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Manganese screening of pineapple by total-reflection X-ray fluorescence (TXRF) spectroscopy

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Summary

Manganese is regarded as an essential trace element for human nutrition and is found in pineapple fruits in elevated concentrations. In this study, the manganese content of pineapple and pineapple juice was analysed by total-reflection X-ray fluorescence (TXRF) spectroscopy. The manganese concentration in whole fruits was highly variable, however, inside the individual fruit there is an increase from the fruit flesh to the skin of the pineapple. Looking at industrially processed pineapple products, purees (n = 12) have the lowest manganese content with a median of 4.9 mg/kg compared with pineapple juices (10.5 mg/kg, n = 17) and concentrates (15.5 mg/kg; n = 48, calculated to 12.8 °Brix). Depending on the concentration of the product, the tolerable daily intake of manganese is nearly reached with only one glass (200 mL) of pineapple juice.

Zusammenfassung

Mangan gilt als essenzielles Spurenelement für die menschliche Ernährung, die Ananas enthält hiervon deutliche Mengen. In dieser Studie wurde der Mangangehalt von ganzen Ananas, Säften und Pürees mithilfe der Totalreflektions-Röntgenfluoreszenzspektroskopie (TRFA) untersucht. Der Mangangehalt der Ananas ist sehr variabel, die Konzentration nimmt jedoch vom Fruchtfleisch zur Schale hin zu. Von den industriellen Verarbeitungsprodukten haben die Pürees (n = 12) mit einem Median von 4,9 mg/kg den geringsten Gehalt, verglichen mit dem Saft (10,5 mg/kg; n = 17) und den Konzentraten (15,5 mg/kg; n = 48, berechnet auf 12,8 °Brix). Abhängig von der Konzentration im Produkt kann die empfohlene tägliche Aufnahme von Mangan mit einem Glas Ananassaft (200 mL) erreicht werden.

1 Introduction

During a routine survey of the levels of some transition metals in commercial fruit juices from *Beattie* und *Quoc* (2000), a surprisingly high level of manganese in pineapple juice was observed. Further investigation revealed that pineapple appears to concentrate manganese to a much greater extent than other fruits. Manganese seems to be selectively accumulated, since other elements, like chromium, iron, nickel or copper are not increased in pineapple fruits. The mean levels of manganese in commercial pineapple juices were also found to be much higher than in other common fruit juices. In the juice manganese is mainly present as the $[Mn(H_2O)_6]^{2+}$ species as shown by electron paramagnetic resonance spectroscopy (*Beattie* und *Quoc*, 2000). Therefore, clarification of pineapple juice by fining agents and filtration cannot remove this water-soluble manganese species as it is not bound to cloudy fruit particles. Accordingly, in a study with Australian pineapples, most of the manganese was found in the filtered juice and only little in the pulp retained on a 0.4 micron filter (*Prasad* et al., 2000).

High levels of manganese in pineapple might reflect the acidic soil conditions in which these fruits are cultivated. The optimum pH has been found to be between 4.5 and 5.5 (*Py* et al., 1987). High water content in soil leads to a reduction of MnO_2 (Pyrolusite) by soil bacteria yielding $Mn(II)^{2+}$. It has been reported that immature pineapple pulp contains as much as 40 ppm of manganese, but this level declines markedly during maturity (*Beattie* und *Quoc*, 2000).

Manganese is essential for many plant functions, like the assimilation of CO_2 in photosynthesis and electron transport: Manganese supports the formation of chlorophyll, acts as a cofactor for plant enzymes and is involved in the biosynthesis of vitamins.

The WHO recommendation for the tolerable daily intake (TDI) is 3.6 mg for adults (60 kg), the upper limit (UL) is

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11 mg, and the recommended daily allowance (RDA) of manganese is 2 mg per person (European Commission Council Directive 90/496/EEC). The toxicity of manganese as an essential element is regarded as low. Higher quantities may lead to diseases as previously found for industrial workers (*Kies,* 1987; *Dörner* et al., 1990). From animal studies there are known neurotoxic effects of excessive exposure to manganese, especially in the basal ganglia, resulting in a dystonic Parkinsonian disorder (*Brouillet* et al., 1993). The authors showed that manganese may produce neuronal degeneration by an indirect excitotoxic process, secondary to its ability to impair oxidative energy metabolism. Neurotoxic effects in rats were found by *Roels* et al., 1997 studying the concentration of manganese in blood, hepatic and cerebral tissues.

There was a concern on the intake of manganese by pineapple juices due to elevated content. This study was focused on the distribution of manganese in the fresh fruit, pineapple juices, concentrates and purees from multiple origins.

