three peel powders (Table 4) have been reported as pancreatic lipase inhibitors and could be responsible for the observed effect [Ray *et al.,* 2014].

Phenolic compounds in the diet have been demonstrated to be effective inhibitors of the digestive enzymes: α-amylase, α-glucosidase, and pancreatic lipase, which is beneficial for diabetic patients or related conditions [Tan *et al*., 2017]. Some common clinical management strategies involve the treatment with enzyme inhibitors, like acarbose and orlistat, for α-amylase and α-glucosidase and pancreatic lipase [Gutiérrez-Grijalva *et al*., 2019]. However, constant consumption of these drugs causes adverse effects related to gastrointestinal pain and oily spotting. Thus, natural alternatives like phenolic compounds from tropical fruits are currently being studied since they can have fewer or no secondary effects.

CONCLUSIONS

The guava, mamey sapote, and passion fruit peel powders showed high TSP content and good antioxidant capacity measured as the DPPH radical and ABTS radical cation scavenging activity. Their extracts also exerted medium α-amylase and lipase inhibition potential and a stronger α-glucosidase inhibitory activity. The excess generation of free radical remains pivotal in the evolution and problems of obesity and diabetes; hence, guava, mamey sapote, and passion fruit peel powders dual-acting antidiabetic and antioxidant capacities suggest they may be suitable for glycemic and overweight management by putting down carbohydrate and lipid digestion. This research proposes that the mamey sapote, guava, and passion fruit peel powders can be applied as functional components according to their technological and physiological characteristics, making them possible candidates to be utilized as ingredients of innovative food products. Depending on these properties, they can be used to improve texture, reduce sugar and calories in foods, such as bakery and meat products. Further, *in vivo* investigations on tropical fruit peel powders' antioxidant and hypoglycemic activities and their potential mechanism of action are necessary.

RESEARCH FUNDING

This investigation is part of the aims of project 997, "Phenols and fiber from tropical fruits. Interactions and bioavailability in *in vitro* digestions" of the "Investigadoras e Investigadores por México del CONAHCYT".

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 322–331
DOI: 10.31883/pjfns/174434
ISSN 1230-0322, e-ISSN 2083-6007

Formulation and Stability of Cellulose Particles Enriched with Phenolic Acids

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Dietary fibers and phenolic acids are recognized for their various health benefits; thus, cellulose was selected as a carrier polymer of phenolic acids, including gallic acid and caffeic acid. Dried cellulose particles were prepared through the complexation of different amounts of cellulose (2.5, 5, 7.5 and 10%, *w/v*) with a constant amount of phenolic acids. Additionally, the complexation time was 15 or 60 min with the goal to determine an impact of the duration of complexation on the adsorption of phenolic acids onto cellulose. The prepared particles were stored at ambient temperature for 12 months to assess their storage stability. Cellulose particles were assessed for the amount of adsorbed phenolic acids and antioxidant activities. For both phenolic acids, the same correlation was established, *i.e.*, reduction of adsorption of phenolic acids occurred with the increase of cellulose amount during complexation. The duration of complexation was not a significant factor in the adsorption of phenolic acids. Antioxidant activity generally followed the trend that was obtained for the amount of adsorbed phenolic acids. Comparing both phenolic acids, cellulose had a higher affinity for caffeic acid (4.665 g/kg) than for gallic acid (3.399 g/kg). However, greater stability of gallic acid/cellulose particles was observed throughout the storage. After storage, the content of gallic acid in cellulose particles slightly decreased (up to 3%), while that of caffeic acid decreased from 10 to 20%. The complexation of phenolic acids with cellulose was proven by recording infrared spectra. Formulated cellulose particles can be a valuable tool for the preparation of plant-based functional additives which can be used for the enrichment of products with phenolic acids in order to improve their antioxidant potential and stability.

Key words: cellulose, gallic acid, caffeic acid, storage stability, antioxidant potential, complexation

INTRODUCTION

The food industry has recently been facing numerous challenges as consumers are looking for high-quality food products with health effects, such as antioxidant and antibacterial activity or prevention of cardiovascular diseases [Magnani *et al.*, 2014]. Many epidemiological researches have revealed a positive correlation among the intake of food abounded in phenolic compounds and possible prevention and regulation of various disorders [Sato *et al.*, 2011]. Dietary phenolics are secondary plant metabolites that are generally divided into two basic groups, *i.e.*, flavonoids and non-flavonoids. Phenolic acids belong to the group of non-flavonoid phenolics which structures are characterized by phenol moiety stabilized by resonance and one carboxylic group [Kadar *et al.*, 2021]. Caffeic acid (3,4-dihydroxycinnamic acid) is a representative of the group of cinnamic acid derivates that are also called phenylpropanoids and it is present in coffee drinks, berries, apples and other food sources [Magnani *et al.*, 2014]. Gallic acid (3,4,5-trihydroxybenzoic acid) is composed of an aromatic ring with three hydroxyl groups and a carboxylic group. It is commonly present in the plant kingdom and found

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Submitted: 14 June 2023
Accepted: 24 October 2023
Published on-line: 6 November 2023

in free form or as a derivate in nuts, tea, grapes, berries, honey and other fruits and vegetables. Its ester derivates are used for the prevention of food spoilage and oxidative rancidity in food packaging materials, processed food and cosmetics [Badhani *et al.*, 2015]. Among the many advantages of phenolic acids, these compounds are reported to have disadvantages related to sensitivity to processing and storage conditions, such as extreme temperatures, oxygen and light [Rashimi & Negi, 2020]. To improve the stability of phenolics, different encapsulation techniques are applied. Freeze-drying is a technique which is often deployed for encapsulation since it can protect thermo--sensitive components. For this purpose, different encapsulating polymers, such as polysaccharides, proteins or lipids, are used as carriers of bioactive compounds and in this way, the stability of phenolic properties can be maintained over a longer period. It was reported previously that dietary fibers have many beneficial impacts on gastrointestinal physiology, and also evoke a hypolipidemic impact since they can influence the expression of enzymes in lipid metabolism [Anderson *et al.*, 2009]. Cellulose and its derivates can be applied as low-calorie substitutions for other carbohydrate additives, but also they can be used as carriers of flavor compounds and stabilizers of dispersed systems in different types of foods. They have also been demonstrated to act as delivery systems and protection carriers of different nutrients and active compounds in food products [Liu *et al.*, 2017; Mu *et al.*, 2014; Vukoja *et al.*, 2021]. The majority of past investigations were organized to investigate on the one hand the release of phenolics from plant cell wall (PCW) components in the gastrointestinal tract and on the other hand the nature of the interaction between those compounds. Phenolics are allocated in the cell vacuoles where they can, during ripening, processing and food consumption, come into contact with PCW components and in this way their bioavailability can be affected [Padayachee *et al.*, 2012a,b]. Cellulose, hemicellulose and pectin are the basic PCW components. The interactions of PCW components and phenolics cause the formation of the complex which can be beneficial in enhancing bioavailability of phenolics. Such complexes prevent the absorption of phenolics in the stomach or small intestine and assist in their transportation to the large intestine where fermentation is carried out with the help of intestinal bacteria [Padayachee *et al.*, 2012b]. Generally, it has been concluded that the structural complexity and properties of phenolics and PCW components were the key elements in the assessment of bioaccessibility and bioavailability of phenolics. Non-covalent interactions (such as hydrogen and hydrophobic interactions) were the driving force between phenolics and PCW components, followed by environmental variables, such as pH and temperature [Le Bourvellec *et al.*, 2004; Padayachee *et al.*, 2012a,b; Phan *et al.*, 2017; Renard *et al.*, 2001; Tang *et al.*, 2003]. Taking all this into consideration, cellulose has great potential to be used as a functional additive in the food products, such as beverages, dairy, fruit, meat and bakery products, hence, cellulose particles enriched with gallic and caffeic acid were formulated in this research.

The aim of this investigation was to formulate cellulose particles that are enriched with phenolic acids including gallic and caffeic acids and to investigate the impact of cellulose amount (2.5, 5, 7.5 and 10%, *w/v*) and time of complexation on the encapsulation of these phenolic acids onto cellulose. Stability of the formulated cellulose particles at ambient conditions over 12 months of storage in terms of retention of phenolic acids and antioxidant activity was also investigated.

MATERIALS AND METHODS

Chemicals

Microcrystalline cellulose and potassium persulfate were purchased from Kemika (Zagreb, Croatia). Gallic acid, caffeic acid, Trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were products of Sigma Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) grade methanol was bought from J.T. Baker (Deventer, Netherlands), and HPLC grade orthophosphoric acid (>85%) was from Fisher Scientific (Loughborough, UK). Cupric chloride, neocuproine and 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Acros Organic (Geel, Belgium), whereas hydrochloric acid (37%) was purchased from Carlo Erba Reagents (Sabadell, Spain).

Formulation of gallic acid/cellulose and caffeic acid/cellulose particles

For the formulation of cellulose particles, cellulose (2.5, 5, 7.5 and 10%, *w/v*) was added into 50 mL of 5 mM gallic acid or caffeic acid solution in 10% (*v/v*) of ethanol. The obtained suspension was agitated at a magnetic stirrer (Stuart US152, Buch and Holm, Hervel, Denmark) at ambient temperature for 15 or 60 min. Afterwards, the mixtures were centrifuged at 2,545×*g* for 15 min to obtain precipitate, whereas the supernatant was discarded to eliminate non-adsorbed phenolic acids. The precipitate was frozen (−18°C) for 24 h and then freeze-dried in an Alpha 1-4 freeze dryer (Christ, Osterode am Harz, Germany). Sample freeze-drying conditions were as follows: the freezing temperature was adjusted to −55°C, the temperature of sublimation was adapted from −35 to 0°C at a vacuum level of 22 Pa, the isothermal desorption was adapted to 0–21°C at a vacuum level of 6 Pa. Part of the obtained particles were analyzed immediately, whereas the other part of the samples was sealed in plastic bags. The sealed samples were stored at ambient temperature for 12 months.

Extraction of phenolic acids from particles

For the extraction of phenolic acids, 0.1 g of each type of cellulose particles was weighed, then 5 mL of acidified methanol (ratio of HCl to methanol was 1:99, *v/v*) were added, and the mixture was well homogenized. After 24-h extraction at room temperature, the samples were filtrated, and clear extract was further analyzed. The extracts were analyzed for gallic acid and caffeic acid concentrations by HPLC, and used to determine antioxidant activity of the particles.

High-performance liquid chromatography of gallic and caffeic acids

The 1260 Infinity II HPLC system (Agilent Technologies, Santa Clara, CA, USA) was applied to determine gallic acid and caffeic acid contents in the prepared particles. The whole system contained a diode array detector (DAD), a quaternary pump, a vial sampler, and a Poroshell 120 EC C-18 column (4.6×100 mm, 2.7 µm). Orthophosphoric acid (0.1%, *v/v*) as a mobile phase solvent A and methanol (100%) as a mobile phase solvent B were used for chromatographic separation. Gallic acid was separated by isocratic elution with 10% of solvent A and 90% of solvent B for 6 min. For caffeic acid, the following gradient elution was applied: 0 min 5% B, 5 min 90% B, and 15 min 5% B. Operating condition were adjusted to the flow rate of 1 mL/min, a column temperature of 28°C and an injection volume of 10 µL. Stock solutions of gallic and caffeic acids in 100% methanol were applied to plot calibration curves in concentrations from 25 to 100 mg/L (r^2=0.9967) and from 15 to 80 mg/L (r^2=0.999), respectively. Peaks were recorded at 270 and 320 nm (for gallic acid and caffeic acid, respectively). Screenings were performed in duplicates, and results were expressed as g of phenolic acid *per* kg of cellulose particles (g/kg).

Determination of the antioxidant activity of particles

Antioxidant activity was determined using the assays with DPPH radicals (DPPH assay), ABTS cation radicals (ABTS assay), as well as ferric reducing antioxidant power (FRAP) and cupric ion reducing capability (CUPRAC) assays. The protocol of DPPH assay was defined elsewhere [Brand-Williams *et al.*, 1995]. The DPPH radical solution was prepared and mixed in a glass tube with 0.2 mL of the extract. After 15 min in dark, absorbance of the mixture was read at 517 nm. The ABTS assay was conducted according to the procedure previously published by Arnao *et al.* [2001]. The ABTS radical cation solution and 0.2 mL of the extract were mixed, kept for 95 min in the dark, and their absorbance was read at 734 nm. The FRAP assay was performed as defined by Benzie & Strain [1996]. Briefly, the FRAP reagent was mixed with 0.2 mL of the extract in a glass tube. After 30 min, the absorbance of the mixture was read at 593 nm. The assay by Apak *et al.* [2004] was adapted in order to evaluate cupric reducing antioxidant activity. To conduct the analysis, 1 mL of $CuCl_2$ solution (10 mM), 1 mL of neocuproine (7.5 mM), and 1 mL of ammonium acetate buffer (pH 7.0) were mixed with the sample and distilled water. The prepared mixture was left in the dark for 30 min, and afterwards absorbance was read at 450 nm. For all four assays, the calibration curves were plotted using Trolox as a standard, and the results were expressed as µmol of Trolox equivalent (TE) *per* kg of gallic acid/cellulose and caffeic acid/cellulose particles (µmol TE/kg). All measurements were done in triplicate using a Cary 60 UV/Vis spectrophotometer (Agilent Technologies).

Recording of infrared spectra

The infrared spectra of the samples were screened by the Cary 630 Fourier transform infrared – attenuated total reflectance (FTIR-ATR) spectrometer (Agilent Technologies) that contained the MicroLab Expert software. The screening of the cellulose and formulated particles was performed at the 4,000–600 cm^{-1} interval.

The following parameters were calculated as a ratio between band intensities: total crystalline index (TCI), lateral order index (LOI), and hydrogen bond intensity (HBI) according to the following Equations (1–3):

$$TCI = A_{1,370\text{-}1,360}/A_{2,900\text{-}2,890} \quad (1)$$

where: $A_{1,370\text{-}1,360}$ is band intensity in the range of 1,370–1,360 cm^{-1} and $A_{2,900\text{-}2,890}$ is band intensity in the range of 2,900–2,890 cm^{-1}.

$$LOI = A_{1,430\text{–}1,420}/A_{897} \quad (2)$$

where: $A_{1,430\text{-}1,420}$ is band intensity in the range of 1,430–1,420 cm^{-1} and A_{897} is band intensity at 897 cm^{-1}.

$$HBI = A_{3,340\text{–}3,330}/A_{1,315} \quad (3)$$

where: $A_{3,340\text{-}3,330}$ is band intensity in the range of 3,340––3,330/1315 cm^{-1} and $A_{1,315}$ is band intensity at 1,315 cm^{-1}.

Statistical analysis

Obtained data were statistically analyzed using the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test (the significance was defined at $p \leq 0.05$). Analysis was performed using the STATISTICA 13.1 software program (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Content of phenolic acids in cellulose particles

Cellulose particles were determined for the content of gallic or caffeic acids, and results after complexation are presented in Table 1. The one-year storage stability of cellulose particles was also evaluated. From the results, it can be concluded that the amount of cellulose used for complexation as well as complexation time affected the adsorption of both phenolic acids. Also, the affinity of cellulose towards selected phenolic acids was different. Generally, cellulose had a higher affinity for caffeic acid. Gallic acid/cellulose and caffeic acid/cellulose particles contained phenolic acids in amounts of 3.399 and 4.665 g/kg, respectively. The highest content of phenolic acids was determined in the cellulose particles prepared with the lowest amount of cellulose, *i.e.*, 2.5% (*w/v*). For both types of particles, a decrease was observed in the content of phenolic acids with the increase of cellulose amount during complexation (regardless of complexation time), thus, the content of gallic acid in particles with 10% (*w/v*) of cellulose was 2.964 g/kg in comparison to content of caffeic acid of 3.493 g/kg. Extended complexation did not have a positive effect on the adsorption of phenolic acids. Comparing particles with the highest content of phenolic acids, the same (p>0.05) content of gallic acid was determined after extended complexation (60 min) as after 15 min of complexation. For caffeic acid, a different trend was observed, so its content

Table 1. Content (g/kg) of gallic acid and caffeic acid in gallic acid/cellulose (GA/C) and caffeic acid/cellulose (CA/C) particles, respectively, prepared by 15-min or 60-min complexation using different amounts of cellulose and stored for 12 months.

Particle	Before storage		After storage	
	15 min	60 min	15 min	60 min
GA/C_2.5	3.399 ± 0.007^{ax}	3.392 ± 0.011^{ax}	3.382 ± 0.008^{ax}	3.381 ± 0.001^{ax}
GA/C_5	3.257 ± 0.009^{by}	3.305 ± 0.007^{bx}	3.190 ± 0.005^{by}	3.251 ± 0.005^{bx}
GA/C_7.5	3.021 ± 0.004^{cx}	2.980 ± 0.001^{cy}	2.950 ± 0.008^{cx}	2.928 ± 0.004^{cx}
GA/C_10	2.964 ± 0.012^{cx}	2.924 ± 0.003^{dx}	2.853 ± 0.019^{dx}	2.855 ± 0.002^{dx}
CA/C_2.5	4.665 ± 0.031^{ax}	4.009 ± 0.059^{ay}	3.800 ± 0.020^{ax}	3.612 ± 0.009^{ay}
CA/C_5	4.105 ± 0.002^{bx}	3.862 ± 0.043^{by}	3.611 ± 0.068^{bx}	3.477 ± 0.023^{by}
CA/C_7.5	3.568 ± 0.056^{cx}	3.637 ± 0.079^{cx}	3.188 ± 0.002^{cx}	3.081 ± 0.044^{cy}
CA/C_10	3.493 ± 0.016^{cx}	3.482 ± 0.065^{dx}	3.133 ± 0.004^{dx}	3.129 ± 0.030^{cx}

Numbers in the particle codes (2.5, 5, 7.5 and 10) indicate the amount (%, *w/v*) of cellulose used for complexation. Values in the same column, separately for particles with gallic and caffeic acid, marked with different letters (a–d) are significantly different at $p\leq0.05$. Values in the same raw, separately for particles before and after storage, marked with different letters (x, y) are significantly different at $p\leq0.05$.

in the particles was significantly ($p\leq0.05$) lower after extended complexation. The stability of particles was also evaluated. Gallic acid/cellulose particles were more stable over the 12 months of storage at ambient temperature than caffeic acid/cellulose particles. After storage, the content of gallic acid in cellulose particles slightly decreased (up to 3%), whereas that of caffeic acid decreased from 10 to 20%.

Interactions between different fibers, including cellulose, and phenolics depend on the molecular structure, physical properties and initial concentration of components used for complexation. It was pointed out that the main mechanism of their interactions was of non-covalent nature, like hydrogen bonds and hydrophobic interaction. Those interactions are also strongly affected by environmental conditions, like pH and temperature and time of complexation [Le Bourvellec *et al.*, 2004; Padayachee *et al.*, 2012a,b; Phan *et al.*, 2017; Renard *et al.*, 2001; Tang *et al.*, 2003]. The main drivers of interactions between cellulose and phenolic acids are the molecular size and structure of phenolic acids [Le Bourvellec *et al.*, 2004]. The basic structural characteristic of all phenolics is the presence of one or more benzene rings and hydroxyl groups by which they bind to specific sites on polysaccharide molecules. Generally, phenolics and fibers bind to each other throughout hydrogen bonds (formed between the OH groups of phenolic compounds and oxygen atoms of polysaccharides), as well as hydrophobic and covalent bonding (between phenolic acids and polysaccharides). Additionally, porosity, particle size and surface properties of dietary fibers can be crucial in the interaction between fibers and phenolic compounds [Quiros-Sauceda *et al.*, 2014; Saura-Calixto, 2011]. Phenolic acids that have more hydroxyl groups in their structure and additionally contain hydrophobic regions ensure hydrogen bonding, thus promoting stronger interaction with molecules of dietary fibers. Phenolic acids can interact by non-covalent interactions with neutral saccharides (xyloglucans) present in the cellulose structure [Padayachee *et al.*, 2012b]. Gallic acid contains 3 hydroxyl groups, whereas caffeic acid has 2; hence, both phenolic acids are candidates for the formation of hydrogen bonds with cellulose. Time of complexation did not play significant part in the adsorption of selected phenolic acids onto cellulose. At the beginning of the interaction between phenolics and cellulose, hydroxyl groups bonded on the cellulose ribbons' surface. After initial interaction, hydrogen bonding and hydrophobic interaction occurred [Liu *et al.*, 2017]. Extended complexation time, which included stirring, did not cause an increase in the adsorption of phenolic acids probably due to the breaking of hydrogen bonds and hydrophobic interactions. Additionally, it is possible that the formation of hydrogen bonds between cellulose molecules occurred due to the higher amount of cellulose during complexation, thereby decreasing the number of free binding sites for phenolic acids. A study on the binding capacity of diverse phenolics and cellulose revealed that binding was affected by the molecular structure of phenolics and it was in the range between 0.4 and 1.4 g/g of cellulose [Phan *et al.*, 2015]. Interaction between cellulose and phenolics (gallic acid, caffeic acid, catechin, ferulic acid, chlorogenic acid, cyanidin 3-glucoside) happened within 1 min and quickly increased over 30 min. Afterwards, the binding between phenolics and cell wall polysaccharides decelerated [Padayachee *et al.*, 2012a; Phan *et al.*, 2015]. Probably, there are specific sites on the cellulose molecular structure that are responsible for the formation of strong biding regions. The second alternative is that cellulose has a certain degree of binding for phenolic molecules and above that degree, phenolic compounds are starting to make the ionic barrier for further binding [Padayachee *et al.*, 2012a]. Our previous study also supported this biding tendency. Cellulose/raspberry particles prepared with 2.5% (*w/v*) of cellulose had the highest content of total phenolics. Higher adsorption of phenolics was achieved in samples prepared throughout 15 min of complexation than in those prepared for 60 min [Vukoja *et al.*, 2021]. Also, our previous study, in which we investigated encapsulation of gallic acid

onto pectin, showed that a higher content of pectin negatively affected encapsulation of gallic acid [Buljeta *et al.*, 2022c].

Antioxidant activity of cellulose particles

Antioxidant activity of cellulose particles was evaluated by four assays, namely DPPH, ABTS, FRAP and CUPRAC, that are the most common for evaluation of this parameter. Results are presented in Tables 2, 3, 4 and 5. Different values of antioxidant activities were obtained depending on the applied assay, *i.e.*, its mechanism of action. Antioxidant activity obtained by the DPPH assay for gallic acid/cellulose particles before storage ranged from 391.6 to 419.4 µmol TE/kg and the values for particles prepared with 2.5, 5 and 7.5% (*w/v*) of cellulose for each complexation time did not differ significantly ($p>0.05$), indicating there was no considerable variation in DPPH radical scavenging activity among the particles (Table 2) even though there was a difference in gallic acid content (Table 1). In the stored particles, a slight change of DPPH radical scavenging activity was noted, and the same trend was retained as before storage (Table 2). Results of antioxidant activity determination for caffeic acid/cellulose particles obtained by the DPPH assay followed the trend that was obtained for caffeic acid content in these particles before and after storage. In the ABTS assay, values of antioxidant activity ranged from 478.6 to 589.7 µmol TE/kg and 874.7 to 1,691.0 µmol TE/kg for gallic acid/cellulose and caffeic acid/cellulose particles before storage, respectively (Table 3). By comparison of the results of the ABTS assay of the samples prepared with the same cellulose amount and different complexation times, it was observed that a complexation time of 15 min was more favorable for the samples of gallic acid with 2.5 and 10% (*w/v*) of cellulose, whereas for the samples with caffeic acid, this effect was observed for the samples prepared with 2.5 and 5% (*w/v*) of cellulose. A complexation time of 60 min had a positive effect only on the gallic acid sample prepared with 5%

Table 2. DPPH radical scavenging activity (µmol TE/kg) of gallic acid/cellulose (GA/C) and caffeic acid/cellulose (CA/C) particles prepared by 15-min or 60-min complexation, after complexation using different amounts of cellulose and stored for 12 months.

Particle	Before storage		After storage	
	15 min	60 min	15 min	60 min
GA/C_2.5	419.4 ± 1.8^{ax}	410.1 ± 3.4^{ay}	411.2 ± 3.3^{ax}	413.3 ± 0.9^{ax}
GA/C_5	418.2 ± 2.3^{ax}	406.4 ± 4.8^{ay}	401.3 ± 4.5^{ax}	409.5 ± 5.4^{bcx}
GA/C_7.5	414.6 ± 2.7^{ax}	408.1 ± 2.8^{ay}	400.4 ± 2.4^{ax}	396.9 ± 4.1^{bx}
GA/C_10	395.4 ± 5.8^{bx}	391.6 ± 3.1^{bx}	357.9 ± 5.1^{bx}	362.2 ± 3.8^{cx}
CA/C_2.5	457.9 ± 2.5^{ax}	429.8 ± 3.1^{ay}	370.7 ± 1.5^{ax}	351.8 ± 1.9^{ay}
CA/C_5	331.7 ± 3.2^{by}	348.0 ± 5.2^{bx}	313.6 ± 2.3^{bx}	306.8 ± 2.5^{by}
CA/C_7.5	313.7 ± 4.1^{cy}	335.2 ± 3.2^{cx}	301.6 ± 3.2^{cx}	299.9 ± 3.5^{bx}
CA/C_10	299.6 ± 1.2^{dx}	294.8 ± 1.1^{dy}	241.5 ± 1.5^{dx}	238.0 ± 3.6^{cx}

Numbers in the particle codes (2.5, 5, 7.5 and 10) indicate the amount (%, *w/v*) of cellulose used for complexation. Values in the same column, separately for particles with gallic and caffeic acid, marked with different letters (a–d) are significantly different at $p\leq0.05$. Values in the same raw, separately for particles before and after storage, marked with different letters (x, y) are significantly different at $p\leq0.05$. TE, Trolox equivalent.

Table 3. ABTS radical cation scavenging activity (µmol TE/kg) of gallic acid/cellulose (GA/C) and caffeic acid/cellulose (CA/C) particles prepared by 15-min or 60-min complexation using different amounts of cellulose and stored for 12 months.

Particle	Before storage		After storage	
	15 min	60 min	15 min	60 min
GA/C_2.5	589.7 ± 2.9^{ax}	575.1 ± 0.39^{ay}	520.1 ± 3.1^{ax}	513.1 ± 0.48^{ay}
GA/C_5	532.6 ± 0.9^{by}	550.6 ± 0.89^{bx}	477.2 ± 5.9^{bx}	454.7 ± 0.71^{by}
GA/C_7.5	508.8 ± 7.3^{cx}	504.8 ± 0.04^{cx}	449.4 ± 4.2^{cx}	433.8 ± 0.65^{cy}
GA/C_10	488.8 ± 0.5^{dx}	478.6 ± 0.51^{dy}	422.6 ± 2.8^{dx}	420.1 ± 0.34^{dx}
CA/C_2.5	$1,691.0\pm9.4^{ax}$	$1,457.9\pm9.2^{ay}$	$1,092.7\pm5.8^{ax}$	969.1 ± 7.5^{ay}
CA/C_5	983.0 ± 5.2^{bx}	958.5 ± 3.4^{by}	945.7 ± 4.2^{bx}	908.8 ± 8.4^{by}
CA/C_7.5	935.3 ± 8.4^{cx}	923.0 ± 0.1^{cx}	888.2 ± 5.9^{cx}	848.6 ± 5.2^{cy}
CA/C_10	874.7 ± 7.4^{dx}	888.1 ± 4.5^{dx}	829.8 ± 7.2^{dx}	823.1 ± 7.4^{dx}

Numbers in the particle codes (2.5, 5, 7.5 and 10) indicate the amount (%, *w/v*) of cellulose used for complexation. Values in the same column, separately for particles with gallic and caffeic acid, marked with different letters (a–d) are significantly different at $p\leq0.05$. Values in the same raw, separately for particles before and after storage, marked with different letters (x, y) are significantly different at $p\leq0.05$. TE, Trolox equivalent.

Table 4. Ferric reducing antioxidant power (μmol TE/kg) of gallic acid/cellulose (GA/C) and caffeic acid/cellulose (CA/C) particles prepared by 15-min or 60-min of complexation using different amounts of cellulose and stored for 12 months.

Particle	Before storage		After storage	
	15 min	60 min	15 min	60 min
GA/C_2.5	155.5±3.1^{ay}	163.0±2.8^{ax}	153.0±3.8^{ay}	163.4±3.1^{ax}
GA/C_5	152.4±2.8^{ay}	159.8±3.4^{ax}	144.1±5.4^{aby}	152.6±2.3^{bx}
GA/C_7.5	142.2±3.6^{bx}	136.9±0.6^{by}	141.6±3.8^{bx}	132.3±3.1^{cy}
GA/C_10	135.4±1.3^{cx}	134.8±2.1^{bx}	123.2±4.7^{dx}	120.9±5.4^{dx}
CA/C_2.5	71.7±2.1^{ax}	64.6±2.0^{ay}	64.4±2.1^{ax}	58.3±3.1^{ax}
CA/C_5	66.4±1.2^{bx}	61.4±1.5^{ay}	51.0±1.2^{bx}	47.8±1.9^{bx}
CA/C_7.5	63.2±3.1^{bx}	54.4±3.1^{by}	48.0±2.9^{bx}	47.5±1.4^{bx}
CA/C_10	61.8±2.1^{bx}	48.9±2.4^{by}	41.6±1.1^{cx}	41.5±0.9^{cx}

Numbers in the particle codes (2.5, 5, 7.5 and 10) indicate the amount (%, *w/v*) of cellulose used for complexation. Values in the same column, separately for particles with gallic and caffeic acid, marked with different letters (a–d) are significantly different at $p \leq 0.05$. Values in the same raw, separately for particles before and after storage, marked with different letters (x, y) are significantly different at $p \leq 0.05$. TE, Trolox equivalent.

Table 5. Cupric reducing antioxidant capacity (μmol TE/kg) of gallic acid/cellulose (GA/C) and caffeic acid/cellulose (CA/C) particles prepared by 15-min or 60-min of complexation using different amounts of cellulose and stored for 12 months.

Particle	Before storage		After storage	
	15 min	60 min	15 min	60 min
GA/C_2.5	2,622.8±5.3^{ax}	2,635.5±6.3^{ax}	2,597.3±7.3^{ax}	2,605.1±3.2^{ax}
GA/C_5	2,513.9±8.7^{by}	2,543.7±0.4^{bx}	2,365.5±6.3^{bx}	2,287.3±8.1^{by}
GA/C_7.5	2,024.8±3.8^{cx}	2,005.2±5.7^{cy}	2,177.6±4.7^{cx}	2,157.9±5.5^{cy}
GA/C_10	1,997.4±8.8^{dx}	1,963.4±9.3^{dy}	1,934.6±8.1^{dy}	1,952.3±1.4^{dx}
CA/C_2.5	2,646.3±10.4^{ax}	2,402.9±8.4^{ay}	2,090.8±9.8^{ax}	2,043.2±9.9^{ay}
CA/C_5	2,380.7±11.1^{bx}	2,284.0±10.4^{by}	2,046.5±7.1^{bx}	2,011.0±7.9^{by}
CA/C_7.5	2,090.5±9.4^{cy}	2,220.1±10.1^{cx}	2,000.0±8.9^{cx}	1,841.6±8.3^{cy}
CA/C_10	1,777.3±7.4^{dx}	1,785.3±8.1^{dx}	1,824.7±7.4^{dx}	1,818.8±7.0^{dx}

Numbers in the particle codes (2.5, 5, 7.5 and 10) indicate the amount (%, *w/v*) of cellulose used for complexation. Values in the same column, separately for particles with gallic and caffeic acid, marked with different letters (a–d) are significantly different at $p \leq 0.05$. Values in the same raw, separately for particles before and after storage, marked with different letters (x, y) are significantly different at $p \leq 0.05$. TE, Trolox equivalent.

(*w/v*) cellulose. ABTS radical cation scavenging activity followed the trend of phenolic acid content before and after storage. The results of the FRAP assay of the particles before storage ranged from 134.8 to 163.0 μmol TE/kg and 48.9 to 71.7 μmol TE/kg for gallic acid/cellulose and caffeic acid/cellulose particles, respectively (Table 4). Interestingly, in this assay, the highest antioxidant activity was determined for the gallic acid/cellulose particles, whereas in DPPH and ABTS assays the highest values were obtained for the caffeic acid/cellulose particles. Additionally, regarding FRAP (as it was the case with DPPH radical scavenging activity), there was not so large difference among the particles with different cellulose amounts even though there was a difference in phenolic acid content. The results obtained by CUPRAC assay followed the trend observed for the content of phenolic acids in cellulose particles (Table 5). These results ranged from 1,963.4 to 2,635.5 μmol TE/kg for gallic acid/cellulose and from 1,777.3 to 2,646.3 μmol TE/kg for caffeic acid/cellulose particles before storage. The highest value of antioxidant activity determined for the samples with gallic acid by the CUPRAC assay before storage was noted for the samples with 2.5% (*w/v*) cellulose regardless of complexation time, whereas 15 min of complexation and 2.5% (*w/v*) cellulose caused the highest antioxidant activity of the samples with caffeic acid.

Throughout our results, it was evident that different values of antioxidant activity were achieved by the application of different assays. Antioxidant activity is one of the many biological activities of phenolics and it is highly dependent on their chemical structure. Two mechanisms are involved in their antioxidant effects and those are single electron transfer (SET) and hydrogen atom transfer (HAT) [Chaillou & Nazareno, 2006; Mercado-Mercado *et al.*, 2020]. The antioxidant activity of phenolic acids is improved with an increased number of methoxy groups, but especially hydroxyl groups, in their structures [López-Martínez *et al.*, 2015]. Craft *et al.* [2012] also stated that polyphenols with

an electron donor group attached to the aromatic ring had considerable antioxidant activity. By studying the chemical structure of gallic acid it can be observed that *para* O–H bond has the lowest binding energy; thus, it is the weakest bond present in its structure and exposed to the reaction with free radicals [Uranga *et al.*, 2016]. Kikuzaki *et al.* [2002] investigated antioxidant activity of caffeic acid and its related compounds (*p*-coumaric, ferulic and sinapic acid). The obtained results showed that caffeic acid exhibited high antioxidant activity determined by the DPPH assay. It is known that the number of hydroxyl groups attached to the benzene ring and the *ortho* substitution with the electron donor methoxy group that caused increased stability of the phenoxy radical affected positively the antioxidant effect of hydroxycinnamic acids (such as caffeic acid) on DPPH radical [Kikuzaki *et al.*, 2002]. Theoretical results obtained in the study by Uranga *et al*. [2016] showed that antioxidant activity of hydroxycinnamic acids, such as caffeic and chlorogenic acid, appeared through SET reaction in DPPH and FRAP assays. Except for the number and position of OH groups attached to the aromatic ring that determine the antioxidant activity of the phenolic acids, it was suggested that hydroxycinnamic acids possessed higher antioxidant potential than hydroxybenzoic acids probably due to the –CH=CH–COOH group which has a better ability to donate H atoms and stabilize radicals than the –COOH group [Mercado-Mercado *et al.*, 2020]. There is an exception for the DPPH assay, where hydroxybenzoic acids, such as gallic acid, show higher activity. In the reaction of phenolic acids with radicals, a quinone derivative may be produced or the original chemical structure can be preserved. Results of a study conducted by López-Martínez *et al.* [2015] showed that caffeic acid formed quinones, whereas gallic acid preserved its structure and managed to neutralize DPPH. Comparing DPPH radicals and ABTS radical cations, DPPH radicals have higher selectivity in reaction with donors of hydrogen than ABTS radical cations [Loncaric *et al.*, 2014]. Apak *et al*. [2004] stated that, additionally to conjugation of the molecule, the number and position of OH groups are directly connected to the antioxidant activity in the CUPRAC method. The binding of phenolic acids on the cellulose probably changed the number of available hydroxyl groups on phenolic acids and also caused changes in reaction with DPPH radicals and ABTS radical cations as well as the reduction of metal ions in the other two assays.

DPPH commonly reacts through SET reactions, but sometimes DPPH radical can be neutralized by either HAT or SET mechanisms as well as unrelated reactions [Craft *et al.*, 2012; Prior *et al.*, 2005]. The DPPH radical scavenging assay is one of the most often used analysis for the determination of antioxidant activity. DPPH is a commercially available stable chromogen radical and it does not need to be generated before the analysis [Shahidi & Zhong, 2015]. In the ABTS assay, an intensely colored ABTS radical cation is formed, and the antioxidant effect is measured as the ability of the compounds to decrease the color reacting with the radical [Prior *et al.*, 2005]. In turn, the FRAP assay measures the reduction of the ferric ion (Fe^{3+})-ligand complex to the blue-colored ferrous (Fe^{2+}) complex by the action of antioxidant [Shahidi & Zhong, 2015]. In our study, tripyridyltriazine (TPTZ) was used as the iron-binding ligand. The FRAP assay is simple, robust and cheap, and is based on SET mechanism; hence, it is recommended to be used in combination with other assays to check the dominant mechanism for different antioxidants [Prior *et al.*, 2005]. Finally, the CUPRAC assay is based on the reduction of cupric (Cu^{2+}) to cuprous ion (Cu^{+}) using antioxidants [Prior *et al.*, 2005]. Analogous to the FRAP assay, a ligand (neocuproine) forms a copper-ligand complex to facilitate absorbance measurement in the CUPRAC assay [Shahidi & Zhong, 2015].

Infrared spectra of cellulose particles

FTIR spectra of cellulose, gallic acid/cellulose particles and caffeic acid/cellulose particles are presented in Figure 1. FTIR spectra of cellulose are in accordance with the spectra obtained by Cichosz & Masek [2002]. The band observed in the region from 3,660 to 2,900 cm^{-1} is connected with the stretching vibrations of O–H and C–H bonds which are present in polysaccharide molecules [Cichosz & Masek, 2020; Hospodarova *et al.*, 2018; Poletto *et al.*, 2011; Popescu *et al.*, 2011; Rosa *et al.*, 2010]. This region was also identified in all our samples but there was a difference in spectra intensity. In fact, the whole IR spectra of gallic acid/cellulose and caffeic acid/cellulose particles had higher intensity than these of cellulose, which could be related to the adsorption of phenolic acids onto cellulose. Caffeic acid/cellulose particles had a higher content of this phenolic acid and higher intensity in comparison to the gallic acid/cellulose particles. The broad band at 3,300 cm^{-1} was connected to the stretching vibration of the OH groups in polysaccharides with inter- and intra-molecular vibrations of hydrogen bonds in cellulose [Poletto *et al.*, 2011; Popescu *et al.*, 2011; Rosa *et al.*, 2010]. Generally, the band at 2,894 cm^{-1} corresponded to CH stretching vibrations of all hydrogen carbon constituents in polysaccharides [Poletto *et al.*, 2011; Popescu *et al.*, 2011]. This band was more pronounced in the case of the tested particles compared to cellulose. Next to the already mentioned region (associated with the formation of hydrogen bonds), another two interesting regions of cellulose are 1,430–1,420 cm^{-1} and 900–890 cm^{-1}. The first one is associated with the amount of the crystalline structure of the cellulose and the second one with the amorphous fraction [Jonoobi *et al.*, 2010; Hospodarova *et al.*, 2018]. Additionally, the following parameters have been calculated: total crystalline index (TCI), lateral order index (LOI) and hydrogen bond intensity (HBI), to allow the interpretation of cellulose structure and changes occurring on it, and respective results are presented in Table 6. HBI increased with the adsorption of phenolic acids on cellulose. For caffeic acid/cellulose particles, this increase was from 1.00 to 1.35, and for gallic acid/cellulose particles from 1.00 to 1.25. This rise could be interpreted by the formation of hydrogen bonds [Cichosz & Masek, 2020]. However, these hydrogen bonds could also be a consequence of cellulose-cellulose interactions. Since TCI parameter decreased with the adsorption of phenolic acids, cellulose-cellulose interactions can be excluded and this proves binding of phenolic acids on cellulose through hydrogen bonds. TCI was 1.21 for cellulose, 1.08 for caffeic acid/cellulose particles,

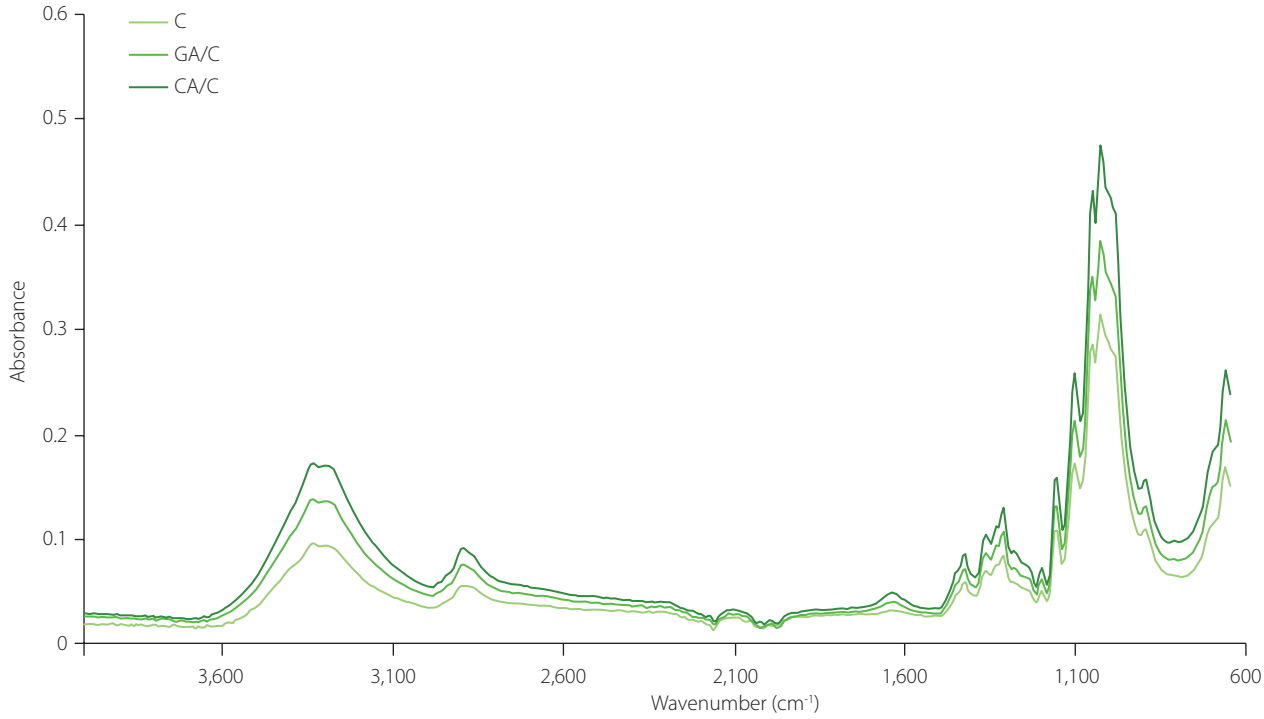

Figure 1. Infrared spectra of cellulose (C), gallic acid/cellulose particle (GA/C) and caffeic acid/cellulose particle (CA/C).

Table 6. Total crystalline index (TCI), lateral order index (LOI) and hydrogen bond intensity (HBI) of cellulose (C), gallic acid/cellulose (GA/C) and caffeic acid/cellulose (CA/C) particles.

Particle	TCI	HBI	LOI
C	1.21	1.00	0.50
GA/C	1.09	1.25	0.58
CA/C	1.08	1.35	0.53

and 1.09 for gallic acid/cellulose particles. The number of OH groups with protons that can be involved in the formation of hydrogen bonds is lower in comparison to the number of oxygen atoms that are able to induced this type of interaction [Cichosz & Masek, 2020; Lindman *et al.*, 2010]. Consequently, there are numerous options for molecules of specific structures to interact with cellulose molecules. Gallic and caffeic acids have phenolic OH groups and one carboxyl group; thus, their structure makes them possible to be involved in hydrogen bonding.

The FTIR analysis also supported the theory that hydrogen binding occurred between phenolic acids and cellulose. Other studies also proved that changes on IR spectra are visible upon the binding of phenolics on cellulose [Vukoja *et al.*, 2021]. The binding of phenolics also revealed changes in the structure on other fibers [Abdelwahab *et al.*, 2013; Buljeta *et al.*, 2021, 2022a,b; Kopjar *et al.*, 2022].

CONCLUSIONS

The aim of this research was to formulate functional cellulose particles enriched with phenolic acids (gallic or caffeic acid). Based on results obtained, it was concluded that cellulose had a higher affinity towards caffeic acid. The amount of cellulose during complexation affected the adsorption of both phenolic acids, and it was concluded that a decrease of the adsorption of phenolic acids occurred with the increase of cellulose amount. Results of the stability testing showed that gallic acid/cellulose particles were more stable during one year of storage at ambient temperature. Considering antioxidant activity of the formulated particles, it was observed that it generally followed the content of phenolic acids on cellulose particles. The formulated cellulose particles can be used for the enrichment of different types of foods (fruit, dairy, and bakery) with cellulose and phenolic acids, additionally improving their oxidative stability. Consequently, these particles could offer a natural, plant-based way of controlling the nutritional value of foods and their stability.

RESEARCH FUNDING

This work was part of the project PZS-2019-02-1595 which has been fully supported by the "Research Cooperability" Program of the Croatian Science Foundation, funded by the European Union from the European Social Fund under the Operational Program for Efficient Human Re-sources 2014–2020. Partially it was supported by IP-2019-04-5749 (financed by Croatian Science Foundation) project. Ina Ćorković acknowledges support from the Croatian Science Foundation program for Training New Doctoral Students (DOK-2020-01-4205).

