Research Article

Received: 15 May 2025 Revised: 21 September 2025 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.70276

Persimmon carotenoid extract rich in β-cryptoxanthin improves glucose homeostasis and reduces liver damage in high-fat-diet-induced type 2 diabetic and metabolic dysfunction-associated steatotic liver disease mice

Bryan Moreno-Chamba,[a,b†] Nuria Martí,[b†] Antonio Cárdenas,[c,d] María Soledad Fernández-Pachón,[e] Blanca Escudero-López,[c,d] Franz Martín,[c,d*] Julio Salazar-Bermeo,[a,b] Manuel Valero,[b] Domingo Saura,[b] María Concepción Martínez-Madrid[b‡*] and Genoveva Berná[c,d‡]

Abstract

Background: Persimmon (*Diospyros kaki* Thunb.) byproducts are a remarkable source of bioactive compounds, particularly carotenoids. Several studies suggest that carotenoids have beneficial effects in prevention of a large number of diseases. This study aims to highlight the effects of a persimmon carotenoid extract (PSCE) rich in β-cryptoxanthin (β-CRX, 51%) in ameliorating type 2 diabetes mellitus (T2DM) and metabolic dysfunction-associated steatotic liver disease (MASLD) in male C57BL6J mice. Animals were fed with a low-fat diet (LFD, $n = 10$), a LFD plus PSCE (0.8 mg kg^{-1} body weight per day; LFD-PSCE, $n = 10$), or a high-fat diet (HFD, $n = 20$), for 6 months. At the third month, 10 random mice from the HFD group were supplemented with PSCE for the remaining 3 months (HFD-PSCE, $n = 10$).

Results: Changes in body weight, glucose homeostasis, serum lipid profile, pro-inflammatory cytokine levels, total liver lipid content and extracellular collagen accumulation were determined. PSCE supplementation reduced body weight in 40% ($P < 0.05$), improved insulin sensitivity and glucose homeostasis ($P < 0.01$) and serum lipid profile ($P < 0.05$), decreased plasma pro-inflammatory cytokine levels in 30% ($P < 0.05$) and reduced liver steatosis and fibrosis ($P < 0.05$). *In vitro*, PSCE also reduced oxidative stress and triglyceride accumulation in 40% and 20%, respectively, in 3T3-L1 adipocytes ($P < 0.001$), supporting its *in vivo* effects.

* *Correspondence to: MC Martínez-Madrid, Institute of Research, Development, and Innovation of Health Biotechnology of Elche (IDiBE), Miguel Hernández University of Elche, 03202 Elche, Spain. E-mail: c.martinez@umh.es; or F Martín, Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), 41092 Seville, University of Pablo de Olavide-University of Sevilla-CSIC, 41013 Seville, Spain. E-mail: fmarber@upo.es*

† *These authors contributed equally to this work.*

‡ *Joint last authors.*

a *University Institute of Food Engineering-FoodUPV, Universitat Politècnica de València, Valencia, Spain*

b *Institute of Research, Development, and Innovation of Health Biotechnology of Elche (IDiBE), Miguel Hernández University of Elche, Elche, Spain*

c *Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), 41092 Seville, University of Pablo de Olavide-University of Sevilla-CSIC, Seville, Spain*

d *Biomedical Research Network on Diabetes and Related Metabolic Diseases-CIBERDEM, Madrid, Spain*

e *Department of Molecular Biology and Biochemical Engineering, University Pablo Olavide, Seville, Spain*

Conclusion: PSCE ameliorated HFD-induced metabolic dysfunction, inflammation and liver injury, suggesting persimmon byproducts represent a promising source of bioactive compounds for managing T2DM and MASLD. Further studies are needed to clarify the mechanistic and molecular role of β-CRX, the main persimmon carotenoid, in mediating these protective effects.

Supporting information may be found in the online version of this article.

Keywords: type 2 diabetes mellitus; β-cryptoxanthin; serum lipid profile; insulin resistance; pro-inflammatory cytokines; liver steatosis and fibrosis

ABBREVIATIONS

ADIPOR	adiponectin receptor 1
ANOVA	analysis of variance
BW	body weight
β-CRX	β-cryptoxanthin
CV	crystal violet
DMEM	Dulbecco's modified Eagle medium
DMSO	dimethylsulfoxide
ELISA	enzyme-linked immunosorbent assay
GLUT1	glucose transporter 1
H_2DCFDA	2′,7′-dichlorodihydrofluorescein diacetate
HDL	high-density lipoprotein
HFD	high-fat diet
HOMA-IR	homeostasis model assessment-insulin resistance
HPLC-DAD	high-performance liquid chromatography using a diode array detector
H&E	hematoxylin–eosin
IFN-α	interferon-α
IFN-γ	interferon-γ
IL-6	interleukin-6
IPGTT	intraperitoneal glucose tolerance test
LDL	low-density lipoprotein
LFD	low-fat diet
MASLD	metabolic dysfunction-associated steatotic liver disease
PPAR	peroxisome proliferator-activated receptor
RD	royal decree
ROS	reactive oxygen species
SEM	standard error of the mean
TC	total cholesterol
T2DM	type 2 diabetes mellitus
TG	triglyceride

INTRODUCTION

Type-2 diabetes mellitus (T2DM) and metabolic dysfunction-associated steatotic liver disease (MASLD) are closely intertwined, chronic metabolic disorders that have become significant global public health concerns.[1-3] MASLD refers to a spectrum of liver conditions characterized by hepatic steatosis in subjects with at least one cardiometabolic risk factor (obesity, T2DM, dyslipidemia or hypertension). Both conditions are progressive and multifactorial (genetic and environmental factors), often coexisting in individuals, exacerbating the risk of severe metabolic and cardiovascular complications and even cancer. These diseases place a burden on healthcare systems worldwide, particularly due to their high prevalence as well as their lifelong intervention management requirement. For instance, according to the International Diabetes Federation's Diabetes Atlas 2021, 10.5% of the adult population (537 million people approximately) have diabetes and about 30% of the Western global population is affected by MASLD.[4-6] For T2DM, pharmacological treatments are widely available. Nevertheless, for both diseases, dietary strategies are the cornerstone of their treatment and, in the case of MASLD, the modification of dietary habits is fundamental. Attention has increasingly turned to dietary strategies to potentially slow the progression of T2DM and MASLD as a part of their treatment. It is important to state that Western high-caloric diets and decreased physical activity play an important role in the pathogenesis of T2DM and MASLD.[3,7]

Dietary interventions, especially those rich in bioactive compounds, as happens with Mediterranean diets rich in nutrients and vitamins, have shown promise in improving metabolic health by regulating blood glucose levels and reducing liver fat accumulation.[8-11] Among bioactive compounds, the potential of carotenoid-rich sources in enhancing insulin sensitivity, reducing hepatic steatosis and mitigating inflammation and oxidative stress (key contributors to the progression of both T2DM and MASLD) has been documented.[12-15] Carotenoids are organic pigments produced by plants, algae and various microorganisms, including bacteria and fungi.[16] Several studies support the protective effect of carotenoids against a range of chronic diseases, including cardiovascular diseases, diabetes and cancer. Thus, a dietary carotenoid intake might mitigate T2DM and MASLD progression and complications.[17-20] Also, carotenoid consumption has been linked to a reduced risk of developing T2DM in both men and women, as well as reducing liver fat accumulation and inflammation.

In this context, persimmon (*Diospyros kaki* Thunb.) has emerged as an interesting reservoir of bioactive compounds, especially due to its high content of carotenoids,[21,22] especially β-cryptoxanthin (β-CRX) which represents about 49.2% of the total carotenoid content found in persimmon byproduct obtained from the juice processing industry.[21] Like other carotenoids, β-CRX exerts a noted antioxidant and free radical scavenger effect, but beneficial effects of carotenoids on human health cannot be simply associated with their antioxidant properties.[23,24] For instance, β-CRX has been associated with stimulating immunoglobulin production as well as the activity of T helper 2 (Th2)-type cytokines and $CD4^+$ T cells in rabbit.[25] Also, the oxidative metabolites of β-CRX have been involved in cancer prevention in animal models through their effects on key molecular targeting events such as nuclear factor kappa-B, retinoic acid receptor, sirtuin-1 and tumor suppressor protein p53 pathways.[26] Thus, β-CRX affects the signaling pathways involved in the pathogenesis of T2DM and MASLD. However, the role of β-CRX, unlike other carotenoids,[13,16,19,20,23] in the pathogenesis of T2DM and MASLD remains unclear, suggesting that additional mechanisms may be involved in its pharmacological effects.

Other important proteins such as adiponectin receptor 1 (ADIPOR), a key mediator of the metabolic effects of adiponectin, such as reducing blood sugar, regulating lipid, resisting atherosclerosis and anti-inflammatory activities,[27-29] has attracted interest in the last decade. ADIPOR downregulation can promote insulin resistance and chronic inflammation, central features of T2DM and MASLD. Glucose transporter 1 (GLUT1) also facilitates glucose uptake in various tissues. Although insulin-independent, GLUT1 regulation in specific scenarios like hyperglycemia can exacerbate lipogenesis, inflammation and fibrosis, worsening liver damage in MASLD.[30-32] Thus, modulating ADIPOR and GLUT1 expression offers potential therapeutic pathways for managing and reducing the progression of metabolic disorders like T2DM and MASLD.

Every year, thousands of tons of persimmon fruits are discarded to a combination of high quality standards of supermarkets, strict government regulations and the high expectations that consumers have when purchasing these fresh fruits based on their size, shape and uniform color.[33,34] The generated persimmon byproduct (often including peduncles, seeds, peels and unused flesh) holds promise as a natural, novel and economical source of extracts rich in carotenoids.[21] Thus, the revalorization of persimmon byproduct to obtain a carotenoid-rich extract with potential effect to improve the progression of T2DM and MASLD would be of interest and could contribute to the United Nations' Sustainable Development Goals for 2030.[35] In this sense, the aim of the study reported here was to investigate the potential of persimmon carotenoid extract (PSCE) in improving clinical parameters related to T2DM and MASLD in a high-fat diet (HFD) mouse model.

MATERIALS AND METHODS

Plant material and carotenoid extraction

Fresh persimmon fruits ('Red Brilliant' variety) were purchased from a local market in Elche, Spain. The byproduct (peels and pulp) derived from the persimmon juice industry was obtained according to Gea-Botella *et al*.[21] The byproduct was subjected to a solid–liquid extraction with acetone (1:3 w/v) in darkness to prevent photolytic degradation according to the method described by Gea-Botella *et al*.[36] All extraction steps were performed with minimal light exposure by using amber glassware and wrapping vessels with aluminium foil. Briefly, the mixture was filtered to remove solid particles; then, the resulting liquid extract was saponified with KOH and decanted with diethyl ether (1:2 v/v). Then, the obtained pellet was washed with water to decant the resins generated by previous saponification. Afterwards, the solvent was evaporated using a rotary evaporator under vacuum (Series R-210, Büchi, Barcelona, Spain) and the remaining water was removed by freeze-drying (Telstar Cryodos-80, Terrassa, Barcelona, Spain). The PSCE yield was 10.41 ± 0.07 g per 100 g of dry persimmon byproduct and the extract was stored under nitrogen at −80 °C, until further use.

Characterization of PSCE by high-performance liquid chromatography (HPLC)

PSCE composition was analyzed by HPLC coupled with a diode array detector (DAD)[21,36] (see supporting information, High performance liquid chromatography analysis). The total content of carotenoids and the composition of the extract were determined by comparing obtained peaks with the chromatogram profile of pure standards.

