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Effect of particle size on functional properties of *Brassica napobrassica* leaves powder. Starch interactions and processing impact

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ABSTRACT

The aim of this work was to determine the physicochemical and functional properties of a *Brassica napobrassica* leaves powder sieved at three particle sizes. Moreover, in order to understand the potential interactions between the *Brassica napobrassica* leaves powder and starch, the pasting properties were assessed and the effect of pH (4–9) and temperatures (70–90 °C) on the phenolic compounds and antiradical activities were also evaluated. Particle size had an effect on physicochemical and functional properties of the vegetable powder. Vegetable fractions affected the apparent viscosity of starch suspension along heating and cooling, with larger effect during heating. The effect of the processing conditions on the functional properties of starch suspensions was influenced by the powder particle sized and the type of starch used. Maize starch seemed to interact more with phenolic compounds than rice starch, which resulted in a protective effect against pH and temperature variations, leading to higher antiradical activities.

1. Introduction

Brassica vegetables are edible leafy-vegetable crops highly appreciated because of their high level of bioactive compounds, particularly flavonoids, hydroxycinnamic acids and glucosinolates (Cartea et al., 2011; Nawaz et al., 2018). The inflorescence, root or bulb is used for human consumption but as a consequence of harvesting or industrial processing great amount of waste is generated, which can reach up to 40% of plant in volume, mostly composed by leaves and stems. Considering the reduction of agri-food waste is one of the United Nations main goals to achieve a more sustainable world by 2030 (United Nations, 2015), it is of great interest to acquire knowledge about those unexploited plant segments.

It is well known the rich bioactive profile of the edible parts of *Brassica* vegetables (Cartea et al., 2011; Clariana et al., 2011; Martínez et al., 2020), but less information exists about potential value of fresh *Brassica* leaves. The environmentally sustainable food production reconsidered the use of agro-industrial waste towards the development of new attractive foods with valuable or bioactive compounds (fibre, proteins, vitamins, minerals and antiradicals) (Carciochi et al., 2017). In fact, some studies have reported that *Brassica* by-products could be a source of bioactive compounds and antioxidant phenolics, particularly in the case of broccoli (Campas-Baypoli et al., 2009; Domínguez-Perles et al., 2010) and cauliflower (Llorach et al., 2003). Therefore, *Brassica* leaves could also be valuable as nutritional and functional food ingredients.

A very convenient process to integrate vegetable ingredients into foods is by previous milling. However, the heterogeneity and the macrostructural, microstructural and compositional characteristics that may exist within the same products constitute a limitation to obtain milled vegetables with adequate nutritional and physicochemical characteristics and with high content in bioactive compounds (Djantou et al., 2011). In fact, the particle size of food products critically influences the degradation of bioactive compounds during processing and determines the interactions established in complex food mixtures, which finally will affect to their bioaccesibility and bioavailability during consumption and digestion (Bornhorst & Singh, 2014). In consequence, fractionation after milling has been reported as a physical alternative to modulate the technological and further physiological performance of different flours and powders from diverse seeds (Tsatsaragkou et al., 2017, De la Hera et al., 2014).

Bakery products are one of the most attractive vehicles for functional ingredients, because they are very affordable and worldwide consumed. In fact, many reports present the enrichment of bakery products on fibers (see review Fendri et al., 2016), proteins by legumes (see review Boukid et al., 2019) and lately by vegetables because their bioactive compounds (Betoret and Rosell, 2020; Krupa-Kozak et al., 2019). The common point of the bakery products is that their main

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constituent is starch, which also is the main ingredient for other foods like baby foods, ice cream, meat products, sauces, soft drinks, soups and smoothies (Copeland et al., 2009). Because of that, it is crucial to understand the interaction of starch with the different functional ingredients. Particularly in the case of phenolic compounds, it has been described their interaction with starch forming helical complexes with amylose or interacting through hydrogen bonds (Zhu, 2015). Whatever type of interaction, processing conditions are going to affect the functional and technological properties of the foods developed. That is even more important in polyphenol rich ingredients because polyphenols are highly reactive species that undergo numerous reactions in the course of food processing, and which, can be readily affected by pH and temperature during processing (Palermo et al 2014). Therefore, initial hypothesis is that size fractionation might be an alternative to modulate by-products physico-chemical properties and in consequence their performance in different starchy systems.

The aim of this work was to determine the physicochemical and functional properties of a *Brassica napobrassica* leaves powder sieved at three particle sizes. Moreover, in order to understand the potential interactions between the *Brassica napobrassica* leaves powder and starch, the pasting properties were evaluated and the effect of pH (4–9) and temperatures (70–90 °C) on the phenolic compounds and antiradical activities were also evaluated.

2. Materials and methods

2.1. Materials

The aerial part of the plant *Brassica napobrassica*, composed by leaves and stems, was provided from local farmer (Valencia, Spain). Maize starch was acquired from Miwon (Seoul, Corea) and rice starch was purchased from Sigma Aldrich (Madrid, Spain). Folin & Ciocalteu's phenol reagent (2 M); 2.2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); (±)-6-Hydroxy-2.5.7.8-tetramethylchromane-2-carboxylic acid (Trolox); Potassium persulfate (≥99.0%); 2.2-Diphenyl-1-picrylhydrazyl (DPPH); Sodium tretraborate decahydrate; Sodium acetate trihydrate; Gallic acid; Boric acid and L (+)-Ascorbic acid were purchased from Sigma Aldrich (Madrid, Spain). Ethanol absolute; Acetic acid glacial and Sodium hydroxide were acquired from Panreac (Barcelona, Spain). All reagents were of analytical grade.

