



Influence of altitude on the physicochemical composition and antioxidant capacity of strawberry: a preliminary systematic review and meta-analysis

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Abstract Several climatic trends are generally associated with altitude, that may influence the nutritional and phytochemical composition of plants. Strawberry is considered a functional food due to biological activities and health benefits. This systematic review and meta-analysis intend to expose possible variations on physicochemical composition and antioxidant capacity of strawberries in regard to altitude. Thirty eligible studies were included for the final meta-analysis. Two altitude ranges were established: 0 – 1000 and 1000–2000 m above sea level. A random-effects model was used to obtain the results. It was discovered that total soluble

solids significantly decreased with altitude. Total titratable acidity increased with altitude. Vitamin C and total anthocyanins showed a significant difference between the groups before we discarded some studies. The analysis of altitude for phenolics and antioxidant capacity evaluated for the DPPH radical scavenging method did not identify any significant differences between the studies. The findings suggest that altitude does not affect the physicochemical composition and antioxidant capacity of strawberries; nonetheless, a more exhaustive study is recommended.

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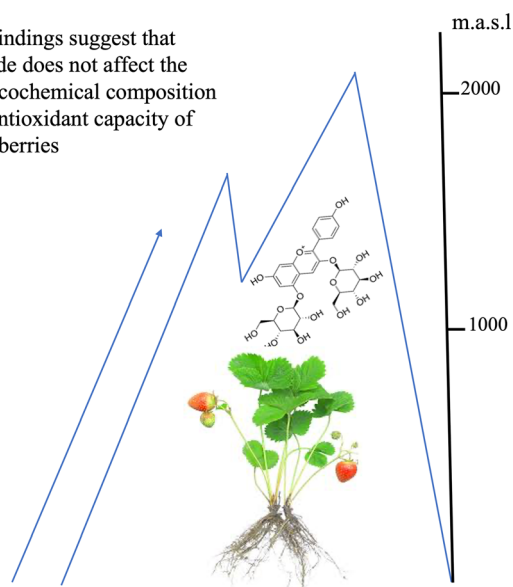
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Graphical abstract

The findings suggest that altitude does not affect the physicochemical composition and antioxidant capacity of strawberries



Keywords Strawberry · Altitude · Physicochemical composition · Antioxidant capacity · Random model · Meta-analysis

Abbreviations

AA	Ascorbic acid
AC	Citric acid
CI	Confidence interval
Cya	Cyanidin-3-glucoside
3-gluc	
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl
F-C	Folin-Ciocalteu
FRAP	Ferric reducing/antioxidant power test
FW	Fresh weight
GAE	Gallic acid equivalent
HCAs	Hydroxycinnamic acids
m.a.s.l.	Meters above sea level
ORAC	Oxygen radical antioxidant capacity
Pg	Pelargonidin
Pg	Pelargonidin-3-glucoside
3-gluc	
Pg 3-rut	Pelargonidin 3-rutinoside

PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses

TTA	Total titratable acidity
TAC	Antioxidant capacity
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
TAcy	Total anthocyanins
TPC	Total phenolic content
TSS	Total soluble solids
Vit C	Vitamin C

Introduction

Strawberry (*Fragaria x ananassa* Duch), one of the most appreciated and popular berries, is considered a functional food due to its potential health benefits, remarkable biological activities (Chaves et al. 2017; Lin et al. 2021), and therefore their rich content of nutrients, with unique color, taste, and flavor. It is consumed on a global scale, both as fresh fruit and in processed forms, such as juices, jams, and jellies (Hossain et al. 2016). The optimal growing conditions for any variety of strawberry are deep, sandy loam soil rich in organic matter with a nearly neutral (6–6.5) soil pH. Also, they need moderate fertilization and irrigation, away from areas that remain wet well into spring. The cultivation site should receive full sunlight and have a gradual slope. This helps prevent frost injury by allowing cold air to drain away from the plants (Siderman 2009).

The agroindustrial strawberry chain is one of the most important to local and global economies. Strawberries are cultivated in nearly all countries around the world (Recamales et al. 2007). In fact, worldwide strawberry production has increased by more than 80% in recent decades, reaching more than 9,125,913 tons in 2020. Certainly, China is the largest strawberry producer in the world with a production volume of 3,801,865 tons per year, while the United States of America ranks second with an annual production of 1,420,570 tons in which California accounts for the highest commercial production of this berry crop, followed by Florida and Oregon (Atlas big 2020). Finally, Mexico ranks third with an annual production of 468,248 tons. It is necessary to mention that China and the United States of America together produce

57% of the total global yield. These countries also have the highest gross income relative to strawberry production, varying between \$7 billion and \$2 billion USD annually (FAOSTAT 2016; Atlas big 2020). It is known that strawberry is currently one of the most studied genomic and agronomic berries due to its commercial, industrial, and economic impacts (Giampieri et al. 2015a, b).

Strawberries possess a unique combination of several phytochemicals, nutrients like vitamin C, and fiber, all of which play a synergistic role in its characterization as a functional food (Hossain et al. 2016; Giampieri et al. 2015a, b; Crespo et al. 2010; Basu et al. 2014; Tulipani et al. 2011). Several studies have identified a wide range of phenolic compounds in strawberry, with high biological potential in humans (Crespo et al. 2010; Giampieri et al. 2015a, b). Phenolic compounds in strawberry were extensively investigated by several authors and classified as belonging to the main group of flavonoids (flavanols, flavonols, and anthocyanins), phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids) and hydrolyzable tannins (gallotannins and ellagitannins) (Guerrero-Chavez et al. 2015; Crespo et al. 2010).

The major class of phenolic compounds is represented by the flavonoids, where anthocyanins are the best known and quantitatively the most important type and are responsible for the bright red color (Giampieri et al. 2015a, b; Mezzetti 2013). Despite a great number of anthocyanins being identified in strawberry, pelargonidin-3-glucoside (Pg 3-gluc) is the most predominant anthocyanin, followed by pelargonidin 3-rutinoside (Pg 3-rut) and cyanidin-3-glucoside (Cy 3-gluc). These three make up over 95% of the total anthocyanin bulk present in most strawberries (Crespo et al. 2010). Free radical severing activity is presumed to be the most potent biological action mode of flavonoids, followed by vasodilatory, anti-inflammatory, and antiproliferative. In addition, some studies have shown specific associations between strawberry flavonoids (anthocyanins) intakes and cardiovascular health, induction of apoptosis, and prevention of DNA damage (Basu et al. 2014; Giampieri et al. 2014).

