

1 Article

2 Oxalate contents of raw, boiled, wok-fried and pesto 3 and juice made from fat hen (*Chenopodium album*) 4 leaves

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11

12 **Abstract:** The total, soluble and insoluble oxalate contents of fresh and wok-fried fat hen
13 (*Chenopodium album*) leaves were extracted and measured using HPLC chromatography. The total
14 oxalate content of the raw leaves was 1112.4 mg/100 g dry matter (DM), and the levels were
15 significantly reduced by boiling (682.8 mg/100 g DM) or cooking the leaves in a wok (883.6 mg/100
16 g DM). The percentages of soluble oxalate contents in the total oxalates of the raw and boiled leaves
17 were similar (mean 75%), while the proportion of soluble oxalate content in the wok-fried leaves
18 were reduced to 53.4% of the total, giving a significant increase in the insoluble oxalate content of
19 the wok-fried leaves. The percentage of insoluble calcium in the total calcium was significantly
20 reduced ($P < 0.05$) when the leaves were boiled but the insoluble oxalate content significantly
21 increased (67.2%) in the wok-fried leaves when compared to the content of the original raw leaves.
22 Processing the cooked leaves into pesto or extracting the juice gave final products that contained
23 significantly reduced total and soluble oxalate contents. The addition of calcium chloride to the juice
24 made a very small reduction in the soluble oxalate content in the juice.

25 **Keywords:** Total, soluble and insoluble oxalates; calcium; boiling; wok frying; pesto; juicing

26

27 1. Introduction

28 The introduction fat hen (*Chenopodium album* L.) is a fast-growing, weedy annual plant found in
29 many parts of the world. Its common names include goosefoot and lamb's quarters. It is extensively
30 cultivated and consumed in Northern India as a food crop where its Hindi name is bathua. The leaves
31 and young shoots of this plant are used in dishes, such as soups, curries and paratha-stuffed breads,
32 and are especially popular in the Punjab. Elsewhere in the world, the plant is considered to be a weed
33 and is rarely eaten. However, the leaves and young shoots may be eaten as a vegetable, either steamed
34 or cooked like spinach. Evidence that the plant has been used as a food plant for centuries was
35 confirmed by the occurrence of carbonized plant remains in storage pits and ovens at Iron Age,
36 Viking Age and Roman sites in Europe, where seeds have been found mixed with conventional grains
37 and, even, inside the stomachs of Danish bog bodies [1].

38 In many developing countries, vegetables are a reliable and rich source of minerals and
39 nutrients, especially for people who adopt a vegetarian lifestyle. It is also important to note that in
40 developed countries a plant or herb can be rediscovered and promoted as a new 'super food'. In this
41 case, it is important to have a record of its nutrient contents and any possible antinutrients that may
42 cause adverse metabolic effects when eaten or interfere with the absorption of other important
43 nutrients from the leaves. Fat hen is classified as a Chenopodiaceae and plants in this family are well
44 known to contain high levels of oxalates.

45 Oxalate is not an essential nutrient for humans and, if possible, it should not be consumed in
46 large amounts. However, oxalates are found in many kinds of edible plants in varying concentrations
47 [2, 3] and, if consumed in large amounts, they may be harmful to human health [2]. Oxalates occur in
48 two forms in plants, water soluble oxalate (bound to Na⁺ or K⁺) or water insoluble oxalate (bound to
49 divalent ions such as Ca²⁺ and Mg²⁺). An intake of large amounts of soluble oxalate can increase the
50 risk of kidney stone development in susceptible people because of the increased concentration of
51 oxalate in the urine. A large increase in the consumption of oxalates in the diet will increase the risk
52 of kidney stone development, so it is important to identify high oxalate-containing foods and, if
53 possible, reduce these levels by processing [3]. In addition, the binding of soluble oxalate to calcium
54 in the intestine may reduce calcium intakes to lower than recommended levels if the diet does not
55 include adequate calcium sources.

