Research Paper

Bene hull oil as a highly stable and antioxidative vegetable oil

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Fatty acid composition, peroxide value, acid value, iodine value, saponification number, unsaponifiable matter content, total tocopherols and phenolics contents, and wax content of Bene hull oil (BHO) were determined and compared to those of Bene kernel oil (BKO) and extra-virgin olive oil (EVOLO). Considering the fatty acid composition and total tocopherols and phenolics contents, the resistance to the production of conjugated diene hydroperoxides and carbonyl compounds during the heating process at 170 °C for BHO was about 4.2 and 7.3 times and about 1.7 and 2.0 times those of BKO and EVOLO, respectively. The antioxidant activity of BHO was exactly the same as that of *tert*-butylhydroquinone at low concentrations (100 ppm).

Keywords: Antioxidant activity / Bene hull oil / Chemical composition / Extra-virgin olive oil / Oxidative stability / *Pistacia atlantica* subsp. *mutica*

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1 Introduction

Pistacia atlantica is one of the most widely distributed wild species of pistachio, and it is considered to be an Irano-Turanian species with distribution from south-west Asia to north-west Africa (Morocco) [1]. It contains four subspecies: *cabulica*, *kurdica*, *mutica*, and *atlantica* [2]. The subspecies of *mutica* is a major variety that grows in the Zagrossian region of Iran and 600–3000 m above sea level [3]. Its oleoresin, known as "Turk terebinth gum", is used to make chewing gum in Iran. The fruits of *P. atlantica*, which have been used traditionally for the treatment of peptic ulcer and as a mouth freshener [4], are called "Bene" in Iran and are used by the natives as food after grinding and mixing with other ingredients. They are round to oval, somewhat flat, and 0.5–0.7 cm in diameter. Their wooden hard shell is covered with a rather dry hull which could be easily removed by pressing between fingers. This soft hull is dark green in color, comprises ~24% of the whole fruit (~25% kernel and ~51% hard shell) and yields up to ~30% oil [5].

Oxidative stability is an important parameter in evaluating the quality of oils and fats, and it is greatly affected by their fatty acid composition and minor components such as tocopherols and phenolic compounds. The oxidation process mainly involves the degradation of polyunsaturated fatty acids and the generation of free radicals, which cause the loss of functional properties and nutritional value [6]. Both tocopherols and phenolic compounds are very important natural antioxidants for the stabilization of unsaturated fatty acids and provide an effective protection against oxidative stress in the human body [7–9].

Previous studies on *P. atlantica* deal with the chemical composition and oxidative stability of the kernel oil from its current subspecies in Iran. Oxidative stability data indicated that the kernel oil from *P. atlantica* subsp. *mutica* had a very high resistance to the formation of lipid oxidation products [10]. Our initial findings showed that its hull oil also has a considerable oxidative stability compared to common vegetable oils. No comprehensive information was found in the literature about the physicochemical characteristics of Bene hull oil (BHO), which can be considered to be a new source of edible oils with some interesting properties. Hence, in our continuing research herein, the chemical composition and oxidative stability of BHO were compared to those of Bene kernel oil (BKO) and extra-virgin olive oil (EVOLO) as a nutritionally healthful edible oil among animal fats and vegetable oils, and also as a relatively stable oil because of the high levels of monounsaturated fatty acids (MUFA) and the presence of natural antioxidants. Furthermore, using the Rancimat method, the antioxidant effect of BHO on EVOLO was investigated and compared to that of *tert*-butylhydroquinone (TBHQ) as a very strong synthetic antioxidant.

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2 Materials and methods

2.1 Materials

The ripe fruit samples of Bene were collected from the fields of Islamabad in the Ilam province, and were stored at –18 °C until use. EVOLO with no added antioxidants in glass bottles was purchased from a local shop. Fatty acid methyl ester (FAME) standards and all chemicals and solvents used in this study were of analytical reagent grade and supplied by Merck and Sigma Chemical Companies.

2.2 Oil extraction

After drying in the shade, the dark green soft hulls and the dark brown wooden hard shells of the Bene fruits were separated from the kernels. The hulls and kernels were ground to powder in a grinder. The powders were extracted with *n*-hexane (1 : 4 wt/vol) by agitation in a dark place at ambient temperature for 48 h. The solvent was evaporated *in vacuo* at 40 °C to dryness.

