Natural plant extracts as inhibitors of potato polyphenol oxidase: The green tea case study

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ABSTRACT

Natural plant extracts have emerged as a potential alternative to sulphites in minimally processed potatoes. The aim of this study was to evaluate the inhibitory capacity of various plant extracts on potato polyphenol oxidase (pPPO) and to optimize the extraction conditions for preventing browning in fresh-cut potatoes (cv. *Monalisa)*. Fifteen aqueous plant extracts were characterized by their total phenolic content (TPC), antioxidant activity (AA) and their pPPO. Clove extract showed the highest TPC and AA among all plant extracts. Garlic, green tea and wheat bran and, clove and marjoram have a pPPO inhibition >50% at the lowest (1–25 g GAE L^{-1} extract) and highest (200–350 g GAE L^{-1} extract) range of TPC, respectively. The green tea extract was selected for further evaluation due to its high capacity to inhibit pPPO activity regardless of the solution concentration. The optimum extraction conditions and treatment concentration were 55 °C, 7 min and 50 mL L^{-1} of original extract solution. These conditions guaranteed the conservation of fresh-cut potato colour mainly preventing the reduction of luminosity parameter. Green tea extract controlled browning in fresh-cut potatoes (cv. *Monalisa*) for 14 days when stored at 4 °C.

1. Introduction

Potato (*Solanum tuberosum* L.) is the world's fifth largest crop in terms of fresh produce after sugarcane, corn, wheat, and rice. The total potato production of the world was estimated at 370, 436, 600 tons in 2019 (FAOSTAT, 2021). For the catering industry, clean and cut potato tubers simplify cooking as well as reduce time and costs (Bilbao-Sainz et al., 2020). However, mechanical damage (peeling and cut) during minimal processing permits oxygen penetration and leads to browning because of melanin formation. Polyphenol oxidase is the main enzyme which participates in this reaction mechanism; it acts on di-phenols with oxygen as an acceptor (Vámos-Vigyázó, 1981). Historically the strategies to prevent enzymatic browning have focused on the elimination of one of the two substrates in the reaction (oxygen or phenolic compounds), the inhibition of the enzyme, or the combination with products of the reaction which can inhibit the formation of the coloured pigments from sub-steps of the non-enzymatic phase.

Currently, risk perceptions of chemicals in food were positively correlated with preference for natural food (Dickson-Spillmann, Siegrist, & Keller, 2011). To face these challenges, natural plant extracts have

emerged as a potential alternative to sulphites in minimally processed potatoes (Bobo, Arroqui, Merino & Vírseda, 2020).

Several studies have been focused on plant extracts as sources of inhibitory compounds in the polyphenol oxidase reaction. In the literature, compounds derived from cinnamic and benzoic acids are the most commonly found competitive inhibitors for PPO in cherries, apples, pears, peaches and potatoes (Parkin, 2008; Vámos-Vigyázó, 1981; Yoruk & Marshall, 2003; Zawistowski, Biliaderis, & Eskin, 1991, pp. 217–273). On the other hand, Chang (2009) indicated that some flavonols could be phenolic inhibitors. They found quercetin to be the most inhibiting, followed by myricetin and kaempferol. A summary of studies with PPO inhibitors based on natural extracts is presented in Table 1.

In the literature, the antibrowning capacity of an extract has been widely found to depend on the food matrix and type of PPO. Thus, while an extract (eg. rice bran) can be effective at inhibiting the browning of potatoes, its efficiency can be lower when applied to apples and may even become inactive when used for bananas (Kubglomsong & Theerakulkait, 2013; Sukhonthara & Theerakulkait, 2011; Theerakulkait & Boonsiripiphat, 2007). Kuijpers et al. (2014) also observed that some plant extracts, for example, the mate extract (*Ilex paraguariensis*)

Table 1
Plant extracts studied as possible antibrowning agents applied on fruit or vegetables.

Extract	Product	Reference
Green tea	Lettuce	Martín-Diana, Rico, and Barry-Ryan (2008)
	Apple	Soysal (2009)
	Fresh-cut peaches	Klimczak and Gliszczyńska-Świgło (2017) Piva et al. (2017)
	Minimally processed potatoes	Bobo, Arroqui, and Vírseda (2009)
Rice bran	Potato, banana and apple	Theerakulkait and Boonsiripiphat (2007)
	Potato	Boonsiripiphat and Theerakulkait (2009)
	Potato, apple and banana	Sukhonthara and Theerakulkait (2011)
	Potato and banana	Sukhonthara and Theerakulkait (2012)
	Potato	Kubglomsong and Theerakulkait (2013)
	Potato and apple	Sukhonthara et al. (2016) Wang, Chen, Fu, Li, and Wei (2017)
Pineapple juice	Banana	Moline, Buta, and Newman (1999)
	Potato, mushroom, apple and banana	Theerakulkait and Saisung (2006)
	Banana	Chaisakdanugull, Theerakulkait, and Wrolstad (2007)
	Apple	Perera, Gamage, Wakeling, Gamlath, and Versteeg (2010)
	Ginger	Lim and Wong (2018)
Papaya peel	Potato	Faiq and Theerakulkait (2018)
	Apple	
	Banana	
Chili pepper	Potato	Mercimek et al. (2015)
	Ginger	Lim and Wong (2018)
Lemon, Red beet	Potato	Mercimek et al. (2015)
Onion	Potato	Lee et al. (2002)
	Pear	Kim et al. (2005)
	Ginger	Lim and Wong (2018)
Garlic	Lettuce	Ihl, Aravena, Scheuermann, Uquiche, and Bifani (2003)
	Mushroom	Bustos, Agudelo-Laverde, Mazzobre,and Buera (2014)
	Avocado	
	Tomato	Ayala-Zavala et al. (2008)
	Potato	Bobo et al. (2009)
Sesame	Apple	Kumar and Singh (2015)
	Banana	
	Potato	
Clove, cardamom, cinnamon, ginger and nutmeg	Apple	Essa, Nadir, and Hamad (2004)
Rosemary essential oil	Potato	Rizzo et al. (2018)
Mushroom "Enokitake"	Apple, mushroom	Jang, Sanada, Ushio, Tanaka, and Ohshima (2002)
Rhubarb juice	Apple	Son, Moon, and Lee (2000)
Pine needle (*Cedrus deodara*)	Apple	Yu, Zhang, and Zeng (2014)
Natural plant extracts from different sources	Potato	Kuijpers et al. (2014)
	Mushroom	

inhibited mushroom PPO, yet could act as an activator of potato PPO. Contradictory results were also observed by Faiq and Theerakulkait (2018) when applying papaya peel extract to distinct vegetables. They achieved higher levels of browning inhibition in potato than in apple or banana.