56 The first report of the total oxalate content of fat hen leaves ranged from 361 to 2027 mg/100 g
57 dry matter (DM) (mean 1100 mg/100 g DM), was for plants grown in Spain [4], this was followed by
58 reported values of 1099 mg total oxalate/100 g dry leaves found growing in Almeria, South East of
59 Spain [5]. Raghuvanshi et al. [6] also reported a value of 883.1 mg total oxalate/100 g DM for fat hen
60 found growing as a weed in wheat fields in Pantnagar, India. While fresh fat hen leaves grown in
61 Tamil Nadu, India contained 539.9 and 375.8 mg/100 g DM for total and soluble oxalate and, for
62 cooked leaves, of 545.7 and 377.5 mg/100 g DM [6].

63 In developing countries, green leafy vegetables, such as fat hen, are reliable and important
64 sources of minerals, particularly calcium and the total calcium in raw and cooked fat hen leaves was
65 539.9 and 545.7 mg/100 g DM, respectively [7]. Amarial and Pius [7] went on to measure the
66 bioavailability using a simulated gastric equilibrium dialysis analysis after a simulated *in vitro* gastric
67 digestion. The mean bioavailability of calcium of the raw and cooked leaves was 32.9%. This was a
68 relatively low value compared with the other green leafy vegetables measured in this study. The low
69 bioavailability observed in this study could be explained by the high content of insoluble oxalate in
70 the leaves. It has been observed that insoluble oxalate is largely made up of calcium oxalate [8] and
71 that the calcium oxalate molecule is 31.3% calcium. So, a large amount of the total calcium content in
72 the leaves of fat hen could be unavailable because of being bound to the insoluble oxalate in leaf
73 tissues.

74 The addition of food ingredients containing a high calcium content to foods containing high
75 soluble oxalate contents has been shown to significantly reduce the soluble oxalate content of the final
76 product [9]. Baking taro leaves with milk [10, 11] and with spinach leaves [12] have also been shown
77 to be effective. The addition of four different calcium salts during the preparation of a green juice
78 made from spinach leaves was also very effective at reducing the soluble oxalate content of the final
79 juice [12]. The preparation of pesto and a juice from fat hen also offers the possibility of reducing the
80 soluble oxalate of the final product by the addition of calcium-containing ingredients.

81 The objective of this study was to investigate the oxalate compositions of raw and cooked leaves
82 of fat hen harvested in New Zealand. In addition, the study investigated the oxalate content of a pesto
83 made from processing the leaves at room temperature and the preparation of a mixed juice using the
84 raw leaves.

85 2. Materials and Methods

86 2.1. Source of materials and preparation

87
88 Fat hen (*Chenopodium album* L.) plants were grown in a Wakanui silt loam soil at the Horticulture
89 Research Area, Lincoln University, Canterbury, NZ (43° 38' 52" S, 172° 28' 0.5" E) at 19 m above sea
90 level. The Wakanui silt loam had a good base fertility and no manure or chemical fertilisers were
91 used. The plants were irrigated as required. Approximately 1.5 kg of leaves was harvested on 15
92 February 2018 when the plants had reached a height of 150 mm. The leaves were cut into 1 - 2 mm
93 pieces with a stainless-steel knife prior to sampling.

94 2.2. *Boiling*

95 Approximately 200 g samples of fresh leaves were added to 400 mL of boiling tap water in a
 96 saucepan. The mixture was stirred and allowed to boil for two minutes. The cooked leaves were then
 97 poured into a colander and allowed to drain for two minutes to remove the excess moisture.

98 2.3. *Wok-frying*

99 Approximately 200 g fresh leaves were added to 200 mL of heated (170°C) canola oil (Pams salad
 100 and cooking oil, Pams Products Ltd., Roskill, Auckland, NZ) and gently stirred while cooking for two
 101 minutes. The mixture was then removed from the steel wok using a slotted spoon and placed on
 102 absorbent kitchen paper to cool for two minutes to allow the excess oil to be removed from the leaves.