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 332–344
DOI: 10.31883/pjfns/173526
ISSN 1230-0322, e-ISSN 2083-6007

Optimization of Cross-Linked Cassava Starch Coating Formulation by Response Surface Methodology and Its Preservation Effects on 'Shatangju' Mandarin

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In this study, cassava starch cross-linked using sodium trimetaphosphate was used as basic material to prepare the preservation coating agent. The addition levels of β-cyclodextrin, phytic acid and glycerol were optimized by response surface methodology with the comprehensive index of oil permeability (OP) and the water vapor permeability (WVP) of starch-based coating. The optimal formulation of the cross-linked cassava starch-based coating was as follows: 3 g/100 g cassava cross-linked starch, 0.7 g/100 g β-cyclodextrin, 0.5 g/100 g phytic acid, and 0.5 g/100 g glycerol. The preservation coating solution prepared by the optimized formula was applied to the postharvest storage of 'Shatangju' mandarins. The coating treatment effectively slowed down the decline of firmness during storage for 20 days at room temperature, reduced the decay rate (by 111.73%) and weight loss rate (by 43.40%), and inhibited the postharvest respiration (by 42.06%) of fruit. The soluble solid content, titratable acidity and ascorbic acid content of the coated 'Shatangju' mandarins were higher 1.32 times, 1.55 times, and 1.52 times, respectively, compared with the corresponding indexes of the control fruits. All these results indicate that the coating treatment could effectively reduce the loss of nutrients, delay the wilting and decay, prolong the shelf life after harvest, and improve the commercial value of 'Shatangju' mandarins.

Key words: modified starch, β-cyclodextrin, phytic acid, glycerol, *Citrus reticulata* Blanco, postharvest storage

INTRODUCTION

Citrus reticulata Blanco is one of the four most cultivated fruits over the world and globally consumed with great popularity [Jurić *et al.*, 2023]. 'Shatangju' mandarin (*Citrus reticulata* Blanco cv. Shatangju) is one of the citrus varieties grown extensively in China, especially in southern Chinese provinces of Guangxi, Guangdong and Fujian [Cao *et al.*, 2020]. Recently, the planting area of 'Shatangju' mandarin in China was 400,000 ha [Guo *et al.*, 2022]. 'Shatangju' mandarin fruits are characterized by attractive appearance, easy-peeling, sweet juiciness, fragrant aroma and other favorable characteristics, and thus become one of the most eagerly consumed fruits [Li *et al.*, 2016]. However, fresh 'Shatangju' mandarin fruits are highly susceptible to mechanical damage and pathogen infection due to the thin and crispy peel, juicy flesh, resulting in a large amount of decay and causing serious economic losses [Majerska *et al.*, 2019]. Therefore, how to delay the deterioration of the fruit quality and flavor during transportation and storage, inhibit the occurrence of various postharvest diseases, and prolong the storage time are urgent problems to be solved. The main storage and preservation techniques for 'Shatangju' mandarin in China is the use of chemical preservatives, such as pyrimethanil, prochloraz,

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Submitted: 1 June 2023
Accepted: 9 October 2023
Published on-line: 8 November 2023

and iminoctadine triacetate [Chen *et al.*, 2020]. Long-term use of these chemical preservatives will cause microbial resistance on the surface of fruits, leading to their reduced efficacy. Moreover, excessive use will lead to pesticide residues on the fruit surface, posing a potential threat to consumer health.

The application of edible coatings with the environment-friendly advantages and remarkable preservation effect has been widely researched to extend the shelf life and preserve the quality of fresh fruits [Jurić *et al.*, 2023]. Starch, chitosan, cellulose, pectin, and their derivatives are the commonly used compounds for fruit coating preservation [Castro-Cegrí *et al.*, 2023]. Edible coatings act as a barrier against oxygen, carbon dioxide and water vapor, delaying the biochemical and physiological changes during storage, reducing microbial growth, and thus effectively maintaining the quality of fresh fruits [Luciano *et al.*, 2022]. The properties of edible coating are largely affected by the type of structural substrate material. The β-cyclodextrin has an amphiphilic, conical, and cylindrical structure, with a hydrophilic outer part (formed by the hydroxyl groups) and a predominantly lipophilic cavity. This special structural property can increase the emulsification of coating agent, making the coating material evenly spread on the surface of fruits to form a film, thus enabling fruit preservation [Matencio *et al.*, 2020]. Glycerol, as a plasticizer of edible starch film, can increase the toughness of film, improve the fluidity of the film liquid, soften the rigid structure of the film, thereby adjust the mechanical properties of the film. Moreover, glycerol also can be used as a polishing agent in food, which can improve the luster of the fruit surface after coating, and improve the transparency of the film [Ben *et al.*, 2022]. Phytic acid has multiple functional groups that can be esterified, complexed and chelated, and can be cross-linked with hydroxyl groups of starch to form esters. The application of phytic acid in fruit and vegetable postharvest preservation coating agents can fully utilize its antioxidant and chelating effects to prevent spoilage caused by oxidation, and seal the stomata of fruits and vegetable epidermis, effectively inhibit the respiration as well as inhibit fungal infection and reproduction [Jiang *et al.*, 2023]. Sodium alginate is a potential biopolymer film or coating component with special colloidal properties, which can thicken, stabilize, suspend, form films, produce gels and stabilize emulsions [Tong *et al.*, 2023]. Therefore, β-cyclodextrin, glycerol, phytic acid and sodium alginate usually can be used as auxiliary materials for edible coating, but there are few studies that have applied them simultaneously in the same coating material.

Due to the high content of amylopectin, cassava starch is highly viscous, and the formed film has the characteristics of high strength, good flexibility, and good barrier [Zhu, 2015]. The cross-linked cassava starch obtained by cross-linking treatment has better shear resistance, better processing strength and stronger film-forming performance [Marques *et al.*, 2006; Wongsagonsup *et al.*, 2014]. However, there are few researches of cross-linked cassava starch-based edible coating on fruits and vegetable preservation, and even fewer researches on the application of cross-linked cassava starch coating in the postharvest preservation of 'Shatangju' mandarin, the application technology of which needs in-depth research.

The purpose of this research was, in the first place, to optimize the formulation of a coating agent containing cross-linked cassava starch, β-cyclodextrin, glycerol, and phytic acid by response surface methodology with the comprehensive index of oil permeability (OP) and water vapor permeability (WVP) of coating as responses, and subsequently to investigate the preservation effect of the cross-linked cassava starch-based coating on 'Shatangju' mandarins, which would provide a useful technical basis for postharvest preservation of these fruits.

MATERIALS AND METHODS

Cassava starch cross-linking

Cross-linked cassava starch was prepared according to the method described by Wongsagonsup *et al.* [2014] with some modifications. Native cassava starch purchased from a local starch manufacturer in Hezhou City in China (100 g) was dispersed in 150 mL of a sodium trimetaphosphate solution with different concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%, based on dry weight of starch) so as to obtain a solid content of 40% (*w/w*). The pH of the starch suspensions was adjusted to 9 with 1 M NaOH solution. The starch suspensions were then incubated at 45°C for 3 h with 120 rpm shaking in a thermostatic oscillating water bath and neutralized to pH 7 with 2 M HCl solution. The modified starches were recovered through centrifugation at 3,200×*g* for 15 min, washed five times with distilled water and dried at 45°C for 12 h. After that, the dried cross-linked cassava starch samples were pulverized with a universal pulverizer (FW100, Tianjin City Taisite Instrument Co., Ltd., Tianjin, China) and sieved through an 80-mesh sifter. All cross-linked cassava starch samples were kept in airtight polyethylene bags for further analysis of their properties including film thickness, film transmittance, oil permeability and water vapor permeability, which were determined according the procedures described below in the subsection "Determination of properties of cross-linked cassava starch-based films". Moreover, the sedimentation volume of cross-linked cassava starch samples was determined according to the methods of Chen *et al.* [2017]. The cross-linked cassava starch with the best properties was selected for further experiments.

Preparation of cross-linked starch coating solution

A portion of 30 g of cross-linked cassava starch with a sedimentation volume of 5.05 mL was dispersed in 970 g of distilled water to obtain a 3 g/100 g starch suspension, then the suspension was incubated at 90°C in a thermostatic oscillating water bath with stirring (400 rpm) to completely gelatinize the starch. The single-factor preparation of cross-linked starch coating solution was performed under the following conditions: the addition levels of β-cyclodextrin of 0.4–1.6 g/100 g, the addition levels of phytic acid of 0.3–1.2 g/100 g, the addition levels of glycerol of 0.3–1.2 g/100 g, and the addition levels of sodium alginate of 0.02–1.00 g/100 g; all the addition levels were based on the dry weight of cross-linked cassava starch. The details of single-factor

experiment design are shown in Table 1. After each factor was completed, the optimal level of the factor would be adopted as the test parameter in the following experiment. All materials were stirred (150 rpm) evenly and held at 90°C for 10 min. Subsequently, all materials were stirred by a magnetic agitator (JJ-1, Skyray Instrument Co., Ltd., Changzhou, China) for 20 min with the speed of 500 rpm and then dispersed by a high-speed organization homogenizer (B-500A, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China) for 2 min with the speed of 15,000 rpm to obtain the coating dispersions, which were then cooled to room temperature and kept in airtight Duran laboratory bottles for further experiments.

Experimental design

The response surface methodology (RSM) was applied to optimize the cross-linked cassava-based coating agent formulation with the comprehensive index (CI) of OP and WVP of cross-linked cassava starch-based coating as the indicator. CI was calculated according to Equation (1):

$$CI = 0.5 \times OP + 0.5 \times WVP \quad (1)$$

Both parameters of OP and WVP were determined as described below.

According to the results of single factor test, Box-Behnken design (BBD) was used to determine the optimal addition of β-cyclodextrin (g/100 g, A), phytic acid (g/100 g, B) and glycerol (g/100 g, C) with fixed dosage of 3 g/100 g cross-linked cassava starch. The coded and decoded values of the three independent variables and their levels were shown in Table 2.

Determination of properties of cross-linked cassava starch-based films

The prepared cross-linked cassava starch-based coating dispersions were poured on a horizontally organic glass board and casted evenly. The surface of the organic glass board was required to be clean, dry, and flat. When the surface of the cross-linked cassava starch-based coating film was dried, the organic glass board was placed horizontally in a hot-air oven (DH411C, Yamato, Tokyo, Japan) and left therein at 45°C for 6 h. Afterwards, the film was removed and kept in a desiccator for the future analysis.

Measurement of film thickness

The cross-linked cassava starch-based film was cut into 50×50 mm pieces, and the film thickness was measured at 5 randomly-selected different positions by a spiral micrometer [Yao *et al.*, 2022]. The average value of the measured results was taken as the thickness value of the film.

Table 2. Factors and their coding levels in the experimental design for the formulation of cross-linked cassava starch-based coating by response surface methodology.

Level	Independent variable		
	A: β-Cyclodextrin (g/100 g)	B: Phytic acid (g/100 g)	C: Glycerol (g/100 g)
–1	0.7	0.5	0.5
0	1.0	0.7	0.7
1	1.3	0.9	0.9

Measurement of film transmittance

The cross-linked cassava starch-based film was cut into a strip of 10×50 mm and attached to one side of a cuvette. The film transmittance was measured by a visible spectrophotometer (722N, Shanghai INESA Scientific Instruments Co., Ltd., Shanghai, China) at 500 nm with an empty cuvette as the control [Dai *et al.*, 2020].

Determination of oil permeability

Oil permeability (OP) of cross-linked cassava starch-based film was determined according to the method described by Ma *et al.* [2017] with slight modifications. Briefly, a sheet of uniform and defect-free cross-linked cassava starch-based coating film was cut into a 40×40 mm square and used to seal the mouth of the tube (the diameter was 20 mm) containing 5 mL of soybean oil. The sealed tube was inverted on the weighed medium-speed quantitative filter paper and then placed in a desiccator for 2 days. The filter paper was then weighed and the changes in the weight of the filter paper were recorded. The OP (g×mm/$/m^2$×d) of cross-linked cassava starch-based coating film was calculated according to Equation (2):

$$OP = \frac{\Delta W \times d}{S \times T} \quad (2)$$

where: ΔW is the change in weight of filter paper (g), d is the thickness of the film (mm), S is the effective area of the film (m^2), and T is the number of storage days.

Determination of water vapor permeability

Water vapor permeability (WVP) of cross-linked cassava starch-based film was determined according to the method used by Dai

Table 1. Design of single-factor experiments for the formulation of cross-linked cassava starch-based coating.

Experiment no.	β-Cyclodextrin (g/100 g)	Phytic acid (g/100 g)	Glycerol (g/100 g)	Sodium alginate (g/100 g)
1	0.4, 0.7, 1.0, 1.3, 1.6	1.0	1.0	1.0
2	0.7	0.3, 0.5, 0.7, 0.9, 1.2	0.7	0.7
3	0.7	0.7	0.3, 0.5, 0.7, 0.9, 1.2	0.7
4	0.06	0.06	0. 06	0.02, 0.04, 0.06, 0.08, 0.10

et al. [2020] with slight modifications. Briefly, anhydrous calcium chloride was dried at 200°C in a hot-air oven (DH411C, Yamato, Tokyo, Japan) to constant weight. After cooling, 5 g of the dried anhydrous calcium chloride was weighed to a 50-mL conical flask (the diameter was 30 mm), the mouth of conical flask was sealed with a uniform and defect-free cross-linked cassava starch--based coating film, then the total weight of the sealed conical flask was recorded. Finally, the sealed conical flask was placed in a desiccator with a saturated NaCl solution at the bottom to maintain a relative humidity of 75% at 25°C. Because the saturation pressure of water vapor at 25°C is 3.169 kPa and the difference in relative humidity on the both sides of the film was 75%, therefore the vapor pressure difference between the both sides of film was 2.377 kPa. The sealed conical flask was weighed every 12 h until the weight was constant. The WVP (g×mm/m^2×d×kPa) of the film was calculated using Equations (3) and (4):

$$\Delta P = p \times \Delta RH \quad (3)$$

$$WVP = \frac{\Delta m \times d}{S \times T \times \Delta P} \quad (4)$$

where: ΔP is the vapor pressure difference between the both sides of film (kPa), p is the saturation pressure of water vapor at 25°C (kPa), ΔRH is the difference in relative humidity (%) on both sides of the film, Δm is the change in weight of sealed conical flask (g), d is the thickness of the film (mm), S is the effective area of the film (m^2), and T is the number of storage days.

Coating treatment of 'Shatangju' mandarin

'Shatangju' mandarins were harvested at the day of experiment in their natural maturation age from a local orchard in Hezhou City, China, and transferred to the laboratory within 2 h. 'Shatangju' mandarins with uniform shape, color and size without bruising or disease symptoms were selected and washed with running tap water and dried at room temperature. All 'Shatangju' mandarins were then randomly divided into two groups with 120 fruits each, among which the group without coating treatment was used as the control group. The experimental 'Shatangju' mandarins were soaked in cross-linked cassava starch-based coating dispersions for 15 s, and then taken out and dried with electric fan. Afterwards, the coating treatment was repeated three times, and the coated samples were stored at room temperature for 20 days.

Determination of quality indicators of 'Shatangju' mandarins during storage period

During the storage period, 'Shatangju' mandarins from each group were randomly selected every 2 days for the determination of quality indicators.

Fruit firmness determination

The 'Shatangju' mandarins were subjected to texture profile analysis using a texture analyzer (TA.XT PLUS, Stable Micro Systems, London, UK) with a P/2 probe used to measure fruit firmness. The pre-test speed and the test speed were set at 1.0 mm/s, the post-test speed was set at 2.0 mm/s, while the penetration depth was 10 mm. Measurements regarding firmness were taken on the equatorial zone on each fruit at 90°.

Assessment of decay rate

Fruits with black spots, brown spots, mold or at least one leakage of juice on the fruit peel were identified as decayed. The decay rate was calculated as the ratio of the decayed fruit number to the total fruit number [Yang *et al.*, 2019].

Weight loss rate determination

The weight of 'Shatangju' mandarins was recorded every 2 days during the storage period. The difference between the initial weight and the weight on the inspection date of the fruit was considered as weight loss, and the results were expressed as percentage [Méndez *et al.*, 2022].

Determination of total soluble solid content

Total soluble solid content of 'Shatangju' mandarins was determined with a digital hand refractometer (PR101-a, Atago Co., Ltd., Tokyo, Japan) following the manufacturers' protocols, and the results were expressed as g *per* 100 g of fruit.

Titratable acidity determination

The titratable acidity of 'Shatangju' mandarins was determined using titration method. In brief, 10 g of 'Shatangju' mandarin flesh was grinded in a mortar with 30 mL of distilled water for 5 min, and then the mixture was filtered using qualitative filter paper with 2.5 μm pore size. The filtrate was collected into a volumetric flask and the volume was fixed to 100 mL. Aliquot of 10 mL of the fixed volume filtrate was titrated with 0.1 M NaOH as titrant and phenolphthalein as color indicator. The results were expressed as g of citric acid equivalent *per* 100 g of fruit [Chettri *et al.*, 2023].

Determination of ascorbic acid content

Ascorbic acid content of 'Shatangju' mandarins was determined using 2,6-dichlorophenol-indophenol titrimetric method as previously described [Chettri *et al.*, 2023; Wang *et al.*, 2016a]. Briefly, 10 g of 'Shatangju' mandarin flesh was grinded in a mortar with 30 mL of 2% oxalic acid solution for 5 min, and then homogenized in a high-speed organization homogenizer (B-500A, Shanghai Yiheng Scientific Instrument Co., Ltd.) at 12,000 rpm for 3 min. After centrifugation and filtration, 10 mL of the filtrate were collected and titrated with 2,6-dichlorophenol-indophenol solution until pink color for 15 s, and 10 mL of 2 g/100 g oxalic acid solutions were titrated as blank control titration. The results were calculated based on the standard curve of ascorbic acid and expressed as mg *per* 100 g of fruit.

Determination of respiration intensity

Static method was used to determine the respiration intensity of 'Shatangju' mandarin fruit during the storage period [Saltveit, 2019]. In brief, the culture dish containing 10 mL of 0.4 M NaOH solution was placed at the bottom of the desiccator, next 1,000 g of 'Shatangju' mandarin were placed on the partition

plate, and then the desiccator was sealed immediately and left static at room temperature (25°C) for 1 h. After standing static for 1 h, the solution in culture dish was transferred into a triangular flask (rinsed with distilled water 4–5 times), the 5 mL of a saturated $BaCl_2$ solution and 2 drops of phenolphthalein were added, the mixture was titrated with 0.2 M oxalic acid, the usage amount of oxalic acid was recorded, and blank control was titrated with the same method. Respiration intensity (RI, mg/kg×h) of 'Shatangju' mandarin fruit was calculated according to the following Equation (5):

$$RI = \frac{(V_1 - V_2) \times C \times 22}{W \times T} \quad (5)$$

where: C is the concentration of NaOH solution (0.2 M), V_1 is the usage amount of NaOH solution for titration of blank control (mL), V_2 is the usage amount of NaOH solution for titration of sample (mL), W is sample mass (g), T is measurement time (1 h), and 22 is mass conversion coefficient of NaOH and CO_2 in the determination.

Statistical analysis

Data were recorded as mean and standard deviation ($n \geq 3$). The analysis of variance (ANOVA) conducted by Duncan's test was used to examine the differences between samples ($p<0.05$). All statistical analyses were done using Data Processing System (7.05 for Windows, Hangzhou Ruifeng Info-technology Co., Zhejiang, China) and Microsoft Office Excel 365 (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSIONS

Effect of different cross-linking degree on properties of the cross-linked cassava starch film

It is difficult to measure the cross-linking degree of cross-linked starch, but the cross-linking degree is negatively correlated with sedimentation volume. In other words, the sedimentation volume decreases in the highly cross-linked starches with increasing amounts of the cross-linking reagent; therefore, the sedimentation volume is usually used to indicate the level of cross-linking degree [Kou & Gao, 2018]. Five cross-linked cassava starch samples with different cross-linking degree were prepared according to the method described in the section of Material and Methods. The sedimentation volume of the prepared cross-linked starches was 7.05 mL, 6.50 mL, 5.90 mL, 5.55 mL, and 5.05 mL, respectively. Correspondingly, the five cross-linked cassava starch samples were named as CS7.05, CS6.50, CS5.90, CS5.55, and CS5.05. The thickness of films of these cross-linked cassava starches was between 0.0358 mm and 0.0376 mm, but the OP and WVP decreased with the decrease of sedimentation volume (Table 3). The smaller the sedimentation volume, the better the performance of the cross-linked cassava starch film to prevent water vapor penetration and oil penetration, and the better the film transparency. Moreover, according to the pre-experimental results, the sedimentation volume of the cross-linked cassava starch did not significantly decrease with further increasing the dosage of sodium trimetaphosphate. Therefore, the cross-linked cassava starch (CS5.05) with the sedimentation volume of 5.05 mL was used as the basic raw material of starch-based coating agent in the subsequent experiments.

Effect of β-cyclodextrin on properties the of cross-linked cassava starch-based film

Adding an appropriate amount of β-cyclodextrin to a cross-linked cassava starch-based solution can effectively increase the emulsification effect and make the coating solution to flow more evenly on the surface of the fruits during the coating process to form a more uniform film. The thickness of the cross-linked cassava starch-based film increased with the increasing content of β-cyclodextrin, while the transparency of the film decreased (Table 4). With β-cyclodextrin content increasing from 0.4 g/100 g to 1.6 g/100 g, the OP of the film decreased from 0.173 to 0.128 g×mm/m^2×d, and then increased from 0.128 to 0.257 g×mm/m^2×d, while the WVP of the film decreased from 2.183 to 2.096 g×mm/m^2×d×kPa, and then increased from 2.096 to 2.965 g×mm/m^2×d×kPa. At the 1.0 g/100 g addition of β-cyclodextrin, the film had the lowest OP and WVP, which was 0.128 g×mm/m^2×d and 2.096 g×mm/m^2×d×kPa, respectively, which is correlated with the molecule structural properties of β-cyclodextrin as β-cyclodextrin itself has an average of 16 crystalline water but still can maintain the crystalline complexation [Liu, 2023].

Effect of glycerol on properties of the cross-linked cassava starch-based film

The thickness of the cross-linked cassava starch-based film fluctuated between 0.0422 and 0.0432 mm with the increasing

Table 3. Thickness, transparency, water vapor permeability (WVP) and oil permeability (OP) of films of cross-linked cassava starch with different sedimentation volume.

Cross-linked cassava starch	Thickness (mm)	Transparency (%)	WVP (g×mm/m^2×d×kPa)	OP (g×mm/m^2×d)
CS7.05	0.0358±0.0004^{c}	85.47±0.15^{e}	2.309±0.002^{a}	0.230±0.003^{a}
CS6.50	0.0366±0.0005^{b}	86.33±0.12^{d}	2.267±0.001^{b}	0.210±0.000^{b}
CS5.90	0.0368±0.0004^{b}	87.37±0.25^{c}	2.194±0.003^{c}	0.205±0.006^{b}
CS5.55	0.0372±0.0004^{ab}	88.07±0.23^{b}	2.123±0.003^{d}	0.189±0.000^{c}
CS5.05	0.0376±0.0005^{a}	88.70±0.17^{a}	2.066±0.001^{e}	0.156±0.004^{d}

All values are the mean ± standard deviation. The means within the same column with different letters are significantly different ($p<0.05$). The number after CS in the sample names indicates sedimentation volume (mL).

Table 4. Thickness, transparency, water vapor permeability (WVP) and oil permeability (OP) of cross-linked cassava starch-based films with different β-cyclodextrin content.

β-Cyclodextrin content (g/100 g)	Thickness (mm)	Transparency (%)	WVP (g×mm/m²×d×kPa)	OP (g×mm/m²×d)
0.40	0.0408±0.0008^{e}	87.37±0.06^{a}	2.183±0.001^{c}	0.173±0.004^{c}
0.70	0.0448±0.0004^{d}	85.33±0.06^{b}	2.146±0.000^{d}	0.162±0.004^{d}
1.00	0.0464±0.0005^{c}	84.07±0.15^{c}	2.096±0.003^{e}	0.128±0.004^{e}
1.30	0.0488±0.0004^{b}	80.87±0.06^{d}	2.610±0.001^{b}	0.205±0.005^{b}
1.60	0.0528±0.0019^{a}	79.80±0.00^{e}	2.965±0.001^{a}	0.257±0.000^{a}

All values are the mean ± standard deviation. The means within the same column with different letters are significantly different (p<0.05).

Table 5. Thickness, transparency, water vapor permeability (WVP) and oil permeability (OP) of cross-linked cassava starch-based films with different glycerol content.

Glycerol content (g/100 g)	Thickness (mm)	Transparency (%)	WVP (g×mm/m²×d×kPa)	OP (g×mm/m²×d)
0.30	0.0432±0.0008^{a}	85.27±0.06^{a}	2.319±0.000^{b}	0.210±0.004^{b}
0.50	0.0430±0.0007^{a}	84.80±0.30^{b}	2.302±0.000^{c}	0.178±0.004^{c}
0.70	0.0424±0.0009^{a}	84.40±0.30^{c}	2.024±0.001^{e}	0.164±0.004^{d}
0.90	0.0422±0.0008^{a}	84.20±0.10^{cd}	2.271±0.000^{d}	0.183±0.004^{c}
1.20	0.0430±0.0007^{a}	83.90±0.20^{d}	2.418±0.001^{a}	0.252±0.004^{a}

All values are the mean ± standard deviation. The means within the same column with different letters are significantly different (p<0.05).

Table 6. Thickness, transparency, water vapor permeability (WVP) and oil permeability (OP) of cross-linked cassava starch-based films with different phytic acid content.

Phytic acid content (g/100 g)	Thickness (mm)	Transparency (%)	WVP (g×mm/m²×d×kPa)	OP (g×mm/m²×d)
0.3	0.0432±0.0004^{a}	85.03±0.21^{a}	2.203±0.000^{b}	0.174±0.004^{b}
0.5	0.0430±0.0007^{a}	84.77±0.15^{a}	2.115±0.000^{c}	0.148±0.004^{c}
0.7	0.0430±0.0007^{a}	85.07±0.29^{a}	2.079±0.001^{e}	0.139±0.004^{e}
0.9	0.0424±0.0005^{a}	83.83±0.21^{b}	2.111±0.000^{d}	0.144±0.004^{d}
1.2	0.0426±0.0005^{a}	83.47±0.21^{b}	2.283±0.000^{a}	0.165±0.004^{a}

All values are the mean ± standard deviation. The means within the same column with different letters are significantly different (p<0.05).

addition of glycerol, while the transparency of the film decreased (Table 5). With the increasing addition of glycerol from 0.3 g/100 g to 1.2 g/100 g, the OP of the film decreased from 0.210 to 0.164 g×mm/m²×d, and then increased from 0.164 to 0.252 g×mm/m²×d, while the WVP of the film decreased from 2.319 to 2.024 g×mm/m²×d×kPa, and then increased from 2.024 to 2.418 g×mm/m²×d×kPa. When the addition of glycerol was 0.7 g/100 g, the film had lowest OP and WVP, which was 0.164 g×mm/m²×d and 2.024 g×mm/m²×d×kPa, respectively. These results are consistent with the findings reported for sweet potato starch films plasticized with glycerol [Ballesteros-Mártinez *et al.*, 2020] and cassava starch-based nanocomposite films [Ma *et al.*, 2017]. The cross-linked cassava starch-based coating solution can transform into polymer winding phase with the added glycerol, and a strong interaction occurred between glycerol and cross-linked cassava starch, resulting in dense structure, thus reducing the OP and WVP of the film [Mali *et al.*, 2006]. However, because glycerol contains three hydrophilic groups, adding excessive glycerol would increase the number of hydrophilic groups of the film, and the WVP would also increase. Excessive glycerol would destroy the starch structure, resulting in the increase of OP and WVP of the film [Ballesteros-Mártinez *et al.*, 2020]. Therefore, in our study, in order to obtain smaller OP and WVP of the film, the suitable addition of glycerol was 0.7 g/100 g.

Effect of phytic acid on properties of the cross-linked cassava starch-based film

Phytic acid can chelate with polyvalent metal ions in food coenzyme, inhibit or delay the enzymatic reaction, thus ensuring the purpose of fruit preservation [Feizollahi *et al.*, 2021]. The thickness of the cross-linked cassava starch-based film fluctuated between 0.0424 and 0.0432 mm with the increasing addition of phytic acid, while the transparency of the film

decreased (Table 6). With phytic acid content increasing from 0.3 g/100 g to 1.2 g/100 g, the OP of the film decreased from 0.174 to 0.139 g×mm/m^2×d, and then increased from 0.139 to 0.165 g×mm/m^2×d, while the WVP of the film decreased from 2.203 to 2.079 g×mm/m^2×d×kPa, and then increased from 2.079 to 2.283 g×mm/m^2×d×kPa. When the addition of phytic acid was 0.7 g/100 g, the film had lowest OP and WVP, which was 0.139 g×mm/m^2×d and 2.079 g×mm/m^2×d×kPa, respectively. Therefore, in order to obtain lower OP and WVP of the film, the suitable addition of phytic acid was 0.7 g/100 g.

Effect of sodium alginate on properties of the cross-linked cassava starch-based film

The thickness of the cross-linked cassava starch-based film increased from 0.0426 to 0.0446 mm with the increasing addition of sodium alginate, while the transparency of the film generally decreased from 85.67 to 81.40% (Table 7). The OP of the film fluctuated between 0.151 and 0.154 g×mm/m^2×d, while WVP slightly decreased first and then increased with the increasing addition of sodium alginate. When the addition of sodium alginate was 0.06 g/100 g, the film had lowest WVP (2.069 g×mm/m^2×d×kPa) and there were no significant differences ($p \geq 0.05$) between the samples in terms of OP values. Therefore, in order to obtain smaller WVP of the film, the suitable addition of sodium alginate was 0.06 g/100 g. Since sodium alginate has the ability to enhance the viscosity of solution and causes the gel property in the medium [Aggarwal *et al.*, 2020; Khoshdouni Farahani *et al.*, 2023], its small amount added would make the viscosity of the cross-linked cassava starch-based coating liquid sharply increased, resulting in difficulty of coating liquid to cast evenly and form well. Therefore, sodium alginate was not selected as an auxiliary agent of the cross-linked cassava starch-based coating in the subsequent research.

Optimization of the cross-linked cassava starch coating formulation by response surface methodology

According to the analysis of the single-factor experiment, the three-factor and three-level test was proceeded by RSM. Design of the experiment and the results of the dependent variable for the formulation of the cross-linked cassava starch coating are shown in Table 8. The comprehensive index of OP and the WVP of the cross-linked cassava starch-based coating was influenced by the addition of β-cyclodextrin, phytic acid and glycerol. A second-order regression equation was established to fit with the experimental data as the following Equation (6):

$$Y=1.710 + 0.054A - 0.037B + 0.120C - 0.099AB - 0.039AC - 0.110BC - 0.400A^2 - 0.230B^2 - 0.240C^2 \quad (6)$$

where: Y is comprehensive index of OP and WVP; and A, B, C are the addition levels of β-cyclodextrin, phytic acid and glycerol, respectively.

The data were analyzed by ANOVA to assess the significance of those factors and their interactions in this model

Table 8. Design of the experiment and the results of the dependent variable for the formulation of cross-linked cassava starch-based coating.

Test no.	Factor			Comprehensive index
	A: β-Cyclodextrin	B: Phytic acid	C: Glycerol	
1	–1	–1	0	0.945
2	1	–1	0	1.322
3	–1	1	0	1.033
4	1	1	0	1.015
5	–1	0	–1	0.864
6	1	0	–1	0.977
7	–1	0	1	1.243
8	1	0	1	1.199
9	0	–1	–1	1.054
10	0	1	–1	1.243
11	0	–1	1	1.472
12	0	1	1	1.203
13	0	0	0	1.726
14	0	0	0	1.657
15	0	0	0	1.692
16	0	0	0	1.698
17	0	0	0	1.752

Table 7. Thickness, transparency, water vapor permeability (WVP) and oil permeability (OP) of cross-linked cassava starch-based films with different sodium alginate content.

Sodium alginate content (g/100 g)	Thickness (mm)	Transparency (%)	WVP (g×mm/m^2×d×kPa)	OP (g×mm/m^2×d)
0.02	0.0426±0.0005^b	84.87±0.35^b	2.091±0.012^b	0.151±0.004^a
0.04	0.0428±0.0008^b	85.67±0.06^a	2.086±0.000^b	0.152±0.004^a
0.06	0.0432±0.0008^b	84.57±0.50^b	2.069±0.010^c	0.151±0.000^a
0.08	0.0434±0.0005^b	82.47±0.23^c	2.104±0.001^a	0.152±0.000^a
0.10	0.0446±0.0005^a	81.40±0.26^d	2.107±0.002^a	0.154±0.004^a

All values are the mean ± standard deviation. The means within the same column with different letters are significantly different ($p<0.05$).

(Table 9). The credible predictive value (R^2=0.9839) showed a high correlation between the predicted value and the experimental value. The adjusted coefficient of the determination was R^2_{Adj}=0.9632. The difference between R^2 and R^2_{Adj} was <0.2, suggesting reasonable agreement between them. The absence of fit term (p=0.0773>0.05) was not significant and the coefficient of variation (C.V.=4.54%) was <5%, indicating that the data had fine fit and reproducibility. In addition, the coefficients of AB, BC, A^2, B^2 and C^2 were significant, with a low p-value (p<0.05), but the coefficient of AC was insignificant with a high p-value (p>0.05).

The interaction of the three independent variables of the addition levels of β-cyclodextrin, phytic acid and glycerol was used to plot the response surface curves and the contour line for the comprehensive index of OP and WVP, as shown in Figure 1. The steepness of the response surface curves reflects the effects between the variables on the response value, and the shape of the corresponding contour plot indicates whether the interactions between the independent parameters are significant [Cai *et al.*, 2019; Wang *et al.*, 2016b]. When the response surface curves have greater inclination, the slope is steeper, it means the variables have greater effects on the response value. An oval contour plot indicates a significant interaction between the two variables, while a circular contour plot indicates an insignificant interaction between the two variables. The response surface plot for β-cyclodextrin and phytic acid as variables was steep, and the contour plot was oval, indicating that the comprehensive index of OP and WVP was sensitive to the changes of the two variables and that the interaction was significant (Figure 1A and B). These plots were similar to those for glycerol and phytic acid as variables (Figure 1E and F). In turn, the response surface plot of β-cyclodextrin and glycerol was relatively steep (Figure 1C), indicating that the comprehensive index of OP and WVP was sensitive to the changes of the two variables. The contour plot for these variables was circular (Figure 1D), suggesting that the interaction was insignificant. Based on the above analyses, the addition of β-cyclodextrin, phytic acid and glycerol was found to be highly important for the OP and WVP of the cross-linked cassava starch-based coating.

The analysis of the response surface method results provided the following optimal conditions using the model equation: the addition of β-cyclodextrin, phytic acid and glycerol was 0.7%, 0.5% and 0.5%, respectively. Under optimal conditions, the minimum predicted comprehensive index of OP and WVP of the cross-linked cassava starch-based coating was 0.455. To verify the predicted result, a verification experiment was carried out under the optimal conditions with a fixed dosage of 3 g/100 g of the cross-linked cassava starch. As a result, actual OP of 0.0883±0.0001 g×mm/m^2×d and actual WVP of 0.8196±0.0004 g×mm/m^2×d×kPa were obtained, meaning that the actual comprehensive index of OP and WVP was 0.454±0.0002. The error between the actual comprehensive index of OP and WVP (0.454±0.0002) and predicted value (0.455) was 0.22%, indicating that the formulation of the cross-linked cassava starch-based coating agent optimized by RSM had good repeatability and that the model designed in this study was

Table 9. Variance analysis of response surface test.

Source of variation	Sum of squares	Degree of freedom	Mean square	F-value	*p*-value
Model	1.49	9	0.17	47.57	<0.0001**
A	0.023	1	0.023	6.59	0.0371*
B	0.011	1	0.011	3.2	0.1166
C	0.12	1	0.12	34.39	0.0006**
AB	0.039	1	0.039	11.17	0.0124*
AC	0.00613	1	0.00613	1.76	0.2263
BC	0.052	1	0.052	15.04	0.0061**
A^2	0.67	1	0.67	192.78	<0.0001**
B^2	0.22	1	0.22	62.33	<0.0001**
C^2	0.23	1	0.23	66.8	<0.0001**
Residual	0.024	7	0.00348		
Lack of fit	0.019	3	0.00641	4.99	0.0773
Pure error	0.00514	4	0.00129		
Correlation total	1.52	16			
R^2	0.9839				
R^2_{Adj}	0.9632				

*significant difference (p<0.05); **extremely significant difference (p<0.01); R^2, regression coefficient; R^2_{Adj}, adjusted regression coefficient; A, β-cyclodextrin; B, phytic acid; C, glycerol.

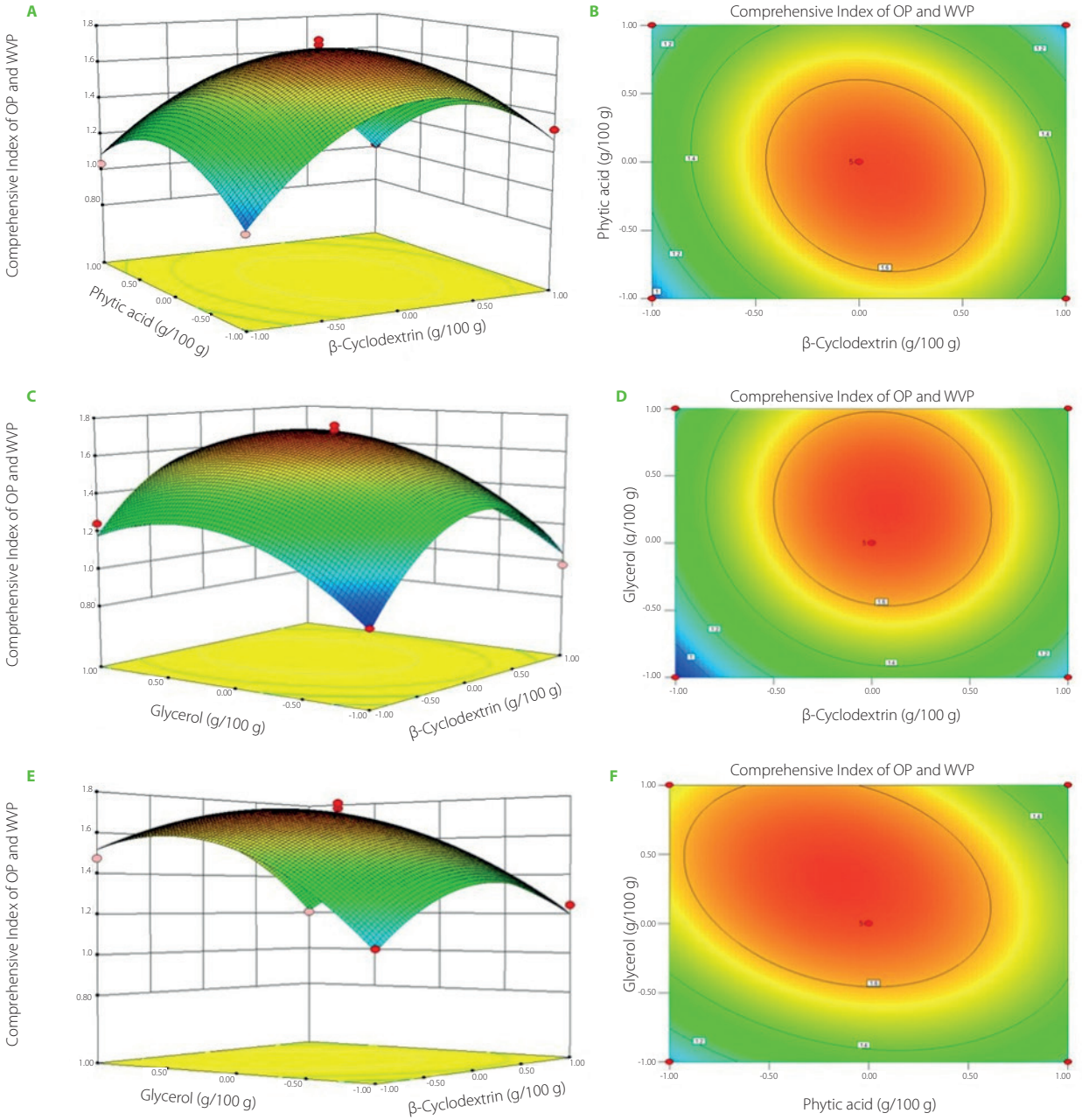

Figure 1. Response surface plots (**A**, **C**, **E**) and contour plots (**B**, **D**, **E**) of the interactions between β-cyclodextrin, phytic acid and glycerol used in the cross-linked cassava starch-based formulation. OP, oil permeability; WVP, water vapor permeability.

suitable for predicting the comprehensive index of OP and WVP of the cross-linked cassava starch-based coating.

■ Effect of the cross-linked cassava starch-based coating on the preservation of 'Shatangju' mandarins

The optimized formulation of the cross-linked cassava starch--based coating had been applied on the fresh 'Shatangju' mandarins to investigate the effects on fruit postharvest preservation, with uncoated 'Shatangju' mandarins as the control. The main function of coating was to reduce the gas exchange of fruit and slow down the postharvest physiological processes [Cao *et al.*, 2022]. Firmness is one of the important indicators of the quality of fresh fruit during postharvest storage. As shown in Figure 2A, the firmness of 'Shatangju' mandarins in both the experimental and control groups decreased with the extension of storage time, which was consistent with the previous articles reporting that edible coating was effective in maintaining fruit firmness of 'Dottato' fig (*Ficus carica L.*) and Huangguan pears [Allegra *et al.*, 2017; Dai *et al.*, 2020]. The 'Shatangju' mandarins in the control group without coating had significantly lower firmness than that of the experimental group with coating since the 2nd day of storage. On the 10th day of storage, the firmness

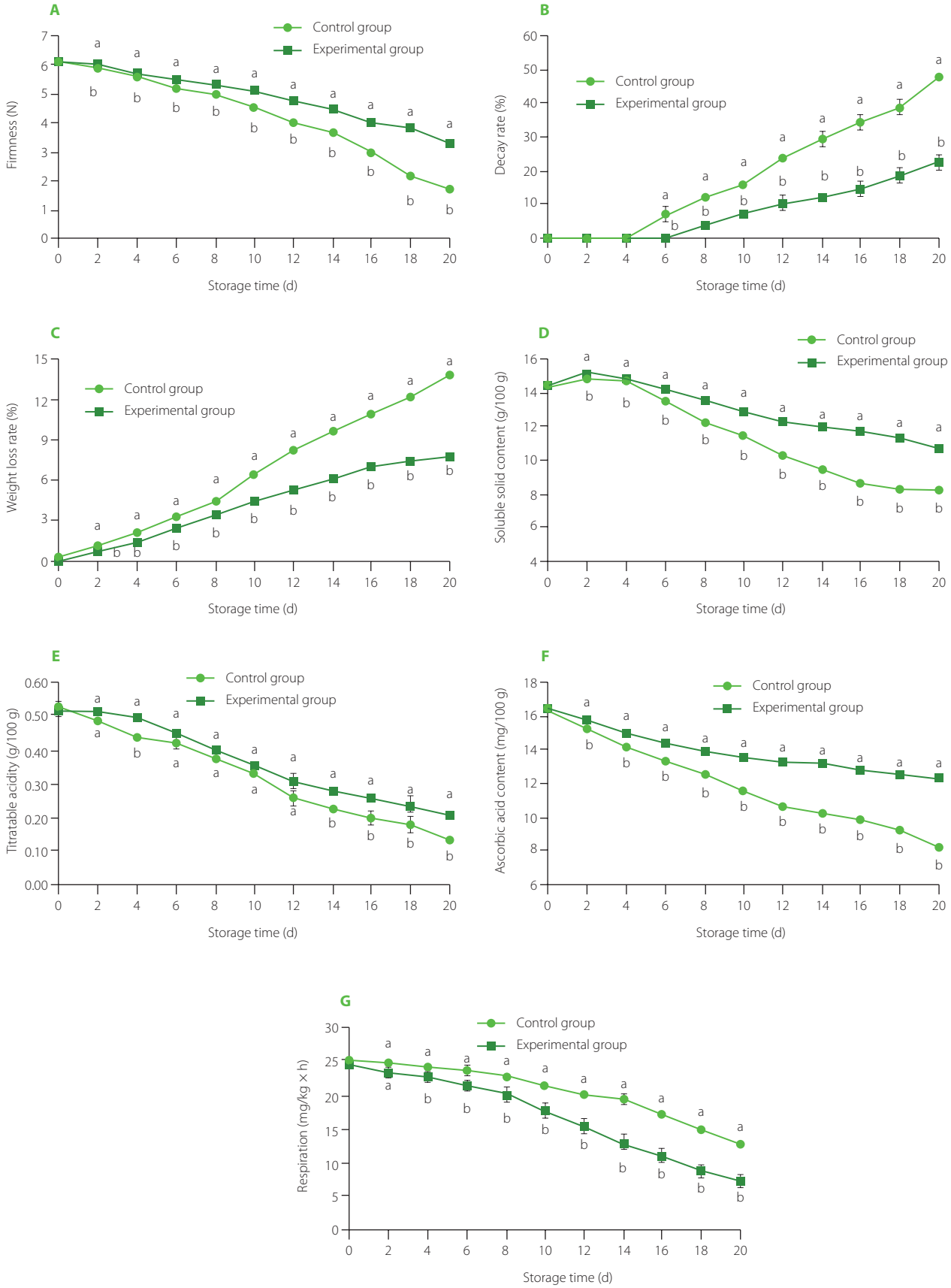

Figure 2. Firmness (**A**), decay rate (**B**), weight loss rate (**C**), soluble solid content (**D**), titratable acidity (**E**), ascorbic acid content (**F**) and respiration intensity (**G**) of 'Shatangju' mandarins uncoated (control group) and coated with the cross-linked cassava starch-based coating agent (experimental group) during storage. All values are presented as the mean and standard deviation. Different letters (a and b) within the same storage time indicate significant differences between experimental and control group ($p<0.05$).

of 'Shatangju' mandarins in the control group was 4.55 N, while the firmness of fruits with the coating was 5.07 N (1.11 times that of the control group). On the 20th day of storage, the firmness of 'Shatangju' mandarins without coating was 1.75 N, while the firmness of coated 'Shatangju' mandarins was 1.93 times higher (3.37 N). These results show that the cross-linked cassava starch-based coating can effectively maintain the firmness of postharvest 'Shatangju' mandarins.