2.2. Food materials and sample preparation

Leaves and stems were cut into pieces of around 4–5 cm length and washed three times changing plant-water proportions (1:3; 1:3 and 1:7) (w/w). Washed samples were dried in an industrial dryer from ControlTecnica Instruments (Wodzislaw, Polska) at 70 °C for 24 h and milled with a domestic mill Moulinex A320R1 (Barcelona, Spain). Milled samples were fractionated with an electromagnetic sieve shaker (Badalona, Spain) according to their particle size (ø > 1 mm; 125 μm < ø < 1 mm; ø < 125 μm) and herein they were referred to large (L), medium (M) and small (S), respectively. The obtained fractions were packaged into sealed pouches and stored at 4 °C until further analysis.

2.3. Chemical and physicochemical characterization of vegetable fractions.

The content in fat, fibre, proteins, water and ash of vegetable fractions was determined. The determination of fats and fibre was carried out following the official AOAC (1990) methods 992.06 and 991.43 respectively. Protein was determined by following official ICC (2000) methods 167. Water content was quantified by vacuum drying at 60 °C until a constant weight.

Water activity was measured with a dew point hygrometer AquaLab Series 3 (Lleida, Spain). The values provided are the average of three replicates. Colour measurements were carried out with a colorimeter Konica Minolta CR-400 (New Jersey, USA). The colour results were provided in a CIELAB system for illuminant D65 and a 10° angle of vision. The registered parameters were as follows: L^* (brightness), a^* (red-green component), b^* (yellow-blue component), h^*_{ab} (hue, attribute related to the differences in absorbance at different wavelengths and considered the qualitative attribute of colour, Eq. 1), and C^*_{ab} (chrome, quantitative attribute of colourfulness, Eq. 2). The global colour difference (ΔE) was calculated by using Eq. 3. The values provided are the average of three replicates.

$$h^*_{ab} = \arctan(b^*/a^*) \quad (1)$$

$$C^*_{ab} = ((a^*)^2 + (b^*)^2)^{1/2} \quad (2)$$

$$\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2} \quad (3)$$

Leaves and stems proportions contained in powders were calculated by using an image treatment analysis. The image treatment analysis was performed with EVO Cam II from Vision Engineering (New Milford, USA). TIFF (Tagged Image File Format)-RGB (Red, Green, Blue) colour images were recorded in conditions of high light intensity (Led-Evo Cam), automatic exposure autofocus and 68 increases. Five samples of each particle size fraction were analysed with a total number of 45 images.

2.4. Functional compounds determination

The content of the ascorbic acid, phenolic compounds and glucosinolates were determined using an Agilent HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump (G1312A), autosampler (G1313A), photodiode array detector (G1312A) controlled by Agilent software (v. A.08.03), and degasser (G1322A) following the methodology and sample preparation described in Vallejo et al., 2003. The values provided are the average of three replicates.

2.5. Pasting properties determination

The pasting properties of the starchy vegetable suspensions were measured using a Rapid Visco Analyzer (RVA-4500, Perten Instruments, Hägersten, Sweden). Maize or rice starches (3 g based on 14% moisture content) were blended with vegetable powder (1.5 g) in an aluminium RVA canister. Then, blends were suspended in 25 mL of distilled water. A controlled heating and cooling cycle, from 50 to 95 °C in 282 s, holding at 95 °C for 150 s and then cooling to 50 °C for 344 s was applied. The initial speed for mixing was 960 rpm for 10 s, followed by a 160 rpm paddle speed that was maintained for the rest of assay. Peak viscosity, final viscosity, breakdown (peak viscosity-trough), setback (final viscosity-trough) and onset temperature for pasting formation were determined from the viscosity plot and recorded using Thermocline software for Windows (Perten Instruments, Häger-sten, Sweden).

2.6. pH treatments and thermal treatments

Solutions (0.1 M) of sodium acetate and glacial acetic acid were used to make buffers at pH 4, 5 and 6. They were adjusted to the desired pH by adding sodium acetate to acetic acid. Solutions (0.01 M) of sodium borate, boric acid and 1 N or 0.25 N NaOH were mixed to prepare buffers at pH 7, 8 and 9. Leaves samples (0.5 g) were mixed with one gram of starch and the blend was suspended in 4 mL of the corresponding buffer and kept under magnetic stirring for 20 min. Treated samples were mixed with 5 mL of absolute EtOH and centrifuged at 1454 × *g* for 3 min. Supernatant was filtered with Whatman 90 mm ø (Maidstone, UK) and used for analysis.

Same type of suspensions, but in water, were used for thermal

treatments. Suspensions were subjected to heat treatment at 70, 80 or 90 °C for 20 min. Then, samples were treated as previously described for pH impact.

2.7. Total phenolic content and antiradical activity

Total phenolic content was assessed using the Folin Ciocalteau method following the modifications proposed by Singleton et al. (1999) and Moraes-de-Souza et al. (2008), using a volume of 30 μL of sample. The absorbance was measured with a spectrophotometer (BioTeK Epoch) at 765 nm every 1 min for a total time of equilibrium at 90 min. The results were expressed as mg/g equivalents of Gallic acid.

The ABTS test was based on the method proposed by Polydera et al. (2005) using a sample volume of 15 μL. The absorbance was measured with a spectrophotometer (Shimazdu UV–VIS-1240) at 734 nm every 30 s for a total time of equilibrium at 30 min. The assay is based on a calibration curve of (±)-6-Hydroxy-2.5.7.8-tetramethylchromane-2-carboxylic acid (Trolox) in the concentration range of 0–6 μg/mL and the results were expressed as mg/g TEAC (Trolox Equivalent Antiradical Capacity).

The DPPH test was based on modifications of the method proposed by Re et al. (1999) using a volume of 40 μL of sample. The absorbance was measured with a spectrophotometer (BioTeK Epoch) at 515 nm every 1 min for a total time of equilibrium at 30 min. The results were expressed as mg/g equivalents of Ascorbic acid. The values provided are the average of three replicates. The assay is based on a calibration curve of L(+)-Ascorbic acid in the concentration range of 0–20 μg/mL as Meq. of Ascorbic acid. The values provided are the average of three replicates.

