Amygdalin Content of Seeds, Kernels and Food Products Commercially-available in the UK

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Abstract

Cyanogenic glycosides are a large group of secondary metabolites that are widely distributed in the plant kingdom, including many plants that are commonly consumed by humans. The diverse chemical nature of cyanogenic glycosides means that extraction and analysis of individual compounds can be difficult. In addition, degradation can be rapid under appropriate conditions. Amygdalin is one of the cyanogenic glycosides found, for example, in Apples, Apricots and Almonds. We have developed and applied a high performance liquid chromatographic procedure for amygdalin quantification to investigate extraction efficiency and to determine levels in a range of foods. Our results show that seed from Rosaceae species contained relatively high amounts (range 0.1 – 17.5 mg/g) of amygdalin compared to seed from non-Rosaceae species (range 0.01 - 0.2 mg/g). The amygdalin content of processed fruit products was very low.

Key words: Cyanogenic glycosides, cyanogenic plants, amygdalin, cyanide toxicity.

1. Introduction

Cyanogenic glycosides are plant secondary metabolites which consist of an aglycone and a sugar moiety (Fig. 1). They are widely distributed in the plant kingdom, being present in more than 2500 plant species (Vetter, 2000). Amygdalin can be found in plant families of the Caprifoliaceae, Mimosaceaw, Oleaceae and Rosaceae; linamarin and lotaustralin are found in the Compositae, Euphorbiacea, Linaceae and Leguminosae; prunasin is found in Polypodiaceae and Rosaceae; and dhurrin is found in the Poaceae family (Vetter, 2000). An important characteristic of cyanogenic plants is the ability to generate toxic hydrogen cyanide. Although cyanogenic glycosides are not toxic when intact, they become toxic when plant enzymes (β-glucosidases and α-hydroxynitrile lyases) come into contact with the cyanogenic glycosides in the plant as a result of tissue damage after bruising or chewing. Enzyme activity results in cleavage of the carbohydrate moiety of the cyanogenic glycoside to yield corresponding cyanohydrins which further decompose to release hydrogen cyanide and an aldehyde or ketone (Poulton, 1990). Following consumption by animals including humans, hydrogen cyanide may also be generated by the action of enzymes from the gut microflora on ingested intact cyanogenic glycosides (Carter, McLafferty, & Goldman, 1980). Several economically important plant foods are highly cyanogenic, among which are cassava, lima beans, butter beans, almond, sorghum, macadamia nut, flax and white clover (Vetter, 2000; Donald, 2009).

Acute cyanide toxicity can occur in humans at doses between 0.5-3.5 mg/kg body weight (Speijers, 1993). Consumption of cyanogenic plants, such as cassava root, almond or apricot kernels, has been reported to cause both acute and sub-acute health problems (depending on dose) such as headache, nausea, vomiting, abdominal cramps, dizziness, weakness, mental confusion, convulsions, cardiac arrest, circulatory and

respiratory failure, coma and in extreme cases death (as reviewed by Geller, Barthold, Saier, & Hall, 2006).

In order to prevent cyanide toxicity, processing procedures such as peeling, crushing, grinding, grating, soaking, fermenting and drying have been used for centuries to reduce potential for toxicity before consumption. The aim of these procedures is to reduce toxicity, either through the loss of water-soluble glycosides, or the maximal production of hydrogen cyanide by the action of plant or microbial enzymes and loss of hydrogen cyanide to the atmosphere before consumption.

Amygdalin (D-mandelonitrile-β-D-gentiobioside) is a cyanogenic glycoside present in kernels and seeds of fruits such as apples, apricots, almonds, cherries, plums and peaches (Donald, 2009). It is one of the most common cyanogenic glycosides. Degradation of amygdalin by enzyme can lead to the production of cyanide when nuts or seeds are macerated or crushed. Enzymatic degradation of amygdalin is divided into three parts. The first part involves the splitting of amygdalin to prunasin and glucose by the enzyme amygdalin lyase. The second part is the hydrolysis of prunasin to mandelonitrile and glucose by the enzyme prunasin lyase and the final stage of the hydrolysis is the breaking down of mandelonitrile to benzaldehyde and hydrogen cyanide (HCN) by hydroxynitrile lyase (Haisman & Knight, 1967). Enzymatic hydrolysis of amygdalin to mandelonitrile usually takes place in slightly acidic condition (pH 5.0-5.8) while the hydrolysis of mandelonitrile to benzaldehyde and HCN proceeds rapidly in alkaline solution (pH 10). The enzymes usually act at temperature of about 20-40°C and can be destroyed at higher temperature. Enzymatic hydrolysis of amygdalin in plant foods takes place within 30 min to 6 hr depending on the degree of maceration of the food sample (Tunçel, Nout & Brimer, 1995).

