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The determination of total germanium in real food samples including Chinese herbal remedies using graphite furnace atomic absorption spectroscopy

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Abstract

This paper outlines the development of a method for the determination of total germanium in foodstuffs utilising graphite furnace atomic absorption. It was found that by varying the drying times interferences could be minimised. Metals including calcium, cobalt, copper, magnesium, nickel, lead and zinc were tested for potential interferences. It was found experimentally that none of the listed metals interfered with this method. The optimal furnace conditions were determined to be; drying for 80 s (85 °C for 30 s, 95 °C for 40 s and 120 °C for 10 s), ashing at 700 °C for 8 s and atomisation at 2600 °C for 3.3 s followed by a tube clean for 2 s at 2800 °C and a lamp current of 5 mA for analysis at 265.2 nm. The method was found to have a linear range of 3.3–125 μg/l with a limit of detection and a characteristic mass of 0.051 and 0.053 ng germanium, respectively. The samples chosen for analysis include vegetables, fruit juices, Chinese herbal remedies and over the counter formulations. It was found that the aloe vera tablet, ginseng tablet and ginger tablet contained 20.83, 5.48 and 9.96 μg/g. Other foods found to contain germanium were potato, garlic and carrot, having 1.85, 2.79 and 0.60 μg/g of germanium. The food found to contain the highest concentration of germanium was Soya mince having 9.39 μg/g.

Keywords: Germanium; Metal; Food; Graphite furnace atomic absorption spectroscopy; Complementary medicine; Herbal remedies; Acid digestion

1. Introduction

In a healthy body cells with cancer potential are continuously formed and destroyed by the bodies own immune system, this is a process known as immunomodulation. Carboxyethylgermanium sesquioxide or Ge-132 exhibits a number of biological activities that have a wide variety of applications in the health care industry (Aso et al., 1985). The immunomodulating activities of Ge-132 have been identified as the production of interferon and the killer T cells and the augmentation of natural killer cells (NK cells) (Montenegro, Bonnia, & Dederen, 1996). It has the overall result of activating the body's own immune system to destroy non-functioning or malfunctioning cells such as tumour cells. Ge-132 exhibits activities other then those immunomodulating ones outlined. It has been shown to have anti-inflammatory, anti-arthritic and photo protective effects (Brutkiewicz & Suzuki, 1987). This work aims to create a direct method for total germanium to identify foodstuffs and over the counter remedies that contain germanium allowing for further extraction of Ge-132.

The intake of micronutrients and antioxidants has been studied with a view to understanding their chemotherapeutic behaviour and their cancer preventative

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properties (Shirai, Asamoto, Takahashi, & Imaida, 2002). Chemotherapeutic behaviour is aimed at destroying an existing tumour while preventative behaviour aims at suppressing the formation of a tumour. A healthy diet provides sustenance and nourishment for growth, development yielding health and well-being (Hambly, Saunders, Rijken, & Rowland, 2002; Bush & Williams, 1999). It has been suggested that the development of tumours is the result of a poor or malfunctioning immune system (Shike, 1999). Studies have reported an inverse relationship between the consumption of green vegetables such as broccoli, cabbage and cauliflower and other vegetables such as garlic, onion, tomatoes and carrots and cancer formation has been well documented (Manson, 2003; Greenwald, Clifford, & Milner, 2001). This tumour suppression has been accounted for by the presence of antioxidants in the vegetables (Ames, 1998). The antioxidants counteract the production of free oxygen radicals as a result of radiation or anaerobic conditions in the body (Stoll, 1998).