2.8. Statistical analysis

In order to evaluate whether the obtained values were significantly different, a multi factorial ANOVA, in pH and temperature treatments, and simple ANOVA both with 95% confidence level, were performed using a Statgraphics Plus 5.1 software (Statgraphics technologies, USA).

3. Results and discussion

3.1. Vegetable fractions characteristics

The leaves of *Brassica napobrassica*, after being dehydrated and ground, were fractioned according to their particle size into three fractions (S, M and L). The percentage of stems contained in each fraction (Table 1) increased with the openings size. Fractionation according to the particle size resulted in significant ($p \leq 0.05$) differences on the powder composition (Table 1). The fibre content progressively decreased as the particle size was reduced, conversely proteins and fat were concentrated in the smaller fractions. Therefore, larger fraction (L) was enriched in fibre content, whereas the smallest fraction (S) was enriched in proteins, due to bigger proportion of stems and leaves, respectively. Water activity in S, M and L samples was 0.44, 0.47 and 0.53, respectively. Therefore, the major content in fibres did not affect the water activity. CIE-$L^*a^*b^*$ coordinates showed, as expected, green colour in S and M samples and a tendency to orange colour in L sample as the a^* coordinate increased because of higher quantity of stems (Table 1). Brightness (L^*) was higher in L sample with no significant differences between S and M samples. This result may be due to bigger particle size and higher water content found in L sample. Colour differences (ΔE), with S sample as a reference, were significant in both cases. In general, colour differences less than 1.5 unit value are not detected visually (Pathare et al., 2013). In our case, visual colour differences were clearly detected in L sample.

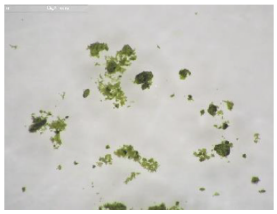
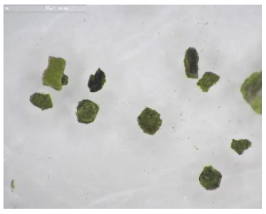
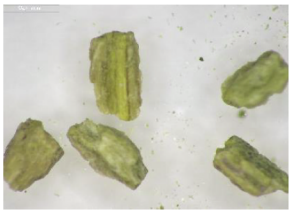
In vegetable samples, 12 phenolic compounds were detected with remarkable content in flavonoids, especially kaempferol and quercetin derivatives, and in hydroxycinnamic acids mostly sinapic and caffeic acids (Table 2). The appearance of p-coumaric acid was probably derived from cinnamic acid and the chlorogenic acid derived from caffeic acid. The content in p-coumaric acid, kaempferol and sinapic acid were significantly higher as the particle size fraction decreased, thus finer fraction concentrated phenolic compounds. Therefore, as it has reported for other *Brassica* crops (Llorach et al., 2003), the leaves contain high content of phenolic compounds. The stems due to their structural functionality in the plant are mostly composed by fibre (Cartea et al., 2011). In our case, the higher presence of leaves in finer fractions could explain the more quantity of phenolic compounds that decreased as the particle size increased together with higher presence of stems.

3.2. Impact of vegetable fractions on pasting properties of starches

In order to identify the effect of those vegetable fraction when integrated in starchy matrices, fractions from *Brassica napobrassica* powders were added to two different types of starch (rice and maize). Two starches were selected to include the potential effect of starch granule size on either the interaction or matrix integration with the vegetable powders. Specifically, rice and maize starches have an average granule diameter of 5 and 15 μm, respectively. The pasting properties of the suspensions were recorded during a heating–cooling cycle in order to understand the interactions that might be established between starch and antiradical/nutritional food compounds (Fig. 1). In general, the three powder fractions greatly affected the pasting properties of starch and the extent of the effect was dependent on the type of starch, being larger on the viscosity of maize starch than that of rice starch. Vegetable fractions affected the apparent viscosity of starch suspension along heating and cooling, with larger effect on apparent viscosity during heating. Regarding the particle size of the vegetable fraction, different behaviour could be displayed depending on the type of starch. In rice starch, a steady increase in the apparent viscosity of starches were observed with increasing the particle size, and that performance was observed during heating and cooling. It must be stressed that with the smallest fraction (S) it was observed a great increase of the apparent viscosity during heating, but the impact was barely noticeable during gel cooling. In the maize starch, the presence of vegetable fractions induced a significant increase of the viscosity during heating, but the diverse particle size of the vegetable powders hardly affected that viscosity. Conversely, during cooling differences associated to the diverse vegetable fractions were readily visible. The swelling of maize starch granules at higher temperatures could favour an increase in protein and fibre destructuring (Ren et al., 2021) that could hinder the effect of vegetable particle size during heating.

When pasting parameters were evaluated (Table 3), the values of peak and final viscosities confirmed the behaviours previously mentioned. It was observed that pasting temperatures of starches decreased when containing vegetables, but in the case of rice starch suspensions, pasting temperature increased as particle size augmented. Some authors (Yildiz et al., 2013) have pointed out that the fibre competition for water could be the reason to increase the pasting temperatures however in the presence of water excess, as was our case, the increasing of pasting temperatures could be due to a more complex phenomenon including competition between starch and solutes by the available water, inhibition of granular starch hydration and the interactions that could be established directly with the starch granules. Nevertheless, the different behaviour observed between rice and maize during heating in the presence of the different vegetable fractions could indicate that antiradical compounds might have more influence than nutritional compounds (Sun et al., 2015) in the case of maize starch, where the increase in fibre content did not have a direct impact on the apparent peak viscosity. In both starches, the incorporation of vegetables increased the final viscosity after cooling, reaching lower values for S and M samples with higher contents in proteins and fat than L samples, which could be associated to the increasing fibre content of the fractions (Sun et al., 2015). In both starchy systems the vegetable fractions

Table 1
Physical characteristics, CIE *L*a*b** colour coordinates and chemical composition in the vegetable fractions (S, M, and L).