The extraction conditions, mainly the temperature and times employed along with the concentration, also affects the antibrowning potential of the extracts. Lee et al. (2002) in potato, Kim, Kim, and Park (2005) in pear and Lim and Wong (2018) in ginger, showed that the inhibitory effect of onion extracts to which a heat treatment had been applied (95–100 °C during 10–15 min) were more effective than those which had not been heated. They found that the non-enzymatic browning products developed during heating of the extract (Maillard reaction products, MRP) have an inhibitory effect on the PPO reaction. This was also observed in several other studies involving MRP (Atrooz, 2008; Lee & Park, 2005; Namiki, 1988; Tan & Harris, 1995). From the previous studies we can see that plant extracts can be interesting anti-browning agents. However, their effectiveness depends on the extraction method, application conditions and the matrix (food) on which they are applied.

The aim of our study is to evaluate the inhibitory capacity of fifteen aqueous plant extracts (cinnamon, clove, garlic, ginger, green tea, marjoram, mint, nutmeg, oregano, pepper (black and white), rosemary, sage, thyme, and wheat bran) on PPO in order to determine the most effective at preventing browning of minimally processed potatoes (cv. *Monalisa*). Thereafter, the extraction conditions of the selected plant extract were optimized and its effect on the quality of fresh-cut potatoes was evaluated.

2. Material and methods

2.1. Experimental design

The experimental design was divided into three phases as it was showed in the scheme of Fig. 1.

2.2. Samples and chemicals

2.2.1. Plant material

Potatoes (cv. *Monalisa*, origin Alava, northern Spain) were bought one week before use from a local potato distributer and stored at 8 °C. The plant parts used for extraction (leaves, buds, bulbs, fruit, rhizome or bran) were obtained at local markets. The plant species employed in the study were as follows: black pepper (*Piper nigrum*) powder, cinnamon (*Cinnamomum verum*) sticks, clove (*Eugenia caryophyllus*) dehydrated, garlic (*Allium sativum*) minced and dehydrated, ginger (*Zingiber officinale*) powder, green tea (*Camellia sinensis*) and marjoram (*Origanum majorana*) dehydrated leaves, nutmeg (*Myristica fragrans*) powder, oregano (*Origanum vulgare*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*) dehydrated leaves, wheat bran (*Triticum* spp.) leaflets and white pepper (*Piper nigrum*) powder.

2.2.2. Plant extraction

An original solution of each plant extract was prepared with a plant/distilled water ratio of 1:6 (w/w) at 55 °C during 15 min by soaking the plant material in the solute water carrier. The extract was filtered with cheesecloth then vacuum filtered. The extract was centrifuged at $8000 \times g$ for 10 min at 4 °C with a 3K30, Sigma centrifuge. The final plant extract was swept with nitrogen and kept at 4 °C until each assay. The concentrations of these original extract solutions were between 181 and 300 µg dry extract per L of water.

2.3. Characterization of plant extracts

2.3.1. Total phenolic content and antioxidant activity

The total phenolic content (TPC, Folin–Ciocalteu method, in g Gallic Acid Equivalents L^{-1}) and antioxidant activity (AA, DPPH· in mmol Trolox Equivalents L^{-1}) were determined as described by Bobo-García et al. (2015). Three samples per plant extraction solution were analysed. The pH of each extract was measured in triplicate in 50 mL aliquots with a Basic 20 Crison pH meter (Crison instruments S.A.).
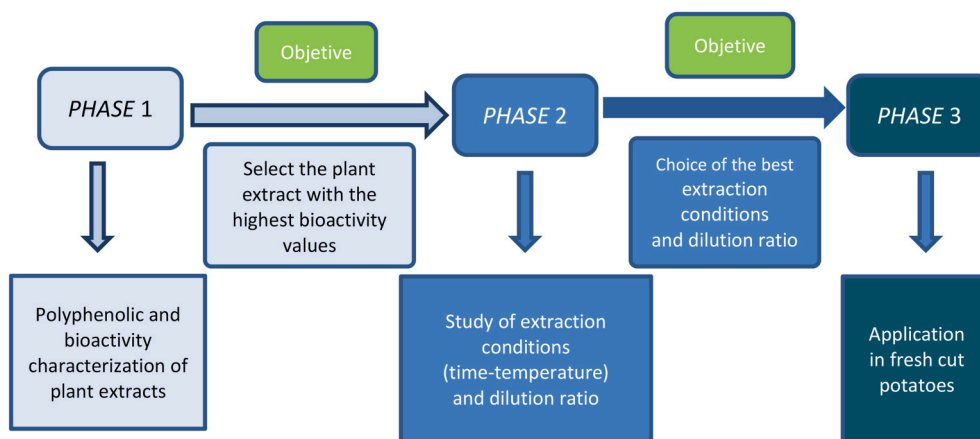
Fig. 1. Scheme of the experimental design.

2.3.2. Plant extracts' capacities to inhibit pPPO activity

2.3.2.1. Extraction of potato PPO.
A crude extract of potato polyphenol oxidase (pPPO) was obtained with the method described by Rojas-Graü, Soliva-Fortuny, and Martín-Belloso (2008) with some modifications. Twenty-five grams of potato were homogenized in 50 mL of McIlvaine buffer (citric acid and di-sodium hydrogen phosphate dehydrate, Panreac, pH 6.5) with NaCl (1 mol L^{-1}, Fluka) and 5% PVPP (poly-vinylpolypyrrolidone, Sigma-Aldrich) with a blender for 2 min at 4 °C. The homogenate was vacuum filtered at 4 °C with Whatman nº 1 filter paper. The filtrate was centrifuged at 25000×g for 20 min at 4 °C. The supernatant of the crude extract, after being swept with nitrogen, was stored at −78 °C until use.

