applied sciences

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In Vitro Bioactivities of Cereals, Pseudocereals and Seeds: Assessment of Antiglycative and Carbonyl-Trapping Properties

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Abstract: Advanced glycation endproducts (AGEs) are the final products resulting from non-enzymatic glycation, which plays a crucial role in diabetes and aging-related health issues. The aim of the present investigation was to examine the inhibitory effects on AGE formation of aqueous and methanolic extracts from cereals (rice, rye, and wheat), pseudocereals (amaranth, quinoa, and buckwheat) and chia seeds. Different in vitro models simulating AGEs induced by glucose (Glc) and methylglyoxal (MGO) were evaluated. The MGO-trapping capacity of extracts was evaluated, alongside their antioxidant capacity and phenolic compound composition, with the aim of exploring any potential correlation with AGEs' inhibitory effects. Extracts (25 mg/mL) demonstrated inhibitory effects on AGEs in protein–Glc and protein–MGO assays, with inhibition levels ranging from below 10% (amaranth extracts) to over 90% (buckwheat extracts) compared with aminoguanidine. Buckwheat methanolic extract exhibited the highest anti-AGE activity (98.3% inhibition in the BSA–Glc and 89.5% inhibition in the BSA–MGO assay), followed by chia seed extracts (80–82% inhibition). Buckwheat aqueous extract showed the greatest capacity to directly trap MGO (IC_{50} = 0.3 mg/mL). Antioxidants and phenolic compounds likely contributed to their antiglycative activity. In conclusion, aqueous and methanolic extracts derived from different natural ingredients such as cereals, pseudocereals, and seeds can be valuable in preventing glycation-related complications.

Keywords: seeds; cereals; pseudocereals; advanced glycation end products (AGEs); glycation; in vitro glycation inhibitors; carbonyl trapping; phenolic compounds

Citation: Mesías, M.; Holgado, F.; Olombrada, E.; Morales, F.J. In Vitro Bioactivities of Cereals, Pseudocereals and Seeds: Assessment of Antiglycative and Carbonyl-Trapping Properties. *Appl. Sci.* **2024**, *14*, 5684. https://doi.org/10.3390/app14135684

Academic Editor: Anna Lante

Received: 9 June 2024
Revised: 26 June 2024
Accepted: 27 June 2024
Published: 28 June 2024

1. Introduction

According to recent data published by the World Health Organization, 41 million individuals die annually due to non-communicable diseases (NCDs), representing 74% of total global mortality [1]. NCDs, commonly denoted as chronic diseases, encompass primary categories such as cardiovascular diseases, cancers, chronic respiratory diseases, and diabetes. Diabetes is characterized by elevated blood sugar levels, known as hyperglycemia, and is associated with various chronic complications arising from vascular damage. These complications include diabetic retinopathy, nephropathy, neuropathy, and an increased risk of cancer or cardiovascular and cerebrovascular diseases, among others [2]. The emergence of these conditions and their associated complications is linked with the presence of glycated proteins and the so-called advanced glycation end products (AGEs). AGEs are the final products of the Maillard reaction formed through a non-enzymatic glycation process. This process is initiated by the interaction between reducing sugars and free amino groups of proteins, lipids, and nucleic acids, being promoted under hyperglycemic conditions in individuals with diabetes [3].

AGEs present in the human body can be divided into exogenous and endogenous origins, including those ingested through processed foods (exogenous) and those generated in vivo within the organism (endogenous). AGEs accumulate in the body under physiological, metabolic conditions and during normal aging [4]. Typically, the body eliminates AGEs

under normal physiological conditions. Nevertheless, during episodes of hyperglycemia or oxidative stress, significant accumulation occurs. This situation is aggravated when diets contain highly processed foods, which are subjected to extensive thermal treatment under low-water conditions, promoting the Maillard reaction and the formation of AGEs [5]. Although AGEs comprise a group of molecules with varying chemical characteristics, their biological effects are very similar by altering the secondary structure of proteins that affect cell biological activity and function, and cause cell damage and cell death [4]. In this context, the search for compounds capable of reducing AGE accumulation through the inhibition of glycation will help to prevent the pathogenesis of diabetes and the associated health complications. Several synthetic compounds have been identified in the literature as inhibitors of AGEs. However, their use has been linked to various adverse effects, emphasizing the need to explore alternative natural products with antiglycative properties [6].

Cereals have been staple foods since time immemorial, and their production is expected to increase by 12% over the next decade [7]. Wheat, rice, and rye stand out for their greater consumption rates, serving as a significant source of nutrients and bioactive components such as dietary fiber, phytochemicals, and antioxidants, which provide different health benefits [8]. Similarly, pseudocereals, including amaranth, buckwheat, and quinoa offer high nutritional value and a rich content of bioactive compounds with antioxidant capacity, enhancing their nutritional and functional significance. These grains exhibit a well-balanced amino acid composition and possess a higher biological value compared to many common cereals. Additionally, they are gluten-free, making them safe for consumption by individuals with celiac disease. As the demand for healthy foods rises, the production of pseudocereals has seen a notable increase in recent years [9]. In addition to cereals and pseudocereals, other seeds, including chia, are noteworthy for their nutritional, phytochemical and therapeutic potential [10]. Phytochemicals contained in all these natural products endow them with well-documented properties, such as anticholesterolemic, hypoglycemic, antioxidant, anti-inflammatory, antimicrobial, antidiabetic, or anticancer capacities [8–11]. However, information regarding the antiglycation properties of cereals, pseudocereals, and other seeds is currently either unknown or quite limited [12,13].

This study investigates the inhibitory effects on the formation of AGEs using aqueous and methanolic extracts from cereals (wheat, rice, and rye), pseudocereals (quinoa, amaranth, and buckwheat) and chia seeds. Various well-established in vitro assays and AGE models were employed for this evaluation. Additionally, the antioxidant capacity and phenolic compound content of all the extracts were examined to discern any potential correlation with the inhibitory activity against AGEs.