2.3 Oil oxidation

The oil samples extracted (200 g) were placed in a fryer (Kenwood DF280, Havant, Hampshire, UK) and maintained at 170 °C during 8 h without stirring. The surface-to-volume ratio of the oil samples in the fryer was about 0.7 cm^{-1}. After every 60 min of heating time, samples were drawn and analyzed.

2.4 Acid value

The acid value (AV) was determined according to the AOCS Official Method Cd 3d-63 [11].

2.5 Carbonyl value

The carbonyl value (CV) of the oils was measured according to the method developed by Endo *et al.* [12] using 2-propanol and 2,4-decadienal as solvent and standard, respectively [13].

2.6 Conjugated diene value

The conjugated diene value (CDV) was measured spectrophotometrically at 234 nm and read against HPLC-grade hexane as blank. The oil samples were diluted 1 : 600 with hexane. An extinction coefficient of 29,000 mol/L was utilized to quantify the concentration of conjugated dienes formed during oxidation [14].

2.7 Fatty acid composition

The fatty acid composition of the vegetable oils was determined by gas-liquid chromatography and was reported in relative area percentages. Fatty acids were transesterified into their corresponding FAME by vigorous shaking of a solution of oil in hexane (0.3 g in 7 mL) with 2 mL 7 N methanolic potassium hydroxide at 50 °C for 10 min. The FAME were identified using an HP-5890 chromatograph (Hewlett-Packard, CA, USA) equipped with a CP-SIL 88 (Supelco, Bellefonte, PA, USA) capillary column of fused silica, 60 m × 0.22 mm ID, 0.2 μm film thickness, and a flame ionization detector (FID). Nitrogen was used as carrier gas with a flow rate of 0.75 mL/min. The oven temperature was maintained at 198 °C, and that of the injector and the detector at 250 °C [15].

2.8 Calculated oxidizability value

The calculated oxidizability (Cox) value of the oils was calculated by the percentage of unsaturated C_{18} fatty acids, applying the formula proposed by Fatemi and Hammond [16]:

$$\text{Cox} = [1(18{:}1\%) + 10.3(18{:}2\%) + 21.6(18{:}3\%)]/100 \quad (1)$$

2.9 Iodine value

The iodine value (IV) was determined according to the AOAC Official Method 920.158 (Hanus method) [17].

2.10 Oil/oxidative stability index

For the determination of the oil/oxidative stability index (OSI), a Metrohm Rancimat model 743 (Herisau, Switzerland) was used. The tests were carried out with 3 g of the oil samples at temperatures of 120 °C at an airflow rate of 15 L/h [18].

2.11 Peroxide value

The spectrophotometric method of the International Dairy Federation as described by Shantha and Decker [19] was used to determine the peroxide value (PV) (thiocyanate method).

2.12 Saponification number

The saponification number (SN) was determined according to the AOAC Official Method 920.160 [20].

2.13 Total phenolics content

The total phenolics (TP) content was determined spectrophotometrically using Foli*n*-Ciocalteau's reagent according to the method described by Capannesi *et al.* [20]. A calibration curve of gallic acid in methanol was performed in the concentration range of 0.04–0.40 mg/mL.

2.14 Total tocopherols content

The total tocopherols (TT) content was determined according to the colorimetric method described by Wong *et al.* [21].

2.15 Unsaponifiable matter content

The unsaponifiable matter (USM) content was determined by the method described by Lozano *et al.* [22].

2.16 Wax content

The wax content was determined according to the method described by Mezouari *et al.* [23]. An accurately weighed quantity of oil (10 g) was taken in an Erlenmeyer flask, and five times its volume of acetone was added. The solution (oil/acetone) was cooled and kept at 4 °C for 24 h to crystallize the waxes. The solid fraction was filtered on a previously weighed Whatman No. 1 filter paper, dried at 45 °C in a vacuum oven and then weighed to obtain the acetone-insoluble matter.

2.17 Statistical analysis

All experiments and measurements were carried out in triplicate, and data were subjected to analysis of variance (ANOVA). ANOVA and regression analyses were performed according to MStatC and SlideWrite software. Significant differences between means were determined by Duncan's multiple range tests; p values less than 0.05 were considered statistically significant.