2.3.2.2. Measurement of plant extracts' capacity to inhibit pPPO activity.
To determine the inhibition capacity of the natural plant extracts, distinct dilutions (ratio of original extract solution:water from 1:12.5 to 1:1 (v:v); 80–1000 mL L^{-1} solution were prepared. These dilutions established a range of TPCs for each plant, for example, (low range TPC: 1–25 g GAE L^{-1} and high range: 100–350 g GAE L^{-1}).

The method used to measure the capacity of plant extracts to inhibit pPPO activity was based on Masuda, Yamashita, Takeda, and Yonemori (2005); it was employed with slight modifications. The crude pPPO extract was diluted in McIlvaine buffer (pH 6.5) at a ratio 1:9. Microplate distribution and quantities in wells were as follows: (A) wells with 120 μL of McIlvaine buffer and 40 μL of pPPO; (B) 160 μL of buffer as a blank of "A" wells; (C) 80 μL of buffer, 40 μL of inhibitor and 40 μL pPPO and (D) 120 μL of buffer and 40 μL of inhibitor as a blank of "C" wells. The zero concentration only contained 200 μL of buffer. The microplate was shaken for 5 min and was incubated for 5 min at 25 °C. After the incubation, catechol 200 mM was added, the microplate was shaken again for 5 min and incubated for 5 min. The samples were measured at 420 nm at 25 °C. The percentage of pPPO inhibition (Equation (1)) was calculated as:

$$\% \ pPPO \ inhibition = [(((A - B) - (C - D)) / (A - B)) \times 100] \tag{1}$$

2.3.3. Characterization of phenolic compound profile from optimized green tea extract

The analysis of the phenolic profile of optimized green tea extract was based on Atoui, Mansouri, Boskou, and Kefalas (2005) with slight modifications. Fifteen millilitres of the extract and 30 mL of ethyl acetate (Panreac, Spain) were agitated for 5 min and centrifuged at 2000×g for 2 min. The supernatant was recovered; the process was repeated three more times. Once all supernatants were combined, the ethyl acetate was evaporated with a rotary evaporator (Büchi, mod. R-200/205) at 40 °C and 17 kPa of pressure. The dry extract was recovered with 5 mL

of methanol and the resulting sample was subjected to the HPLC analysis.

With the resulting sample, the free polyphenols (FP) could be quantified. Alkaline and acid hydrolysis were done to evaluate total polyphenols (PT) (Lin, Chen, & Harnly, 2008). With alkaline hydrolysis the phenolic acids were hydrolyzed; with this acid hydrolysis the flavonols could be analysed. The 3-flavonols could not be hydrolyzed; therefore, to obtain conjugated polyphenols (CP) the amount of free polyphenols was subtracted from the total quantity of free polyphenols. All samples were filtered at 0.45 μm; 20 μl aliquots per repetition were analysed. The phenolic compounds were analysed with a Waters 2695 (Waters, USA) chromatograph coupled to a Waters 996 UV/VIS detector. A LiChrospher RP-18, 5 μm, 250 × 4 mm (Merck; Darmstadt, Germany) column was used. The phenolic acids were quantified at 320 nm and the flavonols at 360 nm as in the method developed by Martínez (2013), and the 3-flavonols were quantified at 280 nm as described by Davidov-Pardo, Arozarena, and Marín-Arroyo (2011). The phenolic compound profile (mg L-1) was determined in triplicate.

2.4. Optimization of green tea extraction

To evaluate the optimal extraction conditions of green tea, a Box-Behnken design (BBD) of three factors was used. The selected BBD had two blocks (run 1 and 2) with 15 assays each block.

The change of the colour parameters L* and a* from the fresh-cut potato (ΔL* and Δa*) were the design response values.

The extraction conditions were temperature at 55, 72.5 or 90 °C and time 5, 10 or 15 min and the green tea concentration applied to potato slices were obtained by diluting the extract to 50, 175 and 300 mL L^{-1} (5, 17.5 and 30% respectively).

Potatoes (40–50 mm size) were sanitized with 300 μg kg^{-1} solution of chlorine for 5 min, washed for 3 min, then peeled and cut into 5 mm thickness slices with a kitchen robot (Robot-Coupe CL 52). Potato slices were immersed in a green extract solution or water (control sample) during 7 min at a rate of 1:3 w/w (potato:solution). After treatment, the slices were superficially dried using paper and stored at room temperature until colour was evaluated.

The colour was measured directly after treatment (0 h) and 7 h later. The L*, a* and b* colour coordinates were determined using a spectrophotometer (Minolta CM-2500d, Minolta CO, Japan) in the CIELab colour space, at D_{65} illuminate and a 10° observer. The colour was measured on the surface at three sites of four different slices of each sample.

2.5. Evaluation of quality of fresh-cut potatoes treated with the green tea optimized extract

Slices of fresh-cut potatoes were treated with the optimized green tea extract or with water (control sample) as described above (section 2.4). The slices were immersed at 4 °C during 7 min at a rate of 1:3 (potato: solution, w/w). Two hundred and 25 g of potato slices were packaged in LDPE bags 0.80 μm thick (22 × 16.5 cm) and sealed in 30% vacuum. The samples were stored for 14 d at 4 ± 1 °C. Three bags each were analysed at 0, 3, 7, 10 and 14 days.

The pH, total soluble solids, water content (g kg^{-1}), colour and texture were measured in the treated and control potato slices as described by Angos, Virseda, and Fernandez (2008) with slight modifications. All the physicochemical parameters were determined in triplicate, with the exception of colour and texture, both with 12 and 20 measurements per sample, respectively.

The pH and soluble solids of the homogenized potato samples were measured with a pH-meter equipped with a penetration probe (Sentron Europe B.V., Netherlands) and a refractometer (North China Optical Instrument Factory, Mod SZJ–A). Water content was determined according to AOAC 930.15 and 934.01 (AOAC 2000).

Texture was performed on cylindrical pieces of potato (27 mm diameter) at room temperature using a *TA.XT2i* texturometer (Stable Micro System LTD., Surrey, UK) equipped with a Warner Bratzler Blade. The Exponent lite V.6.1 software (Stable Micro System LTD., Surrey, UK) and the following test conditions were used: pre-test speed of 10.0 mm/s, test speed of 2.0 mm/s and post-test speed of 10.0 mm/s. The texture parameters were maximum peak strength value (N) and distance (mm) calculated from the force-distance curves. The colour was determined as described above.

