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Characterization of the phenolic profile and *in vitro* antioxidant potential of different varieties of common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.)

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ABSTRACT

Fagopyrum esculentum Moench (common buckwheat) is the predominant type of buckwheat currently cultivated. Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.), which is a promising minor variety of buckwheat, could contribute to biodiversity and sustainable agricultural and food production. This paper evaluates and characterizes the soluble phenolic profile and antioxidant capacity of nine common buckwheat varieties (*Fagopyrum esculentum* Moench) and one tartary buckwheat variety (*Fagopyrum tataricum* (L.) Gaertn.), analyzed in both flour (<250 μm) and semi-finished product (>250 μm) forms. Several different *in vitro* methods were employed: Folin–Ciocalteu assay, DPPH assay, TEAC assay, ORAC assay, HPTLC, HPLC-DAD and LC-HRMS. The results revealed that compared to the common buckwheat varieties, tartary buckwheat showed the highest content of soluble phenolic compounds (10.99 ± 1.35 mg GAE/g d.m.) and the highest antioxidant capacity (respectively 3.44 ± 0.33 mg GAE/g d.m. for the DPPH assay, 21.46 ± 1.74 mg TE/g d.m. for the TEAC assay and 139.14 ± 4.16 mg TE/g d.m. for the ORAC assay). These preliminary findings suggest that tartary buckwheat, with its rich phenolic content and potent antioxidant activity, holds considerable promise as a functional food ingredient which could offer health benefits to consumers.

1. Introduction

Buckwheat (*Polygonum fagopyrum* L.) is an annual herbaceous plant belonging to the Polygonaceae family of the dicotyledonous class (Giménez-Bastida & Zieliński, 2015). The *Fagopyrum* genus comprises fewer than 30 species, which are mostly endemic to southern China. The genus includes two primary cultivated species: common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) (Ohsako & Li, 2020).

Currently, the most widespread species of buckwheat (*F. esculentum*) is distributed across Asia, Europe, Africa, Oceania and North America (Sedej et al., 2012). In Italy, it thrives in the alpine regions of Valtellina and Val Venosta, where it is used for the preparation of typical regional food products (Bonafaccia & Fabjan, 2003).

Tartary buckwheat (*F. tataricum*) is a promising crop that could contribute to biodiversity and sustainable agriculture. Originating in

Abbreviations: SPC, Soluble Polyphenol Content; GAE, Gallic Acid Equivalent; d.m., dry matter; HPLC-DAD, High Performance Liquid Chromatography Coupled to a Photodiode Array Detector; LC-HRMS, Liquid Chromatography High Resolution Mass Spectrometry; AOA, Antioxidant Capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TEAC, Trolox Equivalent Antioxidant Capacity; TE, Trolox Equivalent; ABTS, 2,2′-azino-bis(3-etilbenzotiazolin-6-sulfonic); ORAC, Oxygen Radical Absorbance Capacity; AUC, Fluorescence Decay Curve; AAPH, 2,2′-azo-bis(2-methylpropionamidine) dihydrochloride; HPTLC, High Performance Thin Layer Chromatography; SD, Standard Deviation.

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Table 1
Samples included in this study and the corresponding identification code.

Type of buckwheat	Variety	Code
Common buckwheat	Bamby	BA
	Billy	BI
	Darja	DA
	Grigio-Francese	GF
	Kora	KO
	Lileja	LI
	Nojai	NO
	Panda	PA
	Zirca	ZI
Tartary buckwheat	Siberiano	SIB

western China, it tolerates harsher climates than common buckwheat (Bonafaccia et al., 2003), and is cultivated in the mountainous regions of southwestern China (Sichuan), Bhutan, northern India and Nepal. In Europe, tartary buckwheat is traditionally grown in Luxembourg, Belgium, Germany, Slovenia, Italy, Ukraine, Russia and Serbia (Kreft et al., 2022). Tartary buckwheat is known for its resilience to cold and drought (Luthar et al., 2021) and resistance to UV-B radiation. These characteristics are attributed to its high content of polyphenols, with rutin, quercetin and fagopyrin playing a protective role (Kreft et al., 2022). Studies have shown that the flavonoid content of tartary buckwheat is 23–45 and 25–50 times greater than that of wheat and corn, respectively (Qin et al., 2010; Zhu et al., 2019). The high polyphenolic content might not only protect the plant from adverse environmental conditions, plant diseases and pests, but also provide potential health benefits (Kreft et al., 2022; Luthar et al., 2021), such as anti-inflammatory, antioxidant, and anti-diabetic properties (Bani et al., 2024; Chandramohan et al., 2015; Di Lorenzo et al., 2021; Lee & Choi, 2008; Li & Howard Zhang, 2001; Piazza et al., 2024). In addition to polyphenols, tartary buckwheat is also rich in dietary fiber, protein, vitamins (B and E), minerals and essential amino acids, making it a valuable ingredient for a variety of food products, including noodles, breads, and biscuits. In addition, its nutritional profile and health potential make it an appealing raw material for the development of new functional foods (Li & Howard Zhang, 2001; Zamaratskaia et al., 2023). While previous research has focused on tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.), this study aims to break new ground by evaluating and characterizing the phenolic profile and *in vitro* antioxidant capacity of nine different varieties of common buckwheat in addition to one variety of tartary buckwheat. Additionally, our study compares the particle sizes of different varieties in the form of flour (particle size <250 μm) and semi-finished products (particle size >250 μm) in terms of the quantity of phenolic compounds and antioxidant activity.

2. Materials and methods

2.1. Chemicals

Ethanol, methanol, HPLC grade water, acetonitrile, acetone, toluene, and formic acid were provided by VWR International (Fontenay-sous-Bois, France). Folin-Ciocalteau reagent, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2′-azo-bis(2-methylpropionamidine) dihydrochloride (AAPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), trolox, gallic acid, and rutin were purchased from Sigma Aldrich (Steinheim, Germany). Water was purified using a Milli-Q system (Millipore Billeroca, MA,USA).

2.2. Samples

The study included 9 varieties of common buckwheat (*Fagopyrum esculentum* M.), kindly supplied by Molino Filippini (Sondrio, Italy), and one variety of tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn) (Table 1).

The common buckwheat varieties were provided in the form of flour (particle size <250 μm) and semi-finished products (particle size >250 μm) (Fig. 1). The tartary buckwheat variety was provided only in flour form (particle size <250 μm). The buckwheat samples were stored at 4 °C in the dark.

2.3. Determination of soluble phenolic content

2.3.1. Extraction

The soluble phenolic compounds were extracted using a hydro-alcoholic solution: 0.5 g of sample was suspended in 10 mL of ethanol:

Table 2
Soluble phenolic compound content (SPC) of common (*Fagopyrum esculentum* Moench) and tartary (*Fagopyrum tataricum* (L.) Gaertn) buckwheat varieties.

