EXTRACTS OF JAPANESE QUINCE SEEDS – POTENTIAL SOURCE OF ANTIOXIDANTS

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Abstract
Japanese quince (*Chaenomeles japonica*) is a minor fruit crop in Latvia and Lithuania; it is used for production of juice, aroma and fruit fibers. The seeds are by-products of food processing that could be used further for different purposes. The seeds of Japanese quince contain about 10 to 20% of oil. The composition of this oil is quite unique: nearly 90% of it is formed by two fatty acids - linoleic (52.4%) and oleic (35.6%). We have also found out that the extracts of Japanese quince seeds can be used to improve stability of vegetable oils – 10 wt-% additive of ground seeds to hempseed oil and 5 wt-% additive to rapeseed oil can increase the oxidative stability of these oils about 2.0 and 1.6 times, respectively. Unfortunately, the seeds of Japanese quince contain also amygdalin – toxic cianogenic glycoside. Due to this compound the usage of seeds of Japanese quince are very limited, especially in case of their hydrophilic extracts. Our research was focused on hydrophilic extracts of seeds in order to find out both the best method to prepare polyphenols rich extracts, as well as to determine the amount of toxic amygdalin in the ethanol/water extracts of seeds and in the extracted seeds. We have found out that the largest amount of total polyphenols can be obtained when whole seeds are extracted with the mixture of ethanol and water under reflux.
Key words: Japanese quince, seeds, antioxidants, polyphenols, amygdalin

Introduction

Japanese quince (*Chaenomeles japonica*) is wide spread in Latvia. The chemical composition of the fruit has been studied. A few studies are devoted to the composition of carbohydrates (Barcelo, 2000), pectines (Thomas, 2003), polyphenols (Wojdyło, 2008), organic acids (Ruisa, 1996) and volatile compounds (Jordán, 2003).

Only a few studies are dedicated to the composition of the seeds of Japanese quince. Most of these studies describe fatty acid composition (Gora, 1979; Ruisa, 1996; Granados, 2003; Дейнека, 2005; Mierina, 2009). The compositions of triglycerides (Deineka, 2004), phospholipids and other compounds containing phosphorus (Gora, 1979; Mukhamedova, 1979), as well as phytosterols and α-tocopherol (Gora, 1979) have been analyzed. There are some authors, who detected cyanogenic hydrogen (Ruisa, 1996) and benzaldehyde (Granados, 2003) – degradation products of amygdaline – in the seeds of Japanese quince. Amygdalin itself has been identified in seeds of Japanese quince by us (Moskaļuka, 2010).

This paper is focussed on the characterization of total polyphenol content in seeds of Japanese quince and detection of the amount of cyanogenic compounds in the extracts and extracted seeds.

Materials and Methods

Japanese quince seeds

Fruits of Japanese quince were cut and seeds and pulp were separated. In order to remove damaged seeds, they were washed with water; after that seeds were air-dried at 40±2 °C with forced air circulation (oven *Orakas*). The water content of the seeds was 5.74%. The seeds were packed under vacuum for 2 kg in bags made of polypropylene; they were stored at 18±2 °C in dark until further experiments.

Preparation of the extracts of Japanese quince seeds

The Japanese quince seed oil was obtained (see table 1) from seeds (cut in half or finely ground in coffee grinder and sieved by particle size d<0.069 mm) by extraction with petroleum ether or mixture of $CHCl_3$:MeOH (2:1) under reflux or by mixing at room

temperature (RT). Solvent was removed by vacuum filtration and the extract concentrated by rotary evaporation. Deoiled seed meal was air dried for further analysis.

Hydrophilic extracts of seeds (whole or ground (d<0.069 mm)) were prepared by extraction with ethanol, water or water:ethanol mixture (1:1/v:v) under reflux (method A, B or C, respectively) or at room temperature (temperature about 16 ^{o}C) (method D, E or F, respectively). The ratio "seed:solvent" was 1:5 (g:ml). Duration of extraction was varied from 0.5 to 24 hrs. Mixtures were drained; extracts and air dried seed residues were used for further analysis.

Determination of polyphenol content

Total amount of polyphenols (TAP) was determined according to modified method (Singleton, 1999); amount of polyphenols was expressed as GAE (mg $100g^{-1}$) – mg of gallic acid equivalents per 100 g of Japanese quince seeds.

