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Additional Information

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 Physicochemical, technological and functional properties of upcycled vegetable waste ingredients as affected by processing and storage

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Abstract

 Vegetable wastes are generated during harvesting, processing, and distribution, which implies a wastage of nutrients and evidence inefficiencies in present food systems. Vegetable residues are rich in bioactive compounds, for which their valorisation and reintroduction into the food chain are crucial towards circular economy and food systems sustainability. In this work, upcycled powdered ingredients were obtained from vegetables wastes (carrot, white cabbage, celery, and leek) through a disruption, dehydration and milling process. Disruption pre-treatment at different intensities was followed by freeze-drying or hot- air drying (60 and 70 °C), and final milling to produce fine powders. Powdered products were characterized in terms of physicochemical, antioxidant and technological properties (water and oil interaction), after processing and during four months of storage. Antioxidant properties were generally favoured by hot-air drying, particularly at 70 °C, attributed to new compounds formation combined to less exposure time to drying conditions. The powders showed good water interaction properties, especially freeze-dried ones. Storage had a negative impact on the quality of powders: moisture increased, antioxidant compounds generally diminished, and colour changes were evidenced. Upcycled vegetable waste powders are proposed as ingredients to fortify foods, both processing and storage conditions having an impact on their properties. **Keywords:** vegetable wastes; by-products valorisation; upcycled ingredients; bio-waste processing; storage stability; functional ingredients, functional properties.

Introduction

 Intensive food production implies the use of energy and other resources, for which food discard and food waste generation is a matter of great concern that should be urgently addressed. Fruit and vegetables residues are mainly generated as processing wastes and discards due to high commercialization standards. This plant material is rich in nutrients and has a potential to improve the diets of people facing nutritional disorders. Its reintroduction into the food chain is a step towards circular economy, more sustainable food systems [1], and the achievement of the Sustainable Development Goals approved by FAO (Food and Agriculture Organization), who promotes more nutritious and safe diets with a lower environmental impact. Fresh vegetables perish rapidly after harvesting due to their high moisture content and water activity, which makes them susceptible to microbial spoilage. Drying is a preservation technique commonly applied to reduce water content to safe levels, thus minimizing microbial spoilage and other deterioration reactions, extending shelf life and making food products suitable for safe storage [2]. Hot air-drying (HAD) is the most extended drying method in the food industry and is characterized by lower production and investment costs; freeze-drying (FD) is a more expensive technique which requires qualified staff and implies high energy consumption and longer drying times but yields highest quality final products [3]. Fruit and vegetable dried powders are versatile and stable products which have been proposed as ingredients for functional food development [4] as they can be used to reformulate products to obtain healthier alternatives. Most fruit and vegetable wastes (discards, by-products) consist of edible parts that could also be transformed into powdered products rich in bioactive compounds, thus integrally valorizing these wastes. Processing parameters in powder manufacturing, including pretreatments, drying stage and milling, determine the functional and technological properties of the products [5]. The impact of the drying stage depends on the product, the technique, and the drying conditions applied. The effect of drying on powders characteristics has been evaluated on several fruits and vegetable crops, revealing that heat treatment can induce physical, structural, chemical, and biological changes on the raw material, as well as induce a loss of nutrients and phytochemicals unstable to heat. In contrast, high temperatures have also been reported to have a positive impact on antioxidant properties, due to biochemical reactions or enzymes activation or inactivation, for instance [2, 6, 7]. In addition, milling conditions (pre- or post-drying) influence particle size and thus, powders' properties [8, 9]. Milling can also increase the temperature of the material affecting its quality. Both drying and milling are interdependent [8], size reduction prior to drying modifies drying behavior as it determines mass transfer mechanisms; whereas the structure generated during drying has an influence on milling. Storage and distribution may also have a significant impact on physical, chemical, and biological characteristics of the product, reducing its quality. Powdered products require protection against oxygen, moisture, high temperature or light, since storage may induce reaction mechanisms leading to food degradation with the loss of volatiles and color as well as antioxidants [2], or formation of compounds with pro-oxidant action which may lower the antioxidant capacity [10]. Evaluating the product stability during storage is crucial, since both physicochemical and antioxidant status may be affected.

 The aim of this research was to obtain powdered ingredients from the vegetable wastes generated at the early stages of processing in an agricultural cooperative and evaluate the impact of processing and storage on their properties. To this end, carrot, white cabbage, celery, and leek wastes followed a disruption, drying and milling process to integrally valorize their constituents in powdered form. Then, physicochemical, antioxidant and technological properties of the upcycled vegetable waste ingredients were assessed just after processing and during a four-month storage period.

Materials and methods

The materials and methods section are presented as Supplementary Information

Results and discussion

Impact of processing conditions on physicochemical and antioxidant properties

 Both, previous milling intensity and the dehydration method applied had a statistically significant impact on *particle size* characteristics (Table 1). In line with previous studies, chopping before drying led to coarser particle sizes than grinding [9, 11]. Chopping usually implies shorter drying times due to a less compacted bed which facilitates water migration through the inter-particle spaces; however, faster drying rates together with larger particles being dried have been related to case-hardening phenomena which makes it more difficult to reach low moisture content in the core of the particle and leading to rubbery materials, less crispy and more difficult to mill [12]. FD implied finer particle size powders than HAD. One possible reason for that is that the porous structure generated during FD facilitates milling. On the other hand, air temperature did not have a clear influence on particle size: while drying at 70 °C implied coarser particles in the case of celery and leek residues, the opposite behavior was observed in carrot and white cabbage. This could be attributed to differences among vegetables matrix characteristics and their response to drying. Likewise, in Bas-Bellver et al. [11] the effect of temperature on particle size was more evident in broccoli than in cabbage. Particle size distribution patterns are provided as Fig. S1. Results obtained by the wet procedure slightly shifted to larger values, which could be due to the solubilisation of small particles together with the formation of aggregates when dispersing powders in water. Swelling due to water adsorption could also be a reason for the larger values obtained by the wet procedure.

