1 **Effects of Different Drying Temperatures on the Content of Phenolic Compounds**

2 **and Carotenoids in Quinoa Seeds (*Chenopodium quinoa*) from Finland**

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14 **ABSTRACT**

15 This investigation studied the effects of different drying temperatures on the content of

16 phenolic compounds and carotenoids in quinoa seeds (*Chenopodium quinoa*) from

17 Finland. Five drying temperatures were applied to reach a dry matter content of 94-95%

18 w/w: room temperature, 40, 50, 60, and 70 °C. The process performed at 70 °C allowed

19 the greatest recovery of total phenolic compounds, 994 ± 28 mg kg^{-1}. Ferulic acid and

20 quercetin were the main phenolics identified. The drying process performed at 60 °C

21 allowed the greatest recovery of cumulative carotenoids, 2.39 ± 0.05 mg kg^{-1}. The

22 carotenoids identified were xanthophylls, e.g. lutein, zeaxanthin and neochrome. The use

23 of heat impacted positively on the phytochemical composition of quinoa. The concentration

24 of phenolics and carotenoids increased steadily with the raise of the drying temperature.

25 Results obtained provide scientific knowledge that can be used by producers to increase

26 the availability of such phytochemicals in quinoa seeds.

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31 *Abbreviations:* DW, dry weight; EtOAc, ethyl acetate; RT, room temperature; T,

32 temperature; US, ultrasonic.

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1. Introduction

Quinoa (*Chenopodium quinoa*) is a crop originated in the Andean region of South America about 7000 years ago. Lately, the interest towards the crop has increased worldwide due to its strong tolerance to abiotic stresses (Ismail et al., 2016), and its cultivation has extended to Europe, North America, Asia and Africa. Amongst the various grain crops, quinoa stands out as it is rich in proteins, i.e., the protein content ranges from 13.8 to 16.5% w/w DW, and provides all the essential amino acids, being rich in sulfur-containing amino acids and lysine (Filho et al., 2017). In addition, it is a good source of dietary fiber (about 13.6 to 16.0% w/w DW) and polyunsaturated fats, which are approximately 88% of total fatty acids of the seed (Filho et al., 2017; Tang et al., 2015).

Apart from the macronutrients, quinoa seeds provide non-essential nutrients of biological interest, e.g., phenolic compounds and carotenoids. Phenolic compounds have been the major group of bioactive phytochemicals investigated in quinoa (Tang et al., 2016). They are compounds of hydrophilic nature located mainly in the seed coat (Tang & Tsao, 2017). A great deal of studies performed on quinoa seeds investigated the free and conjugated soluble forms of phenolic compounds (Carciochi et al., 2015; Dini, Tenore, & Dini, 2010; Repo-Carrasco-Valencia, Hellström, Pihlava, & Mattila, 2010), failing to notice most of the bound phenolics that are attached to cell wall structures, e.g., cellulose, hemicellulose, lignin, and structural proteins. Carotenoids are natural lipophilic pigments that can be classified in: (1) carotenes, compounds that are composed of only carbon and hydrogen atoms, e.g., lycopene and β-carotene; (2) xanthophylls, e.g., lutein and zeaxanthin, which contain oxygenated functional groups, e.g. epoxy, carbonyl, hydroxyl, methoxy or carboxylic acid groups (Rivera & Canela-Garayoa, 2012). A limited number of investigations researched the presence of carotenoids in quinoa seeds. Few studies examined the total carotenoid content of quinoa seeds (Dini et al., 2010), while the

60 individual carotenoid composition is largely unknown. Up to now, the investigation

61 performed by Tang, Li, Chen, Zhang, Hernandez, Zhang, *et al.* (2015) is the only reporting

62 the presence of specific carotenoids, e.g., lutein and zeaxanthin, in seeds of three quinoa

63 genotypes.

64 Like other grains, quinoa seeds are dried to low moisture content for storage prior to

65 consumption. Drying prolongs the shelf-life of the raw material as it creates a hostile

66 environment for microorganisms, and impedes chemical and enzymatic reactions that can

67 deteriorate the product. The drying method is adapted to factors such as climate, yield of

68 the harvest, labor availability, machinery cost, etc. In the rural regions of the tropics,

69 farmers traditionally dry quinoa in rectangular slatted granaries having an open side that

70 allow the movement of natural air through the seeds, i.e., ventilation cribs (FAO 2011).

71 Although these ventilation cribs are adaptable structures, suitable for drying all crops

72 requiring ventilation, they do not allow for rigorous control of the drying conditions. For this

73 reason, to preserve and standardize the quality of quinoa seeds after harvesting, industrial

74 farming is moving towards drying the seeds by using hot air. Industrial dryers generally

75 employ air at 50-130 °C to dry moist grains (Odjo, Malumba, Beckers, & Béra, 2015). High

76 temperature drying brings about physical and chemical changes that influence the stability

77 of the phytochemicals found in foods. A number of studies investigated the effects of

78 different drying processes on the phenolic composition of plant materials. Several authors

79 observed decreases in the phenolic content of plant materials after drying (Al-Rawahi,

80 Rahman, Guizani, & Essa, 2013; Calín-Sánchez et al., 2013; Rizzo, Clifford, Brown,

81 Siracusa, & Muratore, 2016). On the contrary, other authors observed no significant

82 changes or increases in the phenolic composition of dried vegetables (Galaz et al., 2017;

83 Lemus-Mondaca, Ah-Hen, Vega-Gálvez, Honores, & Moraga, 2016; Rodríguez et al.,

84 2016). The disagreement found in the literature can be ascribed to great differences in

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85   both the quality of the food matrices investigated and the drying processes, e.g., open air

86   drying, convective drying, microwave drying, etc. The literature provides limited information

87   on the effects of temperature and drying method on the phytochemical composition of

88   quinoa seeds. Carciochi *et al.* (2016) investigated the effects of various roasting

89   conditions, from 100 to 190 °C, on the phenolic content of quinoa seeds and demonstrated

90   that higher roasting temperatures increased the yield of phenolics. To the authors'

91   knowledge, there are no studies examining the effects of drying processes on the

92   phytochemical composition of quinoa seeds. In view of that, we hypothesized that

93   moderate drying temperatures could enhance the release of phytochemicals from the

94   quinoa matrix and facilitate their extraction. The aim of this research was to determine the

95   effects of different drying temperatures: room temperature (RT) and from 40 to 70 °C at 10

96   °C intervals, on the phenolic and carotenoid contents of quinoa seeds from Finland.

