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Stability of total folates/vitamin B_9 in irradiated watercress and buckler sorrel during refrigerated storage

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ABSTRACT

The suitability of post-packaging gamma radiation treatment for preserving total folates or vitamin B_9 in watercress (*Nasturtium officinale* R. Br.) and buckler sorrel (*Rumex induratus* Boiss. & Reut.) during storage at 4 °C was evaluated. Comparable amounts of total folates were found in fresh, non-stored samples of both species. In watercress, the irradiation treatment of up to 5 kGy reduced the loss of total folates caused by 7 days of storage. In turn, the 12-day storage period did not affect the total folate content of buckler sorrel (while the 2 kGy dose decreased the initial levels), evidencing that packaging and refrigeration are enough for preservation. These results suggest that the suitability of post-packaging irradiation for preserving total folates may depend not only on the applied dose but also on the plant matrix under analysis. In addition, new data useful to complete food composition tables or databases is provided.

1. Introduction

Folate or vitamin B_9 is the generic term for naturally occurring folates, a group of water-soluble vitamers of the B-group differentiated by the reduction state of the pteridine ring, the one-carbon substituent linked to R1 and/or R2, and the length of the glutamate chain (Fig. 1). In humans, folate plays a key role as a cofactor in the synthesis of proteins with the amino acids methionine, histidine, serine, and glycine, but also DNA with purines and thymidylate or pantothenate (vitamin B_5) (Delchier, Herbig, Rychlik, & Renard, 2016). Its deficiency can lead to several health related disorders, such as congenital abnormalities (Kao et al., 2014), megaloblastic anaemia (Yadav, Manoli, Vimalraj, & Madhunapantula, 2018), exacerbation of cardiovascular disease (Kolb & Petrie, 2013), certain types of cancer (Chen & Huang, 2018), and neurodegenerative diseases (Kao et al., 2014). Therefore, 400 µg/day of folate is the recommended dietary intake for individuals aged 14 or more years old and 600 µg/day for pregnant women (EFSA, 2014).

Humans cannot synthesize folate and must obtain it from dietary sources, such as green-leafy vegetables (Fajardo, Alonso-Aperte, & Varela-Moreiras, 2017; Morales, Fernández-Ruiz, Sánchez-Mata, Cámara, & Tardío, 2015). However, in addition to the generally low intake of these foods, minimum processing and storage operations can affect the levels of these essential micronutrients (Czarnowska & Gujska, 2012; Delchier et al., 2013), which explains why a folate deficiency can easily occur. Therefore, it is necessary to develop post-harvest preservation methods with a minimal impact on fresh produce quality including folate content.

Today, fresh-cut packaged vegetables are becoming increasingly popular in consumers' market baskets due to their freshness, convenience and health benefits (Baselice, Colantuoni, Lass, Nardone, & Stasi, 2017). At the same time, there is a growing concern surrounding the efficacy of the chemical agents commonly used to sanitize these products, in addition to being perceived negatively by the consumer and harmful to human health and the environment (Castro-Ibáñez, Gil, & Allende, 2017; Pinela & Ferreira, 2017). The adoption of clean, safe and more effective nonthermal technologies, such as ionizing radiation, has been promoted to treat food products (Pinela, Antonio, & Ferreira, 2017; Pinela & Ferreira, 2017); but the available literature on the suitability of this physical treatment for retaining folate levels in fresh vegetables is still very scarce.

Buckler sorrel or French sorrel (*Rumex induratus* Boiss. & Reut.; syn: *Rumex scutatus* subsp. *induratus* (Boiss. & Reut.) Nyman, Fam. Polygonaceae) is an underutilized vegetable traditionally consumed raw in salads and as a snack that is not found in food composition

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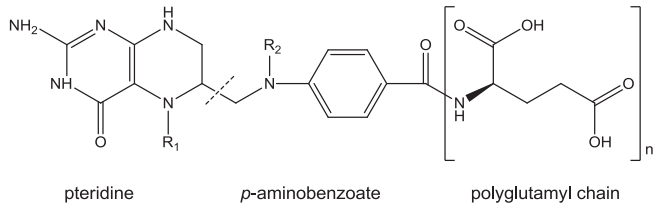

