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RESEARCH ARTICLE

Chromatographic characterization of juice in fruits of different Japanese quince (*Chaenomeles japonica* L.) genotypes cultivated in Sweden

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ABSTRACT

The juice in fruits of 19 genotypes of Japanese quince (*Chaenomeles japonica*), representing plant breeding material, was characterised using high performance liquid chromatography. The juice was extracted by halving and squeezing the fruit. Samples of Japanese quince juice were always analysed fresh. The main compounds found were sugars: glucose (131-1056 mg 100 ml^{-1}), fructose (351-2515 mg 100 ml^{-1}) and sorbitol (10-367 mg 100 ml^{-1}); organic acids: malic acid (2.27-4.84 g 100 ml^{-1}) and quinic acid (0.50-2.50 g 100 ml^{-1}); amino acids: aspartic acid (0.8-10.7 mg 100 ml^{-1}), asparagine (0.2-36.3 mg 100 ml^{-1}) and glutamic acid (6.2-17.7 mg 100 ml^{-1}); the cation potassium (145-214 mg 100 ml^{-1}) and the anion fluoride (21-122 mg 100 ml^{-1}). These results on composition suggest that Japanese quince may be an interesting raw material source of valuable substances and its juice an ingredient for the food industry. A principal components analysis separated the Japanese quince genotypes, thus indicating a clear difference in the chemical composition of the juice.

Keywords: *Chaenomeles japonica*; Japanese quince; Fruits; Juice; Composition

INTRODUCTION

The family Chaenomeles belongs to *Rosaceae*. The genus *Chaenomeles* is originating from East Asia and has four species (Chaenomeles *thibetica*, Chaenomeles *speciosa*, Chaenomeles *japonica* and Chaenomeles *cathayensis*), which are considered as a potential crop in horticulture, due to the firmness of the fruits and the high content in juice (Hellín et al., 2003; Ros et al., 2004), and the fruit dietetic fibres (Thomas et al., 2000; Thomas and Thibault, 2002; Thomas et al., 2003). A horticultural program in North Europe, initially focussed in the Japanese quince (*Chaenomeles japonica*, Fig. 1, Fig. 2 and Fig. 3), was started in 1998 with the European Research Project entitled Japanese quince (*Chaenomeles japonica*) - A new european fruit crop for production of juice, flavour and fibre (FAIR5-CT97-3894, 1998-2001). The purpose was to upgrade the plant by breeding and selection (Rumpunen et al., 1998; Rumpunen, 2002). For these purposes information on the (bio)chemical properties of the fruits is a clear requirement. The juice of Japanese quince can be an interesting ingredient of food, since it is very acidic and no cloudy (Hellín et al., 2003; Ros et al., 2004), being also of interest its antioxidant power, because the high amounts of compounds such as vitamin C and phenolics (Hellín et al., 2003; Ros et al., 2004). It is very important considering its use in industrial food production as an ingredient for acidification having antioxidative characteristics (Durec et al., 2019), making the Japanese quince a healthy fruit Watychowicz et al., 2017).

The scientific references on the properties and constituents of *Chaenomeles* and *Chaenomeles* juice is modest. There are some articles dealing on *Chaenomeles* composition and characteristics: sugars and acids (Lesinska, 1987), volatile and nonvolatile flavor components (Lesinska et al., 1988), complex carbohydrate (Golubev et al., 1990), pectic substances (Rumpunen, 1995; Thomas et al., 2003), *Chaenomeles* fruit juice (Hellín et al., 2003; Ros et al., 2004), fruit characteristics and processing potential (Tarko et al., 2014), tocochromanol composition in the seeds (Górnaś et al., 2014), natural

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Fig 1. *Chaenomeles japonica* plant and fruits.

Fig 2. *Chaenomeles japonica* fruits.

Fig 3. *Chaenomeles japonica* fruits and juice.

ingredient (Wojdyło et al., 2008), element content (Baranowska-Bosiacka et al., 2017; Komar-Tyomnaya and Dunaevskaya, 2017), active compounds (Miao et al., 2018), pentacyclic triterpenoids (Kikowska et al., 2018), antiamoebic and antimicrobial activities (Kikowska et al., 2019a), polyphenol-rich extracts (Kikowska et al., 2019b), industrial characteristics (Lykholat et al., 2019) and the review on *Chaenomeles* – health promoting benefits by Watychowicz et al. (2017).

Focusing on the previous works done on this topic (Lesinska et al., 1988; Rumpunen, 1995; Hellín et al., 2003; Ros et al., 2004; Baranowska-Bosiacka et al., 2017), the objective of this study was to characterise Japanese quince juice using high performance liquid chromatography analyses of sugars, organic acids, amino acids and ions in fruits of different genotypes cultivated in Sweden. An analysis of the principal components (PCA) of the results was also made to show global differences between genotypes on the basis of the results of juice characterization.