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2. Materials and Methods

2.1 Chemicals

General laboratory reagents were purchased from VWR International Oy (Helsinki, Finland). Methyl-*tert*-butyl ether (MTBE), ethyl acetate (EtOAc), diethyl ether, acetic acid (glacial), hexane, ammonium acetate, and sodium sulphate (anhydrous, granular, ≥99%) were of analytical grade. Methanol and acetonitrile were of LC-MS grade. Hydrochloric acid (reagent grade, 37%) and sodium hydroxide (pellets pure) were purchased from Sigma-Aldrich, Inc. (Gillingham, England). Water was purified *in loco* to 18.2 MΩ·cm at 25 °C with a Milli-Q water purification system (Merck KGaA, Darmstadt, Germany). Analytical standards of vanillic acid, gallic acid, *p*-benzoic acid, syringaldehyde, ferulic acid, quercetin, and kaempferol were purchased from Sigma-Aldrich (Gillingham, England). Analytical standards of *(all*-E*)*-lutein and *(all*-E*)*-zeaxanthin were purchased from Extrasynthese (Genay Cedex, France). The analytical standard of *(9*Z*)*-neoxanthin was purchased from Carote*Nature* GmbH (Münsingen, Switzerland).

2.2 Plant Materials and Sample Preparation

Quinoa accession 'Minttumatilda' was sown in June 2016 in Jokioinen (60°48'15''N, 23°29'10''E), Southwestern Finland. Seeds and fertilizer were drilled into the soil at a depth of 1 and 5 cm, respectively. The fertilizer applied was YaraMila 8-24-24 + 5% SO_3 (Yara UK ltd., Lincolnshire, UK), with nutrient composition as follow: nitrogen 8%, potassium oxide 24%, phosphorus pentoxide 24%, and sulphur trioxide 5%. The final soil nitrogen content was 80 kg per hectare. Seeds were harvested in October 2016 fully mature, i.e., leaves and stem of the crop were dry and seeds turned from yellow to brown. About three kg of quinoa seeds were taken from the harvest and used for the experiments. Quinoa seeds were cleaned and divided into subsamples of 500 g. Samples were dried in a

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convection oven (Universal Oven U from Memmert GmbH, Schwabach, Germany) at temperatures of 40, 50, 60, and 70°C until they reached a dry matter (DM) content of 94-95% w/w. One subsample was dried at room temperature (18-22 °C). Dried and unwashed quinoa seeds were milled with a Retsch ZM-100 ultra-centrifugal mill with a 0.5 mm screen insert (Düsseldorf, Germany) and stored at room temperature under vacuum until analysis.

2.3 Determination of Phenolic Compounds

2.3.1. Extraction of Phenolic Compounds

Phenolic compounds were extracted as described by Multari, Neacsu, Scobbie, Cantlay, Duncan, Vaughan, *et al*. (2016). Four independent samples of each treatment were analysed. Phenolics were extracted as free and bound compounds and results are reported accordingly (Table 1). The term total phenolic compounds indicates the sum of the cumulative free and bound phenolics.

Extraction of free phenolic compounds. Samples of milled quinoa seeds (approximately 0.1 g) were suspended in HCl (3 mL; 0.2 M) and extracted into 6 mL of EtOAc (stirred at RT for 10 min and sonicated in US bath for 5 min), and the layers were separated by centrifugation (5 min; 1800g; 18 °C). The extraction was repeated twice. The EtOAc extracts were combined and left to stand over sodium sulphate (anhydrous) and then filtered. The solvent was removed under reduced pressure at a temperature not exceeding 40 °C. Then the EtOAc extracts were dissolved in methanol (1 mL) for UPLC-PDA-ESI-MS analysis. The remaining aqueous fractions, obtained after EtOAc extraction, were neutralized with NaOH (approx. 0.2 mL; 4 M) and freeze-dried.

Extraction of bound phenolic compounds. The freeze-dried pellets were suspended in NaOH (3 mL; 1 M) and stirred at room temperature for 4 h under nitrogen. The pH was

147 reduced to 2 with HCl (approx. 0.4 mL; 10 M), and the samples were extracted into 6 mL

148 of EtOAc (stirred at RT for 10 min and sonicated in US bath for 5 min). This was repeated

149 twice. The EtOAc extracts were combined and the solvent was removed under reduced

150 pressure at a temperature not exceeding 40 °C. The pH of the aqueous fraction was then

151 brought to 7 with NAOH (approx. 1.9 mL; 4 M), and the aqueous fraction was freeze-dried.

152 Then, the freeze-dried fractions were suspended in HCl (3 mL; 2 M) and incubated at 95

153 °C for 30 min with intermittent mixing. The samples were cooled and extracted with 6 mL

154 of EtOAc (stirred at RT for 10 min and sonicated in US bath for 5 min). This was repeated

155 twice. The EtOAc extracts were combined and the solvent was removed under reduced

156 pressure at a temperature not exceeding 40 °C. All extracts were dissolved in methanol (1

157 mL) for UPLC-PDA-ESI-MS analysis.

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159 *2.3.2. UPLC-PDA-ESI-MS Analysis of Phenolic Compounds*

160 The MS analysis of the phenolic compounds was obtained as described by Multari *et al*.

161 (2016). It was performed on an UPLC-PDA-ESI-MS system consisting of a Waters Acquity

162 UPLC® in combination with a Waters 2996 PDA detector and a Waters Quattro Premier

163 mass spectrometer (Waters Corp., Milford, MA). The column used was a Kinetex® C18

164 column (100 × 4.6 mm; 2.6 μm i.d.; 100 Å) from Phenomenex (Torrance, California, USA).