103 2.4. *Pesto*

104 The pesto was made from freshly harvested leaves. The vegetables and fruit, purchased from
 105 the New World Supermarket, Lincoln, Canterbury, NZ (Table 1), were chopped with a stainless-steel
 106 knife and processed in a Breville bottom-drive food processor (Briscoes Homeware Ltd., Auckland,
 107 NZ). The mixture was processed at full speed until it formed a fine paste that was then stored at -
 108 20°C until analysis could take place.
 109

110 Table 1. Recipe for fat hen pesto (g fresh weight).

Ingredients	Fresh weight (g)
Fresh leaves	40
Tap water	60
Garlic cloves	20
Ground walnuts	20
Parmesan cheese ¹	30
Olive oil	5
Salt	3
Pepper	1
Total weight of the pesto	179.0

111 ¹ Grated dried Parmesan cheese
 112 (Puhoi Valley Cheese, Puhoi, NZ)
 113 Containing 1200 mg calcium/100 g DM.

114 2.5. *Juice*

115 All vegetables and fruits except the fat hen leaves, were purchased fresh from the New World
 116 Supermarket, Lincoln, Canterbury, in April 2016. The fruits and vegetables were trimmed of non-
 117 edible parts using a stainless-steel knife and the remaining edible portions were chopped, weighed
 118 and processed using a masticating juicer, together with the fresh fat hen leaves (Oscar 9000, Dongah
 119 Industrial Co., Ltd, Gyeongsangnam Do, South Korea) following the recipe shown in Table 2. A
 120 calcium source, 200 mg calcium chloride (96% CaCl₂ Kirsch Phama GmbH., Salzgitter, Germany), was
 121 added to 100 mL of the juice and the juice was homogenized using a blender (NutriBullet, NBR-
 122 1207M, 600 W, Homeland Housewares LLC, Los Angeles, CA, USA).
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Table 2. Recipe of the fat hen juice mixture (g fresh weight)

Ingredients	Fresh weight (g)
Fresh leaves	140
Apples	40
Cucumber	40
Celery sticks	40
Lemon	25
Total weight of mix	285.0
Weight of juice recovered	212.9

128

129 *2.6. Dry matter*

130 The dry matter contents of each sample of the fresh, boiled and wok-fried leaves were
131 determined by drying in an oven (Watvic, Watson Victor Ltd., NZ) to a constant weight at 105°C [13].

132 *2.7. Extraction of total and soluble oxalic acids*

133 The measurement of total and soluble oxalates followed the method outlined by Savage et al.
134 [3]. Four replicates of 5 g from each sample of raw or cooked fat hen leaves and the extracted juice
135 and pesto were used to measure the total oxalate contents, and four, five g replicates were extracted
136 to measure the soluble oxalate contents. Forty mL of 0.2 M HCl (Aristar, BDH Chemicals, Ltd., Poole,
137 Dorset, UK) were added to conical flasks for the total oxalate extraction and 40 mL of high purity
138 water (Barnstead International, Dubuque, Iowa, USA, 18.2 MΩ·cm) was added for the extraction of
139 soluble oxalates. All flasks were placed in an 80°C shaking water bath for 20 min. The solutions were
140 then quantitatively transferred into volumetric flasks, allowed to cool and then made up to 100 mL
141 with 0.2 M HCl and high purity water, respectively.

142 The extracts in the volumetric flasks were filtered through a cellulose acetate syringe filter with
143 a pore size of 0.45 µm (dismic-25cs, Advantec, California, USA) into 1 mL glass vials. The samples
144 were analyzed with a high performance liquid chromatography (HPLC) system, using a 300 mm x
145 7.8 mm Rezex ion exclusion column (Phenomenex Inc., Torrance, CA, USA) attached to a cation-H
146 guard column (Bio-Rad, Richmond, CA, USA) held at 25°C. Analysis was performed by injecting 20
147 µL of sample or standard onto the column using an aqueous solution of 25 mM sulphuric acid (HPLC
148 grade Baker Chemicals, Phillipsburg NJ, USA) as the mobile phase, then pumped isocratically at 0.6
149 mL/min, with the peaks detected at 210 nm. The HPLC equipment consisted of a Shimadzu LC-10AD
150 pump, CTO-10A column oven, SPD-10Avp UV-Vis detector (Shimadzu, Kyoto, Japan) and a Waters
151 717 plus auto-sampler (Waters, Milford MA, USA). Data acquisition and processing were undertaken
152 using a PeakSimple Chromatography Data System (model 203) and PeakSimple software version 4.37
153 (SRI Instruments, Torrance CA, USA). The oxalic acid peak was identified by comparing the retention
154 time to a standard solution, and by spiking an already-filtered sample containing a known amount
155 of oxalic acid standard. The insoluble oxalate content of each sample was calculated by the difference
156 between the total and soluble oxalate contents.