Determination of total amount of cyanogenic compounds

Total amount of cyanogenic compounds was determined by argentometry (Fend, 2003). In order to determine amount of cyanogenic compounds in extracts, 20 ml of extract was refluxed, the vapour was collected in a flask containing 20 ml 2.5% NaOH solution; 8 ml 6 M NH_4OH solution and 2 ml of 5% KI solution was added to the distillate, followed by titration with 0.02 M $AgNO_3$ solution.

In order to determine the amount of cyanogenic compounds in deoiled seed meal or seed residue, 2 g of seed material was macerated in 20 ml H_2O for 2 hrs at room temperature. The obtained extract was further used as described previously.

The amount of cyanogenic compounds was expressed as HCNeq (mg kg^{-1}) – mg of HCN equivalents per 1 kg of Japanese quince seeds.

Chromatographic analyses

HPLC analysis was carried out with *Agilent Technologies 1200 Series* chromatograph, equipped with UV detector (wave length 210 and 260 nm). *Merck Spherisorb SB C18* reverse phase column (4×150 mm, 5 μm) was used as a stationary phase for HPLC analysis. The column was elueted with mixture of 0.01 M KH_2PO_4 and 6% MeOH (1:15, v/v), flow rate 1 ml min^{-1}.

Fatty acid composition was analyzed with *Agilent Technologies 6890 N* gas chromatograph (Stránský, 2005).

Results and Discussion

The area where Japanese quince is grown in Latvia is nearly 200 ha; the yield is around 1.5 to 2 t ha^{-1} and approximately 50–70 t of fruits of Japanese quince have been sold in year 2009. According to Granados (Granados, 2003), fruits of Japanese quince contain about 5 to 9% of seeds – so, at least 5–7 t of seeds are produced as a by-product of food manufacturing in Latvia every year. These seeds would be a valuable product.

Japanese quince seeds contain about 6 to even 20% of oil (Granados, 2003; Mierina, 2009). The fatty acid composition of the seed oil is unique - nearly 90% of it is formed by two fatty acids − linoleic (52.4%) and oleic (35.6%); the minor fatty acids detected in the oil are palmitic (9.90%), stearic (0.92%), arachidic (0.55%) and linolenic (0.63%) acid. The amount of extracted oil strongly depends on conditions of extraction. We compared the impact of solvent, temperature and duration of extraction on the yield of extracted oil (see table 1). As the yield of oil reached even 20.4% (when finely ground seeds were extracted with petroleum ether at reflux), we tried to obtain oil with oil press (*Taby press type 20*), too. This so called cold-press method (temperature about 60 °C) gave just a few drops of oil (from 50 g of seeds). In order to improve the result, seeds were preheated before the pressing, but the yield of oil did not exceed 2%.

Table 1

The Impact of Method of Extraction on the Yield of Japanese Quince Seed Oil

Method of extraction				Yield of the oil, %	
No.	**Size of seeds**	**Temperature**	**Duration, h**	**Petroleum ether**	**$CHCl_3$:MeOH (2:1)**
1	cut in half	Reflux	6	14.5	9.7
2	d<0.069 mm	Reflux	6	20.4	15.6
3	d<0.069 mm	RT	6	10.6	13.2
4	d<0.069 mm	RT	24	13.3	15.6

Our previous studies show that it is possible to increase oxidative stability of vegetable (rapeseed or hempseed) oil with lipophilic (oil) extracts of Japanese quince seeds. The oxidative stability of vegetable oils was studied (Mierina, 2009) at different conditions with two methods: a) the samples were kept at accelerated oxidation conditions and the oxidative stability was monitored by peroxide values, b) Rancimat method. We used cold pressed rapeseed and hempseed oil to evaluate the antioxidant potential of the seeds of Japanese quince. Variable extracts were prepared from 1 to 20 g of seeds and 100 g oil. In order to find out the optimal concentration that provides highest oxidative stability of vegetable oil, the experiments were carried out into 3 stages; the concentration was scaled down in each next stage. We found out that the highest antioxidant activity can be reached when 5 g or 10 g of seeds were extracted with 100 g of rapeseed or hempseed oil, correspondingly; the antioxidant activity (1.13 and 2.47) of these extracts were detected according to Bandoniene (Bandoniene, 2000).