Strawberries also contain small amounts of other phenolic compounds (Crespo et al. 2010), and these compounds are identified as glycosides of quercetin and kaempferol, esters of hydroxycinnamic acids, especially of ellagic acid, *p*-coumaric acid, and ellagic acid glycoside (Giampieri et al. 2015a, b). These

substances act as antioxidants in the human body and perform antimutagenic, anticarcinogenic, antihypertensive, and anti-inflammatory functions (Nunes et al. 2021; Basu et al. 2014; Hossain et al. 2016). These action mechanisms are directly linked to the modification of etiology of chronic diseases (Basu et al. 2014). On the other hand, ellagitannins were detected only in berries from the Rosaceae family (strawberry). The representative ellagitannin in strawberries is sanguin H-6 (Crespo et al. 2010; Andrianjaka-Camps et al. 2017). Ellagic acid and ellagitannins have been reported as significant contributors to antioxidant and anticarcinogenic effects (Basu et al. 2014). In this way, strawberries stand out among red fruits for their potential health benefits as well as their qualitative and quantitative characteristics that need periodic evaluations (Zhu et al. 2015; Basu et al. 2014).

Scientific studies have shown that the chemical composition of berries is affected by a number of preharvest and postharvest factors (Josuttis et al. 2012). Among the preharvest factors, the variety in composition is considered the main source of variation. In addition, nutritional and nutraceutical quality is also influenced by environmental parameters (viz. temperature, light conditions, fertilization, irrigation, and cultivation systems) (Crespo et al. 2010; Akhatou and Fernández-Recamales 2014), altitude, genotype, harvest time, degree of maturity, geographic origin, organic farming, and growing (Hakala et al. 2003; Gündüz and Özbay 2018; Akhatou and Fernández-Recamales 2014).

Strawberry grows naturally throughout Europe, North and South America, and northern Asia at a minimum altitude of 0 and maximum altitude of 3000 m above sea level (m.a.s.l.) (Cámara de Comercio de Bogotá 2015). Until now, little is known about the variability of bioactive compounds in strawberries affected by growing locations at different altitudes (Josuttis et al. 2012). There are several climatic trends generally associated with altitude, such as increased radiation under cloudless skies, a reduction of atmospheric temperature, a decreasing total atmospheric pressure, and a higher fraction of UV-B radiation (Guerrero-Chavez et al. 2015; Gündüz and Özbay 2018; Josuttis et al. 2012). It has been demonstrated that the combined action of these variables could play a role in determining the final phenolic profile of plants (Guerrero-Chavez et al. 2015; Josuttis et al. 2012). Considering the importance of knowing how external

factors such as altitude gradients can affect the characteristics of strawberries, the aim of this systematic review was to expose the possible variations that exist in the profile of the phenolic content and antioxidant capacity as well as the main quality traits of strawberries regarding altitudinal variation.

Methodology

The present meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (Halvorsen et al. 2002).

Literature search

Investigators searched SCOPUS and Embase for eligible studies up to September 15, 2020. The search terms for studies were “strawberry” combined with “altitude” and “chemistry composition”. The literature search was restricted to articles published in English within the last 21 years. We screened the titles and abstracts of all retrieved publications and then determined the eligibility by checking the full text.

Study inclusion and exclusion

An independent researcher carried out the inclusion and exclusion of studies (Fig. 1). Any discrepancies were resolved by discussion with other team members until a consensus was reached. Studies were included if they met the following criteria: (1) conducted on strawberries; (2) *Fragaria* × *ananassa* D.; (3) strawberry varieties; and (4) provided sufficient data of parameters such as total soluble solids (TSS), total titratable acidity (TTA), vitamin C (Vit C), total anthocyanins (TAcy), total phenolic content (TPC), and antioxidant capacity (TAC). Studies were excluded if: (1) they studied another species of strawberry; (2) did not report the altitude variable; and (3) did not provide dispersion measures regarding the mean values in statistical analysis.

Statistical analysis

Meta-analysis was performed using the RStudio software (Version 4.1.2; <https://rstudio.com/>;

accessed on November 1, 2021), using the *meta* package. The pooled means of each variable under study was reported including confidence intervals (CI) of 95%. The heterogeneity was assessed using the Cochran Q and I² tests. The random-effects model was employed to calculate the pooled means. All *p*-value < 0.05 were considered statistically significant. Egger’s regression test and funnel plot analyses were used to explore publication bias. Funnel plot asymmetry tests were only performed when the number of studies was at least ten ($k \geq 10$) (Sterne et al. 2011). The effect of altitude was evaluated with a sub-group analysis. Altitude was extracted from the articles and in some cases computed by consulting the altitude according to the reported production site.

Results

Study characteristics

A total of 60 studies were retrieved and 36 full texts were reviewed. A total dataset of 30 studies were obtained for the meta-analysis following the eligibility criteria (Fig. 1). Detailed characteristics of included studies can be found in Table 1. The final studies used for meta-analysis were conducted in Europa ($n = 12$), Asia ($n = 7$), and Brazil ($n = 6$). The harvest range was from 2002 to 2019.

Effect of altitude on quality and physicochemical composition of strawberry

Total soluble solids (TSS)

The pooled mean for TSS was 7.73°Brix (CI: 7.50 – 7.96) and the heterogeneity indices computed using the random model were $Q = 447.53$, $I^2 = 82.1\%$, $\tau^2 = 0.81$, and $p < 0.0001$. The forest plot is shown in Fig. S1.1 (Supplementary Materials I). Egger’s linear regression test indicated possible publications bias (p -value = 0.003) that can be noticed in the asymmetry of the funnel plot (Fig. S2.1, Supplementary Materials II). The statistical analysis on the effect of altitude on the total soluble solids (Table 2) suggests a significant difference between the groups. The p -value between the groups was 0.02. The mean at 1000–2000 m.a.s.l. (8.03°Brix) was higher than the mean at 0–1000 m.a.s.l. (7.49°Brix).