Cell culture assays

Mouse fibroblasts (3T3-L1, ATCC® CL-173TM; American Type Culture Collection, Manassas, VA, USA) were used in this study. A stock solution of PSCE in 70% dimethylsulfoxide (DMSO; Merck, Madrid, Spain) was prepared for cell culture assays. The cells were maintained in their respective culture media for up to 15 passages (see supporting information, Cell culture maintenance). For assays, the cell viability of differentiated 3T3-L1 adipocytes was determined by crystal violet staining[22] (see supporting information, Viability of 3T3-L1 cells). To determine the effect of the extract on the intracellular reactive oxygen species (ROS), the adipocytes were labelled with 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) probe[37,38] (see supporting information, Intracellular reactive oxygen species quantification in adipocytes); while the accumulation of triglycerides (TGs) in 3T3-L1 adipocytes was also determined by the Adipored™ assay reagent[30] (see Triglyceride accumulation in adipocytes). A microplate reader (Cytation™ 3 Cell Imaging Multi-Mode, BioTek, Winooski, VT, USA) was used for the measurements.

Animals and procedures

Male C57BL6J mice (5 weeks old) were purchased from Charles River (Cedex, France). Mice were bred and maintained at the Central Animal House of the Andalusian Center of Molecular Biology and Regenerative Medicine (CABIMER). All procedures with animals were approved by the Institutional Animal Care Committee of CABIMER (permission number 06-10-14-138) and performed according to the Spanish law on animal use RD 53/2013 and the European Community policy for Experimental Animal Studies (Directive 2010/63/EU). The mice were housed in groups of five animals per cage with a 12 h:12 h light–dark cycle. All mice were allowed *ad libitum* access to the test diets and water throughout the 24-week research period. Fresh water and a fixed amount of feed were provided three times per week. Body weight (BW), water intake (25.7 ± 2.9 mL per cage per day), food intake (22.6 ± 4.2 g per cage per day) and feed leftovers were recorded once per week throughout the study period. No significant differences were observed in water and food intake among dietetic experimental groups (data not shown). BW was recorded using a calibrated scale by transferring the mice to a clean empty weighing cage. Mice were euthanized by cervical dislocation.

Experimental design and dietary regimen

After a week of acclimatization, mice were randomly separated into the following four groups (Fig. 1): (i) a control group with low-fat or standard diet (LFD) ($n = 10$); (ii) a LFD group supplemented, from the beginning, with PSCE (0.8 mg kg^{-1} BW per day) in drinking water (LFD-PSCE; $n = 10$) to check whether the extract affected the control animals; and (iii) two high-fat diet (HFD) groups ($n = 10$ each). Both LFD and LFD-PSCE control groups were fed standard chow (caloric composition, 4% kcals from fat, 14.3% kcals from protein and 48% kcals from carbohydrate; 2.9 kcal g^{-1} total energy content; 2014 Teklad Global 14% Protein Rodent Maintenance Diet, Harlan, Spain; see supporting information, Table S1) for 24 weeks. The two HFD groups were fed with Teklad TD.06414 chow (caloric composition, 60% kcals from fat, 36% of these kcals from lard, 18.3% kcals from protein and 21.4% kcals from carbohydrate; 5.9 kcal g^{-1} total energy content; Teklad Adjusted Caloric Diet 60/fat; see supporting information, Table S2) for the first 12 weeks. After this period, one of the HFD group of mice were further fed with HFD ($n = 10$) for 12 more weeks while the other group received the HFD plus PSCE

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

(0.8 mg kg^{-1} BW per day) in drinking water (HFD-PSCE; $n = 10$). Before the intervention, mice were individually housed in metabolic cages (Oxylet Physiocage, Panlab, Spain), which allowed automatic monitoring of food and water intake at 1 min intervals. Mice were acclimatized for at least 12 h prior to recording. The body weight of each animal and average water consumption were recorded to adjust and ensure the concentration of PSCE in the drinking water. In total, the nutritional intervention lasted 24 weeks for the four groups. Drinking water was changed every 2 days to avoid oxidation and precipitate formation.

Standard biological parameters, insulin and cytokine measurements

Mice body weights were measured and blood samples were collected every 15 days during the whole nutritional intervention. Animals were fasted overnight prior to different analytical measurements and blood was collected from the tail vein. For plasma isolation, approximately 150 µL of blood was collected into heparinized tubes. The blood was immediately processed by centrifugation for 15 min at 2500 × *g* at 4 °C, and then the plasma was separated, aliquoted and frozen at −80 °C for further analysis. Blood glucose concentrations were measured using an automatic glucometer (Accu-Chek® Aviva System, Roche, Indianapolis, IN, USA). TGs and total cholesterol (TC) concentrations were measured using enzymatic assay kits (Thermo Fisher, Middletown, VA, USA). High-density lipoprotein (HDL)-cholesterol content was determined using a mouse HDL-C enzyme-linked immunosorbent assay (ELISA) kit (Cusabio, Hubei, China), and the low-density lipoprotein (LDL)-cholesterol level was calculated using the Friedewald equation.[39] Interleukin-6 (IL-6) and interferon-γ (INF-γ) were determined using ELISA kits (BD Biosciences, Madrid, Spain). Insulin was assayed by ELISA using a kit from Mercodia (Mercodia AB, Uppsala, Sweden) per the manufacturer's instructions. Standard curves and experimental points were performed in triplicate. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) index.[40]

Intraperitoneal glucose tolerance test (IPGTT)

Mice were fasted overnight. Then they were injected intraperitoneally with glucose (2 g kg^{-1} BW) and blood samples (20 µL) were collected at 0, 15, 30, 60 and 120 min, according to Goikoetxea-Usandizaga *et al.*[41] Blood glucose was measured as previously indicated.

Hepatic lipid extraction and total content determination

Hepatic lipids were extracted from 500 mg of tissue following the method of Folch with minor modifications.[42] The livers were homogenized in chloroform–methanol (2:1, v/v) containing butylated hydroxytoluene as an antioxidant. Then, samples were sonicated for 5 min at an amplitude of 30%, with cycles of 5 s on and 5 s off. Afterwards, samples were agitated overnight and centrifuged at 3000 × *g* for 10 min. After removal of polar lipids, the residual interface was rinsed twice with 4 mL of 50% methanol. The lower chloroform phase with the fat was collected and evaporated in a rotary evaporator under vacuum. The remaining phase was oven-dried at 45 °C for 2.5 h. Finally, the fat weight was determined and the hepatic fat content was expressed as the percentage of the wet liver weight.

Histological evaluation of liver

Paraffin-embedded liver sections and liver cryosections were stained respectively with hematoxylin–eosin (H&E) and Sirius Red to evaluate pathological changes in the livers. The area of fibrosis resulting from the accumulation of collagen fibers was assessed by direct pixel counting on binary images captured at ×40 magnification, and the average area was calculated from 10 images per liver. The analysis was conducted using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and normalized to the area of tissue that was observed.

In silico docking of the main carotenoid found in PSCE with key targets related to physiological improvement

The molecular docking methodology comprised four main steps: ligand preparation, protein selection, docking and result analysis. The major carotenoid identified in PSCE was selected as the ligand for this study. Its three-dimensional structure was obtained from the PubChem database and converted to the mol2 format using OpenBabel. Protein structures were retrieved from the Protein Data Bank (PDB) according to their IDs (supporting information, *In silico* docking experiments). Docking was performed via

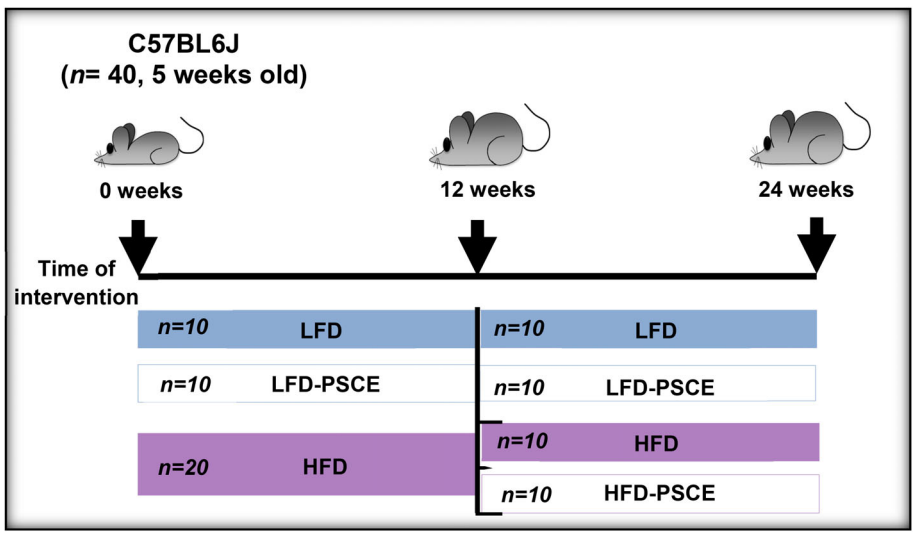


Figure 1. Experimental design. LFD: low-fat or standard diet; LFD-PSCE: standard diet plus 0.8 mg kg^{-1} BW per day of PSCE added in water; HFD: high-fat diet; HFD-PSCE: high-fat diet plus 0.8 mg kg^{-1} BW per day of PSCE, added in water.

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

SwissDock (http://www.swissdock.ch/ (accessed June 2024)) in conjunction with AutoDock Vina, utilizing the 'Attracting cavities' approach. Independent investigations of different structural sections were carried out to ensure a thorough exploration of potential binding interactions. Each run was evaluated based on predicted binding free energies and the quality of interactions at the identified binding sites. The best-scoring complexes were then subjected to further analysis, providing insights into how PSCE may interact with key targets involved in insulin sensitivity, glucose homeostasis, lipid metabolism, inflammatory responses, liver health and general metabolic health signaling pathways.

Statistical analysis

Statistical comparisons among pairs were performed using the unpaired Student's *t*-test when the variables were parametric, normally distributed according to the Shapiro–Wilk test, and when their variances were homogeneous according to Levenne's *F*-test. When the variables were nonparametric, the groups were compared using a Mann–Whitney test. The comparisons among groups with parametric variables were tested using one-way analysis of variance (ANOVA) and Tukey's *post hoc* test. In the case of nonparametric variables, the Kruskal–Wallis test was used. Results in cells were analyzed using ANOVA with Dunnett's test when data were compared to untreated cells or positive control. All tests were two-tailed. The analysis was carried out with GraphPad Prism 5.0 software (La Jolla, CA, USA). All values were expressed as the mean ± standard error of the mean (SEM), where values of $P < 0.05$ were considered statistically significant. Pearson's correlation and principal component analysis, between the composition of the extract and *in vitro* or *in vivo* results, was obtained by PAST (Paleontological Statistics) 4.12b statistical software. Statistical significative correlations were identified when $P < 0.05$.