2. Materials and Methods

2.1. Reagents and Chemicals

D(+)-glucose, bovine serum albumin (BSA), 400 g/L methylglyoxal (MGO) solution, sodium azide, aminoguanidine (AG), 5-methylquinoxaline (5-MQ), o-phenyldiamine (OPD), Trolox, phenolic acid standards, 2,4,6-Tris-(2-pyridyl)-s-triazine (TPTZ), ABTS (2,2′-azino-bis(3-etilbenzotiazoline-6-sulfonic acid) reactive, gallic acid, potassium persulfate, aluminum trichloride hexahydrate and quercetin hydrate were acquired from Sigma (St. Louis, MO, USA). Pyridoxamine dihydrochloride (PM), Folin–Ciocalteu reagent, ferric chloride hexahydrate, sodium acetate, disodium phosphate dodecahydrate, sodium dihydrogen phosphate monohydrate, sodium chloride, potassium chloride, sodium carbonate, ethanol, and methanol (99.5%) were obtained from Panreac (Barcelona, Spain). All other chemicals, solvents, and reagents were of analytical grade.

2.2. Samples

Wheat grains (*Triticum* sp.) originating from Spain (Lot TG070431) were purchased from a local market (Madrid, Spain). Rice grains (*Oryza sativa*) originating from Italy (Lot T W/19PN136), rye grains (*Secale cereale*) from the Netherlands (Lot PHM028), quinoa grains (*Chenopodium quinoa*) from Bolivia (Lot 2139-IRU104-01/111), amaranth grains (*Amaranthus*

caudatus L.) from India (Lot 20810008), buckwheat grains (*Fagopyrum esculentum*) from China (Lot B20011) and black chia seeds (*Salvia hispanica* L.) from Mexico (Lot C-O-AD18-3-V1) were obtained from a company of food ingredients (EcoAndes Import Export, S.L., Madrid, Spain) (Figure 1). All ingredients were certified for origin and organic cultivation. The whole grains and seeds were ground to create a homogeneous mixture using an IKA A11 basic grinder (IKA, Werke GmbH, Staufen, Germany). The resulting material was then passed through a 0.80 mm mesh sieve to ensure standardized particle size.

Figure 1. Cereals (wheat, rice, and rye), pseudocereals (quinoa, amaranth, and buckwheat) and chia seeds selected for the study.

2.3. Preparation of Extracts

Ground samples (250 mg) were extracted in 25 mL of deionized distilled water or 50% (*v*/*v*) methanol solution to obtain aqueous extracts or methanolic extracts, respectively. Samples were vortexed for 15 min at 50 °C. The residue was removed by centrifugation (3950× *g* for 10 min) at 4 °C, and the supernatant was collected and filtered through filter paper (Whatman No. 1). The extraction was repeated twice, and the supernatants were pooled. Methanolic extracts were concentrated by evaporating the organic phase using a TurboVap-LV evaporator (Biotage, Uppsala, Sweden) at a temperature of 40 °C. Final extracts were frozen at −80 °C for subsequent lyophilization. The dried extracts were then weighed and stored at 4 °C prior to analysis. Solubility was expressed as g of solids in the soluble fraction/100 g sample (%, *w*/*w*).

2.4. Measurement of pH

Lyophilized extract (250 mg) was mixed with 10 mL of water for aqueous extracts or 50% methanol for methanolic extracts. The mixture was then vortexed for 3 min, left at room temperature for 1 h and centrifuged (3910× *g* for 10 min) at 4 °C. The pH of the supernatants was measured using a CG-837 pH meter (Schott, Mainz, Germany).

2.5. Determination of Phenolic Acids

Phenolic acids (chlorogenic acid, p-hydroxybenzoic acid, syringic acid, vanillic acid, p-coumaric acid, ferulic acid, gallic acid, protocatechuic acid and caffeic acid) were determined chromatographically according to the method described by Mesías et al. [14] with slight modifications. Briefly, 150 mg of each lyophilized extract was mixed with 2.5 mL of 2 mol/L sodium hydroxide, 2.5 mL of 20 mL/L ascorbic acid solution containing 13.4 mmol/L ethylenediaminetetraacetic acid (EDTA), and 0.5 mL of 0.1 mg/mL of isoferulic acid used as internal standard. The mixture was then flushed with nitrogen and allowed to hydrolyze under agitation for 16 h at room temperature. Following hydrolysis, the sample was centrifuged at 2370× *g* for 10 min at 4 °C, and the resulting supernatant was acidified by adding 0.75 mL of acetic acid. The phenolic acids were then extracted using ethyl acetate (4 mL + 2 mL), and the organic layer containing the liberated phenolic acids was collected by pipetting off the upper organic (supernatant) layer from the bottom aqueous residue layer. The two organic layers were combined and evaporated to dryness in a speed-vac concentrator (Thermo Fisher Scientific, Courtaboeuf, France) for 1 h at 45 °C. The residue was then dissolved in 1 mL of methanol/water (75:25 *v*/*v*) and filtered through a 0.45 µm filter before analysis by HPLC. Results were expressed as µg/g sample. The limit of quantification was set at 4 µg/g sample.

2.6. Determination of Total Phenolic Content

The total phenolic content (TPC) of each lyophilized extract was determined using the Folin–Ciocalteu method as described by Mesías et al. [14] with slight modifications. 75 mg of each extract was dissolved in 3 mL of water for aqueous extracts or 50% (*v*/*v*) methanol for methanolic extracts. For the assay, 250 µL of the appropriately diluted sample solution and 250 µL of Folin–Ciocalteu reagent (diluted 1:1 (*v*/*v*) in methanol) were mixed by vortexing. After exactly 3 min, 500 µL of 75 g/L sodium carbonate solution and 4 mL of water were added to the mixture, which was then vortexed for an additional 10 min and allowed to stand in darkness for 60 min. The absorbance at 750 nm was measured using a BioTek microplate spectrophotometer (Agilent Technologies, Santa Clara, CA, USA), and the results were quantified using gallic acid as a standard. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g extract, and the limit of quantification was set at 4.0 mg GAE/g sample.