Sample Code	Soluble polyphenols (mg GAE/g)	
	Flour	Semi-finished product
BA	$2.34 \pm 0.31^{a, A}$	$4.92 \pm 0.25^{a, B}$
BI	$2.21 \pm 0.21^{a, A}$	$7.33 \pm 0.22^{d, B}$
DA	$2.95 \pm 0.06^{ab, A}$	$5.03 \pm 0.13^{ab, B}$
GF	$2.51 \pm 0.25^{a, A}$	$5.96 \pm 0.65^{bc, B}$
KO	$2.66 \pm 0.14^{ab, A}$	$7.44 \pm 0.49^{d, B}$
LI	$2.96 \pm 0.13^{ab, A}$	$7.62 \pm 0.87^{d, B}$
NO	$2.81 \pm 0.28^{ab, A}$	$6.93 \pm 0.73^{cd,B}$
PA	$2.70 \pm 0.22^{ab, A}$	$7.80 \pm 1.01^{d,B}$
ZI	$3.35 \pm 0.10^{b, A}$	$5.95 \pm 0.37^{bc, B}$
SIB	10.99 ± 1.35^{c}	–

In the same column, samples with different lower-case letters are significantly different ($p < 0.05$). In the same row, upper-case letters indicate statistically significant differences among the different processing treatments ($p < 0.05$).

Fig. 1. Samples included in the study.

Table 3
Compounds identified in flour extracts with the targeted approach.

Peak	Name	RT	m/z sperim	MS/MS	Code									
1	Caffeic acid dihexoside	1.17	503.14007	323-179-221-341-161	SIB	–	–	–	–	DA	BI	BA	PA	GF
2	p-hydroxybenzoic acid 1/ Protocatechualdehyde	1.72	137.02487	93	SIB	–	–	–	KO	DA	BI	BA	PA	GF
3	Epicatechin/Catechin-glucoside isomer 1	2	451.12503	289–245	–	ZI	NO	LI	KO	DA	–	BA	PA	GF
4	Epicatechin/Catechin-glucoside isomer 2	2.6	451.12491	289–245	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
5	Epicatechin/Catechin-glucoside isomer 3	3.2	451.12448	289–245	SIB	ZI	NO	LI	KO	DA	–	BA	PA	GF
6	p-hydroxybenzoic acid 2	3.6	137.0249	93–63	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
7	Epicatechin/Catechin-glucoside isomer 4	3.6	451.12433	289–245	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
8	Caffeic acid hexose isomer a	3.7	341.08755	179-135-125-143-161-269-281-295	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
9	Ferulic acid	3.85	193.05057	–	–	–	NO	LI	KO	–	–	–	PA	–
10	Swertiamacroside	4.06	487.14627	–	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
11	(+)-catechin	4.1	289.07169	245-205-179	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
12	Procyanidin A isomer 1	4.32	575.11902	439-421-451-285-259-467-301	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
13	Sinapic acid glucoside	4.6	385.11346	223–208	SIB	–	–	–	–	DA	–	BA	PA	GF
14	Procyanidin B2	5.41	577.13464	407-425-451-289-245-287-299-541	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
15	Procyanidin trimer 1	5.45	865.19861	–	SIB	ZI	NO	LI	KO	DA	BI	BA	–	–
16	(Epi)afzelechin-(epi) catechin isomer 1	5.8	561.14026	435-407-289-271	–	ZI	–	LI	–	–	BI	–	–	–
17	(Epi)afzelechin-(epi) catechin isomer 2	6.8	561.14056	289-425-271-406-435-285	SIB	–	–	–	–	–	–	–	–	–
18	3-Methylcatechol	6.88	123.04554	–	–	–	–	–	KO	–	BI	BA	–	GF
19	Sinapic acid 2	8.35	385.11346	267	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
20	Procyanidin trimer 2	8.92	865.19904	695-577-407-451-543-713	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
21	(−)-epicatechin	9.2	289.07169	245-247-203-204-227-179	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
22	(Epi)afzelechin–(epi)-catechin isomer 3	9.28	561.14093	289-407-435-205-245-273-271-329-425	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
23	procyanidin B2-3-O-gallate	10.54	729.14624	407-289-441-451-559-577	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
24	Procyanidin trimer 3	11.01	865.19952	543-603-459-457-703-321-374-575-729	–	ZI	NO	–	KO	DA	BI	BA	PA	GF
25	Procyanidin A isomer 2	11.87	575.11945	303-217-243-283-337-379-423-449-513	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
26	Kaempferol-C-hexoside 1	12.38	447.09293	327–357	–	–	NO	LI	KO	DA	BI	BA	PA	GF
27	Kaempferol-C-hexoside 2	13.3	447.09323	327–357	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
28	Epiafzelechin–epiafzelechin–epicatechin 1	15.4	833.20966	543-561-679-706-407-289-271	SIB	–	–	–	–	–	–	–	–	GF
29	Epicatechin-gallate	16.96	441.08252	289-169-193-245-253-271-303-331-397	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
30	Vitexin	17.7	431.09818	–	–	–	–	–	–	DA	BI	BA	PA	GF
31	Epiafzelechin–epiafzelechin–epicatechin 2	18.22	833.20966	707-543-561-435-289-679	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
32	Rutin	18.5	609.14398	301–463	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
33	Isovitexin	18.68	431.09818	311-341-283	–	–	–	LI	KO	DA	BI	BA	PA	GF
34	Isoquercitrin/Hyperoside	19.8	463.08801	301-300-211-179-343-409	–	–	NO	–	KO	DA	BI	BA	PA	GF
35	Epiafzelechin–epicatechin-O-methylgallate	23.72	727.16595	407-435-289-455-543-561-601	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
36	Kaempferol 3-rutinoside	25.78	593.15222	285-257-284	SIB	ZI	NO	LI	KO	–	BI	BA	PA	GF
37	Quercitrin	27.23	447.09299	301-285-284-300	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
38	Procyanidin B2-dimethylgallate	32.54	757.17706	605-587-631-469-437-433-389-287-271	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
39	Apigenin-o-glu	35.31	431.09848	269-311-240-253	SIB	–	–	–	–	DA	–	BA	PA	GF
40	Chrysoeriol-7-O-b-D-glucopyranoside	35.76	461.10901	299-283-255-397-425-253	SIB	–	–	LI	–	DA	–	–	–	–
41	Epiafzelechin–epicatechin-O-dimethylgallate	38.56	741.18134	271-319-407-425-437-465-469-543-573-587-605-615	SIB	ZI	NO	LI	KO	DA	BI	BA	PA	GF
42	Quercetin	44.2	301.0347	179-151-107-121-193-211-229-257-273-301	SIB	–	NO	LI	KO	DA	BI	BA	–	GF
43	Apigenin	52.8	269.04578	185-197-199	SIB	–	–	–	–	–	–	–	–	–
44	Kaempferol	54.7	285.04053	223-239-243-249-257	SIB	–	–	LI	KO	–	–	–	PA	–

water 60:40 (v/v) (Catena et al., 2019) and stirred for 2 h at room temperature in the dark. The samples were then centrifuged at 2000×*g* at 4 °C for 15 min (5810-R, Eppendorf, Hamburg, Germany) and filtered through a paper filter (Whatman grade 1). The extracted solutions were stored at −20 °C until analysis. Each sample was extracted at least in triplicate (Colombo et al., 2023).