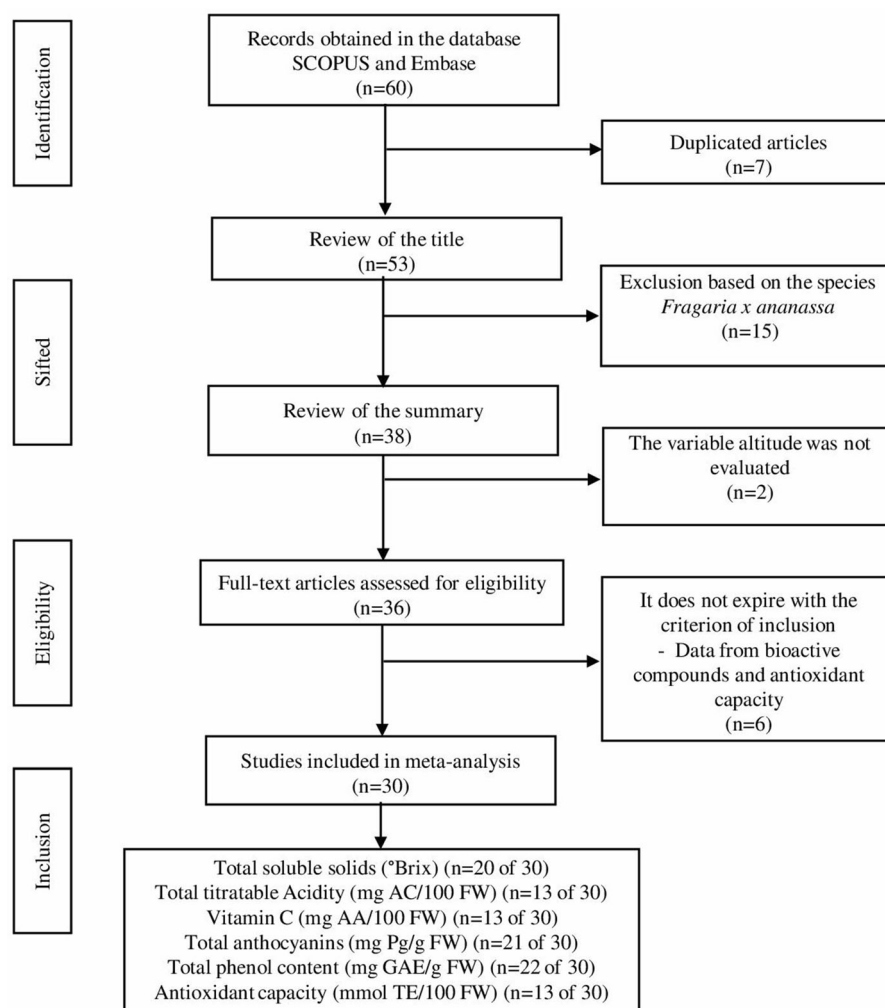


Fig. 1 Prisma flow chart of the eligible studies obtained during the screening process

The I^2 and τ^2 indicated significant heterogeneity and, as the funnel plot suggests, there is also a high asymmetry (Fig. S2.1, Supplementary Materials II). We found studies with similar standard errors (sample size effect) with very different mean effects. In all studies, the sample size was very small and similar across all studies but interestingly the studies with higher and lower variations were well localized. The values of TSS obtained by Capocasa et al. (2008) were the lowest, whereas Diamanti et al. (2009) and Diamanti et al. (2014) found the highest TSS values. Both groups of articles comprise a variety of strawberry collected and study in the same city of Italy (Ancona) but in different years. The authors evaluated the total soluble solids content of 18 different

cultivars. In the first study, corresponding to Capocasa et al. (2008), they analyzed 14 of the 18 cultivars. In the second study they analyzed 8 of the 18 cultivars (Diamanti et al. 2009). Finally, in the third study corresponding to Diamanti et al. (2014) they analyzed only 2 of the 18 cultivars. With that being said, there were 6 matches. That is, the same cultivar was analyzed on at least two different occasions. In the case of the cultivars “Sveva”, “(‘Paros’ × ‘Queen Elisa’)” and “Irma” the concentrations were similar despite having been analyzed in different years. However, in the case of the cultivars “Dora”, “Adria” and “Onda”, the concentrations showed differences between them. This phenomenon could be due to the difference in climatic conditions or even the error of

Table 1 General information extracted from the dataset and selected for the present meta-analysis

	Code [#]	Continent	Country/ settlement	Altitude (m.a.s.l.)	Harvest year	Study variables*	References			
1	1	Asia	Iran/Ardeh	1018	2016	TSS, TTA, Vit C, TAcy, TPC, and TAC ^b	Ghiamati et al. (2020)			
	2		Iran/Ponel	1054						
	3		Iran/Sangdeh	1273						
	4		Iran/Masoleh	1289						
	5		Iran/Zendaneh	1720						
2	a	Europe	Switzerland/ Conthey	480	2008	Vit C, TAcy, and TAC ^b	Crespo et al. (2010)			
	ai									
	aii									
	aiii									
	b		Switzerland/ Bruson	1060						
	bi									
	bii									
	biii									
3	1	South America	Brazil/ Brazlândia	1200	2011	TSS, TTA, Vit C, TAcy, TPC, and TAC ^{a,b}	Pineli et al. (2011)			
	2		Brazil/Brasilia	1100						
4	i	Asia	Turkey/Adana	20	2016	TSS, Vit C, and TPC	Kapur et al. (2018)			
	ii									
5	1	Europe	Italy/Cesena	38	2012	TSS, TTA, Vit C, TAcy, TPC, and TAC ^c	Cocco et al. (2015)			
	2		Italy/Scanzano J	40						
	3		Italy/Verona	42						
6	1	Europe	Norway/Bodø	6	2003	TTA	Davik et al. (2006)			
	2		Norway/Stjørdal	5						
	3		Norway/ Ringsaker	844						
	4		Norway/ Grimstad	4						
7	a	Europe	Italy /Martello	900	2011	TSS, TTA, TPC, TAcy, and TAC ^b	Guerrero-Chavez et al. (2015)			
	a1			1100						
	a2			1200						
	a3			1500						
	b			900	2012					
	b1			1100						
	b2			1200						
	b3			1500						
8	i	Europe	Italy /Ancona	50 ^d	2011	TAcy and TAC ^c	Alvarez-Suarez et al. (2011)			
	ii									
	iii									
9	1	Asia	Antakya	117	2015	TSS, TTA, TPC, TAcy and TAC ^a	Gündüz et al. (2018)			
	2		Turkey/Urumu	443						
	3		Turkey/Saksak	755						
10	-	Asia	Iran/Kamyaran	1572	2018	TSS, TTA, TPC and TAC ^b	Zahedi et al. (2020)			
11	-	Europe	Italy /Ancona	50	2014	TAcy	Giampieri et al. (2014)			