2.7. Total Antioxidant Capacity by Direct ABTS (2,2′-azino-bis(3-etilbenzotiazoline-6-sulfonic acid) Assay

The direct measurement of the total antioxidant capacity was performed according to Gökmen et al. [15] with slight modifications and adapted to the microplate reader. To produce 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid radical cations (ABTS•+), a stock solution of ABTS (7 mmol/L) was reacted with potassium persulfate (2.45 mmol/L) and left to stand in the dark at room temperature for 12–16 h. The resulting ABTS•+ solution was diluted with 50% ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Lyophilized extracts (5 mg) were dissolved in 10 mL of either water or 50% methanol. Next, 200 µL of the sample or Trolox standard was added to 3.8 mL of 50% ethanol and 1 mL of the diluted ABTS•+ solution. After 45 min, the absorbance was read using a BioTek microplate spectrophotometer. Calibration was performed using ethanolic solutions of Trolox with concentrations ranging from 0.08 to 0.25 mmol/L. The total antioxidant capacity was expressed as µmol equivalents of Trolox (TEAC)/g sample, with a limit of quantification of 11 µmol TEAC/g sample.

2.8. Determination of the Reducing Power Using the Ferric Reducing Antioxidant Power (FRAP) Assay

The formation of the Fe^{2+}-TPTZ complex from the Fe^{3+}-TPTZ complex was determined as described by Morales et al. [16] with slight modifications. The lyophilized extracts (50 mg) were dissolved in 10 mL of water or 50% methanol (diluted if necessary). To prepare the FRAP solution, a mixture of 40 mM TPTZ solution, 20 mM ferric chloride hexahydrate solution, and 0.3 mM sodium acetate buffer (pH 3.6) was created. The assay involved adding 40 µL of the sample solution to 200 µL of acetate buffer and 60 µL of the FRAP solution. The resulting mixture was incubated at 37 °C for 30 min, and the absorbance reading (595 nm) was taken using a BioTek microplate spectrophotometer. The Trolox calibration curve was utilized for quantification, and the results were expressed as µmol Trolox equivalent antioxidant capacity (TEAC)/g extract. The limit of quantification was set at 1.8 µmol TEAC/g sample.

2.9. Determination of Flavonoids

Flavonoid content was determined using the aluminum chloride method described by Abdel-Hameed [17]. To prepare the sample solution, 75 mg of each lyophilized extract was weighed and dissolved in 3 mL of water or 50% methanol. For determination purposes, 80 µL of the sample solution was mixed with 80 µL of aluminum trichloride in ethanol and 100 µL of sodium acetate. The resulting mixture was then incubated in darkness for 150 min and centrifuged at 14,926× *g* for 3 min. Flavonoids were determined based on the formation of a flavonoid–aluminum complex with an absorptivity maximum at 440 nm, and absorbance readings were taken using a BioTek microplate spectrophotometer. Quercetin was used as a reference standard, and the results were expressed as µg quercetin

equivalent/100 mg extract. The limit of quantification was set at 0.025 µg quercetin equivalent/100 mg sample.

2.10. In Vitro Glycation Assay with BSA–Glucose and BSA–MGO

The BSA–glucose (BSA–Glc) and BSA–MGO assays, based on Mesías et al. [14], were conducted to assess the inhibitory effects of different extracts on protein glycation induced by Glc and MGO, respectively. To prepare sample solutions, 75 mg of each lyophilized extract was dissolved in 3 mL of water or 50% methanol. For the BSA–Glc assay, 100 µL of the extract solutions was mixed with 200 µL of BSA solution (35 mg/mL) and 400 µL of Glc (175 mg/mL). Blanks containing BSA–Glc but no test sample were kept at −80 °C until measurement. A positive control was created by mixing 100 µL of AG solution (4 mg/mL) with 200 µL of BSA solution and 400 µL of Glc solution. The samples were then incubated for 21 days at 37 °C in an oven, and fluorescence was determined at 360 nm excitation and 420 nm emission using a microplate reader. For the BSA–MGO assay, 100 µL of the extract solutions was mixed with 200 µL of BSA solution (35 mg/mL) and 400 µL of MGO solution (0.4 mg/mL). Blanks containing BSA–MGO but no test sample were kept at −80 °C until measurement. A positive control was prepared by mixing 100 µL of AG solution (4 mg/mL) with 200 µL of BSA solution and 400 µL of MGO solution. The samples were incubated for 14 days at 37 °C in an oven, and fluorescence was measured at 340 nm excitation and 420 nm emission using a microplate reader.

2.11. Evaluation of Direct MGO-Trapping Capacity

Direct MGO-trapping capacity was tested using the method described by Mesías et al. [14]. In this experiment, a 100 µL volume of MGO solution (0.4 mg/mL) was mixed with 750 µL of phosphate buffer (0.1 mol/L, pH 7.4), 50 µL of 5-MQ (internal standard, 1 mg/mL), and 100 µL of either buffer (as a blank), seed extract solutions (0.1–25 mg/mL), or PM solution (as a positive control, 1 mg/mL). The resulting mixture was then incubated at 37 °C for 168 h. After the incubation period, 200 µL of OPD (derivatization agent, 10.8 mg/mL) was added to each sample and control. The mixture was shaken by vortex for 5 s and left to react for 30 min. The amount of residual MGO was quantified based on the amount of the derivatized product, 2-methylquinoxaline (2-MQ), formed in each sample. Quantification was conducted using a Shimadzu HPLC system equipped with an LC-20AD pump, a SIL-10ADvp autosampler, a CTO-10ASVP oven, and an SPD-M20A diode array detector. Chromatographic separation was carried out on a Mediterranea-Sea-ODS2 column (150 mm × 3 mm, 5 µm; Tecknokroma, Barcelona, Spain). The ratio of peak areas of 2-MQ and 5-MQ was used to determine the amount of unreacted MGO in each sample. The percentage decrease in MGO was calculated using the equation: MGO decrease (%) = [(amount of MGO in control–amount of MGO in sample with tested extract solution or PM solution)–amount of MGO in control] × 100%. The IC_{50} values were determined from the dose-response curves of each experiment using Microsoft Excel.

2.12. Statistically Analysis

Statistical analyses were performed using SPSS version 26 (SPSS, Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Scheffe's test was used to identify the overall significance of differences. All statistical parameters were evaluated at $p < 0.05$ significance level. Relationships between the different parameters analyzed were evaluated by computing Pearson and Spearman linear correlation coefficients at the $p < 0.05$ confidence level. The homogeneity of variances was determined using Levene's test.