R1	R2	
H	H	THF
CH_3	H	5-methyl-THF
CHO	H	5-formyl-THF
CH=NH	H	5-formimino-THF
H	CHO	10-formyl-THF
- CH_2		5,10-methylene-THF
= CH -		5,10-methenyl-THF

Fig. 1. Chemical structure of folates and derivatives with associated names.

databases (Carvalho & Morales, 2013). Along with watercress (*Nasturtium officinale* R. Br., Fam. Brassicaceae), a semi-aquatic plant appreciated in salads, soups, smoothies, and other recipes, both species have a high potential to be exploited in the minimally processed food sector because of their characteristic organoleptic properties, namely the sharp, peppery and slightly tangy or sour taste. These leafy vegetables can be collected from the wild flora for culinary purposes. However, while watercress is cultivated in different countries and stocked pre-packaged in supermarkets, the commercially available buckler sorrel is generally wild collected by local farmers and traders who sell it sporadically at local markets. The watercress popularity also comes from its health-promoting effects. A diet supplementation with this functional food has been linked to a reduced risk of cancer (Boyd et al., 2006; Gill et al., 2007) and might be useful in modulating breast cancer progression and recurrence (Ravasco, João, Jorge, et al., 2015; Ravasco, João, Rowland, et al., 2015).

In previous studies, we demonstrated how relevant quality parameters of watercress and buckler sorrel are preserved by post-packaging irradiation (Pinela et al., 2018; Pinela, Barreira, Barros, Cabo Verde, Antonio, Carvalho, et al., 2016; Pinela, Barreira, Barros, Cabo Verde, Antonio, Oliveira, et al., 2016); nevertheless, the impact on total folates was not investigated. Therefore, this study was carried out to determine the folate levels in watercress and buckler sorrel and evaluate the suitability of gamma radiation treatment for preserving this vitamin during refrigerated storage.

2. Materials and methods

2.1. Standards and reagents

Standards of 5-CH_3-H_4 folate monoglutamate (ref. 16252; Schircks Laboratories, Jona, Switzerland) and pteroyl diglutamic acid (ref. 16235; Schircks Laboratories, Jona, Switzerland), pancreatic chicken homogenate (Pel Freeze, Arkansas, USA), rat serum, $NaBH_4$, formaldehyde and octanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (fluorescence grade) was bought from Fisher Scientific (Madrid, Spain). All other general laboratory reagents were purchased from Panreac Química S.L.U. (Barcelona, Spain). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.2. Samples gathering and irradiation

Wild specimens of watercress and buckler sorrel were gathered before flowering in February and April 2014, respectively, in the Bragança region (North-eastern Portugal), considering local consumers' sites and preferences, such as the season and phenological stage (Carvalho & Morales, 2013). The taxonomic identification was confirmed by the botanist Dra. Ana Maria Carvalho from the School of Agriculture of the Polytechnic Institute of Bragança (ESA-IPB), Portugal. Voucher specimens were deposited in the herbarium of this institution. Wild-collected plants were selected instead of those from commercial sources to have better control over the postharvest time period.

Healthy and undamaged edible parts were hand-picked, rinsed in tap water, and a portion was immediately analysed (non-stored control). The remaining fresh material (~20 g) was packaged in sterilized bags of low-density polyethylene (63 μm thickness) and irradiated at 1, 2 and 5 kGy (for watercress) or 6 kGy (for buckler sorrel) (predicted doses) at a dose rate of 1.6 kGy/h in a cobalt-60 experimental chamber (Precisa 22, Graviner Manufacturing Company Ltd., UK) located in the Centre for Nuclear Sciences and Technologies (C2TN) in Bobadela, Portugal. A non-irradiated control followed all the experiments (Pinela, Barreira, Barros, Cabo Verde, Antonio, Carvalho, et al., 2016; Pinela, Barreira, Barros, Cabo Verde, Antonio, Oliveira, et al., 2016). All packaged samples (40 bags for each species, 10 bags per dose) were stored at 4 °C for 7 (for watercress) or 12 days (for buckler sorrel).