157 *2.8. Standard calibration*

158 Two standard curves of oxalic acid (99.99% oxalic acid, Sigma-Aldrich Co., St. Louis, USA) were
159 analyzed using the following concentrations: 1, 2, 5, 10, 15 and 25 mg/100 mL, one prepared in 0.2 M
160 HCl while the other prepared in high purity water. The acid standard curve was used for identifying
161 and calculating the total oxalate content, while the water standard curve was used for calculating the
162 soluble oxalate content. All blanks and standard solutions were passed through a 0.45 µm cellulose
163 acetate filter prior to analysis.

164 2.9. Mineral analysis

165 Four replicates of the raw or cooked leaf samples were weighed into a tared Teflon microwave
166 vessel and the weight recorded. Five mL of 69% nitric acid (BDH Aristar, BDH Chemicals, Ltd., Poole,
167 Dorset, UK) and 1 mL of 30% hydrogen peroxide (BDH Aristar, BDH Chemicals, Ltd., Poole, Dorset,
168 UK) were then added. The Teflon PFA- and Kevlar-shielded vessels were then capped and digested
169 using a CEM Mars Express microwave (CEM Corporation, Matthews, North Carolina, USA). The
170 heating programme was as follows: ramp 10 min to 90°C; hold for 5 min; ramp for 10 min to 150°C;
171 then hold for 5 min. The cooled digest was made up to 25 mL with high purity water (Barnstead
172 International, Dubuque, Iowa, USA, 18.2 MΩ*cm).

173 The concentration of total calcium in each sample was measured on a Varian Axial 720
174 Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES, Varian, Palo Alto, CA,
175 USA) with a SP3 auto-sampler. Minerals were identified and quantitated using an ICP multi-element
176 standard solution (CertiPUR, Merck, KGaA, Darmstadt, Germany) containing 23 elements. The data
177 and the standard curves were processed using ICP-Expert™ II (Varian, Palo Alto, CA, USA). A high
178 purity water test standard, and ICP multi-element standard solutions containing 0.50 µg/g of each
179 element, were run in triplicate to calibrate the ICP-OES instrument and to determine the standard
180 error of the analyses. The overall standard error was ± 0.013 mg/kg. A limit of quantitation (LOQ) test
181 was performed using 10 times the standard deviation of the blank (5% v/v nitric acid) for each
182 mineral. Individual mineral LOQ values ranged from 0.12 to 12.24 µg/L, with a mean of 1.81 µg/L.

183 2.10. Statistical analysis

184 All calculations were performed using Excel 2013 and statistical analysis was carried out
185 using GenStat, Release 15.1 for Windows 7 (VSN International Ltd., Hemel Hempstead,
186 Hertfordshire, UK) to determine the accumulated analyses of variance. The mean values were
187 compared using Duncan's multiple range test ($p < 0.05$). All analyses were carried out in triplicate.
188

189 3. Results

190 3.1. Raw and cooked leaves

191 The total, soluble and insoluble oxalate contents for the raw, boiled and wok-fried fat hen leaves
192 (on a dry weight basis) are shown in Table 3. Boiling the leaves for two minutes significantly reduced
193 the total oxalate content of the cooked leaves but the ratio of soluble oxalate to the total oxalate
194 content remained the same as in the raw leaves (mean 75%).

195 Cooking the leaves in a wok for two minutes resulted increased dry matter content in the cooked
196 leaves from the absorption of cooking oil (from 20.1 to 42.2 g/100 g wet matter (WM), respectively).
197 The proportion of soluble oxalate content to the total oxalate content was reduced compared to the
198 raw and boiled leaves, as a large proportion of soluble oxalate was converted into insoluble oxalate
199 during wok-frying. The total calcium contents of the boiled leaves significantly increased when the
200 leaves were boiled and significantly reduced in the wok-cooked leaves as the leaves absorbed oil
201 during cooking.