87 **Table 1** Particle size characteristic parameters of vegetable waste powders

	Particle size characteristics by the dry procedure				Particle size characteristics by the wet procedure					
TREATMENT	D[4,3]	D[3,2]	d_{10}	\mathbf{d}_{50}	\bf{d}_{90}	D[4,3]	D[3,2]	d_{10}	\mathbf{d}_{50}	\mathbf{d}_{90}
Ca G_HAD60	171 ± 6^g	34.6 ± 1.3 ^g	11.6 ± 0.3^h	$137 + 7^i$	391 ± 10^8	245 ± 24 ^{efg}	$53 + 3^f$	20.6 ± 1.2 ^{hi}	209 ± 20 ^{cd}	530 ± 54 ^{fg}
Ca C_HAD60	$210 \pm 6^{\circ}$	51.2 ± 1.5 ⁱ	17.8 ± 0.5^k	190 ± 7^1	442 ± 12^{i}	$348 + 37i$	$79 + 2^h$	35.4 ± 1.1 ¹	292 ± 16^{gh}	$723 + 97$ ⁱ
Ca G_HAD70	$155 \pm 3^{\text{de}}$	26.0 ± 0.6 ^d	8.6 ± 0.2 ^{cde}	$126 + 58$	358 ± 5 ^{cd}	$228 \pm 8^{\text{def}}$	$48 + 2^e$	18.8 ± 1.1 ^{gh}	$194 \pm 9^\circ$	500 ± 23 ^{efg}
Ca C_HAD70	$200 \pm 6^{\mathrm{i}}$	$50 \pm 3^{\rm i}$	17.36 ± 0.7 ^{jk}	180 ± 7^k	423 ± 9^h	273 ± 18 gh	$67 + 28$	28.8 ± 0.8	249 ± 16 ^{ef}	$562 + 37$ gh
Ca FD	124 ± 3^{b}	33.1 ± 1.7 ^g	12.5 ± 0.8^i	$107 + 3$ ^f	$258 + 6^a$	$156 + 2^a$	39.0 ± 0.4 bcd	15.8 ± 0.3 cdef	123.6 ± 1.4 ^{ab}	350 ± 6^{ab}
WC G_HAD60	161 ± 4 ^{ef}	24.8 ± 0.7 ^{cd}	8.5 ± 0.3 bcd	$134 \pm 4^{\rm hi}$	362 ± 6 ^{de}	218 ± 9 ^{de}	41.5 ± 1.0^d	14.8 ± 0.4 bcde	190 ± 8 c	471 ± 21 ^{def}
WC C_HAD60	214 ± 7^{j}	$48 + 2^h$	17.0 ± 0.9	$197 + 7^{1}$	435 ± 12^{hi}	300 ± 34 ^h	52 ± 3 ^{ef}	18.9 ± 1.4 ^{gh}	257 ± 19 ^{fg}	626 ± 78 ^h
WC G_HAD70	167 ± 6 ^{fg}	28.1 ± 0.7 ^e	9.6 ± 0.2 ^g	$140 \pm 7^{\rm i}$	$370 \pm 13^{\text{def}}$		$228 \pm 12^{\text{def}}$ 37.9 $\pm 1.0^{\text{bcd}}$	12.9 ± 0.4^{ab}	192 ± 9 ^c	505 ± 32 ^{efg}
WC C HAD70	183 ± 5^h	26.1 ± 0.3 ^d	9.2 ± 0.2 ^g	$165 + 5^{j}$	$388 + 9^{fg}$	271 ± 18^{gh}	48 ± 2^e	17.8 ± 0.8 ^{fg}	243 ± 14^{def}	569 ± 46 ^{gh}
WC FD	$102 \pm 3^{\text{a}}$	18.6 ± 1.3^a	$6.7 \pm 0.5^{\text{a}}$	$72 + 4^b$	$246 + 5^a$	171 ± 6^{abc}	40.8 ± 0.4 ^d	16.23 ± 0.14 ef	138 ± 3^{b}	376 ± 15^{abc}
Ce G_HAD60	129 ± 8^{bc}	23.2 ± 1.3 ^{bc}	9.2 ± 0.4 ^{fg}	71 ± 6^b	341 ± 18 c	207 ± 21 ^{cd}	35.9 ± 1.1 bc	13.7 ± 0.3 ^{abc}	129 ± 10^{ab}	508 ± 57 efg
Ce C_HAD60	$262 + 9^k$	$60+2^{j}$	24.5 ± 0.5^1	$220 \pm 7^{\rm m}$	567 ± 21^{j}	381 ± 85 ^{ij}	69 ± 10^8	30 ± 5^k	294 ± 58 ^h	824 ± 132^{j}
Ce G_HAD70		148 ± 27 ^d 23.3 ± 0.9 ^{bc}	9.1 ± 0.3 ^{efg}	75 ± 6^{bc}	$372 + 51$ ^{efg}	164 ± 14^{ab}	30.8 ± 1.4^a	11.8 ± 0.4^a	896 ± 71 ⁱ	418 ± 33 ^{bcd}
Ce C_HAD70	280 ± 16^1	69 ± 3^k	$28 + 2^m$	240 ± 15^n	594 ± 32^k	391 ± 66 ^j	$75 + 7^h$	34 ± 4^1	312 ± 32^h	933±150 k
Ce FD	100 ± 4^a	19.5 ± 1.3^a	7.9 ± 0.6^b	$58 + 4^a$	262 ± 11^a	$154 + 4^a$	38.4 ± 0.6 bcd	15.9 ± 0.2 ^{def}	111 ± 3^{ab}	362 ± 9^{ab}
L G HAD60	125 ± 2^{bc}	22.7 ± 0.2^b	8.23 ± 0.09 bc	$89 + 2^d$	300 ± 5^b	195 ± 10^{bcd}	39.8 ± 1.0 ^{cd}	$14.9 \pm 0.5^{\text{bcde}}$	$146 + 4^{b}$	$450 \pm 30^{\text{cde}}$
L C_HAD60	$158 + 3$ ef	30.8 ± 0.6 ^f	11.8 ± 0.3^h	125 ± 38	359 ± 6 ^{cd}	260 ± 42 ^{fg}	$54 + 5$ ^f	$22 + 3^{i}$	212 ± 34 ^{cde}	564 ± 87 gh
L G_HAD70	134 ± 2 ^c		24.0 ± 0.3 ^{bc} 9.07 \pm 0.14 ^{efg}	97.3 ± 1.4 ^e	319 ± 4^{b}	200 ± 7^{bcd}	38.1 ± 0.6^{bcd}	14.1 ± 0.3^{bcd}	$146 + 4^{b}$	470 ± 19 ^{def}
L C_HAD70	163 ± 4 efg	29.6 ± 0.7 ^{ef}	11.3 ± 0.4^h	$128 + 5^{gh}$	$369 \pm 7^{\text{de}}$	250 ± 9 efg	54 ± 2^{f}	22.2 ± 1.3^i	214 ± 12 ^{cde}	538 ± 15 ^{fg}
L FD	$109 \pm 2^{\text{a}}$	22.5 ± 0.9^b	9.0 ± 0.4 efg	82 ± 3 ^c	$256 \pm 3^{\text{a}}$	$139 + 4^a$	35.0 ± 0.7 ^b	14.1 ± 0.3 bdc	$99 + 3^a$	330 ± 11^a

88 a,b,c... Different letters in the same column for the same residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05). Notes: Dry and wet refers to dispersant, air or water, respectively. Abbreviations: D[4,3]: equivalent volume diameter; D[3,2]: surface area mean diameter; d10, d50, and d90: distribution percentiles. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air dried; FD: freeze-dried. 60 and 70 refer to air drying temperature. Mean and standard deviation of three replicates.