MATERIALS AND METHODS

Plant material and fruits

The origin and denomination of the *Chaenomeles japonica* plants (Fig. 1) from which fruits (Fig. 2 and Fig. 3) were sampled for chromatographic characterisation of its juice (Fig. 3) are given in Table 1. In total, 19 genotypes of Japanese quince (*Chaenomeles japonica*), representing plant breeding material, were extracted and characterised. The plants sampled were cultivated in different test plots (D, NV and RG) at the Department of Crop Science, Swedish University of Agricultural Sciences, Kristianstad, Sweden (56°07’N, 14°10’E). The soil differed between the test plots, in that the NV soil was sandier compared to D and RG soils, which contained considerably more clay. The different populations, from which the plants were sampled, were collected and established during a period of four years. Thus, the plants sampled were of different age. However, all fruits of Japanese quince were sampled at the same developmental stage, when the fruit skin had turned yellow and the seeds had turned brown, indicating maturity (Ros et al., 2004). Following harvesting (September 2000) the fruits were sent to Spain for chromatographic analysis, which was performed immediately once the fruits were received.

Fruit fractionation

Fruits (1 kg of each genotype, 10-12 units) were fractionated into pulp, juice and seeds (Ros et al., 2004). The juice was extracted by halving and squeezing the fruit. For this purpose, a Frutelia AV5 juice extractor from Moulinex (France) was used. Samples of Japanese quince juice were always analysed fresh. To avoid modification of the composition and characteristics of the fresh juice, no thermal treatment was carried-out. Juice samples were

Table 1: Origin and denomination of the Japanese quince (*Chaenomeles japonica*) plants from which fruits were sampled for chromatographic characterization of its juice

Genotypes[a]	Specie	Population	Seed source[b]	Origin
D 3-122	*C. japonica*	9226	orchard, open pollination	Lithuania
D 5-96	*C. japonica*	9236	orchard, open pollination	Lithuania
D 10-19	*C. japonica*	9218	orchard, open pollination	Lithuania
NV 14-73	*C. japonica*	9401	orchard, crossing (9368x9363)	Lithuania
NV 15-97	*C. japonica*	9403	orchard, crossing (9363x9368)	Lithuania
NV 17-18	*C. japonica*	9411	orchard, crossing (9366x9368)	Lithuania
NV 18-34	*C. japonica*	9403	orchard, crossing (9363x9368)	Lithuania
NV 19-27	*C. japonica*	9401	orchard, crossing (9368x9363)	Lithuania
NV 19-44	*C. japonica*	9411	orchard, crossing (9366x9368)	Lithuania
NV 19-64	*C. japonica*	9403	orchard, crossing (9363x9368)	Lithuania
NV 19-108	*C. japonica*	9401	orchard, crossing (9368x9363)	Lithuania
RG 1-27	*C. japonica*	9161	orchard, open pollination	Lithuania
RG 6-104	*C. japonica*	9138	orchard, open pollination	Latvia
RG 6-111	*C. japonica*	9138	orchard, open pollination	Latvia
RG 6-132	*C. japonica*	9138	orchard, open pollination	Latvia
RG 7-50	*C. japonica*	9136	orchard, open pollination	Latvia
RG 7-69	*C. japonica*	9135	orchard, open pollination	Latvia
RG 8-22	*C. japonica*	9134	orchard, open pollination	Latvia
RG 8-25	*C. japonica*	9134	orchard, open pollination	Latvia

[a]Letters D, NV and RG represent different test plots at the Department of Crop Science, Swedish University of Agricultural Sciences, where the plants were cultivated (56°07'N, 14°10'E). [b]In the crossing combinations the numbers 9363, 9366 and 9368 represent three different seed- and pollen-parents of *C. japonica*

frozen (-20ºC) until the chromatographic analyses were made.

Sample treatment before high performance liquid chromatography analysis

Once the juice samples have been thawed and stirred for homogenization, the samples of juice were centrifuged previously to direct injection for high performance liquid chromatography (HPLC) analysis (González-Hidalgo et al., 2019).

High performance liquid chromatography analysis of the Japanese quince juice

Sugars and organic acids were analysed by HPLC according to Hellín et al. (2001). Amino acids and inorganic ions were analysed by HPLC according to Hellín et al. (2003).

Statistical analyses

The samples were analysed in triplicate and an average value was calculated for the juice from a representative sample (1 kg) of each genotype. For each analysed parameter in triplicate an average value, its corresponding standard deviation (s_x) and an analysis of the variance (ANOVA) was also calculated based on the 19 genotypes sampled. Scheffé's homogeneity means test ($p < 0.05$) was used. The statistical computer program used was Statistix 8 for Windows. Pearson's correlation coefficients and a principal component analysis (PCA) of the composition of Japanese quince genotypes fresh juice were obtained using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Sugars content in Japanese quince genotypes fresh juice

The concentration of sugars found in Japanese quince genotypes fresh juice is shown in Table 2. Nine sugars were found: the tetrasaccharide stachyose, the trisaccharide raffinose, the disaccharide sucrose, and the monosaccharides glucose, xylose, rhamnose, fructose, inositol and sorbitol. The sugars content of Japanese quince juice was different between the genotypes. Main sugars were fructose (351-2515 mg 100 ml^{-1}), glucose (131-1056 mg 100 ml^{-1}) and sorbitol (10-367 mg 100 ml^{-1}). The differences between the results of sugars content are statistically significatives ($p < 0.05$) (Table 2).