Graphite furnace atomic absorption spectroscopy (GFAAS) or electrothermal atomic absorption spectroscopy is a microanalytical technique based on the Beer–Lambert's law used for the analysis of total metals (Butcher & Sneddon, 1998; Salvin, 1968). GFAAS is a widely used technique and can be applied to both solid and liquid samples without complex sample manipulation prior to analysis. The versatility of application from environmental samples to food and drug samples coupled with its low limits of detection make it an ideal instrument for the analysis of a wide variety of samples (Deaker & Maher, 1999; Tan & Marshall, 1997). It has been applied to the analysis of metals in samples such as dogfish to baby food (Lima, Barbosa, & Krug, 2000; Silva, Willie, Sturgeon, Santelli, & Sella, 1999; Skoog, Holler, & Nieman, 1998; Viòas, Pardo-Martınez, & Hernández-Córdoba, 1999).

This work aims to develop a method for the analysis of germanium in foods utilising the drying temperature as a means of reduction of interferences. This optimised method was applied to a diverse range of samples including vegetables, liquid extracts and commercially available tablet formulations. Analytical validation is not possible as certified reference materials for the samples tested are not available.

2. Experimental

2.1. Reagents

All experiments were carried out utilising analytical reagent grade chemicals using deionised water. A 1000 mg l^{-1} stock solution of ICP grade germanium was purchased from Reagacon (Shannon, Ireland). Working solutions were prepared by the appropriate dilution of this standard stock solution.

Concentrated sulphuric acid, nitric acid and hydrochloric acid were used for the digestion of samples (Sigma Aldrich, Ireland).

Table 1
Graphite furnace analytical conditions (time in seconds (s))

Step/time	85 °C	95 °C	120 °C	700 °C	2600 °C	2800 °C
Drying	30 s	40 s	10 s			
Ashing				8 s		
Atomisation					3.3 s	
Tube clean						2 s

2.2. Apparatus

Absorbance was achieved and monitored using a Varian GTA 110 equipped with a programmable sample dispenser (PSD 55) and a coated graphite partition tube (Varian) with wall atomisation. The source of radiation used was a germanium hollow cathode lamp. Table 1 shows the GFAAS furnace analysis conditions.

2.3. Samples

Table 2 depicts the samples analysed and the source for each sample.

2.4. Sample preparation

2.4.1. Fresh samples

For the purpose of this study fruit, vegetables and plants are termed fresh solids. The sample was first washed in deionised water and chopped into small

Table 2
The samples analysed and the sources of each

Sample name/type	Source
Fresh tomato	Supermarket – available widely
Onion	Supermarket – available widely
Green pepper	Supermarket – available widely
Yellow pepper	Supermarket – available widely
Red pepper	Supermarket – available widely
Fresh carrot	Supermarket – available widely
Potato	Supermarket – available widely
Garlic	Supermarket – available widely
Peppermint herb	Clinic for alternative medicine
Dong quai	Clinic for alternative medicine
Echinacea	Clinic for alternative medicine
Ginger tablet	Clinic for alternative medicine
Siberian Ginseng extract	Clinic for alternative medicine
Ginseng	Oceanic Supplies Co. Ltd., Limerick
Bamboo shoot	Oceanic Supplies Co. Ltd., Limerick
Soya flour	Healthfood Store
Tvp soya mince	Healthfood Store
Pearl barley	Healthfood Store
Aloe vera tablet	Healthfood Store
Ginseng tablet	Healthfood Store

pieces. A 5 g quantity of the sample was transferred to a 250 ml round bottom flask fitted with a Liebig condenser. To this apparatus 20 ml of a 10:1 ratio of concentrated nitric and sulphuric acid was added. The digest was allowed to stand for 24 h after which time the solution was heated gently to boiling. The digest was refluxed until all the vapour turned from a brown to a white plume at which point the condenser was removed. The sample volume was allowed to reduce to about 1 ml followed by the addition of 10 ml of deionised water. The sample was reduced to approx. 1 ml, allowed to cool and transferred to a 50 ml volumetric flask.