Microorganisms exist widely in nature, and a certain amount of putrefactive microorganisms exist on the surface of fruits after being harvested. In arid and sub-tropical climates, significant postharvest decay in *Citrus reticulata* was reported to be caused primarily by *Penicillium digitatum*, and secondarily by *P. italicum* and *Geotrichum citri-aurantii (syn. G. candidum)* [Hong *et al.*, 2014]. Therefore, the decay rate directly reflects the appearance quality of 'Shatangju' mandarins during the postharvest storage. As shown in Figure 2B, there were no rotten fruits in both the coated experimental group and the uncoated control group on the 4th day of storage, the decay rate of was 0%. With the extension of storage time to the 6th day, 'Shatangju' mandarins in the control group had rotten fruits with a decay rate of 6.67%, while the coated 'Shatangju' mandarins had rotten fruits on the 8th day of storage with a decay rate of 4.00%. The decay rate of 'Shatangju' mandarins of both groups showed an increasing trend with the extension of storage time, but the decay rate of the coated 'Shatangju' mandarins was always significantly (p<0.05) lower than that of the uncoated fruits. In the middle and late storage period (10th–20th day), the decay rate of the 'Shatangju' mandarins in the control group increased rapidly, and was 48.00% on the 20th day of storage, while that in the coated experimental 'Shatangju' mandarins was only 22.67%, which was lower than that in the uncoated fruits on the 12th day of storage (the decay rate was 24.00%). All these results indicate that the cross-linked cassava starch-based coating treatment can effectively reduce the decay rate of 'Shatangju' mandarins and prolong its shelf life at room temperature.

During storage, transpiration and respiration of fruits can cause the loss of water and carbon atoms, leading to a rapid loss of internal moisture, resulting in wilting and shriveling of the fruit surface and continuous reduction of fruit weight, which not only affect the taste, but also reduce the commercial value and shorten the shelf life of the fruit [Mukama *et al.*, 2019]. The weight loss rate of 'Shatangju' mandarins increased during the storage period, and the weight loss rate of coated 'Shatangju' mandarins was significantly (p<0.05) lower than that of the uncoated fruit, especially in the middle and late storage period (10th–20th day) (Figure 2C). The weight loss rate of 'Shatangju' mandarins in the coated group was 7.81%, which was 43.40% lower than that in the uncoated control group (13.8%) at the same time. These weight loss rate results were consistent with literature data showing that the fruit weight loss rate increased during storage and the coating treatment was beneficial in the maintenance of fruit weight [Dai *et al.*, 2020]. Coating treatment can form a uniform and transparent protective film on the surface of fruits. This film may act as a physical barrier on the surface of fruits, and can prevent the water loss of fruits, which was consistent with the results indicating that the film can effectively reduce the WVP.

The changes in the content of soluble solids can affect the taste and the quality of fruit to some extent. Soluble solids are regarded as a substrate of postharvest respiration of fruits, while lower respiration intensity is reasonable to maintain a relatively high soluble solids content. The soluble solid content of 'Shatangju' mandarins in both the coated and uncoated groups increased at the initial stage of storage, and then subsequently decreased with the extension of storage time (Figure 2D), which was consistent with previous results reported for Shatang mandarin fruits with chitosan/nanocrystal cellulose coatings [Cao *et al.*, 2022]. During the storage, the soluble solid content of 'Shatangju' mandarins in the group with the coating was higher than that of the control group without the coating, especially in the middle and late storage, which was consistent with the results indicating that the coated 'Shatangju' mandarins had relatively lower respiration intensity. The results might be due to the fact that the coating treatment can form a film that can effectively inhibit respiration and reduce the decrease in soluble solid content of 'Shatangju' mandarin caused by respiration. On the 20th day of storage, the soluble solid content in the uncoated control group was 8.23%, while in the coated fruits it was 10.32%, which was 1.32 times of that in the control group.

The titratable acidity affects the fruit taste, which reflects the sensory quality and the commercial value of fruit. 'Shatangju' mandarins used organic acids as the substrate for respiration, which led to the decrease of their titratable acidity with the prolongation of the storage time (Figure 2E). The titratable acidity of the coated fruits was significantly (p<0.05) higher than that of the control group after the 14th day of storage. The surface of 'Shatangju' mandarins in the experimental group was coated with transparent protective film which isolated it from air, inhibited the respiration and effectively slowed down the consumption of organic acids, thus ensuring the preservation effects.

Vitamin C is extremely unstable, easily soluble in water and easily to be oxidized. It is a very important nutritional component of 'Shatangju' mandarins [Chen *et al.*, 2020]. The changes of ascorbic acid content in 'Shatangju' mandarins can reflect the nutritional status during the postharvest storage. The content of ascorbic acid of 'Shatangju' mandarins in both the coated and uncoated fruits continuously decreased with storage time prolongation, with the decline in the ascorbic acid content during postharvest storage being consistent with results of a previous study on strawberries stored at 20°C [Shin *et al.*, 2007]. However, the content of ascorbic acid of coated 'Shatangju' mandarins was significantly (p<0.05) higher than that of the control group (Figure 2F). The ascorbic acid content in the uncoated 'Shatangju' mandarins on the 20th day of storage was 8.09 mg/100 g, which was only 50% of the ascorbic acid content of fresh fruits (stored for 0 day). In turn, the ascorbic acid content in the coated 'Shatangju' mandarins was 12.27 mg/100 g on the 20th day of storage, which was 75% of its content in fresh 'Shatangju' mandarin, and it was

1.52 times of the ascorbic acid content in the control group. These results suggest that the cross-linked cassava starch-based coating could slow down the degradation of ascorbic acid, thus may improve the antioxidant capacity of 'Shatangju' mandarins.

The intensity of postharvest respiration of fruits and vegetables is an important characteristic of the rate of metabolism during storage and can be used as an indicator of storage potential, which is directly related to the quality changes, physiological diseases and shelf life of fruits and vegetables [Wantat *et al.*, 2022]. The greater the postharvest respiration intensity of fruits and vegetables, the shorter their shelf life [Khalid *et al.*, 2017]. Figure 2G shows that fresh 'Shatangju' mandarins (stored 0 day) had high respiration intensity, and the respiration intensity of both coated and uncoated 'Shatangju' mandarins showed a decreasing trend with the prolonged storage, indicating that 'Shatangju' mandarin was not a kind of climacteric fruit. The respiration intensity of fruits of the coated experimental group was always lower than that of the control group over the whole storage period, especially at the late stage of storage. The respiration intensity of coated 'Shatangju' mandarins was 7.29 mg/kg×h, which was 42.06% lower than that of the control fruits (12.58 mg/kg×h). This result was consistent with findings from other research showing the ability of an edible coating to lower respiration intensity in fruits [Nasrin *et al.*, 2017]. These results indicated that the cross-linked cassava starch-based coating could effectively reduce the respiration intensity and prolong the postharvest life of 'Shatangju' mandarins.

CONCLUSION

In this study, response surface methodology was successfully applied to optimize the formulation of the cross-linked cassava starch-based coating agent. The optimum conditions for the formulation with a fixed dosage of 3 g/100 g of cross-linked cassava starch were as follows: the addition of β-cyclodextrin was 0.7 g/100 g, the addition of phytic acid was 0.5 g/100 g and the addition of glycerol was 0.5 g/100 g. Optimization and model validation data showed that the experimental results were reliable and the model established within the range of experimental conditions was accurate and effective.

In addition, the results achieved from preservation tests of 'Shatangju' mandarin with the cross-linked cassava starch--based coating agent indicated that the cross-linked cassava starch-based coating solution could form a transparent film on the surface of 'Shatangju' mandarins after the coating treatment. The transparent film effectively improved the changes in firmness, decay rate, weight loss rate, soluble solid content, titratable acidity and ascorbic acid content during the storage period. The coating treatment effectively delayed the postharvest respiration of 'Shatangju' mandarins. All the postharvest experiment results indicate that the cross-linked cassava starch-based coating agent applied on the surface of 'Shatangju' mandarins effectively reduced the loss of nutrients, delayed wilting, shriveling and decay of fruit, prolonged the postharvest preservation period, and thus improved their commercial value.

ACKNOWLEDGEMENTS

The authors would like to thank Guangxi Key Laboratory of Health Care Food Science and Technology for providing laboratory facilities and technical assistance during the study.

RESEARCH FUNDING

This study was financially supported by the Guangxi Key Technologies R&D Program (No. 2022AB20149) and the National Natural Science Foundation of China Regional Fund Project (No. 32160573).

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 345–353
DOI: 10.31883/pjfns/174454
ISSN 1230-0322, e-ISSN 2083-6007

Effect of the Ultraviolet C Light-Emitting Diode Treatment on the Quality of Soil-Grown and Pot-Grown Red Raspberries

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Red raspberries (*Rubus idaeus* L.) are often chosen by consumers for their flavour, taste, and health-promoting properties. A relevant issue related to these fruits is their high perishability. The aim of this study was to investigate the effect of the ultraviolet C light-emitting diode (UVC-LED) treatment on soil-grown and pot-grown raspberries in extending their shelf-life, maintaining their high quality throughout the preservation period, and improving features related to their phenolic compound content. The UVC-LED treatment increased the total phenolic content (158 to 200 mg GAE/100 g) and ferric reducing antioxidant power (55.3 to 78.6 mmol Fe^{2+}/kg) of the pot-grown raspberries when the UVC-LED treatment was followed by storage in a climate-controlled room (20°C). Total anthocyanin content did not increase significantly compared to the control samples. Contrary to expectations, no effect of reducing the microbial count after storage was observed due to the exposure of raspberries to the UVC-LED radiation. The positive outcomes derived from the analyses of bioactive compounds may be implemented in further studies on the same matrix to better manage the treatment and its conditions, such as the exposure time, the distance from the LED lamps, and the UVC-rays' dose, in order to find the best combination in terms of efficacy and efficiency.

Key words: *Rubus idaeus* L., ultraviolet radiation, bioactive compounds, shelf-life extension

INTRODUCTION

Red raspberry (*Rubus idaeus* L.) is a perennial crop of the *Rosaceae* family. The fruit is an aggregate of drupelets with a hollow centre. It is commonly cultivated in at least 30 countries in the world [Kalušević *et al*., 2016; Wang *et al*., 2009], including Italy. Specifically, it is cultivated in the soil or in pots in Piedmont, in the North-West of Italy. Raspberry cultivation in pots is gaining more relevance. First of all, this technique allows for better space and resource management: water is supplied directly to each plant, avoiding waste. In addition, the introduction of additives, minerals, or vitamins is more efficient in pots because all the nutritive substrate – that is to say potting soil or other plant growing support – is in direct contact with the plant roots. Changes in the raspberry production system have led to the use of pots, nutritive substrates, polytunnels and other protective structures, allowing a better administration of the microclimatic conditions and of the overall production system, also limiting the possible spread of diseases, pests, and possible contaminations from the environment. Moreover, the soil or other substrate used for the growth of the raspberry plant can affect berry characteristics, such as the quality and quantity of bioactive compounds or the fruit yield [Balawejder *et al*., 2023]. In addition, the market demands fresh produce all year long, a requirement which is not possible to meet with traditional open-field cultivation. Advantages from pot-grown raspberry cultivation also include the possibility of using the same area for many years, improving

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Submitted: 18 April 2023
Accepted: 24 October 2023
Published on-line: 14 November 2023

yields, fruit quality and size, and favouring a better organisation of working and picking activities [Balawejder *et al*., 2023; Evdokimenko *et al*., 2021; Linnemannstöns, 2020].

Berries are highly appreciated by consumers, who choose to consume them because of the noticeable content of secondary metabolites; they are actually sources of bioactive compounds such as phenolic compounds with antioxidant activity, mainly anthocyanins, which are a subgroup of flavonoids also responsible for the colour of this small berry fruit [Frías-Moreno *et al.*, 2021; Gimeno *et al*., 2022; Kalušević *et al*., 2016; Toshima *et al*., 2021; Villamor *et al*., 2013]. For this reason, they have gained economic importance and their cultivation is more widespread every year [Nile & Park, 2014]. Most berries are deemed potentially beneficial for human health, even if many mechanisms and metabolic actions are not precisely known yet. The emphasis should be placed on attributing significant health-promoting properties to berry fruits, thereby elevating their status above the already acknowledged positive attributes of fruits and vegetables. Numerous studies already show anticancer, antimutagenic, anti-inflammatory, and antioxidant activity, with cardiovascular-protecting characteristics related to the berry consumption [Cardona *et al*., 2013; Paredes-López *et al*., 2010; Rasmussen *et al*., 2005]. Beattie *et al*. [2005] highlight that phytochemicals, as non-nutritive dietary components with broad bioactivity, often elicit health-promoting benefits in the human body. While their absence does not necessarily lead to deficiency conditions, they can potentially exert positive effects on cells as promoters of health, substances that combat diseases, and substances that prevent diseases.

The content of phytochemicals in raspberries depends on (1) intrinsic factors, such as the cultivar, (2) harvesting factors, such as the cultivation area and the environmental conditions, (3) storage temperature and postharvest management, and (4) treatments and processing [de Souza *et al*., 2014; Nile & Park, 2014]. The fragility and perishability of raspberries makes their preservation an issue. Due to their high water content and sugar composition, they are easily subjected to moulds, yeasts, and bacteria development [Kalušević *et al*., 2016; Wang *et al*., 2009]. In the coming years, the industry needs to confront the challenge of discovering novel approaches to maintain the nutritional and microbial integrity of these fruits. Additionally, efforts could be made to enhance the levels of polyphenols and other antioxidants, thus augmenting the value of the end product. An innovative method to accomplish this objective involves using ultraviolet (UV) rays, which belong to the section of the electromagnetic spectrum encompassing wavelengths between 100 and 400 nm. This can be further divided into three sections where UVA, UVB and UVC rays are identifiable. Specifically, the exposure to UV light in wavelength ranges of–280–315 nm (UVB) and 100–280 nm (UVC) has been studied as alternatives to heating treatments, such as pasteurization, and as elicitor treatments to increase the content of bioactive compounds in fruit and vegetables [Darré *et al*., 2022; Koutchma, 2014]. These ultraviolet rays, mainly UVC, are actually used industrially for the disinfection of water, air and surfaces, because their wavelengths alter the DNA of the microorganisms [Darré *et al*., 2022; Kebbi *et al*., 2020; Koutchma, 2014]. A low microbial contamination is an indication of good quality and safety for all products. Small berry fruits were responsible for outbreaks of foodborne diseases in both Europe and North America, and raspberries were specifically linked to 11 outbreaks between 1983 and 2013 [Xu & Wu, 2016]. The main pathogens involved were human noroviruses, including *Salmonella*, which has also been recognised as an issue by European Food Safety Authority (EFSA), and *Escherichia coli* O157:H7 [Butot *et al*., 2018]. The high water and sugar content in berries provide an ideal environment for the growth and proliferation of moulds, for example, *Botrytis cinerea*, an agent of grey mould and responsible for raspberry decay [Butot *et al*., 2018; Gimeno *et al*., 2022; Xu & Wu, 2016].

The most common source of UV rays up to now is constituted by mercury lamps, but some problems can occur with the disposal. Mercury is a dangerous and toxic element, and specific treatment for mercury-containing waste is necessary. Conversely, the light-emitting diode (LED) technology has many advantages and can also be considered for post-harvest treatment. LED lamps offer several advantages, including their affordability in terms of purchase, management, and maintenance. They are also safe for operators and environmentally friendly due to the absence of mercury and their long lifespan, which contributes to reduced environmental impact. LEDs can be adjusted to various wavelengths, do not pose significant heating issues, and provide immediate effectiveness upon being switched on [Chawla *et al*., 2021; Koutchma, 2014].

The application of UV radiation on red raspberries has not yet been investigated in depth; however, it has been discussed in a piece of research by Gimeno *et al*. [2022]. They showcased their study on utilizing UV treatment in conjunction with modified-atmosphere packaging (MAP) to regulate decay and improve quality attributes during the postharvest storage of raspberries. Results showed that the higher CO_2 level is actually helpful in the storage of the fresh product because of reducing its metabolic activity. More vivid colour was achieved, indicating that the anthocyanins were better preserved; the overall decay of the berries was delayed; the loss of firmness was reduced thanks to the coupled effects of MAP and UVC; an increase in the level of flavonoids was also achieved. The best results to improve raspberry storage came from combining the modified atmosphere and light treatment [Gimeno *et al*., 2022].

The objective of this research was to develop a postharvest treatment method for red raspberries that would enhance their preservation and preserve their quality characteristics. This was achieved by employing UVC-LED radiation in both soil and pot cultivations. Specifically, the objective was to understand the effect of UVC light treatment on the quality of raspberries exposed to the rays and later stored at 4°C and 20°C for 72 h, highlighting potential dissimilarities between pot-grown and soil-grown fruits.

MATERIALS AND METHODS

Plant material

Red raspberries of the "Grandeur" cultivar were chosen for the experiments. This cultivar is characterised by berries with

an average weight of 3.5–3.6 g, conical shape and intense red colour [Ackerman & Adams, 2009]. These berries were collected from a local producer from Cuneo, Piedmont (Italy), where the cultivation was managed in soil (S) and in pots (P). All plants of raspberry were grown under hail net. Chemical plant protection was not applied. Irrigation was applied using sprinklers as needed and a fertigation system was applied to guarantee water supply with a commercial fertigation recipe for raspberry. Plots were established by digging up soil and transferring it to 7-L pots. The floricanes were manually pruned out each year and fruits were manually harvested. For both soil-grown and pot-grown raspberries, a treated (TR) and a control (C) sample were considered and two storage temperatures (4°C and 20°C) tested. Raspberries were divided into polyethylene (PE) baskets, 6 for each experimental variant (S C 4, S TR 4, P C 4, P TR 4, S C 20, S TR 20, P C 20, P TR 20) and arranged as a single layer. Each basket contained 12 fruits.

Postharvest ultraviolet light treatment

The ultraviolet light treatment of raspberries was performed with a prototype instrument made for DISAFA by PRO.LUX S.r.l. (Druento, Turin, Italy), a company by MOVE2WEB S.r.l. (Turin, Italy). This instrument consists of a stainless-steel box with 9 tracks inside on which a stainless-steel tray can be placed. The LED plate is positioned on the internal top vault of the prototype; it consists of 20 LED lines (10 emitting UVB rays and 10 emitting UVC, which are switched on separately). Each line is completed by a blue-light LED which turns on when the diodes are functional. Our research activity required only the use of UVC-LED lamps. The treatment was carried out by positioning the baskets with the berries at level 2, 157 mm far from the LED plate. The samples were exposed to UV in a wavelength range between 270 nm and 285 nm and to a power of 14.17 W/m^2 for 14 min, providing a total radiation dose of 11.90 kJ/m^2. The UV-LED treatment was performed on 6 baskets.

Storage conditions

After the radiation treatment (TR), the samples were divided into 2 groups, the first stored in a climate-controlled room at 20°C and the second stored at 4°C for 72 h in a refrigerated room. Every sample was analysed at the starting time of the experiment, which is to say when received in the laboratory, prior to the UVC exposure (start), and after the preservation period. The samples treated with radiation were compared to non-treated samples, referred to as control (C). Control samples from soil and pot cultivation and for each storage condition were considered.

Weight loss determination

Each basket containing raspberries was weighed at start time ($weight_{start}$) and after the storage period ($weight_{final}$) with a precision analytical scale (Kern & Sohn GmbH, Balingen, Germany), and weight loss was evaluated in percentage (WL%) according to the following formula (1):

$$WL\ \% = \frac{weight_{start} - weight_{final}}{weight_{start}} \times 100 \quad (1)$$

Determination of colour parameters

The colour parameters of the red raspberries were evaluated on the surface of the fruit with a CR-400 colorimeter (Konica Minolta, Chiyoda, Japan) in CIELab tristimulus coordinate system: L^* index of brightness ranging from 0 to 100, a^* index showing colour turning from green to red and ranging from −120 to +120, and b^* index showing colour turning from blue to yellow and ranging, again, from −120 to +120. An additional parameter considered was hue angle (h°), suggesting the shade of the colour. It was calculated according to the formula (2):

$$h° = \tan^{-1}(b^*/a^*) \quad (2)$$

Data was processed to obtain the differences (Δ) between the measurement at the start time and the data achieved after the 72-h storage. In this way, ΔL^*, Δa^*, Δb^* and Δh° were obtained.

Texture profile analysis

Texture profile analysis (TPA) of raspberries was performed according to the method described by Giongo *et al*. [2019] with the Texture Analyzer instrument (Stable Micro System, Godalming, United Kingdom). The flat stainless-steel probe had a diameter of 75 mm, test speed was set at 300 mm/min, the application of the charge was 20% over the deformation, and the nominal trigger force was set to 2 g. Of all 7 parameters (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, resilience) obtained with the analysis of all 12 raspberries in the basket, only hardness, chewiness, and resilience were considered.

Total phenolic content, ferric reducing antioxidant power and total anthocyanin content determination

Fruit extracts were prepared for the subsequent quantification of phenolic compounds and ferric reducing antioxidant power (FRAP) of raspberries. A portion of 5.0 g of previously cut raspberries, which were randomly chosen among the 6 baskets prepared for each experimental variant, were added to 12.5 mL of extraction solvent (500 mL of methanol + 24 mL of deionised H_2O + 1.4 mL of 12 M HCl). The preparation was set in the dark for 2 h, then homogenised with Ultra Turrax (Janke and Kunkel, IKA®-Labortechnik, Staufen, Germany) until it became smooth. Samples were then centrifuged at 2,500×*g* for 15 min. The supernatant was collected and used for the quantification of bioactive compounds. Following the method described by Pantelidis *et al.* [2007], total phenolic content (TPC) determination involved the use of Folin-Ciocâlteu's reagent. To the mixture, 40 µL of the extract prepared beforehand (substituted with extraction solvent in the control) was combined with 160 µL of extraction solvent. Additionally, 1 mL of Folin-Ciocâlteu's reagent water solution (in a ratio of 1:9, *v/v*) and 800 µL of Na_2CO_3 solution (prepared by dissolving 1.875 g of Na_2CO_3 in 25 mL of deionised H_2O) were added. The samples were left in the dark for 30 min, and then absorbance at 760 nm was measured with a spectrophotometer. Results were expressed as mg of gallic acid equivalent *per* 100 g of fresh raspberry (mg GAE/100 g).

FRAP of raspberries was determined by Benzie & Strain [1996] method, subsequently modified by Pellegrini *et al*. [2003]. A mixture of sodium acetate buffer pH 3.6 (3.1 g of $C_2H_3NaO_2$ dissolved in 16 mL of CH_3COOH and adjusted to 1 L with deionised H_2O), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) solution (0.0156 g of TPTZ dissolved in 5 mL of 40 mM HCl) and $FeCl_2$ solution (0.135 g dissolved in 25 mL of deionised H_2O) was prepared, following a 10:1:1 (*v/v/v*) ratio. Each sample was then prepared combining 900 µL of the previously prepared FRAP reagent, 90 µL of deionised H_2O, 10 µL of raspberry extract (replaced by extraction solvent in the blank) and 20 µL of extraction solvent. The tubes were left in a water bath at 37°C for 30 min to allow the reaction, then absorbance was read at 595 nm. Results were expressed as mmol of ferrous ion *per* kg of raspberries (mmol Fe^{2+}/kg).

The total anthocyanin content (TAC) determination was based on the method described by Cheng & Breen [1991] with modifications. For each sample, duplicate mixtures were made to measure absorbances at pH of 1 and 4.5. In the first mixture, 50 µL of the extract (replaced with extraction solvent in the control) was combined with 950 µL of pH 1 solution (prepared by dissolving 4.026 g of KCl in 12.45 mL of 12 M HCl and diluted to 1 L with deionised H_2O). In the second mixture, 50 µL of the extract (replaced with extraction solvent in the control) was mixed with 950 µL of pH 4.5 solution (prepared by dissolving 32.82 g of sodium acetate in 18 mL of 12 M HCl and diluted to 1 L with deionised H_2O). Samples were left in the dark to react for 20 min, then absorbance was read at 515 nm and 700 nm. Results were expressed as mg of cyanidin 3-glucoside (C3G) *per* 100 g of fruit fresh weight (mg C3G/100 g), according to the following formula from de Souza *et al*. [2014] with modifications relative to our study:

$$TAC = \frac{A \times MW \times DF \times V \times 10^3}{\varepsilon \times l \times M} \quad (3)$$

where: A is the absorbance calculated as $A = (A_{515} - A_{700})_{pH1} - (A_{515} - A_{700})_{pH\,4.5}$, MW is the molecular weight of C3G (449.384 g/mol), DF is the dilution factor (19), V is the extraction volume, ε is the molar extinction coefficient of C3G (30,400 L/(mol×cm)), l is the optical path length (1 cm), and M is the mass of raspberries used for the extraction.

Microbiological analyses

Microbiological analyses were performed only for the raspberries stored at 20°C, comparing controls and UVC-LED treated fruits. A portion of 20 g of randomly selected raspberries were put into a sterile bag with 180 mL of buffered peptone water (Scharlab, Barcelona, Spain) and individually homogenised with a stomacher (Seward, Worthing, United Kingdom) for 30 s, followed by the preparation of serial dilutions with buffered peptone water, up to 10^{-4}. Dilutions between 10^{-2} and 10^{-4} were plated (spread plate method) in triplicates on 2 different media, plate count agar (PCA, Scharlab) and yeast glucose chloramphenicol agar (YGC, VWR Chemicals, Milan, Italy), to quantify the total aerobic microbial count and yeasts and moulds, respectively. Plates were incubated at 30°C for 48 h in the incubator (Binder GmbH, Tuttlingen, Germany). Results were expressed as $\log_{10}$ colony forming units (CFU) *per* g of raspberries.

Statistical analysis

The data from the quality parameters were further processed with a statistical analysis. The two factors considered were the storage temperatures (4°C and 20°C) and the coupled growing system (soil, S; and pot, P) with the treatment (C, control not treated; TR, UVC-irradiated sample). Analysis of variance (ANOVA) was performed, and statistically significant differences were identified by comparison of average values through Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Weight loss of raspberries during storage

The WL% were lower for raspberries stored at 4°C than those left at 20°C (Figure 1). Water vapour losses are enhanced by a higher temperature, while refrigeration reduces transpiration [Gimeno *et al*., 2022; Nunes & Emond, 2007]. The UVC-LED treatment had a significant effect on the weight losses of berries cultivated in soil and then stored at 20°C (Figure 1). The P samples stored under refrigeration showed a similar trend, even if the statistical analysis shows a lack of significant ($p > 0.05$) difference among data. This suggests a possible intolerable stress for the fruits due to the irradiation, independently from the storage temperature, which results in a higher respiration rate, developing greater weight losses [Nguyen *et al*., 2014]. Moreover, we performed 14-min treatments: this time interval is long from an industrial point of view and also rather stressful for the fruits. The role of the weight losses is particularly important when speaking about fresh fruit, because it is one of the main parameters showing the quality of the product and the economic relevance it may have. Significant weight losses indicate unsuitable storage conditions for berries, leading to wilting and loss of turgidity, which ultimately compromises their visual appeal. Consequently, this results in a less desirable product for consumers.

Colour of raspberries

All ΔL^*, Δa^*, Δb^* and $\Delta h°$ values of the colour analysis of control and UVC-LED treated raspberries after both soil and pot cultivations were above 0 (data not shown), meaning lightness, a^*, b^* and h° decreased during the storage period of 72 h;

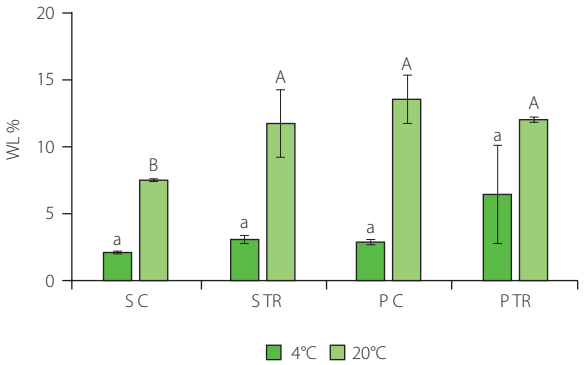

Figure 1. Percentage weight losses (WL%) of raspberries cultivated in soil (S) and in pots (P) not submitted to UVC-LED treatment (C, control) and irradiated (TR, treated), then stored at 4°C and 20°C for 72 h. Mean values with error bars showing standard deviation are represented. Statistically significant differences are marked with different letters ($p \leq 0.05$). Uppercase letters refer to the samples stored at 20°C; lowercase letters refer to the samples stored at 4°C.

however, there were no significant (p>0.05) differences between the samples. This confirms findings earlier described by Gimeno *et al*. [2022], that is to say the scarce influence of the UV radiation treatment on colour. The visual aspect is, of course, influenced by the colour: a brilliant and vivid tone of the fruit makes the product more attractive for the consumer. Challenges may arise, however, from the deterioration of colour pigments known as anthocyanins, which are sensitive to various storage factors, including temperature [Ochoa *et al*., 1999]. This sensitivity can result in alterations in colour, predominantly causing a decrease in redness. Commonly, darkening is a typical effect occurring during storage, as a consequence of changes in firmness and weight losses. Tissues undergo modifications and lose water through respiration and transpiration; thus, changes can be observed also in colour [Nunes & Emond, 2007]. Nunes & Emond [2007] found a linear correlation between weight loss and colour; specifically, high weight losses were related to softer and more coloured berries. Furthermore, the stress conditions, such as the UVC irradiation, during the postharvest period can disrupt the physiological degradation processes of berries, causing their accelerated deterioration, even in relation to colour [Nunes & Emond, 2007; Ochoa *et al*., 1999; Xu & Wu, 2016]. In this case, the treatment was not recognised as visibly altering the colour features of the raspberries.

Texture of raspberries

The indentation in the surface of raspberries and the fact that it is an aggregate of many druplets pose challenges in achieving a uniformly smooth surface for accurate measurements of these fruit texture parameters. Indeed, raspberries show a non-homogeneous structure, with a conical hollow shape, so opposite vectorial forces act on the fruit walls when it is pressed, influencing the measurements. Postharvest textural changes of fruits are related to transpiration. Their extent is different according to the type of fruit and its shape. This discrepancy can commonly be attributed to variations in the composition of the fruit's outer skin (epicarp) and the surface exposed to the external environment [Rodriguez *et al*., 2019]. Hardness, chewiness and resilience were the parameters considered for the analysis in our study, because they are more relevant for raspberries [Nunes & Emond, 2007].

Data showed similar hardness of raspberries at start time and after the storage in the climate-controlled room at 20°C, anyway, a statistically significant (p≤0.05) decrease was observed for fruits cultivated in soil and UVC-LED treated (Table 1). Paying attention to the berries stored at 4°C for 72 h, clear reduction (p≤0.05) of hardness was observed compared to the fruits at start time. This can be explained by the fact that senescence results in the softening of tissues. Water losses and, consequently, weight losses are responsible of changes in the fruits' texture, resulting in a possible wilting of the product. This results in a higher concentration of substances and nutrients in the fruit, changing its structure, causing a possible influence on the consistency of berries [Rodriguez *et al*., 2019]. For this reason, the results should not be considered as a positive textural improvement. Among the UVC-LED treated raspberries, the soil-cultivated and stored at 20°C samples had the lowest hardness of 1,834 N, which was significantly lower compared to the stored control (2,121 N). Looking at the effect of the UVC-LED treatment on pot-cultivated berries, higher values of hardness were noted for the treated fruits, that was 1,684 N at 4°C and 2,465 N at 20°C compared to 1,585 N and 2,169 N for the controls, respectively; however, the differences were not relevant from a statistical point of view (p>0.05), meaning that it is not possible to associate this effect with the UVC treatment. In the case of soil-cultivated raspberries, no significant (p>0.05) difference was identified, with the exception of a S TR 20 sample, compared to the control ones (S C 20). These mild changes may be due to a possible acceleration of the metabolism of the berries, as also reported by Gimeno *et al*. [2022] who linked this behaviour to the UV radiation dose and the fruits themselves. A different outcome may be

Table 1. Texture profile analysis (TPA) results for hardness, chewiness and resilience of raspberries cultivated in soil (S) and in pots (P) at the initial stage (start) and stored at 4°C and 20°C for 72 h after UVC-LED treatment (TR) and without irradiation (C, control).

Treatment	Hardness (N)	Chewiness	Resilience
S start	2,323±283^{aAB}	43.3±4.9^{aAB}	0.12±0.01^{aA}
P start	2,519±400^{aA}	47.8± 5.8^{aA}	0.13±0.01^{aA}
S C 4	1,273±373^{b}	26.8±5.6^{c}	0.13±0.01^{a}
S TR 4	1,285±357^{b}	31.5±4.2^{bc}	0.15±0.03^{a}
P C 4	1,585±331^{b}	34.1±7.7^{bc}	0.14±0.03^{a}
P TR 4	1,684±540^{b}	35.5±8.7^{b}	0.14±0.02^{a}
S C 20	2,121±530^{AB}	45.2±6.4^{A}	0.13±0.01^{A}
S TR 20	1,834±825^{B}	34.0±16^{B}	0.14±0.04^{A}
P C 20	2,169±624^{AB}	40.0±12^{A}	0.14±0.01^{A}
P TR 20	2,465±521^{AB}	52.2±8.6^{A}	0.14±0.01^{A}

Data are reported as mean ± standard deviation. Statistically significant differences are marked with different letters (p≤0.05). Lowercase letters refer to samples stored at 4°C; uppercase letters refer to samples stored at 20°C.

expected as a result of stress, because stressful conditions involve the activation of phenylalanine ammonia lyase (PAL), an enzyme implicated in the synthesis of phenolic compounds and lignin-like polymers, thus resulting in a firmer product. In any case, the great variability and susceptibility of the process with respect to external conditions and intrinsic factors could be the reason why different outcomes were found, showing the necessity of optimisation in relation to the treated matrix [Chawla *et al*., 2021]. The differences among raspberries and the numerous variables affecting UVC treatment do not always lead to comparable results. What we could suggest is a preventive selection of the fruits according to their maturation stage and dimensions. In fact, the effectiveness of the process relies on many factors both in terms of the treatment and the berries: the species, the cultivar, the maturation stage, the shape and dimensions of the fruit, but also the radiation characteristics, wavelength, distance between the product and the lamps, and the presence of systems moving the fruits [Adhikari *et al*., 2015; Koutchma, 2014; Singh *et al*., 2021; Syamaladevi *et al*., 2015].

Chewiness quantifies the product's resistance to being crushed in the mouth and is linked to elasticity and gumminess. Results of raspberry chewiness measurement are shown in Table 1. Fruits stored at 4°C had lower chewiness compared to the berries at the initial stage. Moreover, at 4°C storage, the UVC-LED treatment was responsible for the better preservation of fruit chewiness, showing a slight but significant ($p \leq 0.05$) increment compared to the control samples: 31.5 for the soil-cultivated raspberries (26.8 for the control) and 35.5 for those grown in pots (34.1 for the control). A different situation was definable for the raspberries stored at 20°C, where a less-organised pattern was observed when looking at the results. Insignificant ($p > 0.05$) differences were found between the start values and these determined for the stored samples. In this instance, the irradiated raspberries grown in soil exhibited significantly lower chewiness (34.0) compared to the control fruit (45.2). However, for the pot-cultivated raspberries, chewiness of the irradiated and control berries was statistically comparable ($p > 0.05$). Thus, a correlation can be seen between hardness and chewiness (Table 1). During the UVC-LED treatment, its duration and intensity, together with the characteristics of the fruits themselves, such as the ripening stage, could have acted as enhancers of the senescence process [Gimeno *et al*., 2022].

The last parameter considered in the TPA analysis was resilience, that is to say the capability of the product to gain its shape back after compression. The UVC-LED treatment did not affect this characteristic (Table 1). Results were also not significantly ($p > 0.05$) different both at the beginning and after storage. This highlights the absence of significant effects associated with the application of UVC light.

Total phenolic content, ferric reducing antioxidant power and total anthocyanin content of raspberries

Regarding the total phenolic content at the start time, the soil-grown raspberries showed a significantly ($p \leq 0.05$) higher TPC compared to those cultivated in pots (S start – 197 mg GAE/100 g, P start –131 mg GAE/100 g) (Table 2). This could be attributed to differences in the the composition of the growing substrate: the fact that the pot is a circumscribed space with respect to the field could subsequently result in a different composition in terms of plant bioactive compounds, because water and nutrients are directly in contact with the roots. During refrigerated storage at 4°C, a minor decline in TPC was observed in the soil-cultivated berries compared to the initial measurement (S start). At 4°C storage, the UVC-LED-treated soil-cultivated fruits showed a slightly higher total phenolic content than the control group, but the difference was not statistically significant ($p > 0.05$). In contrast, the plants cultivated in pots exhibited an increase in the TPC during storage, although the final outcome was higher

Table 2. Total phenolic content (TPC), ferric reducing antioxidant power (FRAP) and total anthocyanin content (TAC) of raspberries cultivated in soil (S) and in pots (P) at the initial stage (start) and sored at 4°C and 20°C after UVC-LED treatment (TR) and without irradiation (C, control).

Treatment	TPC (mg GAE/100 g)	FRAP (mmol Fe^{2+}/kg)	TAC (mg C3G/100 g)
S start	197 ± 17^{aA}	79.0 ± 6.5^{aA}	24.0 ± 2.0^{abBC}
P start	131 ± 10^{bC}	56.1 ± 1.1^{bB}	18.4 ± 3.6^{bC}
S C 4	167 ± 8.2^{ab}	67.1 ± 6.3^{ab}	28.9 ± 3.5^{a}
S TR 4	176 ± 17^{a}	63.0 ± 7.4^{ab}	23.7 ± 2.9^{ab}
P C 4	177 ± 10^{a}	69.9 ± 9.7^{ab}	30.0 ± 3.8^{a}
P TR 4	170 ± 25^{ab}	76.2 ± 9.4^{a}	25.5 ± 3.7^{ab}
S C 20	187 ± 10^{AB}	64.8 ± 8.3^{AB}	39.0 ± 1.7^{A}
S TR 20	204 ± 13^{A}	70.2 ± 7.0^{AB}	41.9 ± 6.6^{A}
P C 20	158 ± 10^{BC}	55.3 ± 2.9^{B}	32.3 ± 2.6^{AB}
P TR 20	200 ± 1.3^{A}	78.6 ± 4.4^{A}	34.6 ± 5.1^{AB}

Data are reported as mean ± standard deviation. Statistically significant differences are marked with different letters ($p \leq 0.05$). Data from soil-grown and pot-grown raspberries was not separated for the statistical analysis. Lowercase letters refer to samples stored at 4°C; uppercase letters refer to samples stored at 20°C. GAE, gallic acid equivalent; C3G, cyanidin 3-glucoside equivalent.

for the control group compared to the irradiated sample. This result may not be considered significant due to the storage temperature, which might hinder the activation and complete development of metabolic pathways involved in phenolic synthesis. Conversely, when stored at 20°C, total phenolic content of raspberries from both soil and pot cultivation exhibited increasing trend after undergoing UVC-LED treatment. Considering the raspberries grown in soil, the total phenolic content increased from 187 mg GAE/100 g for the control to 204 mg GAE/100 g for the radiated berries, although this change was not statistically significant ($p>0.05$). The fruits grown in pots showed a significant ($p\leq0.05$) increase in the TPC due to the UVC-LED treatment from 158 mg GAE/100 g determined for the control to 200 mg GAE/100 g for the treated berries. These results may be linked to the effect of temperature on the activation of the secondary metabolism of the fruits.

Regarding the FRAP in our research, the raspberries cultivated in soil did not show any noteworthy changes due to irradiation (Table 2). In fact, at both storage temperatures tested, a similar ($p>0.05$) FRAP was noted for both control and treated samples. In this instance, we cannot observe a discernible effect associated with the exposure of the berries to UVC-LED treatment. Furthermore, the FRAP of berries at the start time was insignificantly ($p>0.05$) higher for the soil-cultivated samples. However, concerning the fruits grown in pots and then UVC-LED-treated, there was a statistically significant ($p\leq0.05$) increase in FRAP of the treated samples compared to the control group at 20°C. This increase correlated with the rise in total phenolic content, indicating that phenolics were responsible for FRAP of raspberries. The refrigerated storage (4°C) of the treated sample led to a final value of 76.2 mmol Fe^{2+}/kg compared to the 69.9 mmol Fe^{2+}/kg reached by the control. The higher storage temperature, 20°C, enhanced the metabolisms, already promoted by the lighting treatment, and led to FRAP of 78.6 mmol Fe^{2+}/kg, while the control showed 55.3 mmol Fe^{2+}/kg. Increased FRAP may be due to the enhancement of the secondary metabolic pathways involved in antioxidant production because of the UVC-LED treatment, which acts as a stressful event on the fruits [Li *et al*., 2019]. The initial measurement of FRAP revealed higher values for raspberries cultivated in the soil than in pots, which was consistent with the findings observed in the TPC quantification.

In line with the TPC and FRAP, the TAC was observed to be higher in S start compared to P start (Table 2). This difference was statistically significant ($p\leq0.05$). The TAC differences at 4°C and 20°C for both soil-grown and pot-grown raspberries reflected what was already discussed for TPC and FRAP. Storage at refrigeration temperature (4°C) was not suitable for the development and improvement of the total anthocyanin content with respect to time start. Moreover, the TAC of the control samples was insignificantly ($p>0.05$) higher than the UVC irradiated berries (S C 4 – 28.9 mg C3G/100 g and S TR 4 – 23.7 mg C3G/100 g; P C 4 – 30.0 mg C3G/100 g and P TR 4 – 25.5 mg C3G/100 g). No differences can be distinguished between the two growing methods. In the case of fruit storage at 20°C, the UVC-LED treatment resulted in a not statistically significant ($p>0.05$) increase of the TAC compared to control soil-grown raspberries. TAC differences between the berries in starting point and after the storage at 20°C were: S C 20 – 39.0 mg C3G/100 g and S TR 20 – 41.9 mg C3G/100 g out of the starting value of 24.0 mg C3G/100 g; P C 20 – 32.3 mg C3G/100 g and P TR 20 – 34.6 mg C3G/100 g out of the starting value of 18.4 mg C3G/100 g. UV light treatment in postharvest has been recognised as a bioactive compound-enhancing technique, able to increase the level of the main phytochemicals in the berries [Gimeno *et al*., 2022].

The results presented and discussed in this paragraph can be only partially compared to other data from literature because the UVC-LED treatment has not been performed widely on raspberries yet. Gimeno *et al*. [2022] treated raspberries with two different UVC doses (2 kJ/m^2 and 4 kJ/m^2) and then stored them at 6°C for 4, 8 and 12 days. Considering the TPC, their results are comparable to ours, since they observed a modest growth after 4 days of storage. Longer storage period, on the contrary, affects negatively the total phenolic content according to the aforementioned authors [Gimeno *et al*., 2022]. The FRAP assay highlighted a reduction in the antioxidant activity of raspberries according to Gimeno *et al*. [2022], which is partially in agreement with the results obtained in our study, where only pot-cultivated samples showed increased FRAP after the UVC radiation treatment compared to the control fruits. UVC radiation treatment has also been applied on other matrices, for example strawberries [Li *et al*., 2019] and blueberries [Perkins-Veazie *et al*., 2008]. Fresh-cut strawberries showed a significant increase in bioactive compound content when treated with a UVC dose of 4 kJ/m^2 and subsequently stored at 4°C for 7 days. Total phenolic content, total anthocyanin content and antioxidant activity, monitored through the DPPH assay, were positively affected by the light treatment [Li *et al*., 2019]. Cultivar-specific behaviour was demonstrated in a study by Perkins-Veazie *et al*. [2008], where Bluecrop blueberries and Collins blueberries showed a different response to the same UVC treatments of 1, 2 and 4 kJ/m^2 followed by a storage at 5°C for 7 days and at 20°C for 2 days. Only Bluecrop blueberries had higher TPC and TAC, conversely Collins blueberries were negatively affected by UVC rays, thus their TPC and TAC decreased. After the UVC treatment, TAC not only grew for fresh-cut strawberries [Li *et al*., 2019] and for Bluecrop blueberries [Perkins-Veazie *et al*., 2008], but also for raspberries, especially after 4 and 8 days of storage [Gimeno *et al*., 2022]. This behaviour acknowledged in the literature data and shared by different fruit matrices is opposite to what we measured in our experiment, that is to say the absence of differences after the 3-day storage at 20°C and a lower total anthocyanin content after the storage at 4°C with respect to control groups. From these data we could suppose a possible impact of the radiation dose, which is particularly high in our study (11.90 kJ/m^2) with respect to the doses of 1, 2 and 4 kJ/m^2 used in the other researches [Gimeno *et al*., 2022; Li *et al*., 2019; Perkins-Veazie *et al*., 2008].