Vegetable fractions	S	M	L
Sample images			
Physical characteristics			
Particle size range	ø < 125 μm	125 μm < ø < 1 mm	ø > 1 mm
% of stems	7.98	11.52	20.75
Chemical composition (%,d.b)			
Moisture content	3.69 ± 0.06[a]	4.1 ± 0.4[a]	9.9 ± 0.3[b]
Protein	24.6 ± 0.2[c]	20.7 ± 0.2[b]	14.13 ± 0.13[a]
Fat	5.32 ± 0.02[c]	3.15 ± 0.06[b]	1.61 ± 0.06[a]
Fibre	1.4 ± 0.3[a]	3.2 ± 0.3[b]	5.9 ± 0.5[c]
Colour parameters			
*L**	20.1 ± 0.2[a]	18.6 ± 0.3[a]	34.3 ± 1.3[b]
*a**	0.3548 ± 0.0007[b]	0.3490 ± 0.0002[a]	0.3648 ± 0.0003[c]
*b**	0.4170 ± 0.0013[c]	0.4046 ± 0.0014[b]	0.3969 ± 0.0004[a]
*C*ab*	0.5475 ± 0.0014[c]	0.5343 ± 0.0009[a]	0.5391 ± 0.0002[b]
*h*ab*	0.76 ± 0.09[a]	0.76 ± 0.08[a]	0.77 ± 0.05[a]
ΔE	–	1.55 ± 0.12[a]	14.1 ± 1.4[b]

* Different superscript letters within a raw indicates significant differences ($P \leq 0.05$).

Table 2
Phenolic compounds identified in each fraction (S, M and L) of *Brassica napobrassica.*

Peak	Rt (min)	Tentative identification	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	S (mg/g)	M (mg/g)	L (mg/g)
1	3.1	Caffeic acid glucoside	341	179	0.928	0.707	0.312
2	3.8	p-coumaric acid derived	405	191	1.954	1.542	1.044
3	4.0	Unknown	372	243	0.456	0.327	0.192
4	7.0	Unknown	292	172	0.949	0.403	0.365
5	7.2	Unknown	292	172	2.289	2.274	0.129
6	11.7	Chlorogenic acid	353	191	0.945	0.495	0.068
7	13.1	Patuletin-glucosil-apiosil-glucoside	787	193	0.733	0.060	0.000
8	14.1	Kaempferol-3,7,4′-triglucoside	772	285	0.965	0.466	0.000
9	16.0	Sinapoyl glucoside	385	223	1.261	0.803	0.117
10	16.4	Sinapoyl glucoside	385	223	1.003	0.518	0.037
11	20.1	Kaempferol-3,7-diglucoside	609	285	0.521	0.215	0.000
12	20.4	Kaempferol-3-O-sophoroside-7-O-glucoside	772	285	0.677	0.330	0.000
13	21.1	Unknown	732	466	1.107	0.573	0.049
14	21.4	Quercetin-7-glucoside	463	301	0.547	0.309	0.046
15	22.1	Kaempferol-3,4′-diglucoside	609	285	1.328	0.652	0.158
16	24.5	1,2-disinapoyl gentiobiose	754	223	0.561	0.202	0.046
17	25.0	1-sinapoyl-2-feruloilgentiobiose	724	301	0.575	0.259	0.024
Total phenolic compounds					**16.799**	**10.134**	**2.588**

* S, M and L refers to the particles size of samples, ø < 125 μm, 125 μm < ø < 1 mm, and ø > 1 mm, respectively.

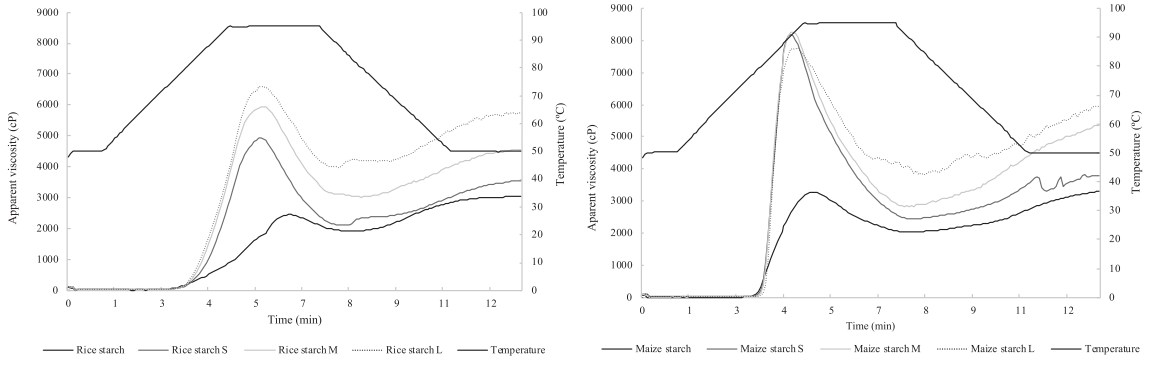


Fig. 1. Pasting properties of starch suspensions containing vegetable powders. S, M and L refers to the particles size of vegetable samples (ø < 125 μm; 125 μm < ø < 1 mm; ø > 1 mm) respectively.

Table 3
Pasting properties of starch suspensions containing vegetable powders.

