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## Comparison of oxalate contents and recovery from two green juices prepared using a masticating juicer or a high speed blender

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#### ABSTRACT

*Background:* Juicing is a popular health trend where green juice is prepared from a range of common vegetables. If spinach is included in the mix then the juice may contain significant quantities of oxalates and these are not safe to consume regularly in large amounts as they predispose some people to kidney stone formation.

Methods: Green juice, prepared from spinach and other common vegetables using a high speed blender that produced a juice containing all the original fiber of the processed raw vegetables, was compared with a juice produced using a masticating juicer, where the pulp containing most of the fiber was discarded in the process. The oxalate contents of both juices were measured using HPLC chromatography.

Results: Two juices were prepared using each processing method, one juice contained a high level of spinach, which resulted in a juice containing high levels of total, soluble and insoluble oxalates; the other was a juice mixture made from the same combination of vegetables but containing half the level of spinach, which resulted in a juice containing considerably (P < 0.001) lower levels of oxalates. Removal of the pulp fraction from the green vegetable juice had resulted in significantly (P < 0.01) higher levels of oxalates in the remaining juices made from both levels of spinach.

*Conclusion:* Green juices prepared using common vegetables can contain high levels of soluble oxalates, which will vary with the type and proportion of vegetables used and whether or not the pulp fraction was retained during processing.

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#### 1. Introduction

Juicing has become a popular health trend in recent years. The term "juicing" refers to a period of three to ten days when a person's diet consists mainly of fruit and vegetable juices. It is widely promoted on the Internet as providing health benefits, such as encouraging weight loss and flushing toxins from the body, even though there is no strong scientific evidence to support these claims. The juicing of a mixture of green leafy vegetables along with several fruits is believed to be the basis of a healthy diet as these fruits and vegetables contain a wide range of essential amino acids, organic acids, vitamins and minerals [1]. It is important to note that the vegetables and the juices are not heated or cooked during juicing and this is seen as an effective way to preserve the positive nutrients in the juices [2], but it does not allow for the possibility of reducing potentially toxic anti-nutrients, such as oxalates.

Oxalate is not an essential nutrient. It is found in many kinds of edible plants in varying concentrations [3,4] and, if consumed in large amounts, may be harmful to human health [3]. An intake of large amounts of soluble oxalate can increase the risk of kidney stone

development in susceptible people because of the increased concentration of oxalate in the urine. As consumption of additional oxalate in the diet would increase the risk of kidney stone development, it is important to identify high oxalate containing foods and, if possible, reduce these levels by processing [3]. In addition, in mammalian metabolism endogenous oxalate is produced by the breakdown of dehydroascorbic acid, glyoxylate, serine and glycine in the liver and is excreted in the urine [5]. At moderate levels of ascorbic acid (vitamin C) intake about 40% of the total oxalate excreted in the urine comes from the breakdown of ascorbic acid in the liver [5]. As oxalate is the end product of ascorbic acid metabolism in mammals [6] and, as it appears that a high percentage of juicers consume large amounts of fruits containing ascorbic acid, the potential to excrete significant amounts of oxalate in the urine is increased during juicing.

A recent report of juicing-induced damage [7] has been publicized. In this case, oxalate nephropathy occurred following six weeks' consumption of a juicing diet that contained large amounts of spinach. Spinach, a common constituent of juicing diets, is known to contain high levels of oxalate, with fresh or frozen spinach contents reported to range from 320 to 1260 mg/100 g wet matter (WM) [3,4,8–10] and higher levels are being reported in fresh, summer-grown plants [9]. Many other vegetables, such as celery, cucumber and parsley also contain moderate levels of oxalates [11].

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Some fruit juices are added to the green juices or they are consumed directly. Fang et al. [12] showed that feeding star fruit (*Averrhoa carambola*) juice, which was known to contain high levels of oxalates to rats, can not only produce acute renal injury through the obstructive effect of crystals of calcium oxalate, but also induce apoptosis of renal epithelial cells. These observations explained the fatal outcomes that have been reported when star fruit have been consumed by uremic patients [13].

Overall, juicing, without considering whether high oxalate fruit and vegetables were included in the mix, was a risky undertaking, particularly if the juices were consumed over a sustained period of time. More consideration should be given to the levels of total oxalates in the main constituents and measurements undertaken of the soluble and insoluble oxalate contents. Most high oxalate-containing foods contained both soluble (bound to Na $^+$ , K $^+$  and NH $^+$ ) and insoluble (bound to Ca $^{2+}$ , Mg $^{2+}$  and Fe $^{2+}$ ) oxalates [4]. The soluble forms were available for absorption from the intestine. However, soluble oxalates can bind to Ca $^{2+}$ , Mg $^{2+}$  and Fe $^{2+}$  ions to become insoluble salts during processing and cooking. There was some evidence to suggest that oxalates can become bound to fiber and if this fraction was removed during processing then the levels in the juice may be significantly reduced.

The objective of this study was to investigate the oxalate composition of green juice prepared using a high speed blender compared with the juice prepared using a masticating juicer where the pulp fraction was discarded in the process. In addition, two juice mixes were prepared using each processor, one containing a high level of spinach and one containing half this amount but with the same combination of vegetables.