Microbial count of raspberries

We decided to perform microbial analyses only for the raspberry groups stored at 20°C because the majority of the microorganisms

representing the fruit microbiota would not grow consistently at 4°C. A positive decontamination effect due to the UVC light exposure of raspberries was not found. No statistically different (p>0.05) results were recorded for C and TR samples (Figure 2). Results from Butot *et al*. [2018] study demonstrated that the UVC treatment of berries was not able to reduce the load of the inoculated pathogens by more than 1 $\log_{10}$ CFU/g. Moreover, additional time of exposure did not cause a further reduction in the contamination, probably because of the structure and shape of the berries, with cavities protecting the microorganisms. Another result consistent with our findings was reported by Gimeno *et al*. [2022], who demonstrated the necessity of coupling the UVC treatment and modified atmosphere packaging to delay the fungal growth and to extend the raspberry shelf-life.

When considering the results from microbiological analysis, it becomes evident that the raspberries cultivated in pots exhibited a higher (p≤0.05) contamination (Figure 2). In the case of the soil-grown berries, both the control and treated fruits exhibited a total aerobic microbial count of 3.98 and 3.77 $\log_{10}$ CFU/g, respectively. These values were approximately 3 $\log_{10}$ lower than the contamination levels observed in the pot-grown berries, which recorded 6.64 $\log_{10}$ CFU/g for the control group and 6.95 $\log_{10}$ CFU/g for the UVC-LED treated group. Similar results were collected for the yeast counts, where the control and irradiated samples from soil cultivation showed a contamination of 3.73 and 3.70 $\log_{10}$ CFU/g, respectively, while the yeast count determined for raspberries grown in pots was 6.50 and 6.73 $\log_{10}$ CFU/g, respectively. The pot-grown raspberries showed a higher microbial load than the soil-grown ones also in this case.

The disparity in microbial counts between the two types (S and P) of raspberries is likely attributable to differences in the pre-harvest management practices used in the cultivations, leading to varying degrees of contamination. A possible problem could be the watering management and the quality of the water used; all the other procedures, manipulation, and transfers followed the same protocols and were performed in the same way for both soil- and pot-cultivated berries. Another possible contamination problem could derive from the potting soil employed. In addition to this, the pot management system could lead to water stagnation, creating the best conditions for the multiplication of bacteria and fungi. In any case, did the results show microbial counts were higher than 3.5 $\log_{10}$ CFU/g, which indicates some critical issues in primary production. This is because literature references present a total bacteria population of 2.5 $\log_{10}$ CFU/g as the normal microbiota for fresh raspberries [Xu & Wu, 2016]. In fact, the aggregate of drupelets composing the small fruit can create shielded places where microorganisms can grow.

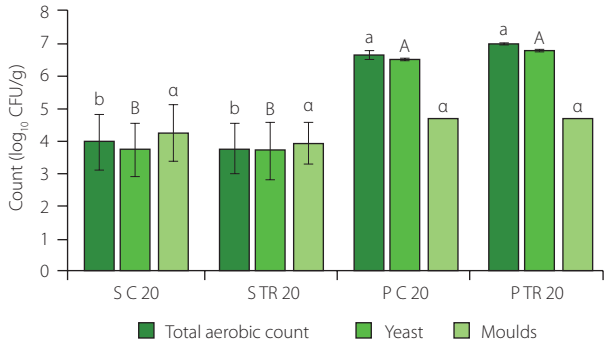

Figure 2. Total aerobic microbial, yeast and mould counts of raspberries cultivated in soil (S) and in pots (P), not submitted to UVC-LED treatment (C, control) and irradiated (TR, treated), then stored at 20°C. Mean values with error bars showing standard deviation are represented. Statistically significant differences are marked with different letters (p≤0.05). Lowercase letters refer to the total aerobic microbial count; uppercase letters refer to the yeast count; Greek letters refer to the mould count.

Regarding the mould contamination, no significant difference was detected among the various samples (Figure 2). Their count was similar in all the raspberries, from both soil and pot cultivation, and the UVC-LED treatment was unable to reduce the mould populations compared to control groups.

CONCLUSIONS

According to weight loss results, the UVC-LED treatment of raspberries for 14 min may be stressful for the fruits. The colour of raspberries was not influenced by UVC radiation, and minimal textural changes were found; hence, a direct link could not be clearly stated between these quality parameters of berries and the UVC light treatment. The outcomes obtained were influenced by the storage temperatures. The treatment with UVC-LED rays influenced the metabolic activity of raspberries, determining positive effects, such as the increment of total phenolic content of raspberries cultivated in soil, UVC-LED treated and stored at 4°C, and for those irradiated and stored at 20°C and the increment of ferric reducing antioxidant power for the pot-grown raspberries. The increased level of anthocyanins was not significant. From the microbiological point of view, no antimicrobial effect was detected due to UVC exposure. The microbial population of pot-grown raspberries was notably higher than the one of the soil-grown ones. Conducting additional experimental activities to evaluate the potential impact of irradiation and to define the best approach to be used for every fruit type could be worthwhile, particularly by dividing the storage period into two stages: an initial adaptation phase at 20°C followed by an extended storage period at 4°C. This approach would allow for the activation of secondary metabolism in the fruit while ensuring effective preservation through refrigeration. Considering the supply-chain perspective, the introduction of this technique could create discontinuities with the cold chain approach. It is, therefore, necessary to demonstrate if a possible longer storability and quality maintenance justifies the set-up of a new and partly different management program, comprehensive of the endorsing costs.

ACKNOWLEDGEMENTS

The authors express their gratitude to the staff of MOVE2WEB S.r.l. for providing the UV-LED furniture prototype.

RESEARCH FUNDING

The research received no external funding.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 354–366
DOI: 10.31883/pjfns/174488
ISSN 1230-0322, e-ISSN 2083-6007

Chemical Composition of Monovarietal Extra Virgin Olive Oils Obtained from Tunisian Mills: Influence of Geographical Origin

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Comprehending the variability in the chemical composition of olive oil from the same olive variety, based on its geographical origin, is essential. This study investigated the chemical composition of Chétoui olive oils from thirteen mills located in Northern Tunisia's Béja region. The chemical composition of olive oil exhibits substantial regional variability. Free acidity ranged from 0.57% to 0.73%, and peroxide values varied between 7.33 and 14.00 meq O_2/kg. Total phenolic content varied as well, with values ranging from 906.53 to 1,298.60 caffeic acid eq/kg (oils from Amdoun and Testour II, respectively). Chlorophyll contents ranged from 2.03 to 7.85 mg/kg, and carotenoids from 1.28 to 3.92 mg/kg. Olive oils from Amdoun and Dogga II were the richest in these compounds. In terms of tocopherols, the range extended from 282.88 mg/kg (oil from Tibar) to 416.79 mg/kg (oil from Testour II). DPPH radical scavenging activity of the polar fraction of all olive oils was higher than that of the non-polar fraction. Fatty acid profiles were mostly similar with exception of the oil from Tibar with lower saturated fatty acid content and higher monounsaturated fatty acid content. Substantial differences in polyunsaturated fatty acid content were found. Triacylglycerol compositions differed as well, with the lowest triolein percentage around 13.66% and the highest at approximately 34.98%. In summary, this study reveals significant regional variations in the chemical composition of Chétoui monovarietal olive oil, highlighting the collection region impact on oil quality and nutritional properties.

Key words: Chétoui olive, olive oil quality, nutritional properties, collection region

INTRODUCTION

Extra virgin olive oil (EVOO) is widely known and consumed globally as a healthy source of dietary fat. It is produced from the fresh ripe olives and is considered as one of the most important agricultural products in the Mediterranean region [Muzammil *et al*., 2021]. The chemical composition of EVOO is highly dependent on various factors such as the cultivar of plant, agronomic practices involved in growing it, and geographical location of the crop [Ben Hmida *et al*., 2022]. EVOO is a staple food in the Mediterranean diet and is widely recognized for its health benefits [Jimenez-Lopez *et al*., 2020]. Olive oil is an essential ingredient in many traditional dishes, making it an important commodity in the food industry [Fernández-Lobato *et al*., 2022]. Its production and consumption are also an important part of the agricultural economy in many countries, including Tunisia [Fernández-Uclés *et al*., 2020]. The country is known for producing high-quality EVOO, with a unique flavor and chemical profile. Monovarietal EVOOs, which are produced from a single cultivar

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Submitted: 8 July 2023
Accepted: 25 October 2023
Published on-line: 28 November 2023

of olive, are gaining in popularity [Hlima *et al*., 2017]. In the Béja region of Tunisia, the Chemlali and Chétoui varieties were widely used to produce monovarietal EVOOs. Monovarietal EVOOs are highly prized for their unique taste and aroma, and considered to be among the best-quality EVOOs produced in Tunisia [Lechhab *et al*., 2022]. Understanding the chemical composition of these oils allows for the evaluation of their quality and identification of the factors contributing to their unique nutritional properties.

Analyzing the composition of monovarietal olive oil is important for several reasons. Firstly, it allows for the characterization and discrimination of different olive oil varieties [Aparicio & Luna, 2002; Kyçyk *et al*., 2016]. This is useful for both consumers and producers, as it helps to ensure the authenticity and quality of the oil. Secondly, analyzing the composition of monovarietal olive oil can provide valuable information for nutritional studies [Uncu & Ozen, 2016]. The health benefits of olive oil are well-known, and understanding the specific composition of different varieties can help to further explore these benefits. Additionally, knowledge about the composition of monovarietal olive oil can be useful in olive breeding projects [Monasterio *et al*., 2013]. For example, by identifying the specific sterol fraction of different varieties, breeders can select parents for new cultivars with improved characteristics [León *et al*., 2011]. Overall, analyzing the composition of monovarietal olive oil is essential for quality control, nutritional research, and the development of new olive varieties.

This study aimed to investigate the chemical composition variations in Chétoui monovarietal olive oils across Northern Tunisia's Béja region. By conducting analysis of oils from thirteen mills, this study sought to uncover notable regional differences in essential chemical parameters, including free acidity; peroxide values; total phenolic content; and contents of chlorophylls, carotenoids, and tocopherols. Additionally, this research assessed the antioxidant potential of these olive oils, shedding light on the varying abilities of the polar and lipidic fractions to scavenge DPPH radicals, along with the fatty acid and triacylglycerol profiles. Ultimately, the findings emphasize the substantial impact of the collection region on the quality and nutritional attributes of Chétoui monovarietal olive oil, offering valuable insights for both scientific exploration and the olive oil industry.

MATERIALS AND METHODS

Chemicals

Reagents and standards used to perform spectrophotometric and titration analyses were purchased from Fluka (Buchs, Switzerland), Sigma-Aldrich (Saint Louis, MO, USA) or Merck (Darmstadt, Germany). The triacylglycerol standards, including trilinolein (LLL), triolein (OOO), tripalmitin (PPP), tristearin (SSS), trilinolenin (LnLnLn), and tripalmitolein (PoPoPo), each with a purity exceeding 98%, were acquired from Sigma-Aldrich (Saint Louis, MO, USA). We employed fatty acid methyl ester (F.A.M.E.) mix (CRM18918) from Supelco (Bellefonte, PA, USA) and α-tocopherol, β-tocopherol, and γ-tocopherol from Fluka (Buchs, Switzerland). All the solvents used for chromatographic methods were of the high performance liquid chromatography (HPLC) grade. *n*-Hexane, diethyl ether, acetone, and acetonitrile were provided by Panreac (Barcelona, Spain).

Sampling of Chétoui olive oils

Thirteen extra virgin olive oils (3×1,000 mL for each oil) of Chétoui olive variety from selected mills in the Béja region of Tunisia were used for the study. Three mills were located in Dogga (Dogga I, Dogga II and Dogga III), two mills each in Medjez El-Bab (Medjez El-Bab I and Medjez El-Bab II), Testour (Testour I and Testour II) and Nefza (Nefza I and Nefza II), and one mill each in Slouguia, Teboursouk, Tibar, and Amdoun (Figure 1). The Chétoui olives

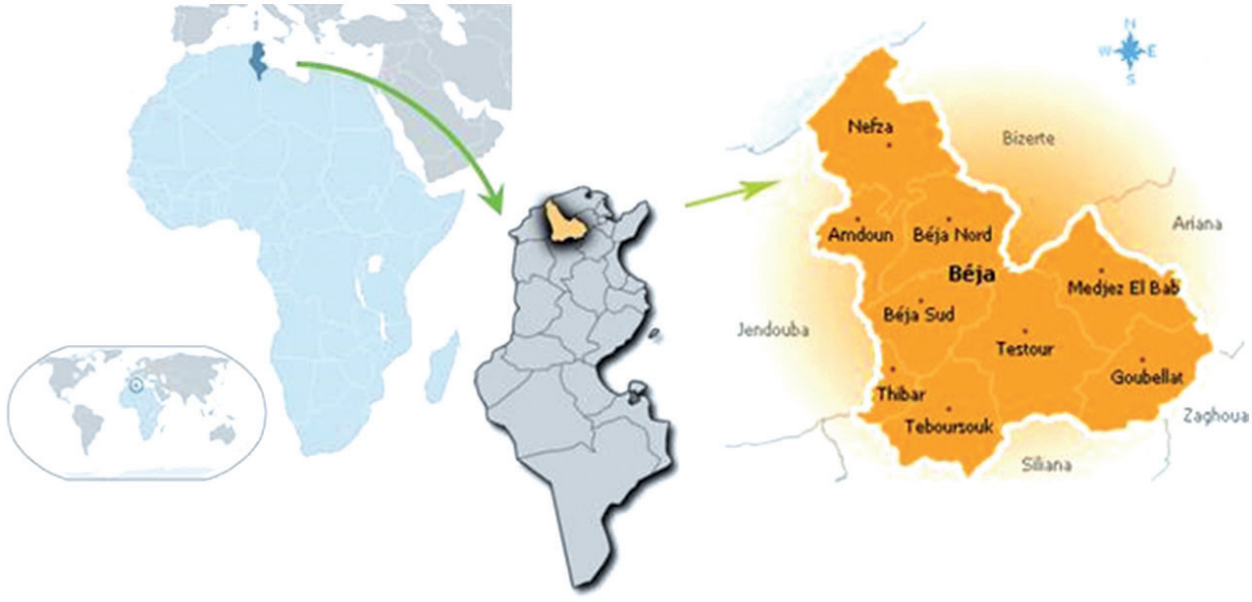

Figure 1. Sampling region of the Chétoui extra virgin olive oil.

were harvested at the optimal stage of ripeness, and the farm operators followed proper grove management and oil processing practices. The olive oils were extracted by a continuous technological plant provided by three-phase centrifugal system. The fresh oils were stored in amber glass bottles at 4°C in the dark until analysis.

Determination of basic indicators of the virgin olive oil quality

Determination of free acidity

The free acidity was determined according the method recommended by the International Olive Council [IOOC, 2017b]. It was performed by dissolving 2 g of olive oil in 50 mL of a neutralized solvent mix (95% ethanol and diethyl ether, 50:50, *v/v*) in an Erlenmeyer flask and titrating the mixture with a 0.1 M ethanolic solution of potassium hydroxide in the presence of phenolphthalein until a lasting pink coloration appeared for at least 15 s. The free acidity was calculated as a percentage of oleic acid by weight (% oleic acid, *w/w*) using Formula (1).

$$\text{Free acidity} = \frac{100 \times (V \times C \times M)}{1{,}000 \times m} \quad (1)$$

where: V, volume (mL) of the KOH solution used, C, concentration (M) of the KOH solution used, M, molar mass of oleic acid (282 g/mol), and m, mass (g) of the sample taken.

Determination of peroxide value

The peroxide value of the examined oils was determined following the IOOC method [IOOC, 2017c]. This involved dissolving 1 g of olive oil in 10 mL of chloroform, 15 mL of glacial acetic acid, and 1 mL of potassium iodide (0.01 N). The mixture was stirred and kept in the dark for 5 min. Subsequently, 75 mL of distilled water and 1 mL of a starch indicator solution (resulting in a violet color) were added. The resulting solution was titrated with a solution of sodium thiosulfate (0.01 N) while vigorously stirring until a color change occurred, transitioning to a transparent color.

The peroxide value, expressed in milliequivalents of active oxygen *per* kilogram of oil, was calculated using Formula (2):

$$\text{Peroxide value} = \frac{(V_E - V_0) \times 10}{m} \quad (2)$$

where: V_E, volume of the sodium thiosulfate solution used for the mixture with test sample (mL), V_0, volume of the sodium thiosulfate solution used for the blank (mL), and m, mass (g) of the sample taken.

Ultraviolet spectrophotometry analysis

Specific extinction coefficients (K232 and K270) were determined following the method adopted by the IOOC [2019]. A 1% oil solution in cyclohexane was prepared (0.1g of oil in 10 mL of cyclohexane), and absorbances were measured at 232 and 270 nm. K232 and K270 were calculated using Formula (3).

$$K_\lambda = \frac{E_\lambda}{C \times S} \quad (3)$$

where: K_λ, specific extinction coefficient at wavelength λ, E_λ, measured absorbance at wavelength λ, C, concentration of the solution (g/100 mL), and S, cuvette thickness (cm).

Absorbance was measured at wavelengths 266 and 274 nm to calculate the change in specific extinction coefficients (ΔK), expressed as in Formula (4):

$$\Delta K = K_{270} - \frac{K_{266} + K_{274}}{2} \quad (4)$$

Preparation of poplar and non-polar olive oil fractions

The separation of polar and non-polar oil fractions was performed according to procedure described by Kalantzakis *et al*. [2006]. This involved dissolving 2.5 g of each oil in 5 mL of *n*-hexane and then extracting it with a mixture of 5 mL of methanol and water (60:40, *v/v*) using a mechanical shaker (Vortex) to ensure thorough mixing. The mixture was than centrifuged at 1,350×g for 10 min to separate the non-polar fraction being obtained by evaporating the *n*-hexane from the upper layer of the supernatant and the polar fraction being collected as is.

Determination of total phenolic content of the extra virgin olive oils

The total phenolic content of the EVOOs was determined through colorimetric analysis using the Folin-Ciocalteu reagent, following the procedures outlined by Psomiadou & Tsimidou [2002]. The total phenolic content was measured in the polar oil fraction. A 13-µL aliquot of appropriately diluted fraction was placed in a tube in the presence of 50 µL of distilled water and 13 µL of the Folin-Ciocalteu reagent. After thorough agitation and a 3-min rest, 125 µL of a 7% Na_2CO_3 solution was added, and the mixture was adjusted with 100 µL of distilled water. The tube was then left to stand at room temperature and in the dark for 90 min, after which the absorbance was measured at 760 nm using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Results were reported as the equivalent of caffeic acid (mg CA/kg oil). Four replicates were performed for each sample.

Determination of chlorophyll and carotenoid contents of the extra virgin olive oils

The contents of carotenoids and chlorophylls of EVOOs were determined using a spectrophotometric method based on the work of Minguez-Mosquera *et al*. [1991], with measuring the absorbance at wavelengths of 470 nm and 670 nm, respectively. The values of the specific extinction coefficients used were 613 L/(g×cm) for pheophytin A as the main EVOO chlorophyll and 2,000 L/(g×cm) for lutein as the main EVOO carotenoid. The pigment contents were calculated by dividing the absorbance at the specified wavelength by the extinction coefficients of a reference compound, spectrophotometer cell thickness (1 cm), and a factor of 100. The result was expressed in mg of pheophytin A for chlorophylls and lutein for carotenoids *per* kg of oil. Four replicates were performed for each sample.

Determination of tocopherol composition of the extra virgin olive oils

The tocopherol composition was determined according to Manai-Djebali *et al*. [2012]. The analysis involved dissolving the oil in *n*-hexane and analyzing the solution using an Agilent 1200 HPLC with a silica gel Lichrosorb Si-60, 5 µm particle size, length (L) × inner diameter (I.D.) of 25 cm × 4 mm column (Agilent Technologies, Santa Clara, CA, USA). The elution was accomplished using a mixture of *n*-hexane and 2-propanol (99:1, *v/v*), and the flow rate was set at 1 mL/min. A fluorescence detector was used for detection, with the excitation and emission wavelengths set at 290 and 330 nm, respectively. The content of individual tocopherols was reported as mg *per* kg of oil. Identification was accomplished by comparing the retention times of α-, β-, and γ-tocopherols. The quantification was performed using a calibration curve for the three tocopherols, and the coefficients of determination (R^2) for α-, β-, and γ-tocopherols were 0.989, 0.999, and 0.991, respectively. Four replicates were performed for each sample.

Determination of fatty acid composition of the extra virgin olive oils

Methyl esters of EVOO fatty acids (FAs) were prepared by vigorous shaking of the oils in *n*-hexane with 0.2 mL of a 2 M methanolic potassium hydroxide solution [IOOC, 2017a]. The gas chromatography separation was performed using an Agilent 6890N gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a flame ion detector (FID) and an HP-1 (polydimethylsiloxane) fused-silica capillary column with an L of 50 m, an I.D. of 0.2 mm and a film thickness of 0.33 mm. The carrier gas was helium, delivered with a constant flow rate of 1 mL/min, and an oven temperature was programmed from 60 to 250°C at a rate of 2°C/min, and then held isothermal for 20 min. The FID temperature was set at 250°C. The identification of FAs was performed by comparing the specific retention time of each compound with those of a fatty acid methyl ester standard (F.A.M.E. Mix, CRM18918-Supelco, Bellefonte, PA, USA). Four replicates were performed for each sample, the results were expressed in area %.

Determination of triacylglycerol composition of the extra virgin olive oils

According to Commission Implementing Regulation (EU) Amending Regulation (EEC) No. 2569/91 [2016], the analysis of triacylglycerols (TAGs) was performed by dissolving 0.12 g of olive oil in 0.5 mL of *n*-hexane and then purifying the triacylglycerol fraction using solid-phase extraction (SPE) with a Silica column and a mixture of *n*-hexane and diethyl ether (87:13, *v/v*). After evaporation, the purified triacylglycerols were dissolved in 2 mL of acetone and analyzed using an Agilent 1200 HPLC Series (Agilent Technology, Palo Alto, CA, USA) equipped with a refractometric detector and a LiChrospher RP-18, 5 µm particle size, L × I.D. of 25 cm × 4.6 mm column (Merck, Darmstadt, Germany). The elution solvent was a mixture of acetone and acetonitrile (50:50, *v/v*), and the flow rate was set at 1.2 mL/min. Triacylglycerols, including LLL, OOO, PPP, SSS, LnLnLn, and PoPoPo, were used as standards (Sigma-Aldrich, St Louis, MO, USA). To determine TAGs, the retention times were graphed by comparing them to reference chromatograms from soybean oil, a 30:70 (*w/w*) mixture of soybean oil and olive oil, and pure olive oil, following the methodology outlined in IOOC [2017d]. It was presumed that the total area of peaks representing different TAGs added up to 100%, and the proportional distribution of each TAG was subsequently computed.

Determination of radical scavenging activity of the extra virgin olive oils

The ability of the of EVOO polar and non-polar fractions to scavenge the DPPH radical was determined according to Kalantzakis *et al*. [2006] by adding 250 µL of each oil fraction in ethyl acetate (10%, *w/v*) to 1 mL of a freshly prepared DPPH radical solution (10 mM in ethyl acetate) in a 2-mL test tube. The mixture was shaken vigorously for 10 s using a Vortex mixer and allowed to stand in the dark for 30 min until a steady state was reached. Its absorbance was then measured at 515 nm and compared to that of a solution only with the DPPH radical (without sample). The DPPH radical scavenging activity of olive oil fractions was expressed in % DPPH radical inhibition.

Statistical analysis

The data analysis and hierarchical cluster analysis (which involved grouping olive oil provenances based on the studied parameters) were performed using JMP 14 software (SAS Institute Inc., Cary, NC, USA). Statistical computations were conducted using one-way analysis of variance (ANOVA) followed by the Newman–Keuls multiple comparison test. A significance level of $p<0.05$ was chosen, indicating a common threshold used to determine statistical significance.

RESULTS AND DISCUSSION

Basic indicators of the extra virgin olive oil quality

The quality assessment of EVOOs from the 13 locations showed significant varying levels of free acidity, peroxide value, K232, K270 and ΔK (Figure 2). The free acidity values offer insights into the quality and freshness of the olive oils produced. Notably, the results revealed variations in free acidity among olive oils from different mills. For instance, oils from Nefza II and Slouguia demonstrated the lowest free acidity, with 0.57 and 0.58% oleic acid (*w/w*), respectively. These findings suggest the possibility of higher quality and lower oxidation in these oils. Conversely, Amdoun and Tibar exhibited the highest free acidity, reaching 0.73 and 0.72% oleic acid (*w/w*), respectively. The acidity level should be less than 0.8% in virgin olive oil and less than 2% in refined olive oil [Jimenez-Lopez *et al*., 2020]. High levels of acidity can affect the taste, odor, and stability of the oil [Issaoui *et al*., 2011].

High peroxide values indicate that the oil has been exposed to light, heat, and/or oxygen, which can lead to a rancid taste and odor. The EVOO with the highest peroxide value was from Dogga II, with a mean value of 14.00 meq O_2/kg, while the lowest

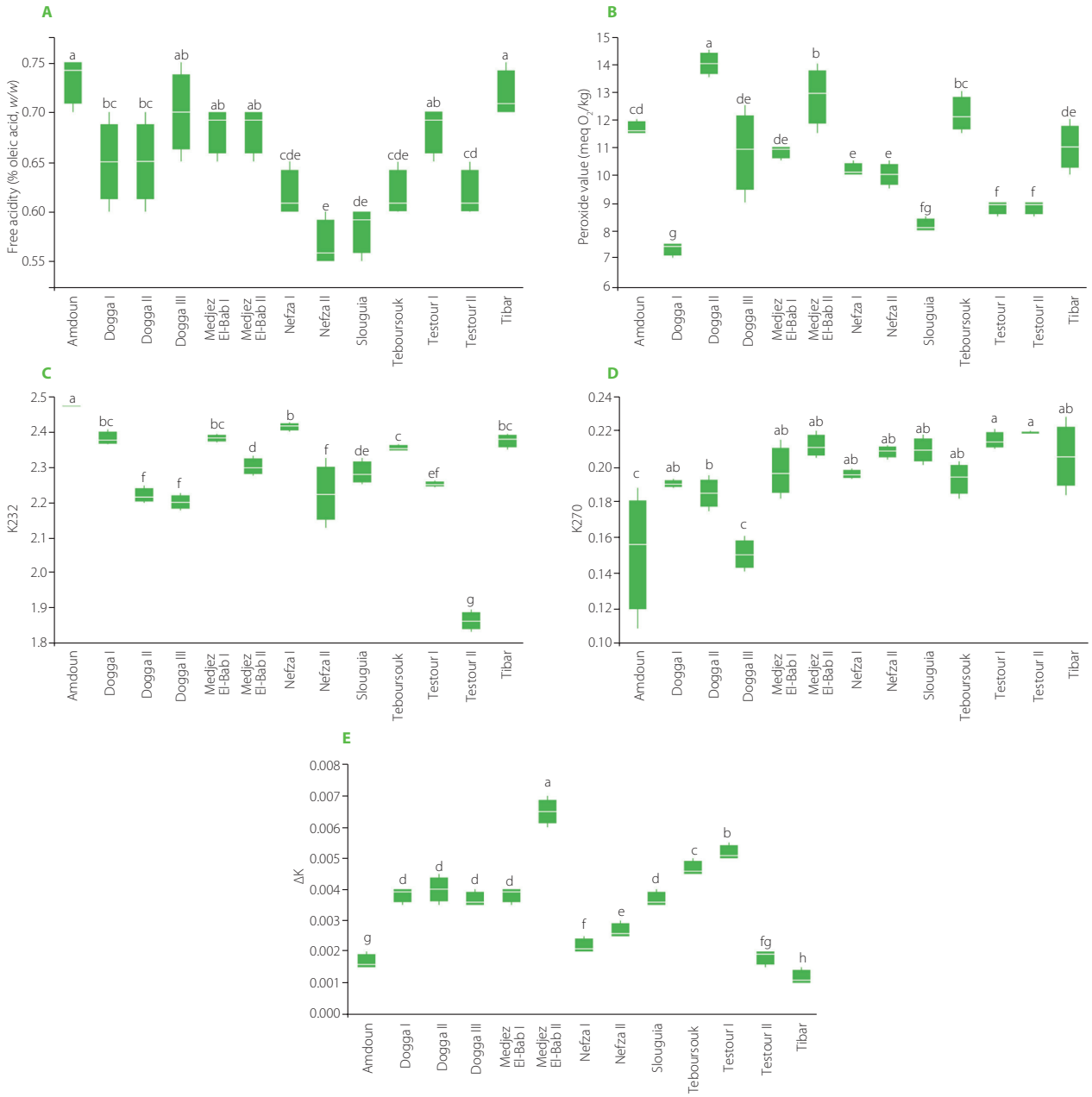

Figure 2. Free acidity (**A**), peroxide value (**B**), and extinction coefficients including K232 (**C**), K270 (**D**) and ΔK (**E**) of the Chétoui extra virgin olive oils from selected mills in the Béja region in Tunisia. Data are expressed as box plots for four replicates. Means in the same box bearing different letters are significantly different at $p<0.05$.

peroxide value was found in oils from Slouguia and Dogga I, with a mean value of 8.17 meq O_2/kg and 7.33 meq O_2/kg, respectively (Figure 2). The specific extinction coefficients (K232, K270 and ΔK) are other indicators of oil oxidation, providing information about its quality and preservation state. K232 indicates the formation of conjugated diene hydroperoxides, primary oxidation products, in the oil. K270 is associated with the formation of secondary products of oxidation, such as aldehydes and ketones. The highest K232 mean value (2.47) was recorded in EVOO from Amdoun, while the lowest value (1.86) was determined in oil from Testour II (Figure 2). The highest K270 values were assayed in EVOO from Testour I and Testour II, with mean values of 0.21 and 0.22, respectively. Additionally, it is worth noting that the olive oils from regions of Tibar, Teboursouk, Nefza I, Nefza II, Slouguia, Medjez El-Bab I, Medjez El-Bab II, and Dogga I exhibited statistically similar ($p\geq0.05$) K270 values, ranging from 0.19 to 0.21. These oils share comparable chemical characteristics in this specific measure. The lowest K270 was recorded in oils from Amdoun and Dogga III, with a mean value of 0.15 for both oils. The highest ΔK value was found for oil from Medjez El-Bab II, with a mean value of 0.0065, while the lowest value was determined for EVOO from Tibar, with a mean value of 0.0012.

The quality of the monovarietal EVOOs, evaluated based on several key indicators, met the standards set for this type of products. All values fell within the standards set for "extra virgin olive oil" as defined by the IOOC [IOOC, 2021], which recommend acidity of less than or equal to 0.8%, peroxide value of less than or equal to 20 meq O_2/kg, K270 of less than or equal to 0.22, K232

of less than or equal to 2.5, and ΔK of less than or equal to 0.01. The quality of the oil is considered higher if the values of these parameters are lower, as this usually indicates that the olives used were fresh and healthy, harvested at the optimal ripening stage, and processed immediately without storage. The results of our study align with those of previous research conducted on the oils of Chétoui variety in Tunisia [Ben Hmida *et al.*, 2022]. This consistency supports the validity of our findings and adds to the existing body of knowledge on the oils of Chétoui variety in Tunisia.

Content of total phenolics

Phenolics are a class of compounds found in olive oil that have antioxidant properties [Bendini *et al.*, 2007]. High content of phenolics is associated with a high oil stability and a low level of oxidation and degradation [El Yamani *et al.*, 2019]. The highest total phenolic content was found in EVOO from Testour II, with a mean value of 1298.60 mg CA/kg, while the lowest value was determined in oils from Amdoun with a mean value of 906.53 mg CA/kg (Figure 3). These differences were found to be statistically significant ($p<0.05$), highlighting the substantial variations in total phenolic content among these regions. The contents of phenolics in olive oil can vary depending not only on the plant cultivar and the region of its production, but also on agricultural practices and the fruit processing method [Ben Youssef *et al.*, 2012; Lechhab *et al.*, 2022]. The total phenolic content was found to be influenced by the irrigation regime, with rain-fed Chétoui olives showing higher levels of total phenolics than those grown with irrigation [Ben Youssef *et al.*, 2010; Haddada *et al.*, 2007].

Content of chlorophylls and carotenoids

Figure 3 displays the variation of chlorophyll and carotenoid contents in EVOOs from different locations in Béja region. The chlorophyll content was found to vary greatly across region, with oils from Dogga II and Amdoun having the highest content (7.85 and 7.54 mg/kg, respectively) and in those from Slouguia having the lowest one (2.03 mg/kg). This variation in chlorophyll content was also reported in previous studies by Ben Youssef *et al.* [2010], who found that the chlorophyll content in Chetoui oils from olives at different stages of maturity varied from 9.90 to 3.35 mg/kg for a ripening index of 1.18 to 5.47. Guerfel *et al.* [2009] also found that the chlorophyll content varied based on the growing region, with values ranging from 2.5 to 9.8 mg/kg. The carotenoid content in the olive oils also exhibited significant variation, with samples from Amdoun and Dogga II displaying the highest content (3.92 and 3.88 mg/kg, respectively), compared to the lowest value noted for Slouguia (1.28 mg/kg). These differences were found to be statistically significant. Ben Youssef *et al.* [2010] reported the carotenoid content in Chetoui oils to vary from 4.34 to 1.49 mg/kg for a ripening index of 1.1 to 5.47. Guerfel *et al.* [2009] also found variation in carotenoid content based on the cultivation region.

Tocopherol composition

Tocopherol is an important factor in determining the quality of olive oil. Tocopherols are a family of vitamin E compounds that

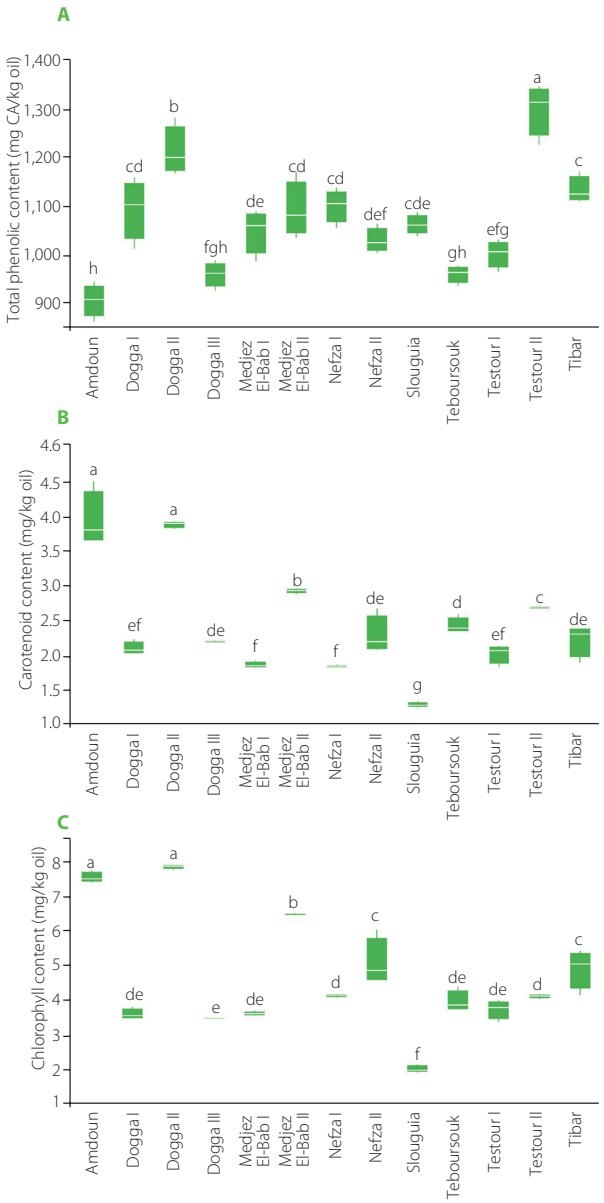

Figure 3. Total phenolic content (**A**), carotenoid content (**B**) and chlorophyll content (**C**) of the Chétoui extra virgin olive oils from selected mills in the Béja region in Tunisia. Data are expressed as box plots for four replicates. Means in the same box bearing different letters are significantly different at $p<0.05$. CA, caffeic acid equivalent.

are found in many vegetable oils, including olive oil [Beltrán *et al.*, 2010]. They play an important role in olive oil quality because they serve as natural antioxidants, which help to prevent oxidation and preserve the freshness of the oil. In particular, α-tocopherol is the most abundant in olive oil and has the highest antioxidant activity [Blekas *et al.*, 1995]. The content of α-tocopherol in olive oil is often used as an indicator of its overall freshness and quality. Oils with higher content of α-tocopherol are less likely to have undergone oxidative spoilage and are therefore less likely to develop off-flavors or odors [Deiana *et al.*, 2002].

The contents of α-, β-, and γ-tocopherols of olive oils from different mills located in the Béja region are shown in Figure 4. The highest total tocopherol content was found in oils from Testour II (416.79 mg/kg), Medjez El-Bab II (410.80 mg/kg),

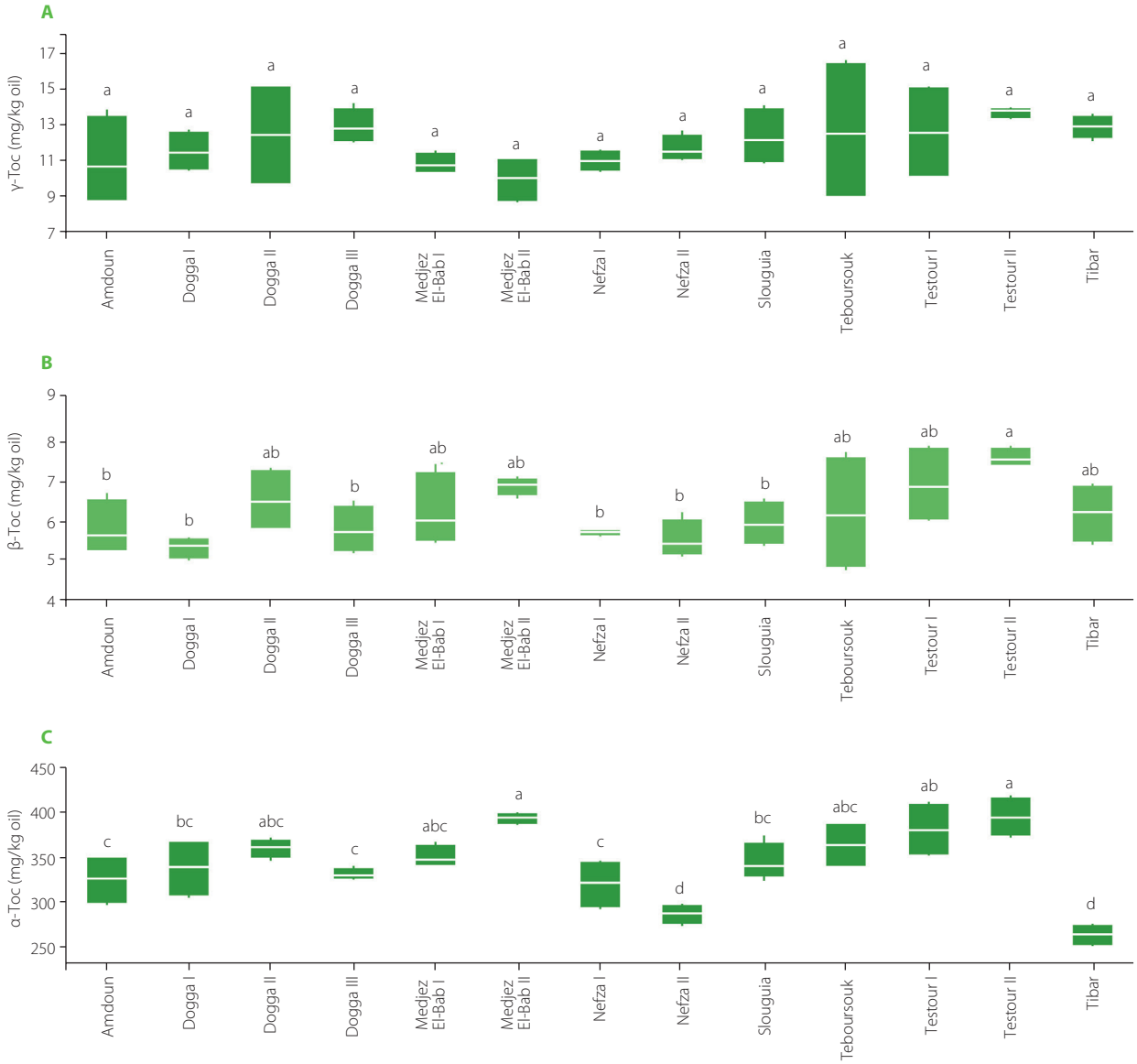

Figure 4. Content of α-tocopherol (α-Toc) (**A**), β-tocopherol (β-Toc) (**B**) and γ-tocopherol (γ-Toc) (**C**) of the Chétoui extra virgin olive oils from selected mills in the Béja region in Tunisia. Data are expressed as box plots for four replicates. Means in the same box bearing different letters are significantly different at $p<0.05$.

and Testour I (400.98 mg/kg). On the other hand, the lowest total tocopherol content was found in EVOOs from Tibar (282.88 mg/kg) and Nefza II (303.89 mg/kg), followed by Nefza I (337.34 mg/kg), and Amdoun (342.03 mg/kg). When comparing the α-, β-, and γ-tocopherol contents, as expected, the highest values were found for α-tocopherols. Similarly, to the total tocopherol content, the highest α-tocopherol content was found in the samples from Medjez El-Bab II (394.00 mg/kg) and Testour II (395.51 mg/kg), whereas the lowest in the oils from the mills in Tibar (263.83 mg/kg) and Nefza II (286.72 mg/kg). Oil from Testour II mill exhibited the highest β-tocopherol content at 7.61 mg/kg, and this value was significantly higher ($p<0.05$) compared to these for oil from mills in Dogga I (with the lowest β-tocopherol content at 5.30 mg/kg,) Nefza II, Nefza I, Dogga III, Amdoun, and Slouguia. Upon conducting ANOVA of all the oil samples, there were no significant ($p\geq0.05$) differences in γ-tocopherol content, which ranged from 9.93 mg/kg in Medjez El-Bab II to 13.67 mg/kg in Testour II. These results suggest that the level of γ-tocopherol does not differentiate monovarietal extra virgin olive oils across the mills and regions.

The tocopherol content of olive oil has been extensively studied and our findings align with the following study. Ben-Temime *et al*. [2006] found that the total tocopherol content was influenced by the production area, ranging from 341.40 mg/kg (Lakhouet) to 405.65 mg/kg (Amdoun). In the cited work, similarly to our study, the analysis of the tocopherols using HPLC showed the presence of their three forms (α-, β-, and γ-tocopherols) with α-tocopherol being the main isomer, and the variation in the total tocopherol contents in oils, which was reflected by changes in α-tocopherol content (from 324.32 mg/kg of Lakhouet oil to 385.35 mg/kg of Amdoun oil); β-, and γ-tocopherols were less represented, with contents not exceeding 12 mg/kg. According to Hassine *et al*. [2015], the *α*-tocopherol content in Testour oil was 477.26 mg/kg and in Medjez El-Bab it was 382.26 mg/kg.

Fatty acid compositions

Fatty acids played an important role in the human diet and had a variety of health effects. They are divided into saturated fatty acids (SFAs) and unsaturated fatty acids (UFA), including monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Previous studies showed that SFAs were implicated in adverse health effects, such as an increased risk of cardiovascular disease, while UFAs were linked to positive health effects, such as reducing the risk of cardiovascular disease [Islam *et al*., 2019]. The ratio of MUFAs to PUFAs was also considered an indicator of the quality of fatty acid content [Chen & Liu, 2020].