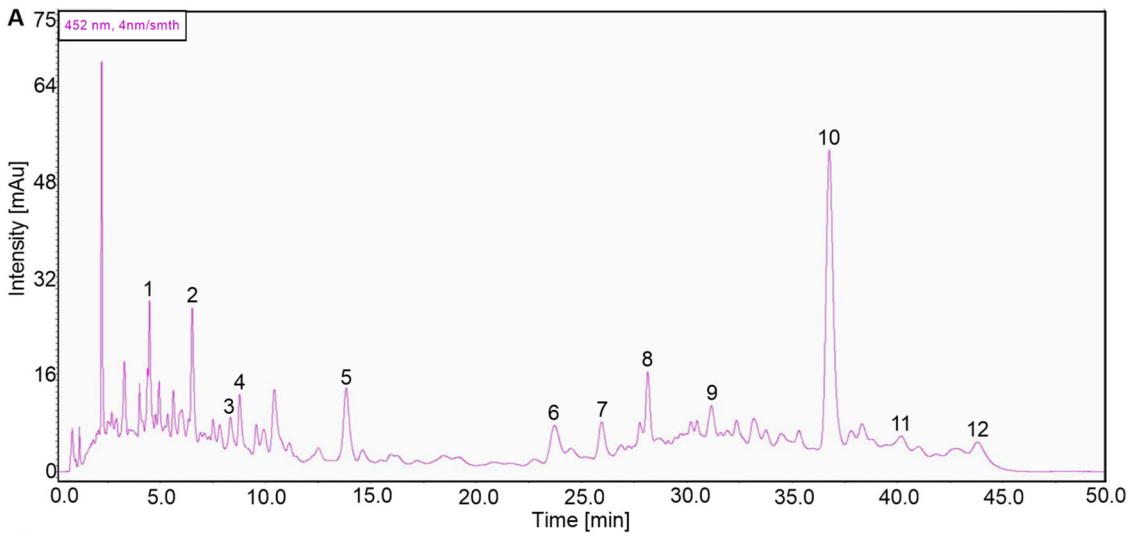
RESULTS

HPLC-DAD analysis revealed that β-CRX is the main carotenoid in PSCE

The results of HPLC-DAD analysis of PSCE are shown in Fig. 2. The chromatogram of the sample revealed 12 carotenoids (Fig. 2(A), (B)). Among them, β-CRX was the main carotenoid in PSCE, forming about 51% of the total carotenoid content of the sample (Fig. 2(C)).

PSCE attenuated oxidative stress and TG accumulation in murine 3T3-L1 adipocytes

Figure 3(A) shows the effect of PSCE on the viability of 3T3-L1 adipocytes. The extract showed no effect on the viability of 3T3-L1 adipocytes below 20 μg mL^{-1} ($P > 0.05$); however, about 10% of cell viability was reduced between 50 and 100 μg mL^{-1} PSCE ($P < 0.001$) in comparison to untreated adipocytes. The loss of cell viability caused by the maximum dose of PSCE was similar to that of DMSO ($P > 0.05$), suggesting that the vehicle and not the



B

Peak	Compound	Ret. Time	λ, nm
1	Lutein	4.531	418/493
2	Neoxanthin	6.543	437
3	Violaxanthin	8.351	405/690
4	Violaxanthin isomer	8.785	418/690
5	Antheraxanthin	13.796	437/690
6	Zeaxanthin	23.620	454/690
7	β-cryptoxanthin 5,6-epoxide	25.949	444/525
8	β-carotene isomer	28.142	449/589
9	β-cryptoxanthin isomer	31.134	439/567
10	β-cryptoxanthin	36.771	452/651
11	β-carotene	40.215	441/567
12	Lycopene	43.807	443/651

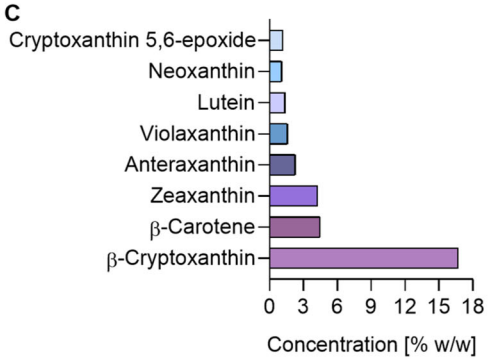


Figure 2. HPLC analysis of PSCE. (A) Representative chromatogram of PSCE. (B) Retention time (Ret. Time) (min) of compounds found in PSCE. (C) Concentration (% w/w) of identified carotenoids found in PSCE. β-CRX constituted 51% of total carotenoid content in the sample. Data shown as mean ($n = 3$).

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

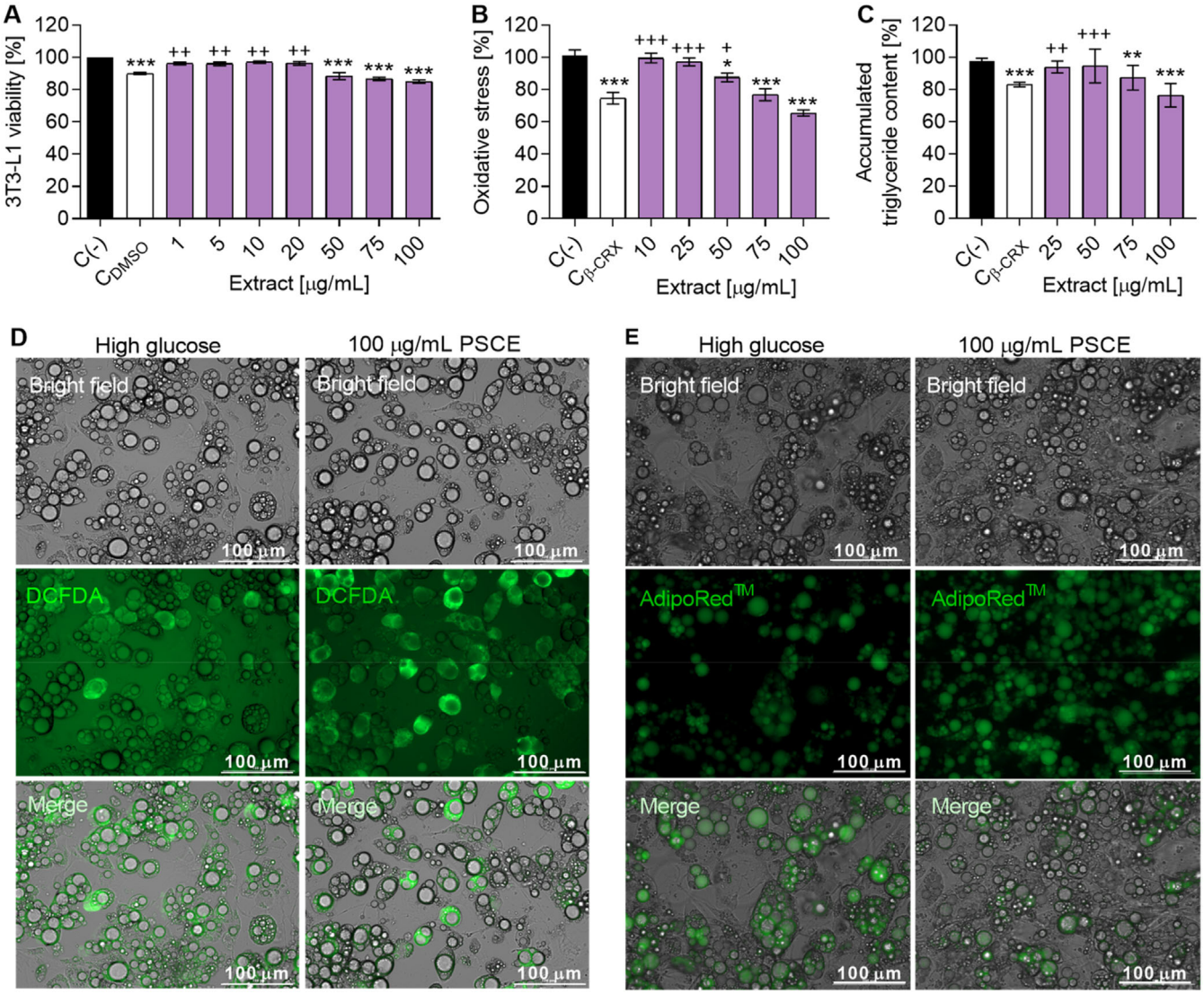


Figure 3. Effect of PSCE on murine 3T3-L1 adipocytes. (A) Cell viability at different doses of PSCE. Untreated cells (C(−)) and dimethylsulfoxide (C_{DMSO}) were included as controls. (B) Intracellular ROS and (C) intracellular TG accumulation in 3T3-L1 adipocytes treated with PSCE. Untreated adipocytes in high-glucose DMEM (C (−)) and a pure form of β-CRX ($C_{\beta\text{-CRX}}$) were included as negative and positive controls, respectively. Representative micrographs of (D) intracellular ROS labelled with H_2DCFDA and (E) intracellular TG accumulation labelled with the Adipored™ assay reagent are also shown. Results were compared to C(−) (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; with one-way ANOVA with Dunnett's *post hoc* test) and to C_{DMSO} or $C_{\beta\text{-CRX}}$ ($^{+++}P < 0.001$, $^{+}P < 0.01$, $^{+}P < 0.05$; with one-way ANOVA with Dunnett's *post hoc* test). Results are shown as mean ± standard deviation ($n = 3$).

persimmon carotenoid extract or its composition (supporting information, Fig. S1(A)), at tested doses, was the cause.

After discarding the toxicity of the extract, the potential effect of PSCE to reduce the intracellular ROS in 3T3-L1 adipocytes was explored (Fig. 3(B)). Results showed that about 40% intracellular ROS were reduced in 3T3-L1 adipocytes at the highest dose of PSCE when compared to untreated cell cultures in high-glucose DMEM ($P < 0.001$). It is known that chronic hyperglycemia contributes to increase ROS production, further exacerbating oxidative stress in diabetes and obesity, and leading to disrupted glucose regulation.[43] The effect of the extract at its highest dose was similar to that of a pure form of β-CRX ($P > 0.05$), its main component. Representative micrographs of intracellular ROS labelled with H_2DCFDA are shown in Fig. 3(D).

The results also show that PSCE exhibited an effective decrease of TG accumulation in murine 3T3-L1 adipocytes at its highest dose ($P < 0.001$) when compared to untreated cells cultured in high-glucose DMEM (Fig. 3(C)). Once again, pure β-CRX reduced the accumulated TG content similarly to PSCE ($P > 0.05$), suggesting that this carotenoid might play a pivotal role in the observed effects of the extract, according to Pearson's correlation ($P < 0.05$) (supporting information, Fig. S1(A)). Results of intracellular TG accumulation labelled with the Adipored™ assay reagent are shown in Fig. 3(E).