In addition to enzymatic degradation, amygdalin degradation can also occur in hot aqueous solution through the process of isomerisation. According to Hwang, Lee,

Lee, & Hong (2002), D-amygdalin can be converted to neoamygdalin (an epimer of amygdalin) after 3 min of heating in boiling water. The products of amygdalin degradation (either through enzymatic or isomerisation process) can be measured by reverse-phase HPLC method (Wasserkrug & Rassi, 1997; Hwang *et al.*, 2002; Koo, Hwang, Cho, Lee, Lee, & Hong, 2005).

Extraction of amygdalin from food plants is a crucial aspect of any analytical procedure due to its potential for rapid degradation. An efficient extraction process would lead to a complete detection of amygdalin without losses or degradation. Koo *et al.* (2005) reported that the use of 4% citric acid solution for extraction of amygdalin in armeniacae semen (a seed of *Prunus armeniaca* Linne, from the Rosaceae family) prevented the conversion of D-amygdalin into neoamygdalin and thus resulted in increased extraction efficiency. Similarly, Wei-Feng, Ding, & Zheng (2005) compared the use of ultrasound, soxhlet extraction with methanol, and reflux extraction with citric acid for extraction of amygdalin from apricot kernels and prunus tomentosa thumb (a traditional Chinese herb medicine). The results showed that reflux extraction with water containing 0.1% citric acid was the best option.

Many of the studies on efficiency of amygdalin extraction were based on the use of water or methanol. Quantification was mostly carried out in almond, apricot and Chinese herbal medicines which contain amygdalin as a major ingredient. There have been few papers on the determination of amygdalin in kernels of other fruits (Voldrich, 1992; Haque & Bradbury, (2002), Viorica-Mirela, Socaciu, Jianu, Florica, & Florinela, 2006). There have been no reports on the amygdalin contents of retail food products. We have optimised the extraction of amygdalin and used a modified RP-HPLC method to measure the amount of amygdalin for the first time in some fruit seeds and processed products available in the UK.

2. Materials and Methods

2.1. Food *samples*

All fruits and processed products used in this study were purchased in Leeds (UK). The following processed products were purchased from local supermarkets: Dole Apple Puree (produced in France), Sainsbury's own-brand Toasted Almond Kernels (produced in USA, packaged in UK by Sainsbury's), EcoMil Almond Milk (7% almond) produced in Australia, EcoMil Almond Cocoa Dessert (8% almond) produced in Spain, Tesco own-brand Almond Flour (produced in USA, packaged in UK by Tesco), Morrison's own-brand White Marzipan (25% almond) produced in UK, Morrison's own-brand Apricot Slices in Juice (produced in UK), Ainsley Harriott Apricot & Honey Cereal Bar (9% apricot) produced in UK, Morrison's own-brand Prune Slices in Juice (produced in UK), Morrison's own-brand Peach Slices in Juice (produced in UK), Toasted Pumpkin Seed (produced in China), California Garden Peach Drink (8% peach dices, 30% peach juice), UHT apple juice (Sainsbury's own-brand Apple Juice (produced in UK), Just Juice Apple Juice (produced in UK) and Premium Apple Juice from concentrate (produced in Egypt), Bramley Pressed Apple Juice (produced in UK), Morrison's own-brand Pressed Apple & Beetroot juice (45% apple juice, 40% apple puree and 15% beetroot juice; produced in UK), and Morrison's own-brand Pure fruit smoothie (47% pressed apples, 16% kiwi fruit, 1.2% lime; produced in UK), Cider (Stella Artois Cider (produced in EU) and Henry Westons Cider (produced in UK). Three packs from different batches were purchased for each brand. All processed products were stored at 4 ^{o}C after purchase prior to extraction.

2.1.1. Fresh fruits

Apricot (from Spain), Plums (Green Plum was from UK, Black Plum from Chile, Purple, Yellow and Red Plums were from South Africa), Peach (from Argentina), Pear

(from Holland), Nectarine (from Chile), Apple (from Brazil), Cherry (Black cherry from UK, Red Cherry from Turkey), Courgette (from Spain), Cucumber (from Spain), Squashes (from UK), Melon (from UK) and Marrow (from Egypt). Three packs of each fruits were purchased from different batches. The fruits were stored at 4 ^{o}C immediately after purchase prior to processing. The seeds of cucumber, courgette, melon, marrow and squash were separated from other tissues with a knife and extracted immediately. The stones from fruits were removed, dried in an oven (37 ^{o} ± 2 ^{o}C) for 3 hours after which individual stones were broken to obtain the intact seeds. The seeds were kept dry overnight in an airtight container and stored at room temperature until extraction.