3 Results and discussion

The chemical composition and characteristics of BKO, BHO, and EVOLO are shown in Table 1. The highest percentage of saturated fatty acids (SFA; mainly palmitic acid, 16:0) was found in BHO (26.45%), EVOLO (17.35%) and BKO (13.15%), respectively. Among the MUFA, the percentage of palmitoleic acid (C16:1) in BHO (13.38%) was about eight times that in BKO (1.78%) and EVOLO (1.55%), whereas their percentages of oleic acid (18:0) ranged from 52 to 61%; thus, BHO had a %MUFA (66.26%) higher than that of EVOLO (62.00%) and BKO (53.83%). The lowest percentage of polyunsaturated fatty acids (PUFA; mainly linoleic acid, 18:2) was observed in BHO (6.60%), followed by olive oil (OLO; 19.82%) and BKO (33.00%). From the information stated above, BHO showed a PUFA/SFA ratio (0.25), Cox value (1.31), and IV (69.81) that were considerably lower than those of EVOLO (1.14, 2.81, and 97.32, respectively) and BKO (2.51, 4.05, and 104.26, respectively), which makes it particularly resistant to oxidation.

The PV and AV of BKO and EVOLO were less than 1.0 meq/kg and 1.5 mg/g, respectively, indicating that these

Table 1. Chemical composition and characteristics of BKO, BHO, and EVOLO.[†]

Parameter	BKO	BHO	EVOLO
Fatty acid [%]			
14:0	0.47 ± 0.10[a]	–	0.40 ± 0.19[a]
16:0	10.39 ± 0.32[c]	23.40 ± 0.28[a]	12.78 ± 0.21[b]
16:1	1.78 ± 0.14[b]	13.38 ± 0.11[a]	1.55 ± 0.17[b]
18:0	2.29 ± 0.03[c]	3.05 ± 0.07[b]	4.17 ± 0.09[a]
18:1	52.05 ± 0.13[b]	52.12 ± 0.17[b]	60.45 ± 0.43[a]
18:2	31.82 ± 0.33[a]	5.65 ± 0.07[c]	18.36 ± 0.22[b]
18:3	1.18 ± 0.07[b]	0.95 ± 0.07[b]	1.46 ± 0.08[a]
20:1	–	0.76 ± 0.06	–
SFA	13.15 ± 0.39[c]	26.45 ± 0.21[a]	17.35 ± 0.08[b]
MUFA	53.83 ± 0.01[c]	66.26 ± 0.34[a]	62.00 ± 0.26[b]
PUFA	33.00 ± 0.40[a]	6.60 ± 0.14[c]	19.82 ± 0.13[b]
PUFA/SFA	2.51 ± 0.10[a]	0.25 ± 0.01[c]	1.14 ± 0.00[b]
Cox value	4.05 ± 0.05[a]	1.31 ± 0.02[c]	2.81 ± 0.00[b]
PV [meq O_2/kg oil]	0.60 ± 0.04[c]	3.72 ± 0.10[a]	0.93 ± 0.04[b]
AV [mg KOH/g oil]	1.31 ± 0.04[b]	5.87 ± 0.24[a]	0.37 ± 0.05[c]
IV [g I_2/100 g oil]	104.26 ± 0.48[a]	69.81 ± 0.39[c]	97.32 ± 0.31[b]
SN [mg KOH/g oil]	106.09 ± 1.83[b]	75.98 ± 5.66[c]	176.86 ± 2.25[a]
USM content [% of oil]	5.22 ± 0.44[b]	6.52 ± 0.16[a]	1.65 ± 0.06[c]
TT content [mg α-tocopherol/kg oil]	818.58 ± 18.25[a]	861.12 ± 57.22[a]	350.25 ± 22.79[b]
TP content [mg gallic acid/kg oil]	81.12 ± 2.12[b]	492.14 ± 30.66[a]	15.20 ± 0.43[c]
Wax content [% of oil]	5.07 ± 1.03[b]	11.59 ± 0.46[a]	4.86 ± 0.57[b]

[†] Means (± SD) within a row with the same lowercase letters are not significantly different at $p < 0.05$.

two oils were unoxidized and of high initial quality (Table 1). In contrast, BHO had a low initial quality (3.72 meq/kg and 5.87 mg/g), probably due to improper storage and handling conditions [24].