PSCE decreased BW and improved plasmatic lipid profile in mice fed with HFD

Figure 4(A) illustrates the effect of PSCE on BW in animals subjected to LFD and HFD. The data showed that animals fed with HFD supplemented with the extract during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in BW with respect to animals fed with HFD during the 6 months of study (HFD group) (18% reduction; $P < 0.001$). The administration of PSCE (LFD-PSCE group) did not affect BW

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

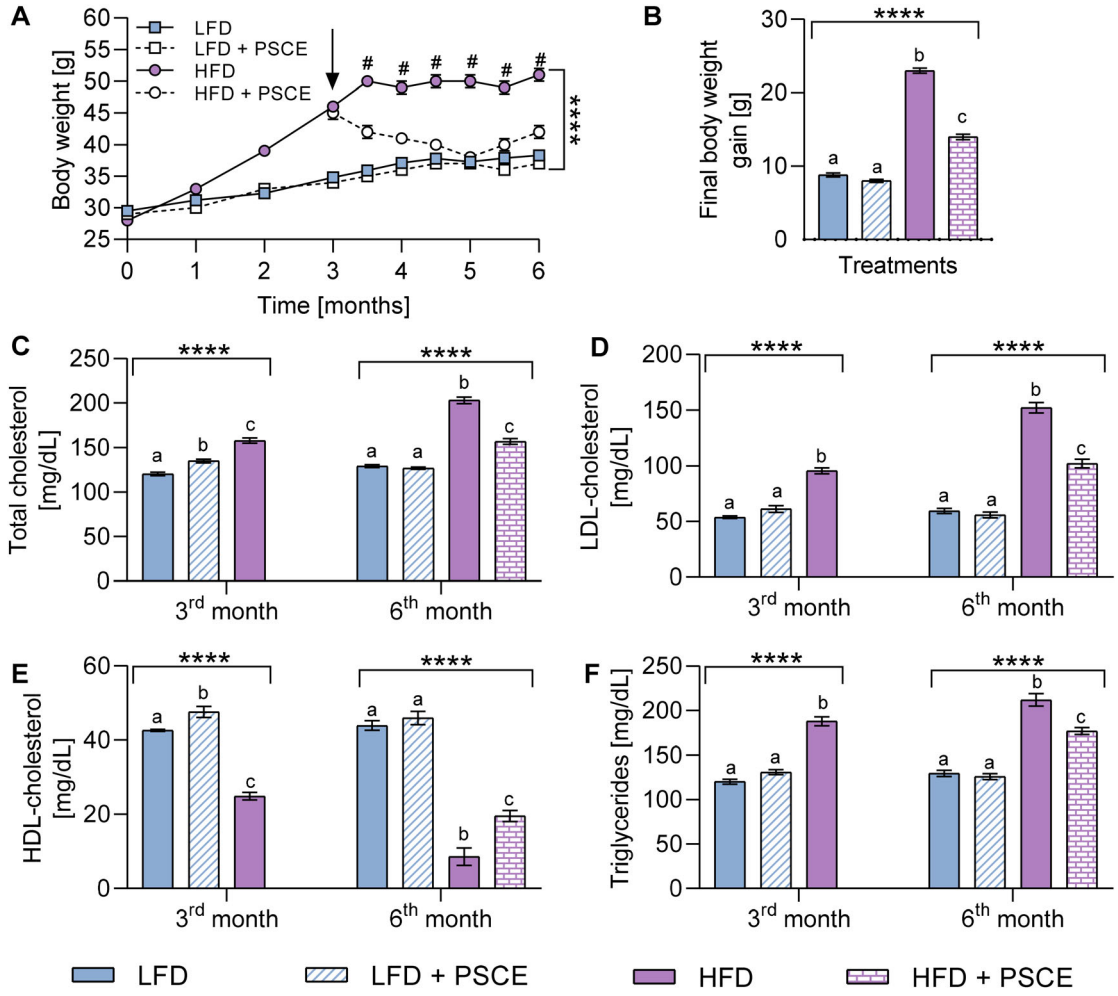


Figure 4. Effect of PSCE along the nutritional intervention study in BW and lipid profile. (A) BW during the 6 months of nutritional intervention study and (B) final BW gain at the end of the nutritional intervention study. Biochemical blood parameters in male C57BL6J mice at the third and sixth month of nutritional intervention with LFD, HFD, LFD supplemented with 0.8 mg kg^{-1} BW per day of PSCE (LFD-PSCE) and HFD supplemented with the same extract concentration in the last 3 months (HFD-PSCE). Changes in (C) total cholesterol, (D) high-density lipoprotein-cholesterol, (E) low-density lipoprotein-cholesterol and (F) triglycerides. The arrow in (A) indicates the beginning of nutritional intervention with PSCE in HFD animals. All data are shown as mean ± SEM ($n = 10$). Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test at 3 and 6 months; ****$P < 0.0001$. Different letters denote significant differences among groups.

of control animals (LFD group) (38.3 ± 0.8 g *versus* 37 ± 0.6; $P > 0.05$). Data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had as expected a significant decrease in BW gain ($P < 0.0001$) with respect to animals fed with HFD during the entire study period (Fig. 4(B)).

Respecting the plasmatic lipid profile, Fig. 4(C)–(F) shows the levels of TC, LDL-cholesterol, HDL-cholesterol and TGs in plasma samples from the four mice groups. The effects of PSCE on TC levels in animals subjected to LFD and HFD are exhibited in Fig. 4(C). Data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in TC values with respect to animals fed with HFD during the 6 months of study (HFD group) (157 ± 9 *versus* 203 ± 11 mg dL^{-1}; $P < 0.0001$). On the contrary, animals fed with HFD showed a significant increase in TC values ($P < 0.0001$) compared to both LFD and LFD-PSCE animal groups, after both 3 and 6 months. The administration of PSCE (LFD-PSCE group) did not affect TC values of control animals (LFD group).

Figure 4(D) highlights the effect of PSCE on LDL-cholesterol levels in animals fed with LFD and HFD. The data showed that animals fed with HFD supplemented with the extract during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in LDL-cholesterol values with respect to animals fed with HFD during the 6 months of study (HFD group) (102 ± 11 *versus* 152 ± 13 mg dL^{-1}; $P < 0.001$). On the other hand, animals fed with HFD showed a significant increase in LDL-cholesterol values ($P < 0.001$) compared to both LFD and LFD-PSCE animal groups, after both 3 and 6 months. The administration of PSCE (LFD-PSCE group) did not affect LDL-cholesterol values of control animals (LFD group).

Figure 4(E) illustrates the effect of PSCE on HDL-cholesterol levels in animals fed with LFD and HFD. Data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had significantly higher HDL-cholesterol levels compared to those fed with HFD during the 6 months of study (HFD group) (19.6 ± 4.2 *versus* 8.6 ± 6.6 mg dL^{-1}; $P < 0.001$). Besides, animals fed with HFD showed significantly lower HDL-cholesterol values ($P < 0.001$) compared

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

to both LFD and LFD-PSCE animal groups, after both 3 and 6 months. The administration of PSCE (LFD-PSCE group) did not affect HDL-cholesterol values of control animals (LFD group).

The effect of PSCE on TG levels in animals subjected to LFD and HFD are displayed in Fig. 4(F). The data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in TG values with respect to animals fed with HFD during the 6 months of study (HFD group) (177 ± 11 *versus* 212 ± 20 mg dL^{-1}; $P < 0.0001$). Moreover, animals fed with HFD showed a significant increase in TG values ($P < 0.01$) compared to both LFD and LFD-PSCE animal groups, after both 3 and 6 months. The administration of PSCE (LFD-PSCE group) did not affect TG values of control animals (LFD group).

Although no significant correlations were observed between PSCE supplementation and the reduction of plasmatic lipid profiles, LFD and HFD supplemented with PSCE mice were grouped with the LFD group, contrary to the results observed in the HFD group, according to the PCA performed (supporting information, Fig. S1(B)).

PSCE improved glucose homeostasis in mice fed with HFD

Figure 5(A) shows the effect of PSCE on fasting glycemia in animals subjected to LFD and HFD. The data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in blood glucose with respect to animals fed with HFD during the 6 months of study (HFD group) (135 ± 6 *versus* 185 ± 8 mg dL^{-1}; $P < 0.0001$). The animals fed with HFD showed a significant increase in blood glucose ($P < 0.0001$) compared to both LFD and LFD-PSCE animal groups. The administration of PSCE (LFD-PSCE group) did not affect the glycemia of control animals (LFD group).

The effects of PSCE on glucose homeostasis, measured by IPGTT, in animals subjected to HFD and LFD are presented in Fig. 5(B). The data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had better glycemic control after glucose overload than animals fed with HFD during the 6 months of study (HFD group). On the contrary, animals fed with HFD showed worse glycemic control after glucose overload ($P < 0.001$) compared to both LFD and LFD-PSCE animal groups. Administration of PSCE (LFD-PSCE group) did not affect glycemic regulation after glucose overload in control animals (LFD group).

Figure 5(C) displays the effect of PSCE on fasting plasma insulin in animals subjected to LFD and HFD. The data show that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in fasting plasma insulin values with respect to animals fed with HFD during the 6 months of study (HFD group) (1.3 ± 0.25 *versus* 4 ± 0.67 ng mL^{-1}; $P < 0.0001$). Otherwise, animals fed with HFD showed an increase in fasting plasma insulin ($P < 0.0001$) compared to both LFD and LFD-PSCE animal groups. The administration of LFD along with PSCE did not affect fasting plasma insulin values of control animals (LFD group).

The effects of PSCE on HOMA-IR in animals subjected to LFD and HFD are displayed in Fig. 5(D). Data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in HOMA-IR index values with respect to animals fed with HFD during the 6 months of study (HFD group) (10.7 ± 0.06 *versus* 45.7 ± 0.33; $P < 0.0001$). Moreover, animals fed with HFD showed an increase in HOMA-IR index value ($P < 0.0001$) compared to both LFD and LFD-PSCE animal groups. The administration of PSCE (LFD-PSCE group) did not affect HOMA-IR index values of control animals (LFD group).

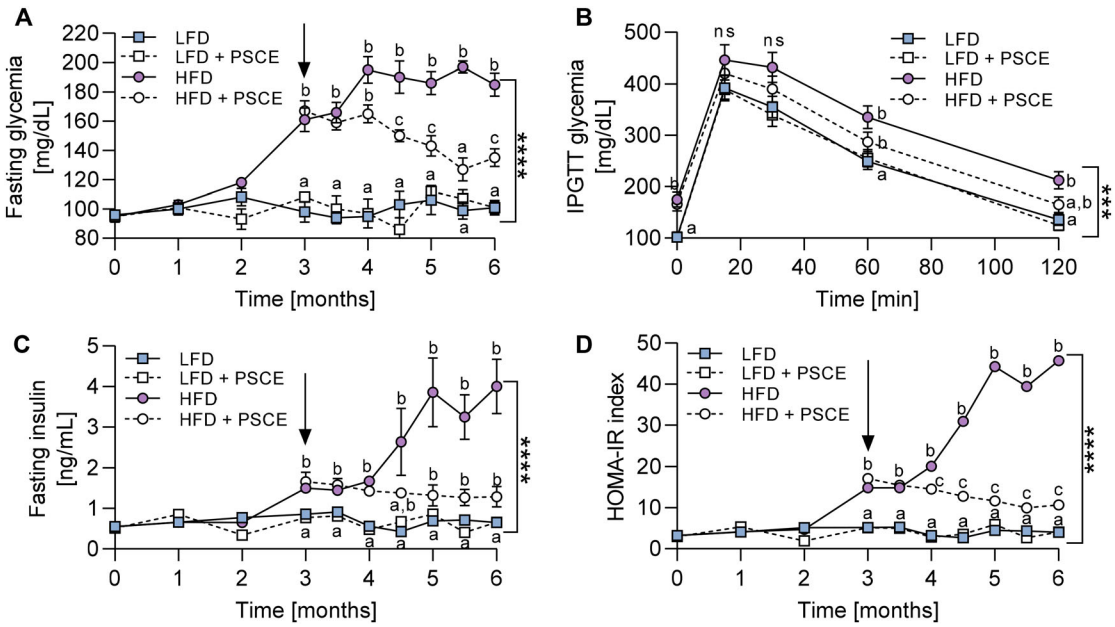


Figure 5. Effect of PSCE in glucose homeostasis along the nutritional intervention study. Male C57BL6J mice were fed throughout the 6 months of nutritional intervention with LFD, HFD, LFD supplemented with 0.8 mg kg^{-1} BW per day of PSCE (LFD-PSCE) and HFD supplemented with the same extract concentration the last 3 months (HFD-PSCE). Changes in (A) fasting glycemia, (B) intraperitoneal glucose tolerance (IPGT) glycemia, (C) fasting insulinemia and (D) HOMA-IR index. All data are shown as mean ± SEM ($n = 10$). The arrow in (A, C, D) indicates the beginning of nutritional intervention with PSCE in HFD animals. Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test at 3 and 6 months; ***$P < 0.001$, ****$P < 0.0001$. Different letters denote significant differences among groups.