2.2. Reagents and standards

Amygdalin, ethanol, diethyl ether, and HPLC-grade methanol were all purchased from Sigma-Aldrich (Dorset, UK). Water was prepared using a Millipore Milli-Q purification system. All other reagents were of analytical grade.

2.3. HPLC procedures

A Shimadzu HPLC consisting of a 20ADXR pump, SIL-20ACXR autosampler, degasser, and SPD-M20A diode array detector set at 214 nm was used. The column was a Phenomenex C18, Type Nucleosile 3, 120 A (150 mm x 4.60 mm, 3 µm) placed in a column oven set at 40 ^{o}C. The mobile phase consisted of methanol and water (25:75, v:v) and the flow rate was 1 ml/min. The mobile phase was sonicated (20 min, 22 ^{o} ± 2 ^{o}C) to remove gas bubbles before use. The injection volume was 5 µl.

2.4. Amygdalin calibration curve

Amygdalin standard was dissolved in water to obtain a stock solution of 100 µg/ml, which was stored at –20 ^{o}C until analysed. A calibration curve was constructed using six standard solutions containing 1, 5, 10, 20, 40, and 50 µg/ml of amygdalin.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated in accordance with ICH (1995); LOD was calculated based on the standard deviation (SD) of the lowest standard analysed as a sample and the slope (S) of the calibration curve; as SD multiplied by 3.3 divided by S, whereas LOQ was calculated as standard deviation multiplied by 10 divided by slope.

2.5. Extraction of amygdalin from almond kernels

2.5.1. Water extraction at 37 ^{o}C

Almond kernel (5 g) was ground in a blender (20 sec; Moulinex Optiblend 2000, France) and 2 g was weighed into a conical flask (200 ml). Water (50 ml) was added, and the flask was placed in a shaking water bath (37 ^{o}C). Amygdalin extractions were carried out for 40, 80, 100, 120 and 180 min. The extracts were filtered (Whatman No. 1 filter paper) and transferred into plastic polypropylene tubes (50 ml). Fat was extracted three times by vortexing (1 min) with *n*-hexane (20 ml). Tubes were centrifuged (10 min, 3,250 x g, using eppendorf 5810R bench top centrifuge). Supernatants were pooled and discarded. Hexane residues were evaporated from the sample with a rotary evaporator (low BP, 35 ^{o}C, 7mbar) and the samples were prepared for HPLC analysis (2.5.5).

2.5.2. Water extraction at 100 ^{o}C

Almond kernels (5 g) were ground as above, and 2 g was weighed into a round bottom flask (500 ml). Water (50 ml) was added, and the mixtures were boiled under reflux for 40, 80, 100, 120 and 180 min. The extracts were filtered and de-fatted as for the water extraction (2.5.1) and prepared for HPLC analysis (2.5.5).

2.5.3. Ethanol extraction at 37 ^{o}C

Almond kernels (5 g) were ground as described above, and 2 g was weighed into a conical flask (200 ml). Ethanol (50 ml) was added, and extractions were carried out in shaking water bath (37 ^{o}C) as for the water extraction (2.5.1). The extracts were filtered as for the water extraction (2.5.1) and ethanol was completely evaporated from the filtrate with a rotary evaporator (low BP, 35 ^{o}C, 7 mbar). Diethyl ether (10 ml) was added to the dried sample and the mixture was vortexed (1 min) at room temperature (20 ^{o} ± 2 ^{o}C) to precipitate amygdalin. The diethyl ether was allowed to evaporate overnight in a fume hood and the precipitated amygdalin was dissolved in water (5 ml) and prepared for HPLC analysis (2.5.5).

2.5.4. Ethanol extraction at 78.5 ^{o}C

The method used for amygdalin extraction in boiling ethanol was as described in Miller, Vandome & McBrewster (2010) with some modifications. Almond kernels (5 g) were ground as above, and 2 g was weighed into a round-bottom flask (500 ml). Ethanol (50 ml) was added, and the mixtures were boiled under reflux for 40, 80, 100, 120 and 180 min. The extracts were treated as for the ethanol extraction above (2.5.3) and prepared for HPLC analysis (2.5.5).

2.5.5. Preparation of extracts for HPLC analysis

Aliquots of the samples were dispensed into eppendorf tubes (1.5 ml), centrifuged (10 min, 22 ^{o}C, 15, 996 x g, using eppendorf 5415C microcentrifuge) and filtered with 0.45 µm PTFE filters (Chromacol, UK).