165 The mobile-phase solvents were (A) water containing 0.1% v/v acetic acid and (B)

166 acetonitrile containing 0.1% v/v acetic acid. The gradient used to separate the different

167 phenolic compounds was: 10% B (0-1.5 min), 55% B (1.5-16.5 min), 80% B (16.5-30.0

168 min), 10% B (30.0-32.0 min). The flow rate was 840 μL min^{-1}, the injection volume was 10

169 μL, and the PDA was set at 210–600 nm. After splitting, the LC eluent (400 μL min^{-1}) was

170 directed into the mass spectrometer equipped with an electrospray interface. The mass

171 spectrometer was run in both negative and positive ion modes with the following source

settings: capillarity voltage, 3.0 kV (ES$^+$) and 5.0 kV (ES$^-$); cone voltage, 15.0 V (ES$^+$) and

22.0 V (ES$^-$); extractor voltage, 3.0 V (ES$^+$) and 4.0 V (ES$^-$); source temperature, 120 °C;

desolvation temperature, 300 °C; desolvation gas flow, 700 L/h; cone gas flow, 100 L/h.

Ions were scanned across the range of *m/z* 120-400. All the phenolic compounds were

identified using the UV-spectra and the parent ions (*m/z* obtained from both positive ion

scan [M + H]$^+$ and negative ion scan [M - H]$^-$). The quantification of the phenolic

compounds by PDA was performed using external standards, according to the individual

absorption maxima. The list of the individual phenolic compounds identified by UPLC-

PDA-ESI-MS, their chromatograms, the limits of detection (LOD) and quantification (LOQ)

are included as Supporting Information (Table S1). Results were expressed as mg kg^{-1}.

2.4.   Determination of Carotenoids

2.4.1. Extraction of Carotenoids

The extraction method for carotenoids was adapted from Delpino-Rius, Eras, Marsol-Vall,

Vilaró, Balcells, Canela-Garayoa (2014). The procedure was performed under dim light

and butylated hydroxytoluene (BHT), 0.1% w/v, was added to the extraction solvents to

minimize carotenoid degradation. Samples of around 700 mg were rehydrated in water (1

mL), vortexed and mixed on an orbital shaker for 10 min at RT. Then, methanol (4 mL)

was added and samples were vortexed and mixed on an orbital shaker for other 10 min.

The mixtures were centrifuged (5 min; 1800*g*; 18 °C), and the organic layers were

recovered and transferred to 50 mL tubes. This step was repeated another time. The

remaining pellets were suspended in hexane (4 mL), vortexed and mixed on an orbital

shaker for 10 min at RT. The mixture was centrifuged (5 min; 1800*g*; 18 °C), and the

supernatants were combined to the methanolic extracts. Next, 5 mL of a solution of NaCl

(8%, w/v) were added. The mixtures were shaken for 15 min at 4 °C, and then centrifuged

197 (5 min; 1800g; 18 °C). The organic layers were recovered, while the aqueous phases were

198 re-extracted three times with 4 mL of hexane:diethyl ether (3:1, v/v) for 10 min, each

199 extraction followed by centrifugation (5 min; 1800g; 18 °C) to separate the organic phase

200 from the aqueous phase. The organic extracts were dried under a stream of nitrogen at

201 RT. The dry residues were dissolved in 0.6 mL of the injection solvent A

202 (acetonitrile/MBTE/methanol, 60/20/20, v/v/v, containing 0.1% of ammonium acetate w/v),

203 (see paragraph 2.4.2 HPLC-DAD Quantification of Carotenoids), and sonicated in US bath

204 for 5 min. All the samples were filtered through 0.22 µm PTEF membranes (VWR

205 International, Finland). Four independent samples of each treatment were extracted. The

206 term cumulative carotenoid content indicates the sum of the individual carotenoid

207 compounds.

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209   *2.4.2. HPLC-DAD Quantification of Carotenoids*

210 The liquid chromatography separation of carotenoids was performed adapting the method

211 from Sheshappa Mamatha, Kumar Sangeetha, Baskaran (2011). Analyses were

212 performed on a HPLC-DAD instrument (Shimadzu Corporation, Kyoto, Japan), equipped

213 with SIL-30AC autosampler, a sample cooler, two LC-30AD pumps, a CTO-20AC column

214 oven, an SPD-M20A diode array detector and a CBM-20A central unit. The system was

215 operated using LabSolutions Workstation software (Shimadzu). Separation was performed

216 at 35 °C using a YMC C30 Carotenoid column (250 x 4.6 mm; 3 µm i.d.) coupled to a C30

217 guard column (20 mm; 4 mm i.d.), both from Waters (Dublin, Ireland). The mobile-phase

218 solvents were (A) acetonitrile/MBTE/methanol, 60/20/20, v/v/v, containing 0.1% of

219 ammonium acetate w/v, and (B) water. The gradient used to separate the carotenoids

220 was: 10% B (0-25.0 min), 0% B (25.0-35.5 min), 10% B (35.5-40.0 min). The flow rate was

221 1 mL min^{-1}, the injection volume was 10 µL, and the DAD was set at 350–500 nm. The

222 quantification of carotenoids by DAD was performed using external standards. The list of

223 the individual carotenoids identified by HPLC-APCI-MS/MS, their chromatograms, the

224 limits of detection (LOD) and quantification (LOQ) are included as Supporting Information

225 (Table S2). Results were expressed as mg kg^{-1}.

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227 *2.4.3. HPLC-APCI-MS/MS Identification of Carotenoids*

228 The MS/MS analysis of carotenoids was performed adapting the methods from Delpino-

229 Rius *et al.*, (2014). The analysis was carried out on a Waters Acquity HPLC in combination

230 with a Waters 2996 PDA detector and a Waters Quattro Premier mass spectrometer. The

231 instrument was operated using an atmospheric pressure chemical ionization source

232 (APCI) in positive ion mode. The APCI parameters were as follows: corona voltage, 4.0

233 kV; extractor voltage, 3 V; source temperature, 120 °C; probe temperature, 350 °C;

234 desolvation temperature, 150 °C; cone gas (nitrogen) flow, 10 L/h; and desolvation gas

235 (nitrogen) flow, 150 L/h. Collision-induced dissociation was achieved using argon as the

236 collision gas at a flow rate of 0.15 mL min^{-1} in the collision cell. Data were acquired using

237 MassLynx 4.1 software (Waters, USA).

238

239 *2.5. Statistical Analysis*

240 Data reported are mean of four independent observations and values are expressed as

241 mean ± SD. The statistical analysis was carried out using SPSS 23.0 for Windows (IBM,

242 Armonk, NY, USA). The Shapiro–Wilk test was applied to verify the normal distribution of

243 the variables. When the statistical distribution was not normal, a logarithmic transformation

244 of the variables was performed. The Levene's test was applied to detect possible non-

245 homogeneity of the variances. The data were analysed using One-Way-Analysis of

246   Variance (ANOVA) to compare the groups and the Tukey's HSD test was performed to

247   allow for multiple comparisons. Differences among groups were considered significant at p

248   < 0.05. Not detected (n/d) values were not included in the statistical analyses.