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

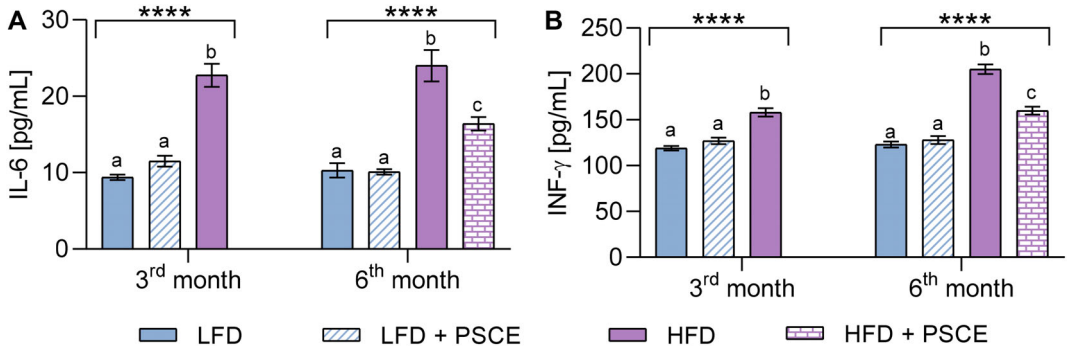


Figure 6. Plasmatic inflammatory cytokines at the third and sixth month of nutritional intervention study. Male C57BL6J mice were fed with LFD, HFD, LFD supplemented with 0.8 mg kg^{-1} BW per day of PSCE and HFD supplemented with the same extract concentration the last 3 months (HFD-PSCE). Changes in (A) IL-6 and (B) IFN-γ. All data are shown as mean ± SEM ($n = 10$). Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test at 3 and 6 months; ****$P < 0.0001$. Different letters denote significant differences among groups.

PSCE reduced inflammatory markers in mice fed with HFD

The effects of PSCE on plasma IL-6 levels in animals subjected to LFD and HFD are presented in Fig. 6(A). Data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in plasma IL-6 values with respect to animals fed with HFD during the 6 months of study (HFD group) (16.4 ± 2.5 *versus* 24 ± 5.8 pg mL^{-1}; $P < 0.001$). On the contrary, animals fed with HFD showed a significant increase in plasma IL-6 values ($P < 0.0001$) compared to both LFD and LFD-PSCE animal groups, after both 3 and 6 months. The administration of PSCE (LFD-PSCE group) did not affect plasma IL-6 values in control animals (LFD group).

Figure 6(B) highlights the effect of PSCE on plasma IFN-γ levels in animals subjected to LFD and HFD. The data show that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in plasma IFN-γ levels with respect to the animals fed with HFD during the 6 months of study (HFD group) (160 ± 12 *versus* 205 ± 15 pg mL^{-1}; $P < 0.0001$). On the other hand, animals fed with HFD showed a significant increase in plasma IFN-γ values ($P < 0.0001$) compared to both LFD and LFD-PSCE animal groups, after both 3 and 6 months. The administration of PSCE (LFD-PSCE group) did not affect IFN-γ values in control animals (LFD group).

PSCE ameliorated liver steatosis and fibrosis in mice fed with HFD

Figure 7(A) illustrates the effect of PSCE on total lipids in livers of animals subjected to LFD and HFD. Data showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in the amount of total hepatic lipids with respect to animals fed with HFD during the 6 months of study (HFD group) (14.5 ± 3.8 *versus* 23.9 ± 5.2%; $P < 0.001$), which is correlated with the high amount of β-CRX in the sample (supporting information, Fig. S1(B)). On the other hand, animals fed with HFD showed a significant increase in the amount of total hepatic lipids ($P < 0.001$) compared to both LFD and LFD-PSCE animal groups, after both 3 and 6 months. The administration of PSCE (LFD-PSCE group) did not affect the amount of total hepatic lipids in control animals (LFD group). Representative micrographs of liver sections stained with H&E are shown in Fig. 7(C).

The effects of PSCE on liver fibrosis in animals subjected to LFD and HFD are displayed in Fig. 7(B). Liver fibrosis was determined by measuring the amount of collagen present in the livers. The data analysis showed that animals fed with HFD supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) had a significant decrease in collagen with respect to animals fed with HFD during the 6 months of study (HFD group) (1.58 ± 0.62 *versus* 3.3 ± 1.1%; $P < 0.0001$). Moreover, animals fed with HFD showed a significant increase in collagen ($P < 0.01$) compared to both LFD and LFD-PSCE animal groups, after both 3 and 6 months. Administration of PSCE (LFD-PSCE group) did not affect the amount of collagen present in livers of control animals (LFD group). Representative micrographs of liver sections stained with Sirius Red are shown in Fig. 7(D).

In silico interactions of β-CRX showed high affinity with proteins involved in glucose metabolism

The interaction of β-CRX, the main carotenoid found in PSCE, with 27 ligands was evaluated using *in silico* docking tools, including attracting cavity (Fig. 8(A)) and SwissParam (Fig. 8(B)) scoring. Among all tested ligands, β-CRX demonstrated the most favorable binding with ADIPOR and GLUT1, exhibiting a strong compatibility with the binding site of both proteins (−6.64 ± 4.15 *versus* −2.33 ± 1.17), according to the attracting cavity score. These results mirrored the values obtained by the SwissParam score, where β-CRX with both ADIPOR and GLUT1 displayed strong molecular stability (−10.32 ± 0.49 *versus* −10.06 ± 0.29). These results highlight the potential of β-CRX as a promising ligand for modulating both ADIPOR and GLUT1 receptor functions with robust and stable interactions (Fig. 8(C)).

DISCUSSION

The results of the study show that dietary supplementation with PSCE rich in β-CRX during 3 months exerted a physiological improvement on HFD-fed mice, reducing the severity and progression of T2DM and MASLD. β-CRX is a carotenoid naturally found in fruits and vegetables such as tangerines, oranges, peaches, nectarines, papaya, mangoes, yellow and orange maize, red peppers, pumpkin and watermelon. In PSCE, β-CRX was present at more than 50% of the total carotenoid content of the sample, which agrees with previous work.[21] *In vitro*, *in vivo* and epidemiological studies suggest that β-CRX, besides as a precursor of vitamin A,[44] provides diverse health-related benefits, particularly protective effects against certain cancers,[26] T2DM,[45-47] MASLD[48,49] and cardiovascular disease.[50] Despite the evidence of the beneficial properties of β-CRX in human nutrition and

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

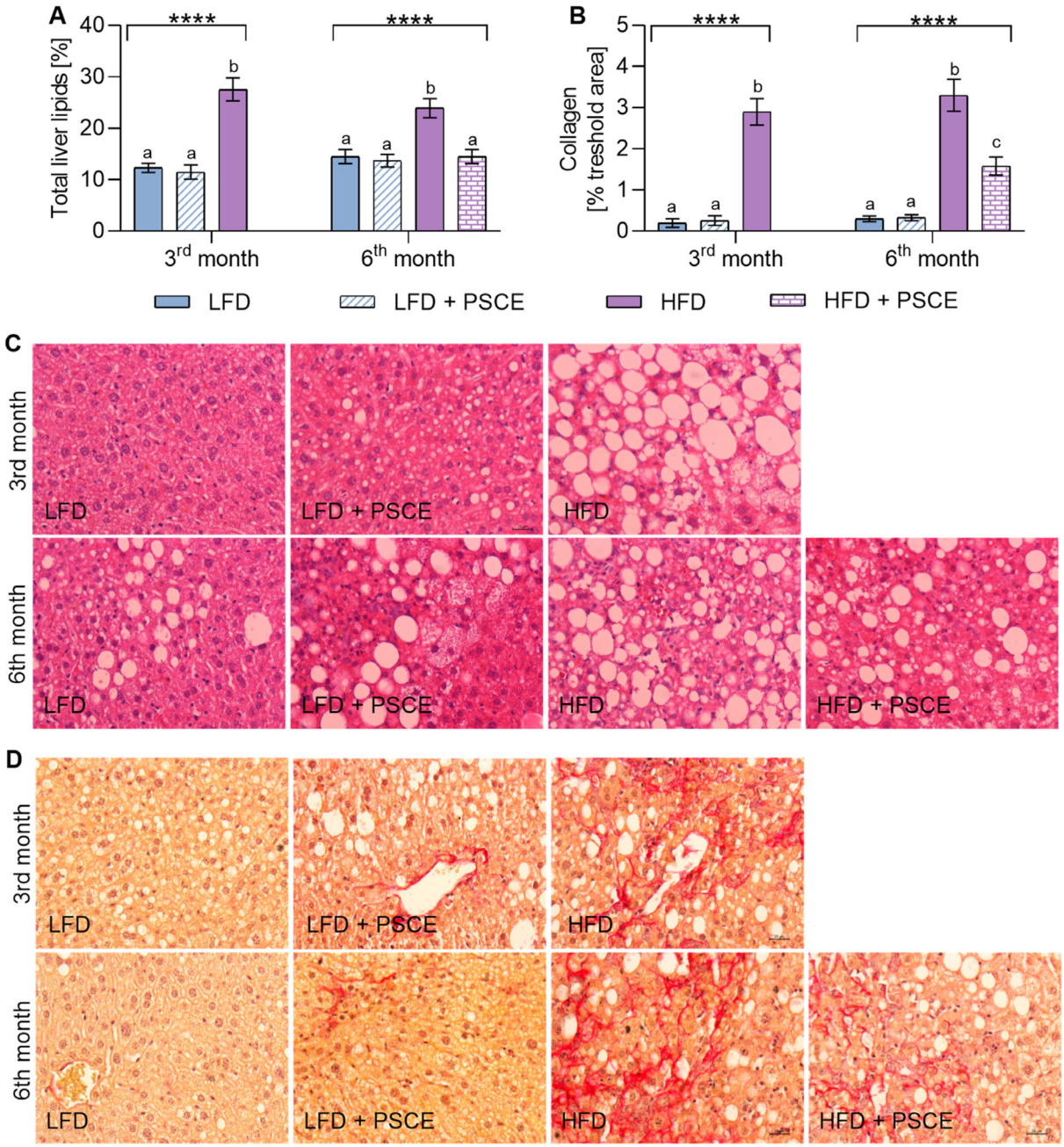


Figure 7. Histological analysis of liver at the third and the sixth month of nutritional intervention. Male C57BL6J mice were fed with LFD, HFD, LFD supplemented with 0.8 mg kg^{-1} BW per day of PSCE (LFD-PSCE) and HFD supplemented with the same extract concentration the last 3 months (HFD-PSCE). Representative micrographs of liver sections stained with (A) H&E or (B) Sirius Red are shown. Image resolution ×40. (C) Total liver lipid content and (D) collagen in male C57BL6J mice at the third and sixth month of nutritional intervention with LFD, HFD, LFD-PSCE and HFD-PSCE. All data are shown as mean ± SEM ($n = 10$). Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test at three and 6 months; ****$P < 0.0001$. Different letters denote significant differences among groups.

health, information about the mechanisms and metabolic pathways related to these beneficial effects is largely unknown.[51]

Here, the dietary intake of *β*-CRX during 3 months significantly reduced BW gain and improved serum lipid profile in male C57BL6J mice fed with HFD. The lipid profile included measurement of TC, HDL-cholesterol, LDL-cholesterol and TGs. This suggests that PSCE supplementation was successful in mitigating key risk factors associated with obesity and dyslipidemia in HFD-fed mice, especially due to its high content of *β*-CRX (supporting information, Fig. S1(B)). Moreover, the absence of these effects in control mice fed with LFD confirms that PSCE does not induce weight loss or metabolic changes under normal dietary conditions, indicating that the positive effects observed happened under specific metabolic disturbances caused by HFD and associated with obesity. The reduction in TGs and LDL-cholesterol suggests that PSCE influences lipid metabolism, which is also supported by the effect of the extract on TG accumulation in 3T3-L1 cells, probably through pathways associated with