2.6. Statistical analysis

Data were subjected to analysis of variance using the software package IBM SPSS Statistics 21 for Windows. In the case of significant differences (P < 0.05), means of treatments were compared using Tukey's test. The Response Surface Methodology was analysing with software Matlab 16. The fitness of the model was evaluated by the coefficient of determination, the fraction of variation that explains the model and the analysis of variance (ANOVA). In addition, the polynomial equations were represented as surface graphs in order to visualize the relationship between the responses and the levels of each experimental factor, to deduce the optimal conditions, and the regression and analysis of variance (ANOVA).

3. Results and discussion

3.1. Characterization of plant extracts

3.1.1. Total phenolic content and antioxidant activity of natural extracts

Total Phenol Content (TPC) results fell into three groups (Fig. 2 A): TPC values ranged from 178.3 to 355.9 g GAE L^{-1} (marjoram, oregano, green tea and mint extracts); a second group contained the herb extracts such as cinnamon, sage, rosemary or thyme which presented values from 93.4 to 58.6 g GAE L^{-1} and the third group contained spice extracts (peppers, ginger, nutmeg, garlic and wheat bran) which gave TPC values lower than 50.0 g GAE L^{-1}. Clove also differed from the rest with the highest value (788.0 g GAE L^{-1}).

A similar pattern was observed with the antioxidant activity (AA, DPPH·) (Fig. 2 B). Clove extract presented the maximum AA (5944.5 ± 0.577 mmol TE L^{-1}). Oregano, green tea, marjoram and mint extract values ranged from 2585.0 to 1374.2 mmol TE L^{-1}, which are the same spices that presented the highest phenolic content. Rosemary, sage, cinnamon, thyme, black pepper, nutmeg, and ginger extracts values range between 652.9 and 103.8 mmol TE L^{-1}. And the plant extracts having the lowest AA (<50 mmol TE L^{-1}) were wheat bran, garlic and white pepper extracts.

There was a linear correlation among TPC and AA in the analysed plant extracts (R^2 = 0.98; AA = 7.5473*TPC-18.678).

These results showed that phenolic compounds were found to be responsible for the antioxidant activity in some of the natural extracts. They are in accordance with those found in literature (Kratchanova, Denev, Ciz, Lojek, & Mihailov, 2010; Rusak, Komes, Likić, Horžić, & Kovač, 2008; Shan, Cai, Sun, & Corke, 2005; Silva, Souza, Rogez, Rees, & Larondelle, 2007). The antioxidant properties of phenolic acids and flavonoids are due to their redox, oxygen suppressing properties and their ability to chelate metals (Rice-Evans, Miller, & Paganga, 1996).

3.1.2. Determination in vitro of the potato polyphenoloxidase inhibition (pPPOI) capacity

Garlic, green tea and wheat bran extracts presented more than 50% of pPPOI at the lowest content of TPC (<25 g GAE L^{-1}) (Fig. 3 A).

Allium is a genus that includes garlic or onion. Both bulbs have several bioactive compounds as quercetin, one of the most abundant flavonoid found in these plants (Moreno-Ortega et al., 2020). This compound exhibits PPO's inhibitory capacity via multiple mechanisms:
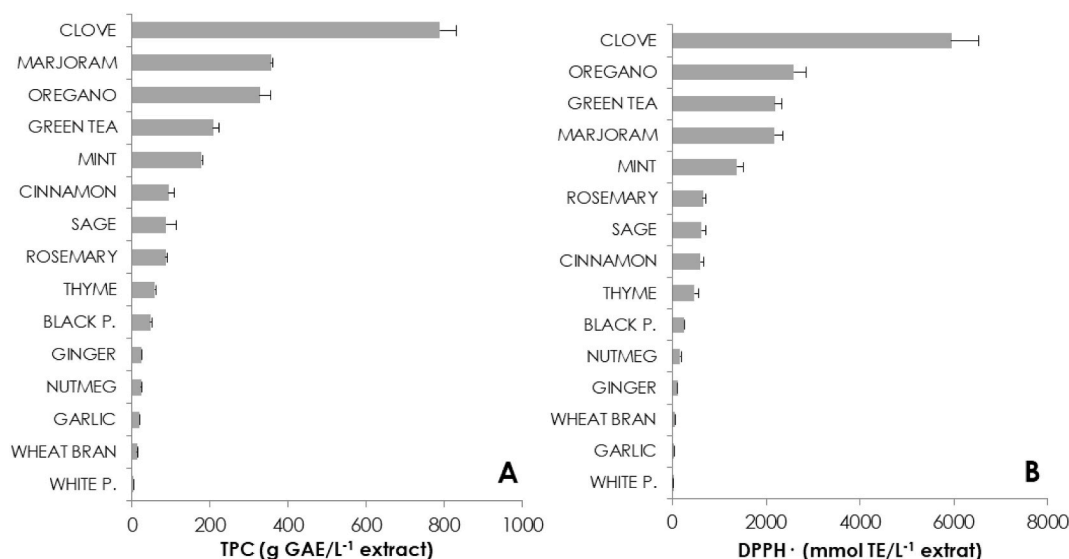