2.3. Folate extraction, deconjugation, derivatisation and purification

Folates were extracted following a procedure optimized by Morales et al. (2015). In brief, the plant samples (~800 mg) previously lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) and reduced to a fine powder (20 mesh) were extracted with phosphate buffer (100 mM, pH 7, 25 ml) at 80 °C for 15 min. After filtration, an aliquot of filtrate (5 ml) was mixed with rat serum (500 μl) and pancreatic chicken solution (5 mg/ml, in phosphate buffer, 1 ml) to hydrolyse terminal glutamate into monoglutamate and internal bonds between glutamate (hydrolysing polyglutamate into diglutamate), respectively, and incubated at 37 °C for 2 h with stirring. $NaBH_4$ and formaldehyde were used as reducing and methylation agents in the derivatisation process, respectively (performed to convert the different folate forms into mono- and diglutamates), and octanol to prevent excessive foaming. The derivatised extracts were then purified using SPE/SAX cartridges (Scharlab, Barcelona, Spain), wetted and activated with methanol followed by distilled water; impurities were drawn out with distilled water. Folates were eluted with 5 ml of sodium acetate (0.1 mol/l) + NaCl 10% (w/v) + ascorbate 1% (w/v).

2.4. HPLC analysis

The folate content was determined using a HPLC-FL system consisting of a Beta 10 (Ecom, Prague, Czech Republic) gradient pump with a Gastorr Degasser HPLC Four Channel BR-14 (Triad Scientific, New Jersey, USA) as a degassing device, joined to an AS-1555 automatic injector (Jasco, Easton, MD, USA), and to an FP-2020 Plus Fluorescence detector (Jasco, Easton, MD, USA). The separation was performed on a Lichrospher 100 RP-18 endcapped column (Merck, Darmstadt, Germany; 250 × 5 mm; 5 μm) at ambient temperature. Data were analysed using the Biocrom 2000 3.0 software (Biocrom, Madrid, Spain). Quantification was performed by comparison of the area of the recorded peaks with calibration curves obtained from commercial standards (5-CH_3-H_4 folate in both mono and diglutamate forms; the HPLC-FL profile of these standards is shown in Fig. 2), and expressed as total folates (from the sum of both compounds) in μg per 100 g of fresh weight.

Chromatographic parameters, namely limit of detection (LOD), limit of quantification (LOQ), linearity, recovery, repeatability and reproducibility were accepted as previously assessed (Morales et al., 2015).

2.5. Statistical analysis

Data results (n = 9) were expressed as mean ± standard deviation. All statistical tests were performed at a 5% significance level using SPSS Statistics software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The differences among treatments were

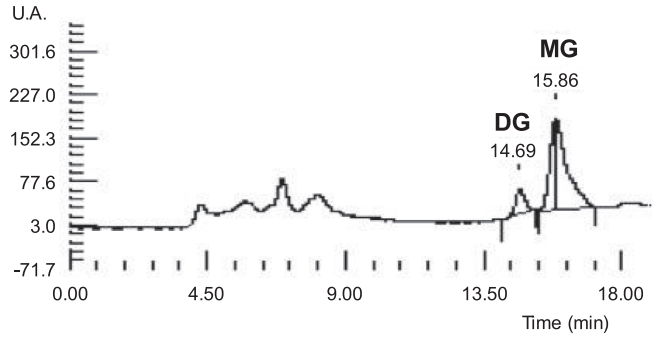

Fig. 2. HPLC-FL profile of 5-CH_3-H_4 folate in both mono (MG) and diglutamate (DG) forms.

analysed using the one-way analysis of variance (ANOVA). The fulfilment of the ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively. All dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

3. Results and discussion

3.1. Folates content in fresh samples

The analysis of folates in foodstuff is a very complex task due to the large number of forms that comprise this vitamin (Fig. 1). Today, different methodologies are available for its analysis, including microbiological and chromatographic techniques. Microbiological methods have been the most commonly used ones but, due to the low selectivity, they are falling into disuse. In fact, the folate content (measured by turbidity) is many times over/underestimated due to interference of other compounds present in the food matrix (Arcot & Shrestha, 2005; O'Hare et al., 2012; Upadhyaya et al., 2017). In turn, the chromatographic techniques have a high sensitivity and specificity towards folate isomers and provide reliable results (Arcot & Shrestha, 2005). For this analysis, the food samples often required an enzymatic treatment to hydrolyse polyglutamates, a derivatisation step to convert the different folate forms into a few ones (mono- and diglutamates), and purification of the deconjugated extract to limit matrix interferences (Morales et al., 2015).