The SN of BHO (75.98 mg/g) was significantly lower than that of BKO (106.09 mg/g) and EVOLO (176.86 mg/g), and also much lower than those of common vegetable oils with an average SN range of 175–250 mg/g (Table 1). Because there is an inverse relationship between SN and weight of fatty acids in the oil, it can be inferred that the Bene oils contain a great number of fatty acids of high molecular weight [25]. However, as can be seen in Table 1, the fatty acid composition of the oils studied cannot be the only cause of the considerable differences in their average molecular weight. In fact, a marked contribution of these differences belongs to their different wax and USM contents. The wax content of BHO (11.59%) was over two times that of BKO (5.07%), EVOLO (4.86%), and even rice bran oil, which is characterized by its high wax content among vegetable oils [23]. Waxes are a group of insoluble high-melting point compounds that occur naturally in crude vegetable oils. These compounds can be disadvantageous since they are primarily responsible for the dark color (turbidity) of refined oil and a high refining loss [26]. On the other hand, these compounds have potential applications in the cosmetic, lubricant, plasticizer, pharmaceutical, food, polymer and leather industries [27].

The USM content of BHO was significantly higher than that of BKO (6.52 *vs.* 5.22%), which was in turn significantly higher than that of EVOLO (1.65%) (Table 1). The USM fraction of vegetable oils naturally contains hydrocarbons, terpene alcohols, sterols and tocopherols, and typically comprises 0.5–2.5% of vegetable oils, although some vegetable oils have exceptional amounts of 5–6% [28, 29]. Tocopherols together with phenolic compounds are particularly important functional constituents of a minor fraction of vegetable oils [30]. Tocopherols have antioxidant properties and they are active as vitamin E, which makes them particularly important for human health. Interest in phenolic compounds is related primarily to their antioxidant activity; nevertheless, they also show important biological activity *in vivo* and may be beneficial in combating diseases related to excessive oxygen radical formation exceeding the antioxidant defense capacity of the human body. There was no significant difference between the TT content of BHO (861.12 mg/kg) and BKO (818.58 mg/kg), but these amounts were considerably higher than that of EVOLO (350.25 mg/kg). BHO is considered to be a rich source of tocopherols since their concentration is greater than those reported for some common vegetable oils, *e.g.* canola, sunflower, cottonseed, and corn oils with a TT content of about 695, 640, 630, and 605 mg/kg, respectively [31]. It was very interesting to find that BHO had a unique TP content (492.14 mg/kg *vs.* 81.12 and 15.20 mg/kg of BKO and EVOLO, respectively) among all common vegetable oils. A study on canola, soybean, sunflower, corn and olive oils indicated that their TP content was 48.19, 45.80, 45.27, 30.80, and 15.27 mg/kg, respectively [15].

Table 2 shows the CDV and CV of the oils studied during the heating process at 170 °C. CDV and CV are good classical indices of primary and secondary oxidative changes in lipids [32, 33]. The initial CDV of BHO (20.18 mmol/L) was significantly higher than that of BKO (10.28 mmol/L) and EVOLO (5.92 mmol/L). This autoxidation indicator increased parallel to the increase in heating time with a lower rate for BHO, so after 8 h of heating, BHO showed an increase of 47% (29.59 mmol/L) whereas this amount for BKO and EVOLO was 197% (30.53 mmol/L) and 343% (26.22 mmol/L), respectively. This indicates that the resistance to the production of conjugated diene hydroperoxides in the heating process for BHO was about 4.2 and 7.3 times that of BKO and EVOLO, respectively. The initial CV of BHO (9.61 μmol/g) was statistically similar to that of EVOLO (10.77 μmol/g) but significantly higher than that of BKO (6.55 μmol/g). The increase in percentage of the CV during the heating process

Table 2. The CDV (mmol/L) and CV (μmol/g) of BKO, BHO, and EVOLO during the heating process at 170 °C.[†]

