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Determination of amygdalin in apple seeds, fresh apples and processed apple juices

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ABSTRACT

Cyanogenic glycosides are natural plant toxicants. Action by endogenous plant enzymes can release hydrogen cyanide causing potential toxicity issues for animals including humans. We have quantified amygdalin in seeds from different apple varieties, determined the effects of processing on the amygdalin content of apple juice and quantified amygdalin in commercially-available apple juices. Amygdalin contents of seeds from fifteen varieties of apples ranged from 1 mg g^{-1} to 4 mg g^{-1}. The amygdalin content of commercially-available apple juice was low, ranging from 0.01 to 0.04 mg ml^{-1} for pressed apple juice and 0.001–0.007 mg ml^{-1} for long-life apple juice. Processing led to juice with low amygdalin content, ranging from 0.01 mg ml^{-1} to 0.08 mg ml^{-1}. The results presented show that the amygdalin contents of commercially-available apple juices are unlikely to present health problems to consumers.

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1. Introduction

Apple (*Malus domestica*) is a member of the *Rosaceae* family that also includes apricots, cherry, peaches, pear and plum and is the most widely consumed fruit in the UK. World apple production in 2011/12 was estimated to be 65.23 million metric tons, out of which 12.2 million metric tons was used for the production of apple juice (Negro & Lojo, 2011). Although apple contains compounds which may confer significant health benefits to humans, apple seeds contain amygdalin (Fig. 1), a potentially toxigenic compound.

Commercial apple juice is usually made from a blend of apples to produce an acceptable juice in terms of flavour. Apples are soaked in water to remove soil and other foreign material. The cleaned apples are then inspected, and damaged or decayed fruit should be removed to avoid patulin contamination from the final product. The sorted apples are ground into mash or pulp for extraction, crushing or cutting the apples to appropriate consistency. The mashed apples are pressed by applying pressure to obtain the juice. In some cases, enzymatic mash treatment is used to improve the pressability of the mash and increase juice yield, achieved by adding a pectinase enzyme such as pectinol specifically developed for apple mash pre-treatment. The enzyme acts mainly by breaking down the structure of the cell wall, thus freeing the juice.

Cyanogenic glycosides, including amygdalin, are naturally-occurring plant toxins. They are widely distributed in the plant kingdom, being present in more than 2500 species (Ganjewala, Kumar, Asha, & Ambika, 2010). Cyanogenic glycosides are stored in vacuoles within plant cells. When tissues are disrupted, for example by crushing, the cyanogenic glycosides come into contact with endogenous enzymes (β-glucosidases and α-hydroxynitrile lyases) resulting in the release of hydrogen cyanide (Zagrobelny et al., 2004). In plants, consequently, cyanogenic glycosides serve as important chemical defence compounds against herbivores (Ganjewala et al., 2010; Zagrobelny et al., 2004). In humans, consumption of cyanogenic plants can cause sub-acute cyanide poisoning with symptoms including anxiety, headache, dizziness and confusion. Acute poisoning results in decreased consciousness, hypotension, paralysis, coma and even death. Acute cyanide poisoning has been reported from the ingestion of apricot kernels (Sahin, 2011), almonds (Sanchez-Verlaan et al., 2011) and cassava (Akintonwa & Tunwashe, 1992).

Cyanogenic glycosides are present in economically important food plants such as apple, almond, various beans, cereals, cassava, taro and sorghum (Donald, 2009; Jones, 1998). Processing techniques such as pounding, crushing, grinding, soaking, fermentation, boiling and drying have been used over the years to reduce the cyanide contents of foods. Processing allows contact between

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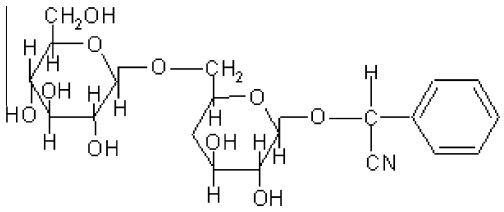

Fig. 1. Structure of amygdalin.

cyanogenic glycosides and endogenous enzymes which results in the hydrolytic breakdown of cyanogenic glycosides to hydrogen cyanide. Because the boiling point of hydrogen cyanide is 26 °C, it easily volatilises during food processing (Montagnac, Davis, & Tanumihardjo, 2009). Quantification of cyanogenic glycosides in plants is carried out either indirectly (by determining the amount of hydrogen cyanide released after hydrolysis) or directly (by determining the intact form). The indirect method is the most commonly used analytical method and usually involves enzymatic hydrolysis followed by colorimetric determination of total cyanide (Bradbury, Egan, & Lynch, 1991; Santamour, 1998). Methods for determination of intact cyanogenic glycosides include liquid chromatography with refractive index detection (Sornyotha, Kyu, & Ratanakhanokchai, 2007), gas chromatography/mass spectrometry (Chassagne, Crouzet, Bayonove, & Baumes, 1996), and HPLC with UV detection (Bolarinwa, Orfila, & Morgan, 2014).

