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Matrix changes driven by cultivar diversity, inulin addition and drying techniques - shaping the antioxidant, antimicrobial and anti-inflammatory properties of blueberry powders

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ABSTRACT

Quality of fruit powders is ambiguously shaped by processing parameters depending on matrix composition. Thus, the objective was to evaluate influence of drying techniques and inulin application on physico-chemical, antioxidant, antibacterial and anti-inflammatory properties of sugar-free juice extract powders prepared from three blueberry cultivars. Drying significantly affected physical attributes, pointed vacuum drying as preferred. The highest phenolics content was ensured by spray- and freeze-drying (9.32–30.36 and 9.25–30.94 mg · 100 g^{-1} dry matter, respectively). Inulin lowered bioactives by 48% and alleviated cultivar-driven differences in powders. Phenolic profile affected antibacterial activity towards *Campylobacter jejuni* stronger than phenolic content. Ten out of fifteen carrier-free powders showed bactericidal effect against *Helicobacter pylori*, while one among inulin-added. Vacuum drying improved anti-inflammatory properties in gastric cell cultures infected with *Helicobacter pylori* of selected blueberry products compared to spray- and freeze-drying. The study provides comprehensive insight into blueberry powders manufacturing with high scientific and practical significance.

1. Introduction

Due to the perishable nature of blueberries (*Vaccinium corymbosum* L.), this fruit is highly prone to mechanical damage and microbiological spoilage, which limits its storability. To prevent post-harvest losses and the associated detriment to earnings, numerous attempts have been made to prolong the presence of blueberriers on the market, regardless of the seasonality of the fruit (Shi et al., 2023). One way to tackle this challenge is to produce powdered formulation towards obtaining natural food additives in a stable and easy-to-handle form (Tao et al., 2018).

Previously, numerous approaches were applied to obtain blueberry powders, among which different blueberry-based matrices i.e., whole fruit, juice, and pomace, were used (Tao et al., 2018). In the case of juice containing low molecular sugars and organic acids, the addition of a high molecular carrier is required so as to make the conversion of liquid to powder feasible. Another possibility to obtain powders from juice is the modification of the fruit-based matrix to remove substances with a low glass transition temperature, including the components that hinder drying (Michalska, Wojdyło, Honke, Ciska, & Andlauer, 2018). Such a procedure not only allows the liquid to be transformed into a powder, but also yields a product with increased bioactive potential compared to juice due to the phenolic-densified matrix. Furthermore, the elimination

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of sugars during the purification stage makes the resulting formulation more versatile in its use as a natural food additive among specific groups of consumers, such as those who need to follow a reduced carbohydrate diet, that is, the diabetics. This kind of treatment significantly alters juice's initial bioactive composition, additionally differentiated in terms of the cultivar used (Herrera-Balandrano, Chai, Beta, Feng, & Huang, 2021), which influences the final quality of blueberry powdered products. Considering the above, the blueberry matrix moderation affects the reactivity of bioactives during further processing (Capuano, Oliviero, & van Boekel, 2018). Additional modification can be made by applying various additives, i.e., carrier agents that facilitate the drying process, but also improve quality and ensure satisfactory production efficiency (Michalska-Ciechanowska, Hendrysiak, Brzezowska, Wojdyło, & Gajewicz-Skretna, 2021). Driven by the increasing consumer awareness of the impact of their eating habits, today an increasing shift is being observed in the scientific and industrial communities towards research on the exploration of functional replacements for the commonly used maltodextrin. Inulin and various fructooligosaccharides are one of the most studied new substances that can improve functionality of powders thanks to their prebiotic properties (Comunian, Silva, & Souza, 2021). Finally, the drying techniques and parameters, which enable the conversion of liquid into powder form, are a key factor that influences physical, chemical, and biological attributes. As the complexity of the matrix combined with numerous factors affecting this matrix during powdering may evoke multidirectional alterations (bioactives release/degradation, bioactives-bioactives interaction, bioactives-carrier interaction, etc.) that determine the characteristics of the final product, it is crucial to properly adjust processing conditions to specific formulations (Michalska-Ciechanowska et al., 2021).

Currently, more and more stress is being placed on the biological aspect that dictates actual impact on human health and well-being. However, to date, little is known about the effects of the various processing steps on changes in the biological properties of plant-based powders during their production. Previously, attempts have been made to determine the impact of drying techniques on the biological properties, including antibacterial and anti-inflammatory activities, of e.g., plum juice extracts (Silvan, Michalska-Ciechanowska, & Martinez-Rodriguez, 2020). However, the difficulties associated with the complexity of the fruit matrix that undergoes drying, additionally magnified by variability between cultivars within a given species, make it impossible to develop a single, unified method of producing powders while maintaining their highest possible quality.

Therefore, it was hypothesized that by appropriate selection of cultivar, drying technique, and process parameters it is possible to moderate the physical, chemical and biological properties of blueberry extract powders towards their improved functionality. Thus, the objective of the study was to evaluate how blueberry juice extract from different cultivars, processed by freeze, vacuum and spray drying with or without inulin, can modify the physical attributes, phenolics composition, antioxidant capacity, antibacterial activity and anti-inflammatory properties in gastric cells.

2. Materials and methods

2.1. Materials

Blueberry cultivars 'Berkey', 'Blucrop', and 'Bluejay' were collected in an experimental orchard located at the Research Station of the Poznan University of Life Sciences (Poznan, Poland). The frozen fruit (15 kg of each cultivar) was thawed by Thermomix (Wuppertal, Vorkwek, Germany) (addition of ascorbic acid and enzymation (Pectinex® BE XXL, Novozymes, Denmark) at 50 °C for 1 h), pressed using a laboratory hydraulic press (SRSE, Warsaw, Poland) and clarified by centrifugation (MPW-251, MPWMed. Instruments, Poland; 10 min, 4,800 rpm, 23 °C). The juice obtained from Berkley, Bluejay, and Bluecrop (12 L, 10°Brix) was loaded on the glass column filled with the amberlite XAD-16 resin (Brentag, Kędzierzyn-Koźle, Poland) to prepare phenolic-rich extracts. Each extract was divided into two parts: the first part was mixed with 5% (*w/w*) of inulin (Beneo-Orafti, Belgium), whereas the second part consisted of phenolic-rich extract without carrier addition (control). Subsequently, powders were obtained from each part in two technological replications. A human gastric epithelial AGS cell line was purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA).

2.2. Drying

To obtain powders of blueberry phenolic-rich extracts, the freeze-drying (FD) (FreeZone freeze dryer; Labconco Corp., MO, USA; 24 h; 65 Pa; −60 °C/+25 °C), vacuum drying (VD) (Vacucell 111 ECO LINE vacuum dryer; MMM Medcenter Einrichtungen GmbH, Germany; drying temperature of 50 (VD50), 70 (VD70) and 90 °C (VD90) at 0.1 mbar for 44, 20 and 16 h, respectively) and spray drying (SD) (B190 spray dryer; Buchi, Flawil, Switzerland; inlet and outlet temperatures: 180 °C and 70 °C; volume flow: 35 $m^3 \cdot h^{-1}$; feed flow: 9 $mL \cdot min^{-1}$) were performed in duplicate (Michalska et al., 2018). The powders were vacuum-packed (PP-5.14, Tepro SA, Koszalin, Poland) in PA/PE film and stored at −20 °C until analyzed.

2.3. Moisture content, water activity, bulk and true density, porosity, and color

The moisture content (*Mc*) of blueberry powders was performed according to the procedure described by Michalska-Ciechanowska, Brzezowska, et al. (2021) and expressed as % of wet basis (wb) ($n = 4$). Dry matter (dm) was calculated as the difference between 100% and wet basis values. Water activity (a_w) was measured at 25 °C in duplicate ($n = 4$) using a Dew Point Water Activity Meter 4TE (AQUA LAB, Pullman, WA, USA). Bulk density (ρb) ($n = 4$) was determined as the ratio of mass (m) to bulk volume (Vb) occupied in the cylinder. The calculation was carried out according to Eq. (1), and expressed as $g \cdot cm^{-3}$:

$$\rho b = m/Vb \tag{1}$$

True density (ρt) ($n = 4$) was indicated by calculating the ratio of dry solid mass (m) to the total volume (Vs) of the powders (air pores excluded) measured using a HumiPyc™/model 2 gas picnometer (InstruQuest Inc., Coconut Creek, FL, USA), according to Eq. (2), and expressed as $g \cdot cm^{-3}$:

$$\rho t = m/Vs \tag{2}$$

Porosity ($n = 4$) was determined as the relation between bulk density (ρb) and true density (ρt) of the powder, according to Eq. (3):

$$\varepsilon = (1 - \rho b/\rho t) \cdot 100 \tag{3}$$

The color measurement ($n = 4$) was made by determining the CIE L^*, a^*, b^* coordinates using a Minolta Chroma Meter CR-410 (Minolta Co. Ltd., Osaka, Japan).

2.4. Identification and quantification of phenolics by UPLC

Blueberry powders were extracted as described by Wojdyło, Oszmiański, and Bielicki (2013). Qualitative and quantitative determination of phenolics was carried out using an Acquity UPLC system (Waters, Milford, USA) with a Q-Tof mass spectrometer (Waters, Manchester, UK). Phenolics were identified and determined at appropriate wavelengths: flavan-3-ols at λ=280 nm (calculated as (+)-catechin), phenolic acids at λ=320 nm (calculated as chlorogenic acid), flavonols at λ=360 nm (calculated as quercetin-3-*O*-galactoside) and anthocyanins at λ=520 nm (calculated as malvidin-3-*O*-glucoside). The results were interpreted using Empower 3 software and expressed as $g \cdot 100\ g^{-1}$ of dm ($n = 4$).

2.5. Antioxidant capacity in vitro

The antioxidant capacity *in vitro* of 80% methanolic (*v/v*) extract powders was measured by TEAC ABTS and FRAP methods as described by Michalska-Ciechanowska, Brzezowska, et al. (2021). Results were expressed in mmol Trolox equivalent per 100 g of dm ($n = 4$) with a Synergy H1 spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA).

2.6. Bacterial strains and culture conditions

The antibacterial activity of blueberry powders was evaluated on five of the most relevant foodborne pathogen bacteria and one of the most important human pathogens: *Campylobacter jejuni* (*C. jejuni*) 11168 obtained from National Culture Type Collection (NCTC), *Escherichia coli* (*E. coli*) 25922, *Salmonella enterica* (*S. enterica*) 14028, *Staphylococcus aureus* (*S. aureus*) 25923 obtained from the American Type Culture Collection (ATCC), *Listeria monocytogenes* (*L. monocytogenes*) 935 obtained from the Spanish Type Culture Collection (CECT); and *Helicobacter pylori* (*H. pylori*) Hp59 obtained from the Institute of Food Science Research collection. *C. jejuni* culture was grown as described by Silvan, Guerrero-Hurtado, Gutierrez-Docio, Prodanov, and Martinez-Rodriguez (2023). Bacteria cultures for *E. coli, S. enterica*, *S. aureus,* and *L. monocytogenes* were prepared as described by Silvan, Michalska-Ciechanowska, and Martinez-Rodriguez (2020). The culture of *H. pylori* was prepared as described by Villalva, Silvan, Guerrero-Hurtado et al. (2022).

2.7. Antibacterial activity

The antibacterial activity of blueberry extract powders (2 mg · mL^{-1} final concentration) was evaluated following the procedure described by Silvan et al. (2020), summarized as follows: 1 mL of blueberry extracts was transferred into different flasks containing 4 mL of BB (*C. jejuni*, *E. coli*, *S. enterica*, *S. aureus*, and *L. monocytogenes*) or 4 mL of BB containing 10% HS (*H. pylori*). Then, the bacterial inoculum (100 μL of ~1 × 10^8 colony formation units (CFU) · mL^{-1}) was placed in the flasks under aseptic conditions and incubated under stirring (150 rpm) under aerobic or microaerophilic (*C. jejuni* and *H. pylori*) conditions at 37 °C for 24 h. After incubation, serial decimal dilutions of mixtures were prepared in saline solution (0.9% NaCl) and plated (20 μL) onto fresh MHB agar. Plates were incubated under the conditions described above and the number of CFU was evaluated after 24 h or 72 h (*C. jejuni* and *H. pylori*). All experiments were carried out in triplicate ($n = 3$) and results were expressed as CFU · mL^{-1}.

2.8. Human gastric epithelial cell cultures

Cells were grown in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12) (Lonza, Madrid, Spain) supplemented with 10% fetal bovine serum (FBS) of South American origin (Hyclone, GE Healthcare, Logan, UK) and 1% penicillin/streptomycin (5000 U · mL^{-1}) (Lonza). Cells were seeded at densities of ~1 × 10^6 cells in 75 cm^2 culture flasks (Sarstedt, Nümbrecht, Germany) and incubated (37 °C, 5% CO_2) in a humidified incubator until 90% confluence. All experiments were performed between passage 5 and 15 to ensure cell uniformity and reproducibility. Before the anti-inflammatory experiments were carried out, it was necessary to evaluate the cytotoxicity of blueberry extracts on the AGS cell line. For that purpose, cell viability was determined by MTT (3,4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide) (Merck, Madrid, Spain) reduction assay as previously described by Villalva, Silvan, Alarcón-Cavero, et al. (2022). Data represent the mean and standard deviation of three independent experiments ($n = 3$).

2.9. Anti-inflammatory activity of blueberry extracts on AGS cells infected with H. pylori

The anti-inflammatory activity of blueberry extracts (2 mg · mL^{-1}) on AGS cells infected by *H. pylori* was evaluated as previously described by Silvan et al. (2021). The amounts of secreted interleukin IL-8 in the collected supernatant of gastric AGS cell samples were determined by an ELISA assay. A commercially available ELISA kit (Diaclone, Besancon, France) was used for the quantification ($n = 3$) of IL-8 cytokine following the manufacturer's instructions. The results of anti-inflammatory activity were expressed as a percentage of IL-8 production with respect to controls infected with *H. pylori*, which represent 100% IL-8 production.