The fatty acid composition of EVOOs from different mills located in the Béja region is shown in Table 1. It was observed that the SFA content was statistically consistent ($p \geq 0.05$) across the region, except for the oil from Tibar, which displayed the lowest SFA content at 14.55% of total fatty acids. When it comes to UFAs, there were no statistically significant ($p \geq 0.05$) differences among the olive oil samples tested, ranging from 83.42% to 85.45%. The MUFA content was also relatively uniform across the region, except for oil from Tibar, which stood out with the highest value of 72.04%. Notably, the PUFA content exhibited significant variability, with the highest value found in the oil from the mill in Amdoun at 18.29% and statistically similar ($p \geq 0.05$) in several other oils, including those from Medjez El-Bab I, Medjez El-Bab II, Dogga III, Dogga I, Slouguia, and Teboursouk ranging from 16.99% to 18.06%. Additionally, the C18:1/C18:2 ratio exhibited significant ($p < 0.05$) differences, with the highest value recorded in the oil from Tibar (5.62) and the lowest in the oils from Amdoun (3.74), Medjez El-Bab I (3.81) and Medjez El-Bab II (3.96). The variation in the amount of fatty acids in olive oils from the Chétoui cultivar (Table 1) was likely due to a combination of genetic factors and environmental conditions during fruit growth and maturity. The fatty acid content in the oil was also influenced by the ripeness of the fruit. If harvesting was delayed, the levels of unsaturated fatty acids, especially linoleic acid, tended to increase while the levels of palmitic acid to decrease [Ben-Temime *et al*., 2006]. The results obtained in our study are in line with previous research conducted by Ben-Temime *et al*. [2006], Ben Youssef *et al*. [2010; 2012], and Yahia *et al*. [2012]. This consistency in findings across various studies reinforces the robustness of our observations and suggests that the patterns in fatty acid composition in olive oils from different regions, especially the distinct characteristics of Tibar and Amdoun, are well-documented and established in the scientific literature.

Triacylglycerol composition

Triacylglycerols (TAGs) are the main constituents of olive oil. TAGs consist of a glycerol molecule attached to three fatty acid chains and are responsible for the oil's physical and chemical properties, such as viscosity, stability, and oxidative behavior [Sánchez & Harwood, 2002]. The fatty acid composition of TAGs is one of the most important factors that affects olive oil quality. It influences the oil's flavor, aroma, and stability, as well as health benefits [Harwood & Yaqoob, 2002]. For example, olive oil with a high MUFA content, such as oleic acid, is considered to have a better flavor and aroma profile, as well as a longer shelf life, compared to oils with a high PUFA content [Garcia-Oliveira *et al*., 2021]. The percentage composition of triacylglycerols detected in Chétoui extra virgin olive oil samples from different locations in Béja region in Tunisia is shown in Table 2. The triacylglycerols included oleodilinolein (LLO), dioleolinolenin (OLnO), dioleolinolein (OLO), palmitolinoleoolein + stearodilinolein (PLO+SLL), dipalmitolinolein (PPL), triolein (OOO), palmitodiolein (POO), dipalmitooleotin (PPO), dioleostearin (SOO), and linoleodistearin + palmitooleostearin (SLS+POS). The data in Table 2 reveals significant disparities in the composition of TAGs in monovarietal olive oils. Notably, the LLO content ranged from 3.56% in the oil from Tibar to 6.36% and 6.92% in the oils from Dogga III and Dogga II, respectively, and these differences could be potentially attributed to variances in environmental conditions for growing olive trees. In the case of OLnO, the highest contents at 5.70% and 5.62% were found in the oils from Dogga II and Dogga III, respectively, while the oil from Tibar presented the lowest at 1.95%. Furthermore, mills located in Amdoun, Testour I, Medjez El-Bab I and Medjez El-Bab II (oils with a peak OLO percentage of 18.02–18.62%) may produce olive oils with enhanced shelf stability, compared to Dogga II and III mills producing oils with the lowest OLO content at 14.31–14.45%. The data indicates that the percentage content of POO was minimal in Amdoun (20.95%) and the highest in Dogga III (22.99%) olive oil, and there were no significant ($p \geq 0.05$) differences among these regions in terms of POO content. For SOO content, there was a significant variability among the regions, with values ranging from 2.47% in Dogga III to 4.79% in Nefza I samples. In general, the triacylglycerol composition of the different olive oil samples showed a wide range of variations, reflecting the diversity of the different regions in terms of climate, soil, and cultivation practices. It was therefore an important factor to consider when evaluating the quality of olive oil [Haddada *et al*., 2007].

DPPH radical scavenging activity of polar and non-polar oil fractions

The results of DPPH radical scavenging activity assay of polar and non-polar fractions of EVOOs taken from different locations are shown in Figure 5. The DPPH radical scavenging activity of the polar fraction was found to be relatively high, ranging from 93.26% for Testour II sample to 95.69% for Dogga III sample. On the other hand, the non-polar fraction showed a lower DPPH radical scavenging activity, ranging from 44.80% to 74.52%, with oils from Medjez El-Bab I and Dogga II having the highest percentage. There was a significant difference in the DPPH radical scavenging activity between the polar and non-polar fractions for all oils, with the polar fraction showing a much higher percentage of DPPH inhibition. This finding is consistent with other studies that have evaluated the antioxidant properties of Chétoui olive oil. For example, a study by Ben-Temime *et al*. [2006] reported that the polar fraction of Chétoui olive oil had a higher total phenolic content and antioxidant activity than the lipid fraction. Another study by Nakbi *et al*. [2010] found that the phenolic compounds in Chétoui olive oil contributed

Table 1. Fatty acid composition (% total fatty acids) of the Chétoui extra virgin olive oils from selected mills in the Béja region in Tunisia.

Fatty acid	Medjez El-Bab I	Medjez El-Bab II	Slouguia	Testour I	Testour II	Teboursouk	Dogga I	Dogga II	Dogga III	Tibar	Amdoun	Nefza I	Nefza II
C16:0	11.94±0.60^{a}	11.98±0.60^{a}	11.41±0.57^{a}	12.76±0.64^{a}	12.71±0.64^{a}	12.07±0.60^{a}	11.39±0.57^{a}	12.46±0.62^{a}	12.05±0.60^{a}	11.03±0.55^{a}	11.73±0.59^{a}	12.12±0.61^{a}	11.13±0.56^{a}
C16:1	0.41±0.02^{b}	0.38±0.02^{bc}	0.32±0.02^{c}	0.55±0.03^{a}	0.44±0.02^{b}	0.43±0.02^{b}	0.36±0.02^{c}	0.46±0.02^{b}	0.43±0.02^{b}	0.27±0.01^{d}	0.33±0.02^{c}	0.46±0.02^{b}	0.33±0.02^{c}
C18:0	3.25±0.16^{a}	3.16±0.16^{a}	3.35±0.17^{a}	3.32±0.17^{a}	3.25±0.16^{a}	3.36±0.17^{a}	3.55±0.18^{a}	3.24±0.16^{a}	3.18±0.16^{a}	3.07±0.15^{a}	3.29±0.16^{a}	3.19±0.16^{a}	3.57±0.18^{a}
C18:1	65.53±3.28^{b}	66.15±3.31^{b}	67.14±3.36^{b}	65.99±3.3^{b}	67.48±3.37^{b}	66.36±3.32^{b}	66.69±3.33^{b}	66.72±3.34^{b}	66.32±3.32^{b}	71.41±3.57^{a}	65.58±3.28^{b}	67.81±3.39^{ab}	68.53±3.43^{ab}
C18:2	17.26±0.86^{a}	16.70±0.84^{a}	16.25±0.81^{a}	15.79±0.79^{ab}	14.52±0.73^{b}	16.19±0.81^{a}	16.44±0.82^{a}	15.52±0.78^{ab}	16.47±0.82^{a}	12.71±0.64^{c}	17.54±0.88^{a}	14.86±0.74^{b}	14.89±0.74^{b}
C18:3	0.80±0.04^{a}	0.79±0.04^{a}	0.74±0.04^{a}	0.78±0.04^{a}	0.78±0.04^{a}	0.81±0.04^{a}	0.75±0.04^{a}	0.8±0.04^{a}	0.79±0.04^{a}	0.70±0.04^{a}	0.75±0.04^{a}	0.76±0.04^{a}	0.74±0.04^{a}
C20:0	0.48±0.02^{a}	0.50±0.03^{a}	0.48±0.02^{a}	0.50±0.02^{a}	0.47±0.02^{a}	0.46±0.02^{a}	0.45±0.02^{a}	0.48±0.02^{a}	0.45±0.02^{a}	0.45±0.02^{a}	0.46±0.02^{a}	0.48±0.02^{a}	0.47±0.02^{a}
C20:1	0.32±0.02^{a}	0.34±0.02^{a}	0.32±0.02^{a}	0.31±0.02^{a}	0.34±0.02^{a}	0.31±0.02^{a}	0.35±0.02^{a}	0.3±0.020^{a}	0.31±0.02^{a}	0.36±0.02^{a}	0.32±0.02^{a}	0.32±0.02^{a}	0.33±0.02^{a}
SFA	15.67±0.78^{a}	15.64±0.78^{a}	15.24±0.76^{a}	16.58±0.83^{a}	16.43±0.82^{a}	15.89±0.79^{a}	15.4±0.77^{a}	16.19±0.81^{a}	15.68±0.78^{a}	14.55±0.73^{b}	15.48±0.77^{a}	15.79±0.79^{a}	15.18±0.76^{a}
UFA	84.33±4.22^{a}	84.36±4.22^{a}	84.76±4.24^{a}	83.42±4.17^{a}	83.57±4.18^{a}	84.11±4.21^{a}	84.6±4.23^{a}	83.81±4.19^{a}	84.32±4.22^{a}	85.45±4.27^{a}	84.52±4.23^{a}	84.21±4.21^{a}	84.82±4.24^{a}
MUFA	66.27±3.31^{b}	66.87±3.34^{b}	67.77±3.39^{b}	66.85±3.34^{b}	68.27±3.41^{b}	67.10±3.36^{b}	67.41±3.37^{b}	67.49±3.37^{b}	67.07±3.35^{b}	72.04±3.60^{a}	66.23±3.31^{b}	68.59±3.43^{b}	69.18±3.46^{b}
PUFA	18.06±0.90^{a}	17.49±0.87^{a}	16.99±0.85^{a}	16.57±0.83^{ab}	15.3±0.77^{b}	17.01±0.85^{a}	17.19±0.86^{a}	16.32±0.82^{ab}	17.25±0.86^{a}	13.41±0.67^{c}	18.29±0.91^{a}	15.62±0.78^{b}	15.63±0.78^{b}
C18:1/C18:2	3.81±0.19^{c}	3.96±0.20^{c}	4.14±0.21^{b}	4.18±0.21^{b}	4.65±0.23^{b}	4.11±0.21^{b}	4.06±0.20^{b}	4.31±0.22^{b}	4.03±0.20^{b}	5.62±0.28^{a}	3.74±0.19^{c}	4.56±0.23^{b}	4.60±0.23^{b}

Data are expressed as average of four replicates ± standard deviation. SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values with different lowercase letters in superscript were significantly different at $p<0.05$

Table 2. The triacylglycerol composition (% total triacylglycerols) of the Chétoui extra virgin olive oils from selected mills in the Béja region in Tunisia.

Triacylglycerol	Medjez El-Bab I	Medjez El-Bab II	Slouguia	Testour I	Testour II	Teboursouk	Dogga I	Dogga II	Dogga III	Tibar	Amdoun	Nefza I	Nefza II
LLnLn	0.03±0.00^{b}	0.03±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.04±0.00^{b}	0.05±0.00^{b}	0.03±0.00^{b}	0.12±0.01^{a}
LLLn	0.08±0.00^{c}	0.10±0.00^{bc}	0.11±0.01^{bc}	0.09±0.00^{c}	0.07±0.00^{c}	0.09±0.00^{bc}	0.08±0.00^{bc}	0.09±0.00^{bc}	0.06±0.00^{c}	0.14±0.01^{a}	0.12±0.01^{a}	0.12±0.01^{a}	0.10±0.00^{bc}
OLLn	0.53±0.03^{d}	0.49±0.02^{de}	0.60±0.03^{d}	0.54±0.03^{d}	0.45±0.02^{e}	0.48±0.02^{e}	0.53±0.03^{d}	1.03±0.05^{a}	0.86±0.04^{b}	0.23±0.01^{g}	0.71±0.04^{c}	0.37±0.02^{f}	0.37±0.02^{f}
LLL	0.48±0.02^{ab}	0.43±0.02^{b}	0.46±0.02^{b}	0.44±0.02^{b}	0.39±0.02^{bc}	0.43±0.02^{b}	0.40±0.02^{bc}	0.47±0.02^{ab}	0.52±0.03^{a}	0.28±0.01^{d}	0.45±0.02^{b}	0.35±0.02^{c}	0.36±0.02^{c}
PLLn	0.20±0.01^{c}	0.16±0.01^{cd}	0.17±0.01^{cd}	0.15±0.01^{c}	0.15±0.01^{c}	0.14±0.01^{c}	0.12±0.01^{d}	0.29±0.01^{b}	0.35±0.02^{a}	0.07±0.00^{e}	0.15±0.01^{c}	0.13±0.01^{d}	0.11±0.01^{d}
LLO	5.44±0.27^{b}	5.41±0.27^{b}	5.44±0.27^{b}	5.50±0.28^{b}	5.19±0.26^{b}	5.39±0.27^{b}	5.36±0.27^{b}	6.92±0.35^{a}	6.36±0.32^{a}	3.56±0.18^{d}	5.96±0.30^{b}	4.67±0.23^{c}	4.84±0.24^{c}
OLnO	2.96±0.15^{b}	2.83±0.14^{b}	2.94±0.15^{b}	2.79±0.14^{b}	2.50±0.13^{c}	2.78±0.14^{b}	2.72±0.14^{b}	5.70±0.28^{a}	5.62±0.28^{a}	1.95±0.10^{d}	2.95±0.15^{b}	2.46±0.12^{c}	2.43±0.12^{c}
PLL	0.93±0.05^{c}	0.94±0.05^{c}	0.94±0.05^{c}	0.98±0.05^{c}	1.12±0.06^{b}	0.92±0.05^{c}	0.74±0.04^{d}	1.19±0.06^{a}	1.04±0.05^{a}	0.72±0.04^{d}	0.55±0.03^{e}	0.70±0.04^{d}	0.79±0.04^{d}
OLO	18.09±0.90^{a}	18.02±0.9^{a}	17.71±0.89^{b}	18.06±0.9^{a}	17.68±0.88^{b}	17.91±0.9^{b}	17.96±0.9^{b}	14.45±0.72^{d}	14.31±0.72^{d}	16.46±0.82^{c}	18.62±0.93^{a}	17.58±0.88^{b}	17.62±0.88^{b}
PLO+SLL	10.88±0.54^{b}	10.89±0.54^{b}	10.82±0.54^{b}	10.56±0.53^{b}	10.18±0.51^{b}	10.31±0.52^{b}	9.58±0.48^{b}	18.37±0.92^{a}	18.2±0.91^{a}	8.42±0.42^{c}	10.97±0.55^{b}	9.55±0.48^{b}	9.69±0.48^{b}
PPL	1.58±0.08^{b}	1.56±0.08^{b}	1.51±0.08^{b}	1.39±0.07^{bc}	1.18±0.06^{c}	1.40±0.07^{b}	1.17±0.06^{c}	4.32±0.22^{a}	4.12±0.21^{a}	1.06±0.05^{d}	1.32±0.07^{bc}	1.24±0.06^{bc}	1.31±0.07^{bc}
OOO	28.52±1.43^{bc}	28.61±1.43^{bc}	28.2±1.41^{bc}	28.9±1.44^{bc}	29.61±1.48^{bc}	29.59±1.48^{bc}	31.45±1.57^{b}	13.66±0.68^{d}	14.13±0.71^{d}	34.98±1.75^{a}	27.79±1.39^{c}	30.97±1.55^{b}	31.43±1.57^{b}
POO	21.45±1.07^{a}	21.65±1.08^{a}	21.66±1.08^{a}	21.47±1.07^{a}	21.95±1.10^{a}	21.49±1.07^{a}	21.08±1.05^{a}	22.11±1.11^{a}	22.99±1.15^{a}	22.31±1.12^{a}	20.95±1.05^{a}	21.62±1.08^{a}	21.57±1.08^{a}
PPO	3.79±0.19^{b}	3.76±0.19^{b}	3.69±0.18^{bc}	3.54±0.18^{bc}	3.53±0.18^{bc}	3.34±0.17^{bc}	3.00±0.15^{c}	7.62±0.38^{a}	7.79±0.39^{a}	3.62±0.18^{bc}	3.91±0.20^{b}	3.73±0.19^{b}	3.34±0.17^{bc}
PPP	0.37±0.02^{d}	0.37±0.02^{d}	0.38±0.02^{d}	0.37±0.02^{d}	0.37±0.02^{d}	0.42±0.02^{c}	0.56±0.03^{a}	0.00±0.00^{e}	0.00±0.00^{e}	0.49±0.02^{b}	0.40±0.02^{c}	0.41±0.02^{c}	0.45±0.02^{bc}
SOO	3.82±0.19^{b}	3.90±0.19^{b}	4.23±0.21^{a}	4.18±0.21^{a}	4.47±0.22^{a}	4.29±0.21^{a}	4.40±0.22^{a}	2.54±0.13^{c}	2.47±0.12^{c}	4.71±0.24^{a}	4.01±0.20^{a}	4.79±0.24^{a}	4.44±0.22^{a}
SLS+POS	0.84±0.04^{d}	0.84±0.04^{d}	1.15±0.06^{b}	1.04±0.05^{c}	1.18±0.06^{ab}	1.02±0.05^{c}	0.86±0.04^{d}	1.26±0.06^{a}	1.19±0.06^{ab}	0.97±0.05^{d}	1.09±0.05^{bc}	1.31±0.07^{a}	1.03±0.05^{c}

Data are expressed as average of four replicates ± standard deviation. The triacylglycerol names were abbreviated by means of three letters corresponding to the fatty acid bound to the glycerol backbone. In alphabetic order: L, linoleic acid (C18:2); Ln, linolenic acid (C18:3); O, oleic acid (C18:1); P, palmitic acid (C16:0); and S, stearic acid (C18:0). Values with different lowercase letters in superscript were significantly different at $p<0.05$

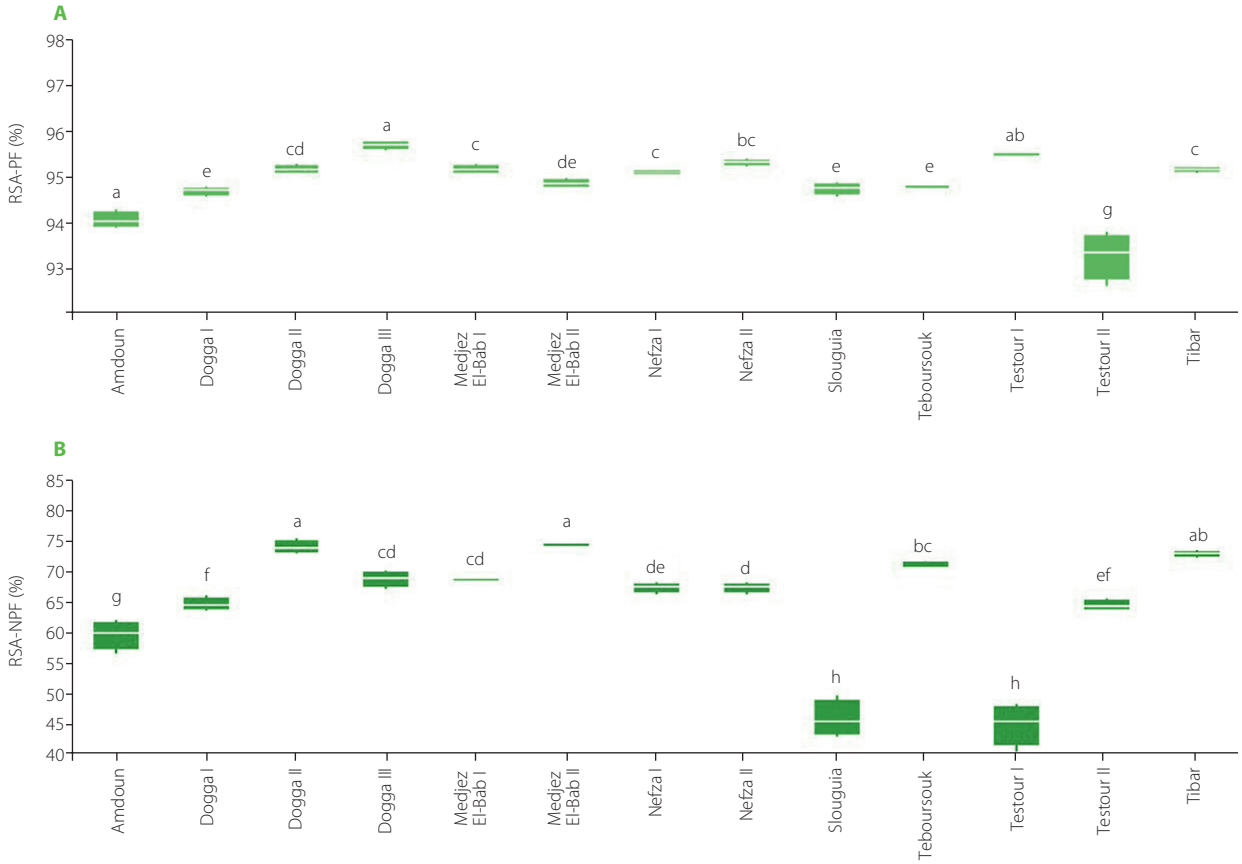

Figure 5. DPPH radical scavenging activity of the polar fraction (RSA-PF) (**A**) and non-polar fraction (RSA-NPF) (**B**) of the Chétoui extra virgin olive oils from selected mills in the Béja region in Tunisia. Data are expressed as box plots for four replicates. Means in the same box bearing different letters are significantly different at $p<0.05$.

to its antioxidant activity. Additionally, a study by Issaoui *et al*. [2015] reported that the antioxidant activity of Chétoui olive oil was influenced by several factors, including the maturity stage of the olives and the oil extraction method [Damak *et al*., 2008].

Hierarchical clustering analysis of olive oils.

After performing hierarchical clustering (Figure 6), it is evident that oils from mills of some regions of olive oil production share common characteristics while others differ significantly.

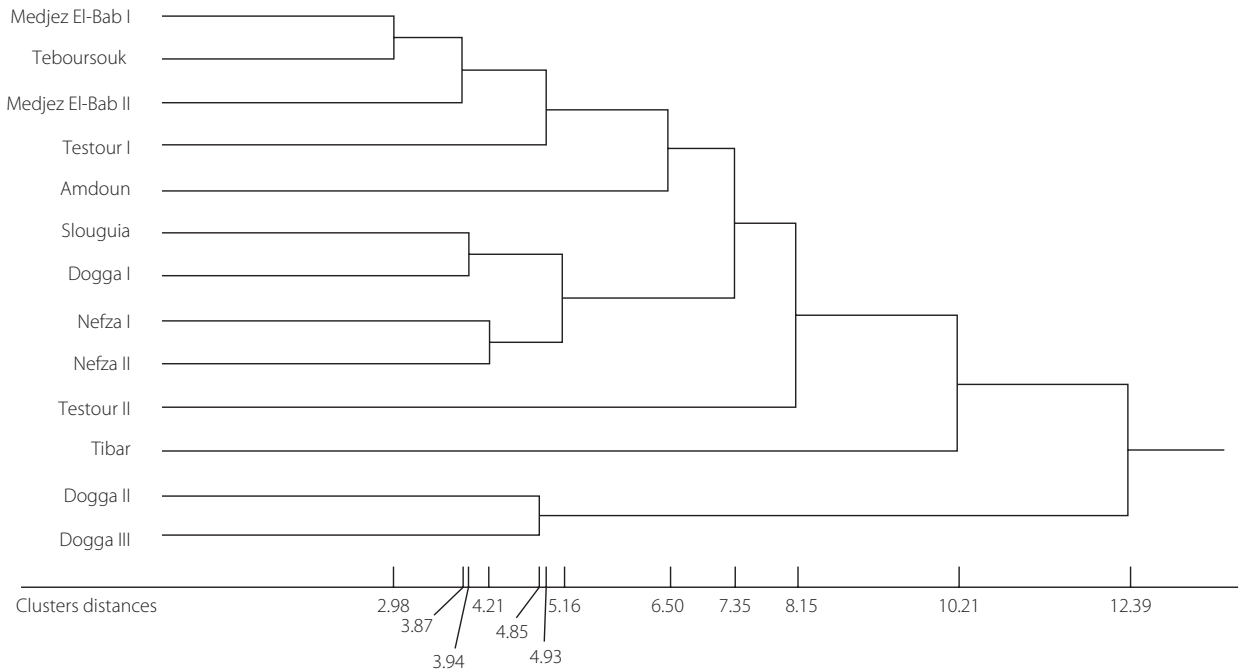

Figure 6. Dendrogram of hierarchical clustering analysis of the Chétoui extra virgin olive oils from selected mills in the Béja region in Tunisia according to their physicochemical properties.

The regions that were most similar in terms of the characteristics of the olive oil collected were Medjez El-Bab I and Teboursouk (cluster distance of 2.98), Medjez El-Bab I and Medjez El-Bab II (distance of 3.87), Slouguia and Dogga I (distance of 3.94), Nefza I and Nefza II (distance of 4.21), Dogga II and Dogga III (distance of 4.85), Medjez El-Bab I and Testour I (distance of 4.93), and finally Slouguia and Nefza I (distance of 5.17). On the other hand, the regions that are furthest apart were Medjez El-Bab I and Amdoun (cluster distance of 6.50), Medjez El-Bab I and Slouguia (distance of 7.35), Medjez El-Bab I and Testour II (distance of 8.15), Medjez El-Bab I and Tibar (distance of 10.21), and finally Medjez El-Bab I and Dogga II (distance of 12.39). These regions had olive oils with characteristics that differed significantly from the other regions. This classification is useful for understanding the differences and similarities between olive oils collected throughout the Béja region and can help identify appropriate strategies to improve olive oil production in this region.

CONCLUSIONS

In conclusion, our investigation of monovarietal extra virgin olive oils from Tunisian mills in the Béja region revealed significant disparities in key chemical parameters among the oils from 13 different mills. These findings underscore the remarkable diversity of Tunisian olive oils, strongly influenced by their geographical origins. Noteworthy, the oils from Testour II displayed the highest total phenolic content, while the oils from Amdoun and Dogga II exhibited high chlorophyll and carotenoid levels. The oil from Amdoun had lower tocopherol content compared to these from Testour II, Medjez El-Bab II, and Testour I mills. Fatty acid profiles were mostly similar across regions, except for the oil from Tibar with the lowest SFA content and highest MUFA content. Furthermore, substantial differences in PUFA content were observed, with the oil from Amdoun having the highest levels. Triacylglycerol compositions also reflected these regional distinctions. Lastly, our study revealed notably higher antioxidant activity in the polar fraction, especially in the olive oil from Dogga III. These results underscore the significant influence of geographical origin on the chemical composition and quality of Tunisian olive oils, emphasizing the importance of considering regional disparities in the evaluation and selection of olive oils. Additionally, they highlight the potential of these oils as natural sources of antioxidants.

ACKNOWLEDGEMENTS

The researchers wish to express their gratitude to the Deanship of Scientific Research at Taif University, Saudi Arabia, for their generous funding of this work. Additionally, the authors would like to extend their appreciation to Hermanos Blanco Barreña S.A. in Córdoba, Spain, for their invaluable support of this research.

RESEARCH FUNDING

This work was supported by Hermanos Blanco Barreña S.A., Córdoba, Spain and the Taif University, Saudi Arabia.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 367–374
DOI: 10.31883/pjfns/174972
ISSN 1230-0322, e-ISSN 2083-6007

Comparative Study on Drying Characteristics and Quality of Apple Cubes Dried in Two Different Microwave Dryers

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A rotary plate microwave dryer (RMD) and a newly-developed microwave convection coupled dryer (MCD) were used to dry apple cubes. The effects of microwave output power on drying, heating characteristics and quality attributes including scorching rate, color parameters, rehydration ratio, shrinkage, hardness, and sensory scores of the apple cubes were investigated and compared. The results showed that the microwave power required to complete drying in RMD was only 1/6 of that in MCD at the same microwave power density. Total drying time was 120, 60 and 30 min at 70, 210 and 350 W in RMD, respectively, while 160, 90, 80 and 60 min at 400; 800; 1,200; and 1,600 W in MCD, respectively. Compared with the products dried using hot air, the apple cubes dried in both dryers at the low microwave power had better rehydration capacity, less shrinkage and lower hardness as well as *a** and *b** value of color. Application of microwave power of over 800 W in MCD and over 210 W in RMD caused the increase in scorching rate as well as decreased the *L** value and the sensory quality of the apple cubes. Microwave drying in MCD with temperature control improved the quality of the dried product. The microwave drying conditions suitable for the apple cubes were 400 W in MCD and 1,600 W in MCD with temperature control followed by 70 W in RMD; the products obtained under these three condition variants had superior or comparable quality to the products obtained upon conventional hot air-drying.

Key words: diced apple, microwave drying, heating characteristics, scorching rate, texture, color parameters, sensory quality

INTRODUCTION

Microwave drying is an advanced method for fast dehydration of food based on dielectric heating [Fu *et al*., 2023; Keser *et al*., 2020]. The characteristics of microwave drying include shorter drying time, reduced energy consumption, improved product quality, and flexibility in the manufacture of various drying products. The application of microwave energy could reduce the drying time by 25–50% compared to conventional drying [Wang *et al*., 2022].

Microwave drying of fruits and vegetables has received considerable attention in the recent years as a tool enhancing both drying rates and product quality [Maftoonazad *et al*., 2022]. Factors affecting microwave drying include construction of the dryer, microwave power size and output mode, material characteristics, temperature control and combination with other drying methods, *etc*. [Aksüt *et al*., 2023; Bhat *et al*., 2022; Heshmati *et al*., 2023; Sun *et al*., 2019; Zeng *et al*., 2022]. Temperature control during drying and combination of drying with the traditional drying technologies such as vacuum drying, air drying and freeze-drying has shown great advantages in improving the quality of dried material, shortening drying time and reducing energy consumption [Chen *et al*., 2021; Joardder & Karim, 2022; Pham & Karim, 2022].

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Submitted: 21 July 2023
Accepted: 8 November 2023
Published on-line: 28 November 2023

Various types of microwave dryers were developed and reported to dry fruits and vegetables using microwave energy. Abderrahim *et al*. [2022] dried Algerian blood orange slices using a domestic digital microwave oven, which had the microwave chamber with the size of 476×272×388 mm; it was observed that the drying time required decreased from 54 to 15.5 min when the microwave power used increased from 200 to 800 W and the specific energy consumption decreased with increased microwave power. Tepe & Tepe [2020] dried the apple slices in a microwave oven with the chamber of 262×452×325 mm, the drying time decreased from 40 to 8 min as the microwave power increased from 120 to 460 W and the rehydration ratio of intermittent-microwave dried-apple slices was higher than hot-air dried-apple slices due to the expansion and puffing of the food by high internal pressure in microwave drying. Okmen & Bayindirli [2000] improved the domestic microwave oven for the study of drying dynamics, which could continuously record the temperature and weight of the sample during the microwave drying process. Zeng *et al*. [2023] developed a microwave hot-air rolling dryer (MHARD) with cylindrical drying capacity to dry ginger slices, and they found that increasing the microwave power from 0.6 to 0.9 W/g resulted in greater damage to the microstructure, promoted the release of starch, and improved the release of bioactive compounds, and once the microwave power further increased to 1.2 W/g, the content of these compounds would degrade. Poogungploy *et al*. [2018] developed a microwave convection combined dryer to dry macadamia nuts and found that the increase in microwave power and temperature had a positive effect on the shortening of drying time and the improvement of drying speed, microwave-assisted hot air drying with surface temperature control provided better product quality in terms of grain brightness and storage conditions, and drying without surface temperature control had the highest energy efficiency. Up to now, the research on fruit microwave drying has been mostly carried out in a single device, and there were few studies on the comparison of fruit drying behavior and product quality in different microwave dryers.

Therefore, this study was aimed to examine and compare the influence of microwave output power and temperature control on the drying kinetics, heating characteristics and quality properties (scorching rate, color parameters, rehydration characteristic, shrinkage, hardness and sensory quality) of the apple cubes in a rotary plate microwave dryer (RMD) and a newly-developed microwave convection coupled dryer (MCD); the related quality attributes of the microwave-dried apple cubes were also compared with these of the conventional hot air-dried (HD) ones.

MATERIALS AND METHODS

Sample preparation

Fresh Red Fuji apples (30 kg) were purchased from a local market in Qingyang (Gansu, China). The apples were peeled, cored and cut into cubes with an edge length of 1 cm for drying experiments.

Drying of apple cubes

A commercial rotary plate microwave dryer, RMD, (M1-211A, Guangdong Midea Manufacturing Co., Ltd, Foushan, China) was employed to dry the apple cubes in the first drying experiment. The equipment had a rectangular chamber with dimensions of 325×315×202 mm and could operate at five differently constant microwave powers. Its maximum microwave output power was 700 W, and the microwave power could be mechanically adjusted by means of pulse. The average microwave power used in the research was 70, 210 and 350 W. The apple cubes of 100 g for each drying were uniformly distributed on a glass turntable with a diameter of 24.5 cm. The microwave power density in terms of chamber volume was calculated as the ratio of microwave power to the volume of drying chamber while the microwave power density in terms of initial material load was calculated as the ratio of microwave power to the initial material load. The initial average power density corresponding to 70, 210 and 350 W was 0.7, 2.1 and 3.5 W/g in terms of material load while its value was 3.3, 10.0, 16.6 W/L in terms of chamber volume. During drying, the samples were taken out, and their moisture loss and temperature were measured quickly using a digital balance with 0.01 g precision (JH2102, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) and an infrared thermal imager (AX8, FLIR Systems Inc, Portland, OR, USA) at every 10-min interval, respectively.

Drying of apple cubes in the newly-developed microwave convection coupled dryer (MCD) was performed in the second drying experiment. MCD was a multifunctional dryer, where hot air drying, microwave drying and combined drying of both could be done. The dryer had a cube chamber with dimensions of 500×500×500 mm. Its maximum microwave output power was 2,000 W and the microwave power could be willfully set between 300 to 2,000 W. The microwave energy could be supplied by either continuous emission at any preset power, or intermittent emission through an automatic on–off controller based on the preset temperature. The apple cubes of 100 g were used and their surface temperature was measured or controlled with an infrared thermal imager (AX8, FLIR Systems Inc). The microwave power used was 400; 800; 1,200; and 1,600 W under the non-temperature control conditions. The corresponding power density in terms of material loading was 4.0, 8.0, 12.0, and 16.0 W/g while its value in terms of chamber volume was 3.2, 6.4, 9.6, and 12.8 W/L. When the temperature of the samples was controlled during drying, the initial microwave power of 1,600 W was set and the highest point of the sample temperature was controlled to 100°C. The weight of the apple cubes was automatically recorded at every 10-min interval.

The conventional hot air-dried (HD) apple cubes were also prepared using MCD at the air temperature and velocity of 60°C and 1 m/s, respectively.

Each drying experiment was carried out in triplicate. After the drying process was complete, the dried product was cooled to room temperature and packed into sealed polyethylene bags for further analysis.

Calculation of drying rate

During the drying process, the drying rate was calculated using the following Formula:

$$\text{Drying rate} = \frac{X_t - X_{t+\Delta t}}{\Delta t} \quad (1)$$

where: X_t, $X_{t+\Delta t}$, moisture content (kg water/kg dry matter) at time t and t+Δt, respectively; and Δt, time interval (min). Drying rate curves were plotted based on the change of the drying rate with moisture content.

Moisture content determination

Moisture content was determined by drying the sample in an oven at 105°C until a constant weight was obtained [AOAC, 1995].

Scorching rate estimation

The scorching rate was defined as the proportion of burnt apple cubes and calculated as the percentage ratio of burnt dried apple cubes to total apple cubes. The burnt cubes were characterized by local or whole carbonization and blackening; they were visually assessed after the drying was over.

Color measurements

Sample surface color was measured at the center of the sample using a colorimeter calibrated with a white standard plate (CR-400, Konica Minolta Co., Japan). Readings were indicated on a CIE1976 $L^*a^*b^*$ scale, where L^* meant lightness, with 100 being very white and 0 being dark; the a^* value represented green (–) to red (+) and b^* represented blueness (–) to yellowness (+). The measurements were carried out with 10 cubes for each treatment. Total color difference (ΔE) was calculated by the difference between values for the dried (L^*, a^* and b^*) and fresh (L_0^*, a_0^* and b_0^*) samples as follows:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (2)$$

Rehydration ratio determination

Rehydration experiment was performed according to the procedure described by Wang *et al*. [2019]. Five dried apple cubes were rehydrated by soaking in distilled water having a temperature of 25°C for 300 min. During rehydration, the samples were withdrawn from the flask at 30-min intervals, drained on the filter paper of a Büchner funnel for 60 s under vacuum to remove surface water, and weighed on an electronic balance. For all experiments, the solid-liquid ratio was kept at 1:50 (*w/v*). The rehydration ratios were calculated as the ratio of the g of rehydrated apple cubes to the g of the dried apple cubes. Rehydration curve was plotted in terms of rehydration ratio versus rehydration time. Each experiment was conducted in triplicate.

Shrinkage estimation

The volume of the sample was measured according to the fluid replacement method described by Wang *et al.* [2019]. The shrinkage percentage was calculated based on the volume of the apple cubes before and after drying. Ten dried apple cubes were randomly selected and determined from each sample lot.

Hardness analysis

The samples were subjected to a puncture test using a texture analyzer (CYHD-1, Penglai Electronic Products Center., Ltd., Shandong, China) fitted with a cylindrical probe 2 mm in diameter. The probe punctured the dried sample placed on a flat base at a constant rate of 1 mm/s. The travel distance of the probe was 20 mm for each test. The maximum force peak in the force-deformation curve of the puncture test was defined as the hardness of the sample. Ten dried apple cubes were randomly selected and determined from each sample lot.

Sensory evaluation

The sensory quality of the dried apple cubes was evaluated based on the sensory attributes, such as color, flavor, taste and texture. A categorized 25-point scale, anchored with "nothing" or "poor" for number 1 and "very intense" or "very good" for number 25, was used to measure the attributed intensity [González-Herrera *et al*., 2016]. All the prepared samples were coded with three-digit random numbers and presented in random order to 10 trained panelists. Final sensory score for each sample was the total score of the four individual attributes.

Statistical analysis

All data obtained in this study were statistically analyzed. The results were expressed as the mean ± standard deviation (SD) of all measurements for each processing. Variance analysis (ANOVA) and Duncan test were used to estimate the difference between the mean values. Mean values were considered to differ significantly at $p<0.05$. Analyses were performed using SPSS 17.0.1 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Drying characteristics

Figure 1 presents the variation of moisture content with time of apple cube drying at different microwave powers in RMD and MCD, respectively. The moisture of apple cubes sharply decreased at the initial drying stage and subsequently slowly decreased as the drying proceeded. With the increase in microwave power in both dryers, the total drying time to reach the required moisture was significantly shortened. This agrees with the trend observed during microwave drying of ginger slices and green bean [Doymaz *et al*., 2015; Zeng *et al*., 2023]. Dryer type had a significant effect on drying time (Figure 1). The total drying time was 120, 60, and 30 min when drying at 70, 210 and 350 W in RMD, respectively. During drying in MCD, the total drying time was 160, 90, 80 and 60 min at 400; 800; 1,200 and 1,600 W. The drying with temperature control at the initial microwave power of 1,600 W took 130 min in MCD. It was observed that RMD had a higher microwave power utilization efficiency than MCD under the same material loading (100 g of apple cubes). When drying at 400 W in MCD and at 70 W in RMD, their microwave power density in terms of chamber volume

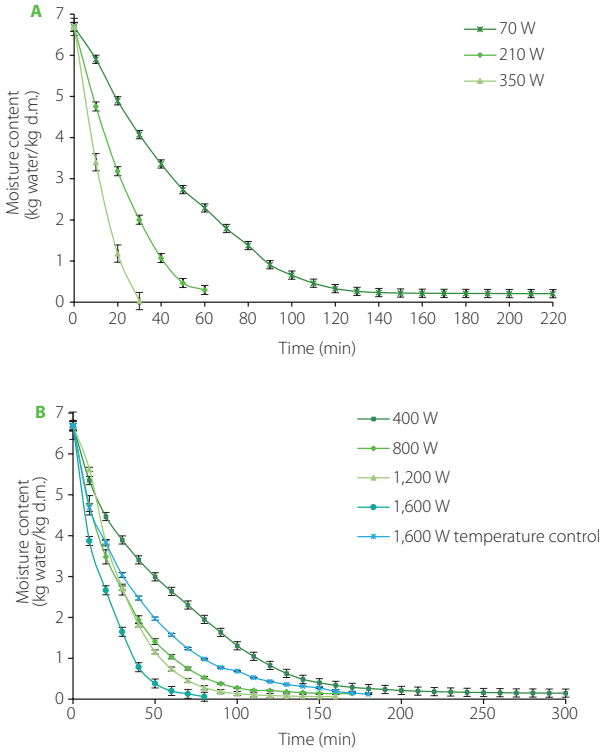

Figure 1. Drying curves of the apple cubes dried at different microwave powers in a rotary plate microwave dryer, RMD (**A**) and a microwave convection coupled dryer, MCD (**B**); d.m., dry matter.

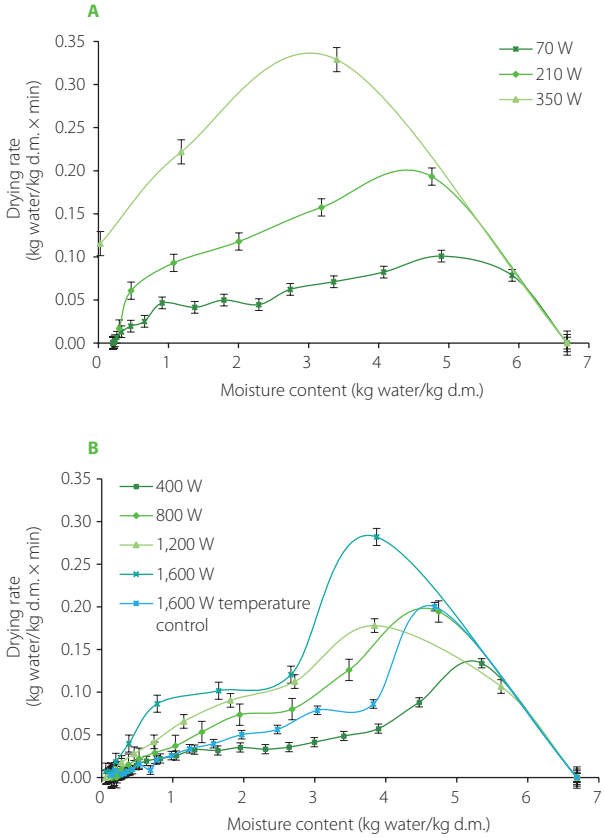

Figure 2. Curves of drying rate *versus* moisture content of the apple cubes dried at different microwave powers in a rotary plate microwave dryer, RMD (**A**) and a microwave convection coupled dryer, MCD (**B**); d.m., dry matter.

was almost the same (3.2–3.3 W/L). Drying at 1,200 W in MCD and at 210 W in RMD also ensured almost the same microwave power density in terms of chamber volume (9.6–10.0 W/L). Thus, the microwave power required to complete drying in RMD was only 1/6 of that in MCD at the same microwave power density of 3.2–3.3 or 9.6–10.0 W/L; moreover, the drying time in RMD was always shorter than in MCD in the two situations. The microwave utilization efficiency during microwave heating and drying was related to the microwave absorption by the material to be dried, which was affected by the shape and size of the drying chamber. It was concluded that the smaller the drying chamber, the better the microwave power absorption and utilization.

Figure 2 depicts the variation of the drying rate with moisture content of the apple cubes dried at various microwave powers in the two dryers. The high microwave power corresponded to the high drying rate and the entire drying process of the apple cubes under all the drying conditions happened at a falling rate. Similar trends were observed for apple and potato slices dried in the RMD [Khan *et al.*, 2018]. In all cases, there was no obvious constant rate drying period, indicating that the internal water diffusion played a decisive role in the drying process, which determined the drying characteristics of the raw material.

Temperature variation of the diced apple in the two microwave dryers

The changes in the temperature of the apple cubes during microwave drying at different microwave powers are shown in Figure 3. The temperature of the apple cubes was significantly

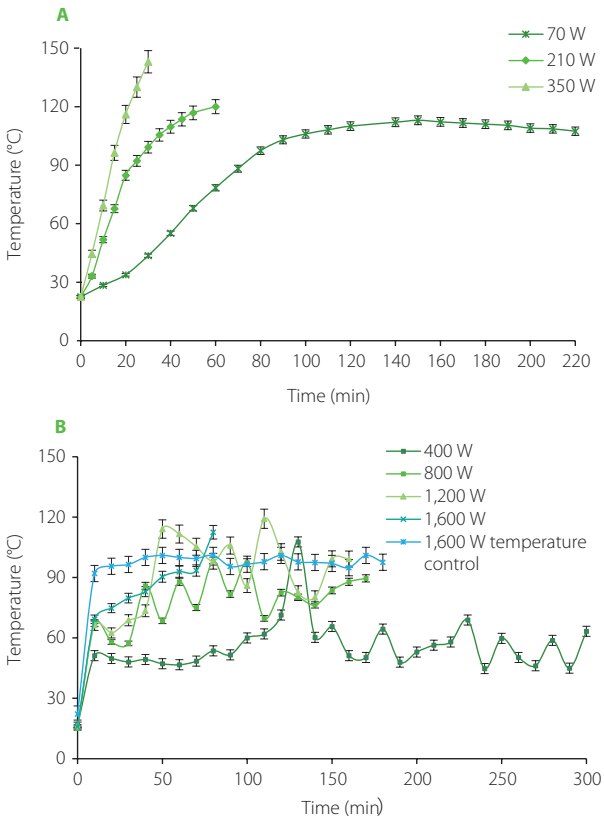

Figure 3. Temperature curves of the apple cubes dried at different microwave powers in a rotary plate microwave dryer, RMD (**A**) and a microwave convection coupled dryer, MCD (**B**).

affected by the microwave power applied in the two dryers. The higher the microwave power, the faster the heating rate, and the more prominent the thermal runaway. The two dryers also exhibited different heating capabilities. At the same power density, such as 3.2-3.3 or 9.6-10.0 W/L, the material temperature increased faster and the highest temperature reached was higher when the drying was done in RMD, indicating that the heating capacity for RMD was better than that of MCD. Compared with the constant microwave power, the microwave drying performed at temperature control could reduce the thermal runaway and temperature fluctuation of the materials to be dried. Meanwhile, high microwave power resulted in a continuous overheating of the samples. Guo *et al*. [2023] dried hawthorn in a pulse-spouted bed microwave freeze-dryer and found that a small amount (0.68 W/g) of microwave energy could lead to thermal runaway and make the temperature of the material rise quickly in the late drying period, which was consistent with the results of this study.