#### 2. Materials and methods

#### 2.1. Source of materials and preparation

All vegetables and fruits were purchased fresh from New World Supermarket, Lincoln, Canterbury, in April 2015. Soil, dead leaves and excess stems were removed using a stainless steel knife and the remaining edible portions were chopped, weighed and processed using a masticating juicer (Oscar 9000, Dongah Industrial Co., Ltd, Gyeongsangnam Do, South Korea) or a high speed blender (Vitamix 5200, Vita-Mix Corp. Cleveland, OH, USA). Each juice type was prepared in triplicate following the recipes shown in Table 1.

#### 2.2. Dry matter

The dry matter (DM) content of each sample was determined by drying in an oven (Watvic, Watson Victor Ltd., NZ) to a constant weight at 105 °C (AOAC method 935.10) [14]

#### 2.3. Extraction of total and soluble oxalic acid

The measurement of total and soluble oxalates was performed following the method outlined by Savage et al. [4]. Three replicates of

**Table 1**Composition of the two juicing mixes.

Ingredients	Low spinach mix (g)	High spinach mix (g)
Spinach	300	600
Apple	300	225
Celery	225	168.75
Cucumber	225	168.75
Green pepper	150	112.5
Red capsicum	150	112.5
Lemon	120	90
Parsley	30	22.5

each juice (5 g) were extracted to measure the total oxalate content and another three replicates were extracted to measure the soluble oxalate contents. Forty milliliters of 0.2 M HCl (Aristar, BDH Chemicals, Ltd., Poole, Dorset, UK) was added to flasks for the total oxalate extraction and 40 mL of Nanopore II water (Barnstead International, Dubuque, Iowa, USA, 18 M $\Omega$ cm) were added for the extraction of soluble oxalates. All flasks were placed in an 80 °C shaking water bath for 20 min. The solutions were then transferred quantitatively into volumetric flasks while still hot. The extracts were allowed to cool and then made up to 100 mL with 0.2 M HCl and Nanopore II water, respectively.

#### 2.4. Sample analysis

The extracts in the volumetric flasks were filtered through a cellulose acetate syringe filter with a pore size of 0.45 µm (Advantec, California, USA) and then placed into glass HPLC vials. The samples were analysed with a high performance liquid chromatography (HPLC) system, using a 300 mm × 7.8 mm Rezex ion exclusion column (Phenomenex Inc., Torrance, CA, USA) attached to a Cation-H guard column (Bio-Rad, Richmond, CA, USA). The equipment consisted of a Shimadzu LC10AD pump (Kyoto, Japan), an autosampler (Waters 717 plus, Milford, MA, USA), and Shimadzu SPD-10Avp UV-VIS detector (Kyoto, Japan) set at 210 nm. Data was captured and processed using a Peak Simple Chromatography Data System (SRI Instruments Model 203, Torrance, CA, USA). An aqueous solution of 25 mM H<sub>2</sub>SO<sub>4</sub> (Mallinckrodt Baker Instra-analyzed®, Kentucky, USA) was used as the mobile phase. Samples (20 µL) were injected into the column and eluted at a flow rate of 0.6 mL/min. The oxalic acid peaks in the samples were identified by comparing with the retention time of a standard solution and by spiking an already-filtered sample with a known amount of oxalic acid standard. The insoluble oxalate content of each sample was calculated by the difference between the total and the soluble oxalate contents [15]. The oxalate data was presented as mg/100 g fresh weight (FW) as this was how these products are commonly consumed.

#### 2.5. Standard calibration

Two standard curves of oxalic acid (99.99% oxalic acid, Sigma-Aldrich Co., St. Louis, USA) were analyzed, with standards of the following concentrations: 1, 5, 10, 15 and 25 mg/100 mL. One batch of standards was prepared in 0.2 M HCl while the other was prepared in Nanopore II water. The acid standard curve was used for identifying and calculating the total oxalate contents, while the water standard curve was used for the soluble oxalate contents. All blank and standard solutions were filtered through 0.45 µm cellulose nitrate filters (Sartorius, Gottingen, Germany) prior to analysis.

#### 3. Results

Three replicate recipes for each juice type were prepared (Table 1). The total weights of the ingredients for the low and high spinach were  $1502.9 \pm 2.4$  and  $1502.5 \pm 0.9$  g, respectively. The mean recovery of juice using the high speed blender was  $98.0 \pm 0.1\%$ , as a small amount of material could not be recovered from the cutting blades and from the inside of the juicer jar (Table 2). The mean recovery of juice using the masticating juicer was  $75.7 \pm 0.2\%$ . This represented the material remaining in the juicer mechanism and the intentional removal of the pulp fraction by the juicer. This also resulted in the mean dry matter of the juice fraction yielded by the masticating juicer to be significantly lower (7.12%) compared to the juice produced by the high speed blender (8.31%).

Overall, the juice prepared using the masticating juicer contained more (P < 0.001) total and soluble oxalates ( $mg/100 \, g \, FW$ ) when compared to the juice prepared using the high speed blender (Table 3). There was a small, significant difference between the insoluble oxalate contents of the juices prepared by the different juicers. The juice

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