2.10. Statistical analysis

The statistical one-way analysis of variance (ANOVA) (HSD Tukey test) and *t*-test were performed to estimate differences ($p < 0.05$), and the Pearson's correlation coefficient was used to examine the dependencies between the selected variables using Statistica 10 (Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Physical properties

The moisture content (*Mc*) of the blueberry extract powders ranged from 0.8 to 7.3% (Table 1). Drying technique differentiates the *Mc* to the highest extent. Samples after FD had approximately 2-times higher *Mc* compared to average values obtained using the other drying techniques, regardless of carrier addition (Silvan, Michalska-Ciechanowska, & Martinez-Rodriguez, 2020). Water activity (a_w) linked to the microbiological and chemical stability of food powders was the highest for products obtained by FD of 'Berkley' with and without inulin (Table 1). Similarly to *Mc*, the drying technique had a stronger influence on a_w than carrier addition. Powders from 'Berkley' had the highest a_w when compared to the 'Bluejay' and 'Bluecrop' cultivars, regardless of carrier addition or the drying technique used. The inulin application resulted in powders with water activity reduced by approx. 33%, 14% and 8%, for 'Berkley', 'Bluecrop' and 'Bluejay' powders, respectively, compared to those without the carrier. No correlation between moisture content and water activity was observed.

Bulk density (ρ_b) ranged from 0.12 to 0.77 g · cm^{-3} (Table 1). The drying techniques had the greatest impact on ρ_b, regardless of carrier addition and blueberry cultivar, with FD and SD resulting in the lowest values, which were, respectively, 2 and 4 times lower than for powders after VD. This is attributable to the structural differences in the powders caused by drying (Michalska & Lech, 2018) as well as *Mc* and a_w ($r = -0.6$). For powders obtained from 'Berkley' by VD at 70 and 90 °C, the addition of inulin resulted in an almost 1.8-fold increase in ρ_b, compared to the control sample. This relationship was not observed for the other cultivars. It may be due to differences in chemical composition between cultivars and thus different transformations of specific constituents and their interactions with inulin that may occur during VD under the aforementioned temperature conditions (Li, Ma, & Liu, 2019).

As for true density (ρ_t), when considering the effect of drying techniques, the only noticeable difference was observed for spray-dried powders ($\bar{x} = 1.15$ g · cm^{-3}), which had the lowest ρ_t when compared to other applied thermal treatments (comparable values) (Table 1). A similar relationship was previously observed for sea buckthorn (Tkacz et al., 2020) and apple powders (Michalska & Lech, 2018), which can be attributed to chemical composition changes (degradation of natively occurring constituents or probable formation of new compounds) under the influence of relatively high temperature during SD, which finally lead to less dense powders. The drying techniques strongly affected porosity (ε), and so the powders can be ranked by increasing values: VD50 (approx. 49%) < VD70 (approx. 54%) ≈ VD90 (approx.

Table 1
Moisture content (%), water activity (a_w), bulk density ($g \cdot cm^{-3}$), true density ($g \cdot cm^{-3}$), porosity (%) and color (CIE $L^*a^*b^*$) ($n = 2$; average ± standard deviation) of blueberry juice extract powders obtained by different drying techniques with or without a carrier.

Cultivar	Drying technique	Mositure content	Water activity	Bulk density	True density	Porosity	L^*	a^*	b^*
				No carrier					
	FD	5.24 ± 1.16 $^{d-h}$	0.210 ± 0.007 ^{jk}	0.163 ± 0.003 ^{ab}	1.13 ± 0.02 ^{ab}	85.47 ± 0.22 ^{pq}	31.36 ± 0.05 $^{b-g}$	10.43 ± 0.01 ^{hi}	0.71 ± 0.02 ^{hi}
	VD50	3.15 ± 0.23 $^{a-f}$	0.111 ± 0.007 ^{a}	0.663 ± 0.030 $^{i-k}$	1.27 ± 0.01 $^{d-f}$	47.26 ± 0.50 ^{bc}	30.06 ± 0.32 ^{a}	3.87 ± 0.24 $^{a-c}$	0.71 ± 0.02 ^{hi}
Bluejay	VD70	2.23 ± 0.77 $^{a-e}$	0.130 ± 0.007 ^{ab}	0.706 ± 0.024 $^{j-l}$	1.33 ± 0.01 $^{f-j}$	46.61 ± 0.29 ^{ab}	30.47 ± 0.00 $^{a-d}$	3.24 ± 0.09 ^{ab}	0.65 ± 0.01 $^{g-i}$
	VD90	1.59 ± 0.07 $^{a-c}$	0.205 ± 0.007 ^{ij}	0.499 ± 0.016 ^{h}	1.34 ± 0.01 $^{f-k}$	62.38 ± 0.32 ^{j}	30.45 ± 0.04 $^{a-d}$	2.24 ± 0.01 ^{a}	0.84 ± 0.00 ^{i}
	SD	2.94 ± 0.19 $^{a-f}$	0.448 ± 0.007 ^{p}	0.280 ± 0.015 ^{de}	1.17 ± 0.02 ^{bc}	75.89 ± 0.40 ^{m}	30.09 ± 0.10 ^{ab}	14.83 ± 0.05 ^{lm}	1.62 ± 0.02 ^{k}
	FD	7.05 ± 0.97 ^{gh}	0.469 ± 0.007 ^{p}	0.117 ± 0.003 ^{a}	1.54 ± 0.04 ^{l}	92.35 ± 0.19 ^{s}	33.15 ± 0.01 $^{j-k}$	17.31 ± 0.06 ^{no}	1.26 ± 0.01 ^{j}
	VD50	3.80 ± 0.11 $^{a-f}$	0.146 ± 0.006 $^{b-d}$	0.690 ± 0.034 $^{j-l}$	1.35 ± 0.02 $^{g-k}$	48.42 ± 0.54 $^{c-e}$	31.86 ± 0.17 $^{e-h}$	5.98 ± 0.58 $^{d-f}$	0.18 ± 0.05 ^{cd}
Berkley	VD70	2.02 ± 0.71 $^{a-d}$	0.169 ± 0.006 $^{c-g}$	0.377 ± 0.011^{fg}	1.30 ± 0.02 $^{e-h}$	70.87 ± 0.45 ^{k}	31.56 ± 0.15 $^{d-g}$	4.34 ± 0.18 $^{b-d}$	0.23 ± 0.01 $^{c-e}$
	VD90	2.54 ± 1.26 $^{a-e}$	0.258 ± 0.006 ^{lm}	0.391 ± 0.012 ^{g}	1.37 ± 0.02 $^{g-k}$	71.29 ± 0.34 ^{k}	31.13 ± 0.10 $^{a-f}$	4.88 ± 0.15 $^{b-d}$	0.50 ± 0.01 ^{fg}
	SD	3.99 ± 1.92 $^{a-g}$	0.359 ± 0.007 ^{o}	0.204 ± 0.006 $^{b-d}$	1.14 ± 0.02 ^{b}	82.01 ± 0.33 ^{o}	32.58 ± 0.10 $^{g-i}$	20.08 ± 0.05 ^{p}	2.15 ± 0.00 ^{l}
	FD	7.33 ± 0.27 ^{h}	0.265 ± 0.007 ^{lm}	0.204 ± 0.004 $^{b-d}$	1.39 ± 0.02 $^{i-k}$	85.27 ± 0.26 ^{pq}	30.90 ± 0.72 $^{a-e}$	11.96 ± 0.87 ^{ij}	0.61 ± 0.05 ^{gh}
	VD50	3.19 ± 0.62 $^{a-f}$	0.166 ± 0.007 $^{c-f}$	0.682 ± 0.027 ^{jk}	1.39 ± 0.02 $^{i-k}$	50.63 ± 0.52 ^{fg}	30.13 ± 0.07 $^{a-c}$	5.31 ± 0.49 $^{c-e}$	0.64 ± 0.06 $^{g-i}$
Bluecrop	VD70	2.83 ± 0.30 $^{a-e}$	0.158 ± 0.007 $^{b-e}$	0.627 ± 0.023 ^{ij}	1.37 ± 0.02 $^{g-k}$	53.98 ± 0.49 ^{h}	30.74 ± 0.48 $^{a-e}$	4.25 ± 0.74 $^{b-d}$	0.47 ± 0.09 ^{fg}
	VD90	2.20 ± 0.08 $^{a-e}$	0.261 ± 0.007 ^{lm}	0.593 ± 0.021 ^{i}	1.38 ± 0.01 $^{h-k}$	56.62 ± 0.35 ^{i}	31.52 ± 0.14 $^{d-g}$	3.54 ± 0.47 $^{a-c}$	0.39 ± 0.02 ^{ef}
	SD	5.39 ± 0.58 $^{e-h}$	0.270 ± 0.007 ^{m}	0.262 ± 0.010 $^{c-e}$	1.23 ± 0.03 $^{c-e}$	78.53 ± 0.55 ^{n}	30.75 ± 0.02 $^{a-e}$	16.64 ± 0.25 ^{mn}	1.95 ± 0.04 ^{l}
				5% inulin					
	FD	3.38 ± 0.99 $^{a-f}$	0.171 ± 0.000 $^{d-h}$	0.174 ± 0.006 ^{ab}	1.34 ± 0.02 $^{f-k}$	86.88 ± 0.19 ^{q}	33.20 ± 0.07 $^{i-k}$	14.29 ± 0.20 ^{kl}	−0.12 ± 0.05 ^{ab}
	VD50	4.35 ± 0.14 $^{c-h}$	0.193 ± 0.006 $^{f-j}$	0.739 ± 0.034 ^{kl}	1.41 ± 0.02 ^{jk}	47.18 ± 0.59 ^{bc}	30.88 ± 0.02 $^{a-e}$	7.60 ± 0.00 ^{fg}	0.12 ± 0.02 $^{c-d}$
Bluejay	VD70	2.78 ± 0.29 $^{a-e}$	0.194 ± 0.006 $^{f-j}$	0.769 ± 0.027 ^{l}	1.41 ± 0.02 ^{jk}	45.19 ± 0.56 ^{a}	31.48 ± 0.18 $^{d-g}$	8.62 ± 0.31 ^{gh}	0.30 ± 0.05^{d-f}
	VD90	0.79 ± 0.35 ^{a}	0.213 ± 0.000 ^{jk}	0.693 ± 0.024 $^{j-l}$	1.39 ± 0.02 $^{i-k}$	49.87 ± 0.51 $^{e-g}$	31.36 ± 0.28 $^{c-g}$	7.59 ± 0.70 ^{fg}	0.26 ± 0.01 $^{c-e}$
	SD	3.86 ± 0.09 $^{a-g}$	0.249 ± 0.006 ^{lm}	0.298 ± 0.008 ^{ef}	1.19 ± 0.02 $^{b-d}$	74.80 ± 0.31 ^{lm}	31.64 ± 0.07 $^{d-g}$	17.33 ± 0.45 ^{no}	1.11 ± 0.04 ^{j}
	FD	7.07 ± 1.68 ^{gh}	0.326 ± 0.008 ^{n}	0.142 ± 0.004 ^{ab}	1.32 ± 0.04 $^{f-i}$	89.12 ± 0.33 ^{r}	35.14 ± 0.41 ^{l}	19.05 ± 0.53 ^{op}	0.72 ± 0.03 ^{hi}
	VD50	4.01 ± 0.18 $^{a-g}$	0.142 ± 0.008 ^{bc}	0.731 ± 0.024 ^{kl}	1.41 ± 0.01 ^{k}	47.96 ± 0.34 $^{b-d}$	34.03 ± 0.03 $^{j-l}$	12.46 ± 0.18 ^{jk}	−0.12 ± 0.03 ^{a}
Berkley	VD70	2.42 ± 0.15 $^{a-e}$	0.101 ± 0.008 ^{a}	0.672 ± 0.021 $^{i-k}$	1.39 ± 0.01 $^{i-k}$	51.22 ± 0.49 ^{fg}	32.19 ± 0.16 $^{f-i}$	7.91 ± 1.21 ^{g}	0.26 ± 0.08 $^{c-e}$
	VD90	1.01 ± 0.66 ^{ab}	0.177 ± 0.008 $^{e-i}$	0.689 ± 0.023 $^{j-l}$	1.38 ± 0.01 $^{g-k}$	49.52 ± 0.40 $^{d-f}$	33.09 ± 0.86 $^{h-k}$	8.66 ± 0.42 ^{gh}	0.26 ± 0.02 $^{c-e}$
	SD	4.20 ± 1.59 $^{b-h}$	0.199 ± 0.008 $^{h-j}$	0.272 ± 0.008 $^{c-e}$	1.06 ± 0.02 ^{a}	74.15 ± 0.40 ^{l}	37.02 ± 0.33 ^{m}	23.91 ± 0.27 ^{q}	1.08 ± 0.14 ^{j}
	FD	6.10 ± 0.40 $^{f-h}$	0.174 ± 0.006 $^{d-h}$	0.193 ± 0.006 $^{a-c}$	1.29 ± 0.03 $^{e-g}$	84.96 ± 0.29 ^{p}	34.24 ± 0.08 ^{kl}	19.04 ± 0.11 ^{op}	−0.25 ± 0.02 ^{a}
	VD50	4.99 ± 0.96 $^{d-h}$	0.195 ± 0.006 $^{f-j}$	0.671 ± 0.027 $^{i-k}$	1.50 ± 0.02 ^{l}	55.00 ± 0.59 ^{hi}	30.70 ± 0.72 $^{a-e}$	7.04 ± 0.92 $^{e-g}$	0.11 ± 0.01 ^{cd}
Bluecrop	VD70	2.19 ± 0.41 $^{a-e}$	0.164 ± 0.006 $^{c-e}$	0.628 ± 0.025 ^{ij}	1.39 ± 0.01 $^{i-k}$	54.36 ± 0.33 ^{h}	30.63 ± 0.15 $^{a-e}$	4.97 ± 0.04 $^{b-d}$	0.14 ± 0.01 ^{cd}
	VD90	2.18 ± 0.41 $^{a-e}$	0.195 ± 0.008 $^{g-j}$	0.676 ± 0.023 ^{jk}	1.40 ± 0.01 ^{jk}	51.32 ± 0.47 ^{g}	31.60 ± 0.20 $^{d-g}$	6.84 ± 0.37 $^{e-g}$	0.09 ± 0.11 ^{bc}
	SD	2.54 ± 0.62 $^{a-e}$	0.237 ± 0.006 $^{k-j}$	0.273 ± 0.010 $^{c-e}$	1.13 ± 0.02 ^{ab}	75.54 ± 0.35 ^{lm}	32.64 ± 0.10 $^{f-j}$	19.36 ± 0.00 ^{p}	1.24 ± 0.05 ^{j}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying; $^{a, b, c, d...}$ - different letters within the column indicated statistical differences ($p < 0.05$; HSD Tukey test).

57%) < SD (approx. 77%) < FD (approx. 87%), regardless of carrier addition or cultivar (Table 1). A similar trend was observed for sea buckthorn powders (Tkacz et al., 2020); however, in the study on apple powders the highest ε was observed for SD products (Michalska & Lech, 2018). This may be attributed to differences in the chemical composition. The impact of the drying technique on porosity was opposite to the impact on bulk density ($r = -0.99$) since porosity is inversely related to bulk density.