202 3.2. Processed raw leaves – pesto and juice

203 The pesto was made from 22.3% fresh fat hen leaves together with the standard ingredients
204 usually added to improve the consistency and taste of the final product. Fresh fat hen leaves made
205
206
207
208
209
210

211 Table 3. Mean dry matter, total, soluble and insoluble oxalates, total calcium and calcium
 212 bound in insoluble oxalate content of fat hen leaves raw, boiled and wok-fried (mg/100 g DM \pm
 213 SE). (Values in brackets are % soluble oxalate in the total oxalate content).
 214

	Raw	Boiled	Wok-fried
Dry matter	20.06 \pm 0.31	11.58 \pm 0.16	42.20 \pm 1.16
Oxalate			
Total oxalate	1112.40 \pm 19.00 ^a	682.79 \pm 29.13 ^b	883.57 \pm 3.45 ^c
Soluble oxalate	866.31 \pm 14.87 ^a (78.0)	496.37 \pm 7.37 ^b (72.7)	472.14 \pm 10.83 ^b (53.4)
Insoluble oxalate	246.09 \pm 28.80 ^b	186.42 \pm 32.53 ^b	411.43 \pm 13.48 ^a
Calcium			
Total calcium	224.60 \pm 1.47 ^b	268.68 \pm 3.62 ^a	162.43 \pm 1.23 ^c
Calcium bound in insoluble oxalate	76.98 \pm 9.01 ^b	58.32 \pm 19.18 ^b	128.70 \pm 4.21 ^c
Soluble calcium/total calcium (%)	65.69 \pm 4.13 ^b	78.14 \pm 4.08 ^a	20.76 \pm 2.58 ^c

215 Means in each row with a different superscript letter are significantly different using Duncan's multiple range
 216 test, $P < 0.05$.

217
 218 up 49.1% of the juice mix and, again, standard ingredients were added to make a pleasant tasting
 219 mix. During preparation the masticating juicer removed a significant portion of plant fiber derived
 220 from the fresh leaves and other ingredients added to the mix. Seventy-five % of the original leaves,
 221 and fruits and vegetables in the mix, were recovered as juice (Table 2). The total, soluble and insoluble
 222 oxalate contents for the pesto and juice (g/100 g juice) are shown in Table 4.

223 Table 4. Dry matter, total, soluble and insoluble oxalate contents of pesto (mg/100 g WM) and juice
 224 (g/100 g juice) made from raw fat hen leaves (Values in brackets are % of soluble oxalate in the total
 225 oxalate content).

	Pesto	Juice	Juice + calcium ¹
Dry matter	30.89 \pm 0.50	12.59 \pm 0.05	12.59 \pm 0.05
Total oxalate	196.25 \pm 23.63	100.75 \pm 3.94	100.75 \pm 3.94
Soluble oxalate	58.50 \pm 1.44 (28.8)	65.75 \pm 1.11 (65.2)	60.50 \pm 1.50 (60.1)
Insoluble oxalate	138.0 \pm 23.74	35.25 \pm 4.59	40.75 \pm 4.94

227 ¹200 mg calcium chloride added to 100 mL of the juice

228 4. Discussion

229 The total calcium contents of the raw and cooked leaves were 224.60 \pm 1.47 and 268.68 \pm 3.62
 230 mg/100 DM, respectively, and this was low compared with the values 539.9 and 545.7 mg/100 g DM
 231 previously reported values for leaves of raw and cooked fat hen grown in Tamil Nadu, India [7].
 232 Amariai and Pius [7] went on to measure the bioavailability of calcium using a simulated gastric
 233 equilibrium dialysis analysis after a simulated *in vitro* gastric digestion. Using this method, the mean
 234 bioavailability of calcium in the raw and boiled leaves grown in India was 32.9%. In this present study
 235 65.69 and 78.14%, respectively, of the total calcium contents of the raw and boiled fat hen leaves were
 236 calculated to be soluble calcium. In contrast, the soluble calcium content of the wok-fried leaves was
 237 20.78%, which corresponds to a marked increase in the proportion of insoluble oxalates in this fried

238 food, which made up 46.6% of the total oxalate fraction (Table 3). The amount of soluble calcium
239 calculated in this study is in contrast to those calculated for raw and cooked fat hen leaves prepared
240 in India [7]. However, this study used very different methods to determine the soluble calcium
241 fraction of the cooked leaves [7].