2.4.2. Dehydrated samples

These include drug formulations and dried foodstuffs such as flour. The sample was ground up to a fine powder. In the case of drug formulations 5 tablets were used in all cases. A 0.1 g weight of the powder was transferred to an evaporating dish and 10 ml of nitric acid was added. The digest was brought to the boil and the volume was reduced to 1–2 ml. To this 1 ml of perchloric acid was added and the digest was reduced to dryness. A 20 ml volume of deionised water was added and the volume was again reduced to almost dryness followed by transfer to a 50 ml volumetric flask.

2.4.3. Liquid samples

This group of samples includes fruit juices. The fruit juice carton was shaken prior to opening and 10 ml of the juice sample was transferred to a glass vile and centrifuged for 40 min. After this time the supernatant was removed and transferred to a 50 ml volumetric flask. The samples were subsequently diluted as required.

3. Results and discussion

This study involved the optimisation of a GFASS method for the determination of germanium in foods. A digestion method was developed for a variety of samples to obtain a consistent matrix, negating the need for complex matching techniques. The optimisation of GFAAS involved the modification of drying times and temperatures and lamp intensity.

3.1. GFAAS drying times and temperature

The drying time is described as the time required for the evaporation of the liquid phase of a sample coating the remaining solid phase onto the inner surface of the tube. If the temperature is too high it results in rapid drying and sputtering of the sample throughout the tube and onto the quartz windows in a random and irreproducible manner. This leads to formation of the atomic cloud in differing region of the tube for subsequent samples, resulting in differing absorption readings for similar concentrations of the metal. It is important to use a temperature that dries the sample but does not cause sputtering. The drying stage can be used to minimise the formation of potential and real interferences, in particular the formation of refractory compounds of the metal. To reduction of errors resulting from sputtering and from the formation of refractory compounds can be achieved by utilising a lower drying temperature for an extended period of time.

The drying temperature used is dictated by the boiling point of the sample matrix, in this case water. The drying times allocated to the stage at 95 °C and at 120 °C were long enough to reduce sputtering and interferences from refractory complex formation and still remain economical. The absorbance and reproducibility of samples were studied for differing times namely 5, 10, 20, 30 and 40 s at 85 °C. The effects of different drying times on the absorbance and %RSD values were studied. It was found that increasing the time has the effect of increasing the absorbance readings and decreasing the relative standard deviation. It has been determined that at drying times of 5 and 10 s the sample absorbance is low and the %RSD is high compared to the results obtained for 20, 30 and 40 s. As a consequence both of these drying times were ruled out for use in the optimised method. Similar responses were achieved after 20, 30 and 40 s in regards to absorbance and %RSD values. To further determine the optimal drying time it was decided to visually inspect the signal output achieved.

Fig. 1A shows that by increasing the drying times causes the formation of a secondary peak after the desired germanium peak. This secondary peak is formed after 20 s drying and increases to give a larger response.

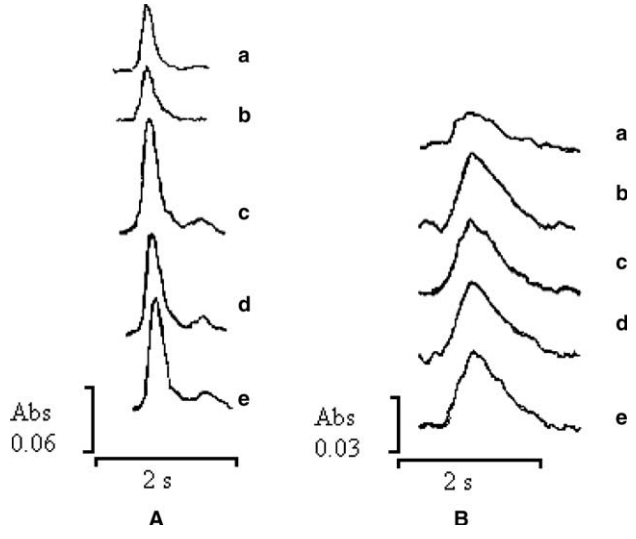

Fig. 1. The responses due to atomisation of the two samples with increasing drying times. The drying times are increasing from top to bottom in the order of (a) 5 s, (b) 10 s, (c) 20 s, (d) 30 s and (e) 40 s. (A) is the response achieved for carrot digest and (B) is the response for Ginseng tablet digest $n = 2$.