Table 1 continued

	Code [#]	Continent	Country/ settlement	Altitude (m.a.s.l.)	Harvest year	Study variables*	References
12	I ii iii	Europe	Spain/Huelva	6	2008	TPC and TAcy	Palencia et al. (2016)
13	I Ii iii iv	Asia	Turkey/Cilicia	20	2018	TAcy, TPC, and TAC ^b	Sarıdaş (2021)
14	-	Europe	Slovenia/ Sevnica	185	2016	TAcy and TPC	Weber et al. (2018)
15	-	South America	Brazil/Passo Fundo	709	2012	TAcy and TPC	Duarte et al. (2018)
16	i ii iii iv v vi vii	South America	Brazil/Passo Fundo	709	2012	TSS, TAcy, and TAC ^b	Chaves et al. (2017)
17	I Ii iii Iv v	Asia	Bangladesh/ Gazipur	14.33	2011	TSS, TTA, and Vit C	Rahman et al. (2016)
18	-	Europe	Čačak/Serbia	225	2009	TSS and TTA	Pešaković et al. (2013)
19	-	Asia	Thailand/Chiang Mai	310	2016	TSS, TTA, TAcy, Vit C, and TPC	Boonyakiat et al. (2016)
20	I Ii iii Iv V vi	South America	Brazil/Paraná State	1116,5	2017	TSS, TTA, TAcy, Vit C, TPC, and TAC ^c	Nunes et al. (2021)
21	-	South America	Brazil/Pelotas	15	2019	TSS, TTA, TAcy, TPC, and TAC ^b	Siebeneichler. et al. (2020)
22	-	Europe	Greece/Athens	30	2009	TSS, TTA, TPC, Vit C, TAcy, and TAC ^a	Roussos et al. (2009)
23	i ii iii iv v vi	South America	Brazil/Atibaia	803	2002	TSS, Vit C, TAcy, and TPC	Cordenunsi et al. (2002)
24	-	Europe	A + Ñ Z	70	2012	TSS, Vit C, TAcy, and TPC	Crecente-Campo et al. (2012)
25	-	Europe	Italy/Ancona	50 ^d	2011	Vit C and TAC ^a	Tulipani et al. (2011)

Table 1 continued

	Code [#]	Continent	Country/ settlement	Altitude (m.a.s.l.)	Harvest year	Study variables*	References
26	i ii	Europe	Italy/Ancona	50 ^d	2014	Vit C, TAcy, TPC, and TAC ^c	Diamanti et al. (2014)
27	i ii	Europe	Italy/Ancona	50 ^d	2013	TSS, TAcy, TPC, and TAC ^c	Diamanti et al. (2013)
28	i Ii Iii Iv V Vi	Europe	Italy/Ancona	50 ^d	2009	TSS, TPC, and TAC ^c	Diamanti et al. (2009)
29	i° ii° iii° iv°	Europe	Italy/Ancona	50 ^d	2009	TSS, TPC, and TAC ^c	Diamanti et al. (2009)
30	i ii iii Iv V vi vii viii ix x xi Xii Xiii Xiv	Europe	Italy/Ancona	50 ^d	2008	TSS, TPC, and TAC ^a	Capocasa et al. (2012)

*TSS: Total soluble solids, TTA: Total titratable acidity, Vit C: Vitamin C content, TAcy: Total anthocyanins, TPC: Total phenolic content, TAC: Antioxidant capacity. ^a Antioxidant capacity evaluated by the ferric reducing/antioxidant power (FRAP) method. ^b Antioxidant capacity evaluated by the radical cation α - α diphenyl- β -picrylhydrazil (DPPH radical) method. ^c Antioxidant capacity evaluated by another method; data not included in the statistical analysis. ^d Altitude corroborated by the authors

[#](1, 2, 3, 4...) same harvest year and cultivar but different country and altitude, (a, ai, aii...) & (b, bi, bii...) same altitude and harvest year but different country and cultivars, (a, a1, a2...) & (b, b1, b2...) same country and cultivar but different altitude and harvest year, and (i, ii, iii, iv...) same country, altitude, and harvest year but different cultivars

the method, which was developed on three different occasions. The funnel plot was also asymmetrical in other analyses presented in this work. Even when the funnel plot and Egger's linear test were used to evaluate publication bias, we should keep in mind that publication bias is not the only explanation for asymmetry (Hunter et al. 2014). In our case, sample size is unlikely to be a publication bias, rather asymmetry and heterogeneity are probably more

related to geographical locations, fruit variety, and other variables that cannot be controlled for lack of systematic information.

Total titratable acidity (TTA)

The heterogeneity indices computed using the random model for TTA were $Q = 41.84$, $I^2 = 0\%$, $\tau^2 = 0.01$, and $p_{\text{heterogeneity}} = 0.54$. The pooled mean was

Table 2 Statistical analysis regarding the effect of altitude on total soluble solids

Altitude	No. of studies	Mean (Brix°)	95%—CI	I ² %	tau ²	P Between groups
0–1000 (m.a.s.l.)	61	7.49	7.23—7.76	85.4	0.84	0.02
1000–2000 (m.a.s.l.)	20	8.03	7.64—8.42	47.3	0.29	

0.94 mg citric acid (AC)/100 g FW (CI: 0.87—1.00). These results indicate a very low heterogeneity between studies. The forest plot is presented in Fig. S1.2 (Supplementary Materials I). The *p*-value obtained with Egger's linear regression test (*p*-value = 0.045) is a borderline value around our significance criterium (*p*-value = 0.05). In the case of titratable acidity, the *p*-value between the groups was 0.0003 (Table 3). Consequently, significant differences between the groups were detected. The mean at 1000—2000 m.a.s.l. (1.10 mg AC/100 FW) was higher than at 0–1000 m.a.s.l. (0.88 mg AC/100 FW). According to these data, a significant increase in total titratable acid content was observed as altitude increased.