Starch	Vegetable Fraction	Peak viscosity (cP)	Trough (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Peak time (min)	Pasting temperature (°C)
Rice	None	2447 ± 1^{a}	1888 ± 32^{a}	559 ± 30^{a}	3020 ± 11^{a}	1133 ± 21^{a}	6.3 ± 0.1^{d}	70.7 ± 0.4
	S	4767 ± 160^{c}	2007 ± 125^{ab}	2760 ± 58^{c}	3407 ± 131^{b}	1400 ± 59^{ab}	5.6 ± 0.0^{c}	67.1 ± 0.5
	M	6117 ± 174^{d}	3048 ± 43^{d}	3068 ± 164^{d}	4552 ± 18^{d}	1504 ± 29^{bc}	5.6 ± 0.1^{c}	70.8 ± 0.4
	L	6528 ± 263^{e}	3937 ± 250^{e}	2592 ± 35^{c}	5725 ± 148^{f}	1788 ± 103^{c}	5.7 ± 0.1^{c}	73.2 ± 1.3
Maize	None	3332 ± 72^{b}	2096 ± 76^{b}	1236 ± 16^{b}	3323 ± 37^{b}	1227 ± 40^{ab}	5.0 ± 0.1^{b}	77.2 ± 0.4
	S	7971 ± 190^{f}	2487 ± 87^{c}	5484 ± 229^{f}	3868 ± 388^{c}	1382 ± 466^{ab}	4.4 ± 0.0^{a}	75.9 ± 0.8
	M	8385 ± 124^{f}	2981 ± 143^{d}	5404 ± 26^{f}	5211 ± 137^{e}	2230 ± 251^{d}	4.4 ± 0.0^{a}	76.1 ± 0.5
	L	7761 ± 58 ^{g}	3753 ± 89^{e}	4008 ± 138^{e}	5935 ± 15^{f}	2181 ± 77^{d}	4.4 ± 0.1^{a}	76.1 ± 0.5

* S, M and L refers to the particles size of samples, ø < 125 μm, 125 μm < ø < 1 mm, and ø > 1 mm, respectively.
Different superscript letters within a column indicates significant differences ($P \leq 0.05$).

weaken the stability of the granules during heating as indicated the higher values for trough and breakdown. But the absence of a direct relationship with the particle size of the fractions, suggests that other interactions apart from the physical impact might take place. Different behaviour between starches was also observed in the setback, that although increased in the presence of the vegetable, only in the case of rice starch was observed a steady increased with the particle size of the fraction. Considering that setback is mainly associated to amylose retrogradation during cooling (Sun et al., 2015), differences could be attributed to their amylose content (rice starch ≈ 5%; maize starch ≈ 8%) (Ali et al., 2016). But also, other factors like different granules swelling might contribute to that difference. Setback results are dependent on the amylose content and also on the extend of granules disruption (related to trough). As it has been mentioned before, the higher gelatinization temperature of maize starch might favour the disruption promoted by proteins and fibres (Ren et al., 2021), as confirmed the higher breakdown observed in this starch. Presumably, the recrystallization of that disrupted structure occurred differently and the larger particle size of L samples somewhat obstruct that process.

3.3. Impact of processing conditions (pH and temperature) on antioxidant properties of vegetable-starchy matrixes

pH and thermal treatments in samples with and without starch were carried out in order to determine whether the processing variables could have an effect on the total phenolic compounds and antiradical activities of *Brassica napobrassica* leaves powders with different particle sizes. Three different antiradical assays were performed to cover a wide range of bioactive compounds: Folin-Ciocalteau methodology as an indicator of total phenolic content, DPPH method due to its high sensitivity towards hydrophilic compounds such as ascorbic acid and ABTS method due to its higher sensitivity to hydrophobic compounds such as some vitamins and flavonoids (Del Caro et al., 2004).

A multifactorial ANOVA analysis indicated that pH, particle size of the vegetable fractions and type of starches had a significant effect on total phenolic and antiradical activities measured by ABTS and DPPH methods (Table 4). Regarding the total phenolic content, expressed on gallic acid equivalents, the statistical analysis also revealed a binary interaction between the factors pH-starch and starch-particle size. Although some variations in the phenolic content was observed within the pH range evaluated, considering the ends values (pH 4 and pH 9), the total phenolic content, showed an increase when decreasing the particle size of the fractions and also with the pH enhancement. It was remarkable the low values obtained at pH 4 in particle size L. The same trend was observed when vegetable fractions were blended with starches. In the presence of starches, the vegetable fractions showed lower antiradical activity, being reduced almost by 40% in the presence of rice starch and only by 10% as average, with maize starch. Phenolic compounds and starch can form non-covalent complexes (Zhu, 2015). The non-covalent interactions between starch and phenolic compounds in food systems involve hydrogen bonds, hydrophobic interaction, electrostatic and ionic interactions (Bordenave et al., 2014). Also amylose can interact with phenolic compounds to form inclusion complex termed V-type amylose in which the driving force is the hydrophobic interaction within the cavity of the helix (Obiro et al., 2012). As pointed out by, Cohen et al., in (2011) the phenolic compounds tightly complexed inside the cavity of amylose helices are resistant to washing with ethanol solution. As in our starches the amylose content did not differ extensively, differences in the gel network but also different hydrophobicity could explain those differences.

Conversely, in the antiradical activity by DPPH measured as the ascorbic acid equivalents, values showed a reduction with the particle size of the vegetable fractions, and the same tendency was observed in the presence of the starches. Regarding the impact of the pH, a gauss curve was envisaged with a maximum value at pH 7 in all vegetable fractions, although at pH 9 values tended to increase, and the same trend was observed in the absence and presence of starches. The type of starch barely impacted the ascorbic acid equivalents.