2.6. Extraction of amygdalin from seeds and food products

The method for extraction with ethanol (2.5.4) was used for all the other fruit kernels, seeds and processed products, apart from the grinding stage which was not included in the procedure for fruits with soft seeds (Courgette, Cucumber, Marrow and Squashes) and processed products. In these cases, the samples (2 g for solid and 10 ml for liquid samples) were extracted directly. Extractions of all the samples were carried out in triplicate.

3. Results and discussion

3.1. Separation of amygdalin by reversed–phase HPLC-UV

HPLC determination of amygdalin from fruit kernels and food products requires the establishment of a good mobile phase with the right dilution ratio. Methanol is a good mobile phase for amygdalin separation by HPLC (Wei- Feng *et al.,* 2005). Having considered this factor, methanol and water were used at a ratio of 25:75, (v:v) in an isocratic elution method. Methanol and water in a ratio of 15:85, (v:v) in a gradient elution method was reported to completely separate amygdalin from apricot and *Prunus tomentosa* thunb within 50 min (Wei-Feng *et al.,* 2005). Our method gave a good separation chromatogram within 15 min with an excellent linearity (correlation R^2 = 0.9998) between the peak area and the concentration of amygdalin (Fig. 2). Amygdalin in extracts of fruit kernels was also clearly separated (Fig. 2). The LOD was 0.1 μg/ml and the LOQ was 0.3 μg/ml. The LOD of amygdalin obtained in this study was lower than that obtained from a micellar electrokinetic chromatography method (2 μg/ml; Kang, Jung, Kim, Shin, & Chung, 2000).

3.2. Optimization of amygdalin extraction from almonds

Generally, the extraction yield of amygdalin from almond kernels with all 4 procedures tested increased with time up to 100 min. After 100 min, there was a decrease in the yield at 120 and 180 min for water extraction at 37 ^{o}C, water extraction at 100 ^{o}C, ethanol extraction at 78.5 ^{o}C, and a constant yield at 100, 120 and 180 min in the case of ethanol at 37 ^{o}C (Fig. 4). The results are an indication that the optimum extraction time for amygdalin with water and ethanol at 37 ^{o}C, and for reflux extraction with water (100 ^{o}C) and ethanol (78.5 ^{o}C) is probably 100 min.

3.2.1. Water extraction at 37 ^{o}C

The maximum extraction yield of amygdalin from almond kernels with water at 37 ^{o}C was obtained after 100 min, followed by a decrease in yield after 120 and 180 min (Fig. 4). Short time extractions at low temperatures resulted in low amygdalin extraction yield (Fig. 4). The amygdalin yield (1.4 mg/100g) obtained from almond with water extraction at 37 ^{o}C for 100mins was lower than that obtained with water extraction at 100 ^{o}C (6.8 mg/100g), ethanol extraction at 37 ^{o}C (2.2 mg/100g) and ethanol at 78.5 ^{o}C (11.9 mg/100g). The decreased yield of amygdalin obtained with water extraction at 37 ^{o}C at 120 and 180 mins could be as a result of losses due to enzyme hydrolysis. Longer incubation times at lower temperatures may have an activating effect on enzymes.

3.2.2. *Water extraction at 100 ^{o}C*

Extraction efficiency of amygdalin from almond kernels in boiling water increased with time and the highest yield was obtained after 100 min, after which a decrease in yield was evidenced (Fig. 4). Boiling water extraction yield (6.8 mg/100g) of amygdalin from almond kernels at 100 min was higher than with water at 37 ^{o}C (1.4 mg/100g) and ethanol at 37 ^{o}C (2.2 mg/100g), but lower than with boiling ethanol (11.9 mg/100g). An

increase in extraction yield of amygdalin at 100 min could be because of the inactivation of enzymes at the boiling temperature (100 ^{o}C), preventing enzymatic degradation of amygdalin and hence resulting in an increased extraction efficiency compared to extracting at 37 ^{o}C. Although there appeared to be no enzymatic degradation when extracting in boiling water, amygdalin can be converted to neoamygdalin at high temperatures (Koo *et al.*, 2005) such as at 100 ^{o}C with long extraction times. Conversion to neoamygdalin could be responsible for the low amygdalin yields observed after 100 min extractions at the higher temperatures. Efficiency of amygdalin extraction with water mainly depends on the water temperature and the extraction time. In addition, water extracts were cloudy as opposed to the clear/transparent extracts obtained with ethanol. Protein precipitation during water extraction was probably responsible for the cloudiness of the water extracts. The longer the extraction times, the more cloudy were the water extracts. Cloudiness could result in column blocking during hplc analysis.