249

3. Results and Discussion

3.1. Cumulative Content of Phenolic Compounds

The effect of different drying temperatures on the cumulative contents of free and bound phenolic compounds is shown in Table 1. Regardless of the treatment, phenolic compounds were mainly identified as bound compounds in quinoa seeds, as they accounted for ≥ 90% of the total phenolics (Figure 1). Total phenolic compounds ranged from 293 ± 9to 995 ± 28 mg kg^{-1}, being greatly affected by the drying process, as the amount of extractable phenolics differed significantly ($p < 0.001$) across the samples. A positive effect of the heat treatment was observed for both the cumulative bound and total phenolics. Drying temperatures of 60 and 70 °C facilitated the extraction of phenolic compounds, as the highest amounts of total phenolics were extracted from samples dried at these temperatures (772 ± 41 and 995 ± 28 mg kg^{-1}, respectively; $p < 0.001$). When quinoa seeds were dried at 70 °C, the amount of extracted phenolics was about 4.5-fold higher than that from quinoa dried at 40 °C. Contrary, drying temperatures ≤ 50 °C did not produce any substantial effect. Quinoa seeds dried at 40 °C showed the lowest content of total phenolic compounds, and no statistically significant differences ($p > 0.05$) were observed with the treatments performed at RT and 50 °C (331 ± 17 mg kg^{-1} and 336 ± 15 of cumulative phenolics, respectively). It is important to point out that the literature provides limited information on the effects of hot drying on the phenolic content of cereals and pseudocerals. Nonetheless, a similar trend can be noticed by examining studies performed on different matrices. Delgado-Nieblas et al. (2017) observed that drying citrus pomace at T ≥ 74 °C increased the extractability of total phenolics by 37%. Sablani, Andrews, Davies, Walters, Saez, and Bastarrachea (2011) reported that total phenolics increased up to 40%, in raspberries and blueberries air-dried at 65 °C compared to the fresh berries. Yang, Chen, Zhao and Mao (2010) reported that the yield of phenolic

275 compounds increased by three times in sweet potatoes dried at 65 °C. It is recognised that

276 higher temperatures generally improve the solubility of phenolic compounds, as the hot air

277 leads to the breakdown of cellular structures, enhancing the release of phenolics that are

278 bound to macromolecules of the cell wall (Guido & Moreira, 2017). Nevertheless, hot air (≥

279 40 °C) can cause losses of free phenolic compounds (Rodríguez et al., 2016). This effect

280 was observed in the present investigation. Indeed, quinoa seeds dried at room

281 temperature showed the highest content of cumulative free phenolics, 34.7 mg kg^{-1}. The

282 loss of free phenolic compounds at high temperatures might be due to the fact that free

283 phenolics are more exposed and more sensitive to the degradative effect of the hot air.

284 Considering the results from this investigation, it is worth mentioning that moderate drying

285 temperatures are suitable for enhancing the extractability of phenolic compounds to a

286 great extent, but their efficacy is dependent on the nature of the vegetal matrix being used

287 and the type of compounds to be extracted (Mamatha et al., 2011). Increases in the

288 extraction of phenolic compounds, due to the use of high temperatures, were reported in

289 studies where cereals were investigated (Van Hung, 2016). These matrices are relatively

290 rich in fibre and provide mainly simple phenolic compounds e.g., phenolic acids, which are

291 covalently bound to cell wall structural components, e.g., cellulose, hemicellulose, lignin

292 (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014). On the contrary, high

293 temperatures might generate loss of phenolics in matrices such as fruits and cocoa

294 providing mostly thermally labile free phenolics such as, flavan-3-ols and anthocyanidins

295 (Francini et al., 2017; Suazo, Davidov-Pardo, & Arozarena, 2014).

296

297 *3.2. Concentration of Individual Phenolic Compounds*

298 The individual phenolic compounds identified in quinoa seeds were four phenolic acids,

299 one phenolic aldehyde, and two flavonoids (Table 1). The presence of *p*-benzoic acid,

vanillic acid, ferulic acid, quercetin and kaempferol in quinoa was already reported by Carciochi *et al.* (2015). In the present investigation, ferulic and *p*-benzoic acids were found as both free and bound compounds, while all the other phenolics were identified only in the bound form. As expected, heat caused a degradation of the free phenolic acids, and drying at room temperature resulted in relatively high concentrations of both *p*-benzoic and ferulic free acids, 1.51 ± 0.13 and 33.0 ± 5.9 mg kg^{-1}, respectively. On the contrary, as the drying temperature increased, no major degradation of the bound phenolics was observed, i.e., no statistically significant differences ($p > 0.05$) were observed in the concentrations of bound vanillic and ferulic acids between quinoa seeds dried at room temperature and those dried at temperatures ≥ 50 °C. Gallic acid was identified when the drying process was performed at 70 °C. This might indicate the presence of gallotannins in quinoa seeds. Gallotannins are esters of gallic acid, which is generally extracted following processes of hot hydrolysis (Newsome, Li, & van Breemen, 2016). It is likely that the drying process at 70 °C might have facilitated the degradation of the hydrolysable tannins into their main constituents, e.g., gallates, which were quantified as bound compounds, i.e., extracted following hydrolysis. In agreement with this hypothesis, Rodríguez *et al.* (2016) found high concentrations of gallic acid in maqui berries that were dried at 80 °C. Quercetin and kaempferol were the most abundant phenolic compounds identified in quinoa seeds. The drying process had a great effect on the two flavonoids that were extracted from quinoa dried at temperatures ≥ 60 °C. In addition, quinoa seeds dried at 70 °C was significantly richer ($p < 0.05$) in quercetin and kaempferol than quinoa dried at 60 °C. Analogous results were described by Tang, Zhang, Li, Chen, Zhang, Liu *et al.* (2016), who reported high concentrations of the two flavonoids as bound compounds in three varieties of quinoa. The derivatives of the hydroxybenzoic acid, e.g. vanillic, gallic, *p*-benzoic acids, and syringaldehyde, were more ubiquitous than the hydroxycinnamates. Nevertheless, from a

