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Physalis peruviana L. production in conditions of the Crimean southern sea shore

ABSTRACT

Intensive climate changes entail the possibility of effective introduction of some tropical plants in the northern hemisphere. Introduction of *Physalis peruviana*, Indian selection, along the Crimean southern sea shore in 2022-2024 revealed the perennial growth character of plants, fully flowering since the first decade of June, starting fruiting in late June, full fruit ripening in the second decade of July, and the possibility of partial ovaries shedding during the period of high temperatures (27-30°C) from mid-July to August. In the mentioned conditions, fruits, leaves and calyx showed high antioxidant status. The efficiency of different extraction methods in polyphenol determination generated high prospects of dry fruit, leaves and calyx extraction with 70% ethanol at 80 oC compared to the application of water, 50% methanol and 98% ethanol extraction at room temperature. The fruits demonstrated similar values of the ascorbic acid (48.5 mg/100 g f.w.), mono- and di-saccharide (35 and 51% per d.w.) content, and higher levels of dry matter (20.2%), phenolics (21.2 mg GAE/g d.w.) and carotenoids (4.51 mg/100 g f.w.), compared to the Colombian fruit randomly sampled at the local supermarket, but had significantly lower values of fruit titratable acidity (49.0 compared to 86.2 mg-eq citric acid/g d.w.). Mineral composition of plants revealed typical Fe, Zn, Cu, Mn and Co distribution between fruit, leaves and calyx with calyx being the richest source of Fe (288.7 mg/kg d.w.). The results indicate high prospects of *P. peruviana* cultivation in Crimea.

KEYWORDS:

***Physalis;* territory product innovation; nutritional value, antioxidants; microelements**

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Производство *Physalis peruviana* L. в условиях культуры Южного Берега Крыма

РЕЗЮМЕ

Интенсивное изменение климата благоприятствует интродукции южных растений в более северные регионы. Интродукция перуанского физалиса *Physalis peruviana*, индийской селекции на южном побережье Крыма в 2022-2024 выявила, что растения ведут себя как многолетние, с началом массового цветения с первой декады июня, начале плодоношения с конца июня, полного созревания плодов со второй декады июля, а также установлена возможность частичного опада соцветий в период высоких температур (27-30°C) со середины июля до августа. Плоды, листья и чехлики плодов растений, выращенных в условиях Крыма проявляли высокую антиоксидантную активность. Проведена оценка эффективности различных условий экстракции на содержание полифенолов в плодах, листьях и чехликах. Наиболее высокие показатели содержания полифенолов были получены при использовании предварительно высушенных образцов и экстракции 70% этиловым спиртом при 80°C в течение часа по сравнению с применением дистиллированной воды, 50% метанола и 98% этанола при комнатной температуре. Содержание аскорбиновой кислоты в плодах крымского физалиса и колумбийского из супермаркета были сходны (48.5 мг/100 г сырой м.), так же, как и содержание моно- и ди-сахаров (35 и 51% сухой м.), однако, крымский физалис характеризовался более высоким содержанием сухого вещества (20.2%), полифенолов (21.2 мг-экв ГК/г сухой м.) и каротиноидов (4.51 мг/100 г сырой массы), в то время как уровень титруемой кислотности в Крымском физалисе составил 49.0 мг-экв. лимонной кислоты/г сухой массы по сравнению с 86.2 мг-экв лимонной кислоты/г сухой массы для Колумбийского физалиса. Анализ минерального состава плодов, листьев и чехлика физалиса (Fe, Zn, Cu, Mn и Co) выявило аномально высокое содержание железа в чехликах плодов (288.7 мг/кг сухой массы). Полученные результаты свидетельствуют о перспективности выращивания *P. peruviana* в открытом грунте на южном побережье Крыма.

КЛЮЧЕВЫЕ СЛОВА:

***Physalis*; инновационный продукт, антиоксиданты; микроэлементы**

1. Introduction

Physalis peruviana L. belongs to the Solanaceae family and is highly valued for the unique biochemical composition, high antioxidant activity and high levels of carotenoids, vitamin C, polyphenols, protein, and dietary fiber [1-3]. Colombia, Kenia, Zimbabwe, Australia, New Zealand, India, and Ecuador are the most important *Physalis* producers in the world, though high level of adaptability of these plants allows to effectively grow them in other countries, such as Peru, Portugal, USA, Brazil, Venezuela, Countries of the Central America, Egypt, Indonesia, Israel, and Great Britain. *P. peruviana* is actively cultivated in Turkey [4,5].

In tropical areas, *Physalis* is a perennial plant, contrary to the continental climate where it is an annual plant. *P. peruviana* was successfully grown in greenhouse conditions in Czech Republic [6,7] with the ascorbic acid reaching 66-102 mg/100 g f.w. and flavonoids 405-526 mg/100 g.