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

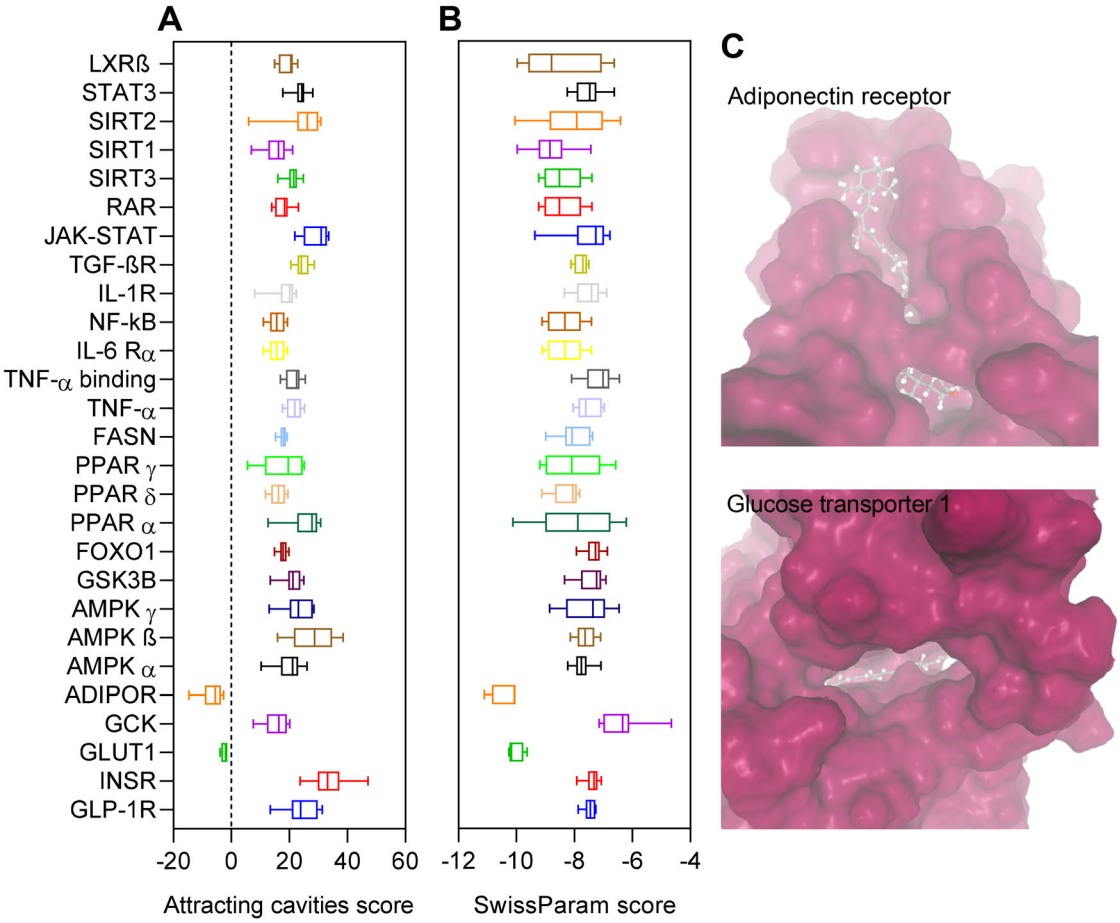


Figure 8. *In silico* docking interactions of β-CRX with targets related to physiological improvement in HFD-induced mice. (A) Attracting cavities score and (B) SwissParam score of the interactions. (C) Three-dimensional binding interaction of β-CRX with the targets that showed the highest affinity scores. The targets that were analyzed: liver X receptor beta (LXRβ), signal transducer and activator of transcription 3 (STAT3), sirtuin-1, -2 and -3 (SIRT1–3), retinoic acid receptor (RAR), janus kinase-STAT (JAK–STAT), transforming growth factor beta receptor (TGF-βR), interleukin 1 beta receptor (IL-1R), nuclear factor kappa-B (NF-κB), interleukin 6 receptor alpha (IL-6 Rα) unit, tumor necrosis factor alpha (TNF-α) binding, tumor necrosis factor alpha (TNF-α), fatty acid synthase (FASN) thioesterase domain, peroxisome proliferator-activated receptor gamma (PPARγ), peroxisome proliferator-activated receptor delta (PPARδ), peroxisome proliferator-activated receptor alpha (PPARα), forkhead box protein O-1 (FOXO1), glycogen synthase kinase 3 beta (GSK3β), activated protein kinase gamma (AMPKγ), activated protein kinase beta (AMPKβ), activated protein kinase alpha (AMPKα), adiponectin receptor 1 (ADIPOR), glucokinase (GCK), glucose transporter type 1 (GLUT1), insulin receptor 1 (INSR), glucagon-like peptide-1 receptor (GLP-1R).

peroxisome proliferator-activated receptors (PPARs), which are known to regulate lipid homeostasis related to obesity or T2DM.[52]

In PSCE, we also identified other carotenoids, albeit at significantly lower concentrations. These carotenoids were mainly β-carotene and zeaxanthin. Published HFD/NASH models indicate that β-CRX is efficacious at lower exposures than zeaxanthin and β-carotene, often needing 20–100 mg kg^{-1} per day of zeaxanthin[53] or 0.5% dietary β-carotene,[54] supporting our interpretation that β-CRX (present at approximately three times the level of the other carotenoids in PSCE) is the principal driver of the observed efficacy.

Regarding other pathways that might be stimulated by PSCE, *in silico* results revealed strong and stable interactions with ADIPOR and GLUT1, key proteins involved in glucose homeostasis and metabolism.[55,56] ADIPOR1 is a key mediator of the metabolic effects of adiponectin. Its dysfunction or downregulation contributes to insulin resistance and chronic inflammation, central features of T2DM.[57,58] Regarding GLUT1, although is not a driver of insulin resistance, its upregulation in hyperglycemia contributes to diabetic complications via increased glucose uptake and oxidative stress in insulin-independent tissues.[31,32] In this sense, PSCE, through β-CRX, might have contributed mechanistically to the regulation of fasting blood glucose levels and glucose homeostasis, measured by IPGTT, in male C57BL6J mice during 24 weeks of nutritional intervention. The data revealed a significant improvement in glucose tolerance, as evidenced by IPGTT values in the HFD-PSCE group compared to the HFD group at the end of the nutritional intervention period. This effect was potentially due to enhanced glucose transport efficiency.[59] Additionally, animals fed with HFD supplemented with PSCE during the final 3 months of nutritional intervention (HFD-PSCE group) exhibited improved fasting glycemic control, which may be linked to GLUT1 modulation as well.[28] PSCE may have also improved insulin sensitivity and glucose homeostasis via ADIPOR activation, as indicated by the noted reduction in HOMA-IR values. This suggest a potential role in slowing the progression of insulin resistance and delaying the onset of T2DM[60] through mechanisms that promote glucose uptake and fatty acid oxidation[27]; however, more evidence is required to confirm these findings.

T2DM typically begins with the onset of glucose intolerance, a prediabetic state of hyperglycemia frequently caused by insulin resistance in liver and muscles. In this study, PSCE supplementation appeared to counteract this process by improving insulin sensitivity. Moreover, low-grade inflammation is a common feature in T2DM patients,[61] and systematic inflammation and BW above a weight considered desirable may synergistically contribute to insulin resistance.[62,63] Thus, the anti-inflammatory potential of the supplementation with PSCE was also explored. T2DM is an important risk factor for MASLD and vice versa.[64] The improvement in insulin sensitivity and lipid profile observed in the PSCE-supplemented mice suggests that the extract may simultaneously reduce the risk of developing both conditions.

The observed reduction in pro-inflammatory cytokines IFN-γ and IL-6 in HFD-fed mice supplemented with PSCE during the last 3 months of nutritional intervention (HFD-PSCE group) is consistent with the known anti-inflammatory effects of carotenoids.[14,65,66] The anti-inflammatory activity of PSCE, or specifically β-CRX, is plausibly mediated through the activation of ADIPOR rather than direct interactions with AMPK or pro-inflammatory cytokines, as *in silico* data demonstrated robust molecular compatibility and stable interactions between β-CRX and ADIPOR rather than with AMPK, IL-6 receptor or TNF-alpha receptor, which agrees as well with the reported anti-inflammatory activity of other carotenoids.[27,28,56] The modulation of inflammatory circulating blood proteins such as IFN-γ and IL-6 helps to restore glucose homeostasis after an overload.[67] The decrease in these markers in serum, coupled with the improved insulin sensitivity, suggests that β-CRX supplementation may mitigate the low-grade inflammation typically associated with T2DM and MASLD; however, this should be corroborated in further studies since a reduction of inflammation can also be achieved by the accumulative improvements in systemic metabolic health facilitated by β-CRX like BW reduction.

The decrease in total hepatic lipids and extracellular collagen deposition in β-CRX-supplemented mice suggests that persimmon extract alleviates liver damage, as evidenced by these key biomarkers of liver health. Fatty liver disease, or hepatic steatosis, refers to the build-up of intrahepatic fats (triacylglycerols) comprising at least 5% of liver weight. This condition marks the initial stage of MASLD and can progress to severe complications such as fibrosis, cirrhosis or hepatocellular carcinoma if inflammation is triggered.[15] In this study, PSCE supplementation appears to mitigate the inflammatory process, potentially reducing the risk of fibrosis progression. In MASLD, GLUT1 facilitates excessive glucose entry into hepatocytes and non-parenchymal cells, feeding lipogenesis, inflammation and fibrosis in an insulin-resistant liver. Its upregulation may represent a maladaptive metabolic adaptation, worsening liver injury and disease progression. Conversely, ADIPOR1 protects against fat accumulation, inflammation and fibrosis. Impaired adiponectin/ADIPOR1 signaling in MAFLD contributes to disease progression, making this pathway a potential therapeutic target.[68] β-CRX supplementation may modulate ADIPOR and GLUT1 expression, improving lipid metabolism and reducing substrate availability for *de novo* lipogenesis.[29,69] This finding aligns with the lipid-lowering effects observed with persimmon extract supplementation. Regarding liver fibrosis, particularly in the context of chronic HFD feeding, our findings highlight the potential of β-CRX in modulating both fat accumulation and fibrosis-related pathways.[67] Nevertheless, pathophysiological mechanisms underlying liver collagen changes in T2DM are still not well clear and warrant further investigation.[41,70]

Several studies have demonstrated the relationships between carotenoid consumption and obesity, T2DM and MASLD, as well as key parameters associated with both diseases, including insulin resistance, glucose homeostasis and lipid metabolism. Our study describes the effects of dietary intake of PSCE rich in β-CRX in an HFD-induced animal model of T2DM and MASLD. Our data provide novel insights into its potential therapeutic effects in these metabolic diseases. The high content of β-CRX gives to this extract an important provitamin A value and suggests a combined contribution in all the beneficial effects detected in the study, which are in line with previous reports that link higher dietary intake of β-carotene and the reduction of the risk of T2DM and MASLD, by improving insulin resistance.[13,14,65,66]

Although β-CRX has been less extensively studied than β-carotene and other carotenoids, the effects observed in this study suggest that it may exert an important role in modulating glucose homeostasis, insulin resistance, inflammation and metabolic pathways associated with T2DM and MASLD. β-CRX can be absorbed in the small intestine after ester hydrolysis and micellarization,[51,71] incorporated into chylomicrons and transported via LDL and HDL, with preferential accumulation in the liver and adipose tissue.[13,72,73] Human studies show a postprandial chylomicron peak at *ca* 6 h and a dose-dependent rise in plasma levels,[73] confirming systemic bioavailability and supporting its capacity to exert direct anti-obesity and metabolic potential. In addition, the *in silico* analysis revealed strong and stable interactions between β-CRX and key proteins such as ADIPOR and GLUT1, highlighting potential mechanistic pathways underlying observed effects; however, this requires validation through targeted *in vitro* and *in vivo* studies.

