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# EXTRACTION OF CAROTENOIDS FROM SELECTED PLANTS

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## Introduction

Carotenoids belong to the most widespread natural dyes, determining the colour of plants and animals. One of the main pigments responsible for the yellow colour of flowers is lutein. Lutein belongs to the dihydroxy-derivatives of carotenoids. It plays an important role in human nutrition due to its strong antioxidant effects[1] in neutralizing free radicals that cause degenerative changes in the retina[2]. It is used as an ingredient in feed for poultry; it is also applied as a colorant in a variety of foods. Lutein content was studied in carotenoid-rich flowers such as *Tagetes* and *Calendula*. The carotenoid content in blossoms of these flowers depends on the cultivar, planting conditions, growing season and vary with regions.

*Tagetes* is one of the richest natural sources of xanthophylls. They occur mainly in the form of esters of fatty acids - lauric, myristic, palmitic and stearic. The extract from the flowers is often added to the poultry feed to achieve intensive color of egg yolks. On the other hand, beneficial effects of lutein esters on human body are known. They are readily absorbed into the bloodstream and can reduce the growth of breast tumour cells, promote the formation of lymphocytes or suppress eye degeneration in an older age[3]. Their strong antioxidant effect, in general, appears to lead to the widespread use of marigold extracts as food supplements and/or functional foods[4,5].

*Calendula* originates from southern Europe and the Orient. In ancient times it was used as a spice, sometimes replacing the scarce saffron. Yellow colour of petals, a mixture of carotenoids, is applied in the food industry to dye rice, butter, cheese and soups. Furthermore, the plant contains essential oils, sterols, triterpenoids faradiol, amyrin and lupeol, and polar flavonoids as isokvercitrin, rutin, narcissin etc. Significant components are also lipophilic substances, especially carotenoids and sterols[6]. All parts of the plant have a pungent smell which repels some insect species. Extracts of *Calendula* flowers are also effective agents against skin inflammations and swellings, accelerate wound healing.

A method for determining lutein and zeaxanthin isomers from marigold flower extract using hexane and ethyl ether, as an extracting solvent, has been reported by Hadden[3]. Numerous organic solvents[7,8] for extraction of carotenoids from vegetable and fruit samples, e.g. n-hexane, have been widely used.

## Materials and methods

Lutein was isolated from deep-frozen mixtures of 7 clones of *Tagetes* and *Calendula* (Institute of Botany of the CAS, v. v. i., Průhonice) and concentrated suspension of fresh brown-orange microalgae *Scenedesmus sp.* (Culture Collection of Autotrophic Organisms CCALA of the Institute of Botany of the CAS, v. v. i., Třeboň). The microalgal cells were cultivated in closed photobioreactor. To reduce water content in cultivated solution, filtration was deployed.

Dry matter in extracted biomass was determined before each extraction to assure the exact content of the dry biomass in the system (110 °C, to the constant weight).

Non-polar extraction agents - hexane and petroleum ether (p. a., Lach-Ner, CR) were applied. Ratio of sample weight/extraction agent 1/6 was used. Extraction of carotenoids was carried out directly from the frozen/fresh samples. Biomass was not dried in order to avoid undesirable losses of unstable substances.

Two procedures of lutein extraction were tested. The first procedure using Soxhlet apparatus was based on repetitive multistage extraction with fresh condensing solvent for 2 and 4 hours. This process is very efficient in terms of intensive contact of the solvent with the extracted material. Its disadvantage is the thermal stress of the sample and the resulting extract, because it operates at the boiling point of the solvent. The second procedure was single-stage extraction process in a stirred batch, at ambient temperature, protected from air and light, under inert atmosphere (nitrogen) for 2 and 4 hours. This procedure is suitable for the separation of thermally unstable materials susceptible to oxidation by atmospheric oxygen. The disadvantages include the necessary subsequent separation of the solid plant material from the extract, low efficiency limited by equilibrium and time consumption.

Individual phases (liquid organic extract and solid biomass or its water suspension) were separated from the obtained solutions by filtration. Part of the extract was then evaporated using the rotary evaporator under vacuum and thus extractable part (dry extract) was prepared.

Spectrophotometric analysis of lutein in liquid extracts and the dry extractable components from the tested plant and algal material was performed at the Department of Food Analysis and Nutrition of the University of Chemistry and Technology Prague. The lutein content in extracts from processed petals and microalgae was related to fresh/deep-frozen biomass.