quantitative standpoint, ferulic acid was the main phenolic acid, especially as bound compound. Regardless of the treatment, the concentrations of bound ferulic acid were higher than the sum of all the individual hydroxybenzoic acids. Ferulic acid levels peaked in quinoa seeds dried at 70 °C (23.6 ± 1.8 and 264 ± 9 mg kg^{-1} for free and bound, respectively). Other authors already observed that the extraction of ferulic acid is facilitated when plant matrices are dried at temperatures ≥ 50 °C (Del Pino-García, González-SanJosé, Rivero-Pérez, García-Lomillo, & Muñiz, 2017; Lemus-Mondaca et al., 2016; Rizzo et al., 2016). Our data are consistent with investigations performed on several cereals and pseudocerals, e.g., wheat, rye, rice, barley and buckwheat, in which bound ferulic acid was identified as a major phenolic compound (Guido & Moreira, 2017; Multari et al., 2016; Pihlava et al., 2015). It is evident that the levels of extractable phenolic compounds in quinoa rose with the increase of the drying temperature. This was clear-cut for gallic acid, syringaldehyde, quercetin, kaempferol, and overall for all the bound phenolics. Quinoa seeds are rich in fibre (Filho et al., 2017), and heat might increase the extractability of the fibre-bond phenolic compounds. In addition, the partial thermal degradation of lignin can lead to the release of phenolic compounds (Del Pino-García et al., 2017). Phenolic acids are intermediates in the phenylpropanoid pathway leading to the synthesis of lignin (Guido & Moreira, 2017). Bound phenolic compounds cannot be extracted by organic solvents, however, they are very beneficial from a nutritional standpoint, as they are released in the colon by action of enzymes or microbiota, contributing to the biological effects of foods (Arruda, Pereira, de Morais, Eberlin, & Pastore, 2018). Results from the current study show that the highest concentration of phenolic compounds was obtained when quinoa seeds were dried at 70 °C; this value needs to be considered when searching for an optimum drying process of quinoa seeds.

3.3 Cumulative Content of Carotenoids

Carotenoids were identified according to their chromatographic and spectroscopic characteristics (UV-vis and MS). Figure 2 shows the cumulative content of carotenoids in quinoa seeds, which ranged from 2.19 ± 0.05 to 2.39 ± 0.06 mg kg^{-1}. The drying process influenced the cumulative carotenoid content of the samples as statistically significant differences ($p < 0.001$) were identified amongst the treatments. The drying process performed at 60 °C facilitated the extraction of carotenoids, as the highest concentration of cumulative carotenoids was obtained following this treatment. The concentrations of carotenoids increased when the drying temperature was raised from 40 to 60 °C. Nevertheless, when quinoa seeds were dried at 70 °C, the lowest amount of carotenoids was yielded. This might be attributed to a breakdown of carotenoids during the treatment at 70 °C. It is reasonable to believe that the drying at elevated temperatures altered the integrity of the matrix, softening the plant tissues (Zhang et al., 2014). In a meta-analysis investigating the effects of cooking techniques on vegetable pigments, Murador *et al.* (2014) argued that heat can modify the structure of cell membranes and cell walls, facilitating the release of pigments from plant tissues. Data from the present investigation suggest that moderate heat (up to 60 °C) enhanced the bioaccessibility of carotenoids from quinoa seeds. Contrary, when seeds were dried at 70 °C, the greater thermal severity might have brought about processes of carotenoid degradation, e.g., oxidation (Shen, Yang, Zhao, Shen, & Diao, 2015).

Despite the growing interest in the phytochemical composition of quinoa, there are very few studies investigating the carotenoid content of quinoa seeds (Dini et al., 2010). To the authors' knowledge, only one study attempted to clarify the carotenoid composition of selected cultivars of quinoa seeds using LC-MS (Tang et al., 2015). Furthermore, no studies have previously investigated the effects of different drying temperatures on the

375 carotenoid composition of edible grains. For the above reasons, it is difficult to make a

376 direct comparison with data from the literature. According to the report of Tang *et al*.

377 (2015), the total carotenoid content in three varieties of coloured quinoa was higher than

378 11 mg kg^{-1}. These values are markedly higher than those obtained from the present

379 investigation. There are many factors influencing the concentration of carotenoids in food,

380 e.g., ecotype, growing conditions, and ripening stage. It is worth mentioning that the

381 ripening stage affects greatly the carotenoid concentrations, as xanthophylls accumulate

382 early during ripening but decline progressively towards the completion of the ripening

383 process (Al-Yafeai, Malarski, & Boehm, 2018). Carotenoids present in quinoa seeds

384 mostly belong to the xanthophyll group (Table 2). It is plausible that the relatively low

385 amounts of carotenoids in the selected quinoa were due to the fact that the crop was

386 harvested at full maturity (information about the maturity stage were not provided by Tang

387 *et al*. (2015)). In addition, the quinoa seeds analysed in the investigation of Tang *et al*

388 (2015) originated from the Andean region in South America, where the climate is ideal for

389 cultivation of quinoa (Filho et al., 2017). The quinoa seeds used in the present

390 investigation originated from Southwestern Finland, where the pedoclimatic conditions

391 might not be ideal for accumulation of carotenoids. The above results demonstrated that

392 the drying process performed at 60 °C was the most suitable to preserve carotenoids in

393 quinoa seeds and to enhance their extractability. As the food industry is constantly looking

394 for new sources of natural products, carotenoids from quinoa seeds might find industrial

395 applications in areas such as natural food additives, bioactive ingredients,

396 pharmaceuticals, and cosmetics.