92 The drying methods applied allowed to reduce a_w values below the target (0.3) (Table 2), thus ensuring stability [13]. Drying technique and temperature, as well as disruption intensity, had an impact on moisture content. Disruption intensity had a different effect depending on drying temperature and residue. Ground samples dried at the highest temperature (70 °C) presented lower moisture content than chopped ones, for 96 all wastes. In contrast, in vegetables dried at 60 °C, only celery and leek samples followed this trend. This would confirm that faster drying rates and larger particles may lead to case-hardening phenomena, thus limiting water migration from the core of the particles [12]. Crusting phenomena are significantly influenced by the vegetable matrix structure but also by soluble solids content, since it implies the

 accumulation of non-volatile compounds carried away by water diffusion [11]. This is evidenced in carrot powders, which exhibited higher moisture and higher soluble solids content. Carrot powders were richer in soluble solids since carrots are relatively rich in sugars among vegetables [14]. Besides, the more brittle structure promoted by FD could have intensified breakage of fibres during milling, releasing more soluble solids in FD powders as compared to HAD ones.

 Regarding antioxidant properties (Table 2) cabbage powders showed the highest phenolic content, whereas carrot ones exhibited the lowest values. HAD produced powders with higher phenolic content, as compared to FD ones. This was confirmed by the multifactor ANOVA analysis, which revealed that drying temperature had a positive significant effect (p-value < 0.05) on total phenolic content. Increasing temperature may reduce the activity of enzymes capable of degrading phenolic compounds [15]; besides, the use of higher temperatures implies a reduction in the time of exposure to drying conditions, thus reducing phenols degradation. Disruption intensity prior to drying showed no clear trend and its impact on phenolic content depended on both the drying temperature and the product structure. In general, ground 113 samples dried at 70 °C exhibited the highest phenolic content, whereas chopped ones resulted in lower values. This fact could be related to a reduced cell tissue damage in chopped samples, so that phenolics remained trapped in the structure during drying, being less susceptible to oxidation. As for total flavonoid content, carrot powders had the lowest values and celery the highest ones. Except for celery, HAD powders had more flavonoids than FD ones. Drying temperature or previous disruption did not have a statistically significant effect on total flavonoid content, although general trends were similar to that of phenols.

 Antioxidant activities (DPPH and ABTS methods) were higher in cabbage and leek powders. Disruption 120 barely affected the antioxidant activity, whereas drying had a significant impact. HAD, especially at 70 \degree C, favoured antioxidant capacity of the powders as compared to FD, for all wastes. It has been previously evidenced that high-temperature and short times may favour antioxidant properties [7]. This could be explained by the formation of new compounds with antioxidant properties like Maillard reaction products, or the incidence of other biochemical reactions which are favoured by high exposure temperatures [13]. High temperatures may also reduce the activity of enzymes with pro-oxidant action. In addition, the use of lower temperatures during air drying implies lengthening the treatment, thus leading to an increased

- 127 exposure time to oxygen. Antioxidant capacity of the powders obtained were in the range of the reported
- 128 for freeze-dried leek powders [10], and higher than the obtained for pumpkin powders [16].

		Moisture		Total phenols	Total flavonoids	DPPH	ABTS
TREATMENT	a_w	(%)	$X_{ss}(g/g_{dm})$	(mg GAE/gdm)	(mg QE/g _{dm})	$(mg TE/gdm)$ $(mg TE/gdm)$	
Ca G HAD60	0.254 ± 0.008^b	$2.9 \pm 0.4^{\rm b}$	0.667 ± 0.017 ^a	1.53 ± 0.12^b	$1.24\pm0.06^{\rm a}$	1.90 ± 0.12^{bc}	$55\pm7^{\rm b}$
Ca C_HAD60	0.239 ± 0.010^{ab}	2.96 ± 0.10^b	0.659 ± 0.017 ^a	2.06 ± 0.16^c	1.464 ± 0.003^b	$2.1 \pm 0.2^{\circ}$	57.5 ± 1.4^b
Ca G_HAD70	0.236 ± 0.011^a	$1.62 \pm 0.32^{\rm a}$	0.685 ± 0.011^{ab}	2.004 ± 0.013 ^c	1.27 ± 0.03^a	1.69 ± 0.10^b	$62 \pm 3^{\circ}$
Ca C_HAD70	0.240 ± 0.005^{ab}	3.26 ± 0.12^b	0.709 ± 0.012^{bc}	2.42 ± 0.15^d	1.45 ± 0.02^b	2.65 ± 0.11 ^c	64.8 ± 1.7^c
Ca FD	$0.236 \pm 0.007^{\rm a}$	2.80 ± 0.11^b	0.724 ± 0.018 ^c	0.74 ± 0.14^a	$1.26\pm0.03^{\rm a}$	$1.01\pm0.11^{\rm a}$	$16.9 \pm 0.3^{\rm a}$
WCG HAD60	0.223 ± 0.003 ^c	2.95 ± 0.02 ^c	0.565 ± 0.017^b	4.69 ± 0.12^b	5.6 ± 0.3^b	2.39 ± 0.14^b	$101 \pm 5^{\rm b}$
WC C_HAD60	0.192 ± 0.006^b	2.55 ± 0.15^b	0.591 ± 0.017^b	4.71 ± 0.06^b	$6.8\pm0.2^{\rm c}$	$2.9 \pm 0.3^{\circ}$	105 ± 3^{bc}
WC G_HAD70	0.223 ± 0.024 ^c	$1.6 \pm 0.3^{\rm a}$	0.512 ± 0.017 ^a	$6.3 \pm 0.3^{\circ}$	7.4 ± 0.3 ^d	3.11 ± 0.12 ^c	110 ± 3^c
WC C_HAD70	0.176 ± 0.008^b	2.27 ± 0.05^b	0.491 ± 0.013 ^a	6.22 ± 0.12 ^c	7.8 ± 0.3 ^d	3.08 ± 0.10^c	$109.0 \pm 0.3^{\circ}$
WC FD	0.121 ± 0.006^a	2.33 ± 0.07^b	0.58 ± 0.02^b	$2.79\pm0.07^{\rm a}$	$3.25\pm0.05^{\rm c}$	$1.15\pm0.13^{\rm a}$	$34.4\pm0.5^{\rm a}$
Ce G_HAD60	0.181 ± 0.008^b	1.45 ± 0.10^{ab}	0.531 ± 0.006^c	2.26 ± 0.02^b	6.9 ± 0.6^{ab}	1.50 ± 0.16^c	63.4 ± 0.3^b
Ce C_HAD60	0.232 ± 0.007 ^d	$2.7 \pm 0.2^{\circ}$	0.49 ± 0.03^{ab}	2.40 ± 0.15^b	$7.8\pm0.8^{\rm bc}$	1.08 ± 0.12^{ab}	68 ± 3^c
Ce G_HAD70	0.205 ± 0.010^c	$1.10 \pm 0.17^{\rm a}$	0.505 ± 0.011 ^{abc}	3.25 ± 0.16^d	$8.2 \pm 0.8^{\circ}$	1.21 ± 0.08^b	77.5 ± 1.0^e
Ce C_HAD70	0.217 ± 0.005^c	1.9 ± 0.6^b	0.51 ± 0.02^{bc}	2.70 ± 0.13^c	$6.29\pm0.13^{\rm a}$	$0.9 \pm 0.2^{\rm a}$	72 ± 2^d
Ce FD	0.150 ± 0.009^a	1.9 ± 0.3^b	0.47 ± 0.03^a	1.88 ± 0.15^a	9.72 ± 0.13^d	1.13 ± 0.13^{ab}	$8 \pm 2^{\rm a}$
L G_HAD60	0.229 ± 0.009^b	1.34 ± 0.04^b	0.620 ± 0.017^b	3.32 ± 0.16^b	7.5 ± 0.2^b	1.6 ± 0.2^b	98.9 ± 1.4^b
L C_HAD60	0.260 ± 0.003 ^c	$1.85\pm0.08^{\rm c}$	0.66 ± 0.02^c	3.26 ± 0.17^b	$7.3 \pm 0.4^{\rm b}$	1.6 ± 0.3^b	99 ± 2^b
L G HAD70	0.230 ± 0.021^b	$1.0\pm0.3^{\rm a}$	$0.479\pm0.006^{\rm a}$	4.34 ± 0.12 ^d	7.3 ± 0.3^b	1.9 ± 0.4^b	$112 \pm 5^{\circ}$
L C_HAD70	0.261 ± 0.006^c	1.6 ± 0.3^{bc}	0.598 ± 0.013^b	$3.8 \pm 0.4^{\circ}$	$6.4 \pm 0.7^{\rm a}$	$2.4 \pm 0.3^{\circ}$	111 ± 4^c
L FD	0.157 ± 0.006^a	1.36 ± 0.06^b	0.59 ± 0.03^b	2.52 ± 0.11^a	6.78 ± 0.16^{ab}	$0.72\pm0.03^{\rm a}$	13.8 ± 1.1^a