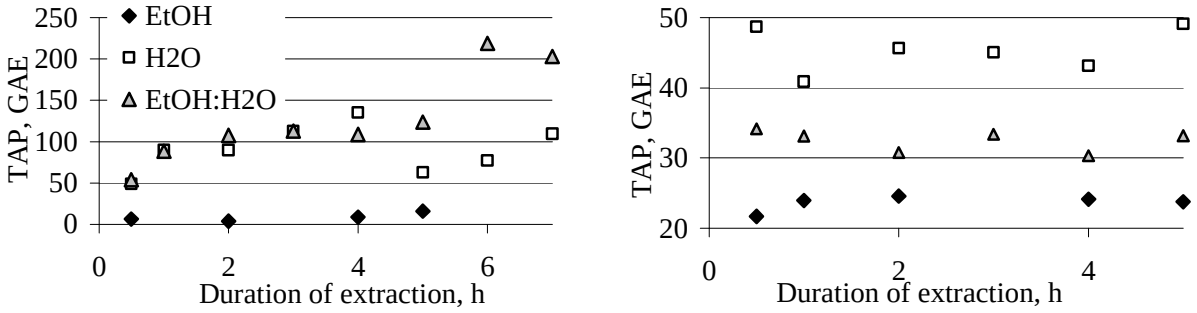
We established that seed oil (obtained with petroleum ether according method No. 2, table 1) contains 238 mg of polyphenols per 100 g of oil; this amount is equal to 35 GAE. We found out that less than 0.5% additive of Japanese quince seed oil can improve the oxidative stability of rapeseed oil.

We approbated different conditions of extraction in order to establish the best method both to prepare extracts rich in polyphenols and to determine the total amount of cyanogenic compounds in extracts and extracted seed material. The whole and finely ground seeds were used for extraction. We have compared six variable extraction conditions: the seeds were extracted with ethanol, water or mixture of water and ethanol (1:1) under reflux (method A, B or C, respectively) and at room temperature (method D, E or F, respectively). In order to optimize the extraction procedure, the impact of the duration of extraction on the total amount of polyphenols and cyanogenic compounds was studied.

We found out that depending from the extraction conditions the TAP varied from 1 to more than 200 GAE (fig. 1 and 2). The lowest result was obtained when whole seeds were extracted with the solvent at room temperature (method D, E and F) even within 24 hrs. When whole seeds were extracted with water, mixture of water and ethanol or ethanol, TAP was only 7 or 1 GAE; due to this whole seeds were not studied further. When ground seeds were extracted at room temperature, the content of polyphenolic compounds was not high – TAP varied from 20 to 50 GAE. The prolongation of extraction from 0.5 to 24 h did not lead to significant increase of TAP. The extraction at room temperature provided the highest amount of polyphenols when solvent was water.

When the extraction of seeds was carried out under reflux, the TAP increased from 50 GAE after 0.5 h to more than 200 GAE after 4 or more hrs. TAP did not change significantly, if extraction time was short (0.5 hrs) and extraction was carried out under reflux or at room temperature; the same situation was observed for all prepared ethanol extracts at any duration of extraction. TAP was remarkably higher, when seeds were extracted with water or mixture of water and ethanol under reflux (in comparison with room temperature). TAP was similar in

case of ethanol and ethanol/water extracts, if extraction was carried out under reflux for less than 5 hrs. Nevertheless, TAP increased nearly twice in case of ethanol/water extracts in comparison with water extracts if the duration of extraction increased up to 6 or 7 h (see fig. 1a and 1b).



a **b**

Figure 1. TAP dependence from solvent and duration of extraction of ground seeds (a – reflux, b – room temperature)

When whole seeds of Japanese quince were extracted under reflux, TAP strongly depended from the solvent used for extraction. The lowest TAP was determined in ethanol extracts; it varied from 8 to 16 GAE depending from duration of extraction. Notably higher TAP was in case of extracts prepared with water and ethanol/water mixture; TAP was similar for both ethanol and ethanol/water extracts, when the duration of extraction was 4 or less hours. When the duration was increased, the total amount of polyphenols was remarkably higher in the extracts prepared with mixture of ethanol and water.