2.3.2. Folin-Ciocalteu assay

The soluble phenolic compounds were quantified using the Folin-Ciocalteu method (Singleton & Rossi Jr, 1965): 300 μL of suitably diluted samples or water for blank sample, were added to 1.5 mL of 0.2 mol/L Folin–Ciocalteau's reagent and 1.2 mL of 7.5g/100 mL sodium carbonate. The samples were maintained for 30 min in the dark and the absorbance was detected at 765 nm in a UV spectrophotometer (Varian Cary 50 SCAN, Palo Alto, CA, USA). The results were expressed as mg gallic acid equivalents (GAE)/g of sample (dry matter, d.m), using a calibration curve of gallic acid in the concentration range of 5–50 μg/mL.

2.3.3. Quantification of phenolic compounds using HPLC-DAD

Since rutin is the most abundant compound, both in common and in tartary buckwheat, the total rutin content was evaluated by HPLC-DAD following the method described by Colombo et al. (2021) (Colombo et al., 2021).

Following the same extraction procedure applied for the Folin-Ciocalteu assay, the extracts were subsequently filtered through a

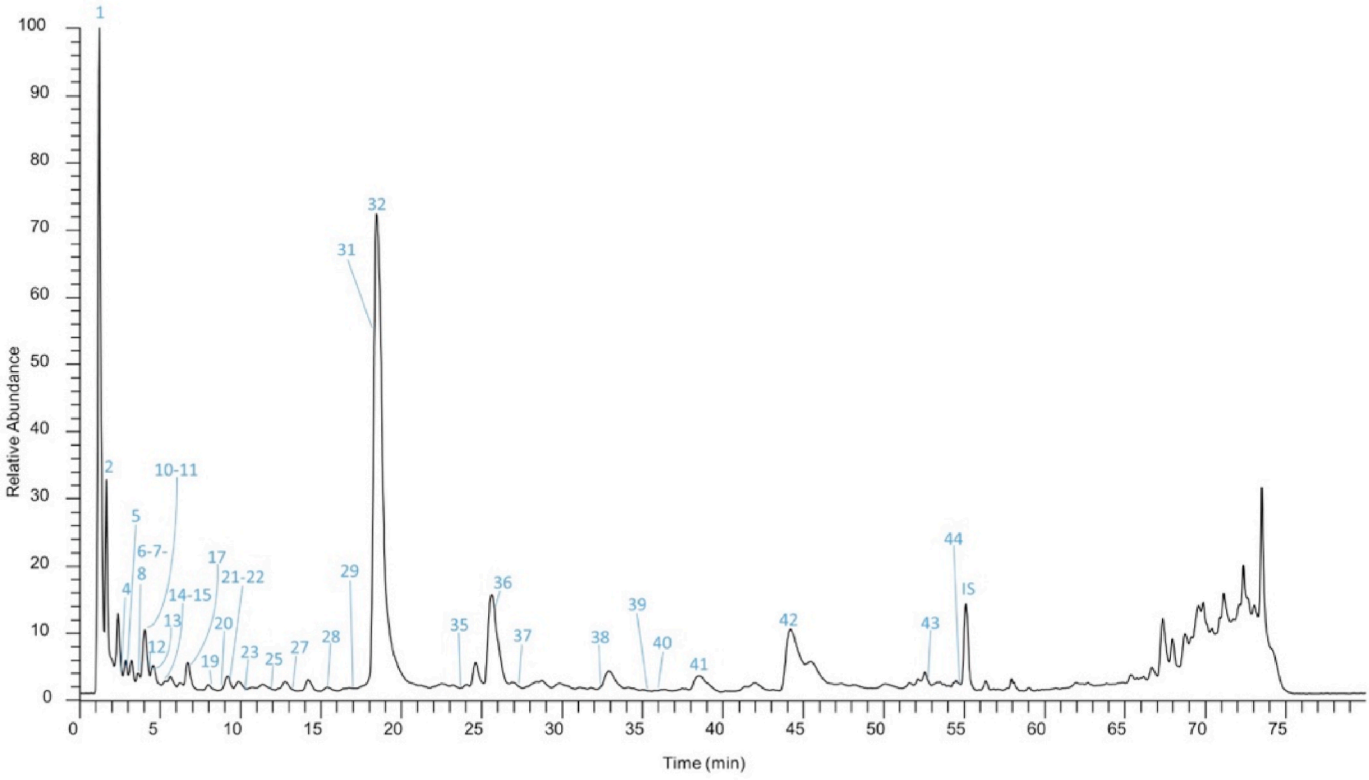
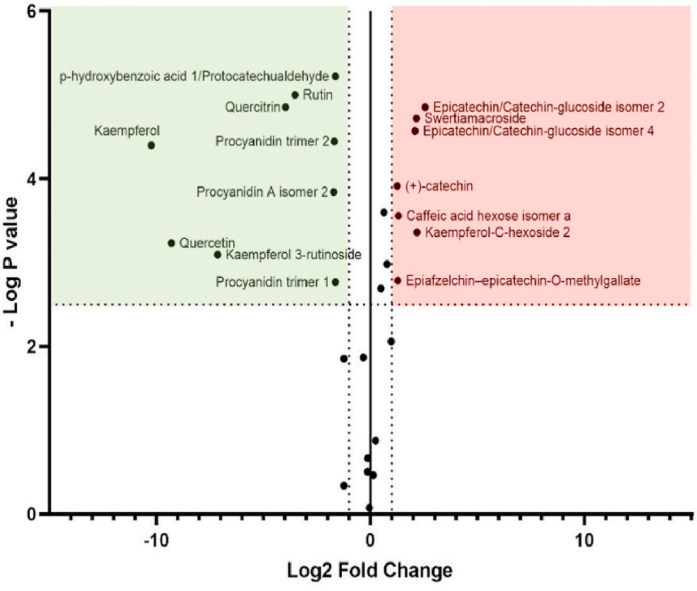


Fig. 2. Total ion currents (TICs) of SIB extracts. The ions identified by the targeted approach are labelled in blue with a progressive number.