3.2.3. Ethanol extraction at 37 ^{o}C

Extraction of amygdalin from almond kernels with ethanol at 37 ^{o}C followed a slightly different trend from water extraction at 37 ^{o}C, water extraction at 100 ^{o}C and ethanol extraction at 78.5 ^{o}C. Amygdalin yield increased with extraction time until 100 min, after which there was no increase in the yield (Fig. 4). Extraction with ethanol at 37 ^{o}C produced a higher yield (2.2 mg/100g) of amygdalin from almond at 100 min than with water at 37 ^{o}C (1.4 mg/100g). However, the 100 min extraction yield of amygdalin in ethanol at 37 ^{o}C (2.2 mg/100g) was lower than with water at 100 ^{o}C (6.8 mg/100g) and with ethanol at 78.5 ^{o}C (11.9 mg/100g). The decrease in the yield of amygdalin extracted from almond kernels with ethanol extraction at 37 ^{o}C for 100 min when

compared with water at 100 ^{o}C and ethanol at 78.5 ^{o}C could be as a result of reduced solubility of amygdalin in ethanol at 37 ^{o}C.

3.2.4. Ethanol extraction at 78.5 ^{o}C

The highest extraction yields of amygdalin from almond kernels were obtained by refluxing with ethanol (Fig. 4). Extraction yields at 100 min (11.9 mg/100g) were higher than those observed with boiling water extraction (6.8 mg/100g), water at 37 ^{o}C (1.4 mg/100g) and ethanol (2.2 mg/100g) at 37 ^{o}C. Ethanol extraction at 78 .5 ^{o}C was, therefore, considered to be a better method for the longer boiling times required for total amygdalin extraction. Consequently, ethanol under reflux was used for the extraction of amygdalin from fruit kernels, seeds and products.

3.3. Amygdalin contents of seeds and processed products

3.3.1. Amygdalin contents of seeds from Rosaceae species

Amygdalin content varied significantly between different Rosaceae species (Table 1). Green Plum had the highest mean amygdalin content (17.5 mg/g) followed by Apricot (14.4 mg/g), Black Plum (10 mg/g), Peach (6.8 mg/g), Red Cherry (3.9 mg/g) and Black Cherry (2.7 mg/g). Amygdalin contents of Purple, Yellow and Red Plums were 2.16, 1.54 and 0.44 mg/g respectively. Nectarine had the lowest amygdalin content (0.1 mg/g) among the stone fruit seeds analysed. The amygdalin content of stone fruit seeds could produce cyanide in the range of 0.01 - 1.1 mg cyanide equivalents/g; this value is relatively high. Thus, ingestion of the kernels either intentionally or accidentally could result in acute or sub-acute health problems (Akyildiz, Kurtoğlu, Kondolot, & Tunç, 2010; Cigolini, Ricci, Zannoni, Codogni, Luca, Perfetti, & Giampaolo, 2011) especially if taking place over prolong periods of time.

Amygdalin content of Apricot in the present study is comparable to the values reported for sweet varieties of Apricot (6.0 – 15.8 mg/g) and lower than the average values (55 - 55.6 mg/g) reported for bitter Apricot varieties (Fermenia, Rossello, Mulet, & Canellas, 1995; Gómez *et al.*, 1998; Yildirim & Askin, 2010). Haque & Bradbury (2002) also reported amygdalin contents of 13.5 mg/g for Apricot kernel. Amygdalin is most abundant in Plum seeds, especially the Green and Black Plums; amygdalin content of Plum seems highly dependent on variety (Table 1). The values reported in this study was similar to the amygdalin content of Plum kernels (12.7 mg/g) reported by Haque & Bradbury (2002). However, a previous study of cyanogenesis in canned stoned fruit (Voldrich & Kyzlink, 1992) reported the amygdalin content of Plum kernels (variety unknown) to be 2.6 mg/g, although this value is similar to the value reported for Purple Plum here, it is lower than the values for Green, Black, Yellow and Red Plums. The Plums used in the 1992 study were of unknown cultivar and the quantification method was based on HCN estimation, which does not usually give an accurate measure of amygdalin due to under-estimation as a result of rapid enzymatic hydrolysis which is difficult to control. Voldrich & Kyzlink, (1992) also reported amygdalin contents of 0.9, 10.2, 4.9 and 11.1 mg/g for Sunhaven, Sunflower, Starking Delicious and Redhaven Peach cultivars respectively. An amygdalin content of 14.7 mg/g and 12 mg/g was also reported for Peach by Sayre (1964) and Haque & Bradbury (2002). These values are comparable to the value reported for Peach here. In contrast, Holzbecher *et al*. (1984) reported a very high (43.3 mg/g) and Viorica-Mirela *et al.* (2006) reported a low (0.02 mg/g) amygdalin value for Peach. The variations in the amygdalin content of Peach could be due to variations in the varieties analysed or environmental factors during fruit formation and cultivation practices. Morello Cherry has been reported to contain 65 mg/g amygdalin (Voldrich & Kyzlink, 1992); this value is higher than the value reported for Red and Black Cherry in Table 1. Amygdalin content of the pome fruit