397

3.4 Concentration of Individual Carotenoids

398

399 A total of six individual carotenoids were identified in quinoa seeds on the basis of

400 authentic standards (when available), UV-Vis and MS data: neochrome A and B tentatively

401 identified as two epimers of neochrome, (*all-E*)-lutein, two lutein isomers (A and B), and

402 (*all-E*)-zeaxanthin (Table 2) (further information are provided as supporting material, Table

403 S2). Samples showed different quantitative profiles as significant differences ($p < 0.05$)

404 were identified in the concentrations of the individual carotenoids. On the contrary, the

405 qualitative profiles were not affected by the drying temperature, as the six carotenoids

406 were detected in all the samples. (*all-E*)-Lutein was the main compound across the

407 samples, ranging from 1.39 ± 0.03 to 1.56 ± 0.04 mg kg^{-1}, in quinoa dried at 40 and 60 °C,

408 respectively. (*all-E*)-Zeaxanthin was the second most abundant carotenoid, ranging from

409 0.22 ± 0.00 to 0.30 ± 0.00 mg kg^{-1}, in quinoa dried at room temperatures and 60 °C,

410 respectively. Neochrome A and B varied to a limited extent among the samples, as for

411 both the compounds, only the quinoa dried at room temperature differed significantly ($p <$

412 0.05) from the other samples. Each of the six identified carotenoids was detected at

413 greatest concentrations when quinoa seeds were dried at 60 °C. Due to very limited

414 number of studies that dealt with the carotenoid profiling of pseudocereals, it is difficult to

415 compare our results on quinoa with data from the literature. Previous investigations that

416 examined the carotenoid content of cereals regarded xanthophylls as the main

417 carotenoids. This was confirmed by our investigation. Data from this study reveal that

418 carotenoid content in quinoa is in the same level as that of wheat and barley (1-3 mg kg^{-1})

419 (Paznocht et al., 2018) but starkly less than millet (about 8 mg kg^{-1}) (Shen et al., 2015). It

420 is acknowledged that thermal treatments can influence the configuration of the conjugated

421 carbon double bonds of carotenoids, which occur mainly as (*all-E*)-isomers and undergo

422 geometric isomerization during thermal processing (Saini & Keum, 2018). From the results

423   presented in Table 2, quinoa seeds dried at RT was found to contain the highest

424   percentage of isomers, i.e., 16.8% of the total lutein content, while in the other samples the

425   lutein isomers accounted for 7-10%. Neochrome A and B were considered isomers of

426   neoxanthin. When these two compounds were added to the analysis, the cumulative

427   content of isomers made up to 27.6% of total carotenoids in quinoa dried at RT, while in

428   the other samples the cumulative isomers ranged from 22 to 25%. Quinoa seeds dried at

429   70 °C resulted in the highest stability, e.g., lutein isomers accounted for 7.11% of total

430   lutein content. As drying quinoa at room temperature took longer time before the dry

431   matter content of 94-95% w/w was reached (used as reference value), the longer

432   exposition to oxygen might have resulted in high degree of isomerization of carotenoids.

433   These results suggest that carotenoids from quinoa seeds are more susceptible to the

434   destabilising action of oxygen than of heat. It can be argued that the drying process might

435   have affected the quality of individual carotenoids from quinoa to a larger extent and might

436   have generated different isomers. Nevertheless, pairs of enantiomers and other isomers

437   can be distinguished only by chiral chromatography, the use of which was beyond the

438   scope of this study. Data from the present investigation indicate that each carotenoid

439   increased when heat was applied to dry the quinoa seeds, peaking when the process was

440   performed at 60 °C, and then declining. It is clear that moderate heat improved the

441   extraction efficiency of carotenoids from quinoa seeds. Moderate heat might have caused

442   the disruption of the carotenoid protein-complexes, and might have inactivated the

443   carotenoid-oxidising enzymes preventing the degradation of the pigments (Kotikova et al.,

444   2016). This might have assisted the release of carotenoids from the plant matrix, and

445   avoided the thermal degradation occurred when quinoa seeds were exposed to the more

446   severe temperature of 70 °C. Studies performed on other carotenoid containing matrices,

447   e.g., basil and sweet potato leaves (Kao, Chiu, Tsou, & Chiang, 2012), lentils (Zhang et

448 al., 2014) and potato tubers (Kotikova et al., 2016) showed that lutein and zeaxanthin were

449 relatively resistant to thermal degradation, as their concentrations were not significantly

450 affected or were higher after cooking than the original level in the raw materials. Reports

451 investigating the relation between carotenoids from plant materials and heat are several

452 (Murador et al., 2014); nevertheless, they provide contradictory results. This is probably

453 due to the fact that the fate of carotenoids during thermal processing is influenced by a

454 range of factors, e.g., processing methods, experimental conditions, type of the food

455 matrix, and the nature of the carotenoids *per se* (Kotikova et al., 2016).

456

4. Conclusion

In the present investigation, UPLC-PDA-ESI-MS and HPLC-DAD were employed to study the content of phenolic compounds and carotenoids in quinoa seeds from Finland. The crop resulted a good source of phenolic compounds, mainly ferulic acid and quercetin, along with xanthophylls, e.g., lutein and zeaxanthin. To increase the bioaccessibility of bioactive phytochemicals from quinoa seeds, different drying temperatures were tested. The study demonstrates for the first time that drying with moderate heat (60-70 °C) is effective to increase the extractability of phytochemicals from quinoa seeds. The drying process performed at 70 °C was the most efficient to extract phenolic compounds, followed by the process performed at 60 °C. As far as carotenoids are concerned, drying quinoa at 60 °C allowed the greatest extractability of xanthophylls, followed by the process performed at 50 °C. It is likely that the heat applied might have inactivated the enzymes responsible for the degradation of phytochemicals, softened the plant tissues, thus enhancing the bioaccesibility of phenolics and carotenoids from the grain. Considering that the highest concentration of phytochemicals was obtained when quinoa was dried at 60-70 °C, the use of hot air to dry quinoa seeds is of nutritional importance, as it may enhance the bioaccessibility of its phytochemicals. Bioaccessibility is the amount of compounds released from the food matrix in the gastrointestinal tract, and becoming available for absorption. According to the results from this investigation, drying at 60-70 °C likely increased the nutritional value of quinoa seeds. The above drying processes are candidates of great potential to establish new industrial methods of grain drying Producers and manufactures of quinoa might consider applying moderate heat when drying the crop, to produce foods richer in bioactive phytochemicals.