Compound	Log2 Fold Change	- Log P Value
Kaempferol	-10.23095166	4.397940009
Quercetin	-9.302449695	3.231361899
Kaempferol 3-rutinoside	-7.140812622	3.092588639
Quercitrin	-3.962980792	4.853871964
Rutin	-3.525549615	5
Procyanidin A isomer 2	-1.726480501	3.841637508
Procyanidin trimer 2	-1.700393019	4.443697499
p-hydroxybenzoic acid 1/Protocatechualdehyde	-1.642133857	5.22184875
Procyanidin trimer 1	-1.641841995	2.767512134
Procyanidin B2	-1.232344594	1.853282964
p-hydroxybenzoic acid 2	-1.232263227	0.338097437
Procyanidin A isomer 1	-0.331772783	1.867452158
(-)-epicatechin	-0.13890021	0.50549199
procyanidin B2-3-O-gallate	-0.129655202	0.664921395
(Epi)afzelchin–(epi)-catechin isomer 3	-0.055058629	0.075197807
Sinapic acid 2	0.137789373	0.464419821
Epicatechin-gallate	0.240941345	0.876167952
Epicatechin/Catechin-glucoside isomer 3	0.488814919	2.691862621
Procyanidin B2-dimethylgallate	0.625153181	3.598599459
Epiafzelchin–epiafzelchin–epicatechin 2	0.774567511	2.98088371
Epiafzelchin–epicatechin-O-dimethylgallate	0.966790884	2.057793458
(+)-catechin	1.250411557	3.913640169
Epiafzelchin–epicatechin-O-methylgallate	1.275424501	2.786216701
Caffeic acid hexose isomer a	1.303342967	3.557520231
Epicatechin/Catechin-glucoside isomer 4	2.07785731	4.568636236
Swertiamacroside	2.140405652	4.721246399
Kaempferol-C-hexoside 2	2.163710842	3.359518563
Epicatechin/Catechin-glucoside isomer 2	2.550693503	4.853871964

Fig. 3. Volcano plot showing the semi-quantitative difference for each metabolite present in the two extracts Kora common varieties (KO) compared with the tartary extract (SIB).

0.45 μm nylon filter (VWR International; Fontenay-sous-Boys, France). A stock solution of rutin (95% purity, Sigma-Aldrich) was prepared, and a solution with a final concentration of 500 μg/mL in methanol was obtained.

Briefly, the analysis was conducted using Jasco HPLC equipment (Jasco, Tokyo, Japan) provided with a pump (PU-980), an interface (LC-NETII/ADC), a diode array detector (MD-2010 Plus), a mixer (LG-150-0.4), a degasser (DG-2080-54), and an injection valve (Rheodyne, Cotati, CA, USA) equipped with a 20 μL loop. A Hibar® 250–4.6 mm column, LiChrospher® 100 RP-18 (5 μm) (Merck KgaA; Darmstadt, Germany) was used for separation. The flow rate was set at 0.8 mL/min^{-1}. The gradient was set up as follows: 0–15 min: 94%–70% A; 15–30 min: 70%–50% A; 30–35 min: 50%–10% A; 35–38 min: 10% A; where (A) had the following composition: water:acetonitrile:formic acid

Table 4
Antioxidant activity (AOA) of common (*Fagopyrum esculentum* Moench) and tartary (*Fagopyrum tataricum* (L.) Gaertn) buckwheat varieties.

Sample Code	Antioxidant activity (AOA)					
	DPPH assay (mg GAE/g)		TEAC assay (mg TE/g)		ORAC assay (mg TE/g)	
	Flour	Semi-finished product	Flour	Semi-finished product	Flour	Semi-finished product
BA	0.68 ± 0.02 c, A	1.86 ± 0.13 bc, B	4.59 ± 0.08 a, A	9.96 ± 0.72 a, B	4.58 ± 0.20 a, A	56.5 ± 1.79 d, B
BI	0.59 ± 0.03 abc, A	2.69 ± 0.13 f, B	4.45 ± 0.18 a, A	15.7 ± 1.50 bc, B	27.3 ± 0.74 e, A	93.2 ± 2.58 h, B
DA	0.64 ± 0.02 bc, A	2.02 ± 0.03 cd, B	5.32 ± 0.33 ab, A	13.9 ± 1.25 b, B	18.6 ± 0.89 c, A	51.4 ± 3.29 c, B
GF	0.56 ± 0.04 abc, A	2.36 ± 0.13 e, B	4.59 ± 0.21 a, A	16.9 ± 0.85 c, B	6.85 ± 0.57 a, A	60.9 ± 2.45 e, B
KO	0.61 ± 0.01 abc, A	2.16 ± 0.17 de, B	6.93 ± 0.24 cd, A	14.7 ± 1.26 b, B	12.5 ± 0.62 b, A	47.0 ± 2.79 b, B
LI	0.63 ± 0.02 bc, A	2.23 ± 0.17 de, B	7.89 ± 0.80 d, A	20.3 ± 2.11 d, B	20.2 ± 0.34 c, A	71.4 ± 2.84 f, B
NO	0.43 ± 0.06 a, A	1.78 ± 0.20 b, B	4.82 ± 0.12 ab, A	13.8 ± 0.64 b, B	19.0 ± 0.63 c, A	85.1 ± 0.87 g, B
PA	0.49 ± 0.02 ab, A	2.00 ± 0.12 bcd, B	4.48 ± 0.19 a, A	14.5 ± 1.04 b, B	14.5 ± 0.65 b, A	82.5 ± 0.87 g, B
ZI	0.63 ± 0.03 bc, A	1.06 ± 0.13 a, B	5.86 ± 0.26 bc, A	7.90 ± 0.96 a, B	23.4 ± 0.43 d, A	25.9 ± 0.31 a, B
SIB	3.44 ± 0.33 d	–	21.5 ± 1.74 e	–	139.1 ± 4.16 f	–

In the same column and for the same assay, samples with different lower-case letters are significantly different ($p < 0.05$). For the same row and same assay, upper-case letters indicate statistically significant differences among the different treatments ($p < 0.05$).

96:3:1 (v/v/v); and (B): acetonitrile:water:formic acid 50:49:1 (v/v/v). Rutin was detected at 360 nm. A calibration curve was constructed for the standard with a concentration range of 0.5–50 μg/mL. The results were expressed as mg rutin/g of sample (d.m).

2.3.4. Qualitative and semi-quantitative profile of phenolic compounds using LC-HRMS

The stock solution (5 mg/mL) of each flour extract was prepared by solubilizing the powder in methanol:water (50:50, % v/v) and subsequently carrying out a 1:2 dilution (v:v) in mobile phase A (Water – 0.1 % formic acid). Trolox was added as an internal standard to obtain a final concentration of 50 μmol/L. The sample (20 μL) was analyzed in triplicate by LC-HRMS using the method described by Baron et al. (2021) (Baron et al., 2021).