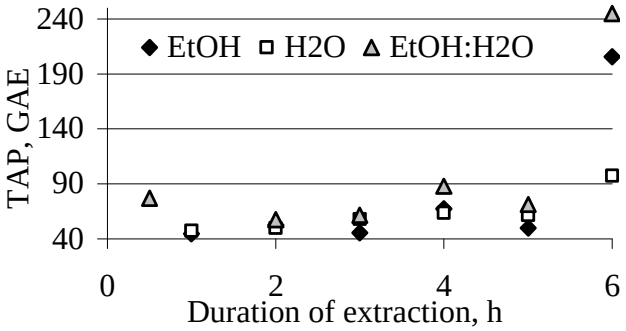


Figure 2. TAP dependence from solvent and duration of extraction of whole seeds

Due to the fact that seeds of Japanese quince contain cyanogenic compounds (Ruisa, 1996, Moskaluka, 2010), we have studied their content (expressed as total amount of HCN) in seeds. We already established (Moskaluka, 2010), that intact seeds contain amygdalin, but extracted seed oil (see Table 1) or mechanically pressed oil do not contain it or its degradation products (benzaldehyde, benzoic acid or mandelonitrile) (according to HPLC analysis).

Now we have determined that the total amount of cyanogenic compounds in Japanese quince seeds is 690 mg kg^{-1}. This amount decreased in deoiled seed meal (see Table 2). It appeared, that the seeds extracted with petroleum ether or mechanically pressed contained benzaldehyde and mandelonitrile; benzoic acid was found in the seeds extracted with CH_3Cl:MeOH.

The aim of this study was both to determine the amount of cyanogenic compounds in the prepared hydrophilic extracts and to elaborate extraction procedure for detoxification of Japanese quince seeds. The highest amount of cyanogenic compounds was determined in water extracts of Japanese quince seeds, followed by extracts prepared with mixture of ethanol and water.

Table 2

The Amount of Cyanogenic Compounds in the Deoiled Seed Meal

Method of extraction	Extractant petroleum ether		Extractant $CHCl_3$:MeOH (2:1)	
	HCNeq	Amygdalin*	HCNeq	Amygdalin*
1	590	–	310	–
2	110	+	400	–
3	250	+	370	–
4	320	+	340	–

* – detected with HPLC

The least amount of cyanogenic compounds was determined in ethanol extracts. The amount of these compounds varied from 270 HCNeq in extract of ground seeds prepared according to the method E (extraction time 0.5 hrs) to 5 HCNeq for the extract prepared from ground seeds according to the method D. The amount of cyanogenic compounds decreased in case of all above mentioned hydrophilic solvents if extraction was carried out under reflux, which is most likely due to enzymatic and/or thermal degradation of amygdalin (Li, 1992). The amount of cyanogenic compounds was 2 to 5 times higher in the extracts prepared at room temperature.

The cyanogenic compounds were determined also in the seed residue obtained by extraction with hydrophilic solvents. The amount of cyanogenic compounds varied from 0 to few hundreds of HCNeq. We have established that in order to detoxify the seeds the best of tested solvents was water. When the whole seeds were extracted for 7 hrs with water or ground seeds were extracted for 4 hrs with water or 6 hrs with ethanol or the mixture of ethanol and water under reflux, the amount of cyanogenic compounds were reduced to 0 HCNeq.

Conclusions

1. An additive of both Japanese quince seeds and their oil can be used to increase the oxidative stability of vegetable oils.
2. The total amount of polyphenols strongly depends on solvent, temperature and the seed particles (degree of grounding), but less - on the extraction time. The highest TAP was determined in extracts of ground seeds when extraction was carried out with ethanol or mixture of ethanol and water under reflux. High TAP was detected in extracts of whole seeds prepared by extraction with water and mixture of water and ethanol under reflux.
3. The highest amount of cyanogenic compounds was found in water extracts of seeds; it decreased with increase of extraction temperature. The prolongation of extraction can reduce the amount of cyanogenic compounds in the extract. The extraction procedure used to prepare extracts rich in polyphenols can be used to detoxify seeds, too; for this purpose the best method is extraction of ground seeds with water under reflux.

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