STUDIES ON *CITRUS* SPECIES FRUITS ASCORBIC ACID CONTENT USING KINETIC, SPECTROPHOTOMETRIC AND IODOMETRIC METHODS

Bungău Simona[*], Fodor Alexandrina[**], Ţiţ Delia Mirela[*], Szabó Ildikó [*]

[*]*Oradea University, Faculty of Medicine and Pharmacy, 23 N. Jiga St., Oradea, Romania Romania, e-mail: simonabungau@gmail.com*
[**]*Oradea University, Faculty of Sciences, 1 University Street, Oradea, 410087, Romania e-mail: afodor@uoradea.ro*

Abstract
Three different method (kinetic, spectrophotometric and potentiometric method) were used to determinate ascorbic acid content in different type of citric fruits. The results obtained have demonstrated that because of various environment factors (vegetal material sources area, meteorological conditions, growth and harvest period, storage conditions and the period before the analysis), the ascorbic acid contain are altering in quite large limits.

Key words: ascorbic acid, determination, titrimetric method, spectrophotometric method, potentiometric method

INTRODUCTION

Citrus species are highly variable, small to medium-size shrubs or trees that are cultivated throughout the tropics and subtropics. They are native to parts of India, China, northern Australia, and New Caledonia. All species are aboriginal, early European, or modern introductions throughout Oceania. *Citrus* is adaptable to many subtropical and tropical environments and soils and has traditionally been cultivated in homegardens together with other important species.

Citrus is primarily valued for the fruit, which is either eaten alone (sweet orange, tangerine, grapefruit, etc.) as fresh fruit, processed into juice, or added to dishes and beverages (lemon, lime, etc.). All species have traditional medicinal value.

The genus *Citrus* is further subdivided into subgenera: *Citrus* and *Papeda,* with the difference being the presence of acrid oil droplets in the pulp vesicles of *Papeda* (Klock P. 2004, Preda M. 1989).

Plants in this genus are shrubs to medium-size trees up to about 6 m in height, although some species can reach 15 m. Rootstocks can greatly affect the height of grafted trees. Trees have thin, smooth, and gray-brown to greenish bark. Most species are single-trunked with very hard wood. Canopy widths range from slender to broad, depending on species. Many cultivated species are pruned so that the canopy is as wide as the tree is tall.

Tree growth and form varies depending on the genetic background and whether the tree was established by seed or grafting.

Flowers are 2-4 cm in diameter, axillary, fragrant, single, few or cymose, and often perfect (having both functional stamens and pistils) or staminate. The calyx is 4-5 lobed and there are usually five petals with oil glands. Stamens number between 20 and 40. Petal colors range from white to pinkish in Kaffir lime to pinkish to purplish externally in citron and reddish in lemon varieties. The subglobose ovary is superior, with 8-18 locules (cavities), with 4-8 ovules per locule in two rows.

Leaves are entire, 4 to 8 cm in length, unifoliate, fairly thick, with winged petioles. Leaves are ovate, oval or elliptical, with acute to obtuse tips, and glands containing oils in glands, which are released when crushed. Young twigs are angled in cross-section, green, and axillary singlespined, while older twigs and branches are circular in crosssection and spineless.

The fruit is a hesperidium, a fleshy, indehiscent berry that ranges widely in size, color, shape, and juice quality. Citrus fruit range in size from 4 cm for lime to over 25 cm in diameter for pummelo. Fruits are globose to ovoid in shape. The fleshy endocarp is divided into 10-14 sections containing the stalked pulp and separated by thin septa. Each section contains pulp (juice vesicles) that contains a sour or sweetish watery juice. A whitish "rag" or mesocarp (also known as the albedo) covers the endocarp. In turn, the thin outer section of the leathery peel or exocarp containing many oil glands is known as the flavedo. Seeds are pale whitish to greenish, flattened, and angular (Preda M.1989, Szabo I., 2009).

Cultivated *Citrus* may be derived from as few as four ancestral species. Natural and cultivated origin hybrids include commercially important fruit such as the oranges, grapefruit, lemons, some limes, and some tangerines (Pârvu C., 2000) as is showed in table 1.