The inulin addition did not significantly affect the brightness (L^*) of powders obtained from the 3 cultivars as a relatively low concentration of inulin was used (5%; *w/w*) (Table 1). SD and FD resulted in higher values of the a^* coordinate compared to VD, regardless of the blueberry cultivar and carrier application. Moreover, carrier addition caused an increase in the value of this attribute. No correlation between a^* parameter and anthocyanins content were found ($r = -0.22$). The inulin application caused a shift of the coordinate b^* values towards negative values (bluish color). When considering the impact of the drying technique, the strongest yellowness was recorded for spray-dried powders. In most cases, the lowest values were recorded for vacuum-dried samples irrespective of carrier addition or cultivar, indicating a tendency towards bluish. However, it is worth noting that the range over which the values change is relatively small and therefore the difference may not be perceptible visually.

3.2. Phenolics and antioxidant capacity

In the blueberry juice extract powders, four major groups of phenolics were identified and quantified, namely, phenolic acids (3 compounds; on average 30.2% of all identified phenolics), anthocyanins (8 compounds; 29.9%), flavonols (11 compounds; 28.2%) and flavan-3-ols (3 compounds; 8.8%) (Table 2a and 2b) (Shen et al., 2014). Their content ranged from 7.32 to 30.9 $g \cdot 100\ g^{-1}$ dm. As regards the cultivar, the highest sum of phenolics was indicated for 'Bluecrop', followed by 'Bluejay' powders (approx. 15% less) and 'Berkley' (approx. 40% lower content). Products without inulin had 48% higher content of phenolics than carrier-added samples. As for the impact of the drying technique, the highest phenolic content in powders was noted after FD followed by SD, while the lowest was after VD90, except for 'Bluecrop' powders with addition of inulin. This could be linked to the probable protective role of inulin during the thermal treatment of these constituents (Michalska-Ciechanowska, Brzezowska, et al., 2021).

3.2.1. Phenolic acids

The content of phenolic acids in blueberry extract powders without inulin was approx. 1.9-fold higher when compared to products with its addition (Table 2a). The highest content of phenolic acids was observed for powder from 'Bluecrop' and was, on average, 35% and 60% higher when compared to 'Bluejay' and 'Berkley'. The phenolic acid content was mainly related to the amount of chlorogenic acid. Chlorogenic acid represented 94% of total phenolic acids in 'Bluecrop' powders, while its share was on average 87% in the other cultivars. This was in line with Pico, Yan, Gerbrandt, and Castellarin (2022), who indicated that 'Bluecrop' had the highest content of chlorogenic acid among the blueberry cultivars analyzed in their study. The content of caffeoyl-glucose was the highest in powders from 'Berkley' and 'Bluejay', whereas the content of feruloyl-glucose was the highest in 'Bluejay' and 'Bluecrop' products. Among drying techniques applied, the highest retention of phenolic acids was found after FD and SD. The strongest degradation of phenolic acids was observed when VD90 was used.

3.2.2. Anthocyanins

The second most abundant group of phenolics in the extract powders were anthocyanins, whose content ranged from 1.5 to 9.8 g · 100 g^{-1} dm (Table 2a). Considering the cultivar, 'Bluejay' powders had the highest average content of anthocyanins – approximately 56% more than 'Berkley' powders, which had the lowest anthocyanin content, regardless of the drying technique or carrier addition. Therefore, from the practical point of view, 'Bluejay' powder can be recommended whenever high anthocyanin content is desired. This cultivar also appeared preferable when the carrier was applied to blueberry juice extract powders production, except of samples after VD90, in which the 'Bluecrop' powders were the most attractive. The addition of inulin caused an approx. 2-fold decrease in the content of individual anthocyanins. The exceptions were powders after VD90: a 1.1-fold decrease was recorded in 'Bluecrop' products, a 2.8-fold decrease in 'Berkley' products, and a 3.5-fold decrease in 'Bluejay' products. This confirms a strong influence of temperature (Zhu et al., 2017) and drying time (Reque et al., 2016) on anthocyanin degradation; however, the different degrees of degradation of the same anthocyanins in powders obtained from different cultivars indicate an ambiguous effect of the blueberry juice extract matrices submitted to drying (Yang et al., 2022). The drying technique influenced the average anthocyanins content in the following order: FD > VD50 > SD > VD70 > VD90, regardless of carrier addition or cultivar. As for individual anthocyanins, 'Bluejay' powders had the highest content of peonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, delphinidin-3-*O*-galactoside, delphinidin-3-*O*-arabinoside, and petunidin-3-*O*-arabinoside. The exceptions were products after VD90 with inulin, while the 'Bluecrop' powders were characterized by the highest amount of malvidin-3-*O*-arabinoside, malvidin-3-*O*-galactoside, and petunidin-3-*O*-glucoside.

Among anthocyanins identified, peonidin-3-*O*-glucoside, not previously indicated as prevailing in blueberry fruit (Herrera-Balandrano et al., 2021), was predominant in all powders tested (approx. 30% higher in 'Bluejay' compared to the other cultivars). Previously, Liu, Liu, Yang, and Zhang (2022) found that among the anthocyanins present in the fruit of greengages (*Prunus mume* Sieb. Et Zucc), only the content of peonidin-3-*O*-glucoside increased under intense UV radiation. This, in turn, may be due to the fact that meteorological parameters can induce different responses of individual anthocyanins depending on the structure (Kovinich et al., 2014), affecting their synthesis differently, and thus the final composition of anthocyanins in the fruit. Moreover, the stability of individual anthocyanin aglycones is also determined by the technological process and their varying persistence, in particular the effects of temperature, light or the presence of oxygen (Cai et al., 2022). Some anthocyanins are more sensitive than others to technological treatment and, consequently, some will be more and others less stable, as observed in the study. Although the contents of cyanidin-3-*O*-glucoside, delphinidin-3-*O*-arabinoside, and petunidin-3-*O*-arabinoside varied, these compounds followed the same path in terms of quantitative fluctuations depending on cultivar, carrier addition, and drying technique, as in case of peonidin-3-*O*-glucoside (Michalska-Ciechanowska, Hendrysiak, et al., 2021). Slight differences deviating from this trend were noted for delphinidin-3-*O*-galactoside, the content of which, depending on the cultivar, was ranked in the following descending order: 'Bluejay' > 'Berkley' > 'Bluecrop' for all of powders, except samples after VD90 with inulin. A different trend was found for malvidin-3-*O*-arabinoside, malvidin-3-*O*-galactoside, and petunidin-3-*O*-glucoside. In the case of malvidin-3-*O*-arabinoside, the second most abundant anthocyanin in powders, the highest content was recorded for 'Bluecrop' products, while 'Bluejay' and 'Berkley' had, respectively, 16% and 47% less of this constituent. The exception were the powders after VD90 without inulin and spray-dried with its application, of which 'Bluejay' powders had the highest content of this compound.

Among inulin-added samples, only in the case of 'Bluecrop' powders after VD90, the content of malvidin-3-*O*-arabinoside, malvidin-3-*O*-galactoside, and petunidin-3-*O*-glucoside was comparable or even higher, compared with products obtained by other drying techniques. This stands in contrast to inulin-free powders, where VD90 resulted in 'Bluecrop' products with the lowest amount of these compounds. These two different trends in the group of anthocyanins may be due to their different stability, which depends, among others, on the anthocyanin chemical structure, the number and position of sugar moieties attached, or the degree of acylation (Ryu & Koh, 2022), as well as environmental factors, mainly pH, temperature, light, oxygen access, presence of ascorbic acid or enzymes, co-pigmentation, etc. (Enaru, Drețcanu, Pop, Stănilă, & Diaconeasa, 2021).

3.2.3. Flavonols

The third identified group of phenolics present in blueberry powders were flavonols, which comprised 11 compounds and their sum ranged from 2.74 to 8.32 g · 100 g^{-1} dm (Table 2b). The highest amount of flavonols was noted for 'Bluecrop' products, while 'Bluejay' and 'Berkley' were characterized by around 19% and 30% lower content, respectively. Similarly to anthocyanins, inulin application resulted in an about 2-fold decrease, regardless of the cultivar and drying technique applied. This was different for samples after VD90 (approx. 1.4-fold decrease). Moreover, in the case of 'Berkley' and 'Bluejay' powders, the fluctuation of the flavonols content depending on the drying technique applied was not so noticeable as in case of 'Bluecrop' powders.

The individual flavonols followed different paths depending on the variables analyzed. The levels of quercetin-3-*O*-galactoside (predominant flavonol in all blueberry powders), quercetin hexuronide, and quercetin-3-*O*-arabinofuranoside were comparable in 'Berkley' and 'Bluejay' powders, and were lower than the levels noted for 'Bluecrop' products. Additionally, in 'Bluecrop' powders obtained by VD90 the lowest content of quercetin-3-*O*-galactoside, quercetin hexuronide, and quercetin-3-*O*-arabinofuranoside was observed, while the addition of inulin resulted in powders with the highest concentration of these compounds compared to other carrier-added samples. For quercetin-3-*O*-glucoside, −(acetyl)hexoside, −rutinoside, and -rhamnoside the course of changes was similar; however, the content of these compounds depending on the cultivar was in the following order: 'Bluecrop' > 'Bluejay' > 'Berkley'. Interestingly, there was also a strong negative correlation between quercetin-3-*O*-rhamnoside with quercetin-3-dimethoxy-rhamnoside and syringetin-3-*O*-rhamnoside ($r = -0.71$) (Fig. S1a; supplementary file). This may be connected with structural changes during fruit maturing which, depending on the blueberry cultivar, occur to different degrees and ultimately result in a different compound formation, as methoxylated derivatives of quercetin glycosides (Buchner, Krumbein, Rohn, & Kroh, 2006; Yan, Song, Falginella, & Castellarin, 2020).

Quercetin-3-*O*-dimethoxyrhamnoside content varied strongly depending on the cultivar; however, it remained relatively constant regardless of the drying technique. Unlike the other flavonols, this is the only compound which was recorded as predominant in powders from 'Berkley'. Quercetin-3-*O*-oxalylpentoside represented 4%, 7% and 10% of all identified flavonols in 'Bluecrop', 'Berkley', and 'Bluejay' powders, respectively. Spray-dried 'Bluejay' products had the highest content of this compound for both inulin-supplemented and inulin-free samples. The variations in its content depending on the drying technique were considerably more noticeable than in other flavonols. Finally, syringetin-3-*O*-rhamnoside and myricetin-3-*O*-galactoside were identified and quantified only in the products obtained from 'Berkley' and 'Bluejay'. The percentage of syringetin-3-*O*-rhamnoside in total flavonols was 6% for both 'Berkley' and 'Bluejay' powders. What is more, its content oscillated on the similar level and remained unchanged independently of the applied drying technique. For myricetin-3-*O*-galactoside, the average percentage was about 1% and 2% in 'Berkley' and 'Bluejay' powders, respectively; however 'Bluejay' powders contained about 2 times more of this constituent than 'Berkley' ones. Slight differences were observed with regard to the drying technique. As in case of the most identified flavonols, the inulin addition resulted in powders with

Table 2a
Phenolic acids and anthocyanins identified in blueberry juice extract powders obtained by different drying techniques with or without a carrier [mg · 100 g^{-1} dm].