The total sugars (up to 4.4 g 100 ml^{-1}) concentrations found for the different Japanese quince genotypes juices are in agreement with the soluble solids (up to 8.8 ºBrix) reported in the same Japanese quince genotypes juices by Ros et al. (2004). It should be considered that the refractometric measure of the soluble solids is a contribution of both soluble sugars and organic acids, which are also in high concentration in these juices (see below). Fructose, glucose and sorbitol are the same major sugars reported by Lesinska (1987) also in Japanese quince. Our results also agree in the range of concentrations in which those sugars are presents in Japanese quince juice, although some differences have been found respect to the results of Lesinska (1987), mainly due to the fact that we determine free sugars soluble in the juice and Lesinska (1987) determine all sugars

Table 2: Sugars content (mg 100 ml^{-1}) in Japanese quince (*Chaenomeles japonica*) genotypes fresh juice (n = 3)

Genotypes	Stach[1]	Raffinose	Sucrose	Glucose	Xylose	Rhamnose	Fructose	Inositol	Sorbitol
D 3-122	nd	39^{a}	55^{b}	1056^{a}	285^{a}	110^{a}	2515^{a}	nd	311^{b}
D 5-96	1^{g}	2^{g}	2^{h}	457^{f}	86^{e}	19^{c}	939^{f}	20^{a}	249^{c}
D 10-19	2^{f}	21^{b}	59^{b}	405^{f}	78^{e}	24^{c}	888^{g}	7^{d}	187^{e}
NV 14-73	9^{c}	1^{h}	33^{c}	335^{g}	71^{e}	16^{d}	841^{g}	10^{d}	173^{e}
NV 15-97	2^{f}	5^{e}	54^{b}	137^{h}	159^{b}	9^{f}	514^{h}	5^{e}	90^{g}
NV 17-18	2^{f}	3^{f}	31^{c}	131^{h}	99^{d}	7^{f}	351^{j}	nd	87^{g}
NV 18-34	nd	9^{d}	21^{d}	553^{e}	115^{c}	nd	1339^{d}	nd	179^{e}
NV 19-27	13^{b}	17^{c}	19^{d}	299^{g}	71^{e}	13^{e}	951^{f}	16^{b}	10^{h}
NV 19-44	6^{d}	1^{h}	8^{f}	399^{f}	75^{e}	16^{d}	972^{f}	9^{d}	118^{f}
NV 19-64	4^{e}	11^{d}	303^{a}	448^{f}	55^{f}	4^{g}	1056^{e}	3^{f}	136^{f}
NV 19-108	22^{a}	20^{b}	12^{e}	137^{h}	131^{c}	18^{c}	490^{h}	5^{e}	124^{f}
RG 1-27	2^{f}	3^{f}	nd	660^{d}	51^{f}	nd	1338^{d}	7^{d}	367^{a}
RG 6-104	2^{f}	4^{f}	13^{e}	619^{d}	169^{b}	32^{b}	1621^{b}	14^{b}	353^{a}
RG 6-111	4^{e}	nd	4^{g}	698^{c}	55^{f}	nd	1326^{d}	nd	303^{b}
RG 6-132	2^{f}	6^{e}	1^{i}	682^{c}	20^{g}	nd	1010^{e}	nd	237^{c}
RG 7-50	nd	nd	nd	732^{c}	53^{f}	nd	1386^{d}	15^{b}	305^{b}
RG 7-69	nd	3^{f}	23^{d}	432^{e}	29^{g}	nd	1013^{e}	12^{c}	116^{f}
RG 8-22	nd	nd	10^{f}	470^{e}	81^{e}	13^{e}	397^{i}	12^{c}	217^{d}
RG 8-25	nd	nd	nd	923^{b}	25^{g}	nd	1490^{c}	9^{d}	228^{d}
Range	1-22	1-39	1-303	131-1056	20-285	4-110	351-2515	3-20	10-367
Mean	6	10	40	504	90	23	1076	10	200
s_x	6	11	73	253	63	28	507	5	99

[1]Stachyose. nd: no detected. Means in column with different letters (a-j) are significantly different ($p < 0.05$)

from the fruit, which includes the sugars components of the polysaccharides included in the structure of the Japanese quince cell wall. The high performance size exclusion chromatography analysis shown the absence of polysaccharides and oligosaccharides in the juice (Ros et al., 2004). No hydrolytic enzymes of polysaccharides are expected to find in the juice (Apolinar-Valiente et al., 2017). The composition of those polysaccharides (from the fruit pulp) was established by Golubev et al. (1990) and Thomas et al. (2000, 2003) and Thomas and Thibault (2002). Valcheva-Kuzmanova et al. (2018) also reported in juice of *Chaenomeles maulei* the absence of soluble polysaccharides, and, as main sugars, glucose (1.7 g 100 ml^{-1}), fructose (1.2 g 100 ml^{-1}), galactose (0.32 g 100 ml^{-1}) and sucrose (0.19 mg 100 ml^{-1}), which is similar, but not the same, to *Chaenomeles japonica*.