Vitamin C (Vit C) content

The pooled mean for vitamin C across all studies was 48.42 mg ascorbic acid (AA)/100 g FW (CI: 45.69 – 51.14). Furthermore, the heterogeneity indices were $Q = 279.80$, $I^2 = 85.3\%$, $\tau^2 = 50.09$ and ($p < 0.0001$) (Table 4). The forest plot is shown in Fig. S1.3 (Supplementary Materials I). The *p*-value of Egger's linear regression test was 0.001, thus publication bias was detected. The *p*-value between the groups for vitamin C was 0.01, which suggests a significant difference between them. The mean at 0—1000 m.a.s.l. (50.99 mg AA/100 g FW) was higher than at 1000—2000 m.a.s.l. (44.59 mg AA/100 g FW), implying that vit C content decreases with altitude. The inspection of the funnel plot (Fig. S2.2, Supplementary Materials II), as well as the outliers analysis, pointed out four studies that clearly have

very different behavior (Roussos et al. 2009; Diamanti et al. 2014; Pineli et al. 2011; Crecente-Campo et al. 2012). The values reported in the four studies are far from the central tendency (pooled mean) as visible in the funnel plot. The Vit C values obtained by Crecente-Campo et al. (2012) (71.20 mg AA/100 g FW) and Diamanti et al. (2014) _ii of “Sveva” cultivar (13.84 mg AA/100 g FW) were the highest (Fig. S2.2), whereas Roussos et al. (2009) (41.22 mg AA/100 g FW) and Pineli et al. (2011) _1 at altitude 1200 m.a.s.l. (31.45 mg AA/100 g FW) found the lowest Vit C values (Fig. S2.2). After removing these studies, the pooled mean did not drastically change but heterogeneity and Egger's linear test did. We found a pooled mean of 47.63 mg AA/100 g FW (CI: 46.30 – 48.95) and the heterogeneity indices were $Q = 35.54$, $I^2 = 0.0\%$, $\tau^2 = < 0.0001$, and *p*-value = 0.54. The *p*-value of Egger's linear regression test was 0.50, suggesting no indication of publication bias. However, with the altitude analysis (Table 5) after removing the previous articles, no significant differences were detected.

Total anthocyanins (TAcy)

First, 67 studies in total were analyzed. For heterogeneity testing of TAcy using the random model, the indices were $Q = 30,497.62$, $I^2 = 99.8\%$, $\tau^2 = 0.05$, and $p_{\text{heterogeneity}} = < 0.001$. In addition, the pooled mean was 0.33 mg pelargonidin (Pg)/g FW (CI: 0.27–0.38). On the other hand, publication bias was not detected using Egger's linear regression test (*p*-value = 0.12). The forest plot is shown in Fig. S1.4 (Supplementary Materials I). With respect to the

Table 3 Statistical analysis regarding the effect of altitude on total titratable acidity

Altitude	No. of studies	Mean mg AC/100 FW	95%—CI	I ² %	tau ²	P Between groups
0–1000 (m.a.s.l.)	24	0.88	0.80 – 0.95	14.1	0.01	
0.0003 1000–2000 (m.a.s.l.)	20	1.10	0.99 – 1.20	0	0	

Table 4 Statistical analysis regarding the effect of altitude on vitamin C content

Altitude	No. of studies	Mean (mg AA/100 g FW)	95%—CI	I ² %	tau ²	P Between groups
0–1000 (m.a.s.l.)	25	50.99	46.95– 55.03	86.0	63.68	0.01
1000–2000 (m.a.s.l.)	17	44.59	41.57– 47.61	79.7	24.66	

Table 5 Statistical analysis regarding the effect of altitude on vitamin C content after removing four articles, namely Roussos et al. (2009), Diamanti et al. (2014), Pineli et al. (2011), and Crecente-Campo et al. (2012)

Altitude	No. of studies	Mean (mg AA/ 100 g FW)	95%—CI	I ² %	tau ²	P Between groups
0–1000 (m.a.s.l.)	22	48.40	46.52– 48.12	13.5	< 0.0001	0.09
1000–2000 (m.a.s.l.)	16	46.32	44.52– 50.28	0.0	< 0.0001	

Table 6 Statistical analysis regarding the effect of altitude on total anthocyanins

Altitude	No. of studies	Mean (mg Pg/g FW)	95%—CI	I ² %	tau ²	P Between groups
0–1000 (m.a.s.l.)	44	0.36	0.28 – 0.44	99.8	0.06	0.001
1000–2000 (m.a.s.l.)	23	0.22	0.21 – 0.24	0	< 0.0001	

statistical analysis related to the effect of altitude on the total anthocyanins in strawberries (Table 6), significant differences were detected between the groups (p -value = 0.001). The mean at 0–1000 m.a.s.l. (0.36 mg Pg/g FW) was higher than at 1000–2000 m.a.s.l. (0.22 mg Pg/g FW). Nevertheless, the heterogeneity tests and values of I^2 and τ^2 clearly indicate a high level of heterogeneity. Looking at the funnel plot (Fig. S2.3, Supplementary Materials II), we can notice an asymmetry not detected in our Egger's test. It is worth mentioning that Giampieri et al. (2014), Diamanti et al. (2013), Diamanti et al. (2014), and Alvarez-Suarez et al. (2011) obtained high levels of anthocyanins. All these studies were carried out at low altitudes, which can be responsible for a low p -value in sub-group comparisons. When these articles were removed and the analysis was carried out again, no significant differences were found between altitude and anthocyanin concentration (Table 7). The pooled mean for TAc was 0.25 mg Pg/g FW (CI: 0.23– 0.26) and the heterogeneity indices were much lower ($Q = 80.35$, $I^2 = 27.8\%$, $\tau^2 = 0.001$, and $p_{\text{heterogeneity}} = 0.03$) with a non-significant asymmetry detection with Egger's linear regression test (p -value = 0.17).

Total phenolic content (TPC)

The heterogeneity indices computed using the random model for TPC were $Q = 59,986.89$, $I^2 = 99.9\%$, $\tau^2 = 0.4$, and $p_{\text{heterogeneity}} = < 0.001$. The pooled mean was 2.56 mg gallic acid equivalent (GAE)/g FW (CI: 2.38–2.75) and the forest plot can be viewed in Fig. S1.5 (Supplementary Materials I). Publication bias was not found according to Egger's linear regression test (p -value = 0.09). The high heterogeneity value is a consequence of the same problems already discussed regarding certain varieties which had been selected to improve antioxidant capacities (Capocasa et al. 2008; Diamanti et al. 2009; Diamanti et al. 2014). In the analysis of altitude, we didn't identify any significant differences between studies (p -value > 0.5) (Table 8).