Fig. 2. Total phenolic content (TPC) (A) and antioxidant activity (DPPH) (B) of different plant extracts. Error bars represent standard deviation (n = 15).
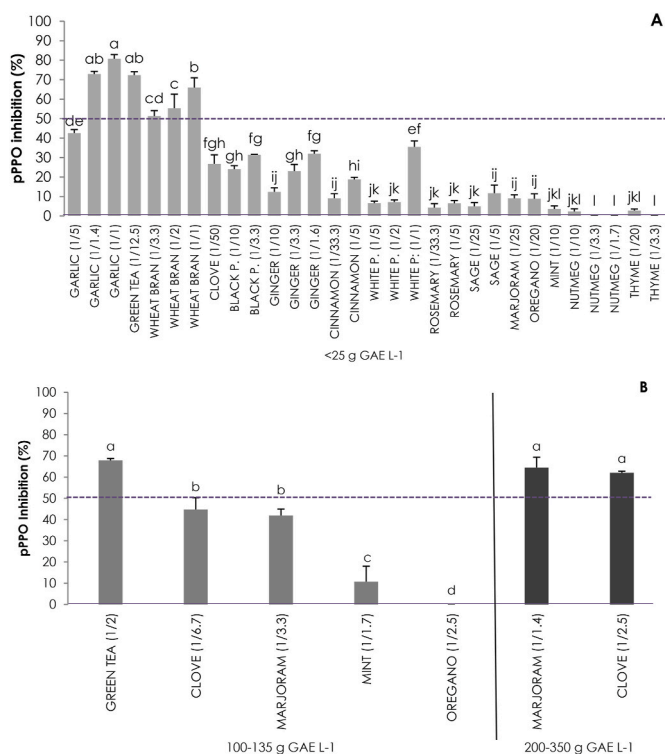
Fig. 3. Potential of plant extracts to inhibit pPPO activity (pPPOI, %)in a range <25 g GAE L^{-1} of extract A) in a range from 100 to 350 g GAE L^{-1} B). In parenthesis the dilution rate (original extract/water, v/v).

by hydrogen peroxide radical scavenging similar to ascorbic acid (Benkeblia, 2005) and by chelating copper on the enzyme active site (Vhangani & Van Wyk, 2021). There are bioactive phenolic compounds related to *Allium* genus that showed synergy with thiol compounds that could increase their inhibitory capacity (Yapi, Gnangui, & Dabonné, 2015).

In the case of wheat bran, it is known that its composition is high in ferulic and *p*-coumaric acid. These compounds showed a certain effectiveness in the specific inhibition of pPPO (Sukhonthara, Kaewka, & Theerakulkait, 2016), as has been observed in this study. It is also important take into account that depending on the raw material from which the studied PPO comes from, a compound could have different role in front of PPO, as activator or inhibitor of its activity (Kuijpers et al., 2014).

The pPPOI of green tea solution in this phenolic range was >50% even though it was so diluted (1:12.5 ratio; 80 mL L^{-1} original extract solution). However, the solutions of garlic and wheat bran only reached the same level of browning inhibition at higher concentrations (ratio higher than mL L^{-1} 1:5 and 1:3.3, 200 and 333 mL L^{-1} original extract solution respectively).

Fig. 3 B presents the pPPO inhibition capacity of the distinct plant extract solutions with higher phenolic content (from 100 to 350 g GAE L^{-1}) and high antioxidant capacity. Besides green tea only clove and marjoram have a pPPOI >50%.

In the cases of clove and marjoram, with TPC levels from 200 to 350 g GAE L^{-1}, their ability to inhibit PPO depended on the total phenolic content, therefore, highly concentrated solutions (ratios of 1:2.5 and 1:1.4; 400 and 250 mL L^{-1} original extract solution, respectively) were necessary to obtain an inhibition percentage greater than 50%.

The antibrowning capacity of green tea was already observed in fruits as apple and peaches (Klimczak & Gliszczyńska-Świgło, 2017; Piva et al., 2017). Soysal (2009) found that 30 mg L^{-1} of green tea extract could inhibit 42% of apple PPO activity *in vitro*. In our work, the capacity of green tea to inhibit pPPO was not affected by its phenolic content;

similar inhibition levels were obtained with all the assayed range of extract dilution ratios. Klimczak and Gliszczyńska-Świgło (2017) found green tea extracts may be antibrowning agent on fresh apple juice, but in this case, the extracts inhibited PPO activity in a concentration-dependent manner. This could be due to different conditions of extraction used and food matrix.

Related to phenolic profile of green tea extract, gallic acid was the major phenolic acid compound (hydroxybenzoic acid) present in the green tea extract (Table 2). A hydroxycinnamic compound, the *p*-coumaric acid was detected although not in a free state. From the flavonols, quercetin and myricetin have been found, the latter was mostly found in the free state.

The flavan-3-ols phenolic compounds are characteristic of green tea. As expected, three of the main flavan-3-ols (epicatechin gallate, epigallocatechin gallate, epigallocatechin) were found. Green tea extract also contains caffeine (xanthine), a central nervous system stimulant which was found to be the major phenolic compound of green tea extract (Atoui et al., 2005; De Dicastillo et al., 2011; Erol, 2013; Kubglomsong & Theerakulkait, 2013; López; Unachukwu, Ahmed, Kavalier, Lyles, & Kennelly, 2010).

In general, flavanols (quercetin, myricetin and kaempferol) (Chang, 2009; Erol, 2013; Kim et al., 2005; Ragaert, Devlieghere, & Debevere, 2007; Ullah, 1991) and phenolic acids (*p*-coumaric acid, ferulic acid, benzoic and cinnamic acid) are the compounds that have been shown to act as pPPO inhibitors. Therefore, gallic acid (phenolic acid), myricetin (flavonoid) and 3-flavanols detected in the green tea extract could probably be responsible for the pPPO inhibition observed when the green tea extract was applied to fresh cut potato (Sang, Lee, Hou, Ho, & Yang, 2005).

These results elucidate the inhibitory effect of the pPPO of some of the natural plant extracts depended on its TPC and AA, whereas other plant extracts, such as the green tea extract, had the same inhibitory effect without such high levels of phenol compounds. This effect could be due to the presence of a specific antioxidant, in certain cases, which can act through distinct mechanisms in the same system (Ishige, Schubert, & Sagara, 2001).

3.2. Green tea extract optimization and characterization

3.2.1. Green tea extract optimization by response surface method

In the framework of this paper, central-composite experimental design, and response surface methodology (RSM) were applied in order to investigate the impact of the maceration parameters on target responses and to optimize extraction processes.

The significant regression coefficients ($p < 0.05$) of the second order

Table 2
Green tea phenolic compounds (mg L-1) identified and quantified by HPLC.