(apple and pear) seeds analysed in this study were 3.0 mg/g and 1.3 mg/g respectively (Table 1).

3.3.2. Amygdalin contents of non-Rosaceae seeds

Amygdalin contents of seeds from Courgette, Melon, Cucumber and Marrow were 0.21, 0.12, 0.07 and 0.06 mg/g respectively (Table 2). Among the squashes analysed, Crown Prince had the highest (0.11 mg/g) amygdalin content, followed by Acorn (0.07 mg/g) and Red Kabocha (0.07 mg/g). Butternut squash contained the lowest amygdalin content at 0.01 mg/g. The amygdalin content detected in the non-Rosaceae fruit seeds analyzed could liberate between 0.001 mg/g to 0.2 mg/g equivalent cyanide; this value is not negligible.

Cyanogenic plants are eaten in different ways; some are often consumed without their seeds (apples, pears) and some are usually consumed with their seeds (courgettes, cucumbers) as the seeds of some food plants are not removed at all during processing. Failure to minimise exposure to amygdalin (and, consequently, hydrogen cyanide) could result in acute and sub-acute health problems to consumers (Shragg, Albertson, & Fisher, 1982; Akyildiz *et al.*, 2010).

3.3.3. Amygdalin contents of processed products

The amygdalin content of various processed products is shown in Table 3. Among the commercially-available apple products analysed, Bramley pressed apple juice had the highest amygdalin content at 0.09 mg/g, followed by pressed Apple and Beetroot juice (0.02 mg/g), Apple Puree (0.02 mg/g) and Fruit Smoothie (0.01 mg/g). UHT apple juice contained the lowest amygdalin content at 0.004 mg/g. Amygdalin was not detected in Cider. In UHT Apple juice and in Cider, amygdalin levels were probably low because of enzymatic degradation and loss during processing. The levels of

amygdalin detected in Peach slices in juice, Apricot slices in juice, Peach drink and Prune slices in juice were 0.06, 0.05, 0.04 and 0.03 mg/g respectively. The presence of amygdalin in Peach drink, Apricot, Prune and Peach slices in juice could be as a result of high amygdalin contents in Apricot and Peach kernels. Fruits with higher concentrations of glycosides in their seeds have been reported to contain higher amounts in their pulp (Voldrich & Kyzlink, 1992). In contrast to this view, Swain, Li, & Poulton (1992) reported the flesh of rosaceous fruits (Apricots, Black Cherry, Peaches, Apple, Pear and Plum) to be acyanogenic. Amygdalin was not detected in Apricot & Honey Cereal Bar probably because the bar contained very low amounts of apricot fruit (see 2.1). Low levels of amygdalin in toasted Pumpkin were low probably because of loss during processing. Amygdalin contents of toasted Almond, Almond Milk, Almond Cocoa Dessert, Almond Flour and Marzipan were 0.12, 0.05, 0.04, 0.03, and 0.02 mg/g respectively. Amygdalin contents of sweet and bitter Almonds have been reported to range from (0 - 11.6 mg/g) and (0.3 – 68.5 mg/g) depending on the cultivar (Berenger-Navarro *et al.*, 2002; Dicenta, Martínez-Gómez, Grané, Martín, & León, 2002; Yildirim *et al.*, 2010). The amygdalin content of toasted Almond (0.12 mg/g) reported here is within the range (0 – 1.7 mg/g) previously reported for sweet Almonds (Dicenta *et al.*, 2002).

In general, the amygdalin content of processed products was lower than that observed in fruit seeds and kernels. Processing normally eliminates, through a number of routes, the potential for toxicity. Although the levels of amygdalin (0.004 – 0.12 mg/g) detected in processed products are not likely to give rise to any toxicity concerns, they are not negligible, and it would be as well to monitor levels periodically.

4. Conclusions

The amygdalin content of different fruit kernels, seeds and processed products was determined following their extraction with ethanol. The results obtained showed that products in the UK market are of low amygdalin content and would unlikely to cause any health problems if consumed normally.