As presented in Tables 1 and 2, fresh watercress and buckler sorrel samples have 13.3 ± 0.3 µg and 13.9 ± 0.5 µg of total folates per 100 g fresh weight, respectively. Therefore, considering the Recommended Dietary Allowances (RDA) of 400 µg/day of folate for individuals aged 14 or more years old (EFSA, 2014), it can be inferred that a 100-g serving of the analysed leafy vegetables provides less than 5% of the RDA. Comparable levels of total folates were already reported in iceberg lettuce (14 µg/100 g) (Westenbrink, Jansen-van der Vliet, & van Rossum, 2012), green bean (11.3 µg/100 g), cauliflower (17.0 µg/100 g), and Brussels sprout (17.6 µg/100 g) (Bureau et al., 2015).

For watercress, a lower folate value (9 µg/100 g) is given by different food composition databases, including the Danish Food Composition Databank (DTU, 2017), the CIQUAL French food composition table (ANSES, 2017), and the USDA Nutrient Database (USDA, 2016); whereas a higher value (40 µg/100 g) is presented by the German Food Code and Nutrient Data Base (MRI, 2018). Fajardo, Alonso-Aperte and Varela-Moreiras (2015) obtained a much higher content (128 µg/100 g) using a microbiological analytical method.

In turn, as far as we know, this is the first report on folate content in buckler sorrel, which provides new data to complete food composition tables or databases. Other *Rumex* sp.pl. were previously identified as having a higher total folate content, namely *R. papillaris* (187 ± 10 µg/100 g) and *R. pulcher* (507 ± 24 µg/100 g) (Morales et al., 2015); whose 100-g portions could cover ~47% of the RDA of folate for adults or exceed 100% of the RDA, respectively. These data highlighted the potential of these underexploited vegetables for being included in contemporary diets as good alternatives to diversify the range of foods rich in folates or vitamin B_9.

Table 1
Total folate content in fresh and irradiated watercress stored at 4 °C for 7 days.

		Folates (µg/100 g fw)
Non-stored fresh sample (day 0)	Fresh control	13.3 ± 0.3[1]
Samples stored at 4 °C for 7 days	0 kGy (control)	8.4 ± 0.3[c]
	1 kGy	10.3 ± 0.3[2]
	2 kGy	10.7 ± 0.5[2]
	5 kGy	10.8 ± 0.1[2]
p-value (n = 45)	Homoscedasticity[1]	0.543
	1-way ANOVA[2]	< 0.001

[1] $p > 0.05$, homoscedasticity among treatments; $p < 0.05$, heteroscedasticity among treatments.
[2] $p < 0.05$ indicates that the mean value of at least one component differs from the others (in this case, multiple comparison tests were performed). Means with different letters differ significantly ($p < 0.05$).

Table 2
Total folate content in fresh and irradiated buckler sorrel stored at 4 °C for 12 days.

		Folates (µg/100 g fw)
Non-stored fresh sample (day 0)	Fresh control	13.9 ± 0.5[1,2]
Samples stored at 4 °C for 12 days	0 kGy (control)	13.9 ± 0.2[1,2]
	1 kGy	13.1 ± 0.7[2]
	2 kGy	9.2 ± 0.4[c]
	6 kGy	14.9 ± 0.1[1]
p-value (n = 45)	Homoscedasticity[1]	0.195
	1-way ANOVA[2]	< 0.001

[1] $p > 0.05$, homoscedasticity among treatments; $p < 0.05$, heteroscedasticity among treatments.
[2] $p < 0.05$ indicates that the mean value of at least one component differs from the others (in this case, multiple comparison tests were performed). Means with different letters differ significantly ($p < 0.05$).