Cultivar	Drying technique	Caffeoyl-glucose	Chlorogenic acid	Feruoylglucose	Sum of phenolic acids	Delphinidin-3-*O*-galactoside	Delphinidin-3-*O*-arabinoside	Cyanidin-3-*O*-glucoside	Petunidin-3-*O*-glucoside	Petunidin-3-*O*-arabinoside	Peonidin-3-*O*-glucoside	Malvidin-3-*O*-galactoside	Malvidin-3-*O*-arabinoside	Sum of anthocyanins
								No carrier						
Bluejay	FD	0.53 ± 0.02 ^{lm}	6.42 ± 0.09 ^{g}	0.30 ± 0.01 ^{h}	**7.26 ± 0.12** ^{e}	0.85 ± 0.01 ^{lm}	0.79 ± 0.02 ^{i}	0.98 ± 0.03 ^{h}	0.46 ± 0.02 $^{h-j}$	0.64 ± 0.01 ^{j}	3.56 ± 0.08 ^{m}	0.83 ± 0.00 ^{fg}	1.69 ± 0.02 ^{kl}	**9.79 ± 0.14** ^{m}
	VD50	0.50 ± 0.01 $^{j-m}$	5.93 ± 0.00 $^{e-g}$	0.28 ± 0.01 ^{gh}	**6.71 ± 0.02** ^{e}	0.87 ± 0.01 ^{m}	0.80 ± 0.01 ^{i}	0.97 ± 0.01 ^{gh}	0.43 ± 0.00 $^{g-i}$	0.62 ± 0.00 ^{j}	3.43 ± 0.01 ^{lm}	0.78 ± 0.00 $^{e-g}$	1.57 ± 0.02 ^{jk}	**9.46 ± 0.01** ^{lm}
	VD70	0.52 ± 0.02 $^{k-m}$	6.17 ± 0.17 ^{g}	0.29 ± 0.01 ^{gh}	**6.98 ± 0.21** ^{e}	0.73 ± 0.04 ^{k}	0.68 ± 0.03 ^{h}	0.83 ± 0.01 ^{f}	0.39 ± 0.00 ^{gh}	0.52 ± 0.01 ^{i}	3.04 ± 0.09 ^{k}	0.72 ± 0.01 ^{ef}	1.40 ± 0.01 ^{j}	**8.31 ± 0.19** $^{i-k}$
	VD90	0.50 ± 0.00 $^{j-m}$	5.99 ± 0.08 ^{fg}	0.25 ± 0.01 ^{g}	**6.74 ± 0.09** ^{e}	0.79 ± 0.00 $^{k-m}$	0.72 ± 0.01 ^{hi}	0.87 ± 0.01 ^{fg}	0.40 ± 0.01 ^{gh}	0.54 ± 0.01 ^{i}	3.18 ± 0.12 ^{kl}	0.76 ± 0.01 $^{e-g}$	1.42 ± 0.04 ^{j}	**8.68 ± 0.22** $^{j-l}$
	SD	0.54 ± 0.01 ^{m}	6.25 ± 0.09 ^{g}	0.30 ± 0.00 ^{h}	**7.09 ± 0.10** ^{e}	0.76 ± 0.05 ^{kl}	0.70 ± 0.04 ^{h}	0.86 ± 0.07 ^{f}	0.39 ± 0.04 ^{g}	0.53 ± 0.03 ^{i}	3.22 ± 0.15 ^{kl}	0.75 ± 0.04 $^{e-g}$	1.46 ± 0.06 ^{j}	**8.68 ± 0.49** $^{j-l}$
Berkley	FD	0.46 ± 0.02 $^{h-j}$	3.68 ± 0.00 ^{c}	0.13 ± 0.01 ^{bc}	**4.27 ± 0.04** ^{c}	0.47 ± 0.06 ^{j}	0.49 ± 0.02 ^{fg}	0.54 ± 0.05 ^{e}	0.12 ± 0.00 ^{b}	0.37 ± 0.04 $^{f-h}$	2.53 ± 0.10 ^{ij}	0.14 ± 0.01 ^{ab}	1.18 ± 0.06 ^{i}	**5.84 ± 0.34** ^{h}
	VD50	0.49 ± 0.02 $^{j-m}$	3.66 ± 0.15 ^{c}	0.13 ± 0.00 $^{b-d}$	**4.28 ± 0.17** ^{c}	0.42 ± 0.04 ^{ij}	0.41 ± 0.00 $^{d-f}$	0.44 ± 0.01 ^{d}	0.08 ± 0.02 ^{ab}	0.30 ± 0.03 $^{d-f}$	2.16 ± 0.01 ^{gh}	0.11 ± 0.01 ^{ab}	0.98 ± 0.02 $^{f-i}$	**4.90 ± 0.09** $^{e-h}$
	VD70	0.48 ± 0.01 $^{j-l}$	3.66 ± 0.14 ^{c}	0.13 ± 0.01 $^{b-d}$	**4.27 ± 0.16** ^{c}	0.40 ± 0.01 $^{h-j}$	0.43 ± 0.03 $^{d-g}$	0.47 ± 0.01 ^{de}	0.10 ± 0.01 ^{ab}	0.30 ± 0.02 $^{d-f}$	2.27 ± 0.05 ^{hi}	0.12 ± 0.00 ^{ab}	1.04 ± 0.04 $^{g-i}$	**5.14 ± 0.17** $^{e-h}$
	VD90	0.47 ± 0.01 $^{i-k}$	3.50 ± 0.08 ^{bc}	0.11 ± 0.01 ^{b}	**4.09 ± 0.10** ^{bc}	0.36 ± 0.02 $^{g-i}$	0.37 ± 0.04 ^{de}	0.44 ± 0.04 ^{d}	0.11 ± 0.02 ^{ab}	0.27 ± 0.01 $^{c-e}$	1.89 ± 0.03 ^{g}	0.11 ± 0.00 ^{ab}	0.80 ± 0.01 $^{d-f}$	**4.36 ± 0.04** $^{d-f}$
	SD	0.48 ± 0.01 $^{j-l}$	3.68 ± 0.05 ^{c}	0.13 ± 0.00 $^{b-d}$	**4.29 ± 0.05** ^{c}	0.44 ± 0.02 ^{ij}	0.44 ± 0.02 $^{d-g}$	0.52 ± 0.02 ^{de}	0.13 ± 0.00 ^{bc}	0.35 ± 0.03 $^{f-h}$	2.33 ± 0.03 ^{hi}	0.15 ± 0.01 ^{ab}	1.07 ± 0.01 ^{hi}	**5.42 ± 0.07** ^{gh}
Bluecrop	FD	0.40 ± 0.03 ^{fg}	10.51 ± 0.53 ^{ij}	0.30 ± 0.03 ^{h}	**11.20 ± 0.59** ^{g}	0.36 ± 0.04 $^{g-i}$	0.51 ± 0.04 ^{g}	0.54 ± 0.04 ^{e}	0.57 ± 0.04 ^{k}	0.41 ± 0.04 ^{h}	2.71 ± 0.18 ^{j}	1.84 ± 0.05 ^{j}	2.20 ± 0.15 ^{n}	**9.15 ± 0.58** $^{k-m}$
	VD50	0.40 ± 0.02 $^{f-h}$	10.05 ± 0.11 ^{i}	0.28 ± 0.00 ^{gh}	**10.74 ± 0.12** ^{g}	0.32 ± 0.01 $^{f-h}$	0.45 ± 0.02 $^{e-g}$	0.49 ± 0.01 ^{de}	0.51 ± 0.00 ^{jk}	0.39 ± 0.01 ^{gh}	2.45 ± 0.03 $^{h-j}$	1.71 ± 0.05 ^{ij}	2.01 ± 0.01 ^{mn}	**8.33 ± 0.02** $^{i-k}$
	VD70	0.42 ± 0.00 $^{g-i}$	10.12 ± 0.04 ^{ij}	0.29 ± 0.00 ^{h}	**10.82 ± 0.05** ^{g}	0.29 ± 0.03 $^{d-g}$	0.41 ± 0.03 $^{d-f}$	0.46 ± 0.02 ^{de}	0.47 ± 0.03 ^{ij}	0.34 ± 0.03 $^{d-h}$	2.31 ± 0.11 ^{hi}	1.57 ± 0.04 ^{i}	1.82 ± 0.08 ^{lm}	**7.66 ± 0.39** ^{i}
	VD90	0.36 ± 0.03 ^{ef}	9.27 ± 0.35 ^{h}	0.19 ± 0.01 ^{f}	**9.81 ± 0.40** ^{f}	0.20 ± 0.01 $^{a-d}$	0.25 ± 0.01 ^{c}	0.28 ± 0.03 ^{c}	0.32 ± 0.02 ^{f}	0.19 ± 0.02 ^{b}	1.39 ± 0.16 ^{de}	1.05 ± 0.12 ^{h}	0.94 ± 0.11 $^{f-h}$	**4.63 ± 0.47** $^{e-g}$
	SD	0.40 ± 0.00 $^{f-h}$	10.74 ± 0.27 ^{j}	0.31 ± 0.01 ^{h}	**11.45 ± 0.27** ^{g}	0.30 ± 0.00 $^{e-g}$	0.44 ± 0.00 $^{d-g}$	0.48 ± 0.00 ^{de}	0.49 ± 0.02 ^{ij}	0.35 ± 0.00 $^{e-h}$	2.48 ± 0.05 $^{h-j}$	1.66 ± 0.06 ^{i}	1.96 ± 0.03 ^{m}	**8.17 ± 0.16** ^{ij}
								5% inulin						
Bluejay	FD	0.28 ± 0.00 ^{cd}	3.34 ± 0.02 ^{bc}	0.16 ± 0.00 $^{c-f}$	**3.77 ± 0.01** ^{bc}	0.49 ± 0.02 ^{j}	0.45 ± 0.02 $^{e-g}$	0.55 ± 0.02 ^{e}	0.25 ± 0.01 $^{d-f}$	0.35 ± 0.00 $^{f-h}$	1.95 ± 0.02 ^{g}	0.46 ± 0.01 ^{d}	0.91 ± 0.01 $^{f-h}$	**5.42 ± 0.08** ^{gh}
	VD50	0.28 ± 0.01 ^{cd}	3.37 ± 0.10 ^{bc}	0.16 ± 0.01 $^{c-f}$	**3.81 ± 0.12** ^{bc}	0.47 ± 0.03 ^{j}	0.43 ± 0.02 $^{d-f}$	0.52 ± 0.02 ^{de}	0.24 ± 0.00 ^{de}	0.33 ± 0.01 $^{d-g}$	1.85 ± 0.10 ^{fg}	0.43 ± 0.03 ^{d}	0.87 ± 0.03 $^{e-h}$	**5.13 ± 0.24** $^{e-h}$
	VD70	0.28 ± 0.01 ^{cd}	3.27 ± 0.09 ^{bc}	0.15 ± 0.00 $^{b-e}$	**3.70 ± 0.11** ^{bc}	0.41 ± 0.03 $^{h-j}$	0.36 ± 0.03 ^{d}	0.44 ± 0.03 ^{d}	0.20 ± 0.00 ^{de}	0.27 ± 0.01 ^{cd}	1.55 ± 0.06 ^{ef}	0.36 ± 0.00 ^{cd}	0.69 ± 0.02 $^{c-e}$	**4.28 ± 0.17** ^{de}
	VD90	0.25 ± 0.01 $^{b-d}$	2.92 ± 0.07 ^{b}	0.13 ± 0.00 $^{b-d}$	**3.30 ± 0.08** ^{b}	0.22 ± 0.01 $^{b-e}$	0.20 ± 0.01 $^{a-c}$	0.26 ± 0.01 ^{bc}	0.12 ± 0.00 ^{bc}	0.16 ± 0.00 ^{ab}	0.90 ± 0.04 ^{ab}	0.25 ± 0.02 ^{bc}	0.38 ± 0.00 ^{ab}	**2.48 ± 0.04** ^{b}
	SD	0.30 ± 0.01 ^{de}	3.57 ± 0.02 ^{bc}	0.17 ± 0.00 ^{ef}	**4.04 ± 0.02** ^{bc}	0.49 ± 0.01 ^{j}	0.45 ± 0.00 $^{e-g}$	0.54 ± 0.00 ^{e}	0.25 ± 0.00 ^{de}	0.33 ± 0.00 $^{d-g}$	1.92 ± 0.02 ^{g}	0.43 ± 0.02 ^{d}	0.88 ± 0.02 $^{e-h}$	**5.29 ± 0.02** $^{f-h}$
Berkley	FD	0.24 ± 0.01 $^{a-c}$	1.93 ± 0.00 ^{a}	0.07 ± 0.00 ^{a}	**2.24 ± 0.01** ^{a}	0.22 ± 0.01 $^{b-e}$	0.24 ± 0.00 ^{bc}	0.27 ± 0.02 ^{bc}	0.07 ± 0.00 ^{ab}	0.17 ± 0.01 ^{b}	1.25 ± 0.03 $^{c-e}$	0.07 ± 0.00 ^{a}	0.57 ± 0.00 ^{bc}	**2.86 ± 0.06** ^{bc}
	VD50	0.25 ± 0.00 $^{b-d}$	1.96 ± 0.02 ^{a}	0.07 ± 0.00 ^{a}	**2.28 ± 0.03** ^{a}	0.22 ± 0.00 $^{b-e}$	0.22 ± 0.00 ^{bc}	0.26 ± 0.00 ^{bc}	0.06 ± 0.01 ^{ab}	0.16 ± 0.01 ^{ab}	1.21 ± 0.00 $^{b-d}$	0.06 ± 0.01 ^{a}	0.55 ± 0.03 ^{bc}	**2.76 ± 0.02** ^{bc}
	VD70	0.25 ± 0.00 $^{b-d}$	1.95 ± 0.01 ^{a}	0.07 ± 0.00 ^{a}	**2.26 ± 0.01** ^{a}	0.23 ± 0.01 $^{b-f}$	0.23 ± 0.01 ^{bc}	0.26 ± 0.01 ^{bc}	0.06 ± 0.01 ^{ab}	0.15 ± 0.00 ^{ab}	1.16 ± 0.00 $^{b-d}$	0.06 ± 0.00 ^{a}	0.52 ± 0.01 ^{bc}	**2.66 ± 0.01** ^{bc}
	VD90	0.24 ± 0.00 $^{a-c}$	1.81 ± 0.00 ^{a}	0.06 ± 0.00 ^{a}	**2.11 ± 0.01** ^{a}	0.13 ± 0.03 ^{ab}	0.13 ± 0.01 ^{a}	0.15 ± 0.00 ^{a}	0.05 ± 0.02 ^{a}	0.09 ± 0.02 ^{a}	0.63 ± 0.04 ^{a}	0.05 ± 0.01 ^{a}	0.26 ± 0.03 ^{a}	**1.49 ± 0.11** ^{a}
	SD	0.27 ± 0.00 ^{cd}	2.09 ± 0.04 ^{a}	0.07 ± 0.00 ^{a}	**2.43 ± 0.04** ^{a}	0.25 ± 0.00 $^{c-f}$	0.26 ± 0.01 ^{c}	0.27 ± 0.01 ^{bc}	0.06 ± 0.01 ^{ab}	0.18 ± 0.00 ^{b}	1.28 ± 0.02 $^{c-e}$	0.07 ± 0.01 ^{a}	0.58 ± 0.02 ^{bc}	**2.95 ± 0.04** ^{bc}
Bluecrop	FD	0.20 ± 0.00 ^{ab}	5.33 ± 0.04 ^{de}	0.15 ± 0.00 $^{c-f}$	**5.68 ± 0.03** ^{d}	0.17 ± 0.01 $^{a-c}$	0.24 ± 0.01 ^{c}	0.26 ± 0.00 ^{bc}	0.26 ± 0.01 ^{ef}	0.20 ± 0.00 ^{bc}	1.29 ± 0.01 $^{c-e}$	0.87 ± 0.02 ^{fg}	1.02 ± 0.02 $^{g-i}$	**4.32 ± 0.05** ^{de}
	VD50	0.21 ± 0.00 ^{ab}	5.27 ± 0.00 ^{d}	0.16 ± 0.00 $^{c-f}$	**5.64 ± 0.00** ^{d}	0.15 ± 0.01 ^{ab}	0.22 ± 0.01 ^{bc}	0.24 ± 0.01 $^{a-c}$	0.25 ± 0.01 $^{d-f}$	0.18 ± 0.01 ^{b}	1.28 ± 0.04 $^{c-e}$	0.84 ± 0.06 ^{fg}	1.02 ± 0.04 $^{g-i}$	**4.20 ± 0.19** ^{de}
	VD70	0.19 ± 0.00 ^{a}	4.84 ± 0.08 ^{d}	0.13 ± 0.01 $^{b-d}$	**5.16 ± 0.07** ^{d}	0.13 ± 0.00 ^{ab}	0.19 ± 0.01 $^{a-c}$	0.21 ± 0.01 $^{a-c}$	0.22 ± 0.01 ^{de}	0.16 ± 0.01 ^{ab}	1.06 ± 0.02 ^{bc}	0.73 ± 0.01 $^{e-g}$	0.83 ± 0.03 $^{d-g}$	**3.54 ± 0.10** ^{cd}
	VD90	0.21 ± 0.02 ^{ab}	5.44 ± 0.29 $^{d-f}$	0.16 ± 0.01 $^{d-f}$	**5.81 ± 0.33** ^{d}	0.17 ± 0.01 $^{a-c}$	0.24 ± 0.02 ^{bc}	0.26 ± 0.02 ^{bc}	0.25 ± 0.02 $^{d-f}$	0.18 ± 0.01 ^{b}	1.30 ± 0.11 $^{c-e}$	0.89 ± 0.08 ^{g}	1.02 ± 0.08 $^{g-i}$	**4.30 ± 0.35** ^{de}
	SD	0.20 ± 0.03 ^{ab}	4.99 ± 0.39 ^{d}	0.15 ± 0.01 $^{c-f}$	**5.35 ± 0.44** ^{d}	0.12 ± 0.01 ^{a}	0.16 ± 0.01 ^{ab}	0.18 ± 0.02 ^{ab}	0.19 ± 0.02 ^{cd}	0.13 ± 0.02 ^{ab}	0.89 ± 0.10 ^{ab}	0.66 ± 0.08 ^{e}	0.64 ± 0.09 ^{cd}	**2.98 ± 0.35** ^{bc}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying; $^{a, b, c, d\ldots}$ - different letters within the column indicated statistical differences ($p < 0.05$; HSD Tukey test).

about 45% lower content of these two constituents when compared to carrier-free products.