2.3.4.1. Targeted analysis of buckwheat extract components. By searching the literature for the known components in buckwheat, a reference database was created (n = 70). The targeted analysis was performed by verifying the presence of the compounds reported in the database, by comparison of their accurate mass ($[M-H]^-$), with a tolerance of 5 ppm. The identification of the compounds was confirmed based on the fragmentation pattern obtained.

2.3.4.2. Semi-quantitative data analysis. A semi-quantitative analysis was carried out in order to compare the relative contents of the compounds in the common buckwheat extract with those of the tartary buckwheat extract (SIB), which was used as a reference. This was based on the integration of the areas of the individual compounds, which were normalized to the area of the internal standard. The ratio thus obtained for each triplicate analysis was averaged, and the fold change was calculated for each compound in common with the reference extract, i.e.:

Compound fold change X= (Average area ratio extract X)/(Average area ratio SIB extract)

The volcano plot was constructed by plotting on the x axis, for each identified analyte, the log2 of the fold change between $(AUC_n)/(AUC_{I.S})_{extract}$ $(AUC_n)/(AUC_{IS})_{Siberiano}$ and on the y-axis, the -Log p value of the average ratios. When metabolites in the two extracts had a log2 fold change ≥ 1 or ≤ -1 and a -Log p value ≥ 2.5, their relative content was considered significantly different.

2.4. Determination of antioxidant capacity

The *in vitro* antioxidant activity (AOA) of the buckwheat samples was measured spectrophotometrically, using 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH), trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity (ORAC) assays and the High-Performance Thin Layer Chromatography (HPTLC).

2.4.1. DPPH assay

The antioxidant capacity of the samples was evaluated as a measure of radical scavenging activity using the DPPH spectrophotometric assay (Brand-Williams et al., 1995). The samples (prepared as described in Section 2.3.1 and suitably diluted) or water, used as a blank (0.5 mL), were added to 1 mL of 0.005% DPPH• in methanol and incubated for 30 min in the dark. The absorbance was measured at 517 nm against methanol. The concentration of antioxidants was calculated using a calibration curve built by plotting the concentration of gallic acid (from 1.0 to 5.0 μg/mL) versus the difference between the absorbance of the blank and the absorbance of standards. The results were expressed as mg GAE/g d.m.

2.4.2. TEAC assay

The Trolox Equivalent Antioxidant Capacity (TEAC) assay was performed as described by Re et al. (1999) (Re et al., 1999), with some modifications. The ABTS radical cation solution was prepared by mixing 2.45 mmol/L potassium persulfate and 7 mmol/L ABTS (1.1 v/v). The mixture was then stored for 12–16 h in the dark at room temperature. Before use, the ABTS+• solution was diluted with ethanol, to obtain an absorbance of 0.7 ± 0.02 at 734 nm. An aliquot of 1.5 mL of ABTS+• solution was mixed with 150 μL of each sample (prepared as described in Section 2.3.1, and suitably diluted), or water was used as a blank. The absorbance was measured after 6 min at 734 nm. The percentage of ABTS+• inhibition was calculated as described below:

$$\% \text{ inhibition of ABTS} + \cdot = [(Ab - At) / Ab] x\ 100$$

where Ab is the absorbance of the blank and At is the absorbance of the sample. Results were calculated using a Trolox standard calibration curve, with concentrations between 10 and 30 μg/mL, and expressed as Trolox equivalents (TE) in mg/g d.m.

2.4.3. ORAC assay

The oxygen radical absorbance capacity (ORAC-FL) assay was performed as previously described by Caceres et al. (2014) (Cáceres et al., 2014). The reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4) for 150 min. The reaction mixtures contained 180 μl of fluorescein at 70 nM, 90 μl of 2,20-azo-bis(2-methylpropionamidine) dihydrochloride (AAPH) at 12 mM and 30 μl of diluted sample or Trolox standard at concentrations ranging from 1 to 8 μmol/L. The reaction