Considering the antiradical activity measured by ABTS and expressed as trolox equivalents, the statistical analysis showed a binary interaction between pH-starch. Less clear was the effect of sample fractions and pH on the antiradical activity derived from hydrophobic compounds. In general, the highest values were obtained at pH 9 and the smallest at pH 8. The presence of starches reduced the antiradical activity of the fractions, particularly rice starch, and the effect was even more accentuated with S fraction. Concerning the interaction pH-starch, vegetable fraction with rice starch presented more antiradical activity at pH 4, but for the rest of pHs, samples with maize starch had higher antiradical activity. Therefore, the L fraction had the lowest total phenolic content and the highest antiradical activity due to hydrophilic compounds. Possibly the less surface/volume proportion, due to its bigger particle size, hindered the extraction of phenolic compounds or the higher proportion of stems in L fraction resulted poorer in this type of compounds and reduced the final obtained values. In fact, some interactions with dietary fibers could result in low extraction levels of bioactive compounds from food matrices (Saura-Calixto, 2011). According to that, the high fibre content of L samples due to great presence of stems together with their structural influence could explain the low levels of phenolic compounds. According to Friedman and Jürguens (2000), conjugated non-phenolic aromatic acids such as trans-cinnamic acids are stable at high pH, but caffeic and chlorogenic acid dramatically changed at high pH, likely due to the two adjacent OH groups in the benzene ring. However, in the presence of other food compounds, antiradical compounds can be partially protected from changes. In the same way, flavonoids are prone to oxidation in conditions such as elevated pH or temperature and protein interactions could protect and prevent them from auto oxidative reactions (Wegrzyn et al., 2008). The haze formation in the plant-based solution showed such interactions (Kardum and Glibetic, 2018). When vegetable fractions were blended with starches, higher levels of phenolic compounds were measured with maize starch compared to rice starch.

Regarding thermal treatments (Table 5), a range of temperatures

Table 4
Effect of pH on total phenolic content and antiradical activities of the three vegetable fractions in the presence of different starches.

		Galic A. Eq. (mg/g)			Ascorbic A. Eq. (mg/g)			Trolox Eq. (mg/g)		
	pH	None	Rice Starch	Maize Starch	None	Rice Starch	Maize Starch	None	Rice Starch	Maize Starch
S	4	4.2 ± 0.3^{e-h}	2.48 ± 0.13^{gh}	2.5 ± 0.2^{b-d}	0.11 ± 0.06^{a}	0.15 ± 0.07^{a-c}	0.17 ± 0.14^{ab}	4.2 ± 0.2^{e-g}	2.7 ± 0.4^{de}	0.80 ± 0.05^{a}
	5	4.553 ± 0.102^{gh}	2.6 ± 0.2^{h}	4.32 ± 0.07^{hi}	0.212 ± 0.107^{ab}	0.05 ± 0.02^{a}	0.10 ± 0.04^{a}	4.5 ± 0.7^{e-g}	2.5 ± 0.3^{de}	4.0 ± 0.5^{h-j}
	6	4.3 ± 0.6^{f-h}	2.24 ± 0.08^{e-g}	4.2 ± 0.2 ^{h}	0.24 ± 0.07^{ab}	0.181 ± 0.114^{a-d}	0.08 ± 0.08^{a}	3.76 ± 0.03^{d-f}	3.0 ± 0.2^{e}	4.218 ± 0.103^{ij}
	7	4.7 ± 0.4^{h}	2.6 ± 0.2^{h}	4.8 ± 0.5^{i}	0.48 ± 0.02^{c-f}	0.64 ± 0.02^{f}	0.51 ± 0.02^{de}	3.9 ± 0.2^{d-g}	2.2 ± 0.2^{de}	3.6 ± 0.2^{f-i}
	8	4.3 ± 0.2^{gh}	2.45 ± 0.13^{gh}	4.0 ± 0.2^{gh}	0.04 ± 0.05^{a}	0.09 ± 0.09^{a-c}	0.06 ± 0.05^{a}	1.6 ± 0.5^{ab}	0.6 ± 0.5^{a}	1.8 ± 0.4^{b-d}
	9	5.3 ± 0.3^{i}	3.2 ± 0.2^{i}	5.6 ± 0.4^{j}	0.15 ± 0.03^{ab}	0.246 ± 0.114^{b-d}	0.24 ± 0.09^{a-c}	4.81 ± 0.08^{fg}	4.40 ± 0.05^{f}	5.2 ± 0.2^{k}
M	4	3.084 ± 0.114^{bc}	1.82 ± 0.12^{c}	2.13 ± 0.15^{bc}	0.27 ± 0.12^{bc}	0.05 ± 0.04^{bc}	0.31 ± 0.09^{a-c}	3.6 ± 0.4^{cde}	2.06 ± 0.12 ^{cd}	1.20 ± 0.08^{a-c}
	5	3.7 ± 0.2^{c-f}	2.15 ± 0.13^{d-f}	3.6 ± 0.4^{fg}	0.13 ± 0.13^{a}	0.15 ± 0.06^{a-c}	0.14 ± 0.14^{ab}	3.807 ± 0.104^{d-f}	2.2 ± 0.2^{de}	3.3 ± 0.4^{f-h}
	6	3.7 ± 0.5^{c-f}	2.03 ± 0.07^{c-e}	3.10 ± 0.12^{ef}	0.24 ± 0.06^{ab}	0.25 ± 0.06 ^{cd}	0.31 ± 0.07^{bcd}	4.38 ± 0.08^{e-g}	2.5 ± 0.2^{de}	3.53 ± 0.07^{f-i}
	7	3.6 ± 0.4^{b-e}	2.15 ± 0.12^{d-f}	3.9 ± 0.2^{gh}	0.59 ± 0.02^{f}	0.67 ± 0.02^{f}	0.582 ± 0.009^{de}	3.53 ± 0.03^{cde}	2.8 ± 0.3^{de}	2.9 ± 0.2^{efg}
	8	3.51 ± 0.09^{b-d}	2.07 ± 0.08^{c-e}	3.25 ± 0.13^{ef}	0.29 ± 0.12^{b-d}	0.117 ± 0.012^{a-c}	0.11 ± 0.08^{ab}	1.3 ± 0.3^{a}	0.7 ± 0.2^{a}	1.5 ± 0.4^{a-c}
	9	4.09 ± 0.14^{d-h}	2.47 ± 0.05^{gh}	3.92 ± 0.04^{gh}	0.26 ± 0.08^{b}	0.31 ± 0.08^{cd}	0.18 ± 0.08^{a-c}	4.94 ± 0.08^{g}	4.37 ± 0.02^{f}	4.853 ± 0.104^{jk}
L	4	1.33 ± 0.06^{a}	0.97 ± 0.04^{a}	1.16 ± 0.14^{a}	0.23 ± 0.08^{ab}	0.08 ± 0.06$^{def\ a-c}$	0.21 ± 0.07^{a-c}	1.48 ± 0.03^{ab}	1.23 ± 0.06^{ab}	1.06 ± 0.03^{ab}
	5	3.64 ± 0.05^{b-g}	1.37 ± 0.02^{ab}	2.297 ± 0.009^{bc}	0.229 ± 0.003^{a-d}	0.244 ± 0.007^{a-d}	0.35 ± 0.04^{a-e}	3.084 ± 0.007 ^{cd}	2.97 ± 0.03^{de}	2.70 ± 0.04^{d-f}
	6	3.27 ± 0.05^{b-d}	1.69 ± 0.04^{bc}	2.69 ± 0.12^{b-e}	0.398 ± 0.008^{b-f}	0.33 ± 0.12^{c-e}	0.34 ± 0.12^{a-e}	4.94 ± 0.03 ^{g}	3.00 ± 0.02^{de}	3.65 ± 0.02^{g-i}
	7	2.69 ± 0.13^{b}	1.8 ± 0.2^{b-e}	3.28 ± 0.02^{d-g}	0.60 ± 0.03^{ef}	0.630 ± 0.012^{ef}	0.59 ± 0.08^{de}	2.51 ± 0.02^{bc}	2.45 ± 0.04^{de}	2.13 ± 0.06^{c-e}
	8	2.72 ± 0.13^{b}	1.8 ± 0.2^{b-d}	2.96 ± 0.07^{c-f}	0.30 ± 0.06^{a-e}	0.134 ± 0.012^{a-d}	0.139 ± 0.05^{a-c}	0.64 ± 0.05^{a}	0.45 ± 0.02^{a}	1.90 ± 0.02^{b-d}
	9	3.747 ± 0.013^{b-h}	2.5 ± 0.2^{f-h}	3.8 ± 0.2^{f-h}	0.553 ± 0.014^{c-f}	0.45 ± 006^{d-f}	0.48 ± 0.06^{c-e}	4.18 ± 0.03^{d-g}	4.36 ± 0.04^{f}	4.770 ± 0.013^{jk}