3. Results and Discussion

Aqueous and methanolic extracts were prepared from various cereals, pseudocereals and chia seeds to evaluate their antiglycative properties. Grains and seeds displayed a wide range of solubility from 7.2% to 25.6% in the extracts (Table 1). Higher solubility

was mostly reached in aqueous extracts (from 10.5% in wheat to 25.6% in chia) than in methanolic extracts (from 7.2% in rice to 11.1% in chia). This fact was probably due to the lower solubility of the major constituents such as carbohydrates and proteins in the organic solvent [18,19]. Minimal variations were observed in the pH values, ranging between 6.4–6.7 in aqueous extracts and between 6.9–7.7 in methanolic extracts.

Table 1. Solubility and pH values of samples extracted in water and 50% methanol [1].

Sample Extract	Soluble Extract (%, *w*/*w*)		pH	
	Aqueous	Methanolic	Aqueous	Methanolic
wheat	10.5 ± 1.4 c	7.8 ± 0.9 ab	6.5	6.9
rice	12.5 ± 0.6 d	7.2 ± 0.7 a	6.7	7.7
rye	17.4 ± 0.6 e	10.2 ± 1.9 c	6.5	7.0
quinoa	16.8 ± 1.0 e	10.4 ± 1.3 c	6.4	7.2
amaranth	16.1 ± 3.4 e	9.2 ± 1.3 bc	6.6	7.4
buckwheat	15.6 ± 0.9 e	9.0 ± 0.9 abc	6.7	7.4
chia	25.6 ± 2.3 f	11.1 ± 2.1 cd	6.5	7.3

[1] Results for soluble extract are expressed as mean ± SD for n = 16. Different letters denote significant differences among all the extracts ($p < 0.05$).

To assess the inhibitory effect of the extracts on AGE formation, fluorescence intensity was measured in different in vitro glycation models, using aminoguanidine as the control for AGE inhibition. Extracts were evaluated at the concentration of 25 mg/mL, corresponding to a final concentration in the reaction medium of 3.6 mg/mL The BSA–Glc model was selected to assess the early stages of protein glycation, as the glycation process initiates the reaction between reducing sugars and proteins. BSA is chosen for these assays due to its resemblance to human serum albumin, while glucose is selected as the sugar source, given its ubiquitous presence in the human body [20]. Conversely, the BSA–MGO model was employed to evaluate the intermediate stages of protein glycation, as MGO is an intermediary compound formed through the auto-oxidation of glucose and lipid peroxidation [21]. In this scenario, MGO is selected as the highly reactive dicarbonyl compound generated under in vivo conditions during protein glycation. MGO binds to the amino groups of proteins, thereby contributing to the production of AGEs [22,23].

In vitro glycation assays revealed significant inhibition of AGE formation by both aqueous and methanolic extracts of all the grains and seeds (Figure 2A,B). Most samples exhibited significant differences in AGEs-inhibitory activity ($p < 0.05$). In the BSA–Glc assay, AGEs-inhibitory rates ranged from 7.8% to 98.3%. The buckwheat methanolic extract demonstrated the highest inhibitory capacity, closely resembling the effect of the AG solution (average inhibitory rate 92.9%), followed by chia with 83.2% and 80.1% inhibition in aqueous and methanolic extracts, respectively. Conversely, amaranth methanolic extracts exhibited the lowest inhibitory activity. Buckwheat and chia methanolic extracts also demonstrated the highest reduction in the formation of fluorescent AGEs in the BSA–MGO assay (over 80–90%), while amaranth, wheat and rye displayed the lowest inhibitory activity (range: 7.8–19.4%). In this model, the highest values for the AGEs-inhibitory rate were below the effect of the AG solution (with an average inhibitory rate of 99.7%).

The proposed mechanisms for inhibiting AGE formation in vivo can be categorized as blocking protein glycation sites, neutralizing oxidative free radicals, modulating AGEs receptors, trapping active dicarbonyl compounds, sequestering metal ions, blocking aldose reductase activity, and decreasing blood glucose levels [6]. Among them, the reduction of the levels of the carbonyl compound and the scavenging of oxidative free radicals have been described as the most important inhibitory mechanisms [6]. MGO is the most extensively studied of the reactive dicarbonyls responsible for the majority of in vivo AGEs [24]. The fate of dicarbonyl compounds in the glycation process is relevant because they are nearly up to 20,000-fold more reactive than glucose and the circulating levels are increased during hyperglycemia [25]. Therefore, an evaluation of the direct MGO-trapping

capacity was conducted to determine whether the grain and seed extracts could scavenge this compound directly. Figure 3 illustrates the varying trapping capacities of the samples in a range of concentrations from 1 to 25 mg/mL. In this assay, pyridoxamine was used as a control because of its capacity to interact with MGO very efficiently, leading to the formation of stable adducts [26]. Both aqueous and methanolic extracts exhibited dose-dependent trapping of MGO except for the buckwheat aqueous extract, which already exhibited inhibition percentages higher than 90% at a concentration of 1 mg/mL. Buckwheat contains quercetin (311 µg/g dry matter) and rutin (4058 µg/g dry matter) at levels 3 to 10 times higher than those in other pseudocereals. These compounds, described as natural antiglycative agents, have their effects further enhanced by the presence of group B vitamins and soluble dietary fiber in the aqueous extract [11]. The activity of pseudocereals aqueous extracts at a concentration of 10 mg/mL was comparable to the effect of 1 mg/mL PM solution (99.1%). For the remaining samples, inhibitions close to 90% were reached at 25 mg/mL, with the exception of wheat and rye methanolic extracts, which did not exceed 80%.