Given its high content in persimmon, dietary β-CRX represents a promising approach for addressing metabolic disorders, particularly in the early stages of T2DM and MASLD, and may complement the effects of other well-known carotenoids.[18] Further preclinical and clinical trials are needed to confirm these mechanism pathways to establish the therapeutic potential of PSCE rich in β-CRX as a nutraceutical in the prevention and management T2DM and MASLD.

AUTHOR CONTRIBUTIONS

BM-C, NM, AC, SF-P and JS-B: Data curation, Investigation, Methodology, Formal analysis, Visualization. FM: Conceptualization, Writing – original draft, Funding acquisition, Project administration. MV: Conceptualization, Writing – original draft, Writing – review & editing. DS: Conceptualization, Supervision, Project administration. MCM-M and GB: Conceptualization, Supervision, Validation.

FUNDING INFORMATION

This research was funded by the Junta de Andalucía (PAI-BIO311) and the Ministerio de Ciencia e Innovación (Spain) through the funded project with code number PID2020-116731RB-C21 to FM.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 American Diabetes Association, 2. Classification and diagnosis of diabetes: standards of medical Care in Diabetes – 2018. *Diabetes Care* **41**:S13–S27 (2018) Available: https://diabetesjournals.org/care/article/41/Supplement_1/S13/30088/2-Classification-and-Diagnosis-of-Diabetes.

2 Ludwig J, Viggiano TR, McGill DB and Oh BJ, Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* **55**:434–438 (1980) Available: https://europepmc.org/article/med/7382552?utm_medium=email&utm_source=transaction&client=bot.

3 Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K *et al*., The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* **55**:2005–2023 (2012) Available: https://onlinelibrary.wiley.com/doi/10.1002/hep.25762.

4 Morrison MC, Mulder P, Salic K, Verheij J, Liang W, Van Duyvenvoorde W *et al*., Intervention with a caspase-1 inhibitor reduces obesity-associated hyperinsulinemia, non-alcoholic steatohepatitis and hepatic fibrosis in LDLR−/−.Leiden mice. *Int J Obes* **40**:1416–1423 (2016) Available: https://www.nature.com/articles/ijo201674.

5 Dongiovanni P, Lanti C, Riso P and Valenti L, Nutritional therapy for non-alcoholic fatty liver disease. *J Nutr Biochem* **29**:1–11 (2016) Available: https://linkinghub.elsevier.com/retrieve/pii/S0955286315002259.

6 Loomba R and Sanyal AJ, The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* **10**:686–690 (2013) Available: https://www.nature.com/articles/nrgastro.2013.171.

7 Buzzetti E, Pinzani M and Tsochatzis EA, The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* **65**:1038–1048 (2016) Available: https://linkinghub.elsevier.com/retrieve/pii/S0026049515003832.

8 Farias-Pereira R, Zuk JB and Khavaran H, Plant bioactive compounds from Mediterranean diet improve risk factors for metabolic syndrome. *Int J Food Sci Nutr* **74**:403–423 (2023) Available: https://www.tandfonline.com/doi/full/10.1080/09637486.2023.2232949.

9 Godos J, Federico A, Dallio M and Scazzina F, Mediterranean diet and nonalcoholic fatty liver disease: molecular mechanisms of protection. *Int J Food Sci Nutr* **68**:18–27 (2017) Available: https://www.tandfonline.com/doi/full/10.1080/09637486.2016.1214239.

10 Al Shamsi M, Amin A and Adeghate E, Vitamin E ameliorates some biochemical parameters in normal and diabetic rats. *Ann N Y Acad Sci* **1084**:411–431 (2006) Available: https://nyaspubs.onlinelibrary.wiley.com/doi/10.1196/annals.1372.033.

11 Shamsi MA, Amin A and Adeghate E, Effect of vitamin C on liver and kidney functions in normal and diabetic rats. *Ann N Y Acad Sci* **1084**:371–390 (2006) Available: https://nyaspubs.onlinelibrary.wiley.com/doi/10.1196/annals.1372.031.

12 Balbuena E, Cheng J and Eroglu A, Carotenoids in orange carrots mitigate non-alcoholic fatty liver disease progression. *Front Nutr* **9**:987103 (2022) Available: https://www.frontiersin.org/articles/10.3389/fnut.2022.987103/full.

13 Clugston RD, Carotenoids and fatty liver disease: current knowledge and research gaps. *Biochim Biophys Acta* **1865**:158597 (2020) Available: https://linkinghub.elsevier.com/retrieve/pii/S1388198119302483.

14 Marcelino G, Machate DJ, Freitas KDC, Hiane PA, Maldonade IR, Pott A *et al*., β-carotene: preventive role for type 2 diabetes mellitus and obesity: a review. *Molecules* **25**:5803 (2020) Available: https://www.mdpi.com/1420-3049/25/24/5803.

15 Nassir F, Rector RS, Hammoud GM and Ibdah JA, Pathogenesis and prevention of hepatic steatosis. *Gastroenterol Hepatol (N Y)* **11**:167–175 (2015).

16 Roohbakhsh A, Karimi G and Iranshahi M, Carotenoids in the treatment of diabetes mellitus and its complications: a mechanistic review. *Biomed Pharmacother* **91**:31–42 (2017) Available: https://linkinghub.elsevier.com/retrieve/pii/S0753332217304523.

17 Elvira-Torales LI, Navarro-González I, Rodrigo-García J, Seva J, García-Alonso J and Periago-Castón MJ, Consumption of spinach and tomato modifies lipid metabolism, reducing hepatic steatosis in rats. *Antioxidants*. **9**:1041 (2020) Available: https://www.mdpi.com/2076-3921/9/11/1041.

18 Jin Y and Arroo R, The protective effects of flavonoids and carotenoids against diabetic complications – a review of *in vivo* evidence. *Front Nutr* **10**:1020950 (2023) Available: https://www.frontiersin.org/articles/10.3389/fnut.2023.1020950/full.

19 Sluijs I, Cadier E, Beulens JWJ, Van Der A DL, Spijkerman AMW and Van Der Schouw YT, Dietary intake of carotenoids and risk of type 2 diabetes. *Nutr Metab Cardiovasc Dis* **25**:376–381 (2015) Available: https://linkinghub.elsevier.com/retrieve/pii/S0939475314003731.

20 Ylönen K, Alfthan G, Groop L, Saloranta C, Aro A and Virtanen SM, Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia dietary study. *Am J Clin Nutr* **77**:1434–1441 (2003) Available: https://linkinghub.elsevier.com/retrieve/pii/S0002916523058057.

21 Gea-Botella S, Agulló L, Martí N, Martínez-Madrid MC, Lizama V, Martín-Bermudo F *et al*., Carotenoids from persimmon juice processing. *Food Res Int*. **141**:109882 (2021) Available: https://linkinghub.elsevier.com/retrieve/pii/S0963996920309078.

22 Salazar-Bermeo J, Moreno-Chamba B, Martínez-Madrid MC, Saura D, Valero M and Martí N, Potential of persimmon dietary fiber obtained from byproducts as antioxidant, prebiotic and modulating agent of the intestinal epithelial barrier function. *Antioxidants* **10**:1668 (2021) Available: https://www.mdpi.com/2076-3921/10/11/1668.

23 Jomova K and Valko M, Health protective effects of carotenoids and their interactions with other biological antioxidants. *Eur J Med Chem* **70**:102–110 (2013) Available: https://linkinghub.elsevier.com/retrieve/pii/S0223523413006351.

24 Seifried RM, Harrison E and Seifried HE, Antioxidants in health and disease, in *Nutrition in the Prevention and Treatment of Disease*. Elsevier, London, pp. 321–346 (2017) Available: https://linkinghub.elsevier.com/retrieve/pii/B9780128029282000163.

25 Ghodratizadeh S, Kanbak G, Beyramzadeh M, Dikmen ZG, Memarzadeh S and Habibian R, Effect of carotenoid β-cryptoxanthin on cellular and humoral immune response in rabbit. *Vet Res Commun* **38**:59–62 (2014) Available: http://link.springer.com/10.1007/s11259-013-9584-8.

26 Lim JY and Wang XD, Mechanistic understanding of β-cryptoxanthin and lycopene in cancer prevention in animal models. *Biochim Biophys Acta* **1865**:158652 (2020) Available: https://linkinghub.elsevier.com/retrieve/pii/S1388198120300445.

27 Coulter AA, Greenway FL, Zhang D, Ghosh S, Coulter CR, James SL *et al*., Naringenin and β-carotene convert human white adipocytes to a beige phenotype and elevate hormone-stimulated lipolysis. *Front Endocrinol* **14**:1148954 (2023) Available: https://www.frontiersin.org/articles/10.3389/fendo.2023.1148954/full.

28 Hsieh YY, Hou WC, Hsu SJ, Liaw CC, Huang C, Shih MCM *et al*., Consumption of carotenoid-rich *Momordica cochinchinensis* (Gac) aril improves glycemic control in type 2 diabetic mice partially through taste receptor type 1 mediated glucagon-like peptide 1 secretion. *Food Funct* **15**:11415–11431 (2024) Available: https://xlink.rsc.org/?DOI=D4FO04316B.

29 Xu W, Tian M and Zhou Y, The relationship between insulin resistance, adiponectin and C-reactive protein and vascular endothelial injury in diabetic patients with coronary heart disease. *Exp Ther Med* **16**:2022–2026 (2018) Available: http://www.spandidos-publications.com/10.3892/etm.2018.6407.

30 Geddo F, Scandiffio R, Antoniotti S, Cottone E, Querio G, Maffei ME *et al*., PipeNig®-FL, a fluid extract of black pepper (*Piper Nigrum* L.) with a high standardized content of trans-β-caryophyllene, reduces lipid accumulation in 3T3-L1 preadipocytes and improves glucose uptake in C2C12 myotubes. *Nutrients* **11**:2788 (2019) Available: https://www.mdpi.com/2072-6643/11/11/2788.

31 Heilig CW, Deb DK, Abdul A, Riaz H, James LR, Salameh J *et al*., GLUT1 regulation of the pro-sclerotic mediators of diabetic nephropathy. *Am J Nephrol* **38**:39–49 (2013) Available: https://karger.com/article/doi/10.1159/000351989.