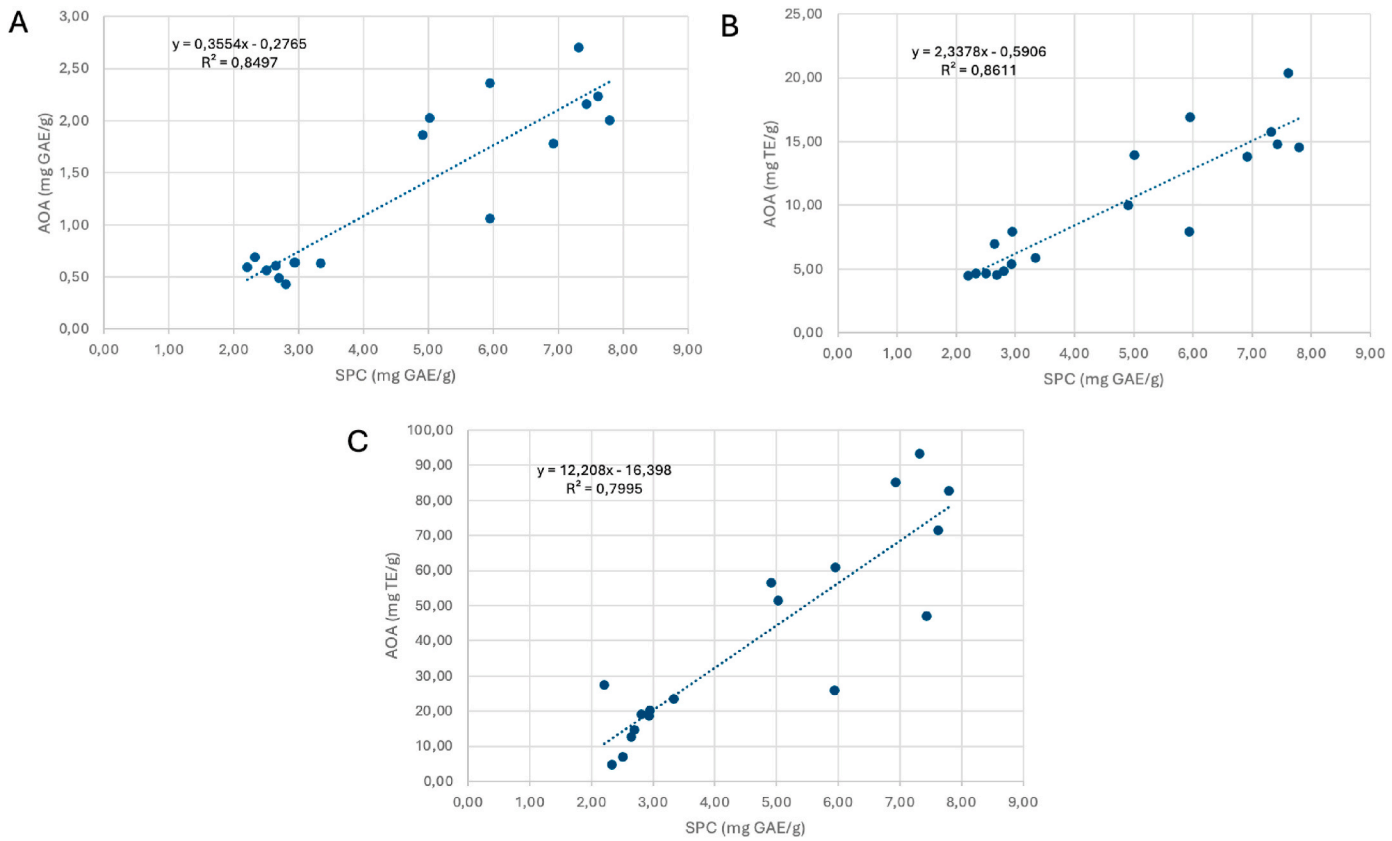


Fig. 4. Correlation analysis between content of total soluble polyphenols (mg/g) and the antioxidant activity (mg/g) (**A**: DPPH method, **B**: TEAC method, **C**: ORAC method) of buckwheat samples.

mixtures were placed in a black 96-well plate (Fisher Scientific) in triplicate. The plate was shaken automatically, and the fluorescence was read in a plate spectrophotometer (Synergy HT, BioTek Instruments) every 2 min at $_{exc}$485 nm and $_{emi}$520 nm. The equipment was controlled by Gene5TM software, version 1.1 (BioTek Instruments). Areas under the fluorescence decay curve (AUC) based on fluorescence values relative to the initial reading were recorded and subtracted from the AUCs of blank samples. The analyses were performed in duplicate. The results were expressed in mg of TE/g d.m.

2.4.4. High Performance Thin Layer Chromatography (HPTLC)

High Performance Thin Layer Chromatography (HPTLC) was used to evaluate the phenolic profiles of the buckwheat varieties studied and their antioxidant activities. This method is an evolution of classical Thin Layer Chromatography (or TLC) and is based on the differential distribution of the analytes between a stationary phase and a mobile phase. HPTLC enabled us to carry out a semi-quantitative analysis and to obtain the fingerprints of the samples (Reich & Schibli, 2007).

The analysis was conducted by detecting the plate at 254 nm and 366 nm, comparing the chromatographic run of the standards with that of the samples and comparing the type and intensity of the coloration of the bands. In addition, the use of 50 mg/10 mL DPPH in methanol as an agent for visualization us to evaluate the antioxidant activity of the individual molecules contained in the sample. DPPH is a radical characterized by a purple colour which in the presence of antioxidants is reduced and develops a yellow colour. The intensity of the discoloration from purple to yellow is proportional to the antioxidant activity of the compound.

The samples were prepared as described in Paragraph 2.3.1. Once the extraction was complete, the organic solvent was evaporated using a rotavapor (Heidolph Instruments GmbH & Co, Schwabach, Germany) and the residual aqueous component was freeze-dried. After freeze-drying, the residue was recovered with 1 mL of methanol and kept at −20 °C until the analysis.

2.5. Statistical analysis

The data were subjected to one-way ANOVA using the least-square difference test (LSD) with the Statgraphic Centurion XVI Program, v. 16.1.17 (Statistical Graphics Corp., Rockville, MD) for Windows. The correlation between the various spectrophotometric assays (Folin-Ciocalteu, DPPH, TEAC, and ORAC assay) was conducted using Excel for Macintosh.

3. Results

3.1. Content of soluble phenolic compounds

Phenolic compounds are present in grains both in soluble form and bound to components of the cell wall (Bani et al., 2023). In this study we focused on the soluble fraction, since several studies have suggested that free phenolic compounds are present in greater amounts in tartary buckwheat than in the bound form (Zhu et al., 2019). Table 2 shows the concentration (mg GAE/g d.m.) of the total soluble polyphenol content found in the flour (particle size <250 μm) and semi-finished products (particle size >250 μm) obtained from the 9 varieties of common buckwheat studied, as well as in the flour (particle size <250 μm) of the tartary buckwheat.

Table 2 shows that the common buckwheat flour has a soluble polyphenol content ranging between 2.21±0.21 and 3.35±0.10mg GAE/g d.m., whereas the semi-finished product showed higher values (4.92±0.25–7.80 ±1.01mg GAE/g d.m.). The data obtained for the buckwheat flour samples are in line with those reported in the literature, showing values between 2.55 and 15.08 mg GAE/g (Inglett et al., 2011).