Due to high antioxidant activity, *Physalis peruviana* L. provides high human antioxidant status due to the consumption of polyphenols, flavonoids, tannins, alkaloids, vitamins C, B3, B6, phytosterols, vitanolids, and physalins. *P. peruviana* fruit record significant antimicrobial activity against gram-positive and gram-negative bacteria, anti-fungal activity against *Aspergillus niger* and *Candida albicans* [8], and anti-carcinogenic activity against lung cancer cells A549, and Caco-2 cells of colon adenocarcinoma [9]. Powerful anti-carcinogenic activity is demonstrated for *β*-carotene, vitamins C and P, vitanolids and physalins, the latter recording also anti-inflammatory, anti-microbial, immunomodulatory, and anti-parasitic properties. Leaves, stems and all plant showed cytotoxic and anti-proliferative effect on different cancer cell lines, such as colon, lung, breast and liver [10-14]. *P. peruviana* L. fruit calyx is successfully used in traditional medicine due to its anti-carcinogenic, antimicrobial, antipyretic, diuretic, anti-inflammatory, and immunomodulatory properties [15]. Fruits are rich in Fe, Ca, Cu, Mn, P, and Zn [16,17].

Physalis biometrical and biochemical characteristics are governed by genetic factors, growth conditions, sowing data, and greatly depend on growth stimulators supply [18-21]. Climate change is another important factor affecting cultivation efficiency especially in temperate conditions.

The present investigation was aimed at evaluating the efficiency of *P. peruviana* cultivation in conditions of the Crimean southern sea shore using Physalis seeds produced by the Indian breeders.

2. Materials and Methods

2.1. Growing Conditions and Experimental Protocol

The research was conducted on *Physalis peruviana* accession, provided by the Veer Kunwar Singh College of Agriculture, India, in 2022-2024 from May to October at the experimental open field of Nikitsky Botanic Garden, situated at the shore of the Black Sea (44°31' N., 34°15' E, 200 m above sea level), characterized by a Mediterranean-type dry subtropical climate, with a mean year temperature of 13.5±1.5°C and average daily temperature above 5°C from the beginning of March to the end of October (Table 1). The experiment was carried out in an agro-brown, slightly carbonate, light clay soil with 3.0% humus, 5.4% carbonates and pH of 7.8. The annual precipitation reached 560-619 mm with a typical predominance in winter-spring period.

Table 1. Mean temperature and total rainfall during the experiment
Таблица 1. Среднемесячные температуры и количество осадков в течение эксперимента

Month	2022		2023		2024	
	Mean temperature (°C)	Rainfall (mm)	Mean temperature (°C)	Rainfall (mm)	Mean temperature (°C)	Rainfall (mm)
May	14.9	24.5	15.7	92.3	14.4	5.9
June	22.9	83.5	20.9	73.0	23.6	40.4
July	24.3	22.3	24.2	20.6	28.5	17.7
August	26.0	20.4	27.2	4.0	25.8	3.1
September	19.6	12.1	22.0	0.4	25.8	64.7

Sowing was practiced on 11 May. The results were expressed as means of the three-year data. The morphological determinations were performed on 10 plants per replicate.

At the level of the productive moisture >40 mm, watering was achieved once a week from May to June. In July-August watering was practiced twice a week according to the production moisture level of 20 mm. Fertilizers were not supplied during the vegetation period, while in Autumn organic mineral fertilizer 'Cherny Zhemchuk' was applied at a dose of 15 g/m^2. The chemical composition of the Fertilizer is presented in Table 2.

Table 2. Chemical composition of 'Cherny Zhemchug' Fertilizer
Таблица 2. Химический состав удобрения Черный Жемчуг

Parameter (Параметр)	Concentration (концентрация), %
Organic matter (Органическое вещество)	>70
SiO_2	7.48-12.98
Fe_2O_3	4.36-5.23
CaO	3.65-4.45
MgO	1.29-2.12
K_2O	2.85-3.5
Na_2O	0.23-0.59
P_2O_5	0.06-0.12
SO_2	0.14-0.30
Total N	0.35-0.77
Water soluble Mn (Водорастворимый Mn)	0.002-0.007
Water soluble B (Водоракстворимый B)	0.30-0.50
pH	8.0
Water content (содержание влаги)	<7-10

This investigation was carried out in accordance with the introduction and selection testing methodology of aromatic and medicinal plants [22]. Morphometric determinations of leaves and fruit were performed using light microscope MSP-1. PC ImageTool v. 2.03 UTHSCSA was used for the metric parameters testing.

2.2. Sample Preparation

After harvesting leaves and fruit were separated and individually weighed. The samples were homogenized, and fresh homogenates were used for the determination of the ascorbic acid. Sample aliquots of leaves and fruit were dried at 70°C to constant weight and used for the determination of total polyphenol content (TP), and mineral composition.

2.3. Biochemical and Elemental Composition Analyses

2.3.1. Ascorbic acid (AA)

The ascorbic acid content was assessed using the manual titration method based on the interaction of the ascorbic acid with sodium 2,6-dichlorophenol indophenolate (Tillman's reagent) [23].