3.2.4. Flavan-3-ols

Flavan-3-ols were the least abundant group identified in blueberry powders, among which procyanidin B1, flavan-3-ol derivative, and (+)-catechin were identified and quantified (Table 2b). In most cases, the lowest content of flavan-3-ols was found in 'Bluejay' products, and the highest content – in 'Bluecrop' powders. The carrier addition affected the content of flavan-3-ols in a way similar to the other groups of phenolics, while the drying technique had divergent effects depending on the cultivar, with SD allowing the greatest retention of these compounds in most cases. Procyanidin B1 was found to be dominant among flavan-3-ols in all powders. All 'Bluejay' powders were characterized by the lowest amount of this constituent, while relatively comparable levels were identified in 'Bluecrop' and 'Berkley' products. The drying technique exerted a considerably different influence on the procyanidin B1 amount, depending on blueberry cultivar and inulin addition. The products of 'Berkley' after VD90 had the lowest, while spray-dried ones had the highest content of this compound. However, in the case of 'Bluecrop' powders, the same drying technique differently influenced inulin-free and inulin-supplemented samples, and the application of SD and VD90 resulted in powders with the highest procyanidin B1 content. A similar trend was observed for the flavan-3-ol derivative. The highest content of (+)-catechin was noted for 'Bluecrop' products, and the lowest for 'Berkley' ones. The only exceptions were observed among the powders after VD90 (inulin-free samples) and SD (inulin-added samples), of which 'Bluejay' powders were characterized by the highest amount of (+) -catechin.

3.2.5. Antioxidant capacity

The antioxidant capacity of powders measured by TEAC ABTS and FRAP methods indicated a strong influence of carrier addition and cultivar, while the drying technique resulted in only minor variations (Fig. 1a and b). The lowest antioxidant capacity was noted for 'Berkley' powders, while products from the other cultivars showed approx. 30% higher TEAC ABTS and FRAP values. The carrier addition caused an approx. 2-fold decrease in antioxidant capacity. Moreover, it was observed that 'Bluecrop' inulin-free and 'Bluejay' inulin loaded powder samples had the highest antioxidant capacity. An increase in temperature during VD resulted in products with antioxidant capacity similar or even higher than freeze- or spray-dried powders. This may be ascribed to the release of particular constituents from more polymerized structures or their structural changes, resulting in compounds with higher antioxidant capacity than parental ones (Liu, Le Bourvellec, Guyot, & Renard, 2021; Michalska-Ciechanowska, Brzezowska, et al., 2021). It can also be linked to the possible formation of Maillard reaction and/or caramelization products during heat treatment, which exhibit antioxidant properties (Nooshkam, Varidi, & Bashash, 2019) that may additionally improve powders properties. After carrier application, drying did not affect the antioxidant capacity of the powders in the same way for all cultivars, which may indicate multiple interactions between inulin and the matrix components during drying, including interactions resulting in enhanced antiradical properties.

Considering all studied cultivars together, although the sum of the identified phenolics had a significant effect on the antioxidant capacity of the powders ($r = 0.93$ for TEAC ABTS and $r = 0.95$ for FRAP) (Fig. S1a, supplementary file), it was flavonols that were the most prominent compounds, the presence of which was most strongly correlated with the values measured by both the ABTS ($r = 0.90$) and the FRAP ($r = 0.92$) method, despite the fact that these compounds were not the dominant ones in the blueberry extract powders. It is worth mentioning that no correlation was found between antioxidant capacity and quercetin-3-dimethyloxyrhamnoside.

3.3. Antibacterial and anti-inflammatory properties

3.3.1. Effect of drying technique, cultivar, and inulin addition on the antibacterial properties of blueberry powders

One of the most relevant bioactivities associated with plant products rich in phenolic compounds, such as blueberry powders, is their antibacterial activity. In the presented study, none of the blueberry powders showed antibacterial activity against *E. coli*, *S. enterica, S. aureus*, and *L. monocytogenes* strains (data not shown). This is contrary to previous studies describing blueberry effectiveness against these microorganisms (Shen et al., 2014; Silva et al., 2015). This confirms that moderation of fruit-based matrices affects biological properties of powdered products (Capuano et al., 2018). Although the phenolics composition may differ depending on the powder production method, involving matrix modification, the most disputable aspect in the previous works was a relatively high concentration of experimental samples used. In this regard, it was reported that blueberry samples showed an inhibitory effect against *L. monocytogenes* and *S. aureus* strains, but this result was obtained using experimental concentrations of 300–900 mg · mL^{-1} (Shen et al., 2014) and of 50–200 mg · mL^{-1} (Khalifa, Kamimoto, Shimamoto, & Shimamoto, 2015; Zhou et al., 2020), respectively. The same applies to *E. coli* and *Salmonella* strains – blueberry sample concentrations of 25–50 mg · mL^{-1} were used to obtain the inhibitory effect against *E. coli* (Khalifa et al., 2015) or even higher concentrations were used against *Salmonella* (450–1800 mg · mL^{-1}) (Shen et al., 2014). Considering that in most cases the strength of the inhibitory effect was dose-dependent, the importance of using experimental samples compatible with their practical application has been highlighted. In this sense, all blueberry powders with and without inulin (2 mg · mL^{-1}) used in the present work significantly inhibited the growth ($p < 0.05$) of the microaerophilic strains *C. jejuni* and *H. pylori* (Table 3), to a different extent, compared to the experimental growth control.

For *C. jejuni* (Table 3), when analyzing the effect of carrier addition, all samples without inulin were significantly more active in reducing bacterial growth (from 0.7 to 2.4 log CFU · mL^{-1} reduction) than those with 5% inulin (from 0.3 to 1.1 log CFU · mL^{-1} reduction), irrespectively of the cultivar and drying treatment applied. This behavior seems consistent with higher content of identified phenolics in the samples without inulin compared to those with carrier addition (Table 2a and 2b). Considering the influence of the blueberry cultivar, 'Bluejay' powders showed the highest antibacterial activity, independently of the drying technique, reducing *Campylobacter* growth between 1.40 and 2.38 log CFU mL^{-1} in absence of inulin, and between 0.70 and 1.09 log CFU mL^{-1} with inulin added.

The highest content of phenolics was obtained from 'Bluecrop' (Table 2a and 2b), which suggests that it is not the phenolic compounds in general, but the presence of some specific ones, which may determine the efficacy of the powder as antibacterial against *Campylobacter* (Silvan et al., 2013). In this respect, specific phenolic compounds present in different cultivars have been shown to be antibacterial agents against *Campylobacter*. In this study, a moderate correlation was found only between the inhibition of the growth of *C. jejuni* and the content of caffeoyl-glucose ($r = 0.62$) and identified anthocyanins ($r = 0.57$) (Fig. S1a; supplementary file), respectively, for all cultivars. However, at a closer look, a strong correlation between caffeoyl-glucose content and *Campylobacter* growth was indicated for 'Bluejay' ($r = 0.79$) and 'Berkley' ($r = 0.93$) (Fig. S1c and d, supplementary file). As for anthocyanins, there was a significant correlation between the growth of *Campylobacter* and the anthocyanin content in 'Bluejay' ($r = 0.65$) and 'Berkley' ($r = 0.85$), but no significant correlation was found for 'Bluecrop' (Fig. S1b, supplementary file).

Finally, when it comes to the impact of the drying technique used, VD treatments produced powders with the highest antibacterial activity from inulin-free extracts (Table 3). For 'Bluejay' and 'Berkley', the most effective treatment was VD90, while for 'Bluecrop', the cultivar richest in phenolics, the antibacterial behavior was observed for all

Table 2b
Flavonols and flavan-3-ols identified in blueberry juice extract powders obtained by different drying techniques with or without a carrier [mg · 100 g^{-1} dm].