The formation of this secondary peak may indicate the formation of a weak refractory compound or may indicate the presence of another metal in the sample (Butcher & Sneddon, 1998). This secondary peak occurs in a region of little importance and has little effect, i.e., a non-read region. The ginseng tablet digest shown in Fig. 1B shows no such secondary peak formation.

The formation of such a secondary peak could lead to potential interferences. Examination of the outputs reveals that the secondary peak formed is further resolved from the main atomic peak after 30 s drying compared with 20 and 40 s (see Fig. 1A). It should also be noted that the secondary peak is less intense after a drying time of 30 s compared to the 20 and 40 s drying times. The combination of these results in conjunction with the absorbance and %RSD achieved at each drying time suggests that a drying temperature of 85 °C for a time of 30 s yields the optimal response (Butcher & Sneddon, 1998).

3.2. Lamp intensity

The intensity of the radiation required to pass through the graphite tube depends on whether the germanium is a high absorbing or weak absorbing species (Skoog et al., 1998). If the intensity is not optimised then maximum absorption of radiation by the atomic cloud will not occur. To this end the absorption of germanium standards in the range 0 to 25 $\mu g\,l^{-1}$ was monitored over five lamp intensities in the range 3–7 mA. It was found that an increase in the lamp intensity from 3 to 5 mA yields an increase in absorption of the germanium standards. It was found also that increasing the lamp intensity above 5 mA has the effect of yielding lower absorbance readings. These results suggest that the lamp intensity yielding maximum absorbance is 5 mA.

3.3. Linearity

The linearity of the method was investigated and the linear range for the optimised method was determined to be 3.37–125 $\mu g\,l^{-1}$, with an R^2 value of 0.9982 ($n = 3$). It was found increasing the germanium concentration above 125 $\mu g\,l^{-1}$ leads to non-adherence of Beers law (Skoog & West, 1996). A positive interference is caused by increasing the germanium concentration above 125 $\mu g\,l^{-1}$. It is not expected that the samples germanium concentration will exceed 125 $\mu g\,l^{-1}$, as a result the linear range of this method is acceptable.

3.4. Limit of detection

The limit of detection was determined to be 3.37 $\mu g\,l^{-1}$ or 0.051 ng germanium based on a 15 μl injection. The limit of detection was determined based on a signal of three times the standard deviation of the blank.

3.5. Limit of quantification

The limit of quantification was determined to be 20 $\mu g\,l^{-1}$ or 0.3 ng germanium based on a 15 μl injection.

3.6. Characteristic mass

The characteristic mass is defined as that mass of element that yields an absorbance of 0.0044 and is calculated (Butcher & Sneddon, 1998). It was found experimentally that the method has a characteristic mass of 0.053 ng germanium.

3.7. Interference study

A number of metals were investigated as possible interferents. These include calcium, cobalt, copper, magnesium, nickel, lead and zinc (Chang, Sung, & Huang, 1999; Denkhaus & Salnikow, 2002; Taylor, Branch, Halls, Owen, & White, 2000).

Two concentrations of each of the seven metals were tested, 1000 and 100 $\mu g\,l^{-1}$. The absorbance and reproducibility of the metal concentration was monitored. It was found that all the metals had low absorbance readings and high %RSD's. The visual inspection of the signal outputs reveal that none of the metals, excluding 1000 $\mu g\,l^{-1}$ magnesium, resulted in a consistent signal output i.e. peak.

The response due to 1000 $\mu g\,l^{-1}$ magnesium, shown in Fig. 2, is similar to the response resulting from 2 $\mu g\,l^{-1}$ germanium with %RSD of 17.1%. It was found that the response due to magnesium occurred after the germanium response in a non-read area. This suggests that magnesium will not interfere with germanium analysis but will appear as a weak peak after the main peak. Reducing the concentration of magnesium to 100 $\mu g\,l^{-1}$ has the effect of eliminating any response. As a result of the interference study it can be concluded that none of the metals tested had the capabilities of being a possible interference.