It is interesting to compare the Japanese quince sugars profile in the juice with that from different fruits. The fructose content in juice from other fruits is: apple cv. Red Delicious (5.3 g 100 ml^{-1}), grape (10.5), pear (8.1), kiwi fruit (8.2), and strawberry (2.2 g 100 ml^{-1}) (van Gorsel et al., 1992). Viljakainen et al. (2002) found a fructose amount in Nordic berries juice in the range 1.8 - 5.6 g 100 ml^{-1}. Hellín et al. (2003) reported the following fructose content in *Citrus* juices: orange 2.4 g 100 ml^{-1}, grapefruit 1.2 g 100 ml^{-1} and lemon 0.9 g 100 ml^{-1}. The glucose content in juice from other fruits is: apple (2.1 g 100 ml^{-1}), grape (9.6), pear (1.7), kiwi fruit (6.9), and strawberry (1.8 g 100 ml^{-1}) (van Gorsel et al., 1992). Viljakainen et al. (2002) found a glucose amount in Nordic berries juice in the range 2.2 - 5.0 g 100 ml^{-1}. Hellín et al. (2003) reported the following glucose content in *Citrus* juices: orange 2.4 g 100 ml^{-1}, grapefruit 2.0 g 100 ml^{-1} and lemon 0.5 g 100 ml^{-1}. The sorbitol content in juice from other fruits is: apple (0.2) and pear (4.1 g 100 ml^{-1}) (van Gorsel et al., 1992). Japanese quince juice can be a source of sorbitol, which is found broadly as a constituent of the vegetables, but in general in smaller amounts. The sweetness power of the sorbitol is half of the sucrose and it is also used as a humectant. The sucrose content in juice from other fruits is: apple (0.8), grape (0.3), pear (0.6), kiwi fruit (1.8), and strawberry (0.2 g 100 ml^{-1}) (van Gorsel et al., 1992). Viljakainen et al. (2002) found a sucrose amount in Nordic berries juice in the range no detected - 0.5 g 100 ml^{-1}. Hellín et al. (2003) reported the following glucose content in *Citrus* juices: sucrose 4.7 g 100 ml^{-1}, grapefruit 2.1 g 100 ml^{-1} and lemon 0.2 g 100 ml^{-1}.

Organic acids content in Japanese quince genotypes fresh juice

The concentration of organic acids found in Japanese quince genotypes fresh juice is shown in Table 3. The chromatographic analysis of organic acids indicated that the Japanese quince genotypes fresh juice is constituted by malic (2.27-4.84 g 100 ml^{-1}), quinic (0.50-2.50 g 100 ml^{-1}) and succinic acid (4-12 mg 100 ml^{-1}). The organic acids content of Japanese quince juice was different between the genotypes. The differences between the results of organic acids content are statistically significatives ($p < 0.05$) (Table 3).

Table 3: Organic acids content (mg 100 ml^{-1}) in Japanese quince (*Chaenomeles)*

japonica) genotypes fresh juice (n = 3)	Malic acid	Quinic acid	Succinic acid
D 3-122	4.84^a	1.48^b	0.004^e
D 5-96	3.10^e	0.90^c	0.008^c
D 10-19	3.97^c	1.29^b	0.006^d
NV 14-73	3.21^e	1.23^b	0.009^b
NV 15-97	2.92^e	1.31^b	nd
NV 17-18	3.26^e	0.86^c	0.005^d
NV 18-34	3.65^d	0.99^c	nd
NV 19-27	3.08^e	1.18^b	0.006^d
NV 19-44	3.44^d	0.98^c	0.012^a
NV 19-64	3.65^d	0.99^c	nd
NV 19-108	2.27^g	1.44^b	0.010^b
RG 1-27	3.00^e	0.60^d	0.005^d
RG 6-104	4.38^b	0.97^c	0.008^c
RG 6-111	3.00^e	0.60^d	0.008^c
RG 6-132	3.10^e	2.50^a	0.009^b
RG 7-50	2.70^f	0.50^d	0.012^a
RG 7-69	3.00^e	0.90^c	0.012^a
RG 8-22	4.13^c	0.84^c	0.009^b
RG 8-25	3.90^c	0.50^d	0.005^d
Range	2.27-4.84	0.50-2.50	0.004-0.012
Mean	3.40	1.06	0.008
s_x	0.63	0.46	0.003

nd: no detected. Means in column with different letters (a-g) are significantly different ($p < 0.05$)

The total organic acids (up to 6.3 g 100 ml^{-1}) concentrations found for the different Japanese quince genotypes juices are in agreement with the titratable acidity (up to 5.6 % anhydrous citric acid) reported in the same Japanese quince genotypes juices by Ros et al. (2004). Malic acid and quinic acid are the same major organic acids reported by Lesinska (1987) and Rumpunen (1995). Our results also agree in the range of concentrations in which those acids are presents in Japanese quince juice. Valcheva-Kuzmanova et al. (2018) reported in juice of *Chaenomeles maulei* a malic acid content of 3.7 g 100 ml^{-1} and a quinic acid content of 1.0 g 100 ml^{-1}, which is the same content of the genotype NV 18-34. They also found citric acid (51 mg 100 ml^{-1}), absent in *Chaenomeles japonica* juice. Baranowska-Bosiacka et al. (2017) reported the presence of oxalate (8.2 mg/100 g), which is of the same order of magnitude that succinic acid (Table 3).