Cultivar	Drying technique	Myricetin-3-*O*-galactoside	Quercetin-3-*O*-rutinoside	Quercetin-3-*O*-galactoside	Quercetin-3-*O*-glucoside	Quercetin-3-*O*-arabinofuranoside	Quercetin-3-*O*-oxalylpentoside	Quercetin-3-*O*-rhamnoside	Quercetin-3-*O*-dimethoxyrhamnoside	Quercetin hexuronide 2	Quercetin-3-*O*-(acetyl) hexoside	Syringetin-3-*O*-rhamnoside	Sum of flavonols	(+)-Catechin	Procyanidin B1	Flavan-3-ol derivative	Sum of flavan-3-ols
									No carrier								
	FD	0.14 ± 0.01 ^{g}	0.32 ± 0.00 ^{fg}	1.57 ± 0.02 ^{h}	0.90 ± 0.02 ^{i}	0.39 ± 0.01 ^{hi}	0.64 ± 0.00 ^{n}	0.20 ± 0.00 ^{de}	0.85 ± 0.01 ^{h}	0.46 ± 0.01 ^{g}	0.47 ± 0.03 $^{f-h}$	0.35 ± 0.03 ^{c}	**6.30 ± 0.08** ^{jk}	0.57 ± 0.00 $^{g-i}$	0.70 ± 0.01 ^{ij}	0.58 ± 0.01 $^{f-h}$	**1.85 ± 0.00** $^{c-g}$
	VD50	0.12 ± 0.01 ^{f}	0.29 ± 0.00 ^{f}	1.50 ± 0.00 $^{f-h}$	0.89 ± 0.02 ^{hi}	0.42 ± 0.01 i	0.58 ± 0.00 ^{m}	0.21 ± 0.00 ^{e}	0.81 ± 0.01 ^{h}	0.45 ± 0.01 ^{g}	0.47 ± 0.01 $^{f-h}$	0.34 ± 0.01 ^{c}	**6.07 ± 0.00** $^{i-k}$	0.52 ± 0.00 $^{f-h}$	0.63 ± 0.00 $^{g-i}$	0.51 ± 0.02 $^{d-f}$	**1.66 ± 0.02** ^{cd}
Bluejay	VD70	0.12 ± 0.00 ^{f}	0.29 ± 0.02 ^{f}	1.50 ± 0.06 $^{f-h}$	0.89 ± 0.03 ^{hi}	0.40 ± 0.01 ^{hi}	0.58 ± 0.03 ^{m}	0.22 ± 0.01 ^{e}	0.83 ± 0.04 ^{h}	0.45 ± 0.01 ^{g}	0.47 ± 0.04 $^{f-h}$	0.36 ± 0.01 ^{c}	**6.11 ± 0.25** $^{i-k}$	0.53 ± 0.04 $^{f-h}$	0.66 ± 0.03 ^{hi}	0.54 ± 0.02 $^{e-g}$	**1.73 ± 0.09** $^{c-e}$
	VD90	0.12 ± 0.00 ^{f}	0.29 ± 0.01 ^{f}	1.53 ± 0.03 $^{f-h}$	0.90 ± 0.00 ^{i}	0.39 ± 0.00 ^{hi}	0.56 ± 0.00 ^{m}	0.23 ± 0.01 ^{e}	0.80 ± 0.00 ^{h}	0.45 ± 0.01 ^{g}	0.48 ± 0.01 ^{gh}	0.35 ± 0.01 ^{c}	**6.09 ± 0.08** $^{i-k}$	0.47 ± 0.01 ^{ef}	0.61 ± 0.01 $^{f-i}$	0.50 ± 0.01 $^{c-f}$	**1.58 ± 0.02** ^{c}
	SD	0.13 ± 0.01 ^{fg}	0.31 ± 0.01 ^{f}	1.53 ± 0.04 $^{f-h}$	0.89 ± 0.02 ^{hi}	0.42 ± 0.01 ^{i}	0.64 ± 0.01 ^{n}	0.22 ± 0.01 ^{e}	0.84 ± 0.01 ^{h}	0.47 ± 0.02 ^{g}	0.48 ± 0.00 $^{f-h}$	0.35 ± 0.02 ^{c}	**6.27 ± 0.11** ^{jk}	0.59 ± 0.01 $^{g-i}$	0.69 ± 0.01 ^{ij}	0.58 ± 0.01 $^{f-h}$	**1.86 ± 0.03** $^{c-g}$
	FD	0.06 ± 0.00 $^{c-e}$	0.11 ± 0.01 $^{a-e}$	1.60 ± 0.01 ^{h}	0.62 ± 0.02 $^{c-f}$	0.41 ± 0.00 ^{i}	0.43 ± 0.01 ^{l}	0.06 ± 0.01 $^{a-c}$	1.08 ± 0.00 ^{j}	0.48 ± 0.03 ^{gh}	0.37 ± 0.01 $^{d-g}$	0.35 ± 0.01 ^{c}	**5.57 ± 0.07** $^{h-j}$	0.43 ± 0.02 ^{e}	0.82 ± 0.10 ^{jk}	0.64 ± 0.11 $^{f-j}$	**1.89 ± 0.23** $^{c-g}$
	VD50	0.06 ± 0.00 ^{cd}	0.11 ± 0.00 $^{a-e}$	1.50 ± 0.05 $^{f-h}$	0.58 ± 0.02 $^{c-e}$	0.37 ± 0.00 $^{g-i}$	0.37 ± 0.01 $^{j-l}$	0.05 ± 0.00 ^{ab}	1.02 ± 0.04 ^{j}	0.46 ± 0.02 ^{g}	0.32 ± 0.04 $^{b-d}$	0.34 ± 0.02 ^{c}	**5.18 ± 0.20** ^{gh}	0.47 ± 0.06 ^{ef}	0.89 ± 0.08 $^{k-m}$	0.69 ± 0.10 $^{h-j}$	**2.05 ± 0.24** $^{e-h}$
Berkley	VD70	0.06 ± 0.00 ^{cd}	0.10 ± 0.01 $^{a-e}$	1.54 ± 0.07 ^{gh}	0.58 ± 0.01 $^{c-e}$	0.39 ± 0.03 ^{hi}	0.39 ± 0.03 ^{kl}	0.06 ± 0.00 $^{a-c}$	1.06 ± 0.06 ^{j}	0.49 ± 0.02 ^{gh}	0.34 ± 0.01 $^{b-e}$	0.34 ± 0.01 ^{c}	**5.35 ± 0.23** ^{hi}	0.47 ± 0.00 ^{ef}	0.89 ± 0.00 $^{k-m}$	0.70 ± 0.00 $^{h-j}$	**2.06 ± 0.00** $^{e-i}$
	VD90	0.05 ± 0.00 ^{bc}	0.08 ± 0.00 $^{a-c}$	1.44 ± 0.02 $^{f-h}$	0.56 ± 0.00 ^{cd}	0.36 ± 0.00 $^{g-i}$	0.35 ± 0.00 $^{i-k}$	0.07 ± 0.00 $^{a-c}$	1.01 ± 0.02 ^{j}	0.46 ± 0.00 ^{g}	0.34 ± 0.01 $^{b-e}$	0.32 ± 0.01 ^{c}	**5.05 ± 0.04** ^{gh}	0.42 ± 0.02 ^{e}	0.86 ± 0.02 ^{kl}	0.69 ± 0.02 $^{h-j}$	**1.97 ± 0.06** $^{d-h}$
	SD	0.06 ± 0.01 $^{c-e}$	0.10 ± 0.00 $^{a-d}$	1.59 ± 0.04 ^{h}	0.64 ± 0.04 $^{c-f}$	0.42 ± 0.01 ^{i}	0.42 ± 0.00 ^{l}	0.07 ± 0.01 $^{a-c}$	1.07 ± 0.00 ^{j}	0.50 ± 0.01 ^{gh}	0.36 ± 0.01 $^{c-f}$	0.34 ± 0.02 ^{c}	**5.56 ± 0.09** $^{h-j}$	0.50 ± 0.00 $^{e-g}$	0.91 ± 0.01 $^{k-m}$	0.75 ± 0.02 ^{j}	**2.16 ± 0.03** $^{f-i}$
	FD	ND	0.77 ± 0.06 ^{jk}	2.51 ± 0.16 ^{j}	1.45 ± 0.06 ^{k}	0.66 ± 0.04 ^{k}	0.35 ± 0.02 $^{i-k}$	0.69 ± 0.04 ^{gh}	0.13 ± 0.00 ^{ab}	0.69 ± 0.03 ^{i}	1.03 ± 0.06 ^{j}	ND	**8.28 ± 0.46** ^{l}	0.61 ± 0.05 ^{hi}	0.98 ± 0.08 ^{lm}	0.71 ± 0.06 $^{h-j}$	**2.30 ± 0.19** ^{hi}
	VD50	ND	0.74 ± 0.02 ^{jk}	2.44 ± 0.05 ^{j}	1.41 ± 0.02 ^{k}	0.63 ± 0.00 ^{jk}	0.32 ± 0.01 ^{hi}	0.67 ± 0.01 ^{gh}	0.12 ± 0.01 ^{ab}	0.67 ± 0.00 ^{i}	0.98 ± 0.02 ^{j}	ND	**7.97 ± 0.09** ^{l}	0.59 ± 0.02 $^{g-i}$	0.94 ± 0.01 $^{k-m}$	0.67 ± 0.00 $^{g-j}$	**2.20 ± 0.04** $^{g-i}$
Bluecrop	VD70	ND	0.71 ± 0.02 ^{j}	2.35 ± 0.05 ^{j}	1.34 ± 0.03 ^{k}	0.59 ± 0.01 ^{j}	0.32 ± 0.01 $^{h-j}$	0.67 ± 0.03 ^{gh}	0.13 ± 0.00 ^{ab}	0.65 ± 0.01 ^{i}	0.95 ± 0.04 ^{j}	ND	**7.71 ± 0.20** ^{l}	0.55 ± 0.01 $^{f-h}$	0.94 ± 0.00 $^{k-m}$	0.67 ± 0.00 $^{g-j}$	**2.16 ± 0.01** $^{f-i}$
	VD90	ND	0.62 ± 0.03 ^{i}	1.88 ± 0.17 ^{i}	1.12 ± 0.08 ^{j}	0.42 ± 0.02 ^{i}	0.27 ± 0.01 $^{f-h}$	0.62 ± 0.05 ^{g}	0.12 ± 0.01 ^{ab}	0.55 ± 0.04 ^{h}	0.82 ± 0.08 ^{i}	ND	**6.41 ± 0.48** ^{k}	0.40 ± 0.03 ^{de}	0.83 ± 0.05 ^{jk}	0.60 ± 0.05 $^{f-i}$	**1.83 ± 0.13** $^{c-f}$
	SD	ND	0.79 ± 0.03 ^{k}	2.53 ± 0.05 ^{j}	1.44 ± 0.01 ^{k}	0.65 ± 0.02 ^{jk}	0.36 ± 0.01 $^{i-k}$	0.71 ± 0.01 ^{h}	0.13 ± 0.00 ^{b}	0.69 ± 0.01 ^{i}	1.02 ± 0.02 ^{j}	ND	**8.32 ± 0.15** ^{l}	0.66 ± 0.04 ^{i}	1.02 ± 0.03 ^{m}	0.74 ± 0.05 ^{ij}	**2.42 ± 0.12** ^{i}
									5% inulin								
	FD	0.07 ± 0.00 ^{de}	0.17 ± 0.00 ^{de}	0.89 ± 0.02 ^{ab}	0.55 ± 0.02 ^{cd}	0.25 ± 0.01 ^{cd}	0.36 ± 0.00 $^{i-k}$	0.12 ± 0.00 ^{bc}	0.48 ± 0.00 $^{c-e}$	0.26 ± 0.01 $^{a-c}$	0.27 ± 0.00 $^{a-d}$	0.20 ± 0.00 ^{ab}	**3.63 ± 0.01** $^{b-e}$	0.29 ± 0.00 ^{bc}	0.36 ± 0.00 $^{a-c}$	0.30 ± 0.00 ^{ab}	**0.95 ± 0.01** ^{ab}
	VD50	0.07 ± 0.00 ^{de}	0.16 ± 0.00 ^{de}	0.88 ± 0.03 ^{ab}	0.54 ± 0.02 ^{cd}	0.22 ± 0.02 $^{a-d}$	0.32 ± 0.02 ^{hi}	0.12 ± 0.01 ^{bc}	0.45 ± 0.01 ^{cd}	0.25 ± 0.01 ^{ab}	0.25 ± 0.01 $^{a-c}$	0.20 ± 0.01 ^{ab}	**3.46 ± 0.13** $^{a-e}$	0.29 ± 0.00 ^{bc}	0.36 ± 0.01 $^{a-d}$	0.29 ± 0.01 ^{ab}	**0.94 ± 0.02** ^{ab}
Bluejay	VD70	0.07 ± 0.00 ^{de}	0.15 ± 0.00 $^{c-e}$	0.86 ± 0.03 ^{ab}	0.52 ± 0.02 ^{bc}	0.21 ± 0.03 $^{a-c}$	0.29 ± 0.03 ^{gh}	0.12 ± 0.02 ^{bc}	0.45 ± 0.03 ^{cd}	0.25 ± 0.02 ^{ab}	0.24 ± 0.05 $^{a-c}$	0.19 ± 0.02 ^{ab}	**3.35 ± 0.25** $^{a-d}$	0.26 ± 0.00 $^{a-c}$	0.34 ± 0.00 ^{ab}	0.27 ± 0.00 ^{ab}	**0.87 ± 0.00** ^{ab}
	VD90	0.06 ± 0.00 ^{cd}	0.13 ± 0.01 $^{b-e}$	0.68 ± 0.02 ^{a}	0.41 ± 0.01 ^{ab}	0.16 ± 0.01 ^{a}	0.25 ± 0.00 $^{e-g}$	0.12 ± 0.00 ^{bc}	0.40 ± 0.02 ^{c}	0.21 ± 0.00 ^{a}	0.22 ± 0.01 ^{ab}	0.17 ± 0.01 ^{a}	**2.81 ± 0.08** ^{a}	0.22 ± 0.01 ^{ab}	0.31 ± 0.01 ^{a}	0.24 ± 0.00 ^{a}	**0.77 ± 0.02** ^{a}
	SD	0.08 ± 0.00 ^{e}	0.17 ± 0.00 ^{e}	0.93 ± 0.03 ^{bc}	0.56 ± 0.02 $^{c-e}$	0.25 ± 0.00 $^{b-d}$	0.38 ± 0.00 ^{kl}	0.13 ± 0.00 ^{cd}	0.50 ± 0.00 $^{d-f}$	0.28 ± 0.01 $^{a-d}$	0.29 ± 0.00 $^{a-d}$	0.22 ± 0.01 ^{b}	**3.79 ± 0.06** $^{c-f}$	0.31 ± 0.00 $^{b-d}$	0.39 ± 0.00 $^{a-e}$	0.32 ± 0.01 ^{ab}	**1.02 ± 0.01** ^{ab}
	FD	0.04 ± 0.00 ^{ab}	0.05 ± 0.00 ^{a}	0.87 ± 0.00 ^{ab}	0.33 ± 0.00 ^{a}	0.23 ± 0.00 $^{b-d}$	0.24 ± 0.00 $^{d-g}$	0.04 ± 0.00 ^{a}	0.59 ± 0.00 ^{g}	0.27 ± 0.00 $^{a-c}$	0.19 ± 0.00 ^{a}	0.19 ± 0.00 ^{ab}	**3.04 ± 0.02** $^{a-c}$	0.24 ± 0.00 $^{a-c}$	0.48 ± 0.00 $^{b-f}$	0.38 ± 0.00 $^{a-d}$	**1.10 ± 0.01** ^{ab}
Berkley	VD50	0.03 ± 0.00 ^{ab}	0.05 ± 0.00 ^{a}	0.86 ± 0.01 ^{ab}	0.33 ± 0.00 ^{a}	0.23 ± 0.02 $^{b-d}$	0.23 ± 0.02 $^{c-f}$	0.04 ± 0.01 ^{a}	0.58 ± 0.02 ^{g}	0.26 ± 0.02 ^{ab}	0.19 ± 0.03 ^{a}	0.18 ± 0.00 ^{ab}	**2.96 ± 0.11** ^{ab}	0.24 ± 0.01 $^{a-c}$	0.48 ± 0.01 $^{c-f}$	0.39 ± 0.01 $^{a-d}$	**1.11 ± 0.02** ^{ab}
	VD70	0.03 ± 0.00 ^{ab}	0.05 ± 0.00 ^{a}	0.85 ± 0.00 ^{ab}	0.33 ± 0.00 ^{a}	0.21 ± 0.00 $^{a-c}$	0.22 ± 0.00 $^{b-e}$	0.03 ± 0.00 ^{a}	0.58 ± 0.00 ^{fg}	0.25 ± 0.01 ^{ab}	0.19 ± 0.01 ^{a}	0.18 ± 0.00 ^{ab}	**2.92 ± 0.03** ^{ab}	0.24 ± 0.01 $^{a-c}$	0.47 ± 0.01 $^{b-f}$	0.37 ± 0.01 $^{a-c}$	**1.07 ± 0.04** ^{ab}

(*continued on next page*)

Table 2b (*continued*)

Cultivar	Drying technique	Myricetin-3-O-galactoside	Quercetin-3-O-rutinoside	Quercetin-3-O-galactoside	Quercetin-3-O-glucoside	Quercetin-3-O-arabinofuranoside	Quercetin-3-O-oxalylpentoside	Quercetin-3-O-rhamnoside	Quercetin-3-O-dimethoxyrhamnoside	Quercetin hexuronide 2	Quercetin-3-O-(acetyl) hexoside	Syringetin-3-O-rhamnoside	Sum of flavonols	(+)-Catechin	Procyanidin B1	Flavan-3-ol derivative	Sum of flavan-3-ols
Bluecrop	VD90	0.03 ± 0.00 ^{a}	0.04 ± 0.00 ^{a}	0.79 ± 0.00 ^{ab}	0.31 ± 0.00 ^{a}	0.18 ± 0.01 ^{ab}	0.19 ± 0.01 $^{a-d}$	0.04 ± 0.00 ^{a}	0.55 ± 0.00 $^{e-g}$	0.24 ± 0.00 ^{ab}	0.18 ± 0.01 ^{a}	0.17 ± 0.01 ^{a}	**2.74 ± 0.01** ^{a}	0.19 ± 0.02 ^{a}	0.44 ± 0.01 $^{a-e}$	0.35 ± 0.01 ^{ab}	**0.98 ± 0.04** ^{ab}
	SD	0.04 ± 0.00 ^{ab}	0.06 ± 0.00 ^{ab}	0.95 ± 0.01 $^{b-d}$	0.37 ± 0.00 ^{a}	0.24 ± 0.00 $^{b-d}$	0.25 ± 0.01 $^{e-g}$	0.04 ± 0.00 ^{a}	0.63 ± 0.01 ^{g}	0.28 ± 0.01 $^{b-e}$	0.20 ± 0.00 ^{a}	0.20 ± 0.00 ^{ab}	**3.25 ± 0.02** $^{a-d}$	0.26 ± 0.01 $^{a-c}$	0.52 ± 0.01 $^{e-h}$	0.41 ± 0.01 $^{b-e}$	**1.18 ± 0.03** ^{b}
	FD	ND	0.39 ± 0.01 ^{gh}	1.31 ± 0.00 $^{e-g}$	0.74 ± 0.01 ^{fg}	0.32 ± 0.00 $^{e-g}$	0.17 ± 0.00 ^{ab}	0.36 ± 0.01 ^{f}	0.06 ± 0.00 ^{ab}	0.34 ± 0.00 $^{d-f}$	0.52 ± 0.01 ^{h}	ND	**4.22 ± 0.04** ^{ef}	0.31 ± 0.00 $^{b-d}$	0.51 ± 0.01 $^{e-g}$	0.36 ± 0.00 $^{a-c}$	**1.18 ± 0.01** ^{b}
	VD50	ND	0.39 ± 0.02 ^{gh}	1.28 ± 0.04 ^{ef}	0.74 ± 0.03 ^{fg}	0.32 ± 0.00 $^{e-g}$	0.16 ± 0.00 ^{a}	0.35 ± 0.01 ^{f}	0.07 ± 0.00 ^{ab}	0.35 ± 0.01 ^{ef}	0.52 ± 0.02 ^{h}	ND	**4.19 ± 0.12** ^{ef}	0.30 ± 0.01 ^{bc}	0.50 ± 0.01 $^{d-g}$	0.36 ± 0.00 ^{ab}	**1.16 ± 0.02** ^{b}
	VD70	ND	0.35 ± 0.01 ^{fh}	1.15 ± 0.01 $^{c-e}$	0.65 ± 0.00 $^{d-f}$	0.27 ± 0.01 $^{c-e}$	0.14 ± 0.01 ^{a}	0.32 ± 0.01 ^{f}	0.05 ± 0.00 ^{a}	0.30 ± 0.00 $^{b-f}$	0.45 ± 0.01 $^{e-h}$	ND	**3.68 ± 0.03** $^{b-f}$	0.27 ± 0.01 $^{a-c}$	0.45 ± 0.01 $^{b-e}$	0.32 ± 0.01 ^{ab}	**1.03 ± 0.02** ^{ab}
	VD90	ND	0.41 ± 0.03 ^{h}	1.37 ± 0.10 $^{e-h}$	0.78 ± 0.05 ^{gh}	0.34 ± 0.02 $^{f-h}$	0.18 ± 0.01 $^{a-c}$	0.38 ± 0.02 ^{f}	0.07 ± 0.00 ^{ab}	0.37 ± 0.03 ^{f}	0.55 ± 0.05 ^{h}	ND	**4.44 ± 0.31** ^{fg}	0.33 ± 0.04 ^{cd}	0.52 ± 0.06 $^{e-h}$	0.36 ± 0.05 $^{a-c}$	**1.21 ± 0.15** ^{b}
	SD	ND	0.36 ± 0.03 ^{fh}	1.18 ± 0.13 ^{de}	0.68 ± 0.07 $^{e-g}$	0.28 ± 0.03 $^{d-f}$	0.16 ± 0.02 ^{a}	0.38 ± 0.05 ^{f}	0.07 ± 0.01 ^{ab}	0.33 ± 0.04 $^{c-f}$	0.50 ± 0.06 ^{h}	ND	**3.93 ± 0.43** $^{d-f}$	0.24 ± 0.02 $^{a-c}$	0.46 ± 0.05 $^{b-e}$	0.33 ± 0.02 ^{ab}	**1.03 ± 0.10** ^{ab}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying; ND – not detected; $^{a, b, c, d, \ldots}$ - different letters within the column indicated statistical differences ($p < 0.05$; HSD Tukey test).