2.3.2. Total Polyphenols (TP)

Total polyphenols were determined in 70% ethanol extract using the Folin–Ciocalteu colorimetric method as previously described [24]. Half a gram of dry *physalis* homogenates of leaves and fruit were extracted with 20 mL of 70% ethanol at 80°C for 1 h. The mixture was cooled down and quantitatively transferred to a volumetric flask, and the volume was adjusted to 25 mL. The mixture was filtered through a filter paper, and 1 mL of the resulting solution was transferred to a 25 mL volumetric flask, to which 2.5 mL of saturated Na_2CO_3 solution and 0.25 mL of diluted (1 : 1) Folin–Ciocalteu reagent were added. The volume was brought to 25 mL with distilled water. One hour later the solutions were analyzed through a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA), and the concentration of polyphenols was calculated according to the reaction mixture absorption at 730 nm. As an external standard, 0.02% gallic acid was used. The results were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE g-1 d.w).

2.3.3. Carotenoids

Determination of carotenoid content in physalis fruit was achieved spectrophotometrically after chromatographic separation of carotenoids [24]. Half a g of homogenized fruit was extracted with small portions of acetone until color disappearance. The combined extract was diluted with 9 mL of hexane and washed 4–5 times with distilled water to remove traces of acetone. The residual extract was quantitatively transferred to a volumetric flask, and the volume was adjusted to 10 mL with hexane. The resulting extract was mixed, filtered through a small portion of anhydrous Na_2SO_4 and subjected to the analysis. The separation of carotenoids was achieved using quantitative thin-layer chromatography on Whatman 3A chromatographic paper in hexane–acetone, 10 : 0.5. Appropriate zones of carotenoid compounds were cut out and extracted with 3 mL of hexane. The determination of carotenoid content in physalis fruit was performed using appropriate specific absorption $E^{1\%}_{1\ cm}$ for β-carotene (2580 at λ = 450 nm), and lutein (2560; λ = 447 nm) through a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA). The internal standards were β-carotene and lutein from Sigma Inc. (Japan).

2.3.4. Sugars

Monosaccharides were determined using the ferricyanide colorimetric method, based on the reaction of monosaccharides with potassium ferricyanide [25]. Total sugars were analogically determined after acidic hydrolysis of water extracts with 20% hydrochloric acid. Fructose was used as an external standard. The results were expressed in % per dry weight.

2.3.5. Titratable acidity (TA)

Titratable acidity was determined by titration of a water extract with a 0.1 M NaOH solution using a ionomer Expert-001 (Econix corp., Russia) and phenolphthalein as an indicator. The results were expressed as percentage of citric acid [26].

2.3.6. Nitrates

Nitrates were assessed using an ion-selective electrode on ionomer Expert-001 (Econix Inc., Russia). One gram of fresh physalis leaves was homogenized with 50 ml of distilled water. Forty-five ml of the resulting extract were mixed with 5 ml of 0.5 M potassium sulfate background solution (necessary to adjust the ionic strength) and analyzed through an ionomer for nitrate determination.

2.3.7. Total Dissolved Solids (TDS)

TDS was determined in water extracts using TDS-3 conductometer (HM Digital, Inc., Seoul, Korea) and expressed in mg kg-1 d.w.

2.3.8. Mineral composition

The determination of element contents (Co, Cu, Fe, Mn, Zn) in plants was conducted in triplicate on the dried, homogenized, mixed samples using atomic absorption spectrometry on Shimadzu GFA-7000 spectrophotometer (Shimadzu, Kioto, Japan) after sample digestion under the temperature increase from 20 up to 425°C and appropriate solution of the residue in 3% HNO_3 [27].

2.4. Statistical Analysis

All determinations were done in triplicate. The data were processed by the analysis of variance and mean separations were performed through Duncan's multiple range test, with reference to 0.05 probability level, using the SPSS software version 29 (Armonk, NY, USA). Data expressed as percentages were subjected to angular transformation before processing.

3. Results and Discussion

3.1. Temperature trends

Significant warming has been typical for the last years. Indeed, comparison of the sum of effective temperatures during the experiment with the long-term average values indicated dramatic changes of the values especially significant in spring and the beginning of summer (Table 3, Fig. 1).

In these conditions the first Physalis shouts appeared on 26 May, most of shoots on 30 May, full fruiting phase since 30 August. Plant height at this stage of development reached 82-89 cm accompanied by a full plant flowering. Plant generative development lasted up to the middle of October. In 2024, the phenophase of general regrowth of perennial plants began on 15 May.

Table 3. The sum of effective temperatures >10°C during the P. peruviana vegetation period of 2023-2024
Таблица 3. Сумма эффективных температур >10°С в период вегетации P. peruviana в 2023-2024)

Month месяц	Decade Декада	Long-term average value Усредненные долговременные показатели)	The sum of effective temperatures (сумма эффективных температур)	
			2023	2024
April	3rd	30	80	216
May	1st	74	110	266
	2nd	134	165	308
	3rd	220	234	410
June	1st	314	371	540
	2nd	428	509	677
	3rd	551	619	817
July	1st	684	765	1002
	2nd	825	900	1213
	3rd	992	1062	1386
August	1st	1147	1229	1538
	2nd	1291	1377	1701
	3rd	1435	1559	1877
September	1st	1541	1672	2009
	2nd	1636	1779	2135
	3rd	1712	1850	2252
October	1st	1771	1924	2337
	2nd	1811	1966	2379