To minimise all possible interferences a digestion blank was created using the same chemicals and digestion technique as used on the samples. It was found that the blank yielded a response similar to that achieved for water. For the purpose of matrix matching it was assumed that the digested samples had the

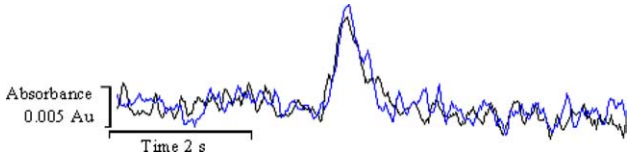

Fig. 2. The signal response achieved when a 1000 $ng\,l^{-1}$ standard of magnesium was run under optimal conditions (drying for 80 s, ashing for 8 s and atomisation for 3.3 s followed by a tube clean for 2 s with a lamp current of 5 mA and analysis at 265.2 nm).

same consistency as water, based on the blank analysis. A certified material has not been used to verify the accuracy of the method, as a suitable sample of known Ge content is not available.

3.8. Sample analysis

Data reliability is maintained through the use of standard additions method for sample analysis. The method outlined previously for the determination of total germanium was applied to the samples listed in Table 3. The type of sample tested included vegetables, herbal remedies, pharmaceutical formulations and Chinese foods. The optimised method was applied with success directly to the digests of this wide variety of samples.

The increase in eastern medicines and herbal treatments has lead to the development of a number of over the counter plant based formulations. It is noticeable that the tablet formulations (aloe vera tablet, ginseng tablet and ginger tablet) have a much higher germanium concentration compared to the fresh samples and required further dilution to allow for accurate analysis. Such high germanium concentrations was expected as tablet formulations, marketed as "one a day", are intended to give a high percentage of the recommended daily allowance of vitamins and minerals.

3.8.1. Herbal remedies

A comparison of some herbal remedies has been conducted including peppermint herb, ginseng tablet, fresh ginseng and bamboo shoot. It was found that the aloe vera tablet contained the highest amount of total germanium having between 20.05 and 21.95 μg g^{-1} (see Fig. 3). Ginger and ginseng tablets contained less germanium having between 6.5 and 12.92 μg g^{-1} and 5.08 and 6.26 μg g^{-1}, respectively.

Table 3
Total germanium content of samples determined by GFAAS under optimal conditions

Sample name	Concentration μg g^{-1}	%RSD
Tomato	0.36	15.16
Onion	0.29	7.64
Green pepper	0.16	6.31
Yellow pepper	0.28	13.03
Red pepper	0.48	15.46
Garlic	2.78	9.27
Carrot	0.60	3.99
Potato	1.85	4.00
Aloe vera tablet	20.83	2.63
Ginseng tablet	5.48	10.77
Ginger tablet	9.96	6.80
Peppermint herb	2.56	32.30
Dong quai	0.43	3.90
Echinacea	5.21	24.70
Siberian Ginseng	0.38	7.00
Ginseng root	0.38	1.40
Bamboo shoot	0.42	2.43
Soya flour	3.64	8.40
Tvp soya mince	9.39	5.15
Pearl barley	1.64	7.10

Three replicates of each sample type each analysed in triplicate to account for natural variance.

