Technological aspects of obtaining ethanol extracts from *Physalis alkekengi* L. leaves

T A Ivanova¹, V T Popova¹, S T Tasheva², N N Mazova¹ and A S Stoyanova²

¹University of Food Technologies, Technological Faculty, Department of Tobacco, Sugar, Vegetable and Essential Oils, 26 Maritsa Blvd. 4000 Plovdiv, Bulgaria
²University of Food Technologies, Technical Faculty, Heat Engineering Department, 4002 Plovdiv, Bulgaria

e-mail: vpopova2000@abv.bg

**Abstract.** The aim of the study was the provision of a technological background for obtaining ethanol extracts with prospective cosmetic application from the leaves of *P. alkekengi* L. Extraction was carried out in laboratory conditions, as a batch static mode process, with four solvents, 95%, 70%, 50%, and 30% ethanol, respectively. The influence of the main technological factors, temperature (20°C, 40°C, and 60°C) and duration (1 h, 3 h, 5 h and 7 h), upon the content of tannins in the extracts was studied by mathematical modelling of the experiment. The equations of extraction, based on the concentration of extracted tannins were derived. The optimal conditions for the extraction procedure of *P. alkekengi* leaves were determined (70% ethanol, 5 h, 60°C). The results from the study, i.e. the dynamics of tannin extraction, the equations of extraction and the technological factors’ combination, could be applied in the obtaining of *P. alkekengi* leaf extraction products intended for use in different personal care products; of course, additional investigations on the subject are necessary, especially in terms of extracts’ individual composition and biological activities.

1. **Introduction**

*Physalis alkekengi* L. (known as Chinese lantern, strawberry groundcherry), family Solanaceae, is a native wild growing plant in Bulgaria, found in habitats up to 1500 m altitude [1]. The plant falls within the scope of the Medicinal Plants Act, regulating the use of the medicinal plant resources on the territory of Bulgaria [2]. *P. alkekengi* aerial parts and roots are used in many folk medicines; recent research has identified a number of specialized metabolites and nutrients, as well as different aspects of their biological and pharmacological activities [3-8] *P. alkekengi* fruit and calyx extracts (CAS No 90082-67-0) are listed in the Cosmetic Ingredient Database (CosIng) of the European Commission, as cosmetic ingredients with skin conditioning properties [9]; water and ethanol extracts from different *P. alkekengi* aerial parts have been included in personal care products, with skin protecting, soothing, anti-aging, melanine (tyrosinase) inhibitory, and other beneficial effects [10, 11]. Bulgarian folk medicine recommends the use of *P. alkekengi* leaves as infusion for tooth pain or as poultice for rheumatism and joint pain [1].

Plant-derived extracts are essential functional ingredients in contemporary medicine and cosmetics, as they are readily accessible and not associated with many of the issues with synthesized components [12]. Therefore, the elucidation of extraction technologies and their optimization is an important aspect in the provision of new natural products for those fast-growing areas [11, 13].
To the best of our knowledge, there is no scientific evidence for the technological aspects of the process of obtaining ethanol extracts with prospective cosmetic application from the leaves of *P. alkekengi*, which is set as the aim of this study.

2. Materials and methods

2.1. Plant material
Leaves of *Physalis alkekengi* L., collected from a natural habitat in the region of Plovdiv (Central South Bulgaria) were used in the study. The plants were identified at the Department of Botany, Plovdiv University “Paisii Hilendarski”. Fresh leaves were air-dried in the shade and kept in tightly closed bags at a temperature of 5-8°C until analysis.

2.2. Extraction procedure
The raw material (air-dried) was characterized in terms of moisture content, by drying to constant weight at 105°C, and tannin content, by titration with potassium permanganate (with indigo carmine used as indicator) [14].

Extraction was carried out in laboratory conditions, as a batch static mode process at a ratio of raw material to solvent of 1:33 (w/v). The ratio (hydro module) was chosen as optimal on the basis of preliminary experiments (data not provided here). Four solvents were used for the extraction, respectively 95%, 70%, 50% and 30% ethanol. The influence of the main technological factors, temperature (varied at three levels, 20°C, 40°C and 60°C) and duration (at four levels, 1 h, 3 h, 5 h, and 7 h), upon the content of tannins in the extracts was studied by mathematical modelling of the experiment (Table 1).

| No | Duration (h) | Temperature (°C) |
|----|--------------|-----------------|
| 1  | 1            | 20              |
| 2  | 3            | 20              |
| 3  | 5            | 20              |
| 4  | 7            | 20              |
| 5  | 1            | 40              |
| 6  | 3            | 40              |
| 7  | 5            | 40              |
| 8  | 7            | 40              |
| 9  | 1            | 60              |
| 10 | 3            | 60              |
| 11 | 5            | 60              |
| 12 | 7            | 60              |

2.3. Statistics
Each experiment in the extraction procedure was performed in a threefold repetition. The experimental data were fitted to mathematical models and the equations of extraction of tannins were derived for each of the solvents. The coefficients of the equations were proved for significance by the *t*-test and for adequacy – by the *F*-test. The statistical significance of the experimental data was evaluated by the *t*-test or ANOVA; differences between means were considered significant at *p*<0.05. The figures were created with MicroCal™ Origin 9.1 software.
3. Results and discussion

The leaves were with moisture content of 9.38±0.05% and tannin content of 3.49±0.02% (on a dry weight basis).

The extracts were clear liquids, green to yellow-brown in color, depending on the solvent.