Antioxidant capacity (TAC) of strawberry in regard to altitude

Initially, four methods were identified in the selected articles: 1) oxygen radical antioxidant capacity (ORAC), 2) Trolox Equivalent Antioxidant Capacity (TEAC), 3) ferric reducing/antioxidant power test

Table 7 Statistical analysis regarding the effect of altitude on total anthocyanins after removing the studies by Giampieri et al. (2014), Diamanti et al. (2013), Diamanti et al. (2014), and Alvarez-Suarez et al. (2011)

Altitude	No. of studies	Mean (mg Pg/g FW)	95%—CI	I ² %	tau ²	P _{Between groups}
0–1000 (m.a.s.l.)	36	0.25	0.23 – 0.27	99.8	38.1	0.20
1000–2000 (m.a.s.l.)	23	0.23	0.20 – 0.26	0	0.0	

Table 8 Statistical analysis regarding the effect of altitude on total phenol content

Altitude	No. of studies	Mean (mg GAE/g FW)	95%—CI	I ² %	tau ²	P _{Between groups}
0–1000 (m.a.s.l.)	53	2.57	2.37—2.77	99.9	0.42	0.45
1000–2000 (m.a.s.l.)	20	2.37	1.86—2.88	38.0	0.23	

Table 9 Statistical analysis regarding the effect of altitude on antioxidant capacity evaluated with DPPH radical scavenging method

Altitude	No. of studies	Mean (mmol TE/100 g FW)	95%—CI	I ² %	tau ²	P _{Between groups}
0–1000 (m.a.s.l.)	18	1.37	1.27—1.48	0.0	0	0.11
1000–2000 (m.a.s.l.)	18	1.26	1.15 – 1.37	54.9	0.02	

(FRAP) and 4) 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH radical) method. However, the studies that utilized the ORAC and TEAC methods were low in number and consequently were eliminated from the statistical analysis. Thus, only two methods were evaluated, as these were the most commonly used ones to evaluate antioxidant capacity: FRAP and DPPH radical. The 30 selected studies comprised several varieties, altitude, regions, methods, etc., which consisted of a total of 57 sub-studies. In this sense, 21 sub-studies evaluated the antioxidant capacity using FRAP and only two were within the altitude range from 1000–2000 (m.a.s.l.). Due to the small sample size for this group, altitude comparison cannot be evaluated in the studies using the FRAP method.

The pooled mean for FRAP at 0–1000 m.a.s.l. was 1.30 mmol TE/100 g FW (CI: 1.14–1.46) and the heterogeneity indices computed using the random model were $Q = 77.43$, $I^2 = 76.8\%$, $\tau^2 = 0.08$, and ($p < 0.0001$). The forest plot is shown in Fig. S1.6 (Supplementary Material I) and Egger's linear regression test did not identify any publication bias (p -value = 0.18). On the other hand, the remaining 36 sub-studies evaluated the antioxidant capacity using DPPH radical. For this method, we had sufficient data

to evaluate the effect of altitude. The heterogeneity indices computed using the random model for DPPH radical were $Q = 62.29$, $I^2 = 43.8\%$, $\tau^2 = 0.02$, and $p_{\text{heterogeneity}} = 0.003$. The pooled mean was 1.30 mmol TE/100 g FW (CI: 1.22 – 1.38) (forest plot is shown in Fig. S1.7, supplementary material I). Publication bias was found according to Egger's linear regression test (p -value = 0.03).

With respect to the statistical analysis related to the effect of altitude on the antioxidant capacity evaluated with DPPH radical in strawberries (Table 9), significant differences were not detected between the groups (p -value = 0.11).

In the funnel plot (Fig. S2.4, Supplementary Materials II), the asymmetry we noticed was not derived from publication bias, at least not bias related to sample size effect. All studies had the same sample size but those studies with lower standard error (associated with lower standard deviation as reported in the articles) correspond to samples from Switzerland and Turkey. These studies contribute to heterogeneity and biases. Given that the number of studies in those regions was not large enough, it was not possible to include them for variable analysis but they probably reflect the influence of sampling variations or fruit

varieties that tend to have low contents of compounds with antioxidant capacity measured as DPPH radical.

Discussion

This meta-analysis included a final set of 30 eligible studies. We analyzed the influence of altitude on the physicochemical composition and antioxidant capacity of strawberry fruit. First, the data were grouped into three ranges; however, no significant results were detected. For this reason, we established two altitude ranges: the first range was 0–1000 m.a.s.l. and the second was 1000–2000 m.a.s.l.. These specific ranges were selected with the aim of summarizing the relationship of the data with respect to the analyzed population. By having two sufficiently representative groups and a significantly high difference between them, the sample size was larger. In this sense, reliable results and better understanding and interpretation of the data can be obtained.

Several studies have characterized the physical, morphological, and chemical properties of different strawberry cultivars. They have even shown that strawberry phenolic compounds and specific fruit quality traits were influenced differently (or not influenced) by altitude and production site, yet these studies are scarce (Senica et al. 2017; Gündüz and Özbay 2018).

Initially, the highest total soluble solids in our dataset were reported at an altitude of 1000–2000 m.a.s.l.. In several studies, it was determined that altitude did significantly affect the content of soluble solids. Sugars in strawberry fruits are mainly mono- and disaccharides (Crespo et al. 2010). A previous study showed that the production site had a significant effect on the content of monosaccharides in different cultivars. However, this research group evidenced significantly lower fructose and glucose contents when plants were grown in the mountain region and hence potentially could have resulted in the strawberries having a low sweetness (Crespo et al. 2010). Additionally, statistical differences were detected between the groups (p -value = 0.02). Pertinent studies have been performed by a group of researchers in Italy. The first study evaluated the quality of strawberries grown in a mountainous zone and determined that the TSS did not differ significantly in relation to the field's altitude, but

strawberries from the site at 1200 m.a.s.l. presented a higher average Brix value than those grown at lower altitudes (Andreotti et al. 2014). The temperature decreased at a rate of about 0.65 °C for every 100 m of elevation. Therefore, low temperatures have been recorded at high altitudes in strawberry production sites. In fact, a study has identified that the synthesis of sugars in berries increases at low temperatures (Davik et al. 2006). Finally, a second study investigated the main quality traits of strawberries with respect to the altitudinal variation of their production sites. The strawberries were cultivated within the same mountain district, under the same rural practice and soil conditions, in an altitude range of between 900 and 1500 m.a.s.l.. As in the previous case, the production site did not significantly affect soluble solids content. Brix values varied between 8.5 and 8.9 degrees and were within the range for this cultivar (Guerrero-Chavez et al. 2015). We suggest that the discrepancy from results obtained with respect to the results reported in other studies could be related to the limited number of studies reported for the altitude range of 1000–2000 m.a.s.l. compared to those for 0–1000 m.a.s.l.. A strawberry fruit's taste is not only influenced by sugars (Mikulic-Petkovsek et al. 2015). It is worth mentioning that sweet strawberries do not necessarily have a high sugar content, but they generally contain low levels of organic acids, especially citric acid (Yang et al., 2009; Krüger et al. 2012).