Effect of various drying conditions on color and scorching rate

Color is an important quality attribute of dried foods, which is the first parameter customers use to judge the quality of dried products [Chong *et al*., 2013]. Desirable dried products are the closest to the fresh fruit in color. The values of color parameters and scorching rate of the apple cubes dried at different microwave powers in two microwave dryers are listed in Table 1. Upon drying, a^* and b^* values increased significantly ($p<0.05$) for all the dried samples, whereas L^* values decreased significantly ($p<0.05$) for the samples dried at medium and high microwave power compared to the fresh samples. The samples dried using HD and microwave at low microwave power and controlled temperature had significantly ($p<0.05$) higher L^* values than the fresh ones. Similar results were observed in dried onion slices, apple and pear [Chong *et al*., 2013; Maftoonazad *et al*., 2022].

The color parameters determined for the dried apple cubes were significantly affected by the microwave power applied in the two microwave dryers (Table 1). In both microwave dryers, L^* values for the dried apple cubes significantly ($p<0.05$) increased with the microwave power while their a^* and b^* values first increased and then decreased ($p<0.05$). The highest a^* and b^* values were noted for the samples dried at the microwave power of 210 W in RMD and for those dried at the microwave power of 1,200 W in MCD. The highest b^* value and higher L^* value were determined for the sample dried at 1,600 W in MCD with temperature control. The ΔE values of all the dried samples were in the range from 51.5 to 136.6, and the sample dried at the controlled temperature had the highest ΔE value, followed by the sample dried using HD and at 210 W in RMD. In contrast, the lowest ΔE value was recorded in the products dried at 70 and 350 W in RMD. We observed that a smaller color difference did not necessarily mean the desired color possessed by a dried product. The browning in the dried fruits may commonly be attributed to oxidation of polyphenols and caramelization, and both drying for a long time and high temperature treatment could promote the occurrence of browning [Chong *et al*., 2013; Maftoonazad *et al*., 2022].

The scorching rates for the samples dried at the different conditions were in the range of 1.2–100.0% and significantly ($p<0.05$) increased with the increase of the microwave power used during drying in both the microwave dryers (Table 1). The scorching rate of the apple cubes dried in RMD and MCD at the maximum microwave power was 100.0% and 87.5%, respectively. The burning phenomena was most likely to occur when drying in RMD due to high heating efficiency. The scorching rate was only 2.3% when drying in MCD with the controlled temperature at the maximum microwave power. The determined results of both color and scorching rate for the dried apple cubes were also confirmed by the appearance of the samples (Figure 4).

TABLE 1. Color parameters and scorching rate of the apple cubes dried at different microwave power in rotary plate microwave dryer (RMD) and microwave convection coupled dryer (MCD).

Drying conditions		L^*	a^*	b^*	ΔE	Scorching rate (%)
Fresh		43.1±1.2^{c}	6.8±0.3^{h}	35.6±1.7^{i}	–	–
HD		49.2±2.4^{a}	26.0±2.5^{d}	121.7±4.3^{e}	134.7±3.2^{ab}	1.2±0.3^{f}
RMD	70 W	46.1±2.2^{ab}	21.0±1.4^{f}	86.4±1.8^{h}	52.8±2.9^{g}	2.3±0.2^{e}
	210 W	25.2±2.5^{e}	52.4±1.3^{a}	159.0±3.3^{b}	132.8±2.1^{b}	45.5±1.3^{c}
	350 W	0.0±0.0^{g}	35.0±2.4^{b}	34.9±2.1^{i}	51.5±2.4^{g}	100.0±0.0^{a}
MCD	400 W	47.2±3.1^{ab}	23.5±1.9^{e}	107.3±3.4^{f}	73.7±1.9^{e}	2.5±0.2^{e}
	800 W	45.9±2.7^{ab}	23.0±1.5^{e}	94.5±3.3^{g}	61.1±3.7^{f}	23.5±2.6^{d}
	1,200 W	38.4±1.3^{d}	35.3±2.3^{b}	151.3±2.8^{c}	119.3±2.4^{c}	44.4±3.1^{c}
	1,600 W	17.8±2.4^{f}	17.8±1.7^{g}	131.3±4.3^{d}	108.8±3.3^{d}	87.9±5.3^{b}
	1,600 W temperature control	44.8±2.7^{b}	32.9±1.3^{c}	169.7±3.6^{a}	136.6±3.1^{a}	2.3±0.1^{e}

Results are mean ± standard deviation (n=3). Values with different superscript letters in the same column are significantly different ($p<0.05$). HD, hot air drying. L^*, lightness; a^*, red(–)/green(+) coordinate; b^*, blue(–)/yellow(+) coordinate; ΔE, total color difference.

Figure 4. Typical visual appearance of the apple cubes dried using different methods. The four pictures in the first row orderly indicate the hot air-dried sample at 60°C and microwave dried samples at 70, 210, 350 W in a rotary plate microwave dryer (RMD) from left to right. The five pictures in the second row orderly indicate the microwave dried samples at 400; 800; 1,200; 1,600 W in a microwave convection coupled dryer (MCD) and at 1,600 W in MCD with temperature control from left to right.

Effect of various drying conditions on rehydration characteristics

Rehydration can be considered as a measure of the damage to the material caused by the drying process [Maftoonazad *et al*., 2022]. Figure 5 presents the variation of the rehydration ratio *versus* time for the apple cubes dried at various drying conditions. In the initial rehydration stage, the rehydration rate increased sharply with the extension of rehydration time, and then gradually decreased to zero; the high absorption rate in the initial stage could be explained by capillaries and cavities near the surface of the material, which were quickly filled with water [Horuz *et al*., 2017]. The microwave power used in the two dryers had a significant effect on the rehydration ratio of the dried apple (Figure 5). At the same time, the higher the microwave power was, the lower was the rehydration ratio, indicating that the high microwave power resulted in microstructure damage. Compared with the apple cubes dried using HD, the apple cubes dried in RMD at 70 and 210 W as well as in MCD at 400; 800; and 1,200 W had better rehydration capacity. Similar results were reported for intermittent-microwave dried-apple slices by Tepe & Tepe [2020]. The stabilized rehydration ratio for the apple cubes dried in RMD at 70, 210 and 350 W was 5.22, 5.00, and 3.39, respectively, while its values noted for the apple cubes dried in MCD at 400; 800; 1,200; and 1,600 W were 5.73, 5.48, 5.11 and 4.53, respectively. The apple cubes dried using HD had a 4.70 rehydration ratio. The apple cubes dried in RMD at 350 W presented the lowest rehydration ratio, followed by the ones dried in MCD at 1,600 W during rehydration. The apple cubes dried at 1,600 W in MCD with temperature control had the highest rehydration ratio during rehydration, which ascribed to less irreversible deformation under the low heating intensity. The structural damage and porous structure development caused by excessive microwave heating led to a poor rehydration capacity of the sample to be dried [Tepe & Tepe, 2020; Wang *et al*., 2014].

Effect of various drying conditions on shrinkage

The shrinkage rates of the apple cubes dried in two microwave dryers are shown in Table 2. The microwave power applied in two microwave dryers had a significant effect on the shrinkage rate of the apple cube, which decreased successively with the increase of microwave power. The shrinkage percent of the apple cubes dried in various conditions was in the range 40.58–68.93%. The most severe shrinkage was observed in the sample dried using HD, followed by the one dried in the MCD with temperature control. The samples dried at 210 and 350 W in RMD had the least shrinkage due to too-rapid mass transport by microwave power, which could cause a 'puffing' phenomenon in the materials to be dried, resulting in the lesser shrinkage [Joardder & Karim, 2022; Mahiuddin *et al*., 2018; Tepe & Tepe, 2020].

Effect of various drying conditions on hardness

The hardness of apple cubes dried using different microwave powers in the two microwave dryers is shown in Table 2. The microwave power applied in two microwave dryers had a significant effect on the hardness of the apple cubes, which was observed to significantly ($p<0.05$) decrease with an increase in the microwave power in both microwave dryers. The hardness values recorded for all the dried samples were in the range of 19.01–39.00 N. The sample dried using HD had the highest hardness. The drying at 350 W in RMD and at 1,600 W in MCD resulted in the minimum hardness of the dried apple cubes. It was noted that the greater hardness of the dried apple cubes was associated with the higher shrinkage rate and greater rehydration ratio (Table 2, Figure 5). The differences in hardness of the dried fruits could be related to the formation of a hard shell on the surface due to the crystallization of sugars as well as the damage of structure in the material to be dried due to excessive heating; surface cracking had a positive influence on hardness while a loose and porous structure could decrease the hardness [Joardder & Karim, 2022; Wang *et al*., 2019].

TABLE 2. Shrinkage rate and hardness of the apple cubes dried at different microwave power in rotary plate microwave dryer (RMD) and microwave convection coupled dryer (MCD).

Drying conditions		Shrinkage rate (%)	Hardness (N)	Sensory evaluation
HD		68.93±1.31^{a}	39.00±1.05^{a}	79.8±1.2^{a}
RMD	70 W	63.37±2.19^{b}	29.89±2.19^{c}	80.3±2.8^{a}
	210 W	42.41±1.13^{f}	21.56±1.13^{f}	33.2±1.4^{e}
	350 W	40.58±0.91^{g}	19.01±0.91^{h}	0.0±0.0^{f}
MCD	400 W	60.51±0.92^{c}	30.87±0.92^{b}	81.8±2.6^{a}
	800 W	57.78±0.86^{d}	25.68±0.86^{d}	55.3±0.7^{b}
	1200 W	51.50±1.11^{e}	24.30±1.11^{e}	47.4±0.9^{c}
	1600 W	50.40±1.02^{e}	19.70±1.02^{g}	34.4±1.3^{d}
	1600 W temperature control	67.73±0.77^{a}	31.16±0.77^{b}	83.1±2.2^{a}

Results are mean ± standard deviation (n=3); values with different superscript letters in the same column are significantly different (p<0.05). HD, hot air drying.

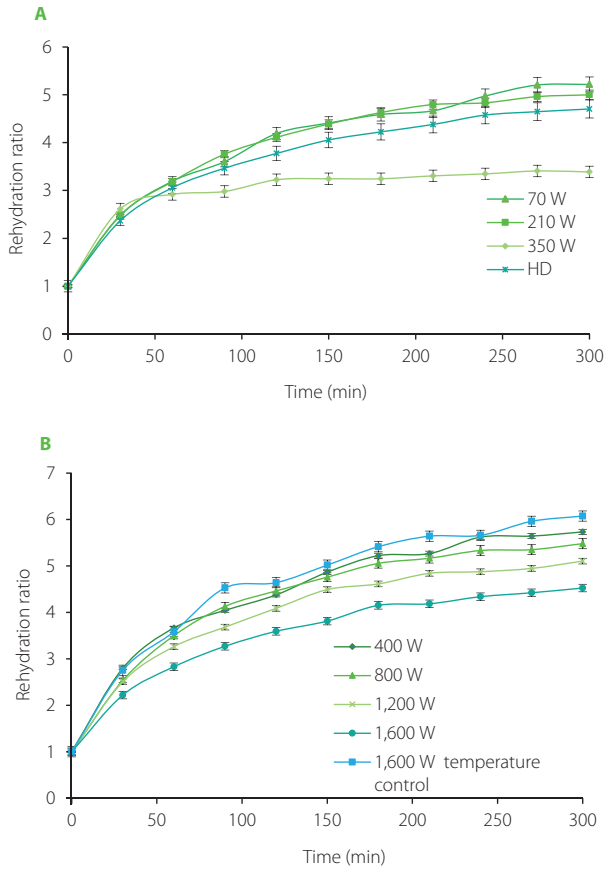

Figure 5. Rehydration ratio curves of the apple cubes dried at different microwave powers in a rotary plate microwave dryer, RMD (**A**) and a microwave convection coupled dryer, MCD (**B**) HD, hot air drying.

Sensory evaluation

The sensory scores and typical visual appearance of the apple cubes dried at the different microwave powers in the two microwave dryers are presented in Table 2 and Figure 4, respectively. It can be observed that the sensory quality of the dried apple cubes was significantly affected by the microwave power applied in the two microwave dryers, and that the sensory score greatly decreased with the increase of microwave power due to the presence of charring in the product. The apple cubes dried at 350 W in RMD were all burnt and had the worst quality, followed by the ones dried at 1,600 W in MCD. The samples dried at 70 W in RMD and at 400 W in MCD as well as at 1,600 W in MCD with temperature control scored above 80 and had excellent sensory quality, which was superior or closest to the one dried using hot air.

CONCLUSIONS

The investigation showed that the microwave dryer construction and microwave power applied had a profound effect on drying time and quality of the apple cubes. RMD had higher drying and heating efficiency than MCD. In both dryers, drying time significantly decreased with the increase in microwave. High microwave power could reduce the shrinkage and hardness of the apple cubes, but weaken the rehydration capacity, leading to serious scorching and decrease in sensory quality. Compared with drying without temperature control, the microwave drying in the MCD with a controlled temperature could greatly decrease scorching rate, and improve color, rehydration capacity and sensory quality of the dried product due to reducing the thermal runaway and temperature fluctuation of the materials to be dried. The products dried at 400 W in MCD and 1,600 W in MCD with temperature control as well as those dried at 70 W in RMD had superior to or comparable color, shrinkage rate, sensory scores and rehydration ratio with the conventional hot air-dried samples. The microwave drying condition suitable for the apple cubes was 400 W in MCD and 1,600 W in MCD with temperature control followed by 70 W in RMD. Further research is required to find out the effects of the preset temperature and convective conditions on drying characteristics and quality attributes.

RESEARCH FUNDING

The authors express their appreciation to the National Natural Science Foundation of China (32060544), Qingyang City Science and Technology Support Project (QNKB2-11) and the Foundation of Local Scientific and Technological Development Project from the Centre Government for supporting our research.

CONFLICT OF INTERESTS

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 375–384
DOI: 10.31883/pjfns/175032
ISSN 1230-0322, e-ISSN 2083-6007

Betaxanthin Profiling in *Beta vulgaris* Leaves and *Gymnocalycium mihanovichii* Grafted Cacti: A Comprehensive Study

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This study was focused on the identification and quantification of betaxanthins using high-performance liquid chromatography with a diode array detector coupled to electrospray ionisation mass spectrometry (HPLC-DAD-ESI-MS) in leaves of various cultivars of *Beta vulgaris* (beet) and *Gymnocalycium mihanovichii* grafted cacti. In *G. mihanovichii* grafted cacti, four betaxanthins, namely histidine-Bx, histamine-Bx, serine-Bx, and proline-Bx, were tentatively identified in the yellow, orange, pink, and red varieties, with contents ranging from 0.09 to 1.55 mg/kg fresh weight (FW). Betaxanthins were not detected in the green cactus. Histidine-Bx was the prevailing betaxanthin compound in the majority of cultivars. Fifteen betaxanthins were successfully identified in the leaves of five *B. vulgaris* cultivars (cv.): Snow Ball, Boldor, Cylindra, Rhubarb, and Round Dark Red. Leaves of yellow beet (cv. Boldor) had the highest total betaxanthin content (20.4 mg/kg FW), while white beet (cv. Snow Ball) had the lowest one (3.43 mg/kg FW). The leaves of red cultivars had comparable betaxanthin contents, ranging from 13.4 to 18.8 mg/kg FW, similarly to the yellow cultivar, indicating their potential as valuable sources of betaxanthins. There was no single dominant betaxanthin in *B. vulgaris* leaves. The leaves of *B. vulgaris* were found to be a richer source of betaxanthin than the grafted cactus *G. mihanovichii*.

Key words: beet, betalains, colorants, grafted cactus, LC-MS, secondary metabolites

INTRODUCTION

Nowadays, as society's interest in healthy nutrition continues to grow, food producers are actively searching for safe alternatives to synthetic food dyes, which frequently raise concerns regarding potential adverse health effects. Natural plant-based pigments are emerging as highly appealing options, not only for the absence of their harmful effects but also for their valuable health-promoting properties. Betalains, anthocyanins, carotenoids, and chlorophyll represent four essential groups of plant pigments abundantly found in nature, playing a significant role in maintaining an excellent overall state of health [Cai *et al*., 2005a; Manzoor *et al*., 2021; Stintzing *et al*., 2002].

Betalains, constituting a group of secondary metabolites found in plants of the Caryophyllales order, exhibit structural diversity, enabling their categorization into two groups: red-violet betacyanins and yellow-orange betaxanthins [Wybraniec *et al*., 2010]. They exert a significant influence on human health due to their various bioactivities, including antioxidant, antibacterial, anticancer, antiviral, and anti-inflammatory potential [Naseer *et al*., 2019]. Betacyanins derive from betanidin, an imine adduct of betalamic acid and *cyclo*-3,4-dihydroxyphenylalanine (*cyclo*-DOPA). Conversely, betaxanthins can be synthesized through the condensation of amino acids or amines with betalamic acid [Gengatharan *et al*., 2015]. It is worth highlighting that

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Submitted: 12 September 2023
Accepted: 9 November 2023
Published on-line: 5 December 2023

betaxanthins can function as a valuable dietary source of essential amino acids [Cai *et al*., 2001].

The only commercial source of betalains is the root of red beet (*Beta vulgaris* L.), which provides pigments in various shades of red and purple betacyanins as well as yellow and orange betaxanthins [Stintzing *et al*., 2002]. Currently, there are only few other known sources of betaxanthins, including the roots of yellow varieties of *B. vulgaris* [Spórna-Kucab *et al*., 2023], flowers of *Portulaca grandiflora* Hook. [Spórna-Kucab *et al*., 2022], fruits of *Hylocereus polyrhizus* [Wybraniec *et al*., 2009], yellow fruits of *Stenocereus pruinosus* [Sandate-Flores *et al*., 2020], yellow pulp of *Opuntia ficus-indica* [Fernández-López *et al*., 2018], tuber skin of *Ullucus tuberosus* Caldas [Mosquera *et al*., 2020], pure yellow genotype of *Celosia argentea* var. *plumosa* [Cai *et al*., 2005b], orange-red genotype of *Celosia argentea* var. *cristata* [Cai *et al*., 2005b], and leaves of *Amaranthus tricolor* L. [Cai *et al*., 2005b]. However, there are promising alternative sources of betaxanthins that have not been studied yet, such as the leaves *of B. vulgaris*.

B. vulgaris roots, commonly known as beetroots, rightfully earn the title of a superfood due to their numerous health benefits and their rich content of biologically active compounds, including betaxanthins, betacyanins, carotenoids, and flavonoids [Bangar *et al*., 2022; Székely & Máté, 2022]. Their bioactive compounds have been demonstrated capable of inhibiting the growth of specific types of cancer cells, scavenging free radicals, mititgating harmful cholesterol levels in the bloodstream, and alleviating inflammation within the body [Bangar *et al*., 2022; Székely & Máté, 2022]. Undoubtedly, one of the most significant advantages of beetroot is the ease of its cultivation, widespread availability, and affordability. Moreover, beet leaves, often considered as mere waste, are increasingly becoming a staple in our diets, as they constitute a valuable source of the above-mentioned bioactive compounds [Székely & Máté, 2022].

Cacti are ornamental plants with a perennial nature, characterized by succulent stems and slow growth rates. They are known for their exceptional ability to survive in dry conditions, displaying a wide variety of shapes and sizes [Perumal *et al*., 2019].

Within a year, betalains derived from beetroot are capable of meeting up to 10% of the global demand for food pigments [Manchali *et al*., 2012; Sadowska-Bartosz & Bartosz, 2021]. The food coloring market is flourishing at an annual growth rate of 4.6%, with estimates projecting it to reach a global market value of 2.3 billion dollars [Prajapati & Jadeja, 2022]. However, cacti, particularly the grafted *G. mihanovichii*, can also function as a source of betacyanins. Utilizing these plants as an alternative betalain source can broaden the spectrum of colorants and pigments [Belhadj Slimen *et al*., 2017]. *G. mihanovichii* grafted cactus has played a notable role in the subtropical regions of South and North America, being responsible for an impressive percentage of cactus production, reaching up to 70% [Belhadj Slimen *et al*., 2017]. This high percentage constituted a significant portion of exported specimens, primarily focused on the markets of the Netherlands and the United States due to their unique colors and forms, which are highly valued as ornamental potted plants. Currently, they do not have significant applications in the food, medical, or industrial sectors. Nevertheless, it is worth noting that the potential use of this cactus as a source of yellow-orange betaxanthins could greatly expand its export opportunities in the food and medical markets [Manchali *et al*., 2012; Sadowska-Bartosz & Bartosz, 2021].

The presented research involved the chromatographic analysis of betaxanthin profiles of the leaves of *B. vulgaris* and grafted cacti of *G. mihanovichii*, utilizing the high-performance liquid chromatography with a diode array detector coupled to electrospray ionisation mass spectrometry (HPLC-DAD-ESI-MS) technique. The main objective of this study was to investigate alternative natural sources of food colorants, betaxanthins. It is important to note that similar studies have not been conducted to date. Previous investigations primarily focused on the betaxanthin profiles of *Beta vulgaris* L., *Chenopodium formosanum*, *Opuntia ficus-indica* L., and *Portulaca grandiflora* Hook. [Gamba *et al.,* 2021; Kugler *et al.,* 2004; Otálora *et al*., 2020; Spórna-Kucab *et al*., 2023, 2022; Xie & Chen, 2021].

MATERIALS AND METHODS

Reagents and reference compounds

Acetone and formic acid purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland) were used for the extraction process. The solvents used were of analytical grade. Liquid chromatography-mass spectrometry (LC-MS) grade methanol and formic acid with a minimum purity of 98% were acquired from Sigma-Aldrich (St. Louis, MO, United States). Deionized water, obtained through a Milli-Q purification system (Merck, Darmstadt, Germany), was utilized in the experiments.

Plant material and extraction process

Plant material from five different cultivars of *B. vulgaris* (Snow Ball, Boldor, Cylindra, Rhubarb, and Round Dark Red) and five varieties of *G. mihanovichii* grafted cacti (orange, green, yellow, red, and pink) was used in the study, as illustrated in Figure 1.

The beet seeds were purchased from the company W. Legutko, located in Jutrosin, Poland. *B. vulgaris* plants were cultivated in the botanical garden of the University of Agriculture in Cracow, Poland, from June to September 2022 by a unit specializing in plant cultivation. Orange, green, and yellow cacti were imported from a Dutch plantation, whereas the red and pink varieties originated from a Polish company named Tomaszewski in Warsaw. After harvesting, the leaves of beets, as well as the upper colored parts of the cacti, were washed, weighed, and directly subjected to the extraction process.

The leaves of beets as well as the upper peeled parts of cacti were individually blended in a household blender. Appropriate extraction procedures were employed to extract betaxanthins from 100 g of beets and cacti. The beets were extracted using the maceration method [Celli & Brooks, 2017], utilizing 200 mL of a 50% (*v/v*) aqueous acetone solution. In contrast, the cacti underwent extraction using 300 mL of a 50% (*v/v*) aqueous

Figure 1. *Beta vulgaris* of selected cultivars - Boldor and Snow Ball (**A**) and *Gymnocalycium mihanovichii* grafted cacti in colors: yellow, green, orange, pink, and red (**B**).

acetone solution enriched with 1% formic acid. The entire extraction process lasted for 90 min, maintaining ambient temperature and ensuring darkness. Upon completing the extraction, the obtained extracts were filtered under reduced pressure to eliminate potential impurities, then evaporated using a vacuum evaporator (Hei-VAP Advantage, Heidolph, Germany) at 25°C, and lyophilized in a freeze dryer (Christ, Osterode am Harz, Germany). After lyophilization, the resulting extracts were weighed. The obtained crude extracts, following appropriate preparation, were employed for analysis using UV-Vis spectroscopy and LC-MS techniques.

Spectrophotometric quantification of total betaxanthins

A quantitative analysis of the total betaxanthin content was conducted using spectrophotometry with the Tecan Infinite 200 microplate reader (Grödig/Salzburg, Austria) [Stintzing *et al*., 2003]. Triple measurements of absorption were performed for the extracts from both beets and cacti, dissolved in water, with each sample having a volume of 200 µL. Spectrophotometric measurements were carried out in the range of 350 to 750 nm with a 1 nm step at a temperature of 25°C. Total betaxanthin content (BC) was calculated for absorbance measured at 474 nm (λ_{max}) according to Equation (1):

$$BC = \frac{A \times DF \times MW \times 1{,}000}{\varepsilon \times l} \quad (1)$$

where: A is the absorbance, DF is the dilution factor, l is the path length (0.53 cm) of the microplate, ε is the molar absorption coefficient for betaxanthin (4.80×10^4 $cm^{-1}M^{-1}$), and MW is molecular weight (339 g/mol) [Stintzing *et al*., 2002]. Results were expressed in mg of pigment *per* 100 g of dry extract (DE) and in mg of betaxanthins *per* kg of fresh weight (FW) of plant material.

HPLC-DAD-ESI-MS analysis of betaxanthins

Before analysis, all samples were carefully diluted in demineralized water (15 mg *per* 500 µL of water) and briefly centrifuged at 3,000×g for 5 min using a centrifuge (Hermle Z323K, Gosheim, Germany). The betaxanthin profiles in beets and cacti were determined using the HPLC-DAD-ESI-MS technique (LCMS-8030 system, Shimadzu, Kyoto, Japan). The LC-MS system included a precise SIL-20ACXR autosampler, an efficient degasser, and a binary pump LC-20ADXR Nexera. Samples were separated using a Kinetex C18 chromatographic column (Phenomenex, Torrance, CA, United States) with dimensions of 100 mm length × 4.6 mm i.d., containing 5.0 µm particles and protected by a 4 mm length × 2 mm i.d. guard column of the same material (Phenomenex). The column temperature was maintained constant at 40°C. Analyses were carried out using a two-component gradient. The mobile phase consisted of methanol (A) and 2% formic acid in water (B). The flow rate was 0.5 mL/min, and 10 µL of the sample was injected for analysis. The solvent gradient system for extracts was as follows: 1% A in B at 0 min, gradient to 11% A in B at 12.0 min, 60% A in B at 24 min, and then gradient to 90% A in B at 24.01 min. UV/Vis spectra were collected using a DAD detector model SPD-M20A (Shimadzu).

In ESI-MS analyses conducted in the positive electrospray ionization mode, the capillary voltage was set at 4.5 kV, and the capillary temperature was maintained at 250°C. ESI-MS data were recorded in the scan mode with *m/z* ranging from 100 to 2,000 Da and the selected ion monitoring (SIM). LabSolution software version 5.91 SP1 (Shimadzu) was used for data acquisition in the HPLC-DAD-ESI-MS configuration.

Reference standards from *B. vulgaris* cv. Chrobry [Spórna-Kucab *et al*., 2023] and *P. grandiflora* Hook. extracts [Spórna-Kucab *et al*., 2022], which contained previously identified betaxanthins, were used to identify individual betaxanthins in the extracts.

The quantitative analysis of individual betaxanthins was carried out by determining peak areas from MS chromatograms of *B. vulgaris* and *G. mihanovichii* extracts. The total betaxanthin content in the examined extracts was previously determined through spectrophotometric method. All samples were analyzed in three independent LC-MS runs.

Statistical analysis

The data were presented as mean and standard deviation (SD) based on three independent analyses. Statistical analysis was conducted using Statistica software version 7.1 (StatSoft, TIBCO Software Inc., Palo Alto, CA, United States), employing one-way analysis of variance (ANOVA) and the Tukey post hoc test, with a significance level of α set at 0.05. *p*-Values below 0.05 were considered statistically significant. The statistical analysis was performed separately for *B. vulgaris* leaves and *G. mihanovichii* grafted cacti.

RESULTS AND DISCUSSION

Betaxanthins in *G. mihanovichii* grafted cacti

Studies on betalain profiles in their numerous sources demonstrate that betacyanins were often found in conjunction with betaxanthins [Cai *et al*., 2005a; Otálora *et al*., 2020; Spórna-Kucab *et al*., 2013, 2018, 2022, 2023; Wybraniec *et al*., 2010; Xie & Chen, 2021]. One of the sources of betacyanins are cacti. Previously, a total of 32 different betacyanins were identified in the red variety of *Gymnocalycium mihanovichii* cv. Hibotan scions [Wybraniec *et al*., 2010]. However, there is a lack of information regarding the presence of individual betaxanthins. Interestingly, according to a previous source [Wybraniec *et al*., 2010], the yellow-orange color is not attributed to the presence of betaxanthins but rather to the synthesis of carotenoids in cacti. In the mentioned study, no betaxanthins were detected in any of the analyzed violet, pink, and red cacti. Here, based on chromatographic, spectrophotometric, and mass-spectrometric data (Table 1), the presence of four polar betaxanthins was indicated in yellow, orange, red and pink varieties of *G. mihanovichii* grafted cacti: histidine-Bx (**1**), histamine-Bx (**2**), serine-Bx (**3**), and proline-Bx (**11**). The chemical structures of compounds **1** (predominant), **2** and **11** are shown in Figure 2. All the betaxanthins were solely identified in the red cactus variety, while they were absent in the green variety. Notably, the yellow and orange varieties lacked histamine-Bx (**2**), while the pink variety contained only histidine-Bx (**1**) and proline-Bx (**11**) (Figure 3). The samples displayed a range of total betaxanthin contents, varying from 0.09 to 1.55 mg/kg FW. The highest levels were determined in the red cactus (1.55 mg/kg FW) followed by the pink variety (1.29 mg/kg FW). Subsequently, the orange and yellow varieties had lower contents (0.22 and 0.09 mg/kg FW, respectively) (Table 2).

The total betaxanthin content determined in *G. mihanovichii* grafted cacti was lower than that of the yellow pulp of the *Opuntia ficus-indica* fruits, which was 275 mg/kg FW [Fernández-López *et al*., 2018]. Some similarities with *G. mihanovichii* grafted cacti were observed regarding the betaxanthin profile of the yellow

Table 1. Chromatographic, spectrophotometric, and mass-spectrometric data of the analyzed betaxanthins of leaf extracts of white, yellow and red *Beta vulgaris* cv. Snow Ball, Boldor, Cylinder, Rhubarb, and Round Dark Red, as well as *Gymnocalycium mihanovichii* grafted cactus (orange, green, yellow, red, and pink) extracts.

No.	Betaxanthin	Trivial name	t_R (min)	λ_{max} (nm)	*m/z* $[M+H]^+$
1	Histidine-Bx	Muscaaurin VII	4.5	470	349
2	Histamine-Bx		5.7	470	305
3	Serine-Bx		6.1	469	299
4	Glutamine-Bx	Vulgaxanthin I	6.2	467	340
5	Ornithine-Bx		6.7	465	326
6	Ethanolamine-Bx		7.0	454	255
7	Lysine-Bx		7.2	458	340
8	Glutamic acid-Bx	Vulgaxanthin II	8.4	469	341
9	Alanine-Bx		10.5	466	283
10	γ-Aminobutyric acid-Bx		12.2	454	297
11	Proline-Bx	Indicaxanthin	13.5	477	309
12	Valine-Bx		19.0	469	311
13	3-Methoxytyramine-Bx		19.6	471	361
14	Isoleucine-Bx	Isovulgaxanthin IV	21.0	469	325
15	Leucine-Bx	Vulgaxanthin IV	21.3	469	325
16	Tryptophan-Bx		22.0	473	398

Bx, betaxanthins; t_R, retention time; λ_{max}, absorption maximum wavelength; *m/z*, mass-to-charge ratio.

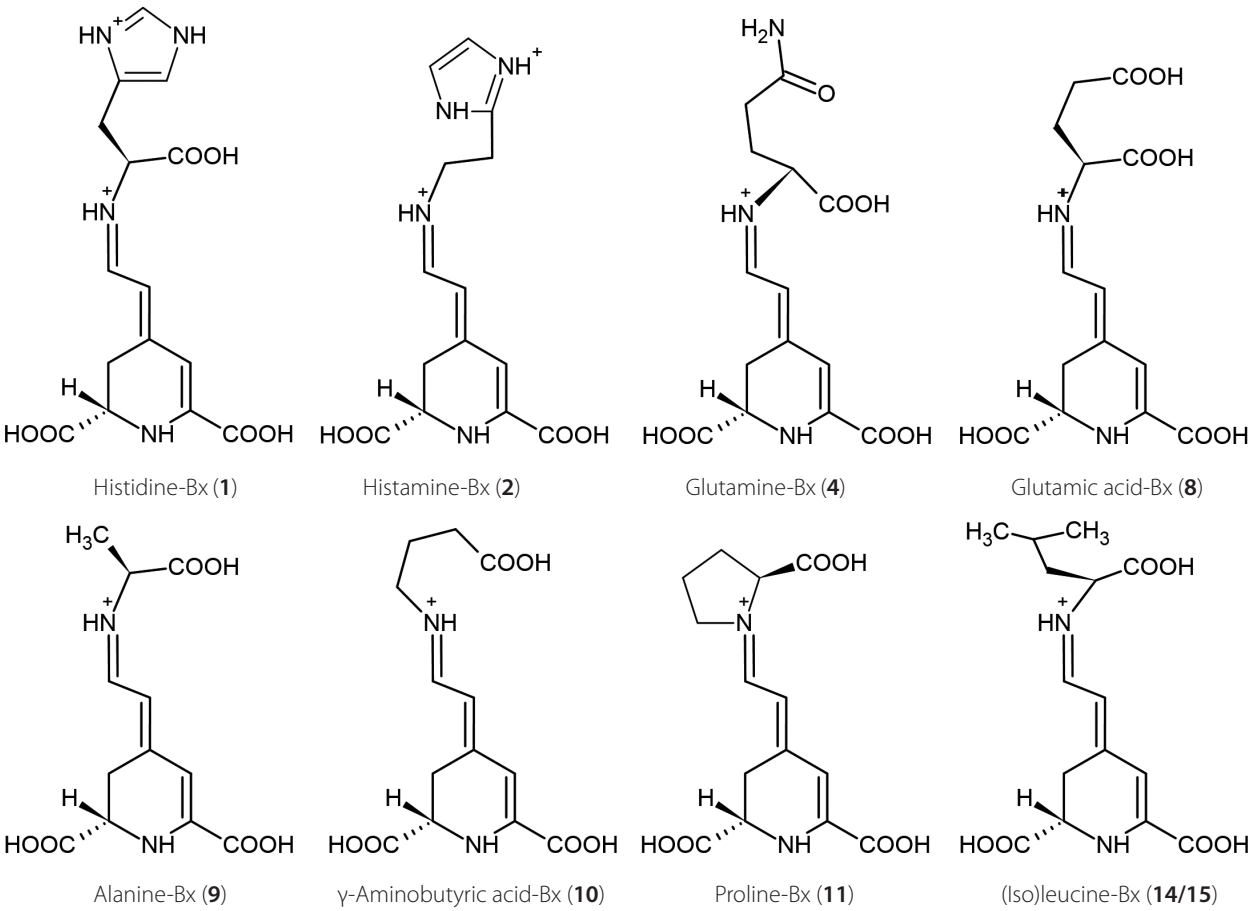

Figure 2. Chemical structures of predominant betaxanthins detected in the leaves of *Beta vulgaris* (compounds **2, 4, 8, 9, 10, 11, 14**, and **15**) and *Gymnocalycium. mihanovichii* grafted cacti (compound **1**).

O. ficus-indica fruits [Fernández-López *et al*., 2018]. Importantly, two specific compounds **1** and **11** were found in both profiles. However, in the case of *O. ficus-indica* fruits, compound **11** prevailed as the dominant pigment. In turn, compound **1** prevailed in *G. mihanovichii* grafted cacti scions, particularly in the orange, red, and pink varieties (Table 2). Additional betaxanthins, namely glutamine-Bx (**4**) and 5-methionine-betaxanthin, were detected in the yellow *O. ficus-indica* [Fernández-López *et al*., 2018], in contrast to their absence in *G. mihanovichii* grafted cacti. In turn, Kugler *et al*. [2007] analyzed the betaxanthin profile of *O. ficus-indica* cv. Gialla and identified a total of 13 distinct betaxanthins. Proline-Bx (**11**) was reported as the major betaxanthin. However, in this complex profile, histamine-Bx (**2**) was absent, in contrast to betaxanthin profile of the red variety of *G. mihanovichii* grafted cacti analyzed in our study (Figure 3, Table 2). Similarly, betaxanthin **2** and two other compounds, histidine-Bx (**1**) and serine-Bx (**3**), were not identified in the betaxanthin profile of *O. dillenii* [Betancourt *et al*., 2017].

The highest total betaxanthin content of *G. mihanovichii* grafted cacti found in the red variety (Table 2) was consistent with a previous study of 35 different cactus varieties, which showed that red varieties generally had the highest content of betaxanthins [Pérez-Loredo *et al*., 2016]. Interestingly, similar results were obtained in the case of beetroots, where red cultivars contained higher amounts of betaxanthins compared to the white and yellow varieties [Spórna-Kucab *et al*., 2023]. The total betaxanthin content in the red *G. mihanovichii* grafted cacti of 1.55 mg/kg FW (Table 2) was higher than that determined in green fruits of *O. ficus-indica* originating from the United States and reaching 1.7 mg/kg DW [Pérez-Loredo *et al*., 2016]. Here, analyses of the green variety of *G. mihanovichii* grafted cacti failed to identify any betaxanthins (Table 2).

The highest total content of betaxanthins in the *G. mihanovichii* grafted cacti extract was determined in the red variety, amounting to 19.5 mg/100 g DE, followed by the pink variety at 13.9 mg/100 g DE, then the orange variety at 5.3 mg/100 g DE, and finally the yellow variety at 1.4 mg/100 g DE. The compound profile of *G. mihanovichii* grafted cacti is not complex. Therefore, the isolation of histidine-Bx (**1**) from the extract of red and pink *G. mihanovichii* grafted cacti is indeed achievable.

Betaxanthins in *B. vulgaris* leaves

For the first time, the identification of betaxanthins has been accomplished in the leaves of *B. vulgaris* cv. Snow Ball, Boldor, Cylindra, Rhubarb Chard, and Round Dark Red. Analysis conducted by utilizing the HPLC-DAD-ESI-MS technique enabled a tentative identification of 15 betaxanthins: histamine-Bx (**2**), serine-Bx (**3**), glutamine-Bx (**4**), ornithine-Bx (**5**), ethanolamine-Bx (**6**),

Table 2. The content of total betaxanthins and individual betaxanthins in extracts from the leaves of five cultivars of *Beta vulgaris* and five varieties of *Gymnocalycium mihanovichii* grafted cactus (mg/100 g dry extract, DE) and the total betaxantin content in fresh weight (FW) of beet leaves and grafted cacti, respectively, (mg/kg FW), determined by HPLC-DAD-ESI-MS.

No.	Betaxanthin	*Beta vulgaris*					*Gymnocalycium mihanovichii*				
		Snow Ball	Boldor	Cylindra	Rhubarb	Round Dark Red	Orange	Green	Yellow	Red	Pink
1	Histidine-Bx	–	–	–	–	–	2.76 ± 0.20^{C}	–	0.99 ± 0.06^{D}	16.1 ± 1.5^{A}	12.5 ± 1.1^{B}
2	Histamine-Bx	14.7 ± 1.3^{b}	0.51 ± 0.03^{e}	19.5 ± 1.3^{a}	3.14 ± 0.20^{d}	5.89 ± 0.47^{c}	–	–	–	0.44±0.03	–
3	Serine-Bx	0.81 ± 0.06^{b}	–	–	0.62 ± 0.04^{c}	1.47 ± 0.10^{a}	2.40 ± 0.18^{A}	–	0.32 ± 0.03^{C}	2.33 ± 0.16^{B}	–
4	Glutamine-Bx	–	25.7 ± 2.3^{a}	–	2.68 ± 0.17^{b}	–	–	–	–	–	–
5	Ornithine-Bx	7.25 ± 0.75^{b}	5.96 ± 0.39^{c}	11.1 ± 0.9^{a}	2.86 ± 0.24^{e}	5.22 ± 0.39^{d}	–	–	–	–	–
6	Ethanolamine-Bx	–	8.28 ± 0.63^{a}	–	1.87 ± 0.17^{c}	6.76 ± 0.55^{b}	–	–	–	–	–
7	Lysine-Bx	–	3.75 ± 0.28^{a}	–	1.24 ± 0.12^{b}	1.13 ± 0.07^{b}	–	–	–	–	–
8	Glutamic acid-Bx	–	24.9 ± 2.1^{a}	18.0 ± 1.2^{b}	1.51 ± 0.13^{d}	8.39 ± 0.67^{c}	–	–	–	–	–
9	Alanine-Bx	–	11.6 ± 0.9^{c}	17.5 ± 1.1^{a}	15.9 ± 1.4^{b}	4.64 ± 0.34^{d}	–	–	–	–	–
10	γ-Aminobutyric acid-Bx	–	42.7 ± 3.6^{b}	32.2 ± 2.8^{c}	26.4 ± 2.3^{d}	44.8 ± 3.2^{a}	–	–	–	–	–
11	Proline-Bx	–	15.5 ± 0.9^{a}	13.3 ± 0.8^{c}	14.6 ± 1.1^{b}	7.32 ± 0.6^{d}	0.12 ± 0.01^{C}		0.04 ± 0.003^{D}	0.55 ± 0.04^{B}	1.36 ± 0.12^{A}
12	Valine-Bx	–	9.34 ± 0.70^{a}	5.57 ± 0.40^{c}	3.14 ± 0.24^{d}	6.07 ± 0.47^{b}	–	–	–	–	–
13	3-Methoxytyramine-Bx	–	3.07 ± 0.21^{a}	1.14 ± 0.08^{c}	2.02 ± 0.14^{b}	0.71 ± 0.09^{d}	–	–	–	–	–
14	Isoleucine-Bx	15.4 ± 1.3^{b}	2.31 ± 0.17^{e}	19.6 ± 1.6^{a}	2.99 ± 0.22^{d}	10.5 ± 0.7^{c}	–	–	–	–	–
15	Leucine-Bx	8.71 ± 0.69^{b}	5.06 ± 0.47^{d}	17.6 ± 1.3^{a}	3.54 ± 0.29^{e}	8.13 ± 0.65^{c}	–	–	–	–	–
16	Tryptophan-Bx	1.11 ± 0.09^{d}	2.62 ± 0.21^{b}	2.94 ± 0.22^{a}	2.99 ± 0.27^{a}	1.54 ± 0.10^{c}	–	–	–	–	–
Total content in extract (mg/100 g DE)		**48.0 ± 4.0^{e}**	**161.2 ± 14.0^{a}**	**158.5 ± 9.8^{b}**	**85.5 ± 5.5^{d}**	**112.5 ± 10.7^{c}**	**5.29 ± 0.50^{C}**		**1.35 ± 0.11^{D}**	**19.5 ± 1.3^{A}**	**13.9 ± 1.1^{B}**
Total content in FW (mg/kg FW)		**3.43 ± 0.35^{e}**	**20.4 ± 1.7^{a}**	**18.8 ± 1.3^{b}**	**15.4 ± 1.1^{c}**	**13.4 ± 0.9^{d}**	**0.22 ± 0.02^{C}**		**0.09 ± 0.01^{D}**	**1.55 ± 0.12^{A}**	**1.29 ± 0.08^{B}**

Data are expressed as mean ± standard deviation (n=3). The superscript letters within each row (a–e or A-D) mean significant differences between results (p<0.05). The statistical analysis was conducted separately for beet leaves and grafted cacti. The assigned betaxanthin numbers correspond to those listed in Table 1. –, Not detected.