Group	Compound	Free	Conjugate	Total
Phenolic Acids	Galic acid	364 (20)	nd	364 (20)
	p-coumaric acid	nd	36.9 (4.6)	36.9 (4.6)
Flavanols	Myricetin	212 (2)	277 (2)	321 (0)
	Quercetin	nd	87.1 (5.8)	87.1 (5.8)
	Kaempferol	nd	4.61 (2.47)	43.6 (2.5)
Flavan-3-ols	Epigallocataquingalate (EGCG)	3259 (51)	nd	3259 (51)
	Epigallocatechin (EGC)	2629 (6)	nd	2629 (6)
	Epicatequingalate (ECG)	686 (2)	nd	686 (19)
Alkaloid	Caffeine	3909 (65)	nd	3909 (65)

Values expressed as mean (n = 3) and standard deviation (SD) nd = no detected.

polynomial equations for the prediction of colour change (ΔL* and Δa* parameters) of treated potatoes at 0 and 7 h at ambient temperature at 0 and 7 h at ambient temperature are shown in Table 3. The ΔL* conforms to a linear + square model and the Δa* conforms to a linear model. Both parameters exhibited adequate fitness with experimental data as designated by high coefficients of determination ($R^2 > 0.87$), and non-significant ($p > 0.05$) lack of fit values, respectively.

The ΔL* and the Δa* close to or slightly lower or higher than zero, respectively, signifies that slices preserved the luminosity and colour of freshly cut slices without browning. In RSM graphics the evolution of the ΔL* and the Δa*parameters from the obtained equations in function of two of the studied factors are presented. They show the range of extraction factors analysed (Fig. 4); the ΔL* varies mainly with the temperature and concentration used, whereas the Δa* has a linear dependency on the three factors.

Rojas-Graü, Sobrino-López, Soledad Tapia, and Martín-Belloso (2006) applied a RSM to evaluate the effect of antibrowning agents on colour of fresh-cut apples storage at refrigerated conditions. They found that L*, a* and ΔE* were the parameters with better R-square, so changes in colour were highly related to storage time.

At the optimum extraction conditions, he colour change was 1.01 for L* and 0.66 for a*. The compound desirability for this optimization was 0.6896. The lowest colour changes were reached in the treated potato slices using a low temperature short time extraction process (55 °C and 7 min) and the more diluted extract tested (50 mL L^{-1}). The optimal extract was characterized: pPPOI of 67.6 ± 0.9%, a TPC of 13.5 ± 0.0 g GAE L^{-1} extract and an AA of 179.2 ± 7.7 mmol TE L^{-1} extract.

In the case of the green tea extract, the temperature of extraction does not seem to be the most important factor for obtaining an extract to achieve high levels of PPO inhibition. Other studies also observed that the increase of temperature was not the most critical factor from the assayed ones (ratio product-solvent, type of solvent) and only improve the diffusion of plant soluble components (Cvetanović et al., 2020).

3.3. Green tea application on fresh-cut potato

3.3.1. pH, total soluble solids and water content

As shown in Table 4, green tea extract application does not have any significant effect on the water content of fresh cut potatoes. However, the green tea treatment slightly controls the changes in pH and total soluble solids observed in the untreated samples during storage. At the end of storage, these parameters were more similar to the fresh ones than to the non-treated control potatoes.

3.3.2. Colour evolution

During sliced potato storage the evolution of colour was affected by the treatment. Potato browning is mainly defined by the decrease in luminosity *L** and the increase in the a* parameter. The reduction of luminosity observed in the control potato during storage is prevented by the green tea extract (Table 5). In the control and the treated fresh-cut potatoes the a* parameter significantly increased; between 0 and 3 d ($p < 0.05$). This phenomenon continued throughout storage. Relating

to the b* parameter, an increase in the values was initially observed in the treated sample. However, its value during storage did not differ very much from that of the fresh cut potato value.

Thus, in this case, the results of the green tea extract and untreated potato coincided with the colour results found by Angos et al. (2008). The parameters L* and *a** showed quite gradual changes over time with respect to the freshly cut potato (d0). In the green tea extract treated potato an increase in *a** over time was observed. However, it was compensated by the parallel increase in *L**. The *b** parameter also presented changes throughout the experiment, mostly on days 1 and 3. However, being that the decrease over time is smaller in this value than in the untreated samples, the b* value of the treated samples during storage did not differ very much from that of the fresh cut potato value. Thereafter, the values of each parameter remained virtually unchanged. Thus, the results obtained, also in accordance with those of Tappi et al. (2017), illustrate how green tea can be used to preserve colour during storage.

The control of browning, during 14 d of storage at 4 °C, of fresh-cut potatoes (cv. *Monalisa*) by the application of green tea extract is illustrated in Fig. 5.

3.3.3. Texture evolution

During storage the maximum strength values of the control and green tea samples increased by 14 and 8% respectively (Table 6); this produces greater hardness of the samples. In regard to the distance parameter, untreated samples showed a change during storage not observed in the green tea treated samples. However, not differences between both samples were detected in this parameter. Angos et al. (2008) also found similar tendency towards hardening during the 14 days of storage of fresh-cut potato slices.

The green tea treatment did not illustrate a remarkable effect on texture parameters. The increase of the maximum strength values of the control and green tea sample may be partly due to the surface dryness detected throughout storage. These phenomena evolved more gradually in the green tea treated samples.

4. Conclusions

Green tea extract optimal extraction conditions were 55 °C during 7 min with a final concentration solution of 50 ml L-1. This extract presented a TPC of 13.5 ± 0.0 g GAE L-1 extract and an AA of 179.2 ± 7.7 mmol TE L-1extract. The main phenolic compounds detected in the green tea extract that could be responsible for the observed pPPO inhibition were 3-Flavanols, myricetin and gallic acid. Using this extract, it was achieved minimally processed potatoes during 14 days at 4 °C. Green tea extract is a promising natural solution that could be recommended to increase the shelf life of fresh-cut potatoes. To complete the characterization of this extract as alternative antibrowning agent, it could be interesting evaluate other aspects. For example, if this natural extract, at this concentration, has antimicrobial activity or study in depth about the compounds that provide that antibrowning activity. Also, it could be interesting develop similar research in other food matrices, both *in vitro* and *in vivo*.

Author contributions

Gloria Bobo: Conceptualization, Methodology, Investigation, Software, Data curation, Visualization, Original draft preparation, Writing-Reviewing and Editing, and Funding acquisition. Cristina Arroqui: Conceptualization, Methodology, Resources, Visualization, Writing-Reviewing and Editing. Paloma Vírseda: Conceptualization, Project administration, Supervision, Resources, Writing- Reviewing and Editing.