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Figure Legend

Figure 1: Chemical structure for the cyanogenic glycosides

(a) A generic structure for the cyanogenic glycosides, where R is variable

(b) Prunasin, found in cherry and peach.

(c) Amygdalin, found in the kernels of apple, apricot, peach, almond, cherry, and plum.

(d) Dhurrin, found in sorghum.

(e) Linamarin, found in cassava, lima beans and flax seeds.

Figure 2: Reversed-phase HPLC of amygdalin. For details of the procedures used, see the text.

(a) amygdalin standard.

(b) ethanol extract of almond kernels after refluxing at 78.5^{0}C.

(c) The amygdalin calibration curve for peak area against concentration of amygdalin

Figure 3: Extraction yield (mg/100g) of amygdalin from almonds using water at (37 ^{o}C and 100 ^{o}C) and ethanol at (37 ^{o}C and 78.5 ^{o}C). Results are ± SD for 3 extractions.

Table 1

Amygdalin content of seeds from Rosaceae species

Fruit Seeds	Amygdalin Content (mg/g)
Apricot	14.37 ± 0.28
Cherry (Black)	2.68 ± 0.02
Cherry (Red)	3.89 ± 0.31
Nectarine (Summer Fire)	0.12 ± 0.01
Peach	6.81 ± 0.02
Plum (Green)	17.49 ± 0.26
Plum (Black; Friar Black)	10.00 ± 0.14
Plum (Purple; Larry Anne)	2.16 ± 0.02
Plum (Yellow; Son Gold)	1.54 ± 0.02
Plum (Red; Laetitia)	0.44 ± 0.04
Apple (Royal Gala)	2.96 ± 0.02
Pear (Conference)	1.29 ± 0.04

Each value is expressed as mean ± standard deviation (n = 3 extractions)

Table 2

Amygdalin content of non-Rosaceae seeds

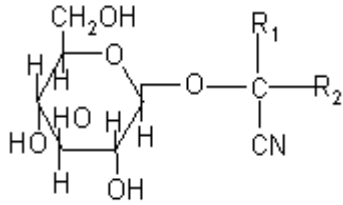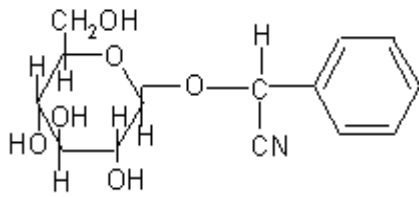| Fruit Seeds	Amygdalin Content (mg/g)
Courgette	0.21 ± 0.13
Cucumber	0.07 ± 0.02
Marrow	0.06 ± 0.01
Melon (Honey Dew)	0.12 ± 0.07
Squash (Crown Prince)	0.11 ± 0.22
Squash (Acorn)	0.07 ± 0.03
Squash (Red Kabocha)	0.07 ± 0.11
Squash (Butternut)	0.01 ± 0.04

Each value is expressed as mean ± standard deviation (n = 3 extractions)

Table 3
Amygdalin content of processed products

Processed Products	Amygdalin content (mg/g)
Almond (Toasted)	0.12 ± 0.06
Almond Milk	0.05 ± 0.01
Almond Cocoa Dessert	0.04 ± 0.02
Almond Flour	0.03 ± 0.01
Apple Juice (100% pressed Bramley)	0.09 ± 0.03
Apple & Beetroot Juice (pressed apple)	0.02 ± 0.02
Apple Juice UHT (3 brands)	0.004 ± 0.01
Apple Puree	0.02 ± 0.01
Apricot & Honey Cereal Bar	nd
Apricot Slices tinned in Juice	0.05 ± 0.07
Cider (2 brands)	nd
Fruit Smoothie (pasteurized)	0.01 ± 0.02
Peach Drink	0.04 ± 0.05
Peach Slices tinned in Juice	0.06 ± 0.01
Prune Slices tinned in Juice	0.03 ± 0.03
Pumpkin (Toasted)	nd
Marzipan	0.02 ± 0.01