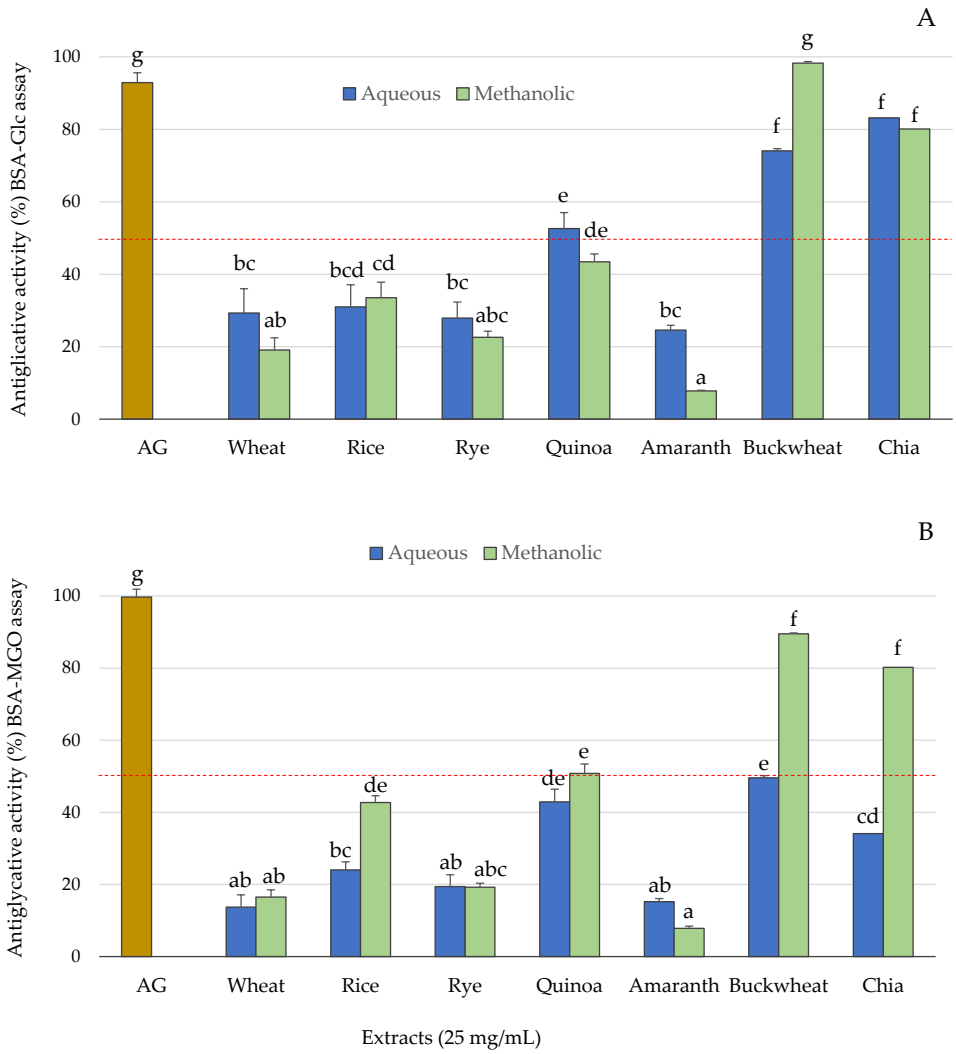

Figure 2. Antiglycative activity of extracts from cereals (wheat, rice, and rye), pseudocereals (quinoa, amaranth, buckwheat) and seeds (chia) at a concentration of 25 mg/mL on the formation of AGEs in the BSA–Glc assay (**A**) and BSA–MGO assay (**B**). Results are presented as mean ± SD (n = 4). Different letters indicate significant differences ($p < 0.05$). The dotted line represents 50% of inhibition.

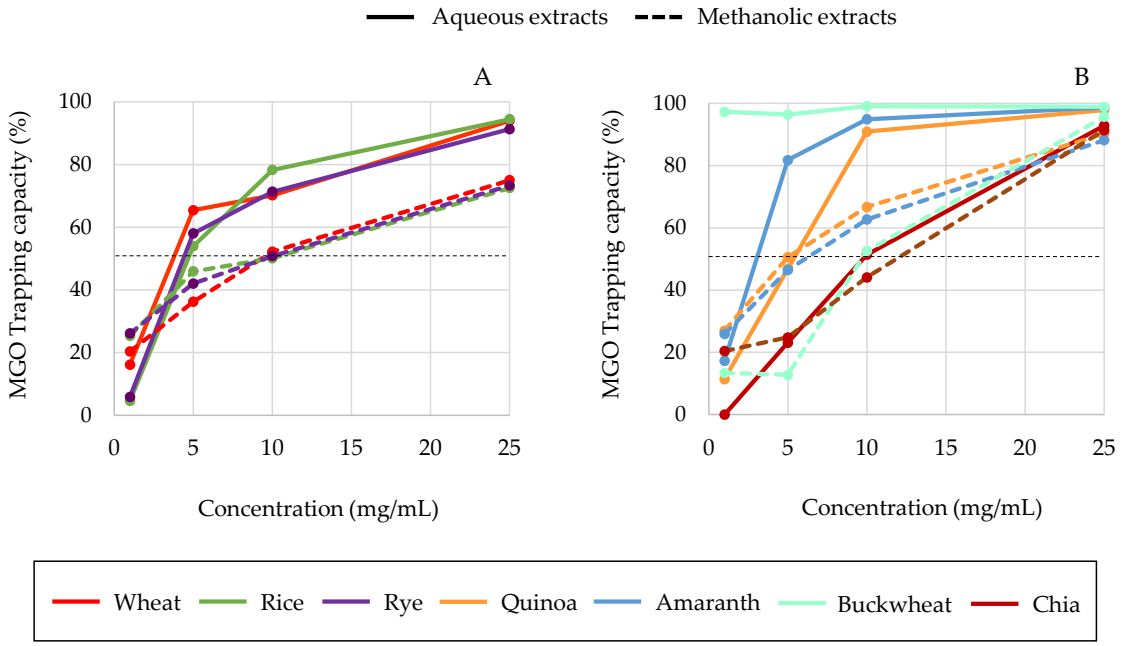

Figure 3. MGO-trapping capacity of extracts from (**A**) cereals (wheat, rice, and rye), (**B**) pseudocereals (quinoa, amaranth, and buckwheat) and seeds (chia) at 168 h. Results are expressed as mean ± SD (n = 4).