Although humans, generally, do not consume apple seeds, apple juice is generally produced from whole apples including the seeds. Apple seeds disintegrate during juice production and contaminate the juice. While there are extensive studies on the antioxidant composition of apple juice (Miller, Diplock, & Rice-Evans, 1995; Spanos, Wrolstad, & Heatherbell, 1990), microbial safety and preservation of apple juice (Evrendilek et al., 2000), there has been no study on the amygdalin content of apple juice, and there is limited information on the amygdalin contents of apple seeds (Haque & Bradbury, 2002; Holzbecher, Moss, & Ellenberger, 1984).

2. Materials and methods

2.1. 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.

2.2. Apples

Fifteen varieties of apples (Braeburn, Bramley, Cox, Elstar, Empire, Egremont Russet, Fuji, Golden Delicious, Granny Smith, Jazz, Pink Lady, Red Delicious, Royal Gala, Rubens and Spartan) were purchased from local supermarkets in Leeds (UK). The apples were stored at 4 °C immediately after purchase prior to processing.

2.2.1. Extraction of amygdalin from apple seeds

Apples were each cut into four equal parts and apple seeds were separated from other tissues with a knife and extracted immediately. Apple seeds (2 g) were disintegrated with a mortar and pestle, and 1 g was weighed into a round-bottom flask (500 ml). Ethanol (50 ml) was added, and the mixture was boiled under reflux for 100 min. The extract was filtered (Whatman No. 1 filter paper) and transferred into plastic polypropylene tubes (50 ml). Ethanol was completely evaporated from the filtrate with a rotary evaporator (low BP, 35 °C, 7 mbar). Diethyl ether (10 ml) was added to the dried sample and the mixture was vortexed (1 min) at room temperature (20° ± 2 °C) to precipitate amygdalin. The diethyl ether was allowed to evaporate overnight and the extracted amygdalin was dissolved in water (5 ml) and prepared for HPLC analysis (2.5).

2.2.2. Apple juice extraction

2.2.2.1. Apple juice from whole apple. Four samples of apple juice were produced from four apple cultivars (Braeburn, Egremont Russet, Golden Delicious and Royal Gala). Whole apples (10) were each washed, cut into 4 pieces and pressed in a commercial juice extractor (Kenwood JE 600). The extracted juice from each apple variety was divided into portions, each of which was subjected to different processing conditions. Apple juice was also produced from either the flesh with skin or the core of the four apple varieties. The flesh with skin and core of the apples were separated with a knife prior to juice extraction. The juice was stored at −20 °C until extraction.

2.2.2.2. Processing of apple juice and determination of amygdalin content. Apple juice was divided into 20 ml portions and treated as follows, (i) extracted for determination of amygdalin content immediately, (ii) boiled immediately, frozen, thawed and then extracted for determination of amygdalin content, (iii) frozen immediately, thawed then extracted for determination of amygdalin content, (iv) held at room temperature (20 ± 2 °C) for 10, 30, 60 or 120 min then frozen, thawed and extracted for determination of amygdalin content, (v) pasteurised at 75 °C for 30 min then held for 10, 30, 60 or 120 min at room temperature (20 ± 2 °C), then frozen, thawed and extracted for determination of amygdalin content, (vi) held for 10, 30, 60 or 120 min at room temperature (20 ± 2 °C) then pasteurised at 75 °C for 30 min then frozen, thawed and extracted for determination of amygdalin content.

2.3. Commercially-available apple juice

The amygdalin contents of apple juices from supermarkets in Leeds (UK) were determined. The following juices were purchased locally: Appletiser (100% concentrate), Aspall Apple juice (100% pressed English apple), Copella Apple juice (hand - picked English apples), Del Monte Quality Long Life Apple Juice (100% concentrate), Innocent Juicy Drink (75% pressed apple) and 100% pressed apple Apple Juice, Jucee Long Life Apple Juice (100% concentrate), Juice Tree Long Life Apple Juice (100% concentrate), Morrisons own-brand Cloudy Apple Juice (100% squeezed apple; Jonagold, Elstar, Golden Delicious) and English Pressed Apple juice (100% fruit), Robinsons Long Life Apple Fruit Shoot Juice Drink (8% concentrate), Sainsbury's own-brand Pressed Apple Juice (100% pressed & squeezed fruit) and Long Life Apple Juice (from concentrate), Sun Grown Cloudy Apple Juice (pure fruit juice) and Long Life Apple Juice (from concentrate), Sun Sip Long Life Apple High Juice (50% fruit juice, 50% concentrate), Tesco own-brand; Fruit Splash Long Life Apple Juice Drink (31% concentrate), Long Life Apple Juice (100% concentrate), Organic Long Life Apple Juice (from concentrate), Long Life Apple Juice (10% concentrate), Pure Apple Juice (100% concentrate; Long Life), Value Apple Juice (100% concentrate; Long Life), Pressed Cloudy Apple Juice, Light Choices Long Life Apple Juice Drink (10% concentrate), Long Life Apple Squash (double strength) and Long Life Apple High Juice (50% fruit juice, 50% concentrate) and Tropicana Pressed Apple Juice (100% pure squeezed apple fruit). All the apple juice was stored at 4 °C after purchase prior to extraction and analysis.

2.4. Extraction of amygdalin from apple juice

The pH of all the apple juices ranged from 3.86 to 3.95. Amygdalin solubility at this pH was tested at room temperature and boiling temperature in our preliminary study. The results showed

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