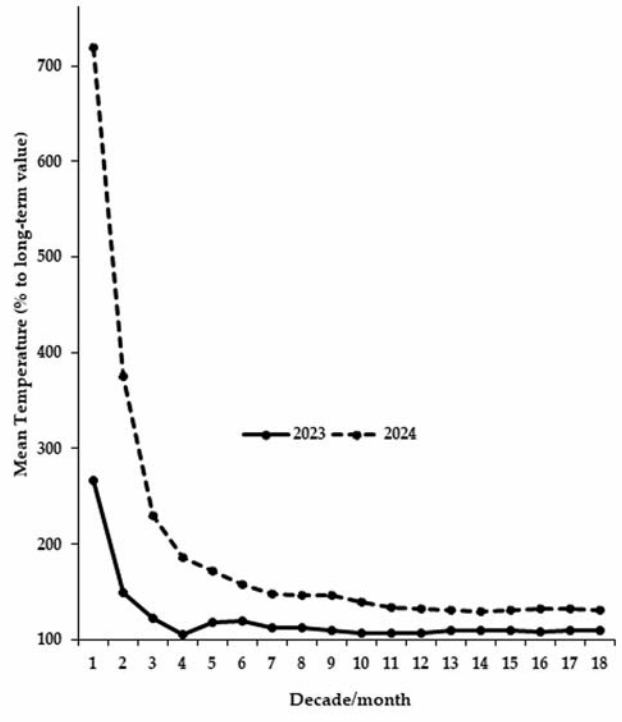

Fig. 1. Dynamics of the sum of effective temperatures at the Southern shore of the Crimea in 2023, 2024
Рис. 1. Динамика изменения показателя эффективных температур на южном побережье Крыма в 2023, 2024)

3.2. Morphological parameters

The levels of resistance and plasticity of plants to specific growth conditions are governed by plant vitality, morphological parameters of the main reproductive organs, and yield in the annual cycle of growth and development.

In conditions of dry subtropical climate of the southern Crimean sea shore morpho-biological parameters revealed that in field conditions *P. peruviana* maintains a perennial type of development. Plants of the 3rd year form annual shoots which become semi-woody in its lower tier. Plant height reached 82 cm at the third year of vegetation with the value 77.2 cm corresponding to the mean value of the three-year experiment. The length of the renewal shoots reached 68-73 cm (Table 4). During growth and development *P. peruviana* stem branched and up to 7 well- developed generative shoots were formed on the bush (mean value is 5.3).

In conditions of the Crimean southern sea shore the phenophase of mass flowering begins in the first decade of June, the beginning of fruiting – from the end of June; mass fruit ripening – in the second decade of July. Temperature greatly affects the growth and development of plants. Biological peculiarities of *P. peruviana* indicate that it belongs to the short day plants. At the Crimean southern sea shore in the middle of July-August in conditions of high temperature (27-30°C) the physalis vegetation period and the period of fruiting expand. The lower fruit located in the places of initial stem branching ripen first followed by fruit on the periphery of the bush. Signs of fruit ripening are the lightening and drying of the calyx as well as the fruit acquiring the color characteristic of the variety. Ripe fruit mostly fall off and in dry weather may remain fresh for up to 10 days. As a result, the period of generative development and fruit ripening continued in the Mediterranean climate of the Crimea until the end of October.

P. peruviana, due to its sweet fruit and its economically valuable characteristics, belongs to the group of berry Physalis. At the stage of ripening, fruit is juicy yellow round, 16.5-24.7 mm diameter (mean value 20.03 mm), and 3.05-6.26 g weight (mean value 4.36 g), with the number of seeds from 133 in small fruits to 145-213 in the large ones (Table 4). The latter parameters of the Crimean *P. peruviana* were 1.8-2.2 times lower than those recorded in Kenia, Colombia and South Africa which may be attributed to different ecotypes and environmental conditions [28].

Table 4. Morphological parameters of the Physalis peruviana L. in conditions of the Crimean southern sea shore
Таблица 4. Морфологические показатели растений физалиса перуанского в условиях Южного берега Крыма