A large variability in folate levels has been described for raw fruits and vegetables. Values ranging from 14 to 46 µg/100 g were found in tomato (Upadhyaya et al., 2017), from 17 to 90 µg/100 g in cauliflower (Bureau et al., 2015; Czarnowska-Kujawska, Gujska, & Michalak, 2017), from 19 to 174 µg/100 g in broccoli (Bureau et al., 2015; Iwatani, Arcot, & Shrestha, 2003), from 24 to 188 µg/100 g in peas (Bureau et al., 2015; Tyagi et al., 2015), and from 27 to 287 µg/100 g in spinach (Bureau et al., 2015; Tyagi et al., 2015). This wide range in folate concentrations can be assigned to the natural variability in the raw material linked to variety or physiological state at harvest time, cultivation/edaphoclimatic and agronomic conditions, and accuracy of the used analytical method (Delchier et al., 2016; Strålsjö, Witthöft, Sjöholm, & Jägerstad, 2003).

3.2. Total folates and postharvest quality: Impact of storage and irradiation

Currently, there is a lack of information about the impact of minimal processing on the final total folate content of minimally processed vegetables. Cooking in boiling water, blanching, steaming, and freezing have been the most investigated operations (Bureau et al., 2015; Delchier et al., 2013; Melse-Boonstra et al., 2002), but a very restricted number of studies evaluated the impact of irradiation (Lester, Hallman, & Pérez, 2010; Müller & Diehl, 1996). Furthermore, these kinds of food products are stored for a certain period of time either prior to sale, during transport and display, or after purchase by the consumer. Therefore, the folate levels in stored foods may differ from

those found in freshly harvested ones (Melse-Boonstra et al., 2002; Octavia & Choo, 2017).

The suitability of gamma-ray irradiation for preserving the total folate contents in watercress and buckler sorrel is presented in Tables 1 and 2. In both cases, this vitamin was affected by at least one of the tested doses ($p > 0.05$). For watercress (Table 1), total folate values were lower in the 7-day stored samples, especially in the non-irradiated control (which showed a decrease of approximately 37%). The post-packaging irradiation treatment had a positive effect on total folate levels; although there was no a significant difference between samples irradiated at 1, 2 or 5 kGy, it seemed that the higher the applied dose, the more effectively this water-soluble vitamin was preserved. Dissimilar effects were observed for buckler sorrel (Table 2). Actually, just the total folate content found in the 2 kGy-irradiated sample differed significantly (~34%) from that of the fresh control analysed immediately after harvest. This demonstrated that packaging and refrigeration are enough for preserving this vitamin for 12 days.

Variations in folate content caused by refrigerated storage have been reported for different fruits and vegetables. Melse-Boonstra et al. (2002) reported folate losses ranging from 0 to 25% in cut and washed samples of leeks, cauliflower and green beans stored at 4 °C for 24 h. Octavia & Choo (2017) found a 21.3–93.2% lower folate content in strawberries stored at 4 °C for 6 days. The authors also verified that more than 50% of this loss occurred on day 3. On the other hand, O'Hare et al. (2012) concluded that it is possible to store choy sum (*Brassica rapa* subsp. *parachinensis*), a dark green leafy vegetable rich in folate, at 4 °C for up to 3 weeks without significantly affecting the contents of this vitamin. In studies regarding the effects of irradiation, Lester et al. (2010) reported that folate levels in baby-leaf spinach irradiated with up to 2 kGy with a cesium-137 source are relatively stable. In turn, Müller and Diehl (1996) noted a dose-dependent decrease in fresh samples of spinach, green cabbage and Brussels sprouts. They also concluded that this vitamin is most sensitive to ionizing radiation when in fresh tissues than in dehydrated ones. These results are consistent with the observations of Araújo et al. (2011), which observed that the physical state of folic acid (a synthetic folate form) plays an important role on its stability toward electron-beam irradiation, being largely unstable in aqueous solution but very stable in powder, even when mixed in a dry food matrix. In the first case, radiation doses over 1 kGy can lead to the formation of pterine-6-carboxilic acid, *p*-aminobenzoyl-L-glutamic acid, and *p*-aminobenzoic acid, among other degradation products (Araújo et al., 2012).