According to the results obtained, the total titratable acidity content was also affected by the different altitudes of the production sites. The p -value between the groups was 0.0003 (Table 3), thus significant differences between the groups were detected. In this sense, we propose that the higher the altitude, the more TTA there will be. Therefore, our results are consistent with Palmieri et al. (2017), Cocco et al. (2015), and Crespo et al. (2010). This research identified that TTA varied significantly for most of the varieties. Higher TTA values were found for all the varieties grown at high altitudes. This high level of acidity in the mountains may be the effect of differences in UV-radiation intensity. On the other hand, there are few studies that did not find a clear correlation with site altitude and TTA content (Guerrero-Chavez et al. 2015; Andreotti et al. 2014).

Content of compounds such as vitamin C are often regarded as one of the main health-related compounds

present in strawberry fruits. It is mainly responsible for the antioxidant capacity of this fruit (Nunes et al. 2021). Vitamin C was determined by HPLC. First, we found that high Vit C in strawberries was positively associated when the fruits were grown at low altitudes, hence a significant difference between the groups was detected (p -value = 0.01). However, with the altitude analysis, after removing four previous articles, no significant differences were detected (p -value = 0.09). Previous studies corroborated our results. They detected that the vitamin C content of strawberries has been affected when plants were grown in a mountainous region at high altitudes, with values ca. 1.2-fold lower than those obtained at lower altitudes (Crespo et al. 2010). In the same way, two varieties of strawberry were analyzed, both grown at different altitudes in Italy. The authors concluded that those fruits cultivated at high altitudes had higher contents of vitamin C than those at lower altitudes, independently of the variety. These differences were attributed to the genetic diversity and properties of the soil, as well as also to the conditions of growth (Nunes et al. 2021). However, the correlation between this parameter and altitude must be further investigated, as was suggested by the inconsistent results reported by Doumett et al. (2011) on wild strawberry.

Berries are the most important dietary sources of anthocyanins (Xu et al. 2021; Lin et al. 2021). To date, very few studies have analyzed the impact that the altitude of production sites has on strawberry anthocyanins (Crespo et al. 2010). In this case, most of the studies included in the meta-analysis evaluated a specific altitude and were classified according to the aforementioned altitude ranges. In most studies, the total anthocyanin content was estimated by a pH differential method, using a UV–VIS spectrophotometer.

Initially, a statistical analysis was performed based on 67 studies. The results we obtained showed statistical differences between the groups (p -value = 0.001). Therefore, total anthocyanins decrease with altitude. However, we infer that those 8 studies influenced the above result. Coincidentally, these studies evaluated ripe strawberry fruits selected in order to increase the total anthocyanin content. These were harvested at the experimental field for genetic improvement at the Azienda Agraria Didattico Sperimentale (Università Politecnica delle Marche, Ancona, Italy) at 50 m.a.s.l. and were classified in

the altitude range of 0–1000 m.a.s.l. We suggest that this could be the reason why these strawberries have a higher concentration of anthocyanins than the others. In addition, this would justify the significant differences between the groups. When these studies were removed, the results changed; a new statistical analysis was performed, and less heterogeneity was observed. In addition, no significant differences were detected between the groups (p -value = 0.20), confirming the aforementioned.

Only a few studies analyzed the influence of altitude on the accumulation of anthocyanins in strawberries cultivated between 900 and 1500 m.a.s.l.. The scientists affirmed that total anthocyanins content was significantly influenced by production site and season. There was a clear trend in this class of compound concentration in both years: the higher the altitude, the lower the final accumulation of anthocyanins, mainly Pel-3-glc and derivatives in strawberry fruits (Guerrero-Chavez et al. 2015). Likewise, when similar studies were performed on other species (*Vaccinium myrtillus* and *Punica granatum*), a lower content of anthocyanins was found in fruits collected at higher altitudes, as also shown in our research (Crespo et al. 2010; Guerrero-Chavez et al. 2015). In fact, in other studies, the anthocyanin contents of *V.myrtillus* fruits were found to decrease with altitudes increasing from 800 to 1200 m.a.s.l. and 1500 m.a.s.l. (Åkerström et al. 2010; Krüger et al. 2021). It is currently known that the total anthocyanin contents in strawberries are strongly influenced, both qualitatively and quantitatively, by external factors such as high or low temperatures and light (photoperiod, quantity, and wavelength including UV-light), and in turn these factors influence the altitude at which the fruits are grown (Krüger et al. 2021).

In the case of total phenolic content, the studies used the Folin-Ciocalteu (F–C) method. Although this method is fairly discussed due to some interferences, it is a widespread method in the field of agrochemistry and the food industry owing to its simplicity, the availability of commercial reagent, and its fairly standardized procedure, which allows a better comparison of the data (Magalhães et al. 2010). The heterogeneity between the studies for this parameter was high. This phenomenon could be explained, since approximately 47% of the studies included in the statistical analysis for this parameter analyzed strawberry varieties with genetic improvements, a product

of Horizon 2020 projects. These varieties did not present high concentrations of the total phenolic content, as in the case of the total anthocyanin content. Although the varieties were selected for an increase in anthocyanins, the content of polyphenols remained the same. It is important to mention that all of them were classified in the altitude range of 0–1000 m.a.s.l.. This explains the notable difference in sample size between one group and another (Table 8). We believe that this could be a limitation and for this reason, no significant differences were detected between the groups (p -value = 0.45). Similar results have been reported in other studies; however, they reported that altitude significantly affected single classes of phenolic compound concentrations in strawberry.