Parameter (параметр)		M±SD	CV (%)	Minimum	Maximum
Plant mass, (g) (Масса растения, г)		628.8±128.0	20.3	438.2	805.4
Plant height (cm) (Высота растения, см)		77.2±3.1	4.0	73	82
Number of generative shoots (количество генеративных побегов)		5.3±1.3	24.5	3	7
Number of inflorescences per plant (количество соцветий на растении)		32.6±5.0	15.3	24	42
Leaf weight (g per plant) (масса листьев, г/растение)		319.2±54.8	17.2	236.6	395.0
Aboveground mass yield (kg/plant) (Урожайность надземной массы, кг/растение)		1.98±0.23	17.9	1.72	2.40
Foliage (%) (% облиственности)		51.1±2.3	4.5	47.8	54.3
Leaf length (cm) Длина листа (см)	Lower tier (Нижний ярус)	13.5±0.9 a	6.7	12.5	15.2
	Higher tier (Верхний ярус)	10.7±1.6	15.0	8.8	12.7
Leaf width (cm) Ширина листа (см)	Lower tier (Нижний ярус)	13.4±1.1	8.2	11.5	15.3
	Higher tier (Верхний ярус)	9.2±1.6	17.4	7.3	11.1
Leaf surface area (cm^2) (Поверхность листа, $см^2$)	Lower tier (Нижний ярус)	160.8±34.5	21.5	113.0	240.2
	Higher tier (Верхний ярус)	82.1±26.4	32.2	54.2	120.3
Petiole length (cm) (Длина черешка, см)	Lower tier (Нижний ярус)	6.9±0.8	11.6	5.5	8.0
	Higher tier (Верхний ярус)	3.5±1.4	40.0	2.1	5.8
Fruit diameter (mm) Диаметр плода, мм		20.03±2.68	13.4	16.5	24.7
Fruit cross-sectional area (cm^2) (Площадь поперечного сечения плода $см^2$)		3.02±0.61	20.2	2.44	4.16
Fruit weight (g) (Масса плода, г)		4.36±0.88	20.2	3.47	6.26
Calyx weight (g) (масса чехлика, г)		0.29±0.04	13.8	0.20	0.36
Seed productivity per one fruit (seed number) Семенная продуктивность в расчете на один плод, шт.		122.1±36.9	30.24	90	213
Mass of 1000 seeds, (g.) (Масса 1000 семян, г)		1.19±0.02	1.68	1.17	1.21
Seed length (mm) (Длина семени, мм)		2.08±0.17	8.2	1.8	2.4
Seed width (mm) (Ширина семени, мм)		1.64±0.14	8.5	1.5	2.0
Seed cross-sectional area (mm^2) Площадь поперечного сечения семени, $мм^2$		2.78±0.45	16.2	2.2	3.9

Lack of significant differences in leaf size and fruit weight was indicated between the Crimean and Indian Physalis plants [18-20]. On the other hand, literature data indicate the possibility of a significant fruit size and yield improvement due to the growth stimulator utilization [18].

P. peruviana leaves are cordate-shaped, developed on renewal branches and shoots differing 1.6-2 times by their size between the lower and higher tiers. The largest leaf surface was recorded at the lower tire of the renewal shoots reaching 240 cm2 compared to 120 cm^2 surface of leaves from the branching shoots of the lower tire (Table 4). Leaf shrinkage coefficient reached 4.85-5.10. According to literature data *P. peruviana* leaves and stems record cytotoxic and anti-prolipherative effects on different cell lines, such as colon cancer cells, chronic myeloid leucosis, lung, breast and liver [10-13].

Physalis calyx is also highly valued both in human health maintenance and plant development. These fused sepals protect fruit from unfavorable climatic conditions from the moment of fruit formation to fruit development and ripening. Its weight composes only 6.65% of fruit weight at a mass ripening stage (less than 0.3 g). During the first 20 days of fruit development calyx supplies fruit with carbohydrates [29].

3.3. Biochemical characteristics

3.3.1. Fruit quality

The most important physalis fruit characteristics are antioxidant parameters, mono- and disaccharide, and carotenoid content (Table 5).

GAE/g d.w.) [38], Peru (4.15 mg GAE/g d.w.) [33], and Northern Chili, Atacama desert (4.43 mg GAE/g d.w.) [8].

Samples of the Crimean *physalis* and *physalis* fruit obtained from the local supermarket (Colombia origin) recorded significantly higher levels of the polyphenol content (Table 5). Such an inconsistancy may be connected with the fact that polyphenol

Table 5. Physalis fruit quality parameters (means of the 2023-2024)
Таблица 5. Показатели качества плодов физалиса, среднее за 2023-2024)

Parameter (Параметр)	Nikitsky Botanic Garden Никитский ботанический сад		Columbia (imported fruit) Колумбия, импорт
	M±SD	Concentration range	
Dry matter (%) (сухое вещества, %)	20.2±1.9 a	18.3 – 22.1	17.75±1.0 a
Polypohenols (TP) (mg GAE/g d.w.) Полифенолы (ТР), мг-экв ГК/г сухой массы)	21.2±1.9 a	19.3 – 23.1	17.5±1.2 b
Flavonoids (mg-eq quercetin/g d.w.) (Флавоноиды, мг-экв. Кверцетина/г сухой массы)	1.5± 0.1 a	1.4 – 1.6	1.4±0.1 a
Total carotenoids (mg/100 g f.w.) (Общее содержание каротиноидов, мг/100 г сырой массы)	4.51±0.31 a	4.20 – 4.81	3.04±0.30 b
Ascorbic acid (mg/100 g f.w.) (Аскорбиновая кислота, мг/100 г сырой массы)	48.5±3.5 a	45.0 - 52.0	47.0±3.7 a
Titratable acidity (TA) (mg-eq citric acid/g d.w.) (Титруемая кислотность (ТА, мг-экв лимонной кислоты/г сухой массы)	49 ±3.2 b	45.8 - 52.2	86.2±6.6 a
Monosaccharides (% per d.w.) (Моносахара, % на сухой вес)	35±3.0 a	33.0 – 38.0	30.4±2.9 a
Total sugar (% per d.w.) (Общий сахар, % на сухой вес)	51±3.9 a	47.2 – 55.0	45.6±4.1 a
Total Dissolved Solids (mg/g d.w.) (Водорастворимые соединения, мг/г сухой массы)	43.6 ±3.3 b	41.3 – 46.9	86.0±6.8 a
Nitrates (mg/g d.w.) (Нитраты, мг/г сухой массы)	2.8 ±0.1 a	2.7 – 2.9	1.2±0.1 b

Values in lines with similar letters do not differ statistically according to Duncan test at p<0.05 Значения в рядах с одинаковыми индексами статистически не различаются согласно тесту Дункана при p<0.05.