For comparison, the content of malic acid in other fruit juices is: apple cv. Red Delicious (0.5), grape (0.3), kiwi fruit (0.5), pear (0.4), and strawberry (0.2 g 100 ml^{-1}) (van Gorsel et al., 1992). In juice from Nordic berries it is reported in the range 0.3 - 1.6 g 100 ml^{-1} (Viljakainen et al., 2002). Concerning quinic acid, van Gorsel et al. (1992) reported 0.8 g 100 ml^{-1} in the juice of kiwi fruit and 0.2 g 100 ml^{-1} in the juice of pear. Heimler and Pieroni (1992) found in apple cv. Summerred low amounts (0.2 mg 100 ml^{-1}) of succinic acid and fumaric acid. The organic acids profile of apple cv. Glockenapfel juice is also similar (Ackermann et al., 1992).

The very high malic acid content of Japanese quince juice makes it undrinkable. In order to obtain a Japanese quince juice with lower acidity, it is possible to carry-out its fermentation with malolactic bacteria, since the acidity of Nordic berries was reduced by fermentation with *Oenococcus oeni* (Viljakainen and Laakso, 2002).

The industry uses malic and succinic acid as agents of acidification. In fact, in the list of allowed additives, malic acid has the E-296 number and succinic acid the E-363. Quinic acid is not included in the list of allowed additives. Quinic acid uses mainly concern organic chemistry, since this acid contain three atoms of carbon with chirality properties and can be used as a building molecule that provide these chirality (Barco et al., 1997; Huang, 1999).

Considering that the content of acids in the juice of Japanese quince is high, it can be used in the same way that lemon juice (Saura et al., 1990) as a natural ingredient for acidification of foods with antioxidative characteristics (Hellín et al., 2003; Ros et al., 2004).

Amino acids content in Japanese quince genotypes fresh juice

The Table 4 provides the concentration of amino acids in the fresh juice of Japanese quince genotypes. As Table 4 shows, the content in amino acids was different between the genotypes. Main amino acids were phosphoserine (1.9-7.9 mg 100 ml^{-1}), aspartic acid (0.8-10.7 mg 100 ml^{-1}), asparagine (0.2-36.3 mg 100 ml^{-1}) and glutamic acid (6.2-17.7 mg 100 ml^{-1}). In most of the genotypes, the concentration of the other amino acids (alanine, phenylalanine, γ-aminobutiric acid (GABA) and lysine) was lower than 2 mg 100 ml^{-1}. The differences between the results of amino acids content are statistically significatives ($p < 0.05$) (Table 4).

Considering the importance of amino acids found in the juice of Japanese quince for human nutrition, threonine, phenylalanine and lysine are essential amino acids. It means that the intake is only from the diet. The γ-aminobutiric acid is a neurotransmitter of the brain. Being amino acids compounds of interest, quantitatively are lower (up to 67 mg 100 ml^{-1}) than vitamin C (up to 112 mg 100 ml^{-1}) and phenols (up to 413 mg 100 ml^{-1}), as was reported in the same genotypes juice by Ros et al. (2004).

It is very interesting to compare the amino acids composition of Japanese quince juices with that from other fruits. Following the analytical results of van Gorsel et al. (1992), expressed as mg of amino acid 100 ml^{-1} of

Table 4: Amino acids content (mg 100 ml^{-1}) in Japanese quince (*Chaenomeles japonica*) genotypes fresh juice (n = 3)