129 **Table 2** Physicochemical and antioxidant properties of vegetable waste powders

130 a,b,c...Different letters in the same column for each residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).
131 Water activity (aw), moisture content (gwater/100 g), solu Water activity (aw), moisture content (gwater/100 g), soluble solids (gsoluble solids/gdry matter), total phenols (mg GAE/gdm), total flavonoids (mg QE/gdm), 132 DPPH and ABTS antioxidant capacity (mg TE/gdm). Ca: carrot; 132 DPPH and ABTS antioxidant capacity (mg TE/g_{dm}). Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air dried;
133 FD: freeze-dried. 60 and 70 refer to air drying temperature. Mean and 133 FD: freeze-dried. 60 and 70 refer to air drying temperature. Mean and standard deviation of three replicates.

134 Optical properties (CIE L^{*}a^{*}b^{*} coordinates) of powders are given in Fig. 1. Carrot powders showed the

135 highest luminosity values. As for the drying technique applied, luminosity was slightly higher in FD

136 samples than in HAD ones, as reported for papaya leaves [17], or cabbage and broccoli powders [18,19].

137 In fact, FD is characterized by providing a whitish appearance due to the reduced incidence of oxidative

138 reactions and the increased porosity. The a* coordinate allowed to distinguish between HAD and FD

- 139 powders, as HAD samples concentrate around zero a* values, whereas FD samples exhibited more negative
- 140 a* values. Carrot powders showed positive a* values approximating to redness.

141 **Impact of processing on water and oil interaction properties**

142 Water and oil interaction properties of powders are given in Table 3. Specific volume was quite similar 143 among powders. Hygroscopicity is related with the ability of a product to absorb water from the

144 environment, it influences caking and stickiness during storage, and thus determines its stability [20]. This

 parameter is related to saccharides content of powders, as reported for raspberry powders [21], which was confirmed by the higher values obtained for carrot ones. Low hygroscopicity values have been related to insoluble fibre components and larger particle sizes, resulting in less surface area for water adsorption. No statistically significant impact of processing conditions on hygroscopicity or wettability was found. In the literature, however, it has been reported that the larger the particle size, the shorter the wettability time, since coarser particles imply a more porous structure increasing wettability [22]. Swelling capacity (SC), water retention capacity (WRC) and water holding capacity (WHC) were, in general, favored by FD as compared to HAD. This increased ability to incorporate water may be explained by the porous structure of FD materials. Martínez-Las Heras et al. [23] reported similar trends for persimmon fibres. Results obtained for HAD powders were in the range of apple pomace, carrot pomace and beetroot pomace powders [24] or pumpkin powders [16]; and higher than goldenberry waste [25] or fig pulp powders [26]. Hydration properties were influenced by porosity and particle size, since SC, WRC and WHC increased as particle size decreased [23]. Solubility is an important physical parameter determining the functional properties of powdered dried products, since it is related to the presence of small hydrophilic molecules and their ability to interact with water. Statistically significant differences were obtained among powders within the same waste. As in Si et al. [21], powders solubility decreased with particle size, values being slightly lower for FD than for HAD powders. White cabbage, celery and leek waste powders solubilities were in the same range; carrot waste powders showed the highest solubility index, as explained by their higher soluble solids content, and in line with the values reported for sugar-rich products such as persimmon pulp (52-77% [27]) or mango peels powders (50-70% [22]). Vegetable wastes powders did not exhibit good oil interaction properties. No results were obtained for emulsifying activity and emulsifying stability, but certain oil holding capacity (OHC) was obtained. Results were in the range of the reported for carrot pomace (2.442 167 ± 0.067 g/g), apple pomace $(2.241 \pm 0.068$ g/g) or beetroot pomace powders $(2.206 \pm 0.064$ g/g) [24]; and higher than peel and pulp fig powders (0.75-0.90 g/g) [26] or pulp pumpkin powders (1.01-1.30 g/g) [16].

170 **a,b,c...** Different letters in the same column for each residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).
171 SC: swelling capacity (mL/g): WHC: water holding capaci 171 SC: swelling capacity (mL/g); WHC: water holding capacity (g/g); WRC: water retention capacity (g/g); OHC: oil holding capacity (g/g). Ca: carrot;
172 WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air dried; FD: freeze-dried. 60 and 70 refers to air drying temperature Mean \pm standard deviation of three repetitions.

 A Principal Component Analysis (PCA) was performed to statistically evidence the relationships between physicochemical characteristics and technological properties of powders (Fig. S2, S3, S4). Three components were required to explain 75% of the variance, whereas 2 components explained 60% of the total variance. The PCA evidenced a close relationship between soluble solids content and water interaction properties such as solubility, wettability and hygroscopicity, whereas other water interaction properties such as WHC and WRC were more related to particle size, having an inverse relationship with that parameter. On the other hand, OHC seemed to be more explained by the specific volume. Plotting the powders according to the components which explain their variance allowed to concentrate FD powders in the region (left) where properties such as specific volume, OHC and water retention and hydration properties are located; whereas HAD powders accumulate on the right side. Carrot powders concentrate in the region explained by soluble solids content and related technological parameters such as solubility, wettability and hygroscopicity.