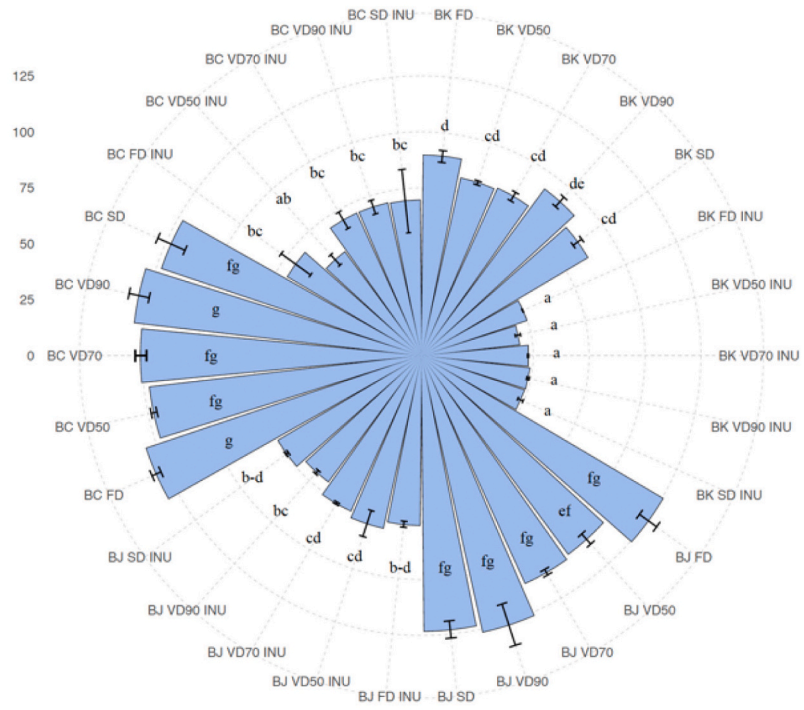
temperatures used (50, 70, and 90 °C). 'Bluejay' products without inulin and those obtained by VD90 achieved the greatest reduction of *Campylobacter* growth (2.4 CFU · mL^{-1} log reduction). For inulin-added samples the behavior was similar. 'Bluejay' products after VD at 70 and 90 °C, 'Bluecrop' products after VD at 50 °C, and 'Berkley' products after VD at 90 °C caused the strongest reduction in *Campylobacter* growth ($p < 0.05$). These results suggest that some compounds produced or released during VD could be involved in the antibacterial activity of these blueberry powders. It was reported that an increase in the temperature during VD could contribute to the enhancement of some bioactive properties, such as antioxidant capacity. This behavior has been attributed to the release of specific compounds from more polymerized structures and to the possible formation of non-enzymatic browning reaction products formed during heat treatment (Liu et al., 2021; Michalska-Ciechanowska, Brzezowska, et al., 2021). These newly formed products could show antibacterial activity (Chua, Chong, Chua, & Figiel, 2019) that may also contribute to improving the properties of blueberry powders. Although the antibacterial activity of blueberry powders against *Campylobacter* was not higher than 3 log reduction with respect to the experimental control, the obtained results can be considered relevant in practical terms. This is because a reduction of 2 log CFU in the number of campylobacters colonizing poultry (main source of human infection by *Campylobacter*) can have a significant impact on consumer health, reducing human infections by a median value of 42% (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2020). In the case of the poultry meat food chain, natural compounds such as blueberry powders could potentially be used at different stages of the food chain, from on-farm feed additives to packaging products.

On the other hand, the most relevant antibacterial effect was observed against *H. pylori*. Just as for *C. jejuni*, but at a higher level, all blueberry powders (with and without inulin) showed a significant antibacterial activity ($p < 0.05$) against *H. pylori* compared to the experimental growth control (Table 3). The antibacterial activity of carrier-free samples was significantly ($p < 0.05$) higher (5.5 log CFU mL^{-1} minimal growth reduction) than that of powders with inulin for each cultivar and same drying technique (2.17 log CFU · mL^{-1} minimal growth reduction), except for 'Bluejay' products obtained by SD, VD at 70 °C, and 90 °C, which showed similar antibacterial efficacy to the samples with inulin. It is noteworthy that ten of fifteen blueberry powders without inulin showed a bactericidal effect against *H. pylori*, while only one sample with 5% inulin was bactericidal ('Bluejay' product obtained by SD), which is linked to higher phenolics content. In terms of cultivar, 'Bluecrop' powders without inulin showed the strongest antibacterial activity, exhibiting bactericidal effects regardless of the drying techniques used. As described above, this blueberry cultivar had the highest content of phenolics (Table 2a and 2b). Bactericidal properties were also identified in 'Berkley' powders obtained by VD (regardless the temperature) as well as in two 'Bluejay' powders (VD50 and SD). However, inulin-supplemented 'Bluejay' powders were the most active in reducing bacterial growth from 5.44 log CFU · mL^{-1} to a total inhibition (bactericidal effect), regardless of the drying technique used. With respect to the influence of the drying techniques, VD treatments turned out to be the most effective in samples without inulin. All blueberry juice extract powders obtained by VD50 showed a bactericidal effect. The products obtained at 70 °C and 90 °C showed the same behavior, except for 'Bluejay'. Finally, for inulin-added powders, those obtained by SD from 'Bluejay', VD70 from 'Berkley', and VD90 and SD from 'Bluecrop' showed high antibacterial activity in each case.

3.3.2. Effect of drying method, blueberry cultivar, and inulin addition on the anti-inflammatory activity of blueberry powders on gastric cells infected with H. pylori

The inflammatory response of the gastric epithelium is directly related to the progression of pathologies associated with *H. pylori* infection. For this reason, it is crucial to modulate the inflammatory process to avoid the occurrence of cell damage (Silvan & Martinez-

(a)



(b)

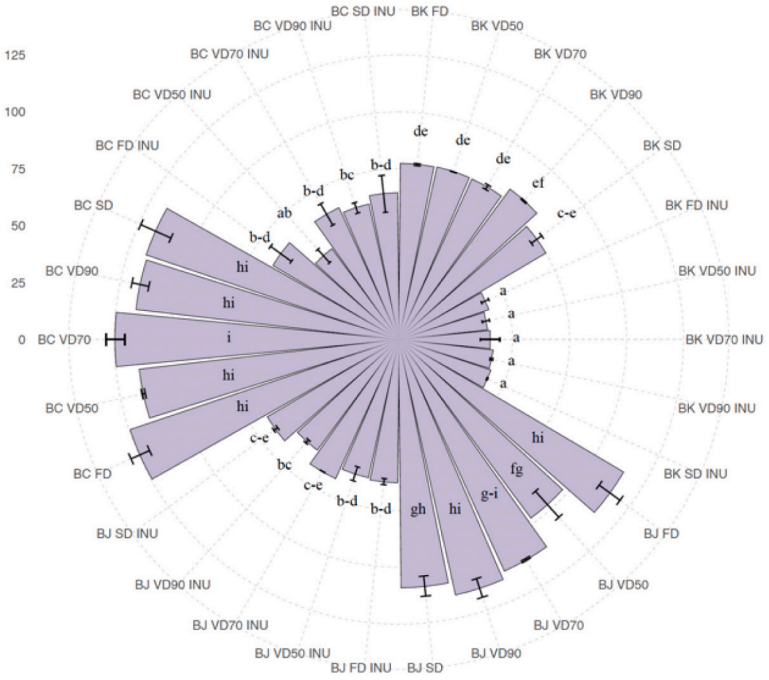


Fig. 1. Antioxidant capacity of blueberry juice extract powders made from *cv.* Berkey (BK), Bluejay (BJ) and Bluecrop (BC) cultivars without and with inulin (INU) after freeze-drying (FD), vacuum drying at 50 °C (VD50), vacuum drying at 70 °C (VD70); vacuum drying at 90 °C (VD90) and spray drying (SD) measured by (a) TEAC ABTS (Trolox Equivalent Antioxidant Capacity by ABTS), and (b) FRAP (Ferric Reducing Antioxidant Potential) methods [mmol Trolox ·100 g^{-1} dm]. $^{a, b, c, d\ldots}$ - different letters indicated statistical differences ($p < 0.05$; HSD Tukey test).

Table 3
Antibacterial activity against *Campylobacter jejuni* and *Helicobacter pylori* of blueberry juice extract powders obtained by different drying techniques with or without a carrier. Results are expressed as Log CFU · mL^{-1} (mean ± SD).

		Cultivar	FD	VD50	VD70	VD90	SD
Campylobacter jejuni	No carrier†	Bluejay	7.82 ± 0.10* ^{aC}	7.75 ± 0.10* ^{abC}	7.31 ± 0.05* ^{aB}	6.84 ± 0.06* ^{aA}	7.69 ± 0.07* ^{aC}
		Berkley	8.12 ± 0.06* ^{bC}	7.89 ± 0.14* ^{bB}	8.00 ± 0.06* ^{cBC}	7.69 ± 0.16* ^{bA}	7.83 ± 0.17* ^{aAB}
		Bluecrop	8.55 ± 0.07* ^{cB}	7.56 ± 0.19* ^{aA}	7.62 ± 0.22* ^{bA}	7.62 ± 0.08* ^{bA}	8.46 ± 0.07* ^{bB}
	5% inulin†	Bluejay	8.52 ± 0.07* ^{aB}	8.47 ± 0.03* ^{aB}	8.13 ± 0.07* ^{aA}	8.18 ± 0.05* ^{aA}	8.42 ± 0.09* ^{aB}
		Berkley	8.76 ± 0.04* ^{bA}	8.94 ± 0.03* ^{bB}	8.99 ± 0.05* ^{cB}	8.83 ± 0.07* ^{bA}	8.75 ± 0.08* ^{bA}
		Bluecrop	8.77 ± 0.06* ^{bB}	8.50 ± 0.17* ^{aA}	8.77 ± 0.11* ^{bB}	8.79 ± 0.08* ^{bB}	8.89 ± 0.03* ^{bB}
Helicobacter pylori	No carrier†	Bluejay	2.15 ± 0.17* ^{bB}	<1.48* ^{aA}	2.33 ± 0.21 ^{bB}	2.24 ± 0.37 ^{bB}	<1.48 ^{aA}
		Berkley	2.60 ± 0.05* ^{bB}	<1.48* ^{aA}	<1.48* ^{aA}	<1.48* ^{aA}	2.57 ± 0.04* ^{bB}
		Bluecrop	<1.48* ^{aA}	<1.48* ^{aA}	<1.48* ^{aA}	<1.48* ^{aA}	<1.48* ^{aA}
	5% inulin†	Bluejay	2.67 ± 0.08* ^{aB}	2.33 ± 0.06* ^{aB}	2.68 ± 0.20 ^{aB}	2.54 ± 0.34 ^{aB}	<1.48 ^{aA}
		Berkley	5.43 ± 0.05* ^{cC}	5.80 ± 0.14* ^{bD}	4.53 ± 0.06* ^{bA}	4.87 ± 0.07* ^{cB}	4.94 ± 0.04* ^{cB}
		Bluecrop	4.70 ± 0.06* ^{bB}	5.95 ± 0.11* ^{bC}	4.69 ± 0.09* ^{bB}	3.18 ± 0.03* ^{bA}	3.26 ± 0.24* ^{bA}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying;
† All samples showed statistical difference in comparison with the control growth ($p < 0.05$, HSD Tukey test).
* Values with asterisk denote statistical difference between the same cultivar with and without inulin for each treatment ($p < 0.05$, *t*-test).
a,b,c - Values with different lowercase letters denote statistical difference within a column ($p < 0.05$, HSD Tukey test) (effect of blueberry cultivar for each treatment).
A,B,C - Values with different capital letters denote statistical difference within a row ($p < 0.05$, HSD Tukey test) (effect of drying treatment for each blueberry cultivar).
Control growth = 9.22 ± 0.03 Log CFU · mL^{-1}.
Growth detection limit = 1.48 Log CFU · mL^{-1}.

Rodriguez, 2022). In this regard, the study evaluated the ability of the blueberry powders to reduce the inflammatory process by decreasing IL-8 production, and the impact of blueberry cultivar, inulin addition, and drying treatment (Table 4). All blueberry powders without inulin showed a significant ($p < 0.05$) inhibition in IL-8 production (from 11.1% to 32.7%) compared to the experimental control group (100% IL-8 production). However, samples with inulin did not exhibit a significant anti-inflammatory activity, except for the 'Bluejay' and 'Bluecrop' powders obtained by VD90 (9.4% and 14.2% of inhibition, respectively), and the 'Bluecrop' powders obtained by VD70 (10.2% of inhibition).