Time [h]	BKO		BHO		EVOLO	
	CDV	CV	CDV	CV	CDV	CV
0	$10.28 \pm 1.59^{c,B}$	$6.55 \pm 0.63^{d,B}$	$20.18 \pm 0.10^{f,A}$	$9.61 \pm 0.56^{e,A}$	$5.92 \pm 0.86^{h,C}$	$10.77 \pm 1.58^{f,A}$
1	$16.52 \pm 0.83^{d,B}$	$7.97 \pm 1.01^{d,C}$	$21.26 \pm 0.28^{e,A}$	$15.47 \pm 1.23^{d,A}$	$7.92 \pm 0.95^{g,C}$	$11.97 \pm 0.60^{ef,B}$
2	$17.33 \pm 0.84^{d,B}$	$11.17 \pm 1.05^{c,B}$	$20.98 \pm 0.95^{ef,A}$	$15.85 \pm 0.53^{cd,A}$	$9.38 \pm 0.71^{f,C}$	$11.75 \pm 1.56^{ef,B}$
3	$18.23 \pm 1.37^{d,B}$	$11.25 \pm 0.89^{c,C}$	$21.92 \pm 0.54^{de,A}$	$15.93 \pm 0.58^{cd,A}$	$10.13 \pm 0.49^{f,C}$	$12.99 \pm 1.51^{e,B}$
4	$22.19 \pm 0.58^{c,A}$	$13.83 \pm 1.01^{b,B}$	$22.29 \pm 0.14^{d,A}$	$16.88 \pm 0.59^{bc,A}$	$12.66 \pm 0.39^{e,B}$	$15.13 \pm 1.19^{d,B}$
5	$22.97 \pm 0.85^{c,A}$	$14.21 \pm 0.60^{b,B}$	$24.26 \pm 0.94^{c,A}$	$16.72 \pm 0.56^{bc,A}$	$14.58 \pm 0.29^{d,B}$	$16.28 \pm 1.51^{d,A}$
6	$23.64 \pm 0.63^{c,A}$	$16.48 \pm 1.52^{a,B}$	$24.10 \pm 0.50^{c,A}$	$16.75 \pm 0.32^{bc,B}$	$17.47 \pm 0.39^{c,B}$	$21.89 \pm 1.67^{c,A}$
7	$28.71 \pm 0.85^{b,A}$	$16.94 \pm 0.54^{a,B}$	$25.59 \pm 0.89^{b,B}$	$17.71 \pm 0.42^{b,B}$	$21.10 \pm 0.74^{b,C}$	$29.29 \pm 0.52^{b,A}$
8	$30.53 \pm 1.87^{b,A}$	$17.50 \pm 1.59^{a,B}$	$29.59 \pm 0.58^{a,A}$	$19.06 \pm 0.98^{a,B}$	$26.22 \pm 0.67^{a,B}$	$31.57 \pm 1.62^{a,A}$

[†] Means ± SD within a column with the same lowercase letters are not significantly different at $p < 0.05$; means (CDV or CV) ± SD within a row with the same uppercase letters are not significantly different at $p < 0.05$.

for BHO, BKO, and EVOLO was 98 (19.06 μmol/g), 167 (17.50 μmol/g), and 193 (31.57 μmol/g), respectively. In other words, the resistance to the production of carbonyl compounds in the heating process for BHO was about 1.7 and 2.0 times that of BKO and EVOLO, respectively. The determination of carbonyl compounds in heated and frying oils is very important for evaluating their quality because these compounds often contribute to rancid and unpleasant flavors, and reduce the nutritional value of fried foods [12, 33, 34]. Thus, BHO is more resistant to sensory deterioration than BKO and EVOLO. In general, based on the formation of primary and secondary lipid oxidation products, the oxidative stability of BHO was obviously higher than that of EVOLO and especially BKO, which in the previous research was introduced as a very stable oil among the common edible oils [10]. This can be attributed to the low levels of the PUFA/SFA ratio and the Cox value, the high content of tocopherols and the unique quantity of phenolic compounds in BHO, although BHO had an initial quality no better than that of the other oils studied (Tables 1 and 2).