2 Material and methods

2.1 Instrumentation

Measurements were done using a total-reflection X-ray fluorescence spectrometer "S2 PICOFOX" (*Bruker* AXS Microanalysis); X-ray tube: 50W metal ceramic, max 50 kV, 5.5 mA, air-cooled with Mo target, multilayer monochromator; detector: Peltier-cooled XFlash® Silicon Drift Detector (–20 °C), no need of liquid nitrogen, high Efficiency Module with 30 mm² detector; sample carrier: Quartz glass with a diameter of 30 mm and a thickness of 3 mm ± 0.1 mm; automatic sample changer with cassette for up to 25 samples.

Internal Standard: 1 g/L selenium (as SeO_2) in 0.5 mol/L HNO_3 (ICP quality, *Bernd Kraft,* Germany); diluted to 10 mg/L selenium with purified water.

The spectra were acquired for 1000 seconds and the element range determined from aluminium to yttrium and palladium to uranium. For the calculation of the TXRF spectra the software Picofox 7.2x (Bruker) was used, the statistical calculations were done with SPSS (IBM).

2.2. Sample preparation

Pineapples coming from Costa Rica, Ghana, Honduras and Spain bought at local German markets, 17 pineapple juices, 48 concentrates and 12 purees from different processing companies and regions were analysed.

In order to study the manganese distribution in the pineapple, the fruits were disposed as follows: divided horizontal into three parts of approximately 3 cm thickness (top, middle and bottom of the fruit), these slices were divided into 3 parts (skin, flesh and core). Samples were crushed with a hand-held blender (MR 6500, *Braun*), filtered (folded filter, diameter 185 mm, DF 520b *Hahnemuehle Fine Art GmbH,* Dassel/Germany) and homogenized 5 min in an ultrasonic bath (Sonorex super RK106, Bandelin) followed by centrifugation 10 min at 4500 rpm (Rotina 35, *Hettich*). Finally, the supernatant of the samples was measured by TXRF. Selenium was added as internal standard for the quantification in concentration of 5 mg per kg of pineapple sample just before grinding. From every part of the fruit three different samples were prepared and measured twice.

Juices, concentrates and purees were first measured for their sugar level (°Brix) and then prepared as follows: adding selenium as internal standard, 4-fold dilution with water for juices and purees and a 11-fold dilution for concentrates. Then the samples were homogenized, centrifuged and measured by TXRF. 5 µL of the prepared sample was pipetted two times on the quartz glass sample carrier (in total 10 µL) and carefully dried at 40–50 °C on a glass coated heating plate for 20 min. Juices, concentrates and purees samples were prepared two times.

3 Results

3.1 Fresh fruit

The first study should look at the manganese distribution inside the fruit. Considering that, four pineapples from the German market originating from Costa Rica, Ghana, Honduras and Spain were analysed. Unfortunately, the cultivation and harvest conditions are unknown. One fresh pineapple from Costa Rica has a superior content of manganese (54 mg/kg) in comparison with the other analysed fruits and was therefore excluded from the following examination.

Figure 1 shows the concentration of manganese in three parts of the pineapple fruit (skin, flesh and core). The median for the horizontal slices of the top, centre and bottom are for skin 15.1, flesh 7.4 and core 9.4 mg/kg manganese. On the vertical axes the manganese content on top is 10 mg/kg, on bottom 9.5 mg/kg and the lowest in the centre of the fruit with 7.5 mg/kg. Even if there is a large variation in the samples, the skin samples are significantly different from those of the flesh and the core (see Fig. 1). The concentration is increasing from the pure fruit flesh to the skin region of the fruit. Expanding the data with other pineapples from German supermarkets the following manganese concentration in the fruit flesh can be found: Six different pineapples from Costa Rica have a mean of 18.4 mg/kg and a concentration range from 1.5 to 54 mg/kg (one outstanding sample), four pineapples from Ghana have a mean of 12.1 mg/kg (range from 5.4–21.3 mg/kg), one from Honduras contains 8.6 and one from Spain 7.2 mg/kg manganese. With one exception mentioned above, the pineapples have a concentration range between 1.5–21.3 mg/kg, nevertheless the concentration level in different pineapple fruits is very high compared to other fruits. More information about the growing conditions and the variety are necessary to interpret these values.