Most of the phenolic compounds identified in the samples have been associated with a relevant anti-inflammatory response (Puangpraphant, Cuevas-Rodríguez, & Oseguera-Toledo, 2022), so their reduced content related with the use of inulin would explain the decrease in anti-inflammatory capacity. It was previously observed for vacuum-dried plum juice powders that a higher anti-inflammatory capacity was influenced by both the type of treatment used and the temperature, finding that products treated by vacuum drying at 80 °C showed the highest anti-inflammatory capacity (Silvan, Michalska-Ciechanowska, & Martinez-Rodriguez, 2020). This behavior may apparently be associated with the detachment of specific compounds from more complex structures and/or the formation of Maillard reaction products, which have shown to be particularly effective in inhibiting IL-8 production in intestinal epithelial cells (Kitts, Chen, & Jing, 2012). Regarding the impact of blueberry cultivar, reduction in IL-8 production values below 80% was only obtained for all 'Bluejay' inulin-free powders. Anthocyanins, which have been predominant in 'Bluejay' powders (Table 2a), were positively correlated with the inhibition of IL-8 production by AGS cells infected with *H. pylori* ($r = 0.65$) (Fig. S1a; supplementary file), and it agrees with the anti-inflammatory activity of anthocyanins previously described for *H. pylori*-infected human gastric cells (Kim et al., 2013). However, the main reduction in IL-8 production by the infected AGS cells was observed for the 'Bluecrop' powders obtained by VD90 and VD70 (30.8% and 32.7% of inhibition). In these cases, as mentioned above, other compounds produced during VD seem to be involved.

3.4. Chemometric analysis

The Principal Components Analysis (PCA) indicated the relationships between chemical composition (phenolics profile and content, antioxidant capacity) and *in vitro* biological properties of blueberry juice extract powders obtained from three different cultivars with or without the addition of inulin. In the case of whole data set (Fig. 2a), the two main principal components identified (PC1 and PC2) explained 84.22% of the total data variance. In Fig. 2a two groups can be distinguished between variables and factors, i.e., powders with and without inulin addition. Moreover, among carrier-free samples, more clear distinction according to the cultivar was visible, while inulin addition partially masked these differences. When scrutinizing the data in more depth, compared to inulin-free samples (Fig. 2b), the PCA showed how its application (Fig. 2c) differentiates the phenolics content and bioactive properties of blueberry powders. A closer look at Fig. 2b indicated that 3 groups of powders can be distinguished in terms of similarities of

Table 4
Effect of blueberry juice extract powders on pro-inflammatory cytokine IL-8 production in AGS cells infected by *Helicobacter pylori*. Results are expressed as % production of IL-8 with respect to the untreated infected cells.

	Cultivar	FD	VD50	VD70	VD90	SD
No carrier	Bluejay	78.2 ± 1.2†* ^{aA}	76.8 ± 1.0†* ^{aA}	74.5 ± 0.7†* ^{bA}	72.9 ± 2.1†* ^{bA}	78.1 ± 4.8†* ^{aA}
	Berkley	82.9 ± 3.4†* ^{aA}	84.1 ± 2.2†* ^{bA}	80.4 ± 4.3†* ^{cA}	77.8 ± 4.3†* ^{bA}	83.0 ± 1.9†* ^{bA}
	Bluecrop	81.5 ± 4.0†* ^{aB}	88.9 ± 2.6†* ^{bB}	67.3 ± 5.0†* ^{aA}	69.2 ± 0.7†* ^{aA}	85.3 ± 0.2†* ^{bB}
5% inulin	Bluejay	95.9 ± 5.1* ^{aB}	95.8 ± 5.9* ^{aB}	97.9 ± 2.9* ^{bB}	90.6 ± 1.9†* ^{aA}	97.2 ± 2.5* ^{aB}
	Berkley	99.2 ± 1.1* ^{aA}	98.1 ± 2.7* ^{aA}	94.1 ± 1.5* ^{bA}	99.3 ± 0.9* ^{bA}	95.0 ± 2.6* ^{aA}
	Bluecrop	95.4 ± 4.6* ^{aB}	96.8 ± 0.2* ^{aB}	89.8 ± 2.3†* ^{aA}	85.8 ± 3.1†* ^{aA}	97.2 ± 4.0* ^{aB}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying;
† − Values denoted statistical difference in comparison with control groups (untreated AGS cells; 100% IL-8 production) ($p < 0.05$, HSD Tukey test).
a,b,c - Values with different lowercase letters denote statistical difference within a column (blueberry cultivar in presence or absence of inulin) ($p < 0.05$, HSD Tukey test).
A,B,C - Different subscript letters denote statistical difference within a row (drying treatments) ($p < 0.05$, HSD Tukey test).
* - Values with asterisk denote statistical difference between the same cultivar with and without inulin for each treatment ($p < 0.05$, t-test).

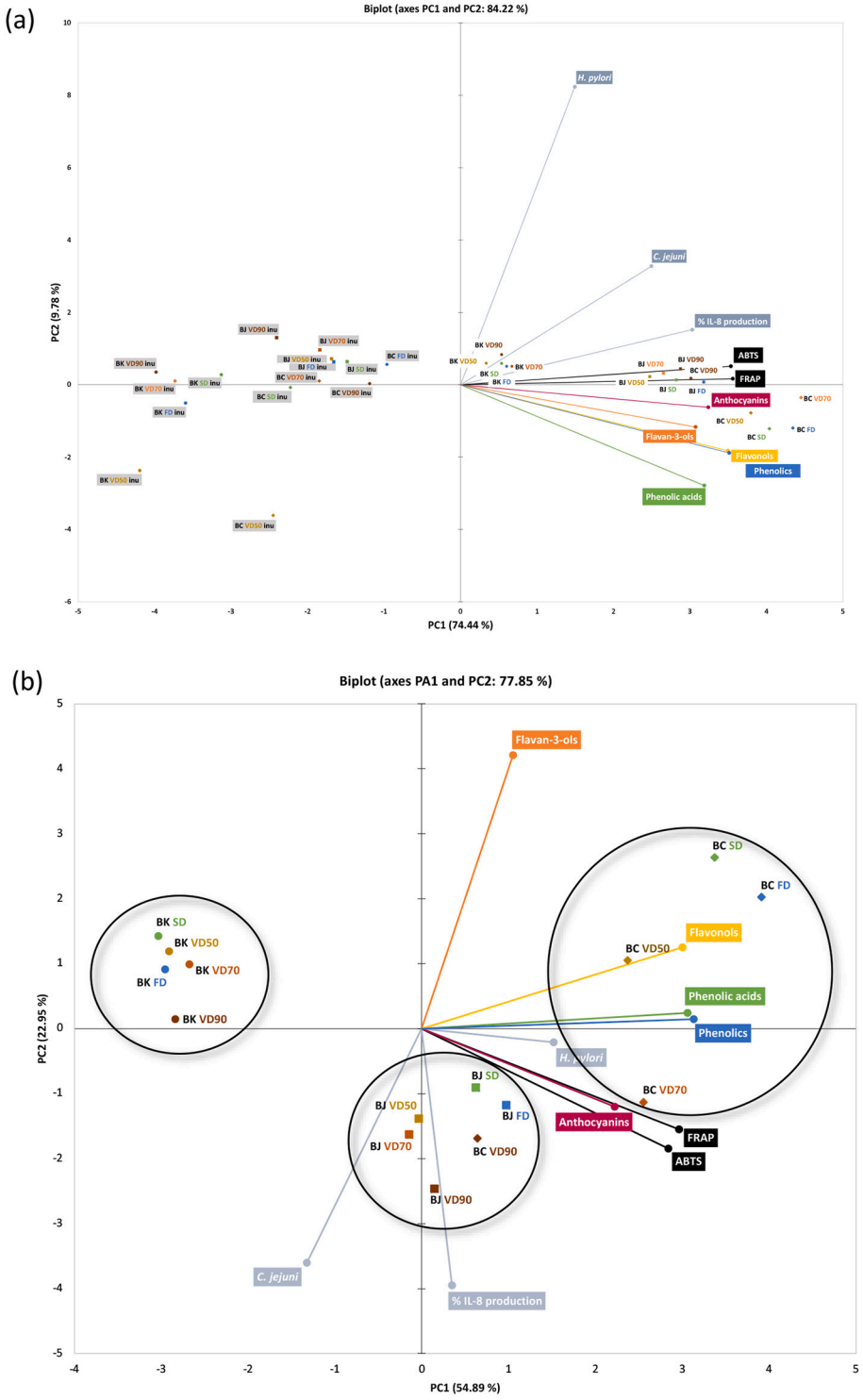


Fig. 2. Principal Components Analysis (PCA) biplot that indicates principal components (PC) scores of blueberry juice extract powders with and without inulin (inu) addition gained after freeze-drying (FD), vacuum drying at 50 °C (VD50), vacuum drying at 70 °C (VD70); vacuum drying at 90 °C (VD90) and spray drying (SD) obtained from Berkley (BK; ●), Bluecrop (BC; ◆) and Bluejay (BJ; ■) for: (a) whole data set; (b) only inulin-free powders data set; (c) only inulin-added powders data set. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; *C. jejuni* – inhibition of *Campylobacter jejuni* growth; *H. pylori* – inhibition of *Helicobacter pylori* growth; Anti-inflammatory activity – pro-inflammatory cytokine IL-8 production in AGS cells infected by *H. pylori*.

chemical and biological properties linked to the cultivar, except 'Bluecrop' powders after VD90, which were grouped together with 'Bluejay' samples. It implied that only this product had similar quality as all 'Bluejay' samples. Drying technique had stronger influence on 'Bluecrop' samples compared to 'Berkley'. Addition of inulin grouped the samples as followed: (1) Berkley' powders and (2) 'Bluejay' and 'Bluecrop' powders (Fig. 2b and c). Moreover, in the second group, 'Bluecrop' powders were similar in terms of phenolics content and anti-inflammatory properties, while 'Bluejay' ones were comparable in terms of antioxidant capacity and inhibition of *C. jejuni* and *H. pylori*

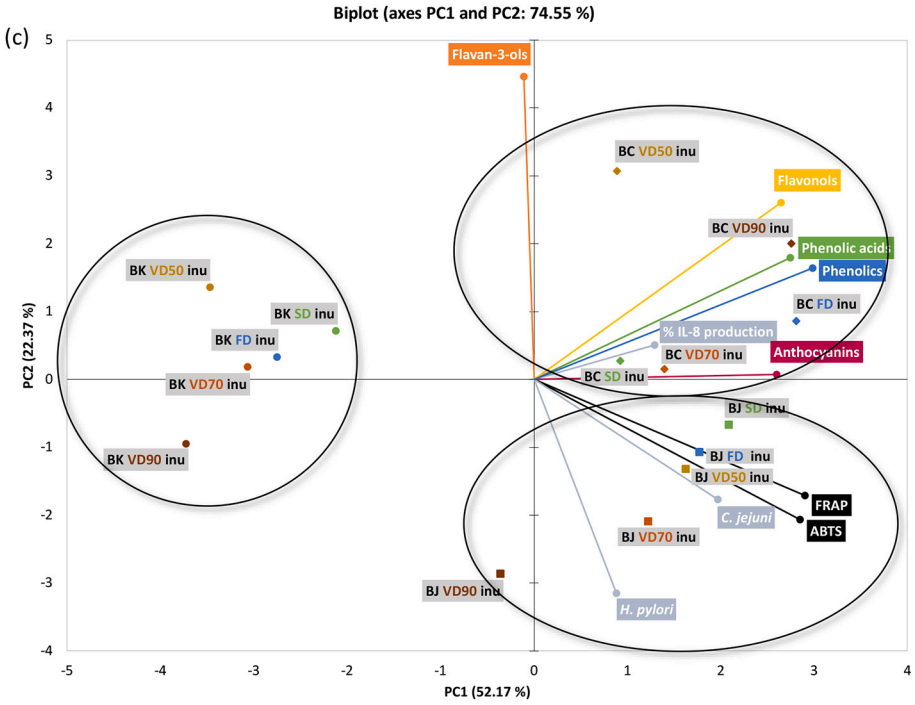


Fig. 2. (*continued*).

growth. This indicated that not the content, but the specific compounds are responsible for selected bioactive properties.

4. Conclusions

The study demonstrated a novel approach towards tailor-made powder manufacturing based on natural bioactives in blueberry juice phenolic-rich extracts, taking into account the influence of multistep processing.

The physical properties of the blueberry powders were strongly affected by the drying technique, with the vacuum drying being the preferred treatment resulting in the lowest moisture content and water activity as well as the highest bulk density values. Among the analyzed cultivars, 'Bluecrop' powders were the richest source of phenolics. Whereas syringetin-3-*O*-rhamnoside and myricetin-3-*O*-galactoside were identified only in 'Berkley' and 'Bluejay' samples. Although the application of inulin resulted in powders with about 2-fold lower phenolics content and antioxidant capacity compared to carrier-free samples, for those with inulin, it appeared to provide protection for selected variants. In general, spray-dried powders were of similar or better quality compared to freeze-dried ones. Antibacterial activity towards *C. jejuni* was found to depend on the presence of particular bioactives, rather than on their total amount. As many as ten out of fifteen blueberry inulin-free products showed a bactericidal effect against *H. pylori*, whereas the same was true for only one inulin-supplemented product. Cultivar was shown to strongly affect the anti-inflammatory properties of blueberry products, and vacuum drying at higher temperatures was found to even enhance this effect for some powder variants, unlike the other treatments (freeze- and spray drying).

The study indicated that the blueberry cultivar composition and its matrix modifications through inulin addition and drying technique can serve as a tool for designing products with programmed antimicrobial and anti-inflammatory potential with possible application in customized food production. However, this approach should be adapted to specific plant-based matrix composition as numerous bioactives components (qualitative and quantitative differences) may diversely interact under specific processing conditions.

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CRediT authorship contribution statement

Jessica Brzezowska: Funding acquisition, Investigation, Writing – original draft, Writing – review & editing. **Adolfo J. Martinez-Rodriguez:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Jose Manuel Silvan:** Methodology, Investigation, Writing – original draft, Writing – review & editing. **Grzegorz P. Łysiak:** Writing – review & editing. **Aneta Wojdyło:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Krzysztof Lech:** Methodology, Investigation. **Anna Michalska-Ciechanowska:** Supervision, Funding acquisition, Project administration, Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2023.103481.

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