Values presented in Table 5 indicate high content of the ascorbic acid, polyphenol, carbohydrates, and high total antioxidant activity of Physalis fruit which is in accordance with the literature data [14]. Thus, vitamin C concentrations were close to the values described for physalis fruit of India [30,31], Argentina [32], and Peru [33], though according to the Kumar data [19] the ascorbic acid biosynthesis may be enhanced at least by 17% in Physalis fruit under growth stimulator supply.

Contrary, total sugar content in the Crimean Physalis was more than 1.5 times lower than the values described for the Peru *physalis* fruit (75-85% d.w.) [33]. The latter phenomenon may relate to significantly higher altitude of plant growth in Peru (>3000 m above the sea level) compared to the Nikitsky Botanic Garden (200 m above the sea level) as well as significant differences in plant ecotypes.

3.3.2. Polyphenol content

P. peruviana provides a wide spectrum of polyphenols, including gallic, p-cumaric, hydroxybenzoic acids, and kaempherol. These compounds increase glucose tolerance and antioxidant status of diabetic rats, decreasing the level of oxidative stress in brain [34,35]. According to literature data, mean levels of polyphenols in *P. peruviana* fruit vary from 0.3 mg GAE/g d.w. in Poland [36] to 7.3 mg GAE/g d.w. in Turkey [37] and with intermediate values typical for Portugal (0.4-0.6 mg

levels in plants may be greatly affected by environmental factors, genetics, and the chosen method of the extraction conditions [39]. Thus, to explain the obtained phenomenon of great discrepancy between the present results and physalis polyphenol levels published earlier special investigation has been achieved based on a comparison between different extraction methods both on dried and fresh physalis fruit.

3.3.3. Effect of extraction conditions on the results of the polyphenol (TP) levels

Optimization of polyphenol analysis for different plant species and organs solicits the necessity of the development of individual extraction conditions for the provision of maximum extraction efficiency. The complexity of the problem, intensively discussed by numerous authors [40,41], includes the possibility of polyphenol degradation both under elevated temperature and as a result of significant duration of the extraction process at moderate temperatures due to enzymatic degradation, enhancement of the determined polyphenol levels by virtue of polyphenol complexes decay, and deactivation of the enzymes during heating. Besides, solvent polarity greatly affects the efficiency of polyphenol extraction depending on the chemical structure of the target compounds, while small sampling size arises the problem of probe representativity confirming

great advantages of dry fruit power utilization compared to fresh samples. Most of the published *physalis* data was obtained on fresh fruit homogenates with the determined concentration range of 0.3-7.3 mg GAE/g d.w. [2,33,36,37,42,43]. Guine et al. [38] demonstrated significant variations in the results of polyphenol determination in *physalis* fruit under water, acetone, and methanol supply for the extraction. Taking into account the high toxicity of methanol and acetone, diluted ethanol at 80℃ was used in the present investigation as the main modification of the extraction process. The efficiency of 50% methanol, water, and 98% ethanol extract utilization at room temperature was also evaluated for a comparison. And all the analyses were achieved on dry and fresh *physalis* fruit.

The data presented on Fig. 2 indicate several peculiarities of polyphenol analysis.

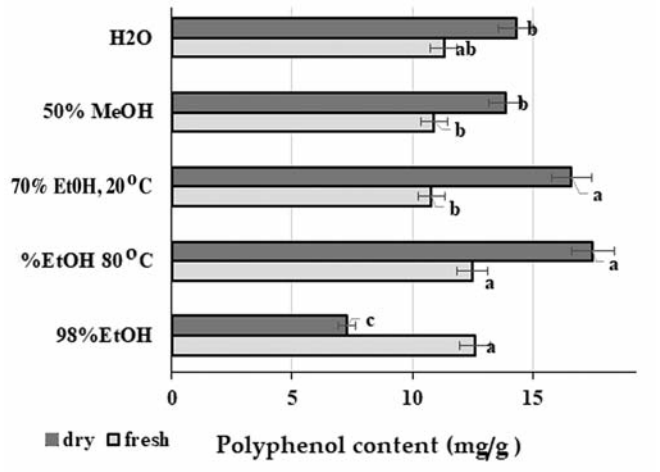

Fig. 2. Fruit polyphenol levels before and after sample drying at 70°C. For each target (dry-fresh) values with similar letters do not differ statistically according to Duncan test at p<0.05
Рис. 2. Уровни полифенолов, полученные без и после высушивания образцов при 70°С. Значения с одинаковыми индексами для сухих и свежих образцов статистически не различаются согласно тесту Дункана при p<0.05

Thus, except 98% ethanol, all solvents used for the extraction provided significantly higher levels of polyphenols for dry fruit powder compared to fresh fruit with the highest levels typical for 70 % ethanol both at 80°C and room temperature. High polarity of water and 50% methanol extracts recorded lower values of polyphenol content in dried fruit compared to 70% ethanol, while fresh homogenates were characterized by relatively close values of phenols. Supposedly, the beneficial effect of the preliminary fruit drying is connected with the deactivation of the enzymes participating in polyphenol degradation. Contrary, 98% ethanol application provided higher polyphenol levels in fresh fruit compared to preliminary dried ones.