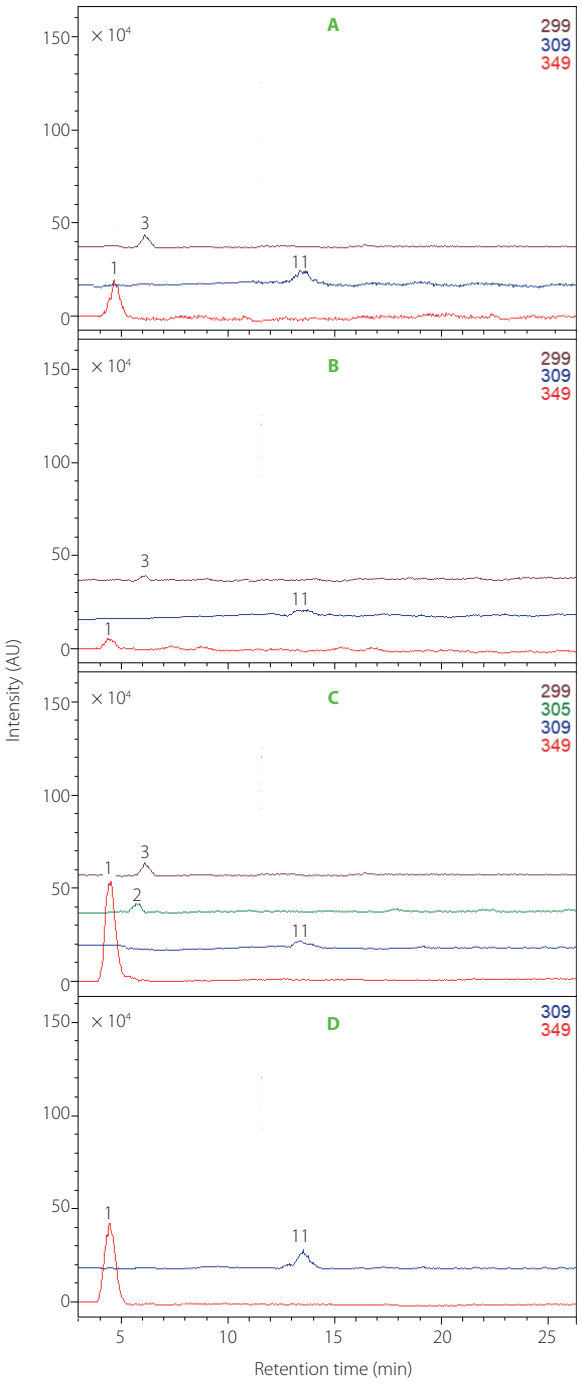

Figure 3. Chromatogram monitoring selected ions (ESI-MS) in the positive ion mode for betaxanthins from *Gymnocalycium mihanovichii* grafted cacti varieties: (**A**) orange, (**B**) yellow, (**C**) red, and (**D**) pink. The numbers and names are available in Table 1.

lysine-Bx (**7**), glutamic acid-Bx (**8**), alanine-Bx (**9**), γ-aminobutyric acid-Bx (**10**), proline-Bx (**11**), valine-Bx (**12**), 3-methoxytyramine-Bx (**13**), isoleucine-Bx (**14**), leucine-Bx (**15**), and tryptophan-Bx (**16**) (Figure 4, Table 1). The chemical structures of the dominant betaxanthins in beet leaves are shown in Figure 2. The presence of all compounds, except for histidine-Bx (**1**), was confirmed based on the root extract of *B. vulgaris* cv. Chrobry obtained in our previous study [Spórna-Kucab *et al*., 2023]. In turn, the extract from *P. grandiflora* analyzed previously [Spórna-Kucab *et al*., 2022] played a crucial role in betaxanthin **1** identification.

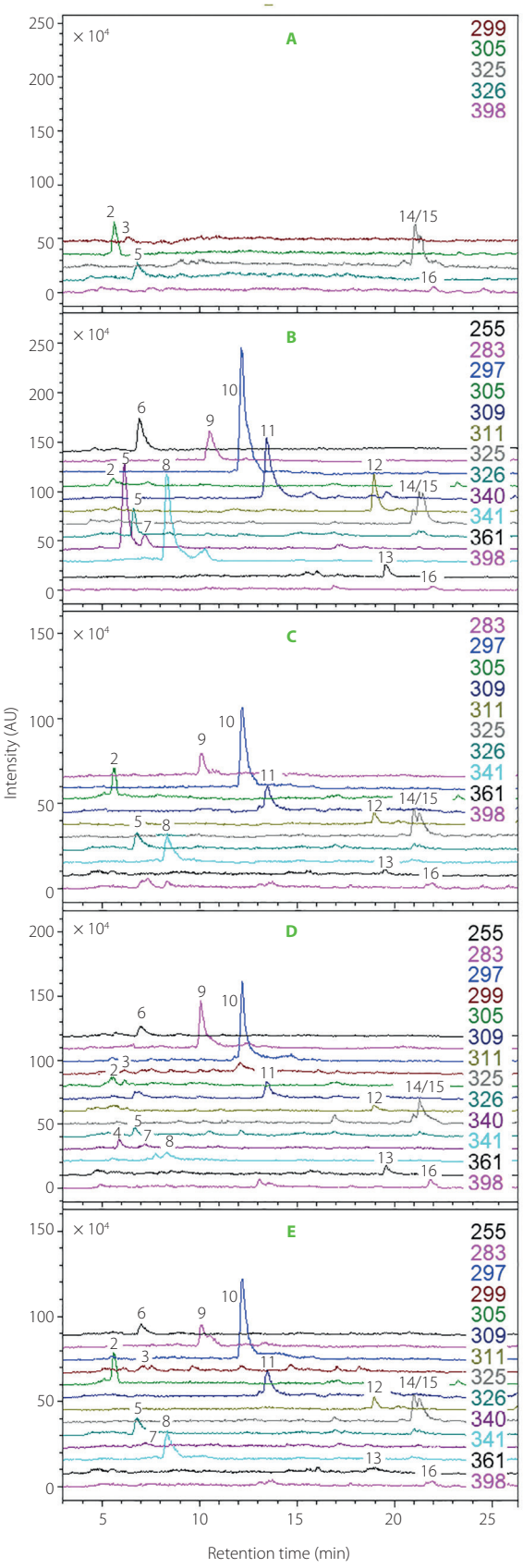

Figure 4. Chromatogram monitoring selected ions (ESI-MS) in the positive ion mode for betaxanthins from leaves of *Beta vulgaris* cultivars: (**A**) Snow Ball, (**B**) Boldor, (**C**) Cylindra, (**D**) Rhubarb and (**E**) Round Dark Red. The numbers and names are available in Table 1.

The qualitative profile of betaxanthins varied depending on the beet cultivar. Fifteen betaxanthins were identified only

in the Rhubarb cv. (Figure 4). In the other cultivars, various combinations of betaxanthins were noticeable. Remarkably, the Snow Ball cv. had the smallest diversity of betaxanthins, including 6 compounds.

Research on chemical compound profiles in different parts of a plant contributes to a more comprehensive understanding of the influence of chemical composition on plant properties and the selection of appropriate sources of specific natural compounds. The *B. vulgaris* cv. Boldor and Cylindra have already been analyzed for their betaxanthin profiles in their root systems in our previous study [Spórna-Kucab *et al*., 2023], which revealed a complex profile, including 23 betaxanthins. Additionally to most compounds indentified in *B. vulgaris* leaves in the current study, the presence of histidine-Bx (**1**), asparagine-Bx, arginine--Bx, glycine-Bx, threonine-Bx, dopa-Bx, dopamine-Bx, tyrosine-Bx, methionine-Bx, and phenylalanine-Bx was detected. It is worth noting that histamine-Bx (**2**) was not detected in the root samples. In contrast, it was present in the leaves of all *B. vulgaris* cultivars (Figure 4, Table 2). This observation is in agreement with previous research on the roots of red and yellow beet cv. Burpees Golden, as well as the leaves of Swiss chard cv. Bright Lights, which unequivocally established the presence of histamine--Bx (**2**) solely in the leaves, and its absence in the beet roots [Kugler *et al*., 2004, 2007].

Research on Swiss chard revealed the betaxanthin profile in leaves, emphasizing their potential as an equally rich source of betaxanthins [Kugler *et al*., 2004, 2007]. The presence of a total of 25 betaxanthins was established, including compounds that were not identified in the current study in the leaves of *B. vulgaris*. Specifically, these compounds were histidine-Bx (**1**), asparagine-Bx, aspartic acid-Bx, glycine-Bx, threonine-Bx, dopa-Bx, tyrosine-Bx, dopamine-Bx, methionine-Bx, tyramine--Bx, and phenylalanine-Bx. Whereas most of betaxanthins have previously been documented in Swiss chard [Kugler *et al*., 2004, 2007], ornithine-Bx (**5**) has been reported in the current study for the first time in leaves of all *B. vulgaris* cultivars. Interestingly, its presence was recently confirmed in roots of *B. vulgaris* Forono, Tytus, Ceryl, Boldor, and Chrobry cultivars [Spórna-Kucab *et al*., 2023].

The total betaxanthin content was the highest in fresh leaves of the yellow beet variety cv. Boldor (20.4 mg/kg FW), followed by the red cultivars: Cylindra, Rhubarb, and Round Dark Red (18.8 15.4, and 13.4 mg/kg FW, respectively), with the lowest value determined in the white cultivar Snow Ball (3.43 mg/kg FW). This study revealed lower total betaxanthin content in the leaves, ranging from 3.34 to 20.4 mg/kg FW, compared to 107 mg/kg FW assayed in the case of Swiss chard [Gamba *et al*., 2021; Kugler *et al*., 2007]. Here, the red beet cv. Cylindra had a similar betaxanthin content of 18.8 mg/kg FW compared to the yellow cv. Boldor which had 20.4 mg/kg FW. This may emphasize the underestimated significance of betaxanthins in red beets.

Dried extracts obtained from the leaves of the yellow beet cv. Boldor had the highest total betaxanthin content, reaching 161.2 mg/100 g DE. In contrast, the white cv. Snow Ball accumulated the lowest betaxanthin quantities, 48.0 mg/100 g DE. Red cultivars, on the other hand, had contents between these values (158.5, 112.5, and 85.5 mg/100 g DE for cultivars Cylindra, Round Dark Red, and Rhubarb, respectively). Betaxanthin content in dried extracts from the *B. vulgaris* roots of five cultivars (four red: Ceryl, Chrobry, Forono, and Tytus, as well as one yellow: Boldor) has been previously studied [Spórna-Kucab *et al*., 2023]. The results of that study revealed notably elevated betaxanthin content, with the highest levels noted in red beet cultivars: Ceryl, Chrobry, Forono, and Tytus (from 669 to 1231 mg/100 g DE for peel and 528 to 609 mg/100 g DE for flesh), rather than in the yellow ones (317 and 574 mg/100 g DE for flesh and peel, respectively). These findings suggest that, in contrary to the common perception that yellow beets are a rich source of betaxanthins, their value may be surpassed by those found in red cultivars. Within the leaves of *B. vulgaris*, substantial levels of betaxanthins were likewise detected in the red cultivars (Table 2).

Different pigments were found to predominate in various beet cultivars. In leaf extracts of all examined cultivars of *B. vulgaris*, γ-aminobutyric acid-Bx (**10**) was predominant, except for cv. Snow Ball. In the extract of cv. Boldor, there were notable levels of γ-aminobutyric acid-Bx (**10**) at 42.7 mg/100 g DE, glutamine-Bx (**4**) at 25.7 mg/100 g DE, and glutamic acid-Bx (**8**) at 24.9 mg/100 g DE. In the extract of cv. Rhubarb, the dominance of three pigments was noted: γ-aminobutyric acid-Bx (**10**) at 26.4 mg/100 g DE, alanine-Bx (**9**) at a level of 15.9 mg/100 g DE and proline-Bx (**11**) at a level of 14.6 mg/100 g DE. Compound **10** also dominated in the leaf extract of cv. Round Dark Red, with a quantity of 44.8 mg/100 g DE and in cv. Cylindra, with a quantity of 32.2 mg/100 g DE. Isoleucine-Bx (**14**) and histamine-Bx (**2**) were the major pigments in the extract of cv. Snow Ball, with contents of 15.4 mg/100 g DE and 14.7 mg/100 g DE, respectively. In leaf extract of cv. Cylindra, γ-aminobutyric acid-Bx (**10**) clearly dominated, reaching a level of 32.2 mg/100 g DE. In this cultivar, the following compounds stand out as well: isoleucine-Bx (**14**) with a content of 19.6 mg/100 g DE, histamine-Bx (**2**) with a content of 19.5 mg/100 g DE, glutamic acid-Bx (**8**) with a content of 18.0 mg/100 g DE, alanine-Bx (**9**) and leucine-Bx (**15**) with contents of 17.5 mg/100 g DE and 17.6 mg/100 g DE, respectively. These betaxanthins contribute unique properties to each cultivar, arousing curiosity about their potential health benefits.

Glutamine-Bx (**4**) and γ-aminobutyric acid-Bx (**10**) were predominant betaxanthins in *B. vulgaris* cv. Boldor, both in the roots [Spórna-Kucab *et al*., 2023] and in the analyzed leaf extracts. Moreover, betaxanthin **4** prevailed in all studied root extracts (Snow Ball, Boldor, Cylinder, Rhubarb, and Round Dark Red), except for the peel of yellow *B. vulgaris* (cv. Boldor) where betaxanthin **11** predominated. This underscores the significance of these compounds in the overall composition of beetroot, encompassing both its roots and leaves.

CONCLUSIONS

In conclusion, this study has provided valuable insights into the presence and distribution of betaxanthins in different varieties of *Gymnocalycium mihanovichii* grafted cacti and the leaves of *Beta vulgaris* cultivars. The research revealed distinct betaxanthin

profiles in different varieties of these plants, shedding light on their potential as sources of these pigments. In *G. mihanovichii* grafted cacti, preliminary investigations indicated the presence of four polar betaxanthins, *i.e.*, histidine-Bx (**1**), histamine-Bx (**2**), serine-Bx (**3**), and proline-Bx (**11**). The total betaxanthin contents varied among these varieties with the highest levels observed in the red one, followed by the pink, orange, and yellow ones. Betaxanthins were not identified in the green cactus variety. When comparing *G. mihanovichii* grafted cacti to other studied cacti, it becomes evident that while the betaxanthin content in *G. mihanovichii* grafted cacti was lower, its profile was unparalleled in other plants.

Betaxanthins in the leaves of various of *B. vulgaris* cultivars, including Snow Ball, Boldor, Cylindra, Rhubarb Chard, and Round Dark Red, were identified for the first time ever. The qualitative profile differed among cultivars, with the greatest diversity found in the Rhubarb cultivar. Their contents in *B. vulgaris* leaves varied among cultivars as well, with yellow cultivar Boldor exhibiting the highest total betaxanthin content. It is worth noting that the contents found in the red cultivars were also very high, confirming that the leaves of these beet cultivars are also an excellent source of betaxanthins.

In summary, this research has expanded our knowledge of betaxanthins in *G. mihanovichii* grafted cacti and leaves of *B. vulgaris*, highlighting them as sources with diverse pigment profiles.

RESEARCH FUNDING

This research was financed by the Polish National Science Centre for years 2019-2020; Project No. 2019/03/X/ST4/00968.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 4, 385–401
DOI: 10.31883/pjfns/175550
ISSN 1230-0322, e-ISSN 2083-6007

Cricket Flour and Pullulan Microparticle Formation *via* Electro-Blow Spinning as a New Method for the Protection of Antioxidant Compounds from Fruit Extracts

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Cricket flour was evaluated as an encapsulation material for protecting phenolic-rich fruit extracts (cranberry fruit and pomegranate peel extracts) and compared to pullulan. Electro-blow spinning (EBS) was used as a high throughput technique for encapsulation and compared to freeze-drying. The particles' morphology was analyzed *via* scanning electron microscopy (SEM). Fourier transform infrared and UV-vis spectroscopy were used for chemical characterization and encapsulation efficiency determination, respectively. The extract stability and antioxidant activity of the microparticles were studied by exposing samples to UV light irradiation for 30 h. Both extracts were successfully encapsulated in all encapsulating materials. SEM analysis showed that the obtained materials were micro-sized with a shape of capsule. Encapsulation efficiency was between 58.5 and 88.1% for the samples made *via* EBS and 51.2 to 79.3% for those made *via* freeze-drying. Encapsulation brought a significant improvement of extract stability and antioxidant activity. The non-protected extracts lost 50% of their antioxidant activity after 30 h of UV light radiation, while those protected with pullulan and cricket flour filtrate mixture experienced a 20% activity reduction. These findings indicate EBS to be a successful technique for the encapsulation of bioactive molecules, and cricket flour to be a new potential encapsulating material candidate that proves best when using a copolymer, such as pullulan.

Key words: electro-hydrodynamic processing, pomegranate peel extract, cranberry fruit extract, edible insect, polysaccharide, encapsulation

INTRODUCTION

Antioxidant compounds, such as flavonoids, phenolic acids, carotenoids, and tocopherols, are heavily used in the food, cosmetic, and pharmaceutical industries because of their therapeutic and disease-preventing properties, namely their antimicrobial, anticarcinogenic, antidiabetic, antihypertensive and anti-inflammatory activities [Cilek *et al.*, 2012]. They are known to prevent or reduce oxidation, mitigate the adverse effects of free radicals in tissues, and prolong the shelf life of other products and bioactive molecules [Yağmur & Şahin, 2020]. Natural antioxidants can be extracted from plants, for example cranberries and pomegranates. Cranberry extract is highly ranked for its antioxidant quality and quantity [Niesen *et al.*, 2022; Tsirigotis-Maniecka, 2020]. Its antioxidant activity is attributed to the interdependent actions of its organic acids, carbohydrates, and flavonoids. Among the many benefits of cranberry extract, some of the most notable include its ability to inhibit oxidative processes and growth of several tumor cells, and to aid in preventing

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Submitted: 24 May 2023
Accepted: 16 November 2023
Published on-line: 6 December 2023

urinary tract infections by inhibiting adhesion of *Escherichia coli* to urinary tract tissues [Tsirigotis-Maniecka, 2020].

Pomegranates are another natural source of antioxidants, specifically flavonoids, condensed tannins, ellagitannins, and gallotannins [Shirode *et al*., 2015]. The peels and seeds of this fruit contain most of these antioxidant molecules but are often discarded during the juice production process [Banerjee *et al*., 2017]. Specifically, pomegranate peels contain more than 30% of the total anthocyanins in the fruit [Azarpazhooh *et al*., 2019] and the highest content of punicalagin – a water-soluble ellagitannin. When hydrolyzed in the small intestine, punicalagin has been found to exhibit antioxidant, antifungal, and antibacterial properties [Kaderides *et al*., 2020]. When recovered, pomegranate peel extracts have also been successfully used as natural antimicrobial agents against *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Yersinia enterocolitica* and have been also proven to elicit beneficial effects against urinary tract infections [Cui *et al*., 2020]. Furthermore, pomegranate extracts, including peels, can be used in the cosmetic and pharmaceutical industry as therapeutic agents against a broad range of conditions, such as skin inflammation, acne, psoriasis or even skin cancer [Kalouta *et al*., 2020].

Despite these many benefits, antioxidant-containing fruit extracts have also been found to be very sensitive to environmental conditions, like oxygen, pH, ultraviolet (UV) light, temperature, and humidity. To circumvent these limitations, various protection methods have been used, of which, encapsulation is one of the most common [Kaderides *et al*., 2019]. By encapsulating bioactive molecules, they can be shielded from the environments that act to degrade them. However, not all encapsulating materials or techniques provide the same degree of protection. The type of encapsulating material and encapsulating technique can profoundly impact the lifetime and stability of bioactive molecules.

Amongst the encapsulation techniques, some common ones include spray-drying, freeze-drying, self-assembly of polymers, emulsification, and liposome preparation [Rostami *et al*., 2019]. Some of these involve critical temperatures (spray-drying) which can degrade certain bioactive molecules. Others require equipment that is very expensive or produces unsatisfactory yields. Electrohydrodynamic (EHD) processing has been of recent interest because it may be performed at ambient conditions and also because its start-up cost is low. EHD processing is a technique in which a polymeric solution is pumped through a circuit and ejected towards a collector. Because of an electrical potential difference between the polymeric solution and the collector, the polymeric solution is rapidly ejected to the collector. The solvent evaporates and, due to strong electrostatic forces, nano-/micro-particles or microfibers are formed [Rostami *et al*., 2019]. However, EHD processing is limited by low throughput. Typical flow rates for particle formation are less than 1mL/h, which hinders its application in the industry [Perez-Masia *et al*., 2015]. This limitation has been addressed by many researchers [Vass *et al*., 2020] but proposed solutions mostly apply to the industrial space. A new method that can be easily used in lab as well as industrial settings and that drastically improves the throughput is electro-blow spinning (EBS). EBS is a form of EHD processing improved by involving two driving forces during material production instead of one: electric force and air force. This combination enhances productivity, stabilizes the production process, and can also improve properties of the resulting products. With high solution feed rates, EBS is expected to be an important method for mass production of micro- and nano-materials. The EBS technique has already been successfully used to create fibers from polytetrafluoroethylene, hyaluronic acid, or oxide ceramic fibers [Zhou *et al*., 2017], but, to the authors' knowledge, it has not been used for microparticle formation.

To better understand how the broad space of particle formation may benefit from EBS, equipment costs, energy costs, and amounts of time needed were compared for three common microparticle formation techniques: freeze-drying, electrospinning, and electro-blow spinning. Comparing first equipment costs, the lab-scale equipment for freeze-drying cost starts from around 15,000 €. There are no commercial set ups for electro-blow spinning, but equipment prepared in-house can cost around 3,600 €. Similarly efficient electrospinning set ups can be assembled for around 3,500 €. Therefore, these numbers suggest that the EBS technique has economic advantages compared to other techniques. Comparing next the energy consumption during microparticles production, we find that the production of the same amount of material (around 15 g of dry material) costs 25 €, 2.5 € and 6 €, for freeze-drying, electrospinning, and electro-blow spinning, respectively. Lastly, when comparing the time required to produce similar amounts of material, EBS consumes around 8 h, while freeze-drying and electrospinning need around 24 and 500 h, respectively. Hence, electro-blow spinning is a much less time-consuming technique.

Of the biomaterials for encapsulation, some of the most widely used are zein, gelatin, whey protein concentrate, silk, collagen, and pullulan [Rostami *et al*., 2019]. Pullulan is an extracellular, linear polysaccharide produced by the dimorphic fungus *Aureobasidium pullulans* in starch and sugar crops [Yang *et al*., 2020]. It is not only used in its pure form for encapsulation, but also to facilitate the ease of use of other biopolymers for encapsulation [Aguilar-Vázquez *et al*., 2018]. It is a very desirable encapsulating material for tissue engineering and drug delivery for the following reasons: it is non-toxic, odorless, tasteless, and edible; it is easily modified *via* its backbone of hydroxyl groups; and accepted by the American Food and Drug Administration (FDA) [Carvalho *et al*., 2020].

One material that, to the authors' knowledge, has not yet been studied for the encapsulation of bioactive molecules is cricket flour. It is a flour made by dehydrating or roasting insects followed by grinding them into a fine powder. This means that processed cricket flour could be used to formulate supplement food protein powders [David-Birman *et al*., 2018]. Though crickets and other insects have been slow to disseminate into the Western consumption culture, they offer many great nutritional and environmental benefits. Nutritionally, insects are not only rich in essential amino acids, but also valuable sources

of unsaturated fatty acids, dietary fiber, vitamins, and minerals [Mlček *et al.*, 2019; Montowska *et al.*, 2019; Orkusz, 2021]. Furthermore, they are naturally gluten-free, making them desirable for the production of foods for celiac patients [Wieczorek *et al.*, 2022]. Studies have also shown that cultivating crickets with diets equal in quality to that of traditional livestock, results in a food conversion ratio that is about twice as high as that achieved in broiler chicken and pig production [Zielińska *et al.*, 2018]. From an environmental standpoint, cricket farming has been found to produce 80% less greenhouse gas emissions than cattle. Crickets thus pose great potential for use in the food and pharmaceutical industries looking to improve their nutritional and environmental benefits. Presenting them in the form of flour or an encapsulating material may make them more readily accepted by Western consumption culture. Moreover, owing to a high protein content, its good solubility and capacity to form gels [Ndiritu *et al.*, 2019; Stone *et al.*, 2019], cricket flour can be considered as a potential encapsulating material of bioactive compounds.

Protection of bioactive materials from harsh environmental conditions is one of the most important topics for the extension of their shelf life and activity over long storage times. Expanding upon the technologies and materials available for prolonging bioactive viability can contribute to ways of improving human health and quality of life. Furthermore, the possibility of protecting bioactive molecules with materials that have an increased nutritional and environmental value is crucial for proper development. Cricket flour and other food products that can be categorized as superfoods are of high interest in this respect. Therefore, this work has many aims surrounding the protection of bioactive molecules from harsh environmental conditions. Firstly, cricket flour was evaluated as a novel encapsulating material for the protection of cranberry fruit and pomegranate peel extracts. Additionally, considering the many advantages of the EBS technique, EBS was extended for particle production and compared to the commonly used freeze-drying technique.

MATERIALS AND METHODS

Materials and reagents

Pullulan was purchased from Hayashibara Co., Ltd. (Okayama, Japan). Cricket flour, made from finely milled crickets (*Acheta domesticus*) was purchased from Crunchy Critters (Derby, UK). Cranberry extract (*Vaccinium macrocarpon* fruit extract) and pomegranate peel extract (*Punica granatum* peel extract) were purchased from Zrób Sobie Krem (Prochowice, Poland). Distilled and deionized water was used as the solvent for all formulations.

Preparation of pure biopolymers and extract-containing biopolymer solutions

All solutions were prepared at room conditions, in glass bottles and were processed directly after preparation. Cranberry fruit and pomegranate peel extracts and biopolymers (cricket flour and pullulan) were used without further purification. First, solutions of each extract and biopolymer were prepared. Fruit extract solutions were prepared by dissolving in water separately pomegranate peel extract and cranberry extract at the concentration of 25 g/L. To prepare the solution of pullulan (PU), 50 g/L of this biopolymer was dissolved in water. For the preparation of the pullulan-cricket flour blends (PU-CFF), first 100 g/L of cricket flour was suspended in water under ambient conditions. After 2 h of stirring, the mixture was filtrated through lab cellulose filter with thickness of 0.22 mm for removing coarse sediments. The filtrate was dried to determine the dry weight (DW) of cricket flour that passed through the filter. The total solid content of cricket flour filtrate (CFF) was 20 g/L. This filtration allowed for insect debris to be removed so that only the water-soluble compounds, or small dispersed particles which would not affect electro-blow spinning process, remained in the aqueous solution. After this dissolution and filtration of cricket flours, 50 g/L of pullulan was added to the filtrate and dissolved at ambient conditions. For preparation of CFF sample, no additional steps were added. Water solution of CFF was used at a concentration of 20 g/L.

For the preparation of fruit extract-containing pullulan particles, 25 g/L of cranberry or pomegranate extracts were added to pullulan water solutions (at concentration of 50 g/L) and mixed at ambient conditions for about 1 h until completely incorporated. These pullulan-cranberry (PU-Cranberry) and pullulan--pomegranate (PU-Pomegranate) solutions were later processed *via* electro-blow spinning (as described in a subsequent section) or freeze-dried. For the preparation of the pullulan-cricket flour blends containing the fruit extracts, also 25 g/L of fruit extracts were added to the water solution of pullulan (50 g/L) and cricket flour filtrate (20 g/L). Pullulan-cricket flour filtrate-cranberry (PU--CFF-Cranberry) and pullulan-cricket flour filtrate-pomegranate (PU-CFF-Pomegranate) solutions were prepared acc. to this procedure and later processed by electro-blow spinning or freeze--dried. Pure cricket flour filtrate microparticles containing the fruit extracts were prepared in the same way as PU-CFF particles, but without adding pullulan.

Chemical composition analysis of biopolymers and fruit extracts

Protein content

Total protein content was measured following the Lowry method with some minor modifications [Benito-González *et al.*, 2019]. Briefly, 1 mL of modified Lowry reagent was well mixed with 0.2 mL of each previously prepared solution and incubated for 10 min at room conditions. After incubation, 0.1 mL of the Folin-Ciocalteu reagent (mixed with ultrapure water 1:1, *v/v*) was added and vortexed. Mixed solutions were then incubated for 30 min at room temperature and covered from light. The blank was prepared by mixing 0.2 mL of water with respective amounts of Lowry and Folin-Ciocalteu reagents. After incubation, the absorbance was measured with UV-1800 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan) at 750 nm. The standard curve was prepared with serial dilutions of bovine serum albumin (BSA). Total protein content was expressed as g of proteins *per* 100 g of the analyzed dry material. Determination was carried out in triplicate.

Lipid content

Total lipid content was determined following the sulpho-phospho--vanillin method, with some minor modifications [Benito-González *et al.*, 2019; Frings & Dunn, 1970]. A phospho-vanillin reagent was prepared by dissolving vanillin in water at a concentration of 6 g/L. A portion of 350 mL of the vanillin solution was then mixed with 50 mL of water and 600 mL of concentrated phosphoric acid. For sample analysis, 20 µL of each previously prepared material solution was mixed with 200 µL of concentrated sulphuric acid, well stirred, and incubated in boiling water for 10 min. After conditioning samples in cold water for 5 min, 10 mL of the phospho-vanillin reagent were added, mixed, and the mixture was incubated at 37°C for 15 min. A blank was prepared with 20 µL of 96% ethanol and respective amounts of reagents. The absorbance was measured at 540 nm using a UV-1800 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). A calibration curve was plotted using known concentrations of sunflower oil, and total lipid content was expressed as g of lipids *per* 100 g of the analyzed dry material. Determination was carried out in triplicate.

Characterization of physical properties of biopolymer and extract-containing biopolymer solutions

All dynamic viscosity measurements were taken at 20°C and atmospheric pressure using the Rotavisc – rotational viscometer (IKA, Königswinter, Germany) equipped with a low-viscosity adapter. Conductivity measurements were performed with a multifunction device (CX-705, Elmetron, Zabrze, Poland) equipped with a conductivity electrode (ECF-1, Elmetron, Zabrze, Poland). Surface tension was measured following the Wilhemy plate method using an Easy-Dyne K20 tensiometer (Krüss GmbH, Hamburg, Germany). All physical property determinations for PU, PU-Cranberry, PU-Pomegranate, CFF, CFF-Cranberry, CFF-Pomegranate, PU-CFF, PU-CFF-Cranberry and PU-CFF-Pomgranate solutions were carried out in triplicate.

Preparation of biopolymer encapsulated extracts

Electro-blow spinning

Electro-blow spinning (EBS) equipment was assembled in-house and used for the encapsulation of pomegranate and cranberry extracts with PU, PU-CFF and CFF. The EBS apparatus consisted of a feeding system, 0–30 kV power supply (Acopian, Easton, PA, USA), air flow system (air compressor) (Airpress, Przeźmierowo, Poland), and grounded collector equipped with lab cyclones, where all produced materials were collected. The anode was connected to a coaxial needle system where the 0.8 mm internal needle was connected to a syringe containing the sample solution and the 1.0 mm external needle was connected to an air flow system. The processing of each extract-containing biopolymer solution was performed in room conditions. Dried particles (PU--Cranberry-EBS, PU-Pomegranate-EBS, PU-CFF-Cranberry-EBS, PU-CFF-Pomegranate-EBS, CFF-Cranberry-EBS and CFF-Pomegranate-EBS) were collected to glass vials and stored in them under 0% of relative humidity (RH) and temperature of 4°C (enclosed in a desiccator with silica gel which was left in the fridge). An air compressor with maximum air compression up to 800 kPa was used to create air pressure that dried the formed particles and helped them reach the collector. All samples were prepared under the same air pressure (200 kPa). Flow rates ranged from 15 to 17 mL/h, and the applied voltage varied from 13 to 17 kV (Table 1). In one working cycle, *ca.* 20 g of the material were obtained from 300 mL of the used solution.

Freeze-drying

The freeze-drying (FD) technology was used to compare encapsulation technologies as well as to quantify and compare extract stability in the PU, CFF and PU-CFF blend. Before freeze-drying, extract-containing biopolymer solutions were frozen at –80°C for 24 h, and then placed into a VirTis Genesis 35 EL freeze-dryer (SP Scientifics, Warminster, PA, USA) at the pressure of 10 Pa for the next 24 h. The obtained powders (PU-Cranberry-FD, PU-Pomegranate--FD PU-CFF-Cranberry-FD, PU-CFF-Pomegranate-FD, CFF-Cranberry-FD, CFF-Pomegranate-FD) were stored at 0% RH and 4°C, enclosed in a desiccator with silica gel and left in the fridge until use.

Encapsulation efficiency evaluation

The amount of cranberry or pomegranate extract incorporated into pullulan, pullulan-cricket flour filtrate and cricket flour filtrate particles was analyzed by UV-vis spectroscopy according to a protocol adapted from the article by Alehosseini *et al.* [2019]. Specifically, all materials were dissolved in ultra-pure water (20 g/L), and the absorbance was measured at 280 and 292 nm for solutions of microparticles with cranberry fruit and pomegranate peel extracts, respectively, using a UV-1800 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). Calibration curves for each extract were obtained by tracking the absorbance of varying concentrations of the pure extracts at 280 and 292 nm (R^2>0.995). These curves were used to determine the total encapsulated fruit extract. Encapsulating materials produced no peaks around 280 and 292 nm, confirming that they did not contribute to absorbance values. Theoretical encapsulated fruit extract was taken as the amount of extract present in the solution before EBS or FD. Encapsulation

Table 1. Electro-blow spinning (EBS) processing parameters of pullulan (PU), cricket flour filtrate (CFF) and pullulan-cricket flour filtrate (PU-CFF) polymers that were used to encapsulate cranberry juice and pomegranate peel extracts.

Sample	Applied voltage (kV)	Solution flow rate (mL/h)	Air pressure (kPa)	Working distance (cm)
PU	13	15	200	100
PU-Cranberry	15	15	200	100
PU-Pomegranate	15	15	200	100
CFF	13	15	200	80
CFF-Cranberry	13	15	200	100
CFF-Pomegranate	13	15	200	100
PU-CFF	15	17	200	100
PU-CFF-Cranberry	16	16	200	120
PU-CFF--Pomegranate	17	16	200	120

efficiency (EE) was then calculated using Equation (1) with analysis of three independent replicates of each sample.

$$EE\ (\%) = \frac{\text{Total encapsulated fruit extract}}{\text{Theoretical encapsulated fruit extract}} \times 100 \quad (1)$$

Moisture content determination

To estimate moisture content of the extracts and microcapsules, all materials (*ca*. 0.5 g) were placed in a lab dryer and dried at 110°C. The drying process stopped when the weight of tested materials attained constant [Hamdan *et al.*, 2020]. Measurements were performed in triplicate.

Morphological and size distribution characterization of materials formed *via* EBS

Scanning electron microscopy (SEM) was conducted using an S-4800 microscope (Hitachi, Tokyo, Japan) with an accelerating voltage of 10 kV for SEM and a working distance of 40 mm. Materials created *via* EBS analyzed by SEM, were not sputtered with any conductive coating. Particle size diameters of all materials were measured by means of ImageJ software (version 1.52n, NIH & LOCI, University of Wisconsin, Madison, WI, USA) from the SEM micrographs in their original magnification [Çanga & Dudak, 2019; Saud *et al*., 2023]. In all cases, a minimum of 150 particles were analyzed to determine their size distribution (or average sizes).

Infrared spectroscopy analysis

Fourier transform infrared spectra (FTIR) were collected for the chemical characterization of pure biopolymers, pure and encapsulated *via* EBS and FD fruit extracts by using the attenuated total reflection (ATR) attachment on Invenio S FT-IR (Bruker, Billerica, MA, USA) spectrometer. Single spectra were averaged over 24 scans at 4 cm^{-1} resolution in the wavelength range from 400 to 4,000 cm^{-1}. All analyses were performed in duplicate.

Total phenolic content determination

Total phenolic content was analyzed using the method with a Folin-Ciocalteu reagent, according to a method adapted from Cilek *et al*. [2012]. To 0.5 mL of pure and encapsulated in biopolymers fruit extract solutions (1 mg/g), 2.5 mL of the Folin-Ciocalteu reagent (10 times diluted with water) were added and, followed by 2 mL of Na_2CO_3 (75 g/L). The sample was incubated for 5 min at 50°C and then cooled. For a control sample, 0.5 mL of distilled water was used. The absorbance was measured at 760 nm. The results were expressed in g of gallic acid equivalent *per* g of dry weight of sample (mg GAE/g DW). Three-replicate analyses were performed for each type of material.

Antioxidant activity analysis

ABTS radical cation scavenging activity

Antioxidant activity of the fruit extracts and microparticles, and changes of this activity after UV light radiation were analyzed by an improved 2,2-azino-di-(3-ethylbenzothiazoline-sulphonic acid radical cation ($ABTS^{\bullet+}$) method as described by Re *et al*. [1999] and Qabaha *et al*. [2019]. In brief, the $ABTS^{\bullet+}$ stock solution (7 mM) was prepared through the reaction of 7 mM ABTS and 2.45 mM of potassium persulphate as the oxidant agent. The working solution of $ABTS^{\bullet+}$ was obtained by diluting the stock solution in phosphate-buffered saline (PBS) to produce an absorbance of 0.70±0.02 at λ=734 nm. Samples were dissolved in water at a concentration of 1 mg/mL, and 100 µL of the solution was added to 900 µL of the $ABTS^{\bullet+}$ solution. Absorbance readings at wavenumber of 405 nm were taken at room conditions exactly 10 min after initial mixing. A calibration curve was plotted by using 6-hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid (Trolox). The antioxidant activity of pure and encapsulated cranberry and pomegranate fruit extracts was expressed as µmol Trolox equivalents (TE) *per* g sample DW. Determination was carried out in triplicate.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined following the method described by Okonogi *et al*. [2007]. The tested pure and encapsulated in biopolymer fruit extracts were mixed with ethanol to prepare the ethanolic test solution (1 mg/mL). $DPPH^{\bullet}$ (100 µM) was dissolved in ethanol and mixed with an aliquot of 100 µL of the sample solution. After vigorous shaking, the mixtures were left to stand for 30 min in the dark at room temperature. Then, the absorbance was measured at 517 nm. Trolox was used as a standard, and the results were expressed as mmol TE/g sample DW.

Accelerated degradation studies

To accelerate the oxidation of fruit extracts and more rapidly evaluate the protecting effect provided by the biopolymers, all produced microparticles were exposed to UV light radiation for a time span of 30 h, using an Ultra-Vitalux lamp (OSRAM Licht AG, Munich, Germany). This lamp operates with a power of 300 W that is generated by a quartz discharge tube and a tungsten filament. As described by the manufacturer, it produces a blend of radiation very similar to that of natural sunlight where the radiation of 315–400 nm after 1 h of exposure is of 13.6 W, and the radiation of 280–315 nm after 1 h of exposure is of 3.0 W. Sample stability was determined using a UV-1800 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan) after various UV light exposure times (from 0 to 30 h). The absorbance was measured in the wavenumber range from 220 to 700 nm. All measurements were performed in triplicate.

Statistical analysis

Data analysis was carried out using SPSS by IBM Corporation (Redmont, WA, USA). One-way analysis of variance (ANOVA) was completed to determine the significant differences between sample means, at a significance level of $p<0.05$. Mean values were compared using the Tukey test.

RESULTS AND DISCUSSION

Chemical composition of encapsulating and bioactive materials

In this study, protein, and lipid contents were analyzed in pullulan (PU), cricket-flour filtrate (CFF), and pullulan-cricket flour filtrate (PU-CFF), and respective results are presented in Table 2.

Table 2. Total protein and lipid content of extracts and encapsulating materials including pullulan (PU), cricket flour filtrate (CFF) and pullulan with cricket flour filtrate (PU-CFF).

Sample	Protein content (g/100 g)	Lipid content (g/100 g)
Cranberry fruit extract	3.77±0.23	0.80±0.14
Pomegranate peel extract	2.87±0.31	1.02±0.10
PU	3.6 ±1.35	3.29±0.60
CFF	39.71±2.50	19.03±1.71
PU-CFF	45.32±1.14	20.41±1.77

Additional cricket flour information from the provider reveals that it contained 56.8 g of proteins, 29.3 g of lipids and only 5.5 g of carbohydrates in 100 g of flour. Because of the very low carbohydrate content in cricket flour (according to provider's information and already published data [Montowska *et al*., 2019; Orkusz, 2021]) and the fact that pullulan consists of 99.9% maltotriose, carbohydrate composition was not analyzed for these materials.

Data presented in Table 2 show that CFF and PU-CFF both contained a comparably high protein content. Though being a highly pure maltotriose-chain polymer, pullulan was still found to contain residual amounts of proteins. However, this protein content was 10 times smaller than CFF. The slightly higher protein content in PU-CFF compared to CFF was attributed to this small amount of protein in PU. The protein content determined from cricket flour filtrate was lower than that provided by the supplier for cricket flour. This fact can be explained by material loss during filtration. Despite this, literature data provide protein content in cricket powders to range from 42 to 45 g/100 g [Montowska *et al*., 2019], which represent similar values to data presented in this research.

The protein content in pomegranate peel extract and cranberry fruit extract was 2.87 and 3.77 g/100 g, respectively. These contents were similar to the fruit extracts providers' information. Moreover, a high content of lipids was not detected in any of the analyzed fruit extracts, which also coincides with the information from the provider.

In the case of pullulan, small contents of protein and lipids were detected. This was probably an effect of the presence of some residuals after polymer purification from microorganisms producing this material. The presence of lipids in materials with cricket flour filtrate was observed at levels of approximately 19 g/100 g for pure CFF and 21 g/100 g for CFF mixed with PU. Total lipid content was lower when compared to provider's information, but similar to data presented by another scientific group for cricket powder – around 25 g/100 g [Montowska *et al*., 2019].

This information suggests that the main encapsulating macromolecules of PU were polysaccharides and that lipids and proteins which for CFF and PU-CFF were dominating encapsulating materials, were residual molecules in the encapsulation process.

Physical properties of biopolymer and extract-containing biopolymer solutions

The conductivity, surface tension and dynamic viscosity of the solutions of biopolymers and biopolymers with pomegranate peel and cranberry fruit extracts are shown in Table 3. All solutions presented conductivity in the range of 0.04–2.34 mS/cm. Pure PU solution showed the lowest values of conductivity of 0.04 mS/cm, which was similar to already published data [Tomasula *et al*., 2016]. When cricket flour filtrate was added to the pullulan solutions, the conductivity increased up to 1.21 mS/cm, suggesting that the filtered cricket flour contributed by increasing the concentration of ions in the solution. This result was not surprising as the protein content of the CFF was significant, and proteins are charged macromolecules with high conductivity. Similar conductivity behaviors of solutions containing proteins (like casein solutions) have been previously observed [Tomasula *et al*., 2016]. Moreover, the conductivity increased when fruit extracts were added to the solutions (Table 3), which was also likely an effect of an increased ion concentration coming from polyphenols of the extract solutions.

Table 3. Physical properties of solutions of cranberry fruit extract and pomegranate peel extract mixed with encapsulating materials including pullulan (PU), cricket flour filtrate (CFF) and pullulan with cricket flour filtrate (PU-CFF).

Solution	Conductivity (mS/cm)	Surface tension (mN/m)	Dynamic viscosity (cP×s)
PU	0.04 ± 0.006^{f}	35.16 ± 0.04^{d}	42.11 ± 0.03^{b}
PU-Cranberry	1.71 ± 0.01^{c}	33.18 ± 0.03^{h}	41.28 ± 0.07^{c}
PU-Pomegranate	2.05 ± 0.09^{b}	34.29 ± 0.07^{g}	40.97 ± 0.08^{d}
CFF	0.93 ± 0.07^{e}	37.23 ± 0.03^{a}	11.59 ± 0.12^{f}
CFF-Cranberry	1.74 ± 0.03^{c}	36.92 ± 0.06^{b}	11.92 ± 0.03^{e}
CFF-Pomegranate	2.04 ± 0.08^{b}	36.03 ± 0.04^{c}	11.95 ± 0.08^{e}
PU-CFF	1.21 ± 0.07^{d}	35.04 ± 0.13^{e}	43.48 ± 0.09^{a}
PU-CFF-Cranberry	2.12 ± 0.09^{b}	34.81 ± 0.08^{f}	41.53 ± 0.11^{c}
PU-CFF-Pomegranate	2.34 ± 0.03^{a}	34.87 ± 0.04^{ef}	42.04 ± 0.05^{bc}

Different letters in column indicate significant differences between the samples ($p<0.05$).

Surface tension of the solutions ranged from 33.18 to 37.23 mN/m. The addition of extracts caused a slight reduction in its value, which was significant ($p<0.05$) in the solutions with CFF and pure pullulan as encapsulating materials. Similar reductions have been reported previously [Torres-Giner *et al*., 2017], where mixing biopolymers with *Aloe vera* extract caused slight changes in solution's surface tension. The dynamic viscosities of the solutions varied with values between 11.59 and 42.11 cP×s when fruit extracts were added to biopolymer solutions. Much higher ranges of viscosity were determined in the solutions where pullulan was added as the encapsulating material. When CFF was used to protect fruit extracts, solution viscosities presented 4 times lower dynamic viscosity range. It should be noted that all analyzed physical properties of the prepared solutions where typical for solutions that have been used for various encapsulating techniques, *e.g.*, electro-spinning, blow-spinning, and spray--drying. For the electrospinning technique, solution conductivity should not increase above 500 mS/cm [Ramakrishna, 2007]. Best surface tension and conductivity values for successful solution electrospinning are 20-45 mN/m and 170-21,500 cP×s, respectively [Williams *et al*., 2018]. Moreover, for electrospraying, solution surface tension and conductivity should be similar, as in the case of electrospinning, but dynamic viscosity should not be lower than 2 cP×s to successfully obtain microparticles [Zhang *et al*., 2019].