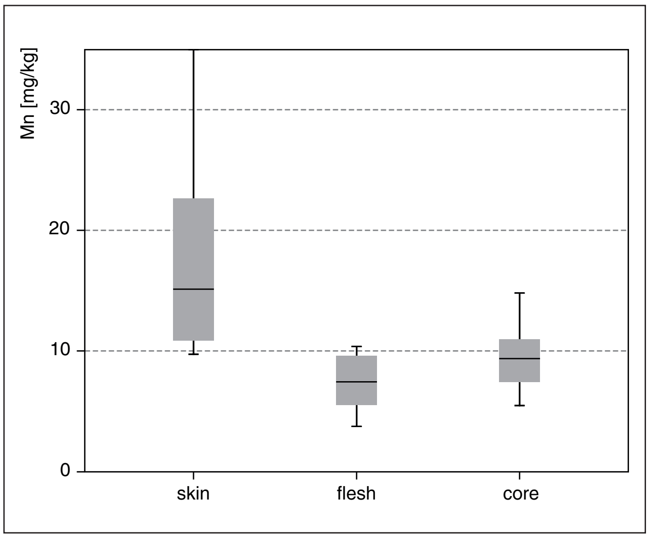

Fig. 1 Contents in manganese in the skin, the flesh and the core of pineapples (n = 3 pineapples × 3 rep.)

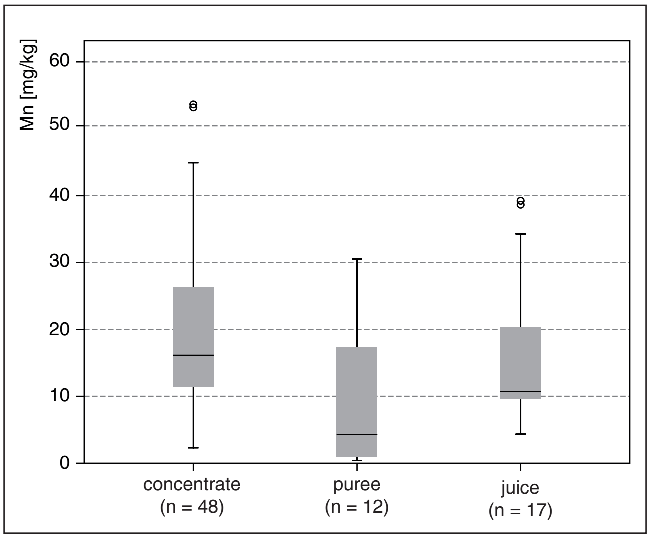

Fig. 2 Contents in manganese of purees, juices and concentrates adjusted to 12.8 °Brix.

3.2 Concentrate, juice and puree

To compare the manganese concentrations of industrially produced juices, purees and concentrates, the concentrations were calculated to a total sugar content of 12.8 °Brix. The manganese distribution of 12 purees, 17 juices and 48 different concentrates are shown in Figure 2.

The juices and the reconstituted concentrates have the highest manganese concentrations up to 53.6 mg/kg whereas the purees have the biggest variation starting from very low amount from 0.4 up to 31.5 mg/kg manganese. Looking at the median the purees have the lowest manganese concentration (4.2 mg/kg) followed be the juice with 10.2 mg/kg and the reconstituted concentrates show highest amounts with 16.4 mg/kg.

The variability of the manganese level is extremely high, starting from 0.4 for purees going up to 54 mg/kg in one outstanding sample of a concentrate. Despite this variability, some distinctions can be made. The median of juices (10.5 mg/kg) and concentrates (15.5 mg/kg) are not significantly different. However, the purees have a significant lower concentration of manganese, with a median of 4 mg/kg (range from 0.4 to 33 mg/kg Mn). Table 1 shows the manganese concentration from puree, juice and concentrate in dependency of the origin of the products. The variation of the data is very high and cannot be explained only by the origin and the processing technology. Looking only at the concentrates from different countries – shown in Figure 3 – the concentrates from Kenya, Costa Rica (only

Tab. 1 Manganese content from concentrate, puree and juice from different origins adjusted to 12.8 °Brix (n: number of samples measured twice)

product	country	n	mean	minimum	maximum
concentrate	Brazil	5	11.7	6.2	18.4
	Costa Rica	2	25.7	25.4	26.2
	Indonesia	4	10.5	5.4	13.2
	Kenya	5	34.0	27.8	41.4
	Philippines	3	9.2	3.0	12.7
	South Africa	2	11.7	7.6	15.8
	Thailand	16	26.7	15.3	53.6
	Vietnam	3	11.2	6.1	14.1
	total	40	20.8	3.0	53.6
puree	Brazil	1	30.5		
	Guatemala	4	0.8	0.4	1.6
	Thailand	4	17.3	16.4	18.1
	total	9	11.5	0.4	30.5
juice	Costa Rica	7	13.8	6.4	39.2
	Ghana	2	10.7	10.0	11.3
	Guatemala	2	4.7	4.4	5.1
	South Africa	1	14.1		
	Thailand	1	34.2		
	total	13	13.5	4.4	39.2