Table 3
Significant regression coefficients (P < 0.05) of the 2-nd order polynomial equations for colour changes (ΔL* and Δa*) in green tea treated potato slices.

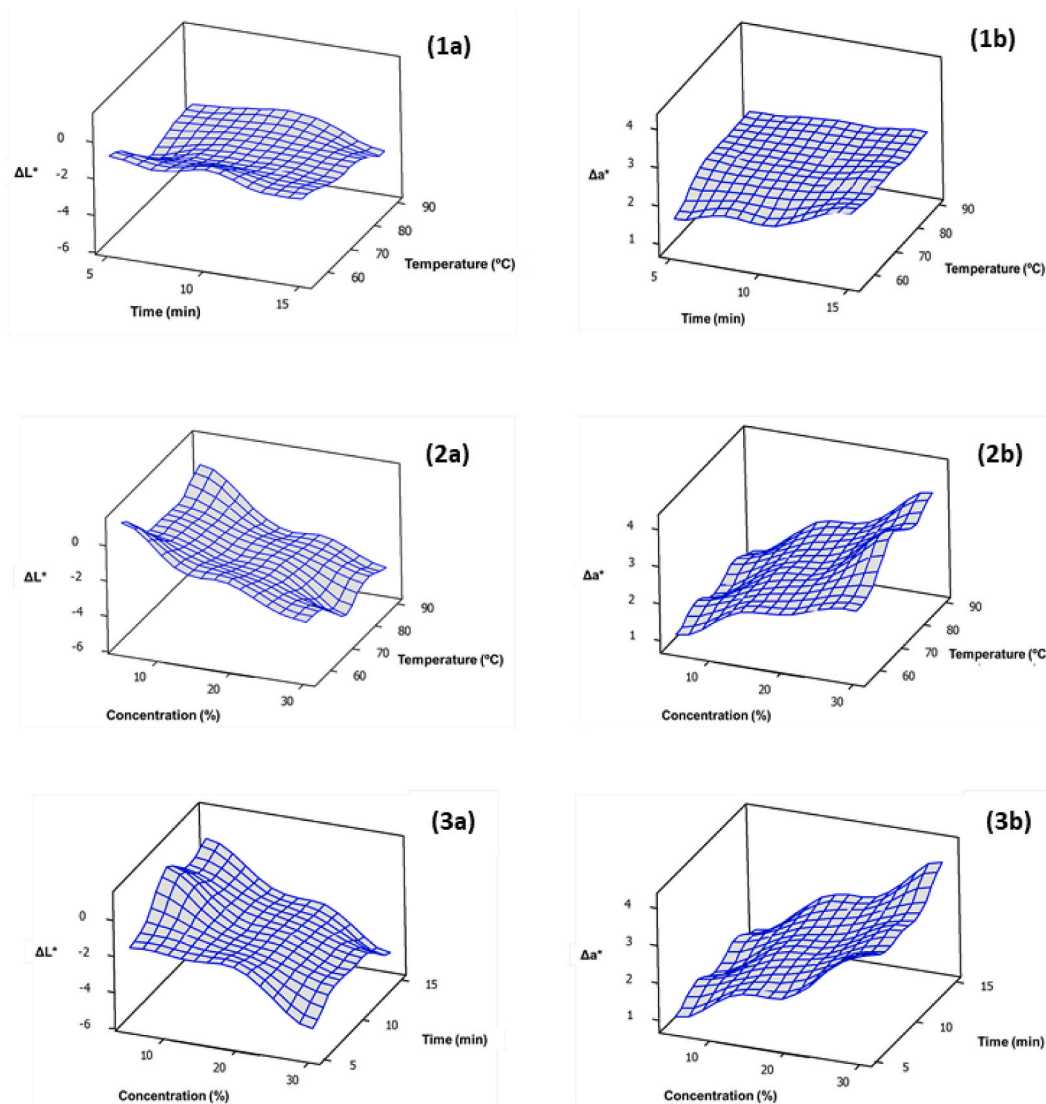| Model term	Coefficients	
	ΔL*($R^2 = 0,874$)	Δa*($R^2 = 0,867$)
Constant	13.4374	−2.36031
Temperature	−0.374501	0.0744417
Time	ns	−0.0709813
Concentration	−0.174618	0.0838448
Temperature* Temperature	0.00229266	ns
Time*Time	−0.0226939	ns
Concentration*Concentration	ns	ns

ns = no significant.

Fig. 4. Tridimensional surface graphics of the potato slices ΔL*(a) and Δa* (b) parameters versus extraction conditions and solution concentration: time and temperature (1) concentration and temperature (2) concentration and time (3).

Table 4
Physicochemical analyses of control and green tea treated potato slices during the storage at 4 °C.

	0 d	3 d	7 d	10 d	14 d
pH					
Control	5.89 [Cb]	5.82 [Cb]	5.83 [Cc]	5.76 [Bc]	5.68 [Aa]
	(0.08)	(0.04)	(0.03)	(0.11)	(0.06)
Green tea	5.85 [Cab]	5.75 [Aa]	5.80 [BCbc]	5.83 [Ca]	5.73 [Aa]
	(0.04)	(0.08)	(0.02)	(0.04)	(0.02)
Water content (g kg $^{-1}$)					
Control	804,2 [Aa]	809.5 [Aa]	827.0 [Aa]	807.2 [Ab]	822.7 [Ab]
	(1.4)	(11.5)	(28)	(4.1)	(0.93)
Green tea	794.0 [Aa]	811.0 [Aa]	806.5 [Aa]	805.2 [Ab]	805.5 [Aab]
	(10)	(0.9)	(10.2)	(1.2)	(11.53)
Total Soluble Solids (°Bx)					
Control	5.0 [Aa]	5.0 [Aa]	5.7 [Cc]	5.5 [BCc]	5.3 [Bb]
	(0.0)	(0.0)	(0.29)	(0.0)	(0.3)
Green tea	5.0 [Aa]	5.0 [Aa]	5.0 [Aa]	5.0 [Aa]	5.0 [Aa]
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

Different lower case letters (a-c) showed significant differences (p < 0.05) between treatments (column) for each day. Different upper case letters (A-D) showed significant differences (p < 0.05) between storage days for each treatment (rows). Values expressed as mean (n = 3) and standard deviation (SD).