Genotypes	Phosphoserine	Aspartic acid	Threonine	Serine	Asparagine	Glutamic acid	Alanine	Phenylalanine	GABA[a]	Lysine
D 3-122	5.0^{d}	5.4^{c}	0.6^{b}	2.1^{d}	0.9^{j}	9.3^{f}	0.4^{d}	nd	nd	0.5^{c}
D 5-96	2.0^{h}	2.4^{e}	nd	0.7^{g}	nd	6.2^{h}	0.3^{e}	nd	nd	nd
D 10-19	2.8^{f}	3.8^{d}	0.5^{c}	1.1^{f}	1.0^{j}	10.8^{e}	0.5^{d}	nd	0.3b	0.1^{d}
NV 14-73	3.6^{e}	4.2^{d}	0.4^{d}	1.0^{f}	4.7^{f}	8.3^{f}	0.6^{d}	0.3^{d}	0.3b	nd
NV 15-97	5.7^{c}	2.9^{e}	0.6^{b}	1.7^{e}	1.3^{j}	10.9^{e}	0.9^{c}	1.3^{b}	0.7ab	0.8^{b}
NV 17-18	7.9^{a}	0.9^{g}	0.2^{e}	1.4^{f}	0.4^{k}	17.3^{a}	1.3^{b}	1.0	0.9a	0.5^{c}
NV 18-34	5.4^{c}	0.8^{g}	0.5^{c}	2.1^{d}	2.8^{h}	14.6^{b}	2.6^{a}	4.4^{a}	1.0a	0.3^{d}
NV 19-27	6.8^{b}	3.0^{e}	0.5^{c}	1.8^{e}	1.0^{j}	11.3^{e}	1.5^{b}	1.2^{b}	0.8a	0.8^{b}
NV 19-44	5.9^{c}	1.4^{f}	0.5^{c}	4.0^{b}	2.1^{i}	17.7^{a}	1.3^{b}	1.0^{b}	0.8a	0.4^{cd}
NV 19-64	4.8^{d}	4.7^{c}	0.4^{d}	1.1^{f}	3.5^{g}	8.7^{f}	0.5^{d}	nd	0.8a	0.3^{d}
NV 19-108	5.7^{c}	4.7^{c}	0.6^{b}	2.2^{d}	1.1^{h}	11.0^{e}	0.7^{d}	1.0^{b}	0.9a	0.5^{c}
RG 1-27	2.2^{g}	9.9^{a}	1.2^{a}	4.2^{b}	36.3^{a}	12.2^{d}	0.7^{d}	nd	nd	nd
RG 6-104	3.8^{e}	5.1^{c}	0.7^{b}	2.9^{c}	6.6^{e}	9.9^{f}	0.6^{d}	nd	0.5b	1.3^{a}
RG 6-111	2.4^{g}	4.9^{c}	0.2^{e}	0.2^{h}	2.0^{i}	6.3^{h}	0.4^{e}	nd	nd	0.7^{b}
RG 6-132	2.6^{f}	7.5^{b}	1.2^{a}	4.9^{a}	8.7^{d}	11.1^{e}	0.7^{d}	nd	nd	nd
RG 7-50	1.9^{h}	10.7^{a}	1.1^{a}	3.8^{b}	15.9^{b}	11.0^{e}	1.0^{c}	0.1^{d}	0.4b	0.1^{d}
RG 7-69	2.2^{g}	5.0^{c}	0.3^{e}	0.9^{f}	0.2^{k}	7.7^{g}	0.3^{e}	nd	nd	0.7^{b}
RG 8-22	3.6^{e}	5.7^{c}	0.8^{b}	1.9^{e}	4.5^{e}	13.2^{c}	0.7^{d}	nd	nd	nd
RG 8-25	2.2^{g}	4.2^{d}	0.6^{b}	1.8^{e}	10.9^{c}	11.1^{e}	0.5^{d}	0.6^{c}	0.3b	nd
Range	1.9-7.9	0.8-10.7	0.2-1.2	0.2-4.9	0.2-36.3	6.2-17.7	0.3-2.6	0.1-4.4	0.3-1.0	0.1-1.3
Mean	4.0	4.6	0.6	2.1	5.5	11.0	0.8	0.6	0.4	0.4
s_x	1.8	2.6	0.3	1.3	8.6	3.1	0.6	1.0	0.4	0.4

[a]GABA: gammaaminobutiric acid. nd: no detected. Means in column with different letters (a-k) are significantly different ($p < 0.05$)

fruit juice, aspartic acid appears in many fruit juices and in considerable quantities: apple cv. Red Delicious (16), grape (8), kiwi fruit (10), pear (13) and strawberry (11). Grape juice mainly contains proline (101), arginine (59), and alanine (17). Kiwi fruit contains a broad profile of amino acids (threonine, serine, proline, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, cysteine, lysine, histidine, and arginine), all of them at a maximum of 5 mg 100 ml^{-1}. Strawberry juice mainly contains alanine (14) and serine (10). Apple juice (Ackermann et al., 1992) mainly contains asparagine (89), aspartic acid (11), and γ-aminobutiric acid, alanine and serine (1 mg 100 ml^{-1}). Hellín et al. (2003) reported that *Citrus* juices contain a high amount of aspartic acid (up to 470 mg 100 ml^{-1} in grapefruit), glutamic acid (up to 280 mg 100 ml^{-1} in grapefruit), serine (up to 28 mg 100 ml^{-1} in lemon), proline (up to 295 mg 100 ml^{-1} in orange) and arginine (up to 150 mg 100 ml^{-1} in lemon and orange). In general, no glutamic acid has been reported in the juice of those fruits (except *Citrus*), while in Japanese quince was the major, in agreement with Lesinska (1987).

Ions content in Japanese quince genotypes fresh juice

The concentration of inorganic cations and anions found in Japanese quince genotypes fresh juice is shown in Table 5. Five cationic and two anionic components were found: sodium, ammonium, potassium, magnesium and calcium; fluoride and chloride. The cations and anions content of Japanese quince juice was different between the genotypes. Main cations were potassium (145-214 mg 100 ml^{-1}), calcium (8.0-15.1 mg 100 ml^{-1}) and magnesium (3.9-8.7 mg/100 ml^{-1}). Main anions were fluoride (21-122 mg 100 ml^{-1}) and chloride (1.4-9.4 mg 100 ml^{-1}). The differences between the results of ions content are statistically significatives ($p < 0.05$) (Table 5).

The low found sodium content (up to 12 mg 100 ml^{-1}) is interesting in human nutrition, since sodium is responsible for blood pressure. Baranowska-Bosiacka et al. (2017) reported the presence of potassium (249.7 mg/100 g dry weight), phosphorus (64.1 mg/100 g dry weight), calcium (22.9 mg/100 g dry weight), magnesium (16.7 mg/100 g dry weight), and sodium (2.8 mg/100 g dry weight), in *Chaenomeles japonica*. These data are lower than what we found in *Chaenomeles japonica*. Komar-Tyomnaya and Dunaevskaya (2017) reported average values of potassium (1.4 g 100 g^{-1}), calcium (290 mg 100 g^{-1}), and magnesium (92 mg 100 g^{-1}) in different *Chaenomeles* fruits species. These data are higher than what we found in *Chaenomeles japonica*.