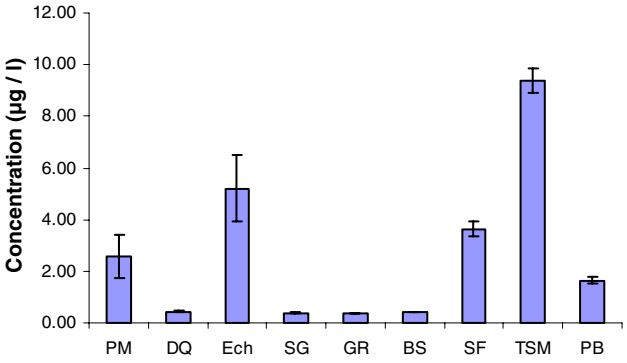

Fig. 3. Comparison of germanium concentration of plants used in Chinese herbal remedies. PM – Peppermint herb, DQ – Dong quai, Ech – Echinacce, SB – Siberian ginseng extract, GR – Ginseng root, BS – Bamboo shoot, SF – Soya flour, TSM – Soya mince and PB – Pearl barley.

3.8.2. Ginseng

The comparison of the germanium content of ginseng tablets, fresh ginseng and Siberian ginseng extract was conducted. It was found that ginseng root contains between 0.35 and 0.41 μg g^{-1} total germanium and the Siberian ginseng extract contains 0.38 μg g^{-1} germanium. The difference between the upper and lower germanium concentration in the ginseng root results from the analysis of different roots. It is interesting to note that the Siberian ginseng extract has a germanium concentration in between the range achieved for the fresh root. This may suggest that the maximum amount of germanium retained within ginseng is within the range.

These concentrations of ginseng root and extract are much lower then those achieved for the tablet formulation. This was to be expected as the tablets are much more concentrated being formed from the dehydrated plant. Only one tablet is required daily whereas 100 g of fresh ginseng could be consumed on a daily basis. Taking this into consideration the amount of germanium in the tablet is almost equivalent to the daily intake of germanium from fresh ginseng. The germanium concentration of bamboo shoot was also conducted and it was found experimentally to contain 0.42–0.43 μg g^{-1} germanium. This concentration of germanium is within a similar range as that achieved for the fresh ginseng and the ginseng extract.

3.8.3. Soya products

It was found that soya mince contained a concentration of germanium similar to the tablet formulations. It was found to contain between 9.35 and 9.45 μg g^{-1} germanium. Soya flour was found to contain between 3.42 and 3.85 μg g^{-1} germanium. Both soya mince and soya

flour are products derived from the soya bean. Other samples to be tested include pearl barley (1.35–1.95 μg g^{-1} germanium), peppermint oil extract (2.56 μg g^{-1} germanium), Echinacea (5.21 μg g^{-1} germanium) and Dong quai (0.43 μg g^{-1} germanium).

The samples chosen have all been reported to yield some therapeutic properties in patients. These properties range from anti-viral to anti-inflammatory and are used in the treatment of a diverse range of ailments such as irritable bowel syndrome (peppermint herb) and the common cold (Kang, Ansbacher, & Hammoud, 2002; Pittler & Ernst, 1998).

3.8.4. Fresh vegetables

The comparison of the average germanium concentration of widely available and commonly used fresh vegetables was conducted, shown in Fig. 4. The comparison of this group has revealed that garlic contains the largest concentration of total germanium having 2.79 μg g^{-1}. Garlic has been reported to have a number of medicinal properties, in particular the ability to boost the immune system. Other foods studied include tomatoes (0.3–0.39 μg g^{-1} germanium), onions (0.27–0.32 μg g^{-1} germanium) and carrots (0.57–0.62 μg g^{-1} germanium). It is interesting to note that potato was found to contain 1.85 μg g^{-1} germanium. The determination of total germanium in potato has not been carried out previously.