481   ***Authors Information***

482   The research is part of the project "Perucrop" planned jointly by Suomela J.-P., Baoru Y.,

483   Keskitalo M., and other researchers from the University of Turku and the Natural Resource

484   Institute Finland (Luke). Multari S. designed and performed all the experiments reported in

485   this study, carried out the chromatographic and spectroscopic analyses (HPLC and

486   MS/MS), analysed data, performed the statistical analysis, and wrote the manuscript;

487   Marsol-Vall A. shared with Multari S. all the aspects of the carotenoid analysis, Suomela

488   J.-P. supervised the analytical work, and revised the manuscript; Keskitalo M. provided the

489   quinoa samples; Yang B. revised the manuscript. All the authors approved the final version

490   of the manuscript for publication. The authors declare no competing financial interest.

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637 .

638   *5.   Tables and Figures*

639   **Figure 1. Total Content of Phenolic Compounds from Quinoa Samples.**

640   **Figure 2. Total Content of Carotenoids from Quinoa Samples.**

641

642 **Table 1**. Content of Individual Phenolic Compounds.

drying tempe-rature	vanillic acid		gallic acid		p-benzoic acid		syringaldehyde		ferulic acid		quercetin		kaempferol		cumulative	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
RT	n/d	32.3 ± 3.3a	n/d	n/d	1.51 ± 0.13a,b	7.38 ± 0.75c,b	n/d	1.85 ± 0.18a	33.0 ± 5.9a,c	259 ± 17a	n/d	n/d	n/d	n/d	34.7 ± 5.8 a	297 ± 23 c
40 °C	n/d	24.3 ± 3.0b	n/d	n/d	1.22 ± 0.28 b,d	6.54 ± 0.59c	n/d	n/d	20.1 ± 3.1b,d	241 ± 7a	n/d	n/d	n/d	n/d	21.2 ± 3.5 b,c	272 ± 74c
50 °C	n/d	34.0 ± 3.5a	n/d	n/d	1.83 ± 0.13c,a	8.05 ± 0.75 b,d	n/d	n/d	31.9 ± 3.6c	260 ± 12a	n/d	n/d	n/d	n/d	33.7 ± 3.6 a	302 ± 15 c
60 °C	n/d	33.0 ± 3.2a	n/d	n/d	1.19 ± 0.28d,a	15.6 ± 0.9 a	n/d	n/d	24.7 ± 2.2b,c	243 ± 15a	n/d	288 ± 24b	n/d	171 ± 23a	25.8 ± 2.4 c	746 ± 42 b
70 °C	n/d	30.6 ± 3.0a,b	n/d	140 ± 16	1.20 ± 0.09d,a	9.03 ± 0.60d	n/d	4.72 ± 0.22a	23.6 ± 1.8 d,b	264 ± 9a	n/d	339 ± 23a	n/d	182 ± 11a	24.7 ± 2.0 c	970 ± 30 a

643 Data (mg kg^{-1}) is presented as mean ± SD and represents mean of four independent measurements. n/d = not detected (i.e., below

644 the detection level). Values with unlike letters (a-d) within the same column differ significantly ($p < 0.05$).

645

646   **Table 2**. Content of Individual Carotenoids.

drying temperature	zeaxanthin	lutein	lutein isomer A	lutein isomer B	neochrome A	neochrome B
RT	0.22 ± 0.00 **d**	1.47 ± 0.04 **c,b**	0.09± 0.00 **a**	0.21 ± 0.01 **a**	0.24 ± 0.01 **b**	0.11 ± 0.01 **b**
40 °C	0.27 ± 0.00 **c**	1.39 ± 0.03 **c**	0.04 ± 0.01 **b**	0.10 ± 0.00 **c**	0.29 ± 0.02 **a**	0.14 ± 0.01 **a**
50 °C	0.28 ± 0.00 **b**	1.50 ± 0.04 **b,a**	0.04 ± 0.00 **b**	0.12 ± 0.01 **b**	0.29 ± 0.01 **a**	0.14 ± 0.01 **a**
60 °C	0.30 ± 0.00 **a**	1.56 ± 0.04 **a**	0.04 ± 0.00 **b**	0.09 ± 0.00 **c**	0.27 ± 0.01 **a**	0.13 ± 0.00 **a**
70 °C	0.28 ± 0.00 **b**	1.40 ± 0.04 **c**	0.04 ± 0.01 **b**	0.07 ± 0.00 **d**	0.28 ± 0.01 **a**	0.13 ± 0.00 **a**

647   Data (mg kg^{-1}) is presented as mean ± SD and represents mean of four independent measurements. Values with unlike letters (a-d)