Comparison of the results for the *physalis* fruit with those obtained for dry leaves and calyx revealed even higher differences in the results of polyphenol determination depending on the chosen solvent (Fig. 3). The data presented on Figure 3 indicate that application of 70% ethanol for polyphenol extraction of physalis leaves under elevated temperature provided the best results with the polyphenol concentrations decreasing according to: 70% ethanol, 80℃ > 70% ethanol, 20℃ > 50% methanol > water > 98% ethanol.

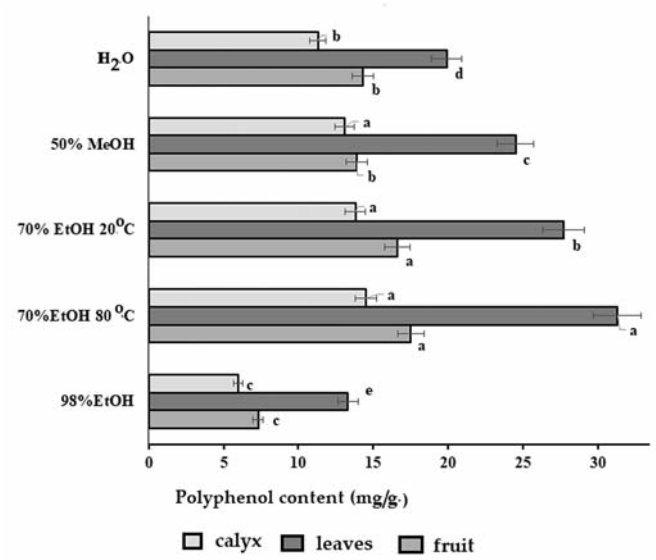

Fig. 3. Effect of extraction conditions on polyphenol levels in fruit, leaves, and calyx of Physalis peruviana. For each organ values with similar letters do not differ statistically according to Duncan test at p<0.05
Рис. 3. Влияние условий экстракции на содержание полифенолов в плодах, листьях и чехликах Physalis peruviana. Для каждого органа значения с одинаковыми индексами статистически не различаются согласно тесту Дункана при p<0.05

As far as calyx is concerned, phenolic levels in this organ were only slightly lower than the appropriate concentrations in fruit preserving the same tendency of the concentration decrease from the highest in case of 70% ethanol (80℃) to the lowest for 98% ethanol application. These results confirm that the utilization of elevated temperature during the extraction with 70% ethanol on dry *physalis* fruit, leaf and calyx homogenates suits the demands of obtaining high yield of polyphenols and is in accordance with the known relative stability of polyphenols in these conditions for certain objects [40].

3.3.4 Carotenoid profile

The total carotenoid content in *physalis* fruit was usually characterized by the predominance of beta-carotene [33] with the rather wide range of concentrations 1.12-4.00 mg/100 g f.w. [2,33, 36,42, 43]. In general, according to literature data, the number of carotenoids in *physalis* fruit reaches 45 [42] including lutein, *β* -cryptoxantine, *β*- and α-carotene [36]. The ripening

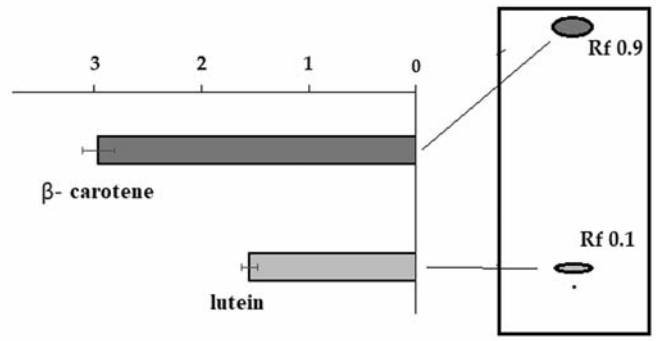

Fig. 4. Carotenoid composition of Physalis peruviana fruit. TLC: Chromatographic paper Watman 3A; hexane : acetone, 10:0.5
Рис. 4. Каротиноидный состав плодов Physalis peruviana.ТСХ: хроматографическая бумага Ватман 3А, гексан: ацетон,10:0.5

process is characterized by the conversion of lutein to beta-carotene [33].