*Different superscript letters within a column indicate a significant difference ($P \leq 0.05$). S, M and L refers to the particles size of samples (ø < 125 μm; 125 μm < ø < 1 mm; ø > 1 mm), respectively.

Table 5
Effect of temperature on total phenolic content and antiradical activities of the three vegetable fractions in the presence of different starches.

		Galic A. Eq. (mg/g)			Ascorbic A. Eq. (mg/g)			Trolox Eq. (mg/g)		
Sample	Temp. °C	None	Rice Starch	Maize Starch	None	Rice Starch	Maize Starch	None	Rice Starch	Maize Starch
S	–	2.556 ± 0.003^{f}	2.000 ± 0.005^{e}	2.130 ± 0.003^{d}	0.583 ± 0.002^{h}	0.694 ± 0.005^{h}	0.550 ± 0.005^{i}	2.81 ± 0.03^{ce}	1.61 ± 0.02^{e}	2.82 ± 0.04^{h}
	70	4.323 ± 0.008^{j}	2.965 ± 0.004^{h}	4.740 ± 0.008^{k}	0.024 ± 0.005^{a}	0.099 ± 0.006^{a}	0.458 ± 0.007^{h}	3.64 ± 0.03^{f}	1.61 ± 0.02^{e}	3.267 ± 0.012^{j}
	80	4.528 ± 0.006^{k}	3.174 ± 0.006^{i}	4.700 ± 0.007^{k}	0.24 ± 0.02^{e}	0.200 ± 0.004^{c}	0.177 ± 0.006^{a}	3.50 ± 0.02^{f}	2.43 ± 0.04^{g}	1.93 ± 0.02^{e}
	90	5.441 ± 0.007^{l}	2.043 ± 0.007^{f}	3.921 ± 0.008^{i}	0.051 ± 0.005^{b}	0.294 ± 0.003^{e}	0.258 ± 0.003^{e}	3.16 ± 0.02^{e}	1.62 ± 0.04^{e}	2.502 ± 0.012^{g}
M	–	2.245 ± 0.012^{e}	1.269 ± 0.009^{c}	2.189 ± 0.003^{e}	0.673 ± 0.008^{j}	0.764 ± 0.005^{i}	0.689 ± 0.006^{j}	2.64 ± 0.02^{c}	1.49 ± 0.02^{d}	2.397 ± 0.012^{f}
	70	3.314 ± 0.002^{g}	2.131 ± 0.004^{g}	3.114 ± 0.005^{g}	0.307 ± 0.005^{d}	0.189 ± 0.007^{c}	0.444 ± 0.002^{h}	3.06 ± 0.02^{de}	1.57 ± 0.02^{de}	3.11 ± 0.02^{i}
	80	3.893 ± 0.002^{i}	2.489 ± 0.009^{h}	3.82 ± 0.02^{h}	0.15 ± 0.02^{f}	0.165 ± 0.007^{b}	0.233 ± 0.004^{d}	3.01 ± 0.02^{de}	2.39 ± 0.02^{g}	1.82 ± 0.03^{d}
	90	3.752 ± 0.003^{h}	1.69 ± 0.02^{d}	2.424 ± 0.008^{f}	0.02 ± 0.02^{a}	0.268 ± 0.06^{d}	0.415 ± 0.002^{g}	2.69 ± 0.04^{c}	1.36 ± 0.02^{c}	2.84 ± 0.02^{h}
L	–	1.04 ± 0.02^{a}	0.627 ± 0.009^{a}	1.170 ± 0.009^{b}	0.639 ± 0.003^{i}	0.615 ± 0.005^{g}	0.751 ± 0.005^{k}	1.36 ± 0.02^{a}	0.89 ± 0.02^{a}	1.34 ± 0.04^{c}
	70	1.437 ± 0.003^{b}	0.827 ± 0.004^{b}	1.171 ± 0.009^{b}	0.487 ± 0.003^{g}	0.19 ± 0.02^{a-c}	0.197 ± 0.009^{b}	1.55 ± 0.04^{b}	0.87 ± 0.02^{a}	1.13 ± 0.03^{ab}
	80	1.517 ± 0.006^{c}	0.843 ± 0.006^{b}	1.432 ± 0.007^{c}	0.329 ± 0.006^{de}	0.188 ± 0.004^{c}	0.218 ± 0.005^{c}	1.7 ± 0.3^{b}	1.91 ± 0.02^{f}	1.190 ± 0.004^{b}
	90	1.793 ± 0.003^{d}	0.847 ± 0.009^{b}	1.095 ± 0.009^{a}	0.087 ± 0.002^{c}	0.320 ± 0.005^{f}	0.302 ± 0.002^{f}	1.53 ± 0.02^{b}	1.03 ± 0.03^{b}	1.11 ± 0.03^{a}