The dynamics of extracted tannin content, according to the experimental matrix (process duration and temperature combinations) (Table 1), are presented on Figures 1-4, respectively for the four solvent concentrations in the study.

![Figure 1](image1.png)

**Figure 1.** Concentration of tannins (%) in the extracts from *P. alkekengi* leaves with 30% ethanol (temperature, 20-60°C; duration, 1-7 h).

![Figure 2](image2.png)

**Figure 2.** Concentration of tannins (%) in the extracts from *P. alkekengi* leaves with 50% ethanol (temperature, 20-60°C; duration, 1-7 h).

![Figure 3](image3.png)

**Figure 3.** Concentration of tannins (%) in the extracts from *P. alkekengi* leaves with 70% ethanol (temperature, 20-60°C; duration, 1-7 h).

![Figure 4](image4.png)

**Figure 4.** Concentration of tannins (%) in the extracts from *P. alkekengi* leaves with 95% ethanol (temperature, 20-60°C; duration, 1-7 h).

As seen from the obtained results, the two technological factors of the process, duration ($x_1$) and temperature ($x_2$), had strong positive impact on the content of extracted tannins, for each of the solvents used.

Independent of solvent concentration, the content of extracted tannins increased parallel to the increase of temperature, from 20°C to 60°C and duration, from 1 h to 5 h. The highest level of process duration in the study, 7 h, was virtually excessive, as no significant increase in tannin extraction was detected.
The maximal values of extracted tannin content under the conditions of the study were achieved by variant 11 (Table 1), at a temperature of 60°C and extraction duration of 5 h; the obtained discrete values for the four solvent concentrations are presented in Table 2.

**Table 2.** Tannin content in ethanol extracts from *P. alkekengi* leaves (60°C; 5 h).

| Solvent concentration | Tannin content (%) |
|-----------------------|--------------------|
| 30% ethanol           | 0.0553             |
| 50% ethanol           | 0.0675             |
| 70% ethanol           | 0.0747             |
| 95% ethanol           | 0.0450             |

The functional dependency of the extracted tannin content (*y*) on the technological factors of extraction (duration, *x*₁ and temperature, *x*₂) was confirmed by the respective equation of extraction (equations 1-4). The derived equations of extraction were proved adequate and with significant coefficients, and were as follows:

- **30% ethanol:**
  \[ y = 0.037 + 0.011x_1 + 0.007x_2 - 0.002x_1x_2 + 0.003x_1^2 \]  \[ (1) \]

- **50% ethanol:**
  \[ y = 0.049 + 0.009x_1 + 0.006x_2 + 0.003x_1x_2 + 0.002x_1^2 - 0.001x_2^2 \]  \[ (2) \]

- **70% ethanol:**
  \[ y = 0.045 + 0.007x_1 + 0.011x_2 + 0.003x_1x_2 + 0.012x_1^2 - 0.003x_2^2 \]  \[ (3) \]

- **95% ethanol:**
  \[ y = 0.033 + 0.006x_1 + 0.005x_2 + 0.002x_1x_2 - 0.001x_1^2 \]  \[ (4) \]

where *y* was the content of tannins (%); *x*₁ – the duration of the process (h); and *x*₂ – the temperature (°C).

The highest concentration of tannins in the extracts was achieved by 70% and 50% ethanol; in turn, the lowest tannin concentrations were observed for 95% ethanol, for the entire range of technological factors’ variation. The differences in the dynamics of extracted tannins on ethanol concentration basis confirmed the decisive impact of solvent selection in plant extraction technology, as the selectivity of the organic solvent-water mixture does not follow unambiguously the change in concentration [13]. Those results were in full compliance with previous studies on the technological aspects of tannin extraction from the leaves of different genotypes of another *Physalis* species, *P. peruviana* L. [15], as well as from the leaves of various medicinal and essential oil bearing plants, for example tobacco [16], mint [17], thyme [18], and rosemary [19]. Similar influence trends were observed for the regarded technological factors (temperature, duration, ethanol concentration) in the optimization of the extraction procedure for flavonoids from *P. alkekengi* stems [11].

The maximal tannin concentration in the crude extracts of *P. alkekengi* leaves, 0.075%, was secured by the following combination of the technological factors in the study: solvent 70% ethanol, temperature 60°C, and duration 5 h, which could be considered as the optimal conditions under the scope of the
study. As stated previously, the efficacy of extraction was assessed on the basis of extracted tannins, which represent phytochemicals with recognized astringent, antioxidant and other beneficial effects in cosmetics. Therefore, the defined technological matrix in this study could be further practically applied in the targeted obtaining of crude or refined extraction products (concentrated, dried) with realistic prospective for use in different cosmetic products, such as skin and hair lotions, tonics, mouth waters, etc.

4. Conclusions
In compliance with the aim of the study, a technological background for obtaining crude ethanol extracts from *P. alkekengi* leaves was provided. The maximal content of extracted tannins was secured with 70% ethanol in a 5-hour extraction at 60°C (0.075%). To the best of our knowledge, these are the first results defining the influence of the technological factors (temperature, duration, solvent concentration) and the optimal technological conditions for the extraction procedure. The results from the study, i.e. the dynamics of tannin extraction, the equations of extraction and the technological factors’ combination, could be applied in the obtaining of *P. alkekengi* leaf extraction products intended for use in different personal care products. Of course, additional investigations on the subject are necessary, especially in terms of extracts’ individual composition and biological activities, which are set as future objectives.

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