Table 1

Cytrus importants species and hybrids

IMPORTANT SPECIES	IMPORTANT HYBRIDS
Citrus aurantifolia – Key Lime	*Citrus × latifolia* – Persian Lime
Citrus maxima – Pomelo	*Citrus × limon* – Lemon
Citrus medica – Citron	*Citrus × limonia* – Rangpur
Citrus aurantium – Orange	*Citrus × paradisi* – Grapefruit *Citrus decumana*
Citrus reticulata – Mandarin Orange & Tangerine *Citrus nobilis*	*Citrus × sinensis* – Sweet Orange
	Citrus × aurantium – Bitter Orange

Citrus fruits are notable for their fragrance, partly due to flavonoids and limonoids (which in turn are terpenes) contained in the rind, and most are juice-laden. The juice contains a high quantity of citric acid giving them their characteristic sharp flavour. They are also good sources of vitamin C and flavonoids (Mozăceni A.V, 2002)

Citrus limon

ASCORBIC-ACID Fruit 5,208 - 5,566 ppm

Citrus aurantifolia

ASCORBIC-ACID Fruit 291 - 4,444 ppm

Citrus auranti

ASCORBIC-ACID Fruit 420 - 3,947 ppm Leaf 3,000 ppm;

Citrus reticulata

ASCORBIC-ACID Fruit 280 - 3,684 ppm

Citrus paradisi

ASCORBIC-ACID Fruit 337 - 3,862 ppm

Citrus medica

ASCORBIC-ACID Fruit 884 - 11,130 ppm Fruit Juice 300 - 445 ppm

MATERIAL AND METHOD

Extraction of ascorbic acid was made from fresh fruits with a solution of oxalic acid, a solvent commonly used for vitamin C (Bungău S.2002, 2003).

Vegetal materialul

Fresh citrus fruits were analyzed for their day of purchase. Ascorbic acid solution samples are obtained by extraction of plant product with oxalic acid. For all the fruits analyzed, taking the fruit and get a juice by squeezing. 50 g fruit pulp is mixed with 30 mL 1% oxalic acid and shaking the flask mix of 10 in 10 minutes, 2-3 seconds, requiring an hour minimum time of contact. Then filter through a frit Por 160, and the filtrate is centrifuged one hour at 4000 rpm to separate solids, insoluble (Bungău S., 2003). The extract obtained is brought to 100 mL volumetric flask.

Procedure

Each reaction mixture had a total volume of 25 mL. The order of adding solutions in the reaction vessel, concentration and initial basic solution of the mixture are presented in Table 2. The initiation of reaction was performed by rapid injection of 0.5 mL hydrogen peroxide solution 3.10^{-2} M, the zero moment of the reaction being defined as the moment of rapid injection of 0.5 mL hydrogen peroxide solution.

Table 2

Composition of reaction mixture in order reagents added (Bungău S.2002, 2003)

Chemical Species	Volume added (mL)	Initial concentration in the reaction mixture (mol/L)
KI 0,15 M	1	$6\cdot10^{-3}$
$HClO_4$ $3{,}75\cdot10^{-2}$ M	5	$7{,}5\cdot10^{-3}$
Ascorbic acid $2\cdot10^{-3}$ M	between 0,1-2,0	between $8\cdot10^{-6}$-$1{,}6\cdot10^{-4}$
H_2O	between 18,4-16,5	
H_2O_2 $3\cdot10^{-2}$ M	0,5	$6\cdot10^{-4}$

Apparatus

Evolution of the concentration of iodide was made by potentiometric measurements (Bungău S.2002, 2003). Measure redox electrode consists of a Pt plate immersed in the reaction mixture, the reference electrode is a saturated calomel one. The process was followed in a thermostated vessel equipped with stirring. To register potential-time curves was used a potentiometer Digitronix DXP-2040 (Seiko) connected through data acquisition card to a computer.

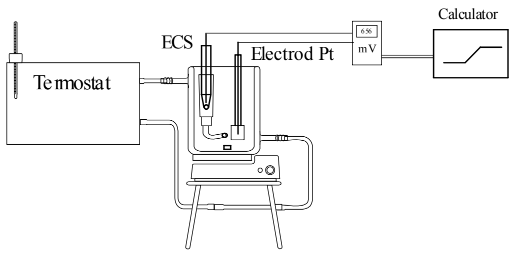

Fig.1. Equipment scheme (Bungău S., 2003)

RESULTS AND DISSCUSION

The experimental data were processed with data acquisition program Table Curve and Origin 5.0.

Table 3 includes data from the literature (Klock P., 2004, Schirarend C.2001) on vitamin C content of citrus species and hybrids.

Table 3

Vitamin C content of citrus species and hybrids (data from literature)

NR. CRT.	NAME	ASCORBIC ACID CONTENT IN [PPM] / 100 G FRESH FRUIT
1.	*Aurantifolia*	4,450
2.	*Aurantia*	4,150
3.	*Limon*	5,566
4.	*Paradisi*	3,862
5.	*Reticulata*	3,684
6.	*Sinensis*	4,150

The experimental values obtained in this work by the proposed titration method were compared (figure 2) with values obtained by potentiometric and spectrophotometric method (Table 4).