186 **Evolution of physicochemical and antioxidant properties of vegetables waste powders during storage**

187 During storage, both moisture content and water activity increased, more markedly in the case of celery 188 and cabbage waste powders (Fig. 2). There was significant variability between disruption pre-treatments 189 and the drying techniques applied, this suggesting a different impact of storage on a_w and x_w values 190 depending on the structure of the processed material. Overall, there was about $2.4\% \pm 1.5\%$ moisture gain 191 and 0.14 ± 0.08 a_w gain during 4 months of storage at room conditions. Similar results have been reported for orange juice powders (1.5% moisture gain in 6 months, a^w gain from 0.264 to 0.448) [28]; dehydrated pumpkin soup mix (moisture increase from 4.91 to 5.18% and a^w from 0.341 to 0.342) [29], apricot fruit bar (3% moisture gain in 6 moths) [30], soursop fruit powder stored 91 days [31], or apple peel powders with moisture increases depending on temperature, time and packaging conditions [2]. Hence, powders must be stored and packed in suitable conditions to avoid moisture gain and loss of stability. Evolution of soluble solids content was variable: for some powders, there was a slight increase over time, whereas others exhibited a decrease. Simple sugars may experiment variations during storage since sucrose may invert to glucose and fructose, and fructose may be consumed in Maillard reactions. Slight fluctuations in the soluble solids content were also observed in tomato powders stored for 5 months, with no significant changes [32]. 201 standard deviation of three repetitions. a,b,c Different letters within the same residue indicate statistically 202 significant differences at the 95% confidence level (p -value < 0.05).

 Color is a very important quality factor in fruit and vegetable products since it influences consumer acceptability. Fig. 3 shows color differences (ΔE) during the storage period, with respect to initial values. Color changes during powders storage have been extensively reported. Color changes are normally attributed to chemical and physical reactions such as non-enzymatic browning [28,31] and related to aw, moisture or sugar content in the stored food products [31]. The lowest ΔE was obtained for celery powders, while carrot and white cabbage powders showed the highest color differences. A stabilization of color changes was observed after 2-3 months of storage, but the fourth month implied a significant change. In some powders, particularly carrot waste ones, color differences decreased or maintained during the fourth 211 month. In general, color changes were less significant in FD powders than in HAD ones. Chroma (C_{ab}^*) 212 and hue (h_{ab}) parameters are presented in the SI section (Table S1). In carrot and celery waste powders, storage implied a slight decrease in colour purity, whereas cabbage and leek waste powders experimented a slight increase. Carrot waste powders exhibited an orange hue which increased during storage. In contrast, in cabbage, celery and leek powders, with a tendency towards green, the hue generally decreased. Hue

216 decrease was also observed by Tavares et al. [33] in jambolan juice powder stored at 25 °C and 35 °C; Fernández-López et al.[28] reported no changes throughout the storage period.

 Maximizing nutrients and bioactive compounds retention not only during processing but also during storage is a prevailing matter. The evaluation of postharvest processing and the impact of subsequent storage on vegetable antioxidants properties is of great practical importance [10]. Storage negatively affected phenol and flavonoid content of the powders, particularly after the third month (Fig. 4). Similar results have been reported for apple peel powders [2], and for HAD and FD berries [34] during 10 months of storage. Similarly, storage had a negative effect on the antioxidant capacity (ABTS) which was more noticeable in HAD powders. FD celery and leek powders were an exception, since antioxidant capacity slightly increased. A decrease in the ABTS antioxidant capacity had been also observed on FD and HAD kale leaves [3]. DPPH antioxidant activity evolved differently during storage depending on the dehydration technique, increasing in FD powders and decreasing in HAD, although after a slight increase in the second month. An increase during storage was also observed by del Caro et al. [35] on HAD prunes, who attributed it to the formation of Maillard compounds even after long storage periods. Other authors [34] have also reported a slight increase in the DPPH antioxidant activity during storage in HAD and FD strawberry and raspberry.

Conclusions

 Vegetable wastes have been successfully transformed into powdered products through a simple but efficient transformation process involving a disruption pre-treatment, a dehydration step and final milling. The processes described could be easily adopted by industry to upcycle these wastes and obtain new ingredients which could be used to improve the nutritional value of foods. The study has demonstrated that both processing and storage imply quality changes in the powdered products. Processing parameters have conditioned physicochemical, antioxidant and technological properties of vegetable waste powders. Besides, physicochemical attributes such as soluble solids content, particle size or specific volume have been related to technological characteristics such as hydration and oil interaction properties. During storage, changes in quality attributes, and a general decrease in the antioxidant properties of powdered products were revealed, although the behaviour of FD and HAD powders was different. Thus, processing conditions must be chosen considering not only their impact on product characteristics, but also their influence on

storage stability.

 The feasibility of transforming these wastes into functional food ingredients by means of affordable and technically viable processes which can be easily implemented has been presented. Results may help IV- range producers make decisions on how to give added value to their residues. Capital investment and production costs are critical issues when developing waste valorisation processes; according to the latter, HAD can be considered the most suitable drying technique for upcycling these vegetable wastes.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Lucía Seguí, Cristina Barrera and Noelia Betoret contributed to conception and design. Claudia Bas-Bellver

did the experimental work supervised by Lucía Seguí and Cristina Barrera. Lucía Seguí, Cristina Barrera

and Noelia Betoret contributed to funding acquisition. Lucía Seguí, Cristina Barrera and Claudia Bas-

Bellver performed the analysis and interpretation of data. Claudia Bas-Bellver draft the paper. Lucía Seguí

wrote and edit the final version. The paper was revised and approved by all co-authors.

Data availability

Data supporting the findings of this study are available upon reasonable request.

Ethical Approval and Consent to Participate

- Not applicable.
- **Consent for publication**
- Not applicable.
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FIGURES

380
381 **Fig. 1** CIEL*a*b* coordinates of waste powders. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G:

ground; C: chopped; HAD: hot-air dried; FD: freeze-dried. Mean and standard deviation of three replicates.

 Fig. 2 Water activity, moisture and soluble solids content during 4 months of storage. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying. Mean and

390
391 Fig. 3 Total color difference of (A) carrot, (B) white cabbage, (C) celery and (D) leek waste powders stored

392 during 4 months, with respect to time zero values. Mean and standard deviation of three repetitions.