Encapsulation efficiency and moisture content of microparticles

Encapsulation of pomegranate peel and cranberry fruit extracts was carried out using EBS and FD methods. The processing conditions for the in-house electro-blow spinning of PU, CFF, PU-CFF solutions and extract-containing biopolymer solutions were optimized in a preliminary study and are presented in Table 1. The most effective material drying was achieved when the working distance was set up at 100 cm. In the case of cricket flour filtrate (CFF), smaller working distance had to be used, up to 80 cm, because with longer distances, the material did not reach the collector but instead spread over the working space. In the case of PU-CFF-Cranberry and PU-CFF-Pomegranate samples, the working distance was set up to 120 cm. When a shorter distance was used, the material reaching the collector was still wet. This could probably be caused by the higher concentration of the encapsulating material used to protect fruit extracts and the need for greater distance to evaporate all the water. A slightly higher voltage was needed for particle formation for the samples with higher conductivity and dynamic viscosity ranges of the used solutions. The same situation was also observed in the case of electrospinning method applied for particle formation [Ramakrishna, 2007].

EE results of all analyzed samples are presented in Table 4. Obtaining such a high EE for microcapsules made with the EBS technique (above 80% for PU blends and around 60% where CFF was used as an encapsulating matrix in comparison to 51–79% EE for the samples made by FD) supported that the constructed EBS equipment was working properly and allowed for effective encapsulation of fruit extracts in all biopolymers. Moreover, EE values obtained for the freeze-dried materials were in line with findings from previous research, where EF of fish oil encapsulation in whey protein concentrate *via* FD and EE was about 63% [El Ghannam *et al*., 2015]. In the case of the EBS technique, EE values were similar to those obtained through other techniques such as spray-drying or electro-spinning For example, saffron encapsulated in gelatin *via* electro-spinning presented EE at 63–74 % and *via* freeze-drying – at 51–65% [Golpira *et al*., 2021].

Moisture content of all produced materials is presented in Table 4. All samples made *via* EBS or FD contained residual amount of water after processing. The lowest water content was observed in CFF-Cranberry sample made *via* EBS. Moisture content of the other materials produced *via* EBS varied from 2.3 up to 2.7 g/100 g, and there were no significant differences between them in this respect. In the case of the samples made *via* FD, the moisture content was significantly higher in PU-Cranberry (3.2 g/100 g), PU-Pomegranate (3.2 g/100 g), and PU-CFF-Pomegranate (3.1 g/100 g). These data were similar to those presented for beads of blueberry juice encapsulated in maltodextrins *via* FD [Wilkowska *et al*., 2016].

Morphological characteristics of electro-blow spun particles

The particles obtained *via* electro-blow spinning (EBS) were characterized with scanning electron microscopy (SEM). Figures 1, 2, and 3 display representative micrographs of the various particles

Table 4. Encapsulation efficiency (EE) of cranberry juice and pomegranate peel extracts in pullulan (PU), cricket flour filtrate (CFF) and pullulan-cricket flour filtrate (PU-CFF) by electro-blow spinning (EBS) and freeze-drying (FD) methods and moisture content (MC) of obtained microparticles.

Microparticle	EE EBS (%)	EE FD (%)	MC EBS (g/100 g)	MC FD (g/100 g)
PU-Cranberry	88.1±5.8^{a}	79.3±2.1^{b}	2.5±0.3^{ab}	3.2±0.5^{a}
PU-Pomegranate	85.6±7.2^{ab}	74.8±5.9^{b}	2.3±0.3^{b}	3.2±0.3^{a}
CFF-Cranberry	58.5±6.9^{c}	51.2±3.4^{c}	2.0±0.4^{b}	2.5±0.3^{ab}
CFF-Pomegranate	61.1±4.6^{c}	52.4±3.5^{c}	2.7±0.2^{ab}	2.8±0.1^{ab}
PU-CFF-Cranberry	82.0±4.9^{ab}	73.8±5.8^{b}	2.4±0.3^{b}	2.8±0.3^{ab}
PU-CFF-Pomegranate	80.3±6.9^{ab}	69.5±5.1^{bc}	2.6±0.3^{ab}	3.1±0.5^{a}

Different letters indicate significant differences between the samples ($p<0.05$) in two data groups – encapsulation efficiency and moisture content values.

without extract, with cranberry extract, and with pomegranate extract. These micrographs prove the successful particle formation with the EBS technique.

EBS-processed pullulan particles were relatively homogenous, smooth, and round (Figure 1A). No fiber structures were observed. Mean microparticle size was about 3.5 µm, and about 80% of all microparticles had diameters that were less than 4 µm (Figure 1D). When encapsulating fruit extracts, the structure, shape, and size of the pullulan microparticles varied depending on the chosen extract. In the case of PU-Cranberry-EBS, very tiny fiber structures were observed in combination with round, disk-shape with concave center microparticles (Figure 1B). Mean microparticle size of PU-Cranberry-EBS was about 5.6 µm, and more than 70% of them presented sizes below 6 µm (Figure 1E). For this bioactive-biopolymer combination, microparticles smaller than 1 µm were not observed. In the micrograph of PU with pomegranate extract (PU-Pomegranate-EBS), no fiber-structures were observed (Figure 1C). The resulting EBS microparticles of this material were like the ones made from PU-Cranberry material. Mean microparticle size diameter was about 5.3 µm, with almost 80% of the microparticles having sizes lower than 6 µm (Figure 1F).

When pullulan with cricket flour filtrate were mixed and used as the encapsulating material through EBS technology, the resulting microparticles were slightly larger than those made with pure PU material. Specifically, the average PU-CFF-EBS microparticle diameter was around 6.2 µm and no microparticles smaller than 2 µm were observed (Figure 2D). In addition to the microparticles, some very tiny fiber structures were observed. With cranberry extract, PU-CFF-Cranberry-EBS microparticles were like pure PU-CFF-EBS microparticles in terms of size and shape (Figure 2B and 2E). With pomegranate extract (PU-CFF-Pomegranate-EBS), the mean microparticle size was about 5.9 µm with the majority (around 80%) of the produced microparticles being less than 8 µm in diameter (Figure 2C and 2F).

In the case of cricket flour filtrate materials processed by EBS, round and slightly deformed microparticles were observed, where the mean size diameter was 3.94 µm, 4.9 µm and 4.6 µm for pure cricket flour filtrate, CFF microparticles loaded with cranberry extract and CFF microparticles loaded with pomegranate extract, respectively (Figure 3). About 70% of the microparticles produced from CFF were less than 10 µm (Figure 3D).

When analyzing microparticle size diameter for all the materials produced with the EBS technology, 90% of the microparticles presented size less than 9 µm for PU-EBS and PU-Pomegranate-EBS samples. The widest microparticle size distribution was observed for the pure cricket flour filtrate and CFF-Pomegranate-EBS samples. Only 55% and 65% of those samples, respectively, presented size distribution lesser than 9 µm. When dealing with a polymer solution with low electrical conductivity, the electrostatic force has two components: normal and tangential to the surface of the drop. The effect of these forces on a droplet of polymer solution and on the coaxial stretching of the stream of material is the formation of various forms of nanomaterials and it could affect the material size and shape [Ramakrishna, 2007].

Considering an effect of solution dynamic viscosity on the final material shape and size, we can observe that with increasing viscosity, the mean size of microparticles obtained with EBS also increased. With lower solution dynamic viscosity, materials made by EHD form mostly microparticles and with increasing viscosity the solution can form fibers instead of microparticles [Ramakrishna, 2007]. This solution behavior was observed in all solutions formed with pullulan, where dynamic viscosity varied between 41 and 43 cP×s. Mean microparticle size of the materials made with higher viscosity numbers was greater, and the materials tended to form microparticles with some tiny fibers between them. Moreover, solutions with lower dynamic viscosity, like the materials formed from cricket flour without pullulan, displayed smaller mean microparticle size. This observation can be explained by distribution of forces that affect a solution droplet leaving an injector. A greater distribution of forces is needed to stretch a more viscous solution and, conversely, less forces are needed to stretch less viscous solutions; thus, the obtained materials' size can be smaller [Ramakrishna, 2007].

FTIR spectra characteristics of biopolymers, fruit extracts and extract-containing biopolymer microparticles

All materials made from biopolymers containing pomegranate and cranberry extracts, as well as the pure biopolymers, were characterized using FTIR spectroscopy. These spectra are shown in Figure 4 and 5. Characteristic peaks of the spectra for the different encapsulated materials (typical for functional groups of polysaccharides, proteins, or lipids) were chosen to highlight similarities in the chemical composition of the microparticles created with non-processed extracts and to confirm the presence of the extracts in the microparticles.

In the case of FTIR spectra corresponding to both fruit extracts (cranberry and pomegranate), characteristic spectral region for polysaccharides was presented: region 1,500–1,200 cm^{-1} – including deformational vibrations of groups with local symmetry, like CH_2 and C–OH deformations encountered in carbohydrates and region 1,200–800 cm^{-1} [Wan *et al.*, 2021]. Those typical for polysaccharides regions were also easy to observe on FTIR spectra of pullulan and its mixture with cricket flour filtrate (Figure 4). No characteristic bands from proteins and lipids were observed on the FTIR spectra for either fruit extracts or pullulan spectra. These observations agree with data presented previously, where the total protein and lipid contents in the fruit and biopolymers were analyzed (Table 2).

In the case of cricket flour filtrate spectra, many regions characteristic for fatty acids and proteins were observed (Figure 4), proving the presence of those compounds in the samples, and thereby confirming previously presented data in Table 2. One of the most characteristic peaks detected on the spectra of pure cricket flour filtrate were those at wavenumbers of 2,850 and 2,917 cm^{-1}. Those peaks correspond to C–H stretching vibrations, or more precisely, to the symmetric and antisymmetric (respectively) methylene stretching vibrations in the chemical compounds [Sinclair *et al.*, 1952]. A characteristic peak for fatty acids (1,737 cm^{-1}) was strongly

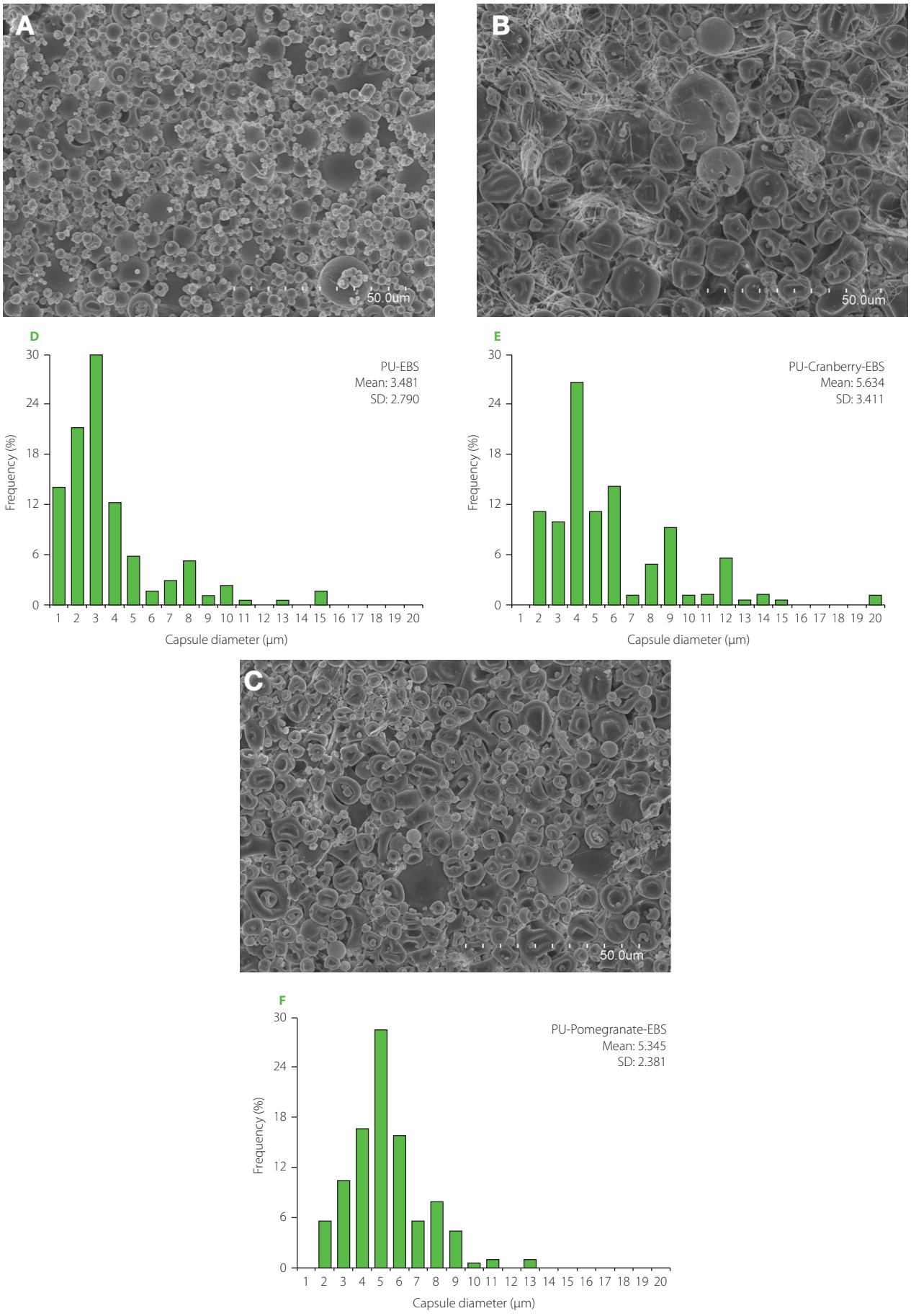

Figure 1. Scanning electron microscopy (SEM) micrographs of pure pullulan microparticles, PU-EBS (**A**), cranberry fruit extract encapsulated in pullulan, PU-Cranberry-EBS (**B**) and pomegranate peel extract encapsulated in pullulan, PU-Pomegranate-EBS (**C**), as well as microparticle size diameter distribution of PU-EBS (**D**), PU-Cranberry-EBS (**E**) and PU-Pomegranate-EBS (**F**).

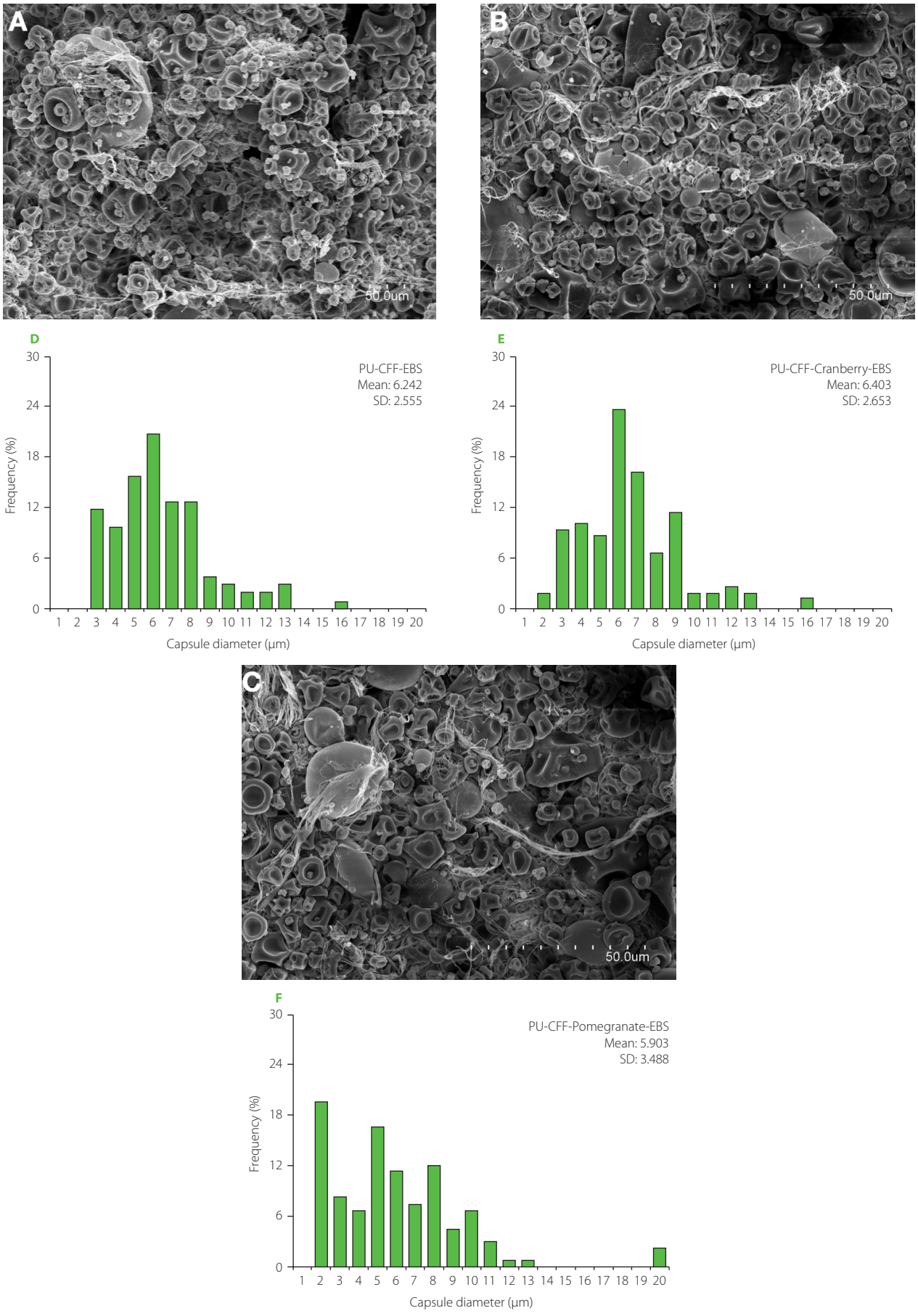

Figure 2. Scanning electron microscopy (SEM) micrographs of pure pullulan-cricket flour filtrate microparticles, PU-CFF-EBS (**A**), cranberry fruit extract encapsulated in pullulan-cricket flour filtrate, PU-CFF-Cranberry-EBS (**B**) and pomegranate peel extract encapsulated in pullulan-cricket flour filtrate, PU-CFF-Pomegranate-EBS (**C**), as well as microparticle size diameter distribution of PU-CFF-EBS (**D**), PU-CFF-Cranberry-EBS (**E**) and PU-CFF-Pomegranate-EBS (**F**).

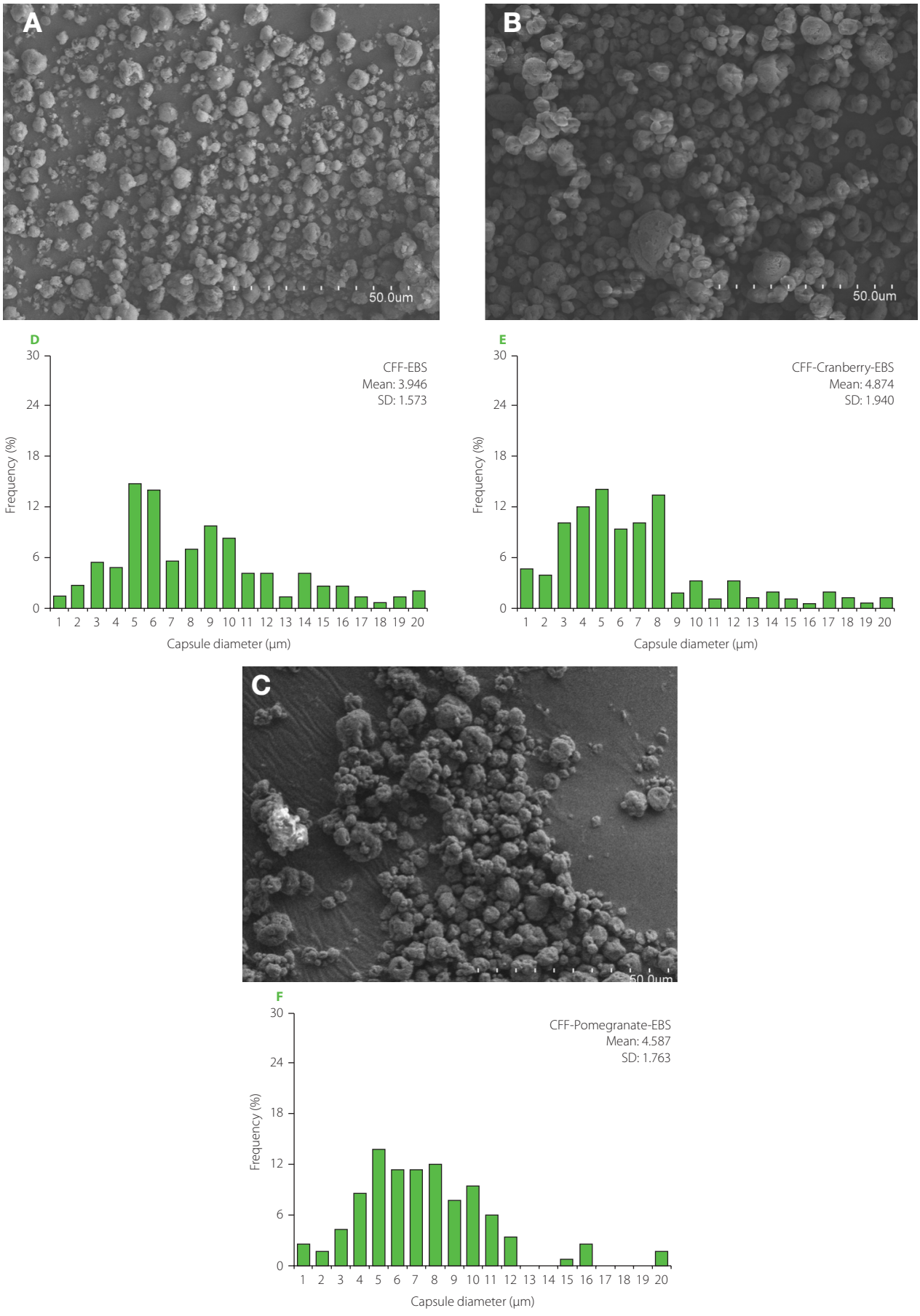

Figure 3. Scanning electron microscopy (SEM) micrographs of pure cricket flour filtrate microparticles, CFF-EBS (**A**), cranberry fruit extract encapsulated in cricket flour filtrate, CFF-Cranberry-EBS (**B**) and pomegranate peel extract encapsulated in cricket flour filtrate, CFF-Pomegranate-EBS (**C**), as well as microparticle size diameter distribution of CFF-EBS (**D**), CFF-Cranberry-EBS (**E**) and CFF-Pomegranate-EBS (**F**).

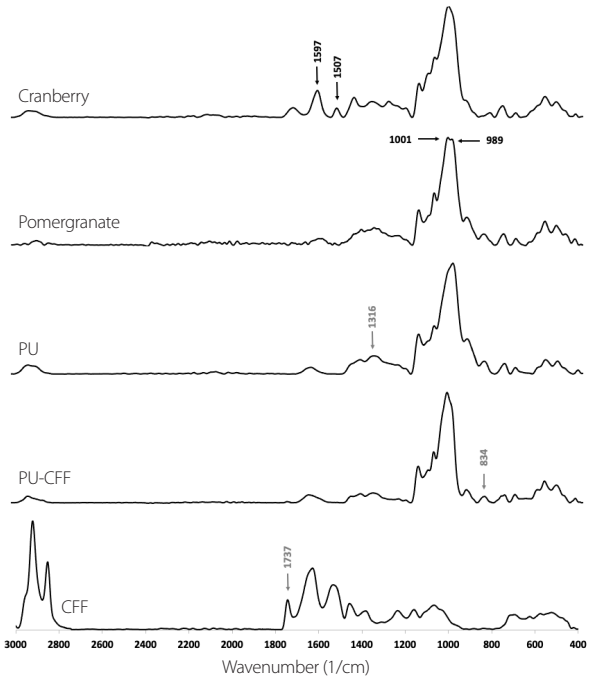

Figure 4. Fourier transform infrared spectra of cranberry fruit extract, pomegranate peel extract and encapsulating materials: pullulan (PU), pullulan mixed with cricket flour filtrate (PU-CFF) and cricket flour filtrate (CFF).

presented in the FTIR spectra of cricket flour filtrate but less present in the spectra of pure fruit extract. This peak corresponds to a strong carbonyl bond [Sinclair *et al*., 1952].

In the case of fruit extracts, we compared encapsulation in pure pullulan (PU), pullulan-cricket flour filtrate mixtures (PU-CFF) and pure cricket flour (CF) *via* EBS and FD. When using pure pullulan as the encapsulating material, characteristic peaks for pomegranate and cranberry extracts were observed in FTIR spectra at wavenumbers of 1,001 and 1,507 cm^{-1}, respectively, confirming the presence of extracts in these materials (Figure 5). Additionally, in the FTIR spectra of all analyzed materials, the presence of characteristic regions for carbohydrates were observed at wavenumbers of 1,500–1,200 cm^{-1} and 1,200–800 cm^{-1}, that correspond to deformational vibrations of CH_2, C–OH groups in polysaccharides and monosaccharides, respectively, that come from pullulan. All presented peaks in PU-Cranberry-EBS, PU-Cranberry-FD, PU-Pomegranate-EBS and PU-Pomegranate-FD spectra suggest successful pomegranate extract encapsulation with pullulan *via* both EBS and FD.

When analyzing the spectra of cranberry and pomegranate extract encapsulated by the mixture of pullulan with cricket flour filtrate (*via* EBS and FD), characteristic peaks were observed at wavenumbers of 989 and 1,597 cm^{-1} (Figure 5). These peaks can be observed on spectra corresponding to pomegranate peel extract and cranberry fruit extract, respectively. Peaks at those wavenumbers correspond to strong stretching C=C bonds, suggesting the presence of common polyphenol compounds in the extracts [Hong *et al*., 2021; Nawrocka *et al*., 2020]). The presence of those peaks in the spectra of PU-CFF-Cranberry-EBS, PU-CFF-Cranberry-FD, PU-CFF-Pomegranate-EBS and PU-CFF-Pomegranate-FD, but not in the pure

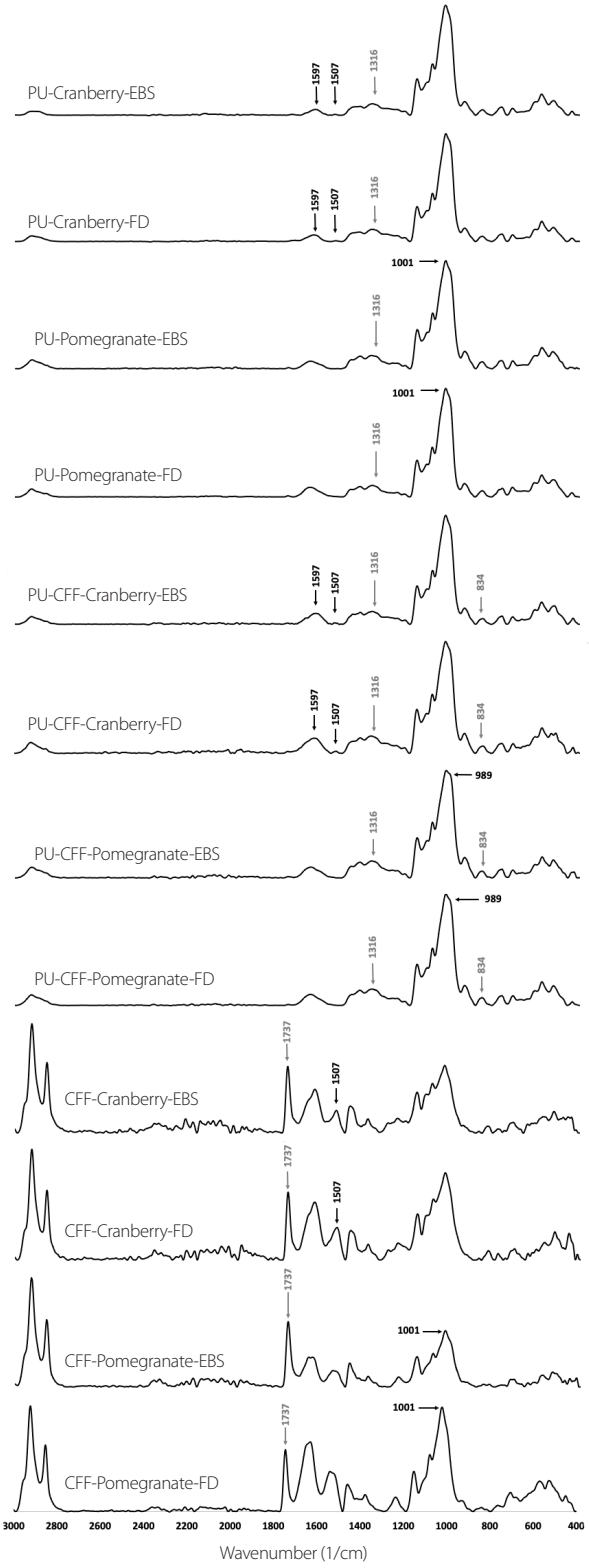

Figure 5. Fourier transform infrared spectra of cranberry fruit extract and pomegranate peel extract encapsulated *via* electro-blow spinning (EBS) and freeze-drying (FD) in pullulan (PU-Cranberry-EBS, PU-Cranberry-FD, PU-Pomegranate-EBS and PU-Pomegranate-FD), pullulan mixed with cricket flour filtrate (PU-CFF-Cranberry-EBS, PU-CFF-Cranberry-FD, PU-CFF-Pomegranate-EBS and PU-CFF-Pomegranate-FD) and cricket flour filtrate (CFF-Cranberry-EBS, CFF-Cranberry-FD, CFF-Pomegranate-EBS and CFF-Pomegranate-FD).

encapsulating material spectra (Figure 4), suggests successful encapsulation with both techniques. Moreover, in all analyzed samples we have observed the presence of peaks in regions

typical for carbohydrates (1,500–1,200; 1,200– 800 cm^{-1}) and for proteins (1,650–1,550 cm^{-1}) [Hong *et al*., 2021; Sinclair *et al*., 1952], which suggests that the dominant encapsulating materials were both carbohydrates and proteins from both the pullulan and cricket flour filtrate.

For the samples of fruit extracts encapsulated with filtered cricket flour by freeze-drying and EBS (Figure 5), peaks were observed in the regions typical for fatty acids (2,950–2,850 cm^{-1} and 1,800––1,700 cm^{-1}). These regions correspond to C–H and C=O stretching vibrations, respectively. Another interesting region detected on those spectra occurred at wavenumbers of 1,650–1,500 cm^{-1}, which is typical for the presence of protein, amide I and II region [Barth, 2007]. This suggests that the main encapsulating materials for pomegranate peel extract were proteins and lipids coming from the cricket flour extract. Moreover, in the FTIR spectra of pure extracts and encapsulated materials, peaks were observed in the regions typical for carbohydrates (1,500–200 and 1,200–800 cm^{-1}), which proves the presence of fruit extracts in the samples encapsulated with cricket flour filtrate *via* EBS and FD.

■ Stability of electro-blow spun and freeze-dried pure and encapsulated fruit extracts after UV light radiation

Antioxidant activity analyzed *via* ABTS and DPPH assays and total phenolic content of cranberry fruit and pomegranate peel extracts and microparticles loaded with extracts are presented in Table 5. The total phenolic content and antioxidant activity of microcapsules were around three times lower than those of the extracts. Considering encapsulation efficiency and the encapsulating material-to-extracts ratio, the lower antioxidant activity determined in the case of immobilized pomegranate of cranberry extracts seems to be understood. Moreover, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activities and the total phenolic content of pomegranate peel extract and cranberry extract were similar to those previously presented in various research [Elfalleh *et al*., 2009; Fischer *et al*, 2011; Qabaha *et al*., 2019; Wang *et al*., 2023]. Analyses of the total phenolic content and antioxidant activity were also performed with encapsulating materials (data not shown); phenolic content and antioxidant activity were undetectable for biopolymers.

Degradation process of cranberry fruit and pomegranate peel extracts was analyzed *via* measuring the reduction in normalized absorbance and $ABTS^{\bullet+}$ scavenging activity as a result of 30 h of UV light radiation (Figures 6 and 7, respectively). For both fruit extracts encapsulated in different biopolymers, significantly higher stability was observed with UV light exposure than for the extracts that were not encapsulated. After 30 h of UV light exposure, unprotected cranberry and pomegranate extracts exhibited a reduction of absorbance at wavelengths of 280 and 292 nm, respectively, by around 53% (Figure 6A) and over 60% (Figure 7A). When comparing absorbance changes data to antioxidant activity reductions after 30 h of UV light radiation, similar behavior of fruit extracts was observed (Figure 6B and 7B). The initial $ABTS^{\bullet+}$ scavenging activity of unprotected cranberry fruit extract reached 34.91 µmol TE/g (Table 5), which was comparable with data published by other research groups [Baranowska & Bartoszek, 2016]. After 30 h of UV light radiation, the unprotected extract lost up to 50% of its antioxidant activity (Figure 6B). The initial

Table 5. Total phenolic content and $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activity of pomegranate peel and cranberry juice extracts in free forms and encapsulated in pullulan (PU), cricket flour filtrate (CFF) and pullulan-cricket flour filtrate (PU-CFF) by electro-blow spinning (EBS) and freeze-drying (FD) methods.

Extract/microparticle	Total phenolic content (mg GEA/g DW)	$ABTS^{\bullet+}$ scavenging activity (µmol TE/g DW)	$DPPH^{\bullet}$ scavenging activity (µmol TE/g DW)
Pomegranate	59.78±4.21^{a}	40.78±3.15^{a}	0.94±0.07^{a}
PU-Pomegranate-EBS	13.87±1.73^{c}	15.62±1.64^{c}	0.33±0.05^{bc}
PU-Pomegranate-FD	14.28±0.80^{c}	17.65±1.15^{c}	0.29±0.02^{c}
PU-CFF-Pomegranate-EBS	12.17±0.96^{d}	18.23±1.04^{c}	0.33±0.02^{bc}
PU-CFF-Pomegranate-FD	11.45±1.07^{d}	16.25±0.44^{c}	0.26±0.04^{c}
CFF-Pomegranate-EBS	39.45±2.36^{b}	40.62±2.25^{a}	0.39±0.03^{b}
CFF-Pomegranate-FD	37.05±1.71^{b}	39.55±1.43^{a}	0.40±0.05^{b}
Cranberry	15.53±0.80^{c}	34.91±1.60^{b}	0.92±0.03^{a}
PU-Cranberry-EBS	4.87±0.32^{e}	19.07±0.98^{c}	0.32±0.06^{bc}
PU-Cranberry-FD	4.90±1.15^{e}	18.95±0.20^{c}	0.29±0.02^{c}
PU-CFF-Cranberry-EBS	4.67±0.29^{e}	18.55±0.49^{c}	0.34±0.02^{b}
PU-CFF-Cranberry-FD	5.45±1.43^{e}	18.24±1.00^{c}	0.31±0.03^{c}
CFF-Cranberry-EBS	10.85±0.73^{d}	31.33±0.91^{b}	0.43±0.01^{b}
CFF-Cranberry-FD	10.83±0.29^{d}	32.50±1.36^{b}	0.35±0.04^{bc}

Different letters in column indicate significant differences between the samples (p<0.05). GAE, gallic acid equivalent; DW, dry weight; TE, Trolox equivalent.

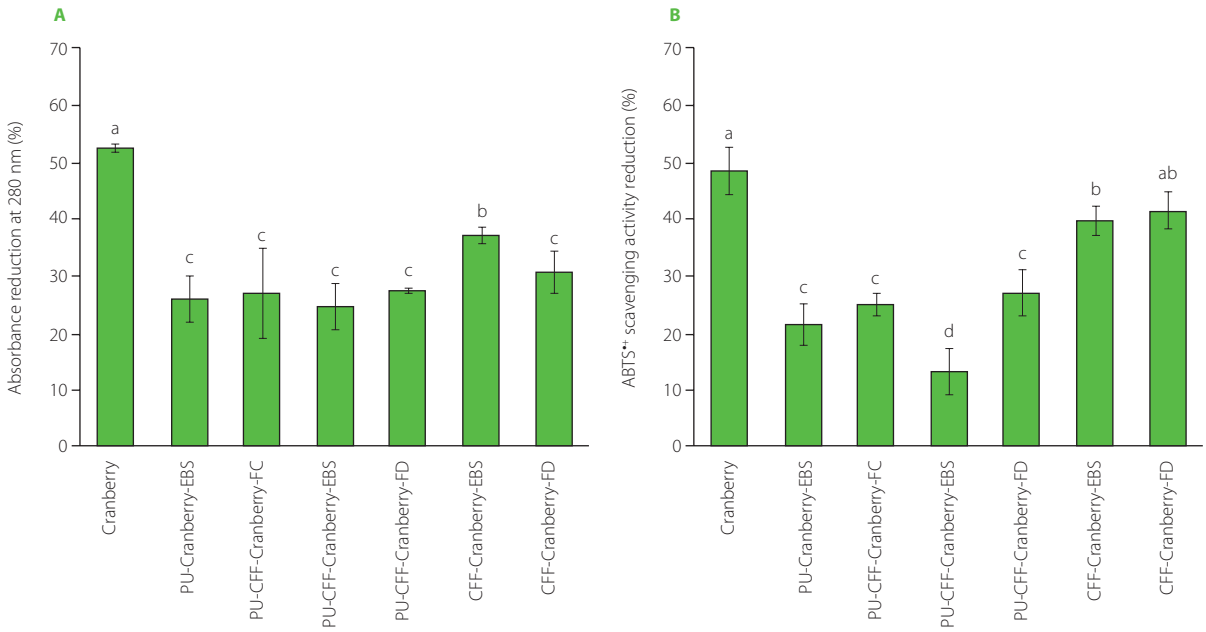

Figure 6. Reduction in absorbance at a wavelength of 280 nm (**A**) and reduction of $ABTS^{\bullet+}$ scavenging activity (**B**) of pure and encapsulated cranberry fruit extract after 30 h of UV light radiation. Different letters above bars indicate significant differences between the samples ($p<0.05$). PU, pullulan; CFF, cricket flour filtrate; PU-CFF, pullulan mixed with cricket flour filtrate.

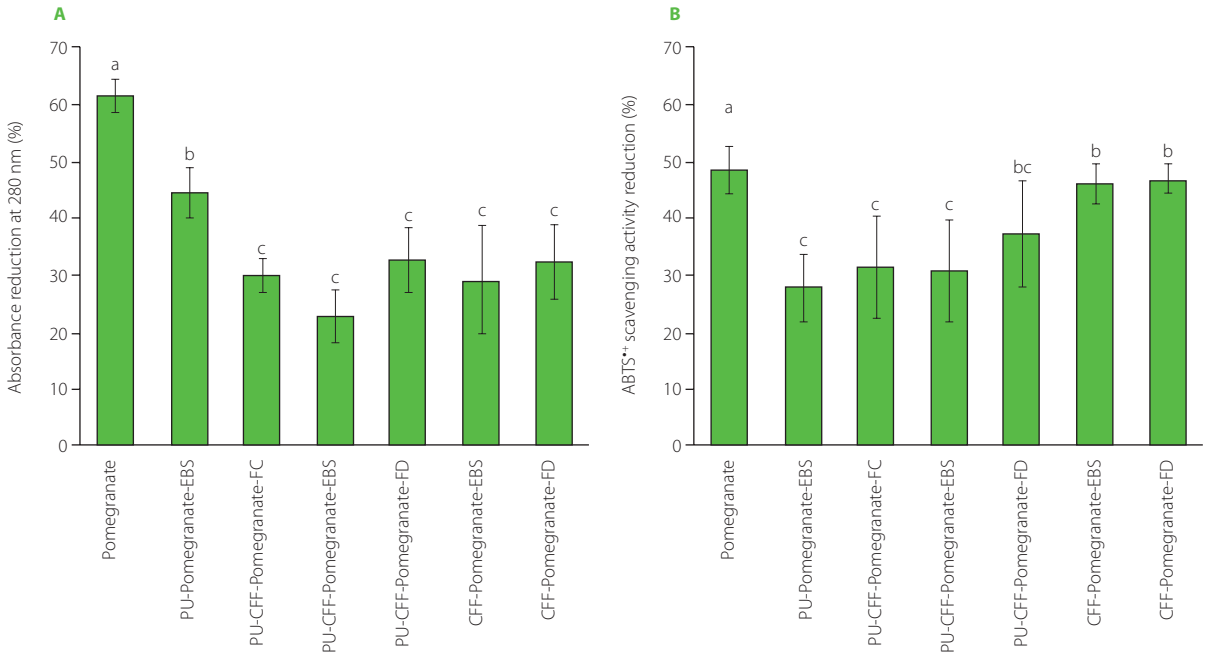

Figure 7. Reduction in absorbance at a wavelength of 292 nm (**A**) and reduction of $ABTS^{\bullet+}$ scavenging activity (**B**) of pure and encapsulated pomegranate peel extract after 30 h of UV light radiation. Different letters above bars indicate significant differences between the samples ($p<0.05$). PU, pullulan; CFF, cricket flour filtrate; PU-CFF, pullulan mixed with cricket flour filtrate.

$ABTS^{\bullet+}$ scavenging activity of pomegranate peel was 40.78 µmol Trolox/g (Table 5) and dropped by more than 55% after 30 h of UV light radiation (Figure 7B).

Conversely, the fruit extracts encapsulated in pure pullulan and pullulan with cricket flour filtrate *via* EBS and FD degraded around 13–25% (cranberry fruit extract) and 25–40% (pomegranate peel extract) after the same UV light exposure (Figure 6A and 7A). The differences in reductions between these samples were statistically not significant ($p\geq0.05$). This can lead to a conclusion that the cranberry fruit extract encapsulated with pullulan and its mixture with a cricket flour extract *via* both – EBS and FD, exhibited similar protective behavior. Like for the absorbance changes, much smaller antioxidant activity reduction was observed when the fruit extracts were encapsulated with pullulan and its mixture with cricket flour filtrate *via* EBS and FD (Figure 6B, PU-Cranberry-EBS, PU-Cranberry--FD and PU-CFF-Cranberry-FD; Figure 7B, PU-Pomegranate-EBS, PU-Pomegranate-FD, PU-CFF-Pomegranate-EBS and PU-CFF--Pomegranate-FD). The reduction of $ABTS^{\bullet+}$ scavenging activity after 30 h of UV light radiation in the case of these samples was

about 20–25% and 37–45% for cranberry and pomegranate extracts, respectively, and differences between the obtained data were not statistically significant ($p \geq 0.05$). The reduction of $ABTS^{\bullet+}$ scavenging activity after 30 h of UV light radiation was statistically significant ($p<0.05$) only in the case of the cranberry fruit extract encapsulated with pullulan mixed with the cricket flour extract *via* EBS (Figure 6B). In this case, the sample lost only 13% of its antioxidant activity. These results indicate that all encapsulated samples (prepared by both, electro-blow spinning and freeze-drying) present excellent antioxidant protection from UV light. Moreover, adding a filtrate of cricket flour to pullulan can improve protective properties of the polysaccharide itself. Also from these data, electro-blow spinning can be considered to ensure the same efficiency as freeze-drying with respect to encapsulation and protection of bioactive compounds.

To sum up, pullulan itself and mixed with cricket flour filtrate were the best protecting materials for antioxidants in the extracts exposed to UV radiation. It was not possible to explicitly define a single material that proved most successful in protecting the fruit extracts from harsh environmental conditions. We did, however, clearly see differences when comparing data to the materials protected only by cricket flour filtrate. Data obtained from encapsulated fruit extract stability and antioxidant activity analysis suggest that the cricket flour filtrate can still be used as an effective encapsulating material for antioxidant components, but its protective property significantly increases when mixed with polysaccharides from pullulan. Moreover, there was no significant difference in protecting activity, when extracts were encapsulated *via* electro-blow spinning or freeze-drying. This result indicates the EBS to be an effective and efficient method for bioactive compounds protection from harsh environment conditions and comparable to freeze-drying.

CONCLUSIONS

In summary, two bioactive extracts, namely cranberry fruit extract and pomegranate peel extract, were successfully encapsulated *via* two methods: electro-blow spinning and freeze-drying. The encapsulating materials used were pullulan, cricket flour filtrate, and a mixture of pullulan and cricket flour filtrate. UV-vis spectroscopy and an ABTS assay demonstrated that encapsulation of the fruit extracts significantly improved extract stability and reduced the loss of antioxidant activity after 30 h of UV light radiation. There were no significant differences in the level of protection when EBS or FD was used as an encapsulating technique or when pullulan and cricket flour were used. Data presented in this paper suggest that cricket flour can be considered as a new encapsulating material. Furthermore, mixing cricket flour with some extracts that present desired properties in food or cosmetic industry (*e.g.*, antioxidant activity) could lead to greater acceptance of this material in the Western market. These findings indicate that electro-blow spinning can be used as an effective method for the protection of bioactive molecules. The greatest advantage of using the EBS technique is its low equipment and consumption cost, compared to other encapsulation techniques.

ACKNOWLEDGEMENTS

Special thanks are given to Dr. Amparo Lopez-Rubio, from the Institute of Agrochemistry and Food Technology (IATA-CSIC), Valencia, Spain, for support, constructive critique and help in editing this paper. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors.

RESEARCH FUNDING

S. Wilkanowicz acknowledges support by the Warsaw University of Technology Internal Grants Foundation (504/04328/7192/44.000000).

CONFLICT OF INTERESTS

The authors declare no competing interests.

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