With regard to the very high oxidative stability of BHO and also its high TT and TP contents, it was expected that the addition of BHO to other common vegetable oils can improve their oxidative stability. Therefore, different percentages (up to 15%) of BHO were added to EVOLO, and using the Rancimat test, its antioxidant activity was compared to that of a synthetic antioxidant of wide commercial use such as TBHQ, which has a very strong antioxidant activity, especially at high temperatures. As shown in Fig. 1, a power equation could well describe the antioxidant behavior of BHO with high determination coefficient (R^2 = 0.9865); that is, the OSI of EVOLO initially rose steeply from 3.11 (0% BHO) to 7.49 h (2% BHO), but then increased less rapidly at the higher percentages of BHO (2–15%). As can be seen in Fig. 2, the OSI of EVOLO linearly increased as the TBHQ concentration (up to 200 ppm) increased (R^2 = 0.9842). With regard to the common levels of 100 or 200 ppm for TBHQ in edible oils, their corresponding OSI were calculated to be 3.97 and 4.91 h, respectively, from the equation shown in Fig. 2. Substituting these quantities into the equation shown in Fig. 1, we could

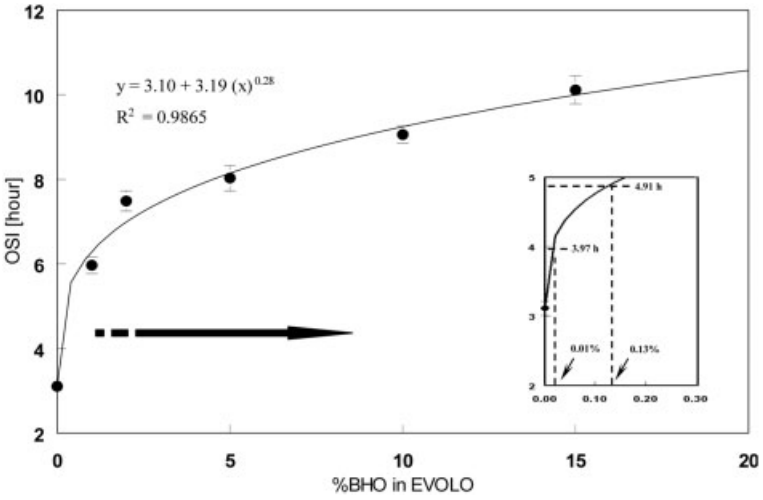

Figure 1. The effect of different percentages of BHO on the OSI of EVOLO.

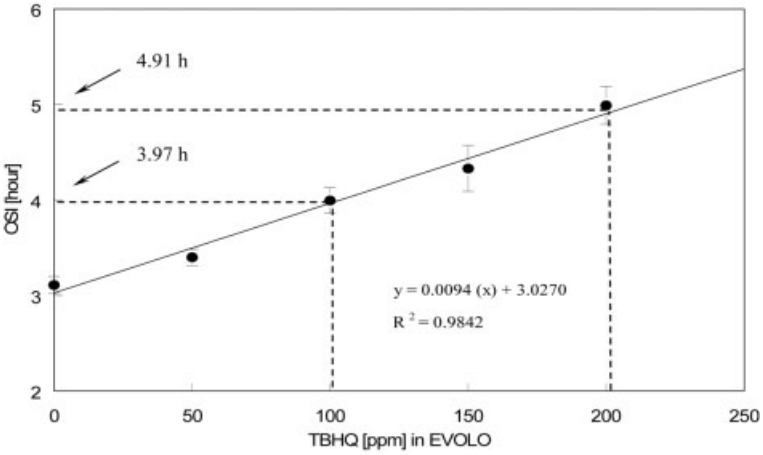

Figure 2. The effect of different concentrations of TBHQ on the OSI of EVOLO.

calculate the corresponding percentages of BHO in EVOLO to be 0.01% (or 100 ppm) and 0.13% (or 1300 ppm), respectively. It was very interesting to find that the antioxidant activity of BHO was exactly the same as that of TBHQ at low concentrations (0.01% or 100 ppm).

Our results in this research indicate that BHO can be used as a source of safe and effective natural antioxidants. More studies regarding separation and identification of its antioxidative components are still being performed in our research group. The search for natural antioxidants has been increasing in recent years, since they can protect the human body from free radicals and retard the progress of many chronic diseases [35]. Furthermore, the application of the most widely used synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), TBHQ and propyl gallate (PG) has been questioned because of possible toxic and carcinogenic components formed during their degradation [36].

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The conflict of interest statement

The authors have declared no conflict of interest.

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