242 A similar mixture of ingredients was used to make a juice from three cultivars of bitter gourd
243 fruits [14]. The mean recovery of juice from the mix was 75%, meaning that a significant amount of
244 fiber was discarded by the masticating juicer. They also noted that the soluble oxalate contents of the
245 juices they prepared were very low, and the insoluble content was even lower. They commented that
246 the other ingredients in the mixture would have effectively diluted the oxalate contents in the juice.
247 In addition, the soluble and insoluble oxalates would tend to bind to the discarded fiber fraction. In
248 this experiment, the total and soluble oxalate contents of the juice prepared (Table 4) were also
249 relatively lower than in the raw fresh leaves, for the same reason. It is interesting to note that the
250 addition of a food source of calcium (dry powdered Parmesan cheese) to the pesto increased the
251 insoluble oxalate content and, thus, decreased the available soluble oxalate, whereas the addition of
252 calcium chloride to the juice had little effect. The addition of Parmesan cheese to the pesto mix had a
253 similar effect as the addition of milk to taro leaves [10, 11] and spinach leaves [12] while the addition
254
255 of calcium chloride during the preparation of juice from fat hen leaves was not as effective as the
256 addition of calcium chloride during the processing of rhubarb stems [9]. In this present experiment,
257 it is possible that the addition of the other ingredients containing high fiber contents during the
258 preparation of juice from fat hen leaves may have had the effect of reducing the overall soluble oxalate
259 contents of the juice. While these added ingredients had the effect of reducing the overall soluble
260 oxalate content, the effect of the addition of fiber, which the soluble oxalate may have bound to during
261 juice preparation, possibly had the most important effect. This fiber was then removed in the
262 discarded fiber fraction. The addition of calcium chloride to the juice was only marginally effective
263 (Table 4).

264 5. Conclusions

265 Overall, this experiment has shown that raw, boiled and wok-fried fat hen leaves contained high
266 levels of total and soluble oxalates. Boiling the leaves significantly reduced the soluble oxalate content
267 by leaching, while the levels in wok-fried leaves were effectively diluted by the absorption of the
268 salad oil into the leaves. The insoluble oxalate fraction in the wok-cooked leaves contained a
269 significant proportion of the total calcium content with the proportion of calcium locked in the
270 insoluble oxalate ranging from 34 to 79% of the total calcium content in the leaves. Compared with
271 raw leaves, the proportion of calcium locked in insoluble oxalate was reduced by boiling (34 v 22%
272 of the total calcium content) and markedly increased by wok-frying (79%). This effect of wok-cooking
273 decreases soluble oxalate availability for absorption but also decreases the availability of the calcium
274 in the fat hen leaves.

275 The soluble oxalate content of the pesto and juice made from the leaves was relatively low
276 because the leaf content was reduced by the addition of the other fruits and vegetables used to make
277 a balanced mix. These products would have little negative effect in the diet because of their low
278 soluble oxalate content. In contrast, the leaves, if consumed regularly or in large amounts, could be
279 of considerable risk in the diet due to absorption of soluble oxalate from the raw and boiled leaves.
280 The reduced proportion of soluble oxalates in wok-cooked leaves because of binding to calcium
281 within the leaves is advantageous as long as other adequate calcium sources are present in the diet.

282

283 **Acknowledgments:** This study was funded by Lincoln University; the authors wish to thank Janette Busch for
284 proof reading this paper.

285 **Author contributions:** Both authors contributed to all parts of this paper.

286 **Conflicts of Interest:** The authors declare no conflict of interest.

287

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