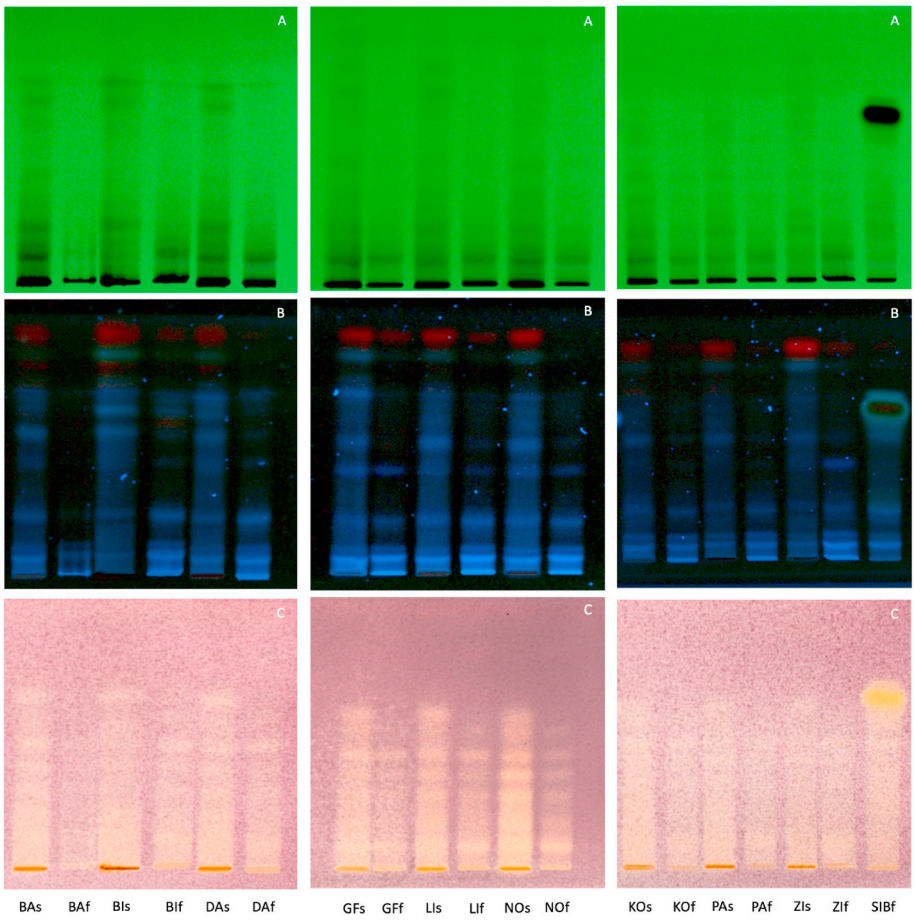


Fig. 5. HPTLC analysis of buckwheat samples Bamby (BA), Billy (BI), Darja (DA), Grigio-Frencese (GF), Lileja (LI), Nojai (NO), Kora (KO), Panda (PA), Zirca (ZI), Siberiano (SIB); f: flour; s: semi-finished A) detection at 254 nm, B) detection at 366 nm, C) detection in visible light after derivatization with 0.05% DPPH.

Among the analyzed samples, tartary buckwheat flour (SIB) had the highest amount of soluble phenolic compounds (10.99±1.35 mg GAE/g d.m.). These results are in line with the paper by Kreft et al. (2016), where the tartary buckwheat variety had a higher content of total soluble polyphenols than common buckwheat varieties. Similarly, Kitabayashi et al. (1995), reported that the tartary buckwheat had an almost 100 times higher rutin (flavonol-3-*O*-rutinoside) content than the species *Fagopyrum esculentum* Moench.

Rutin is the predominant compound found in both common and especially in tartary buckwheat varieties (Wang et al., 2024); other phenolic compounds such as catechins, phenolic acids and procyanidin B2 are also found although in a lesser amount. We thus focused our attention on rutin (Table 1S of the supplementary material). The analyses were performed on flour and semi-finished samples of each variety by setting the UV detector at 360 nm. The results obtained to date appear to be in line with those found by Folin-Ciocalteu's assay. Additionally, in this case, a higher rutin content was observed in the semi-finished sample (average 0.28±0.06 mg/g) compared to the flour (average 0.18±0.02 mg/g).

The tartary buckwheat sample (7.93±0.16 mg/g) also showed a significantly higher rutin content than common buckwheat varieties. The results on rutin agree with those reported in the literature in similar samples (common buckwheat 0.06±0.00 mg/g and tartary buckwheat 7.6±1.1 mg/g) (Zhu et al., 2019). For some semi-finished samples, the peak associated with rutin was not quantifiable, since it partially coeluted with other compounds, and therefore no value is reported.

The qualitative phenolic profile of the flour extracts was determined using a targeted approach, which consisted of the construction of a database of buckwheat components already reported in the literature and containing 70 entries (Table 2S of the supplementary materials). The targeted analysis was performed by verifying the presence of the compounds reported in the database using their accurate mass ($[M-H]^-$), with a tolerance of 5 ppm. The identities of the compounds were confirmed based on the fragmentation patterns obtained.

Table 3 shows the 44 identified metabolites. Seven were phenolic acids (16%), 14 were catechins/epicatechins (32%), 13 were other flavonoids (30%), 8 were procyanidins (18%) and 2 other phenols (5%). The majority was present in all extracts, except for (epi)afzelechin-(epi) catechin isomer 2 and apigenin, which were only detected in tartary buckwheat (SIB), and epiafzelechin–epiafzelechin–epicatechin 1, which was only found in the flour extract of the common Grigio-Francese variety (GF) and in the tartary buckwheat extract (SIB). In addition, chrysoeriol-7-*O*-β-D-glucopyranoside, which was previously found only in the flour extract of the common Lileja variety (LI) and in the tartary buckwheat extract (SIB), was identified based on the exact mass and fragmentation spectrum as no data were available in the literature.

Fig. 2 shows the chromatogram obtained for the tartary sample (SIB), where each identified peak reports a progressive number following the elution order (the relative name is shown in Table 3)

Fig. 3 shows the data in graphical form (Volcano plot) of Kora common varieties (KO) compared with the tartary extract (SIB) and the related tables containing Log2 Fold change and –Log p value for the compounds in common.

Compounds that are significantly more abundant in the Tartary buckwheat extract (SIB) are highlighted in green, while those more abundant in the common Kora extract (KO) are highlighted in red. Compounds such as rutin, quercetin, and kaempferol are significantly more abundant in the Tartary buckwheat extract (SIB). Conversely,

compounds such as epicatechin/catechin-glucoside isomer 2, kaempferol-c-hexoside 2, and swertiamacroside are present in higher concentrations in the common Kora variety (KO). Compounds not highlighted in any color show Log2 Fold Change values close to 0, suggesting that their abundance is comparable between the two extracts.

3.2. Antioxidant capacity

Three different spectrophotometric methods were used to evaluate the *in vitro* antioxidant activity of the samples: DPPH assay, TEAC assay, and ORAC assay. *In vitro* antioxidant activity (AOA) is one of the most investigated properties since oxidative stress is a key factor in the induction of chronic diseases and their progression. Many assays have been developed for the *in vitro* evaluation of AOA. For a complete evaluation, different methods need to be applied in parallel and compared (Alam et al., 2013).

The DPPH assay is an excellent tool for monitoring chemical reactions involving radicals. DPPH• is a stable free radical with a characteristic absorption at 517 nm. This absorption significantly decreases when the active compound reacts with proton radical scavengers (Gulcin, 2020).

The Trolox equivalent antioxidant capacity (TEAC) method is based on the measurement of colour loss. This colour loss occurs when an antioxidant is added to the blue-green chromophore ABTS+• (2,2-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid). Antioxidant compounds reduce ABTS+• to ABTS, thus leading to decolorization (Gulcin, 2020).

In the ORAC assay, the reaction is based on the transfer of a hydrogen atom from the antioxidant to the free radical. AAPH is used as the initiator radical to generate the peroxyl radical ROO (Gulcin, 2020).

The *in vitro* antioxidant activity (AOA) of the buckwheat samples, determined by the DPPH, TEAC and ORAC assays, is reported in Table 4.