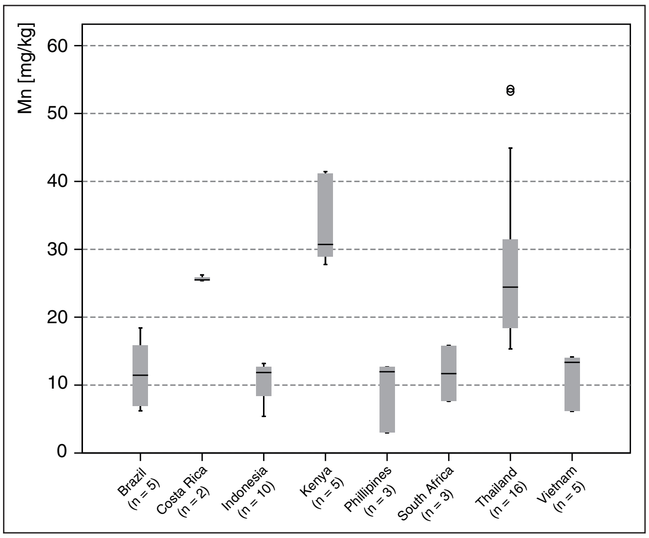

Fig. 3 Manganese concentration in concentrates of different origins calculated to a total sugar content of 12.8 °Brix

2 samples) and Thailand have the highest concentrations. The concentrates from Brazil, Indonesia, Philippines, South Africa and Vietnam have concentrations less than 20 mg/kg. For further interpretation details about the variety, the growing and the processing conditions are necessary.

4 Discussion

Pineapple fruits, pineapple juices and purees contain significantly higher concentrations of manganese in comparison to other fruits and juices. Clearly, this is not related to contaminations during processing. Several reasons could be responsible for this high concentration, like soil, fertilizers and plant species. Indeed, the pineapple cultivation requires an acid soil that increases the mobility of manganese and its availability by the plant (*Beattie* und *Quoc,* 2000; *Scheffer* et al., 2010). In addition, other factors may also contribute to the accumulation of manganese by the plant, such as period of growth and maturation of the fruit. The influence of pH on manganese mobility in plants is known since a long time (*Sims,* 1986). At a pH below 5.2 elements like zinc and manganese have a higher mobility and will be easier accessible by the plants. Therefore similar problems with higher manganese contents are to be expected with peaty soils and Vaccinium fruits like wild blueberries.

Analyses of fresh fruit revealed that manganese content is significantly higher in the skin of the fruit than in the flesh and the core (Fig. 1). This may indicate a horizontal phenomenon of mineral transfer from the inside to the outside of the fruit. This fact could be partially explained by the mechanism of plant transpiration from the core to the leaves and thus the skin of fruit (*Cote* et al., 1993). However, no significant differences were observed vertically in the fruit from the top to the bottom.

The manganese content in purees is significantly lower than in juices and concentrates (Fig. 2) whereas no significant differences were found between juices and concentrates. Higher manganese content in juice and concentrate can be explained by the possible use of the skin of the fruit during the juice production. The solids left from the canned pineapple production, like part of the skin and the core is crushed by a hammer mill and pressed to a juice (*Hodgson* und *Hodgson,* 1992). These products, beside the whole fruit will be used for the fruit juice production. To produce a good puree it is recommended to use only the pure fruit flesh without peels.

Manganese is regarded as an essential element for humans. There are some indications that it is important for bone health and collagen metabolism and acts against osteoporosis and diabetes mellitus. In nutrients the manganese level is very different. Higher amounts can be found in wheat, cacao, nuts, legumes, some vegetables and fruits, like blackberry, wild blueberries and pineapples. Tea (*Camellia sinensis)* contains up to 900 mg/kg in the tea leaves; a cup of tea contains 1.4–3.6 mg/L manganese.

As discussed in the introduction, with one glass of pineapple juice (0.2 L) with a manganese concentration of 15 mg/L the WHO recommended daily intake of 3.6 mg per day is nearly reached. Nevertheless manganese is not a toxic trace element and the health benefit is frequently discussed (*Kies,* 1987; *Schuchardt* und *Hahn,* 2011). Manganese is an activator and constituent of several enzymes like superoxide dismutases and pyruvate carboxylases, it is discussed in the prevention of chronic diseases like osteoporosis and is important for the normal growth of the body. Manganese plays an important role in the carbohydrate metabolism and might be important to prevent diabetes mellitus (*Walter* et al., 1991). The oral consumption is much less toxic than the exposition with airborne dust over the lung (*Howe* et al., 2004). The oral absorption rate is between 2–20 % and is reduced by elements like iron.