Table 2 describes the IC_{50} values (mg/mL) for the MGO-trapping capacity of the extracts. Aqueous extracts demonstrated lower IC_{50} values compared to methanolic extracts, which involve a higher MGO-trapping capacity. Notably, buckwheat exhibited the lowest IC_{50} (0.3 mg/mL), indicating its highly effective capacity to trap MGO, followed by amaranth (2.8 mg/mL) and wheat (3.4 mg/mL). Conversely, chia exhibited the highest IC_{50} value (10.0 mg/mL), suggesting its more limited MGO-trapping capacity. Previous studies have reported high levels of dicarbonyl compounds in chia flour, which were not detected in wheat flour [27]. Considering this, the possible contribution of MGO by chia seeds should not be discarded, which could explain why no decreases were observed in the trapping of this compound. Among methanolic extracts, quinoa and amaranth showed the highest activity, with IC_{50} values of 4.9 and 5.8 mg/mL, respectively, while the remaining samples displayed values close to 10 mg/mL. Contrary to expectations, there were no significant correlations between the IC_{50} values obtained from the MGO-trapping assay and the antiglycative activity observed in the BSA–MGO assay ($r = -0.3574$, $p = 0.2096$) or the BSA–Glc assay ($r = 0.2680$, $p = 0.3542$).

Table 2. MGO-trapping capacity of different grain and seed extracts.

Sample Extract	IC_{50} (mg/mL)	
	Aqueous	**Methanolic**
Wheat	3.4	9.0
Rice	4.6	10.0
Rye	4.2	9.6
Quinoa	5.4	4.9
Amaranth	2.8	5.8
Buckwheat	0.3	9.4
Chia	9.6	11.8

These results suggest that the mechanism through which AGE formation is inhibited is not solely reliant on the direct trapping of the active dicarbonyl compounds. However, the systems are not fully comparable since the glycation reaction proceeds slower in the

BSA–Glc system since the conversion of glucose to the open-chain form is necessary, as well as its autoxidation to generate reactive dicarbonyl compounds. In parallel, some natural pure compounds exert their antiglycative activity by binding to protein residues through hydrogen bonds or van der Waals forces, and effectively protecting the structural integrity of the proteins and inhibiting glycation [4]. MGO reacts with BSA via the arginine and lysine residues to generate AGEs. Then, the coexistence of antiglycative and proglycative compounds in the extracts is plausible. The presence of high levels of reducing sugars other than glucose or reactive dicarbonyl compounds could boost the reaction and participate in the glycation process as promoters [4].

Previous research has emphasized that antioxidant compounds are potent inhibitors of the formation of AGEs through the scavenging of oxidative free radicals and chelating of metal ions [6,28]. In the present study, the antioxidant capacities of the extracts were evaluated using FRAP (electron donating ability) and ABTS (free radical-scavenging capacity) assays. The results showed significant variations, ranging from 29.7 to 282.9 µmol TEAC/g for the FRAP method and from 113.1 to 684.2 µmol TEAC/g for the ABTS method (Table 3).

Table 3. Characterization of aqueous and methanolic extracts of grains and seeds [1].

Sample Extract	FRAP (µmol TEAC/g)	ABTS (µmol TEAC/g)	TPC (mg GAE/g)	Flavonoids (mg/QE/g)
wheat				
aqueous	29.7 ± 0.4 a	128.6 ± 12.9 a	5.2 ± 0.2 a	0.7 ± 0.0 ab
methanolic	43.2 ± 1.9 a	191.1 ± 9.1 ab	5.4 ± 0.1 a	0.6 ± 0.0 ab
rice				
aqueous	87.7 ± 5.8 d	226.7 ± 11.9 bc	8.0 ± 0.1 abc	0.3 ± 0.0 a
methanolic	148.9 ± 8.8 e	212.3 ± 19.3 b	10.1 ± 0.1 c	1.8 ± 0.1 d
rye				
aqueous	66.8 ± 2.0 bc	113.1 ± 14.2 a	5.2 ± 0.1 a	0.7 ± 0.0 ab
methanolic	48.1 ± 1.8 ab	135.7 ± 11.7 a	5.5 ± 0.1 a	0.7 ± 0.0 ab
quinoa				
aqueous	70.0 ± 4.9 cd	159.6 ± 19.7 ab	7.7 ± 0.2 abc	2.8 ± 0.1 e
methanolic	73.9 ± 5.8 cd	178.8 ± 7.4 ab	8.9 ± 0.2 bc	4.7 ± 0.2 g
amaranth				
aqueous	41.6 ± 0.6 a	300.6 ± 23.4 d	6.6 ± 0.1 ab	0.8 ± 0.0 b
methanolic	41.1 ± 1.0 a	217.1 ± 11.9 bc	7.6 ± 0.2 abc	3.5 ± 0.1 f
buckwheat				
aqueous	165.9 ± 1.6 e	521.3 ± 36.2 e	22.0 ± 4.1 e	0.4 ± 0.0 a
methanolic	297.7 ± 13.5 g	684.2 ± 43.5 f	28.9 ± 3.1 f	1.4 ± 0.1 c
chia				
aqueous	68.8 ± 5.9 cd	190.0 ± 9.4 ab	20.2 ± 0.4 de	0.6 ± 0.1 ab
methanolic	226.1 ± 6.7 f	295.6 ± 16.8 cd	16.4 ± 1.2 d	0.7 ± 0.0 ab

[1] FRAP: Ferric-Reducing Antioxidant Power. TEAC—Trolox equivalent antioxidant capacity. ABTS—2,2′-azino-bis(3-etilbenzotiazoline-6-sulfonic acid, TFC—Total phenolic content. GAE—Gallic acid equivalent. QE—Quercetin equivalent. Different letters indicate significant differences ($p < 0.05$).

With the exception of rye and amaranth, methanolic extracts exhibited higher electron-donating capacity, with buckwheat having the highest activity (282.9 µmol TEAC/g) and amaranth the lowest (41.1 µmol TEAC/g). In aqueous extracts, buckwheat again showed the highest results (165.9 µmol TEAC/g), while wheat had the lowest activity (29.7 µmol TEAC/g). Similar to the FRAP results, buckwheat displayed the highest free radical-scavenging activity in the ABTS assay, while amaranth exhibited the lowest activity in both aqueous and methanolic extracts. Once more, methanolic extracts showcased superior antioxidant capacity compared with aqueous extracts, except in the case of rice and amaranth. Significant correlations were found between FRAP and ABTS ($r = 0.7549$, $p < 0.0001$).