393
394 Fig. 4 Total phenol (mg GAE/g_{dm}), total flavonoid (mg QE/g_{dm}), and antioxidant activities (mg TE/g_{dm}) by 395 the DPPH and ABTS methods of the vegetable waste powders during four months of storage. a,b,c Different 396 letters within the same residue indicate statistically significant differences at the 95% confidence level (p-397 value < 0.05). Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot air-398 drying; FD: freeze-drying. Mean and standard deviation of three repetitions

SUPPLEMENTARY INFORMATION

Materials and methods

 Raw materials (vegetable wastes) were generated in the processing lines of the agricultural cooperative Agrícola Villena, Coop. V. (Alicante, Spain). These consisted of wastes of the ready-to-eat lines in the case of carrot (*Daucus carota*, L.) and celery (*Apium graveolens*, L.); and wastes of the fresh pre-packed vegetables lines, in the case of cabbage (*Brassica oleracea* var. capitata, L.) and leek (*Allinum porrum*, L.). Carrot and celery discards were sticks which did not meet size stardards, whereas cabbage and leek wastes consisted of outer leaves or upper leaves, respectively.

 Once received at the laboratory facilities, vegetable wastes were processed freshly so that the plant material was disrupted with a food processor (Thermomix® TM6, Vorwerk, Madrid, Spain) to reduce particle size 409 to pieces of ≤ 10 mm diameter (chopped, C), or pieces of ≤ 5 mm diameter (grounded, G). Conditions for tissue disruption were set according to previous experiences [1]. Disruption was followed by a dehydration treatment, either hot-air drying (HAD) or freeze-drying (FD). HAD was conducted in a convective tray dryer (Pol-eko Aparatura, Katowice, Poland) until water activity (aw) was reduced below 0.3, to guarantee powders' stability. To this aim, ground and chopped residues were distributed on the dryer trays (~200 g 414 of residue/tray) in 10 mm thick layers, and dried at 60 °C or 70 °C of temperature with an air stream velocity of 2 m/s. FD was carried out in a freeze dryer (Lioalfa-6, Telstar, Terrasa, Spain) for 24 h under freezing 416 conditions (-45 °C) and sub-atmospheric pressure ($P = 0.1$ mbar), with previous sample grinding and freezing in a deep freezer (Matek CVN-40/105) at −40 °C during 24 h. After drying processes, dried materials were milled (10,000 rpm for 2 min at 30 s intervals) (Thermomix® TM6, Vorwerk, Madrid, Spain) to obtain the final fine-grained powder. Powders were packed into glass containers with aluminium 420 lid in a light-free environment and stored during 4 months at room temperature (24-27 °C). Particle size characteristics and technological properties (water and oil interaction properties) were measured after powder manufacturing. Physicochemical characteristics (including water activity, moisture content, total soluble solid content and optical properties) as well as antioxidant properties (total phenol and flavonoid content and antioxidant capacity) were measured both, just after being processed and after 2, 3 and 4 months of storage.

426 The experimental design yielded 20 types of powders, which will be identified next by type of waste: Carrot 427 (Ca), Celery (Ce), White cabbage (WC) and Leek (L); the pre-treatment applied: Ground (G) or Chopped 428 (C); and the drying method used: Hot air-drying at 60 °C (HAD60) or at 70 °C (HAD70), or Freeze-drying

429 (FD).

430 **Physicochemical and antioxidant determinations**

 Moisture content (xw) was measured according to the official method 934.06 of the AOAC [2], based on 432 water removal of samples during vacuum drying (Vaciotem, JP Selecta) ($P = 10$ mmHg) at 60 °C until 433 constant weight. Water activity (a_w) was obtained with a dewpoint hygrometer at 25 °C (Aqualab 4TE; 434 Decagon devices Inc., USA). Total soluble solids content (x_{ss}) was determined by a thermostatic refractometer (Abbe Atago 3-T, Japan) through the measurement of Brix degrees at 20 °C, according to the ISO 1743:1982 method. When necessary, Brix measurements were obtained from an aqueous extract of soluble solids in a 1:10 (w/v) ratio. Particle size distribution was determined in dry and wet conditions, using a Malvern Mastersizer equipment (Model 2000; Malvern Instruments Limited, UK). For the dry method, the equipment was coupled to a dispersion unit Scirocco 2000 with air as dispersant at 2.5 bar of pressure and 60% speed. For the wet method, the equipment was coupled to a unit Hydro 2000, setting the particle absorption index at 0.1, and using refractive indexes of 1.52 and 1.33 for the sample and for the dispersed phase (deionized water), respectively. Results were obtained as equivalent volume mean diameter 443 D[4,3] and surface area mean diameter D[3,2], as well as the distribution percentiles d_{10} , d_{50} , and d_{90} . Optical properties were measured with a spectrocolorimeter (Minolta CM 3600D, Konica Minolta Sensing, Inc, Japan), using the illuminant D65 and an observer angle of 10° as reference. Color coordinates of the 446 CIEL*a*b* color space, and resultant C_{ab} * (chroma) and h_{ab} (hue), were obtained by reflectance from the [absorption spectrum](https://www.sciencedirect.com/topics/nursing-and-health-professions/absorption-spectroscopy) provided by the equipment in the 380-770 nm range. Readings were made on a 448 black background, placing samples in standardized-size plastic cuvettes $(37 \times 50 \times 22 \text{ mm})$. Color changes during powders storage was calculated by means of Equation 1 [3].

450
$$
\Delta E = \sqrt{(L_1^* - L_n^*)^2 + (a_1^* - a_n^*)^2 + (b_1^* - b_n^*)^2}
$$
 (1)

451 where L_{i}^{*} , a_{i}^{*} , and b_{i}^{*} are the color parameters of the powders after processing and L_{n}^{*} , a_{n}^{*} , and b_{n}^{*} are 452 color parameters of the stored powder at month n.

 Antioxidant properties of vegetable wastes powders were measured by determining phenol and flavonoid compounds, and antioxidant activity by the DPPH (2,2-diphenyl-1-picryl hydrazyl) and ABTS (2,2-azobis- 3-ethyl benzthiazoline-6-sulphonic acid) methods. Determinations were accomplished on extracts of samples, using an 80% (v/v) methanol/water solution as the extracting solvent, and an extraction ratio of 1:20 (w/v). Extracts were obtained by stirring the powder and solvent during 1 h in a horizontal stirrer (COMECTA WY-100, Comecta, Barcelona, Spain), and then centrifuged for 5 min at 10,000 rpm (Eppendorf Centrifuge 5804/5804R, Eppendorf SE, Hamburg, Germany). Measurements were carried out on the separated supernatants (extracts). An 80% (v/v) methanol/water solution replacing the extract was used as a blank in all analyses.