Table 5
Colour (L*, a* y b*) of control and green tea treated potato slices during the storage at 4 °C.

	0 d	3 d	7 d	10 d	14 d
L*					
Control	70.54 [Ba]	66.39 [Aa]	67.74 [Aa]	67.74 [ABa]	65.18 [Aa]
	(3.13)	(6.70)	(5.58)	(4.73)	(3.98)
Green tea	73.09 [Cb]	70.49 [BCb]	71.21 [ABc]	71.21 [ABb]	71.02 [Ac]
	(2.10)	(2.55)	(2.37)	(2.28)	(2.18)
a*					
Control	0.026 [Aa]	1.03 [Bb]	1.18 [Ba]	1.14 [Ba]	1.31 [Ba]
	(0.48)	(0.97)	(0.639)	(0.67)	(0.71)
Green tea	0.020 [Aa]	1.28 [Bb]	1.59 [Cb]	1.81 [Cb]	1.85 [Cb]
	(0.37)	(0.66)	(0.629)	(0.54)	(0.54)
b*					
Control	25.69 [Ba]	20.68 [Aa]	19.70 [Aa]	20.51 [Aa]	19.66 [Aa]
	(2.32)	(3.36)	(3.74)	(3.04)	(3.77)
Green tea	28.51 [Bb]	26.10 [Ac]	26.40 [Ac]	26.99 [Ac]	26.79 [Ab]
	(2.16)	(2.08)	(2.53)	(1.96)	(1.83)

Different lower case letters (a-c) showed significant differences (p < 0.05) between treatments (column) for each day. Different upper case letters (A-D) showed significant differences (p < 0.05) between storage days for each treatment (rows). Values expressed as mean (n = 3) and standard deviation (SD).
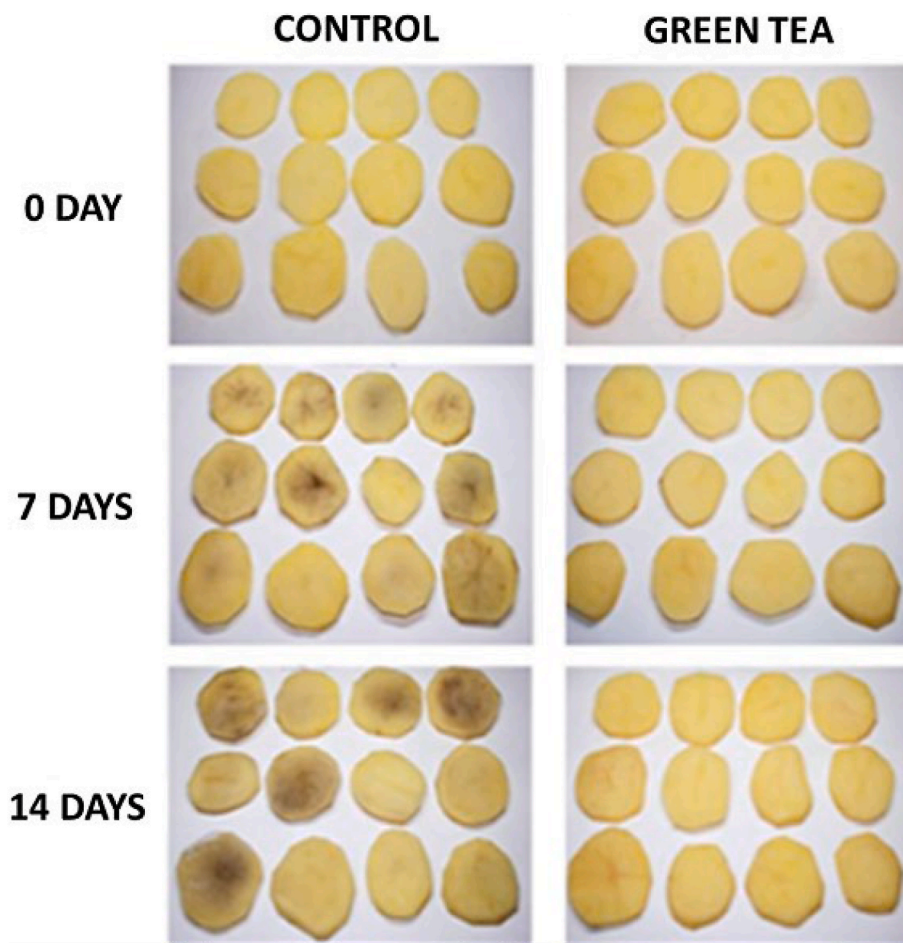
Fig. 5. Aspect of fresh-cut potatoes (cv. *Monalisa*) during 14 d of storage at 4 °C.

Table 6
Maximum strength (N) and distance (mm) of control and treated potato slices during storage at 4 °C.

	0 d	3 d	7 d	10 d		14 d
Maximum strength (N)						
Control	61.36 [Ab]	63.14 [Aab]		73.60 [Bb]	73.07 [Bb]	70.07 [Bb]
	(8.35)	(8.31)		(10.0)	(9.20)	(9.76)
Green tea	66.07 [Ac]	65.25 [Ab]		68.58 [ABb]	71.82 [Bb]	71.54 [Bb]
	(7.55)	(5.87)		(8.29)	(9.67)	(8.30)
Distance (mm)						
Control	3.42 [Aa]	3.49 [ABab]		3.63 [BCa]	3.73 [Cb]	3.69 [Cb]
	(0.27)	(0.27)		(0.27)	(0.30)	(0.25)
Green tea	3.51 [Aa]	3.56 [Ab]		3.60 [Aa]	3.61 [Aab]	3.6 [Aab]
	(0.22)	(0.23)		(0.30)	(0.29)	(0.16)

Different lower case letters (a-c) showed significant differences ($p < 0.05$) between treatments (column) for each day. Different upper case letters (A-D) showed significant differences ($p < 0.05$) between storage days for each treatment (rows). Values expressed as mean (n = 3) and standard deviation (SD).

CRediT authorship contribution statement

Gloria Bobo: Conceptualization, Methodology, Investigation, Software, Data curation, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition. **Cristina Arroqui:** Conceptualization, Methodology, Resources, Visualization, Writing – review & editing. **Paloma Virseda:** Conceptualization, Project administration, Supervision, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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