Table 4

Comparative data, chemometric and recovery criteria for ascorbic acid obtained from fruit in mg/50 g plant material (fresh fruit)

Fruit	**Potentiometric method**		**Spectrophotometric method**		**Titrimetric method**		**Rec. %**
	Content	s % n=5	Content	s % n=5	Content	s % n=5	
Grapefruit [9] *Citrus decumana* *Citrus x paradisi*	19.6	0.5	19.6	0.3	19.7	1.1	99.4
Mandarin – tangerine *Citrus nobilis* *Citrus madurensis* *Citrus reticulata*	26.75	0.4	26.6	0.4	26.7	1.2	100.2
Orange [9] *Citrus aurantium* *Citrus sinensis*	28.17	0.8	28.25	0.8	28.2	0.7	99.8
Lemon [9] *Citrus limon*	24.53	0.7	24.4	0.4	24.5	1.7	100.1
Bergamot *Citrus bergamia*	19.6	0.8	19.5	0.5	19.6	1.1	99.6
Chitra *Citrus aurantifolia*	19.4	0.7	19.5	0.8	19.5	1.1	99.7
Pomelo *Citrus maxima*	20.4	0.8	20.4	0.8	20.4	0.8	100.0
Kiwi *Actinidia chinensis*	15.1	0.3	15.2	0.5	15.2	0.8	99.3

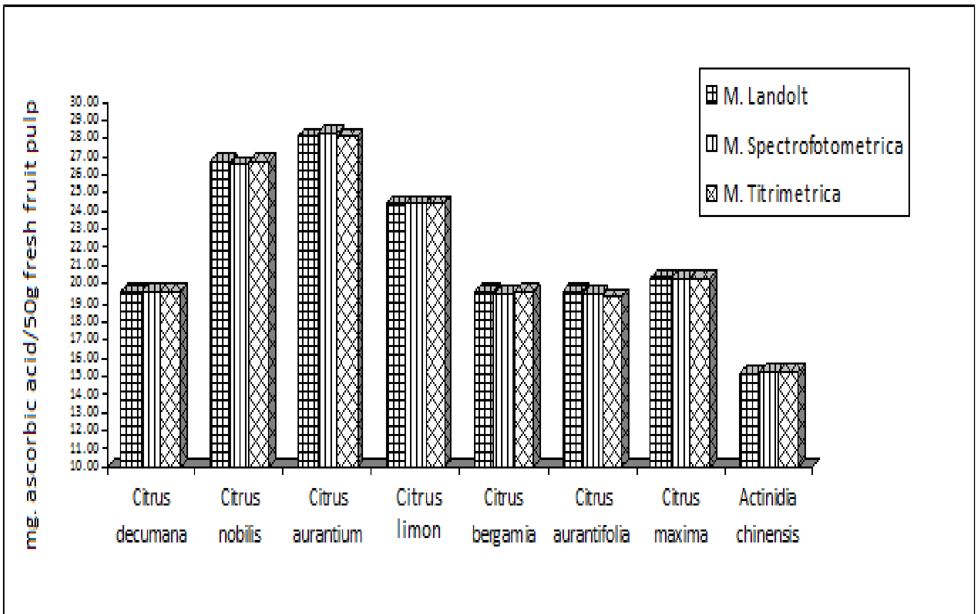

Fig.2. The results obtained by three methods for presented citrus

CONCLUSION

The kinetic measurements based on Landolt type system hydrogen peroxide – iodide (in acidic media), spectrophotometrical measurements and iodometric measurements were used for ascorbic acid determination in different citric fruits. All the three type of measurements have shown very similar results. The results obtained were used in statistic studies that have demonstrated that the difference between the scattering and averages for the two methods (kinetic and spectrophotometric) are not significant to those obtained from the so cold "true values" obtained using STAS method (iodometric titration), so the three methods have very closer precision and can be used successfully in that type of determination.

Also, all the values obtained were used in studies concerning ascorbic acid content in fruits with high ascorbic acid content and to demonstrate that, because of many factors (provenience area of vegetal material, meteorological conditions, sowing period, period of harvesting, the storage conditions and the period before the analysis etc.), data obtained varied between quite large limits.

In conclusion, we can say that the limits in which ascorbic acid concentration are varying are quite large and to present an exactly contain value is not correct, just in case that all environmental factors that can interferes are mentioned.

Also, according to experimental data from Table 4, we can see that, of citrus, tangerine, orange and lemon have a higher vitamin C content.

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