32 Wang Y, Heilig K, Saunders T, Minto A, Deb DK, Chang A *et al*., Transgenic overexpression of GLUT1 in mouse glomeruli produces renal disease resembling diabetic glomerulosclerosis. *Am J Physiol*

Renal Physiol **299**:F99–F111 (2010) Available: https://www.physiology.org/doi/10.1152/ajprenal.00466.2009.
33 Hosseininejad S, González CM, Hernando I and Moraga G, Valorization of persimmon fruit through the development of new food products. *Front Food Sci Technol* **2**:914952 (2022) Available: https://www.frontiersin.org/articles/10.3389/frfst.2022.914952/full.
34 Direito R, Rocha J, Sepodes B and Eduardo-Figueira M, From *Diospyros kaki* L. (persimmon) phytochemical profile and health impact to new product perspectives and waste valorization. *Nutrients* **13**:3283 (2021) Available: https://www.mdpi.com/2072-6643/13/9/3283.
35 Transforming our world: the 2030 Agenda for Sustainable Development (2015). Available: https://www.refworld.org/legal/resolution/unga/2015/en/111816.
36 Gea-Botella S, Moreno-Chamba B, De La Casa L, Salazar-Bermeo J, Martí N, Martínez-Madrid MC *et al.*, Carotenoids from persimmon (*Diospyros kaki* Thunb.) byproducts exert photoprotective, antioxidative and microbial anti-adhesive effects on HaCaT. *Pharmaceutics* **13**:1898 (2021) Available: https://www.mdpi.com/1999-4923/13/11/1898.
37 Moreno-Chamba B, Salazar-Bermeo J, Narváez-Asensio M, Navarro-Simarro P, Saura D, Neacsu M *et al.*, Polyphenolic extracts from *Diospyros kaki* and *Vitis vinifera* by-products stimulate cytoprotective effects in bacteria-cell host interactions by mediation of transcription factor Nrf2. *Phytomedicine* **134**:156020 (2024) Available: https://linkinghub.elsevier.com/retrieve/pii/S0944711324006779.
38 Salazar-Bermeo J, Moreno-Chamba B, Hernández-García M, Saura D, Valero M, Martí N *et al.*, Optimization of hypobaric and ultrasonic processing of persimmon rhamnogalacturonan-I to enhance drug-digestion interactions. *Int J Biol Macromol* **295**:139453 (2025) Available: https://linkinghub.elsevier.com/retrieve/pii/S0141813025000029.
39 Friedewald WT, Levy RI and Fredrickson DS, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**:499–502 (1972) Available: https://academic.oup.com/clinchem/article/18/6/499/5676160.
40 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC, Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**:412–419 (1985) Available: http://link.springer.com/10.1007/BF00280883.
41 Goikoetxea-Usandizaga N, Bravo M, Egia-Mendikute L, Abecia L, Serrano-Maciá M, Urdinguio RG *et al.*, The outcome of boosting mitochondrial activity in alcohol-associated liver disease is organ-dependent. *Hepatology* **78**:878–895 (2023) Available: https://journals.lww.com/10.1097/HEP.0000000000000303.
42 Mopuri R, Kalyesubula M, Rosov A, Edery N, Moallem U and Dvir H, Improved Folch method for liver-fat quantification. *Front Vet Sci* **7**:594853 (2021) Available: https://www.frontiersin.org/articles/10.3389/fvets.2020.594853/full.
43 Caturano A, D'Angelo M, Mormone A, Russo V, Mollica MP, Salvatore T *et al.*, Oxidative stress in type 2 diabetes: impacts from pathogenesis to lifestyle modifications. *Curr Issues Mol Biol* **45**:6651–6666 (2023) Available: https://www.mdpi.com/1467-3045/45/8/420.
44 Burri BJ, β-Cryptoxanthin as a source of vitamin a. *J Sci Food Agric* **95**:1786–1794 (2015) Available: https://onlinelibrary.wiley.com/doi/10.1002/jsfa.6942.
45 Takayanagi K, Morimoto S, Shirakura Y, Mukai K, Sugiyama T, Tokuji Y *et al.*, Mechanism of visceral fat reduction in Tsumura Suzuki obese, diabetes (TSOD) mice orally administered β-cryptoxanthin from satsuma mandarin oranges (*Citrus unshiu* Marc). *J Agric Food Chem* **59**:12342–12351 (2011) Available: https://pubs.acs.org/doi/10.1021/jf202821u.
46 Sugiura M, Nakamura M, Ogawa K, Ikoma Y and Yano M, High-serum carotenoids associated with lower risk for developing type 2 diabetes among Japanese subjects: Mikkabi cohort study. *BMJ Open Diabetes Res Care* **3**:e000147 (2015) Available: https://drc.bmj.com/lookup/doi/10.1136/bmjdrc-2015-000147.
47 Montonen J, Knekt P, Järvinen R and Reunanen A, Dietary antioxidant intake and risk of type 2 diabetes. *Diabetes Care* **27**:362–366 (2004) Available: https://diabetesjournals.org/care/article/27/2/362/28291/Dietary-Antioxidant-Intake-and-Risk-of-Type-2.
48 Ni Y, Nagashimada M, Zhan L, Nagata N, Kobori M, Sugiura M *et al.*, Prevention and reversal of lipotoxicity-induced hepatic insulin resistance and steatohepatitis in mice by an antioxidant carotenoid, β-cryptoxanthin. *Endocrinology* **156**:987–999 (2015) Available: https://academic.oup.com/endo/article/156/3/987/2423104.
49 Ota T, Prevention of NAFLD/NASH by astaxanthin and β-cryptoxanthin, in *Carotenoids: Biosynthetic and Biofunctional Approaches*, Vol. **1261**, ed. by Misawa N. Springer, Singapore, pp. 231–238 (2021) Available: http://link.springer.com/10.1007/978-981-15-7360-6_21.
50 Gammone M, Graziano Riccioni G and D'Orazio N, Carotenoids: potential allies of cardiovascular health? *Food Nutr Res* **59**:26762 (2015) Available: http://foodandnutritionresearch.net/index.php/fnr/article/view/759.
51 Burri BJ, La Frano MR and Zhu C, Absorption, metabolism, and functions of β-cryptoxanthin. *Nutr Rev* **74**:69–82 (2016) Available: https://academic.oup.com/nutritionreviews/article-lookup/doi/10.1093/nutrit/nuv064.
52 Takayanagi K, Prevention of adiposity by the oral administration of β-cryptoxanthin. *Front Neur* **2**:1–6 (2011) Available: http://journal.frontiersin.org/article/10.3389/fneur.2011.00067/abstract.
53 Jin Z, Liu M, Zhao H, Xie J, Yin W, Zheng M *et al.*, Effects of zeaxanthin on the insulin resistance and gut microbiota of high-fat-diet-induced obese mice. *Foods* **13**:3388 (2024) Available: https://www.mdpi.com/2304-8158/13/21/3388.
54 Suzuki S, Umegaki K and Chiba T, High-dose β-carotene suppresses non-alcoholic steatohepatitis progression in a mouse model. *J Food Sci* **90**:e70363 (2025) Available: https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.70363.
55 Liu X, Wang N and Gao Z, β-Carotene regulates glucose transport and insulin resistance in gestational diabetes mellitus by increasing the expression of SHBG. *Clin Exp Pharma Physio* **49**:1307–1318 (2022) Available: https://onlinelibrary.wiley.com/doi/10.1111/1440-1681.13712.
56 Sergi D, Melloni M, Passaro A and Neri LM, Influence of type 2 diabetes and adipose tissue dysfunction on breast cancer and potential benefits from nutraceuticals inducible in microalgae. *Nutrients* **16**:3243 (2024) Available: https://www.mdpi.com/2072-6643/16/19/3243.
57 Anderson PD, Mehta NN, Wolfe ML, Hinkle CC, Pruscino L, Comiskey LL *et al.*, Innate immunity modulates adipokines in humans. *J Clin Endocrinol Metab* **92**:2272–2279 (2007) Available: https://academic.oup.com/jcem/article/92/6/2272/2597866.
58 Abranches MV, Oliveira FCED, Conceição LLD and Peluzio MDCG, Obesity and diabetes: the link between adipose tissue dysfunction and glucose homeostasis. *Nutr Res Rev* **28**:121–132 (2015) Available: https://www.cambridge.org/core/product/identifier/S0954422415000098/type/journal_article.
59 Olson AL and Humphries K, Recent advances in understanding glucose transport and glucose disposal. *F1000Res* **9**:639 (2020) Available: https://f1000research.com/articles/9-639/v1.
60 Holst JJ, The physiology of glucagon-like peptide 1. *Physiol Rev* **87**:1409–1439 (2007) Available: https://www.physiology.org/doi/10.1152/physrev.00034.2006.
61 ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D *et al.*, 3. Prevention or delay of diabetes and associated comorbidities: standards of care in diabetes – 2023. *Diabetes Care* **46**:S41–S48 (2023) Available: https://diabetesjournals.org/care/article/46/Supplement_1/S41/148039/3-Prevention-or-Delay-of-Diabetes-and-Associated.
62 Garofalo RS, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL *et al.*, Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKBβ. *J Clin Invest* **112**:197–208 (2003) Available: http://www.jci.org/articles/view/16885.
63 Sugiura M, Nakamura M, Ikoma Y, Yano M, Ogawa K, Matsumoto H *et al.*, The homeostasis model assessment-insulin resistance index is inversely associated with serum carotenoids in non-diabetic subjects. *J Epidemiol* **16**:71–78 (2006) Available: http://www.jstage.jst.go.jp/article/jea/16/2/16_2_71/_article.
64 Bhala N, Liver steatosis linked to type 2 diabetes outcomes. *BMJ* **384**:q309 (2024) Available: https://www.bmj.com/lookup/doi/10.1136/bmj.q309.
65 Lampousi AM, Lundberg T, Löfvenborg JE and Carlsson S, Vitamins C, E, and β-carotene and risk of type 2 diabetes: a systematic review and meta-analysis. *Adv Nutr* **15**:100211 (2024) Available: https://linkinghub.elsevier.com/retrieve/pii/S2161831324000450.
66 Jang SH, Lim JW and Kim H, Mechanism of beta-carotene-induced apoptosis of gastric cancer cells: involvement of ataxia-telangiectasia-mutated. *Ann N Y Acad Sci* **1171**:156–162 (2009) Available: https://www.ncbi.nlm.nih.gov/pubmed/19723050.

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

67 Eckersall PD and Bell R, Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. *Vet J* **185**:23–27 (2010) Available: https://linkinghub.elsevier.com/retrieve/pii/S1090023310001176.
68 Polyzos SA, Kountouras J, Zavos C and Tsiaousi E, The role of adiponectin in the pathogenesis and treatment of non-alcoholic fatty liver disease. *Diabetes Obes Metab* **12**:365–383 (2010) Available: https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/j.1463-1326.2009.01176.x.
69 Moraes-Vieira PM, Saghatelian A and Kahn BB, GLUT4 expression in adipocytes regulates de novo lipogenesis and levels of a novel class of lipids with antidiabetic and anti-inflammatory effects. *Diabetes* **65**:1808–1815 (2016) Available: https://diabetesjournals.org/diabetes/article/65/7/1808/16104/GLUT4-Expression-in-Adipocytes-Regulates-De-Novo.
70 Bataller R and Brenner DA, Liver fibrosis. *J Clin Invest* **115**:209–218 (2005) Available: http://www.jci.org/articles/view/24282.
71 Granado-Lorencio F, Donoso-Navarro E, Sánchez-Siles LM, Blanco-Navarro I and Pérez-Sacristán B, Bioavailability of β-cryptoxanthin in the presence of phytosterols: in vitro and in vivo studies. *J Agric Food Chem* **59**:11819–11824 (2011) Available: https://pubs.acs.org/doi/10.1021/jf202628w.
72 Randolph GJ and Miller NE, Lymphatic transport of high-density lipoproteins and chylomicrons. *J Clin Invest* **124**:929–935 (2014) Available: http://www.jci.org/articles/view/71610.
73 Wingerath T, Stahl W and Sies H, β-Cryptoxanthin selectively increases in human chylomicrons upon ingestion of tangerine concentrate rich in β-cryptoxanthin esters. *Arch Biochem Biophys* **324**:385–390 (1995) Available: https://linkinghub.elsevier.com/retrieve/pii/S0003986185700525.

Journal of the Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.