In previous studies we evaluated the suitability of post-packaging irradiation for preserving relevant quality attributes of watercress (Pinela, Barreira, Barros, Cabo Verde, Antonio, Carvalho, et al., 2016) and buckler sorrel (Pinela, Barreira, Barros, Cabo Verde, Antonio, Oliveira, et al., 2016) during refrigerated storage. Based on these results, it was possible to conclude that the impact of post-packaging irradiation on total folates was comparable to that observed for the overall postharvest quality of these vegetables, i.e., the non-irradiated buckler sorrel sample stored for 12 days had the most similar profile to the fresh control (which was analysed immediately after harvest); in the case of watercress, the 2 kGy dose was suitable for preserving the overall quality, while the 5 kGy dose preserved well the antioxidant activity and total flavonoid content.

4. Conclusions

Current literature lacks data on folate content in raw vegetables and on the impact of irradiation on this biologically important B-group vitamin. Herein, it was demonstrated that fresh samples of watercress and buckler sorrel have comparable amounts of total folates. In watercress, although the content of this vitamin decreased during storage, the irradiation treatment had a positive effect on its preservation. In turn, the 12-day storage period did not affect the total folate content of buckler sorrel, evidencing that packaging and refrigeration are enough for its preservation. However, a folate loss was noted in the 2 kGy-irradiated sample of this vegetable. These results suggest that the suitability of post-packaging irradiation for preserving total folates may depend not only on the applied dose but also on the plant matrix under analysis.

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Conflict of interest

The authors declare no conflicts of interest.

References

ANSES. (2017). CIQUAL French food composition table. https://ciqual.anses.fr/ (accessed 18 March 2018).
Araújo, M. M., Marchioni, E., Bergaentzle, M., Zhao, M., Kuntz, F., Hahn, E., & Villavicencio, A. L. C. H. (2011). Irradiation stability of folic acid in powder and aqueous solution. *Journal of Agricultural and Food Chemistry, 59*(4), 1244–1248.
Araújo, M. M., Marchioni, E., Zhao, M., Kuntz, F., Di Pascoli, T., Villavicencio, A. L. C. H., & Bergaentzle, M. (2012). LC/MS/MS identification of some folic acid degradation products after E-beam irradiation. *Radiation Physics and Chemistry, 81*(8), 1166–1169.
Arcot, J., & Shrestha, A. (2005). Folate: Methods of analysis. *Trends in Food Science & Technology, 16*(6–7), 253–266.
Baselice, A., Colantuoni, F., Lass, D. A., Nardone, G., & Stasi, A. (2017). Trends in EU consumers' attitude towards fresh-cut fruit and vegetables. *Food Quality and Preference, 59*, 87–96.
Boyd, L. A., McCann, M. J., Hashim, Y., Bennett, R. N., Gill, C. I. R., & Rowland, I. R. (2006). Assessment of the anti-genotoxic, anti-proliferative, and anti-metastatic potential of crude watercress extract in human colon cancer cells. *Nutrition and Cancer, 55*(2), 232–241.
Bureau, S., Mouhoubi, S., Touloumet, L., Garcia, C., Moreau, F., Bédouet, V., & Renard, C. M. G. C. (2015). Are folates, carotenoids and vitamin C affected by cooking? Four domestic procedures are compared on a large diversity of frozen vegetables. *LWT – Food Science and Technology, 64*(2), 735–741.
Carvalho, A. M., & Morales, R. (2013). Persistence of wild food and wild medicinal plant knowledge in a Northeastern region of Portugal. In A. P. Pardo de Santayana, & R. Puri (Eds.). *Ethnobotany in the New Europe: People, health and wild plant resources* (pp. 147–171). Oxford, UK: Berghahn Books.
Castro-Ibáñez, I., Gil, M. I., & Allende, A. (2017). Ready-to-eat vegetables: Current problems and potential solutions to reduce microbial risk in the production chain. *LWT – Food Science and Technology, 85*, 284–292.
Chen, W.-J., & Huang, R.-F. S. (2018). Low-folate stress reprograms cancer stem cell-like potentials and bioenergetics metabolism through activation of mTOR signaling pathway to promote in vitro invasion and *in vivo* tumorigenicity of lung cancers. *The Journal of Nutritional Biochemistry, 53*, 28–38.
Czarnowska, M., & Gujska, E. (2012). Effect of freezing technology and storage conditions on folate content in selected vegetables. *Plant Foods for Human Nutrition, 67*(4), 401–406.
Czarnowska-Kujawska, M., Gujska, E., & Michalak, J. (2017). Testing of different extraction procedures for folate HPLC determination in fresh fruits and vegetables. *Journal of Food Composition and Analysis, 57*, 64–72.
Delchier, N., Herbig, A.-L., Rychlik, M., & Renard, C. M. G. C. (2016). Folates in fruits and vegetables: Contents, processing, and stability. *Comprehensive Reviews in Food Science and Food Safety, 15*(3), 506–528.
Delchier, N., Ringling, C., Le Grandois, J., Aoudé-Werner, D., Galland, R., Georgé, S., ... Renard, C. M. G. C. (2013). Effects of industrial processing on folate content in green vegetables. *Food Chemistry, 139*(1–4), 815–824.
DTU. (2017). Danish Food Composition Databank. https://frida.fooddata.dk/?lang=en (accessed 18 March 2018).
EFSA (2014). Scientific opinion on dietary reference values for folate. *EFSA Journal, 12*(11), 3893.
Fajardo, V., Alonso-Aperte, E., & Varela-Moreiras, G. (2015). Folate content in fresh-cut vegetable packed products by 96-well microtiter plate microbiological assay. *Food Chemistry, 169*, 283–288.
Fajardo, V., Alonso-Aperte, E., & Varela-Moreiras, G. (2017). Total folate content in ready-to-eat vegetable meals from the Spanish market. *Journal of Food Composition and Analysis, 64*, 223–231.
Gill, C. I. R., Haldar, S., Boyd, L. A., Bennett, R., Whiteford, J., Butler, M., ... Rowland, I. R. (2007). Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy adults. *American Journal of Clinical Nutrition, 85*(2), 504–510.
Iwatani, Y., Arcot, J., & Shrestha, A. K. (2003). Determination of folate contents in some Australian vegetables. *Journal of Food Composition and Analysis, 16*(1), 37–48.