Each value is expressed as mean ± standard deviation (n = 3 extractions). nd - not detected.
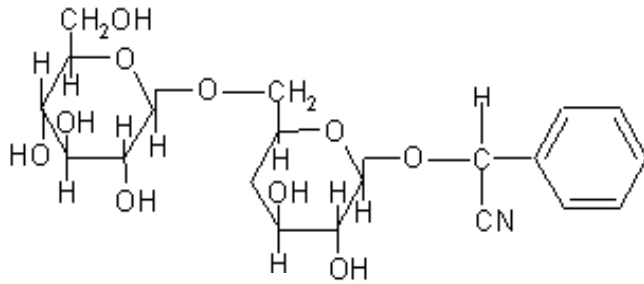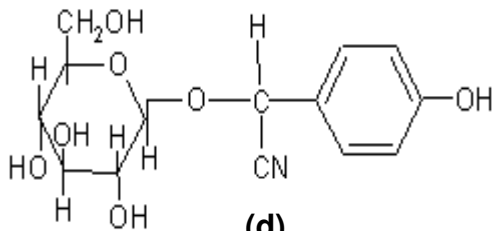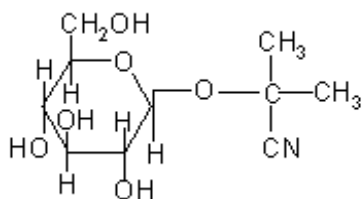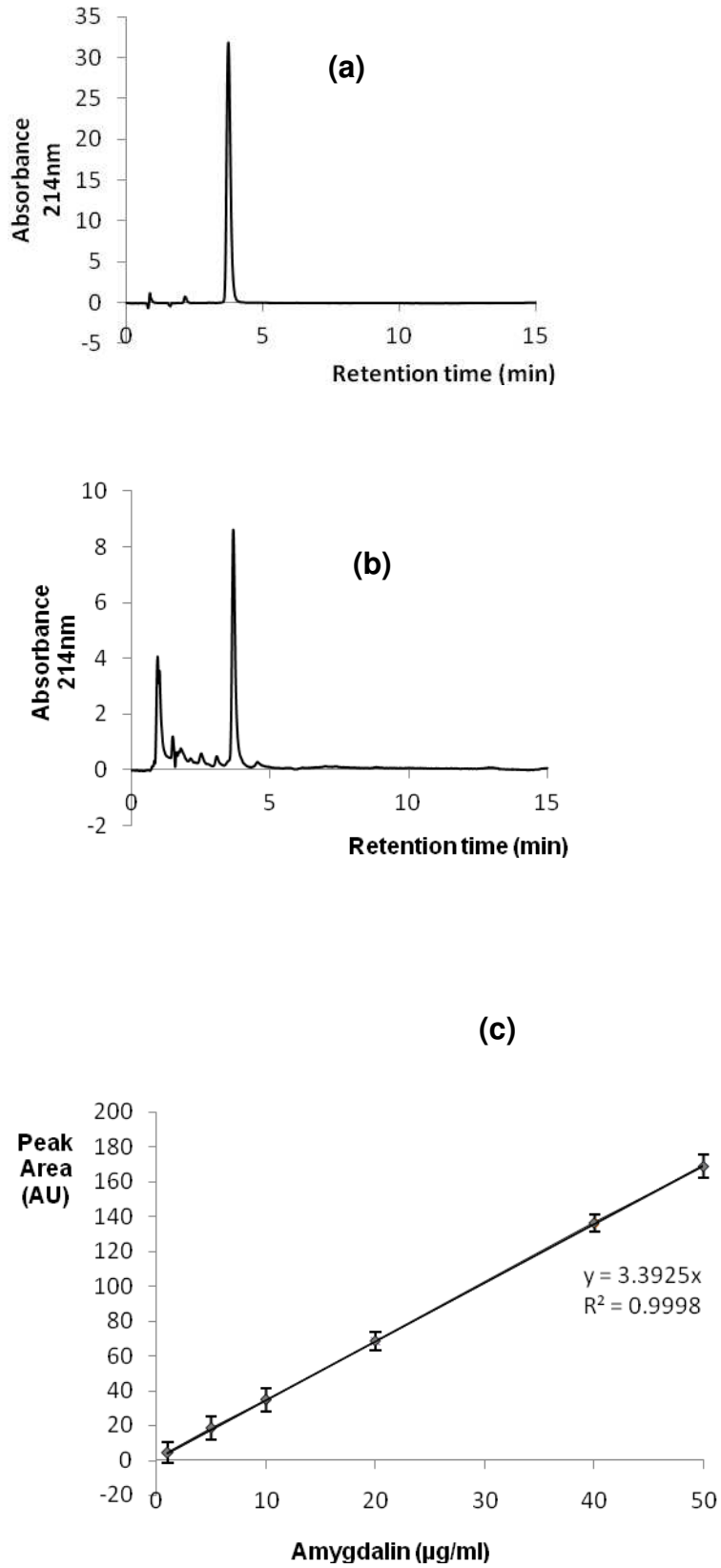
Figure 1

(a)

(b)

(c)

(d)

(e)

Figure 2

(a)

(b)

(c)

Figure 3