A comparison among the inorganic ions content of Japanese quince juice and other fruits can be done considering that the content of inorganic ions of orange juice is (Hendrix and Redd, 1995): potassium (116-265 mg 100 ml^{-1}), calcium (6-29 mg 100 ml^{-1}), magnesium (10-17 mg 100 ml^{-1}), sodium (0.2-2.4 mg 100 ml^{-1}), fluoride (0.1-0.2 mg 100 ml^{-1}) and chloride (3.6-13.2 mg 100 ml^{-1}). The content of inorganic ions of apple juice is (Lea, 1995): potassium (90-150 mg 100 ml^{-1}), calcium (3-12 mg 100 ml^{-1}), and magnesium (4-7 mg 100 ml^{-1}). The content of potassium of Japanese quince juices was similar to that of Citrus juices (Hendrix and Redd, 1995). This result is important for the potassium intake.

Statistical analyses

Pearson's correlation coefficients calculated among the total constituents of Japanese quince genotypes fresh juice are presented in the Table 6. The content of sugars and organic acids was slightly correlated (0.42*). The content of cations and anions was also slightly correlated (-0.46*).

From the point of view of the horticultural breeding and plants selection it is interesting the result that no strong correlations have appeared between the constituents of the Japanese quince juice (sugars, organic acids, amino acids and inorganic ions). This result indicates the possibility for the development of some genotypes containing high amounts of sugars, amino acids and inorganic ions and low amounts of organic acids, in order that the taste of the juice is no acid. Ros et al. (2004) reported the possibility to develop varieties of Japanese quince with high content of vitamin C and phenolic compounds, and low acid content.

The analysis of principal components (Fig. 4) of the genotypes of Japanese quince shows that they are separated along axis. The composition of the juices extracted from the genotypes of the Lithuanian Japanese quince is different to the composition of the juices extracted from the genotypes of the Latvian Japanese quince, since they are separated along the horizontal and vertical axis. The different location of the genotypes of the Japanese quince in the principal components analysis (Fig. 4) indicates differences between the genotypes that are useful when varieties with specific characteristics of the juice are developed. It indicates interesting possibilities for plant selection and further improvement by horticultural breeding. Since it is need to separate factors due to the environment from factors due to the genetic of the plants, is necessary to have other approach based in the use of clones of Japanese quince. This work of research is still in development.

Table 5: Ions content (mg 100 ml^{-1}) in Japanese quince (*Chaenomeles japonica*) genotypes fresh juice (n = 3)

Genotypes	Sodium	Ammonium	Potassium	Magnesium	Calcium	Fluoride	Chloride
D 3-122	5.1^{b}	1.2^{a}	175^{c}	3.9^{f}	11.9^{d}	96^{b}	8.1^{b}
D 5-96	3.4^{d}	nd	145^{d}	4.9^{d}	13.2^{c}	nd	nd
D 10-19	4.1^{c}	0.6^{c}	158^{c}	5.3^{d}	15.1^{b}	82^{c}	4.8^{d}
NV 14-73	3.4^{d}	0.4^{d}	177^{c}	7.3^{b}	12.4^{d}	120^{a}	5.2^{d}
NV 15-97	2.6^{e}	1.4^{a}	173^{c}	8.7^{a}	13.0^{c}	101^{b}	3.7^{e}
NV 17-18	2.7^{e}	0.3^{d}	146^{d}	4.4^{e}	9.2^{e}	70^{d}	4.9^{d}
NV 18-34	5.3^{b}	0.9^{b}	150^{d}	4.4^{e}	10.3^{e}	74^{d}	8.1^{b}
NV 19-27	4.2^{c}	1.5^{a}	167^{c}	4.2^{e}	9.1^{e}	87^{c}	5.1^{d}
NV 19-44	3.4^{d}	1.0^{ab}	165^{c}	6.5^{c}	11.0^{d}	25^{e}	1.5^{f}
NV 19-64	4.3^{c}	0.7^{b}	163^{c}	5.1^{d}	8.0^{e}	80^{c}	5.1^{d}
NV 19-108	1.9^{f}	0.2^{e}	149^{d}	3.8^{f}	9.8^{e}	122^{a}	9.4^{a}
RG 1-27	3.7^{d}	0.4^{d}	188^{b}	6.6^{c}	11.7^{d}	nd	nd
RG 6-104	2.6^{e}	0.6^{c}	187^{b}	4.2^{e}	10.8^{e}	65^{d}	6.8^{c}
RG 6-111	3.7^{d}	1.2^{a}	157^{c}	5.4^{d}	13.7^{c}	nd	nd
RG 6-132	12.3^{a}	0.8^{b}	187^{b}	7.7^{b}	18.5^{a}	nd	nd
RG 7-50	3.3^{d}	0.8^{b}	182^{b}	7.4^{b}	11.5^{d}	nd	nd
RG 7-69	0.4^{g}	0.4^{d}	206^{a}	4.1^{e}	11.8^{d}	nd	nd
RG 8-22	3.2^{d}	0.3^{e}	214^{a}	5.5^{d}	12.7^{d}	21^{e}	1.4^{f}
RG 8-25	5.5^{b}	0.6^{c}	208^{a}	6.4^{c}	11.3^{d}	nd	nd
Range	0.4-12.3	0.2-1.5	145-214	3.8-8.7	8.0-15.1	21-122	1.4-9.4
Mean	4.0	0.7	174	5.6	11.8	79	5.3
s_x	2.4	0.4	21	1.5	2.4	32	2.5

nd: no detected. Means in column with different letters (a-f) are significantly different ($p < 0.05$)