Polyphenols are natural compounds extensively described in the literature that potentially inhibit the formation of AGEs [4]. Phenolic compounds and flavonoids were also examined for their role in antiglycative activity (Table 3). TPC varied widely, from

5.2–5.5 mg GAE/g in rice and wheat to 16.4–20.2 mg GAE/g in chia and 22.0–28.9 mg GAE/g in buckwheat. The high TPC values found in buckwheat and chia samples compared to wheat are consistent with findings in the literature. Szawara-Nowak et al. [29] reported a nearly threefold increase in TPC in breads when 50% of wheat flour was replaced with buckwheat flour, and Mesías et al. [27] observed a significantly higher TPC content in biscuits when 5% of wheat flour was replaced by chia flour. Significant correlations were found between FRAP and TPC (r = 0.7698, $p < 0.0001$) and ABTS and TPC (r = 0.8600, $p < 0.0001$), indicating a relationship between the outcomes of these assessments and the involvement of TPC in the antioxidant capacity of the extracts. Lower variability was observed in the flavonoid content, ranging from 0.3 mg QE/g in rice aqueous extract to 4.7 mg QE/g in quinoa methanolic extract (Table 3).

The significant relationship between antioxidant capacity and the inhibition of total AGE formation has been described by different authors. Ramkissoon et al. [30] reported significant correlations between FRAP, TPC and the antiglycation potency in ethanolic extracts derived from common household condiments/herbs. Lin and Zhou [31] also demonstrated an increase in both the antioxidant capacity and the in vitro antiglycation capacity in quercetin-fortified bread extracts. Similarly, the multifunctional roles of flavonoids against the formation of AGEs and AGEs-induced harmful effects have been reported [32]. Consistent with these observations, extracts showing the highest antiglycative activity (buckwheat, chia, quinoa, and rice) were those with the highest FRAP and TPC results but not with the highest ABTS and flavonoid results. Significant correlations were observed for the BSA–MGO assay and FRAP (r = 0.9001, $p < 0.0001$), ABTS (r = 0.6391, $p < 0.0001$), and TPC (r = 0.7552, $p < 0.0001$) and between the results of the BSA–Glc assay and FRAP (r = 0.7814, $p < 0.0001$), ABTS (r = 0.6822, $p < 0.0001$), and TPC (r = 0.8734, $p < 0.0001$). The correlations were also significant when both water and methanolic extracts were analyzed independently (Table 4). However, the strongest relationship was observed in the methanolic extracts, probably because polar organic solvents are more effective at extracting phenolic compounds, which are mainly responsible for the antioxidant capacity of the extracts [33].

Table 4. Correlations between antiglycative, antioxidant capacities, and TFC for aqueous and methanolic extracts.

	BSA–Glc		**BSA–MGO**	
	Aqueous	**Methanolic**	**Aqueous**	**Methanolic**
FRAP	r = 0.5984 $p = 0.0010$	r = 0.8242 $p < 0.0001$	r = 0.7355 $p = 0.0010$	r = 0.9401 $p < 0.0001$
ABTS	r = 0.4533 $p = 0.0261$	r = 0.8389 $p < 0.0001$	r = 0.5691 $p = 0.0037$	r = 0.7229 $p = 0.0002$
TPC	r = 0.8393 $p < 0.0001$	r = 0.9529 $p < 0.0001$	r = 0.6412 $p = 0.0007$	r = 0.9124 $p < 0.0001$

The distribution of individual phenolic acids within the total phenolic acids was also determined in the tested extracts (Table 5). As expected, and considering the previous results, buckwheat and chia displayed the highest levels in 6 of the 9 phenolic acids analyzed, followed by quinoa, which, in turn, coincides with the greater antiglycative activity of these samples. Given the fundamental contribution of free radicals to the development of glycated proteins, it is plausible to anticipate the potential involvement of phenolics and various antioxidants in the inhibition of the initial protein glycation process. In this context, previous studies have reported the significant antiglycative capacity of phenolic acids in in vitro glycation systems through different mechanisms of action. Chlorogenic acid (CGA) has been shown to have potent antiglycative activity, inhibiting the formation of AGEs due to its capacity to interact with reactive dicarbonyl compounds like MGO [34,35]. Dietary supplementation with caffeic acid has been reported to prevent and

delay AGEs-induced vascular dysfunction in diabetes by inhibiting the formation of AGEs in vitro, which was demonstrated in a BSA–Glc system [36]. Ferulic acid has been noted to inhibit the formation of fluorescent AGEs and mitigate the AGEs-induced inflammatory response in culture cells [37,38]. The inhibitory effect of other phenolic acids, such as gallic acid [39], p-coumaric [40] or syringic acid [41], on AGE formation has also been previously documented. All these observations may potentially elucidate the results observed for the tested extracts in the antiglycation and MGO-trapping assays.

Table 5. Phenolic acid content (μg/g) in each grain and seed extract [1].