Flavan-3-ol accumulation was significantly lower at 1300 m.a.s.l. in relation to production sites located at low altitudes (Andreotti et al. 2014). In berries, the increase of hydroxycinnamic acids (HCAs) is not gradual along the altitudinal gradient; nonetheless, their levels were higher on the hilltop than in the foothills. Differences in HCAs values between different altitudes may be a result of the long-term abiotic stress effect. HCAs (particularly caffeic acid derivatives) increased with the rising altitude as a response to higher light intensity (especially UV radiation) (Rieger et al. 2008; Häkkinen and Törrönen 2000; Senica et al. 2017; Doumett et al. 2011). On the other hand, p -coumaric acid was the main compound detected in strawberry and its concentration was generally higher at a lower altitude (900 m.a.s.l.) (Guerrero-Chavez et al. 2015). In addition, a moderate increase in the levels of isorhamnetin and kaempferol glycosides was detected at higher altitudes (Senica et al. 2017). Finally, ellagic acid derivatives were significantly more concentrated in strawberries grown in sites at high altitude (approximately 0.28 mg/g FW) compared to strawberries grown at lower altitudes (0.22 mg/g FW) (Osuttis et al. 2010). Other studies into berries have suggested that increasing solar radiation at higher altitudes causes elevated biosynthesis of *ortho*-hydroxylated flavonoids in contrast to the flavonoids lacking this feature. Different flavonoids and hydroxycinnamic acids function as important UV shields and seem to be a part of the plant's protective mechanism that is stimulated at high irradiation sites. Also, other studies reported that fruits growing in a low-light forest environment displayed lower phenolic content, with particularly

low amounts of flavonols (Escobar-Bravo et al. 2017; Mikulic-Petkovsek et al. 2015; Rieger et al. 2008).

Previous studies mentioned that some species of berries, like strawberry (*Fragaria x ananassa* D.), black raspberry (*Rubus occidentalis* L.), red raspberry (*Rubus idaeus* L.), and thornless blackberry (*Rubus* sp.) contained high levels of total antioxidant compounds (TAC), which provide protection against harmful free radicals and have been associated with cardioprotective effects, producing positive effects on human health (Battino et al. 2009). The principal reports of these species' high antioxidant capacity state that they protect against peroxy radicals (ROO^\cdot), superoxide radicals (O_2^\cdot), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^\cdot) (Wang and HsinShan 2000). In this case, two statistical analyzes were performed, one for each method used in the evaluation of antioxidant capacity. These methods are complementary, and they follow the same principle (electron transfer). However, they evaluate different action mechanisms: the FRAP method measures the reducing capacity based upon the reduction of ferric ion, while the DPPH radical scavenging method evaluates the sample's ability to neutralize the free radical 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (Alvarez-Suarez et al. 2009). During the statistical analysis of the antioxidant capacity evaluated by the FRAP method, it was determined that approximately 91% of the studies were classified in the lower altitude range (0–1000 m.a.s.l.), the remaining 9% belonging to the higher altitude range (1000–2000 m.a.s.l.). Insufficient data were found to perform a statistical analysis comparing the two altitudinal groups. Nonetheless, a clear trend is evident in the studies analyzed, where the antioxidant capacity of strawberries grown in the lower altitude range was only assessed using the FRAP method. As regards the analysis of the DPPH radical method to evaluate the antioxidant capacity of strawberries, enough data were found to perform an altitudinal statistical analysis. However, no significant differences were detected between the groups.

One group of investigation collected wild strawberries between 2650 and 3300 m.a.s.l.. The main objective of this study was to determine the principal differences in the chemical composition of strawberries cultivated at high altitude from those grown at low altitude, in this case including the factor of exposure to ultraviolet radiation. The relationship between altitude

and all measurements was examined by linear and nonparametric correlation analysis. The results indicated that wild strawberries at a high altitude presented higher total antioxidant capacity content in comparison with cultivated strawberries at a lower altitude (Peñarrieta et al., 2009). A previous study carried out in Italy corroborates this event. They analyzed two varieties of strawberry: *Regina delle Valli* (911 m.a.s.l.) with a higher content of phenolic acid and vitamin C and *Alpina* (178 m.a.s.l.), whereby *Regina delle Valli*—found at higher altitudes—had a higher antioxidant capacity than the variety found at a lower altitude, namely *Alpina* (Doumett et al. 2011).

According to Guerrero-Chavez et al. (2015), altitudinal variation affects the quality of *Elsanta* strawberries harvested at altitudes at 900 and 1500 m.a.s.l.. In this case, fruits grown at the higher altitude presented approximately 36% less antioxidant capacity than those at the lower altitude. These results are consistent with the lower concentrations of compounds like anthocyanins that present high antioxidant capacity, registered in strawberries from high elevation sites (Rieger et al. 2008). In another study, strawberries were cultivated at two production sites in Switzerland: at 1060 and 480 m.a.s.l.. The variation of chemical composition in these strawberries was related to the difference in the harvesting site and the specific genotype. The antioxidant capacity correlated strongly with total vitamin C content. The antioxidant content was negatively affected when strawberries grown at the higher altitude, as there is a relationship between lower vitamin C concentration in fruits at higher altitudes (Crespo et al. 2010; Shioh et al., 2000).

Conclusion

Specific strawberry quality traits like total soluble solids and total titratable acidity showed significant differences according to the altitude. A significant increase in the content of the both parameters was observed as altitude increased. In the same way, the accumulation of vitamin C and total anthocyanin content in strawberry fruits cultivated at higher altitudes had lower values before we discarded some studies. However, after discarding such studies no significant differences were detected. This effect can be explained as the result of a combination of several

climatic factors (temperature, humidity, daily average radiation, rain, etc.) acting differently at various altitudes. On the other hand, the effect of altitude on the antioxidant capacity evaluated with DPPH radical in strawberries, significant differences were not detected between the groups. An altitude analysis was not performed for the FRAP method, due to the small sample size for the altitude range of 1000–2000 (m.a.s.l.). Similarly, no significant differences were detected between the groups in the case of total phenol content. This is attributed to a high heterogeneity between the studies, due to a notable difference in the size of the sample between one group and another. The findings suggest that altitude may or may not affect the physicochemical composition and antioxidant capacity of strawberries.

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Code availability Not applicable.

Declarations

Conflicts of interest The authors declare no conflict of interest.

Consent to participate All authors consented to participate in the development of the work.

Consent for publication All authors consented to publish the work.

Ethics Approval Not applicable.

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