 Total phenolic content was determined using the modified method of Folin-Ciocalteu [4][5]. For the analyses, 0.125 mL of the extract were mixed with 0.5 mL of bidistilled water and 0.125 mL of the Folin- Ciocalteu reagent (Sigma Aldrich). The mixture was kept 6 min in darkness, followed by the addition of 1.25 mL of sodium carbonate solution (7%) and 1 mL of bidistilled water. After 90 min in darkness, absorbance was measured at 760 nm with a spectrophotometer (Helios Zeta UV/Vis, Thermo scientific, UK). Results were expressed in mg of Gallic Acid Equivalents (GAE) per g of dry matter. Total flavonoid content was measured following the modified colorimetric method of aluminium chloride [19]. Accordingly, 1.5 mL of the extract were mixed with 1.5 mL of a 2% w/v aluminium chloride in methanol solution. After reaction for 10 min in darkness, absorbance was measured at 368 nm. Results were expressed in mg of Quercetin Equivalents (QE) per g of dry matter.

 Antioxidant activity was measured by the DPPH and ABTS radical methods. The ability to scavenge the DPPH radical was determined applying Brand-Williams et al.[20] method, with some modifications. Thus, 0.1 mL of the extract were mixed with 2 mL of a 0.1 mM solution of DPPH (2,2-diphenyl-1-picryl hydrazyl) in methanol and 0.9 mL of methanol. The mixture reacted during 60 min in darkness and the absorbance was measured at 575 nm in a spectrophotometer (Helios Zeta UV/Vis, Thermo Scientific, UK). The ability 477 to scavenge the ABTS radical was measured following the method described by Re et al. [6]. ABTS⁺ free radical (2,2-azobis-3-ethyl benzthiazoline-6-sulphonic acid) was obtained by preparing a solution of 7 mM of ABTS and 2.45 mM of potassium persulfate and left to react during 16 h in darkness at room temperature. 480 ABTS⁺ solution was mixed with phosphate buffer (pH 7.4) until an absorbance of 0.70 ± 0.02 at 734 nm

was reached. Measurements were performed by mixing 0.1 mL of the extract and 2.9 mL of the ABTS⁺ solution. Absorbance was measured at 734 nm after 7 min of reaction. Regardless of the method used, the antioxidant activity was expressed in mg of Trolox Equivalent (TE) per g of dry matter.

 Analytical determinations of samples were performed at least in triplicates. When extraction was needed, determinations were performed on two different extracts, with three repetitions per extract.

Water interaction and oil emulsifying properties

 Specific volume of powders was determined by measuring the volume of 5 g of sample in a 10 mL test tube. Solubility, which is the mass fraction of dissolved solids (DS) in the hydrated sample, was obtained following the method described by Mimouni et al.[7] as the ratio between the total soluble solids content 490 (x_{ss}) and the total solids content (1- x_w). Hygroscopicity was determined by the method proposed in [8] based on water gain when the product is kept inside an airtight container with a saturated solution of sodium 492 sulphate at room temperature (25 °C) during one week. Results were expressed in g of water/100 g of sample. Wettability was defined as the time in which 2 g of powder in 20 mL of distilled water get fully wet [9]. Swelling capacity (SC) was calculated following the Raghavendra et al. [10] method, as the ratio 495 between the volume of the sample when immersed in water excess after 18 h at 25 \degree C, and the initial weight of the sample. Results were given in mL/g. Water holding capacity (WHC) was determined as the amount of water retained by the sample without the application of any external force, except gravity and atmospheric pressure [10]. WHC was calculated as the ratio between the amount of water contained in the 499 hydrated powder (0.2 g of powder hydrated with 10 mL of water, during 18 h at 25 °C) (HR) and the dry weight of the powder after freeze-drying (DR). Water retention capacity (WRC) was obtained as the amount of water retained by the sample when subjected to an external force such as pressure or centrifugation [10]. 502 Around 1 g of powder was hydrated with 10 mL of water during 18 h at 25 °C. Then, the mixture was centrifuged at 2000 rpm for 30 min, discarding the supernatant and obtaining the weight of the decanted residue (W), which was then freeze-dried and weighed (R). WRC was calculated as the ratio between the water retained by the powder (W) and the dry weight of the residue (DR).

 Oil holding capacity (OHC) was obtained according to Garau et al. [11]. Around 0.2 g of powder were mixed with 1.5 g of sunflower oil and kept overnight at room temperature. Mixture was centrifuged at 1500 x g for 5 min, discarding the supernatant and obtaining the weight of the residue. Results were expressed in g of oil absorbed per g of powder. Emulsifying activity (EA) was carried out following Yasumatsu et al. [12] method. A 2% (w/v) aqueous powder solution was mixed with sunflower oil and homogenised with a vortex (Reax top, Heidolph, Germany) during 5 min at 2400 rpm; then, the resulting emulsion was centrifuged at 10,000 rpm for 5 min. Volume of emulsion formed was calculated according to equation 4, 513 where V_{EL} is the emulsion volume (mL) and V is the total fluid volume (mL). Emulsion stability (ES) was determined by the modified method of Yasumatsu et al. [12]. A 2% (w/v) aqueous powder solution was 515 mixed with sunflower oil and vortexed at 2,400 rpm during 5 min. The emulsion was heated up to 80 °C 516 for 30 min, tempered at room temperature, and then centrifuged at 2,000 rpm for 5 min. ES was calculated by means of as the ratio between the volume of the emulsion layer (mL) and the total fluid volume (mL).

Statistical analysis

 All analytical determinations were determined at least in triplicate. Results were statistically analysed using Statgraphics Centurion software (Centurion XVII.I, StatPoint Technologies, Inc.). One-way ANOVA and Multifactor ANOVA were carried out to analyse statistical significance of results, at the 95% confidence

level. SPSS 16.0 statistics software (IBM SPSS) was used for principal Component Analyses (PCA).

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Supplementary figures

 Fig. S1 Particle size distribution of vegetable waste powders. A) Determination by the dry procedure. B) Determination by the wet procedure. Error bars represent the standard deviation of five replicates. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying

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 Fig. S2 3D graph of the PCA (Principal Component Analysis) performed on vegetable waste powder properties plotting 3 components (75% of the total variance explained). WHC: water hydration capacity; WRC: water retention capacity; SC: Swelling Capacity; OHC: oil holding capacity; SpVol: Specific volume; PartSizeW: particle size by the wet method; PartSizeD: particle size by the dry method; xw: moisture content; xss: soluble solids content

 Fig. S3 2D graph of the PCA (Principal Component Analysis) of vegetable waste powder properties plotting 2 components (60% of the total variance explained). WHC: water hydration capacity; WRC: water retention capacity; SC: Swelling Capacity; OHC: oil holding capacity; SpVol: Specific volume; PartSizeW: particle size by the wet method; PartSizeD: particle size by the dry method; xw: moisture content; xss: soluble solids content

 Fig. S4 2D graph of the PCA (Principal Component Analysis) of vegetable waste powders as described by components 1 and 2. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot air-drying; FD: freeze-drying. Error bars represent the standard deviation of three replicates

583 **Supplementary tables**

584 **Table S1.** Color parameters Cab* (chroma) and hab (hue) of powders along four months of storage. Ca: 585 carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot air-drying; FD: freeze-586 drying. Mean \pm standard deviation of three replicates