Sample Extract	CGA	pHB	SYN	VA	pCU	FA	GA	PCA	CA
wheat									
aqueous	ND	50.0 ± 3.3 b	5.4 ± 0.7 a	ND	19.7 ± 0.5 a	262.1 ± 9.6 e	ND	24.0 ± 0.1 c	14.4 ± 0.4 b
methanolic	ND	63.1 ± 0.2 b	16.3 ± 2.4 c	ND	51.7 ± 1.7 c	481.9 ± 19.7 g	ND	40.7 ± 0.8 d	49.0 ± 1.3 d
rice									
aqueous	ND	194.2 ± 4.0 d	17.7 ± 1.1 c	5.1 ± 0.5 ab	96.9 ± 1.9 e	674.3 ± 11.1 i	ND	ND	7.5 ± 0.5 a
methanolic	ND	318.0 ± 0.8 f	18.7 ± 0.0 c	ND	191.1 ± 0.8 g	1218.7 ± 15.0 k	ND	6.7 ± 0.4 ab	39.4 ± 0.8 cd
rye									
aqueous	13.3 ± 0.6 b	33.4 ± 0.8 ab	29.5 ± 0.6 d	8.0 ± 0.4 b	56.3 ± 0.1 cd	551.6 ± 2.5 h	ND	9.7 ± 0.4 b	15.8 ± 0.3 b
methanolic	5.6 ± 0.2 a	33.4 ± 0.1 ab	17.8 ± 0.0 c	3.7 ± 0.3 a	93.2 ± 3.2 e	630.0 ± 15.1 i	ND	5.8 ±0.3 a	47.8 ± 1.6 d
quinoa									
aqueous	ND	200.3 ± 13.4 d	7.1 ± 0.5 a	ND	231.4 ± 11.8 h	464.3 ± 35.7 g	ND	8.9 ± 0.9 b	14.9 ± 0.7 b
methanolic	ND	261.1 ± 20.9 e	ND	ND	364.5 ± 42.9 i	802.2 ± 124.0 j	ND	ND	34.5 ± 2.6 c
amaranth									
aqueous	ND	539.3 ± 5.5 g	4.2 ± 0.2 a	ND	17.2 ± 0.2 a	73.6 ± 1.71 c	5.7 ± 0.0 a	ND	11.3 ± 1.0 ab
methanolic	ND	732.1 ± 11.2 h	ND	ND	26.9 ± 0.3 b	140.8 ± 2.3 d	ND	7.7 ± 0.2 b	22.9 ± 4.9 bc
buckwheat									
aqueous	28.1 ± 0.3 c	126.1 ± 2.3 c	11.6 ± 0.7 b	71.7 ± 2.1 c	60.4 ± 2.2 d	20.7 ± 0.5 a	48.3 ± 0.3 b	ND	85.3 ± 3.6 e
methanolic	185.4 ± 2.3 d	434.7 ± 25.1	187.1 ± 9.7 f	ND	132.8 ± 5.2 f	47.3 ± 1.0 b	90.6 ± 1.0 c	4.6 ± 0.3 a	89.4 ± 2.4 e
chia									
aqueous	219.2 ± 16.7 e	26.9 ± 0.1 a	16.1 ± 0.7 c	ND	31.9 ± 1.1 b	147.8 ± 6.4 d	ND	40.6 ± 1.5 d	1485.4 ± 77.1 f
methanolic	25.0 ± 1.3 c	48.1 ± 5.4 b	45.1 ± 2.3 e	ND	64.2 ± 6.6 d	301.9 ± 28.3 f	ND	72.3 ± 4.7 e	4017.2 ± 323.4 g

[1] CGA—Chlorogenic acid. pHB—p-hydroxybenzoic acid. SYN—syringic acid. VA—vanillic acid. pCU—p-coumaric acid. FA—ferulic acid. GA—gallic acid. PCA—protocatechuic acid. CA—caffeic acid. ND—not detected (<4 μg/g extract). Different letters indicate significant differences ($p < 0.05$).

In agreement with the findings of this study, buckwheat has been reported to exhibit significant amount of phenolic acids [42], which play a significant role in the antioxidant properties and health benefits associated with buckwheat consumption [43]. Szawara-Nowak et al. [29] assessed the inhibition of AGE formation in both BSA–Glc and BSA–MGO systems using extracts obtained from wheat bread enriched with or without buckwheat flour. These researchers noted that the percentage of inhibition increased when wheat flour was substituted with buckwheat by 50%, and this increase was correlated with the levels of total phenolic content. The high content of certain phenolic acids observed in chia, quinoa and rice, as reported by other authors [44–46] could also be responsible for the high anti-AGE activity of the extracts. Specifically, quinoa contains bioactive compounds such as phenolic compounds and flavonoids, which have been reported to increase the activity of antioxidant enzymes in rats. Therefore, incorporating quinoa into new products could be beneficial in the development of functional foods [47]. These findings imply that the antioxidant and antiglycation properties of grains and seeds may be attributed, at least partially, to the synergistic effect of phenolic compounds present in the extracts. However, it is also evident that other factors could contribute to the antiglycative activity, such as the trapping of carbonyl compounds like MGO as a possible mechanism of action.

4. Conclusions

The present study demonstrated that extracts from cereals (rice, rye, and wheat), pseudocereals (amaranth, quinoa, and buckwheat), and chia seeds have in vitro antiglycative effects. In the BSA–Glc and BSA–MGO assays, all the extracts exhibited inhibitory activity on the development of AGEs, with buckwheat and chia extracts showing particularly notable effects. Specifically, the aqueous extracts from buckwheat and the methanolic extracts from quinoa seemed to hinder the formation of AGEs by directly trapping reactive carbonyl compounds such as MGO. The antioxidants and phenolic compounds naturally present in the extracts could explain these activities. A significant relationship between antioxidant capacity and the inhibition of AGE formation was identified. However, it cannot be completely ruled out that natural compounds in the extracts from the studied cereal, pseudocereals, and seeds exert their antiglycative activity by acting at multiple

points. These findings could be applicable to the cosmetic, pharmaceutical and nutraceutical industries, as they identify natural sources of extracts with potential uses in preventing glycation-related complications and human aging. Future research should focus on evaluating the specific mechanism of action of the antiglycative effects of the different compounds, as well as their structural modifications, to better understand the structure–antiglycative activity relationship. Further assessment of synergistic or antagonistic interactions between compounds within extracts is necessary to uncover the mechanisms behind their antiglycative activity and identify multiple potential targets. Additionally, more studies are needed, particularly in vivo research, to investigate the antiglycative and carbonyl-trapping properties of these natural ingredients, aiming to recognize them as inhibitors of AGE formation and to prevent glycation-related complications.

Author Contributions: Conceptualization, M.M. and F.J.M.; Methodology, F.H. and E.O.; Software, M.M.; Validation, M.M.; Formal analysis, F.H. and E.O.; Investigation, M.M. and F.J.M.; Resources, M.M. and F.J.M.; Writing—original draft, M.M.; Writing—review & editing, F.J.M.; Supervision, M.M.; Project administration, F.J.M.; Funding acquisition, M.M. and F.J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was part of the R&D project ACRYREVAL (PID2022-137697NB-I00), funded by MCIU/AEI/10.13039/501100011033/ and by ERDF: A Way to Make Europe.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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