Table 6: Pearson's correlation coefficients calculated among the total constituents of Japanese quince (*Chaenomeles japonica*) genotypes fresh juice

Of Japanese quince (*Chaenomeles japonica*) genotypes fresh juice		1	2	3	4
1	Total sugars				
2	Total organic acids	0.42*			
3	Total amino acids	0.13	-0.20		
4	Total cations	0.24	0.22	0.28	
5	Total anions	-0.17	0.29	-0.23	-0.46*

*$P<0.05$

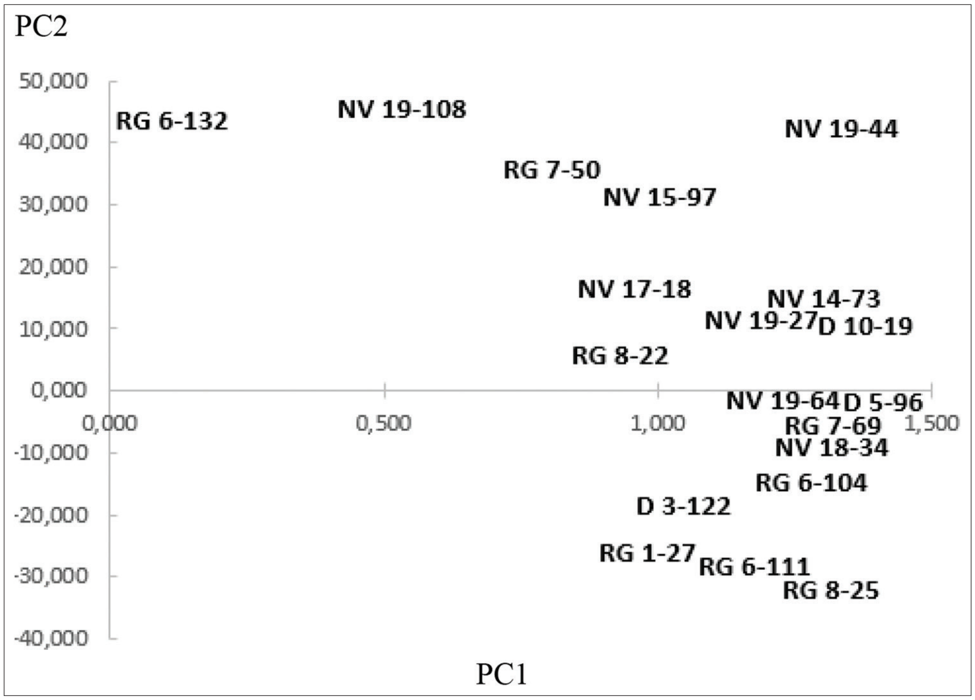

Fig. 4. Principal component analysis of the composition of Japanese quince (*Chaenomeles japonica*) genotypes fresh juice. For the Principal Component 1 (PC1) the Eigenvalues, Variance (%) and Accumulated variance (%) were 1.79, 35.9 and 35.9, respectively. For the Principal Component 2 (PC2) the Eigenvalues, Variance (%) and Accumulated variance (%) were 1.50, 30.0 and 65.9, respectively.

CONCLUSIONS

The different genotypes in the specie *Chaenomeles japonica* produce fruits rich in juice. The juice is very acidic, with a high concentration of organic acids (malic and quinic acids), a mean concentration of sugars (fructose, glucose and sorbitol), and a moderate concentration of amino acids (glutamic acid, asparagine and aspartic acid) and inorganic ions (potassium and fluoride). Due to its characteristics and composition, *Chaenomeles japonica* fruit juice should be useful for the food industry, especially as an acidulant with high antioxidant properties (Hellín et al., 2003; Ros et al., 2004), previous results on acidulant with high antioxidant properties of *Chaenomeles japonica* fruit juice. The characteristics of *Chaenomeles japonica* juice reported previously and also these described in this manuscript suggest that *Chaenomeles japonica* juice could be stabilized by High Hydrostatic Pressures (Hurtado et al., 2019a,b), with a better preservation of the thermolabile nutritional compounds. The variability in juice characteristics with statistically significative differences revealed between genotypes offer a possibility for selection and further development through plant breeding.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' contributions

This manuscript contains a part of the results from the project 'Japanese quince (*Chaenomeles japonica*) - a new European fruit crop for production of juice, flavour and fibre'. K. Rumpunen cultivated the *Chaenomeles japonica* genotypes in Sweden, which were sent to Spain. M. P. Hellín and M. J. Jordán made the extraction of the *Chaenomeles japonica* genotypes fresh juice. M. P. Hellín and J. M. Ros made the chromatographic analysis of the juices and the statistical analysis of the data. Finally, the manuscript has been prepared by J. M. Ros, with the approval of all the authors.

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