* Results expressed as mean ± standard deviation. Different superscript letters within a column indicate a significant difference ($P \leq 0.05$). S, M and L refers to the particles size of samples (ø < 125 μm; 125 μm < ø < 1 mm; ø > 1 mm), respectively.

from 70 °C to 90 °C was selected to evaluate the impact of temperature and to ensure the gelatinization of both starches. A multifactorial ANOVA analysis determined that temperature, particle size and starch had a significant effect on total phenolic and antiradical activities measured by ABTS and DPPH methods. In the total phenolic content, the statistical analysis also revealed a binary interaction between temperature-starch and temperature-particle size of the vegetable fractions. Again, the larger fraction (L) showed the lowest phenolic content independently of the temperature. For a given particle size, an increase in temperature produced an increase in the obtained gallic acid equivalents. Therefore, the extraction of phenolic compounds from *Brassica napobrassica* dried leaves powder increased with temperature. This effect was lower with particle size increase, likely, as it was mentioned for the pH, the bigger size reduced the extraction of total phenolic compound probably due to matrix effect and minor proportion surface/volume in contact with water. At the different temperatures, the presence of starches reduced significantly the content of phenolic acids in the fractions. Again, the phenolic content of the blend vegetable-maize starch was higher than that of vegetable-rice starch.

A significant reduction of the ascorbic acid equivalents was observed when heating the blends, more noticeable in the smallest fraction. As mentioned above, DPPH methodology focuses on hydrophilic compounds, which could be easily dissolved in water and degraded by temperature. This effect was lower in the samples containing starch revealing possible interactions, and some kind of protection of the antiradical activity, but that effect was barely detected in the largest vegetable fraction (L). In general, maize starch samples showed higher ascorbic acid equivalents than rice starch, particularly at 90 °C, thus, exerting some type of protection. In ABTS obtained values, the statistical analysis confirmed interaction effect between particle size-starch and starch-temperature. In vegetable solutions without starch the extraction of hydrophobic compounds increased with temperature and a slight decrease at 90 °C was observed, possible due to a degradation effect at this temperature. It was remarkable the low values of Trolox equivalents obtained in L for all temperature treatments. Conversely to results obtained with DPPH, in the ABTS, vegetable-starch blends did show lower values in all the fractions, thus starches did not protect this antiradical activity. Anyway, at 80 °C the biggest and smallest antiradical activity values were obtained in samples containing rice and maize starch, respectively.

Maize starch seemed to protect better the antiradical activities against thermal lost than rice starch. The higher apparent viscosity reached with maize gels might help to protect the bioactive compounds and their antiradical activity. Therefore, results show that changes in bioactive compounds after thermal treatments may result from three phenomena: (1) thermal degradation, which reduces their concentration, (2) a matrix un-structuring effect, which increases the extractability of bioactive compounds, resulting in higher concentration with respect to the raw material, (3) interaction with other ingredients of the food formulation, and the interaction ingredient-bioactive compound might protect them from degradation.

4. Conclusions

This study demonstrated the feasibility of using *Brassica napobrassica* leaves powder as a source of bioactive and valuable compounds in the development of new starchy products. The particle size had an effect on physicochemical and functional properties of the vegetable powder. The addition of vegetable powder in a starch suspension had an effect on the pasting properties of the suspension. The effect of the processing conditions on the functional properties of starch suspensions was influenced by the powder particle sized and the type of starch used. Maize starch seemed to interact more with phenolic compounds which resulted in a protective effect against degradation at all processing conditions studied and resulting in higher antiradical activities. However more studies are needed to understand the mechanism of action.

CRediT authorship contribution statement

Ester Betoret: Investigation, Methodology, Data curation, Formal analysis, Writing - original draft. **Cristina M. Rosell:** Conceptualization, Funding acquisition, Methodology, Supervision, 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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