648   within the same column differ significantly ($p < 0.05$).
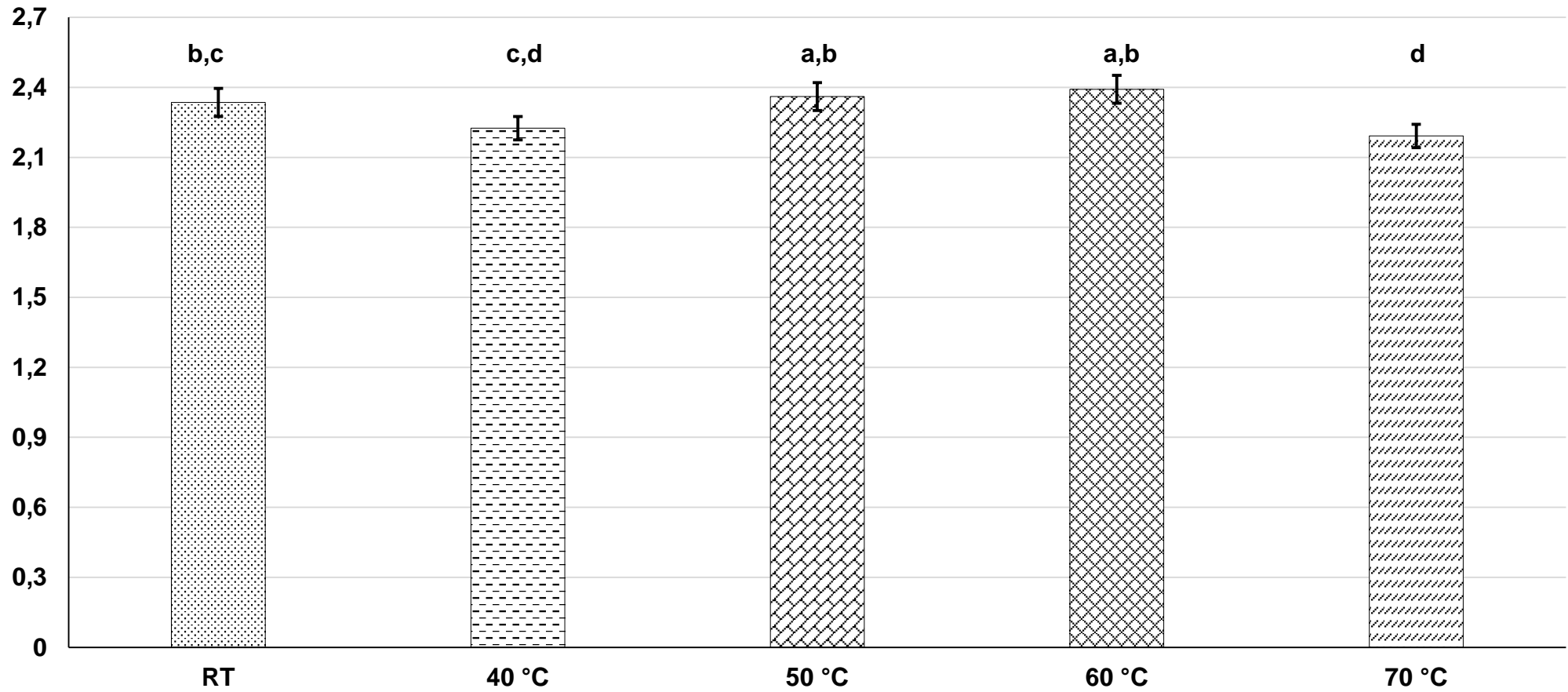
649

650

651 **Figure 1.**
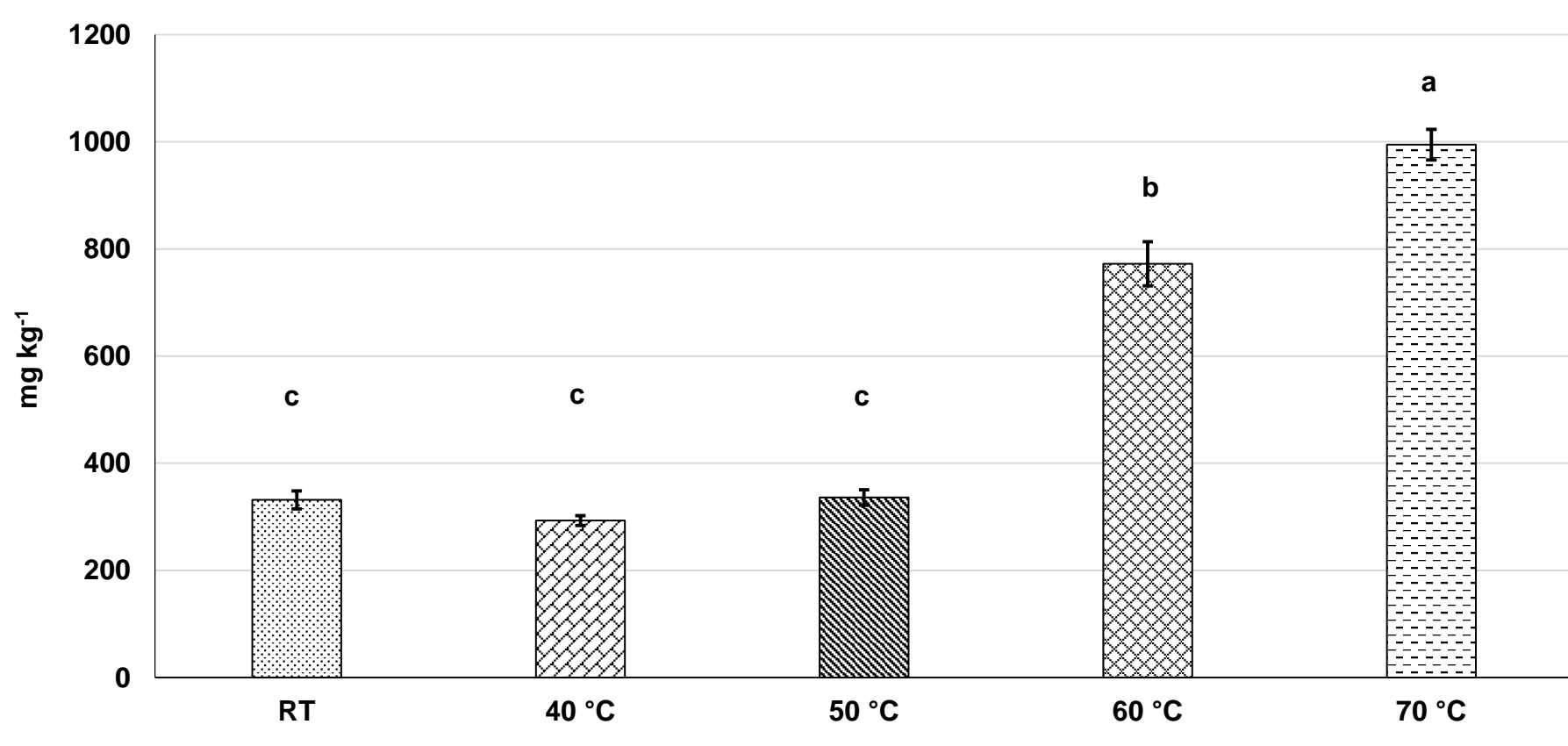
652

653 Data is presented as mean ± SD and represents mean of four independent measurements. Values with unlike letters (a-c) differ

654 significantly ($p < 0.05$).

655

656 **Figure 2.**

657

658 Data is presented as mean ± SD and represents mean of four independent measurements. Values with unlike letters (a-d) differ

659 significantly ($p < 0.05$).