Kao, T.-T., Chu, C.-Y., Lee, G.-H., Hsiao, T.-H., Cheng, N.-W., Chang, N.-S., ... Fu, T.-F. (2014). Folate deficiency-induced oxidative stress contributes to neuropathy in young and aged zebrafish – Implication in neural tube defects and Alzheimer's diseases. *Neurobiology of Disease, 71*, 234–244.

Kolb, A. F., & Petrie, L. (2013). Folate deficiency enhances the inflammatory response of macrophages. *Molecular Immunology, 54*(2), 164–172.

Lester, G. E., Hallman, G. J., & Pérez, J. A. (2010). γ-Irradiation dose: Effects on baby-leaf spinach ascorbic acid, carotenoids, folate, α-tocopherol, and phylloquinone concentrations. *Journal of Agricultural and Food Chemistry, 58*(8), 4901–4906.

Melse-Boonstra, A., Verhoef, P., Konings, E. J. M., van Dusseldorp, M., Matser, A., Hollman, Peter C. H., ... West, C. E. (2002). Influence of processing on total, monoglutamate and polyglutamate folate contents of leeks, cauliflower, and green beans. *Journal of Agricultural and Food Chemistry, 50*(12), 3473–3478.

Morales, P., Fernández-Ruiz, V., Sánchez-Mata, M. C., Cámara, M., & Tardío, J. (2015). Optimization and application of FL-HPLC for folates analysis in 20 species of mediterranean wild vegetables. *Food Analytical Methods, 8*(2), 302–311.

MRI. (2018). German Food Code and Nutrient Data Base. https://www.vitamine.com/lebensmittel/ (accessed 18 March 2018).

Müller, H., & Diehl, J. F. (1996). Effect of ionizing radiation on folates in food. *LWT – Food Science and Technology, 29*(1–2), 187–190.

O'Hare, T. J., Pyke, M., Scheelings, P., Eaglesham, G., Wong, L., Houlihan, A., & Graham, G. (2012). Impact of low temperature storage on active and storage forms of folate in choy sum (*Brassica rapa* subsp. *parachinensis*). *Postharvest Biology and Technology, 74*, 85–90.