3.8.5. Naturally coloured food

The relationship that food colour has on germanium absorption and retention was studied by the comparison of germanium content of three coloured peppers, Fig. 5. It was found the green pepper contained the least amount of germanium (0.15–0.17 μg g^{-1}) and red pepper contained the most (0.43–0.56 μg g^{-1}) with yellow pepper falling in between (0.25–0.32 μg g^{-1} germanium). The pigments contained within the peppers are β-carotenes and the differing colours result from different pigments. It has been reported that green peppers contain lutein, yellow peppers contain zeaxanthin and red peppers contain capsanthin. Upon examination of the structure of these three compounds it is apparent that capsanthin is more polar compared to luetin and zeaxanthin. Zeaxanthin is more polar than luetin, the polarity of the compounds may be the key to germanium absorption and retention. This relationship between β-carotenes and germanium uptake is again suggested by the high concentration of germanium in the carrot samples (Breithaupt, 2000; Hornero-Medez & Minguez-Mosquera, 2001). Excluding the garlic and potato samples it was found that the concentration of germanium in the fresh vegetables was similar to the concentrations of the fresh Chinese samples.

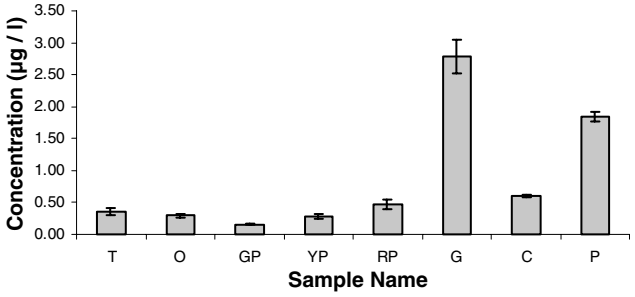

Fig. 4. Comparison of germanium content of the fresh samples using average germanium concentrations. T – Tomato, O – Onion, GP – green pepper, YP – yellow pepper, RP – red pepper, G – garlic, C – carrot and P – potato. All values are calculated using mean values where $n = 6$ except for garlic $n = 4$ and potato $n = 3$.

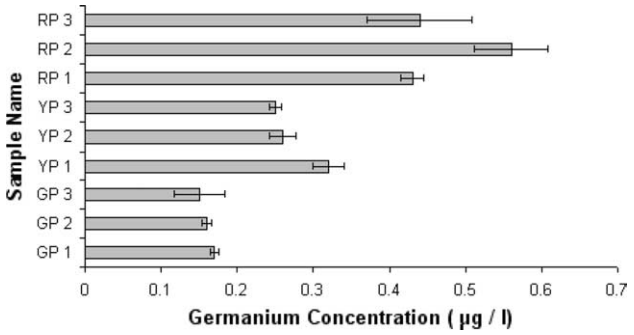

Fig. 5. Comparison of germanium concentration of green pepper (GP), yellow pepper (YP) and red pepper (RP). All values shown are mean values with $n = 2$.

3.8.6. Fruit juices

The germanium content of a number of commercially available fruit juices was examined. Fruit juices are a suspension of fruit particles and juice. The pretreatment process utilised aims to remove the particulate matter from the liquid phase. In doing so the solid particulate is discarded, this means that only the liquid phase is kept. The results obtained for the study show that grapefruit and orange juice contain similar total germanium concentrations. These germanium concentrations are higher then the other two samples. Both oranges and grapefruit are both classed as citrus fruit while apples are classed as malaceous fruits and pineapple classed as miscellaneous fruits. It has been suggested that germanium forms a complex with natural sugars such as fructose and mannitol (Chen, Mou, Yan, & Ni, 1997) such a result may suggest a complexation with natural acids.

4. Conclusion

Given the recent increase in health awareness the content of food and pharmaceuticals is being brought into question. It is necessary to develop methods to allow

the public to make informed decisions regarding foods/pharmaceuticals based on their content. A method for the quantitative determination of germanium in a wide variety of foodstuffs and pharmaceutical formulations was developed. Utilising this method, germanium was found to occur in 24 samples tested. These samples include over the counter tablet formulation of aloe vera, ginseng root, garlic and potato. It was found that the concentration of germanium in the fresh food samples was in a similar range as that of the fresh Chinese vegetables, 0.29–2.78 $\mu g\ g^{-1}$.

Further investigation into the relationship between pigment and germanium retention is being carried out following the comparison of the germanium concentration of three different coloured peppers.

Acknowledgements

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