			Chroma (C_{ab}^*)		Hue (h _{ab})				
	Month 0	Month 2	Month 3	Month 4	Month 0	Month 2	Month 3	Month 4	
Ca G_HAD60	25.8 ± 0.3 ^{cd}	24.69 ± 0.08 bc	23.7 ± 0.7 ^{ab}	25.8 ± 1.3 ^d	$71.7 \pm 0.5^{\rm de}$	75.93±0.17fgh	77.3 ± 0.5 ^{ghi}	75 ± 6 ^{fg}	
Ca C_HAD60	30.4 ± 0.3 ^h	26.4 ± 0.7 ^{de}	$26.1 \pm 0.5^{\text{de}}$	28.6 ± 0.5 ^g	$67.7 \pm 0.3^{\text{a}}$	75.4 ± 0.5 ^{fg}	77.9 ± 0.8 hi	78.3 ± 0.3 ⁱ	
Ca G_HAD70	23.4 ± 0.2^a	23.1 ± 0.4^a	23.0 ± 1.4^a	24.1 ± 0.5^{ab}	69.0 \pm 0.4 ^{ab}	71.67 ± 0.16 ^{de}	73.9 ± 1.0 ^{ef}	74.8 ± 0.5 ^f	
Ca C_HAD70	27.2 ± 0.6 ^{ef}	26.5 ± 0.2 ^{de}	$26.7 \pm 1.3^{\text{de}}$	28.0 ± 0.7 ^{fg}	69.9 ± 0.3 ^{abcd}	75.8 ± 0.3 ^{fgh}	78.5 ± 1.3^i	$78.9 \pm 0.3^{\rm i}$	
Ca FD	$26.8 \pm 0.5^{\text{de}}$	26.4 ± 0.8 ^{de}	28.2 ± 0.7 ^{fg}	28.3 ± 0.5 ^{fg}	69.2 ± 0.7 ^{abc}	71.3 ± 0.3 ^{cd}	71.2 ± 0.8 bcd	71.71 ± 0.09 ^{de}	
WC G_HAD60	23.5 ± 0.2^a	24.74 ± 0.04 bc	24.85 ± 0.12 ^c	25.40 ± 0.15 ^d	91.5 ± 0.2^i	88.88 ± 0.02 ^f	87.7 ± 0.3^e	86.6 ± 0.2 ^c	
WCC HAD60	24.55 ± 0.11 bc	24.8 ± 0.2 bc	24.6 ± 0.2 ^{bc}	25.4 ± 0.3 ^d	92.01 ± 0.09	90.88 ± 0.10^h	89.56±0.09g	88.66 ± 0.09 ^f	
WC G_HAD70	23.5 ± 0.3^a	27.0 ± 0.2 ^{efg}	27.2 ± 0.4 ^g	27.15 ± 0.08 ^{fg}	88.7 ± 0.3 ^f	86.1 ± 0.3^b	86.00 ± 0.12^b	84.51 ± 0.05^a	
WC C_HAD70	24.3 ± 0.5^b	26.7 ± 0.2 ^{efg}	26.7 ± 0.3 ^{ef}	26.6 ± 0.2^e	89.0 ± 0.2 ^f	87.01 ± 0.06 ^d	86.9 ± 0.2 ^{cd}	86.7 ± 0.3 °	
WC FD	23.4 ± 0.5^a	25.4 ± 0.2 ^d	26.6 ± 0.4^e	25.5 ± 0.4 ^d	105.95 ± 0.19 ⁿ	103.41 ± 0.09 ^m	101.72 ± 0.12 ¹	101.1 ± 0.5^k	
Ce G_HAD60	23.4 ± 0.4 cde	23.5 ± 0.4 ^{def}	22.3 ± 0.2^b	21.2 ± 0.2^a	93.42±0.14 ^g	92.41 \pm 0.14 ^{ef}	92.5 ± 0.2 ^f	93.06±0.05g	
Ce C_HAD60	$24.2 \pm 0.4^{\rm h}$	23.1 ± 0.3 ^{cd}	22.25 ± 0.15^b	21.33 ± 0.10^a	94.05 \pm 0.19 ^h	92.0 ± 0.3^e	92.1 ± 0.3 ef	92.27 ± 0.15 ef	
Ce G_HAD70	23.5 ± 0.2 cdef	23.5 ± 0.2 ^{def}	23.3 ± 0.2 cde	23.05 ± 0.12 ^c	90.6 ± 0.2 ^d	89.84 ± 0.18 c	89.77 ± 0.15 ^c	89.6 ± 0.4 °	
Ce C HAD70	24.1 ± 0.3 ^{gh}	23.8 ± 0.2 efg	23.9 ± 0.5 ^{fgh}	23.3 ± 0.4 cde	87.9 ± 0.6^b	88.1 ± 0.4^b	$87.4 \pm 0.8^{\rm a}$	87.9 ± 0.4 ^{ab}	
Ce FD	24.78 ± 0.06 ⁱ	24.2 ± 0.3^h	24.15 ± 0.06 ^{gh}	23.1 ± 0.4 ^{cd}	103.45 ± 0.05	103.09 ± 0.06^{i}	102.62 ± 0.04^i	$102.86 \pm 0.06^{\mathrm{i}}$	
L G_HAD60	26.7 ± 0.3 ^{bc}	$27.5 \pm 0.5^{\text{de}}$	27.04 ± 0.13 ^{cd}	27.5 ± 0.4 ^{de}	91.55 ± 0.10^i	90.31 ± 0.08 ^g	90.2 ± 0.4 ^g	89.53 ± 0.18 ^f	
L C_HAD60	26.45 ± 0.05 bc	24.6 ± 0.4^a	25.1 ± 0.4^a	26.4 ± 0.3 bc	92.50 ± 0.12^k	92.12 ± 0.07	91.62 ± 0.19 ⁱ	91.05 ± 0.13^h	
L G_HAD70	27.01 ± 0.06 cd	27.8 ± 0.2 ^{ef}	27.8 ± 0.7 ef	28.03 ± 0.04 efg	89.31 ± 0.08 ^f	88.6 ± 0.4 ^{de}	88.48 ± 0.15 ^{cd}	88.23 ± 0.13 c	
L C_HAD70	26.1 ± 0.3^b	28.1 ± 0.9 ^{efg}	27.9 ± 0.4 ^{ef}	28.2 ± 0.2 ^{fg}	88.8 ± 0.4^e	86.94 ± 0.13^b	86.86 ± 0.15^b	86.01 ± 0.14 ^a	
L FD	28.54 ± 0.05 ^{gh}	27.9 ± 0.3 ^{efg}	29.1 ± 0.4^h	28.9 ± 0.3^h	99.35±0.10°	98.93 ± 0.07 ⁿ	97.68 ± 0.05 ^m	97.03 ± 0.05 ¹	

587 a,b,c... Different letters in the same column for a similar residue indicate statistically significant differences at the 95% confidence level (*p*-value < 0.05).

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