Octavia, L., & Choo, W. S. (2017). Folate, ascorbic acid, anthocyanin and colour changes in strawberry (*Fragaria* × *annanas*a) during refrigerated storage. *LWT, 86*, 652–659.

Pinela, J., Antonio, A. L., & Ferreira, I. C. F. R. (2017). Methods combined with irradiation for food preservation. In I. C. F. R. Ferreira, A. L. Antonio, & S. Cabo Verde (Eds.). *Food irradiation technologies: Concepts, applications and outcomes* (pp. 237–279). Cambridge: Royal Society of Chemistry.

Pinela, J., Barreira, J. C. M., Barros, L., Cabo Verde, S., Antonio, A. L., Carvalho, A. M., ... Ferreira, I. C. F. R. (2016). Suitability of gamma irradiation for preserving fresh-cut watercress quality during cold storage. *Food Chemistry, 206*, 50–58.

Pinela, J., Barreira, J. C. M., Barros, L., Cabo Verde, S., Antonio, A. L., Oliveira, M. B. P. P., ... Ferreira, I. C. F. R. (2016). Modified atmosphere packaging and post-packaging irradiation of *Rumex induratus* leaves: A comparative study of postharvest quality changes. *Journal of Food Science and Technology, 53*(7), 2943–2956.

Pinela, J., Barros, L., Barreira, J. C. M., Carvalho, A. M., Oliveira, M. B. P. P., Santos-Buelga, C., & Ferreira, I. C. F. R. (2018). Postharvest changes in the phenolic profile of watercress induced by post-packaging irradiation and modified atmosphere packaging. *Food Chemistry, 254*, 70–77.

Pinela, J., & Ferreira, I. C. F. R. (2017). Nonthermal physical technologies to decontaminate and extend the shelf-life of fruits and vegetables: Trends aiming at quality and safety. *Critical Reviews in Food Science and Nutrition, 57*(10), 2095–2111.

Ravasco, P., João, D., Jorge, M., Rowland, I., Giallourou, N., Swann, J., ... Harbourne, N. (2015). MON-LB027: Watercress in breast cancer patients undergoing radiotherapy: Relevance in DNA damage of dermatitis? *Clinical Nutrition, 34*, S260–S261.

Ravasco, P., João, D. R., Rowland, I., Giallourou, N., Swann, J., Dias, L. M., ... Harbourne, N. (2015). MON-LB019: Does watercress influence phase angle in breast cancer patients during radiotherapy? *Clinical Nutrition, 34*, S257.

Strålsjö, L. M., Witthöft, C. M., Sjöholm, I. M., & Jägerstad, M. I. (2003). Folate content in strawberries (*Fragaria* × *ananas*a): Effects of cultivar, ripeness, year of harvest, storage, and commercial processing. *Journal of Agricultural and Food Chemistry, 51*(1), 128–133.

Tyagi, K., Upadhyaya, P., Sarma, S., Tamboli, V., Sreelakshmi, Y., & Sharma, R. (2015). High performance liquid chromatography coupled to mass spectrometry for profiling and quantitative analysis of folate monoglutamates in tomato. *Food Chemistry, 179*, 76–84.

Upadhyaya, P., Tyagi, K., Sarma, S., Tamboli, V., Sreelakshmi, Y., & Sharma, R. (2017). Natural variation in folate levels among tomato (*Solanum lycopersicum*) accessions. *Food Chemistry, 217*, 610–619.

USDA. (2016). USDA National Nutrient Database for Standard Reference. https://www.usda.gov/ (accessed 13 March 2018).

Westenbrink, S., Jansen-van der Vliet, M., & van Rossum, C. (2012). Updated folate data in the Dutch Food Composition Database and implications for intake estimates. *Food & Nutrition Research, 56*(1), 5449.

Yadav, M. K., Manoli, N. M., Vimalraj, S., & Madhunapantula, S. V. (2018). Unmethylated promoter DNA correlates with p53 expression and apoptotic levels only in Vitamin B_9 and B_{12} deficient megaloblastic anemia but not in non-megaloblastic anemia controls. *International Journal of Biological Macromolecules, 109*, 76–84.