This study shows variations of the manganese concentration in pineapples, purees, juices and concentrates ranging from 1–54 mg/kg. The concentration of manganese might be influenced by the origin which can be seen on the data from the concentrates (see Tab. 1). For further studies it is essential to get detailed information about the raw material, the variety, the growing conditions, the soil, the maturity and the production conditions for purees, juices and concentrates.

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Beeinflussen Thaumatin-ähnliche Proteine im Wein dessen Aromaprofil?

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Zusammenfassung
Ein Thaumatin-ähnliches Protein (TLP) wurde nach Ultrafiltration und FPLC bis zur Homogenität aus einem deutschen Weißwein der Sorte Riesling angereichert. Nach Quantifizierung wurde die ursprüngliche Konzentration dieses Proteins auf etwa 3 mg/L Wein bestimmt. Unter der Annahme einer ähnlichen Süßkraft dieses TLPs wie die vom Süßstoff Thaumatin (E 957) aus *Thaumatococcus danielii*, könnte das einem Zuckergehalt von bis zu 6 g/L entsprechen. Organoleptische Tests mit einem trockenen Wein der Sorte Riesling zeigten, dass zugesetztes käufliches Thaumatin in der hier vorgefundenen Konzentration (3 mg/L) eine deutliche Veränderung des Aromaprofils bewirkte. Der auf diese Weise behandelte Wein zeigte ein harmonischer empfundenes Säureprofil bei gleichzeitiger Zunahme der Fülle im Vergleich zur Kontrolle.

Summary
A thaumatin-like protein (TLP) was isolated from a German white wine of the grape variety Riesling and purified to homogeneity by ultrafiltration and FPLC. The concentration of this protein in the original wine was determined to be approximately 3 mg per liter. Assuming that this TLP from the investigated wine sample is a sweetener with a similar intensity like the intensely sweet protein thaumatin (E 957) from the tropical flowering plant *Thaumatococcus danielii* it may correspond to a sugar content of up to 6 g per liter. Organoleptic tests with a Riesling dry wine showed that a commercial thaumatin caused a clear change of the sensory profile after addition in a similar concentration (3 mg per liter) as found in the investigated wine sample. The wine sample supplemented with commercial thaumatin exhibited a more harmoniously perceived acid profile with a simultaneous increase of the body in comparison to the control.

Abbildungen 1 bis 3 finden Sie unter
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Einleitung

Thaumatin-ähnliche (thaumatin-like, TL) Proteine weisen in ihrer Struktur große Homologien mit dem intensiv süß schmeckenden Thaumatin-Protein aus den Beeren der westafrikanischen Katamfe-Pflanze *Thaumatococcus danielii* auf (*van der Wel* and *Loeve,* 1972). Bei Thaumatin handelt es sich um ein Stoffgemisch aus sechs Proteinen, die als Thaumatin I, II, III, a, b und c bezeichnet werden. Die Varianten bestehen aus jeweils genau 207 Aminosäuren und unterscheiden sich nur geringfügig in ihrer Sequenz (*Iyengar* et al., 1979; *Edens* et al., 1982). Ihr Molekulargewicht beträgt etwa 22,3 kD. Bezogen auf die Masse ist die Süßkraft von Thaumatin etwa 2000- bis 3000-fach stärker als die von Saccharose. Thaumatin wird deshalb auch als Süßstoff verwendet und ist als solcher unter der Bezeichnung E 957 in der EU-Süßungsrichtlinie ausgewiesen (*Roth* and *Lück,* 2012). Für die Funktion als Süßstoff scheinen 5 Lysin-Reste entscheidend zu sein, da die Phosphorylierung der Aminogruppen in deren Seitenketten zur Abnahme der Süße führt (*Kaneko* and *Kitabatake*, 2001).
TL-Proteine kommen in relativ großer Anzahl vor und werden der Gruppe der PR-5 (pathogenesis-related)-Proteine zugeordnet. Diese sind stabil bei niedrigen pH-Werten und weitgehend resistent gegenüber proteolytischen Enzymen. Sie werden von vielen Pflanzen als Abwehrstoffe bei Stress und Infektionen gebildet, häufig besitzen sie eine antifungale Wirkung (*Grenier* et al., 1999). In Most und Wein aus der Traube *Vitis vinifera* wurden sowohl PR-5-

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