The antioxidant activity of the common flour variety, determined by the DPPH assay, ranged between 0.43±0.06 and 0.68±0.02 mg GAE/g d.m., whereas that of the semi-finished product ranged between 1.06 ±0.13 and 2.69±0.13 mg GAE/g d.m. These results indicated that the flour of each common buckwheat variety had a lower antioxidant activity than the corresponding semi-finished product. Similarly, according to the TEAC test, the semi-finished component of each variety expressed a greater antioxidant activity (range 7.90±0.96 - 20.3±2.11 mg TE/g d.m.) than the corresponding flour (range 4.48±0.19 - 7.89 ±0.80 mg TE/g d.m.). Lastly, the ORAC assay showed a similar trend to the other two spectrophotometric assays, with the semi-finished samples having a higher antioxidant activity (25.9±0.31 - 93.2±2.58 mg TE/g d.m.) than the corresponding flour samples (4.58±0.20 - 27.3±0.74 mg TE/g d.m.).

Notably, in all three spectrophotometric tests applied, the tartary buckwheat (SIB) flour showed a greater antioxidant activity than any sample analyzed (3.44±0.33 mg GAE/g d.m. for the DPPH assay, 21.5 ±1.74 mg TE/g d.m. for the TEAC assay and 139.1±4.16 mg TE/g d.m. for the ORAC assay, respectively). These results can be explained by the fact that tartary buckwheat has a higher polyphenol content than common buckwheat (Table 2). In fact, polyphenols are the main molecules that contribute to the antioxidant activity of the samples, and consequently, as their concentration increases, the antioxidant power also increases in parallel (Žilić et al., 2012).

Fig. 4 shows the correlations between the different samples of common buckwheat (both flour and semi-processed), in terms of soluble polyphenol content and antioxidant activity.

The comparison of the methods highlighted a clear variability between the flour and the semi-finished product. The correlations between the content of soluble polyphenols and the antioxidant activity using the DPPH method (Fig. 4A), TEAC method (Fig. 4B) and ORAC method (Fig. 4C) yielded R^2 values of 0.8497, 0.8611, and 0.7995, respectively. This finding indicates good linear correlation in all samples between the total polyphenol content and *in vitro* antioxidant activity. The phenolic compounds are therefore directly associated with the antioxidant activity (Žilić et al., 2012).

In addition, the *in vitro* antioxidant activity of the samples was characterized using the HPTLC technique. To obtain a semi-quantitative evaluation of the *in vitro* antioxidant capacity of samples, the DPPH was selected as the derivatization agent and the plate was exposed at 254 nm, 366 nm, and with visible light. The variation in colour, from violet to yellow, was proportional to the AOA of each compound contained in the samples.

Fig. 5 shows the results of the HPTLC analysis conducted on buckwheat samples, both as flour (<250 μm) and semi-finished product (>250 μm), detected at the wavelengths of 254 nm and 366 nm, before derivatization, and by visible light after derivatization with DPPH•. Before derivatization, using both wavelengths (254 nm and 366 nm) allows for a comprehensive analysis, as different compounds may absorb or fluoresce at one or both wavelengths. This approach provides a more complete picture of the composition of the samples analyzed, especially in the case of complex matrices such as buckwheat samples.

As shown in Fig. 5, the samples have different profiles (fingerprints). In particular, the semi-finished samples show more compounds than the corresponding flours, as indicated by the intensities of the bands detected at 254 and 366 nm (panel A–B).

All semi-finished samples showed a greater antioxidant capacity than the corresponding flours, as indicated by the greater discoloration of the bands in visible light (panel C). As explained by Klepacka et al. (2021), this could be attributable to the fact that the dehulling of buckwheat flours leads to a notable decrease in the rutin content, the main flavonoid involved in the antioxidant activity of this pseudocereal.

Note that the tartary buckwheat sample (SIBf) showed a different profile compared to all the other analyzed samples. It seems to have a comparable, if not superior, antioxidant capacity, to that of the semi-finished samples.

These observations are supported by the results obtained with the spectrophotometric assay. The samples that had a high antioxidant activity with the application of the colorimetric methods also show a high antioxidant capacity in the chromatographic test.

4. Conclusions

Today, the most widespread variety of buckwheat is *Fagopyrum esculentum* Moench (common buckwheat). However, due to its richness in bioactive compounds, *Fagopyrum tataricum* (L) Gaertn., also known as “Tartary buckwheat", has emerged in Europe, including Italy. In recent years, dishes based on tartary buckwheat are increasingly enjoyed by consumers. This offers the opportunity to develop new food products, based on ancient culinary traditions, with a re-evaluation of the quality and health potential of tartary buckwheat through contemporary scientific knowledge. Given the increasing interest in tartary buckwheat, the present study characterizes the phenolic composition and the relative *in vitro* antioxidant activity of nine varieties of common buckwheat (*Fagopyrum esculentum* Moench) as flour (particle size <250 μm) and semi-finished products (particle size >250 μm) and a variety of tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) as flour (particle size <250 μm). This comparison is crucial for identifying differences in phenolic profiles and antioxidant activity between the two species, providing insights into how the nutritional and functional properties of buckwheat-based products can be optimized. By examining these aspects, our study aims to contribute to a deeper understanding of the potential applications of different buckwheat types in modern food products.

Although the tartary buckwheat variety demonstrated the highest content of phenolic compounds and antioxidant capacity compared to the common buckwheat varieties, it is important to highlight that the analyses of the nine varieties of common buckwheat are also important. The data obtained from these varieties provide a comprehensive overview of the phenolic content and antioxidant activity across different strains of *Fagopyrum esculentum* Moench, which is essential for defining

the nutritional quality based on its varieties.

The results from the spectrophotometric assays were corroborated by chromatographic techniques, which confirmed the reliability of theused methods, as the findings were consistent across the different analytical approaches. The preliminary results suggest that tartary buckwheat is a promising matrix due to its high levels of phenolic compounds with antioxidant activity, potentially offering beneficial health properties. However, these findings regarding tartary buckwheat need to be confirmed, as they were obtained from a limited number of samples compared to the data collected from common varieties (9 varieties, 18 different samples). Additionally, it is essential to evaluate the organoleptic characteristics of tartary buckwheat, as these play a crucial role in determining its overall acceptability and potential applications in the food industry.

In addition, fingerprinting the various buckwheat varieties could be used as a valuable tool for comparing and contrasting different geographic origins in terms of secondary metabolite content. These insights not only contribute to the understanding of the nutritional quality of different buckwheat varieties but also pave the way for further studies to validate these findings and explore their implications for health and food product development.

CRediT authorship contribution statement

Corinne Bani: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Elena Peñas:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Giovanna Baron:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Cristina Martínez-Villaluenga:** Writing – review & editing, Supervision, Methodology, Investigation. **Francesca Mercogliano:** Methodology, Investigation. **Giancarlo Aldini:** Supervision, Methodology, Investigation. **Stefano Piazza:** Writing – review & editing. **Chiara Di Lorenzo:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Patrizia Restani:** Supervision, Project administration, Methodology, Investigation, Conceptualization.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.117261.

Data availability

Data will be made available on request.

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