Carotenoid content and carotenoid profile in the physalis fruit is governed to a large extent by fruit maturity and carotenoid distribution between peel, pulp, and calyx [2,43]. Thus, according to literature data, the main physalis carotenoids are beta-carotene and lutein with the highest content of beta-carotene for mature fruit and thrice higher concentrations in peel, where lutein prevails. The highest level of lutein was recorded in salyx [2].

The present results indicate the predominance of only two carotenoids: *β*-carotene and lutein in the Crimean *physalis* fruit (Fig. 4) with the total carotenoid level 1.51 times higher compared to the data of the Colombian imported product (Table 5) with the predominance of beta-carotene reaching 65.6% and 57.9% from the total content respectively. Taking into account that the dietary intake of beta-carotene may reduce the risk of developing chronic and age-related diseases [44], while lutein is highly effective in treatment of age-related macular degeneration [45] the obtained results confirm high medicinal value of *physalis* fruit.

3.4. Mineral composition

P. peruviana is known to be rich in potassium, magnesium and copper [8,32]. In this respect, the results obtained for fruit harvested at the southern Crimean shore indicated higher levels of these elements compared to the appropriate data for the Chilean product [8] (Table 6) recording valuable differences in the mineral availability between these regions.

The mineral distribution between fruit, leaves and calyx of the Crimean physalis revealed significantly higher levels of Zn, Cu, Mn and Co in leaves, while Fe concentrations in calyx happened to exceed the appropriate values in leaves and fruit by 2.6 and 4.2 times respectively (Fig. 5).

The phenomenon of Fe predominance in physalis calyx may record both physiological and medicinal importance since this organ is valuable for fruit ripening and is highly valued due to its antioxidant, anti-inflammatory, and anti-carcinogenic properties [46,47].

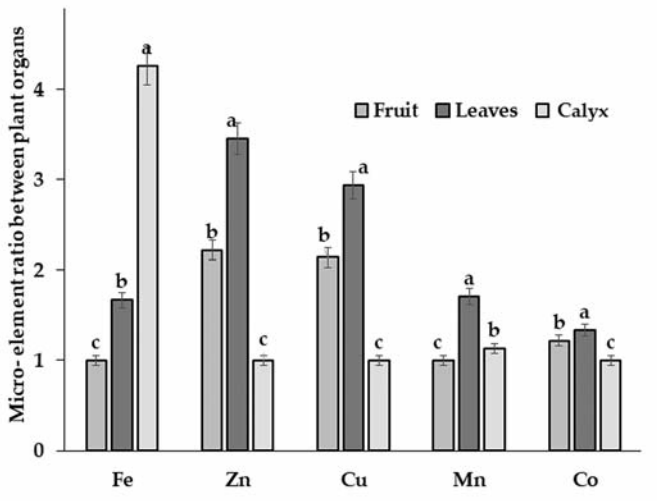

Fig. 5. Mineral distribution between fruit, leaves and calyx of P. peruviana. Within each element values with similar letters do not differ statistically according to Duncan test at p<0.05
Рис. 5. Распределение элементов (Fe, Zn, Cu, Mn, Co) между плодами, листьями и чехликами плодов P. peruviana. Для каждого элемента значения с одинаковыми индексами статистически не различаются согласно тесту Дункана при p<0.05

Table 6. Micro-element accumulation in the fruit Physalis peruviana in conditions of the Crimean southern sea shore (mg/kg d.w.)
Табица 6. Содержание микроэлементов в плодах Physalis peruviana в культуре Южного берега Крыма, мг/кг.с.в.

Organ	Fe	Zn	Cu	Mn	Co
Fruit плоды	67.8±6.0 c	11.59±0.81 b	7.77±0.73 b	5.36±0.50 b	2.11±0.19 a
Leaves Листья	113.0±10.1 b	18.03±1.20 a	10.69±0.98 a	9.17±0.82 a	2.32±0.21 a
Calyx Чехлики	288.7±25.2 a	5.21±0.49 c	3.63±0.31 c	6.05±0.55 b	1.73±0.11 b
M±SD CV (%)	156.5±116.7 74.6	11.61±6.41 55.2	7.36±3.66 48.2	6.86±2.03 29.6	2.05±0.30 14.6
Chili fruit (*) Чилийские плоды	25.7	7.13	4.28	8.08	-

(*) literature data for Atacama P. peruviana fruit [8]. Values in columns with similar letters do not differ statistically according to Duncan test at p<0.05 Литературные данные для плодов P. peruviana fruit пустыни Атакама [8]. Значения в столбцах с одинаковыми индексами статистически не различаются согласно теста Дункана при p<0.05

4. Conclusions

The results of the present investigation revealed high nutritional properties of *P. peruviana* fruit grown at the southern Crimean Sea shore and showed peculiarities of Fe, Zn, Cu, Mn, and Co distribution between fruit, leaves and calyx with the predominance of Fe in the latter plant part. The Crimean climate promotes the development of *P. peruviana* as a perennial plant with high carotenoid content in fruit and high polyphenol concentrations in leaves, fruit and calyx. Comparison of different polyphenol extraction methods allowed to indicate high benefits of 70% ethanol application at 80°C using dried fruit powder. The phenomenon of global warming makes it suppose a good suitability of *P. peruviana* production at the southern Crimean Sea shore.

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