**Results and discussion**

The effect of experimental procedure, both in Soxhlet and batch arrangement, on extraction efficiency of lutein from *Tagetes* petals is compared in Table I. Extractables represent overall components which can be recovered from processed material to extract solution. Lutein content (the 3rd column of the Table I) in the extract is calculated to fresh material. Lutein concentration in dry extract after solvent removal from extract solution is stated in the 4th column of the Table I.

Table I
Extract composition of *Tagetes* petals (dry mass 11.8 wt. %)

| Processing | Extractables [wt. %] | Lutein content in extract [mg/kg] | Lutein content in dry extract [mg/kg] |
|---|---|---|---|
| Soxhlet, 2 hrs, hexane | 10.5 | 193 | 361 |
| Soxhlet, 2 hrs, petroleum ether | 6.0 | 198 | 313 |
| Soxhlet, 4 hrs, hexane | 15.4 | - | - |
| Stirred batch, 2 hrs, hexane, inert, lab. temp. | 5.1 | 478 | 477 |
| Stirred batch, 2 hrs, hexane, inert, lab. temp. | 5.9 | 452 | 591 |

It is evident that Soxhlet extraction process strongly depends on time (in case of hexane application). Also, the amount of extractable components is lower in case of petroleum ether application in comparison with hexane. Therefore, subsequent test has been made using stirred batch extractor and hexane as a solvent. But, in this single-stage extraction the efficiency of the process is only 50 % in comparison to a Soxhlet one. On the other hand, lutein yield obtained at mild conditions exceeds Soxhlet arrangement.
If we compare literature data, for instance, the lutein content of *T. erecta* was reported[9] in the range of 0.216–0.976 g/kg based on the weight of fresh flowers, which equals 0.02–0.1 wt. %.

Similar extraction experiments were conducted under comparable conditions using *Calendula* petals. The results are given in Table II.

Table II
Extract composition of *Calendula* petals (dry mass 13.6 wt. %)

| Processing | Extractables [wt. %] | Lutein content in extract [mg/kg] | Lutein content in dry extract [mg/kg] |
|---|---|---|---|
| Soxhlet, 2 hrs, hexane | 5.0 | 25 | 66 |
| Soxhlet, 2 hrs, petroleum ether | 7.6 | 26 | 142 |
| Soxhlet, 4 hrs, hexane | 6.6 | - | - |
| Stirred batch, 2 hrs, hexane, inert, lab. temp. | 3.7 | 80 | 99 |
| Stirred batch, 2 hrs, hexane, inert, lab. temp. | 4.6 | 108 | 153 |

The content of all extractables using hexane was in this case lower. It is interesting that by application of a Soxhlet apparatus and petroleum ether as an extraction agent concentration of extractables was higher than using hexane.
In comparison with *Tagetes*, *Calendula* provides yield approx. 50 % of extractables using a Soxhlet apparatus, and 75 % in stirred batch extractor arrangement under mild conditions (ambient temperature, under inert atmosphere and absence of light). Comparing *Calendula* results to data in Table I (*Tagetes*) lutein content achieved far lower concentration.

Table III brings similar data from experiments using fresh dense water suspension of brown-orange microalgae *Scenedesmus sp.* The tests were made using stirred batch extractor at ambient temperature under inert atmosphere and in the absence of light.

Table III
Extract composition of *Scenedesmus sp.* suspension (dry mass 21.3 wt. %)

| Processing | Extractables [wt. %] | Lutein content in extract [mg/kg] | Lutein content in dry extract [mg/kg] |
|---|---|---|---|
| Stirred batch, 2 hrs, hexane, inert, lab. temp. | 2.5 | 24 | 22 |
| Stirred batch, 2 hrs, petroleum ether, inert, lab. temp. | 2.1 | 15 | 18 |

Concentrated suspension of *Scenedesmus sp.* was processed gently to obtain an extract enriched in carotenoids. Only small amount of lutein (mg of lutein to kg of suspension) was isolated from studied samples. It is necessary to add, no direct comparison of lutein content in marigold flowers and microalgae has been reported in literature[10].

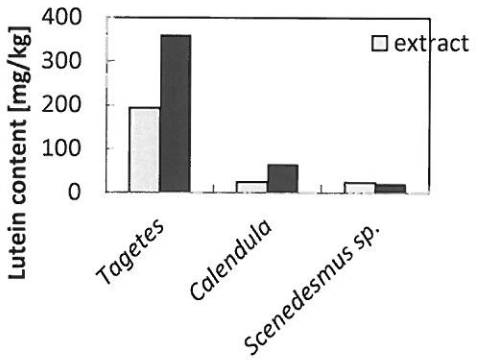


Figure 1. Comparison of lutein yield obtained from individual processed biomass (□ extract, dry extract)

Summary comparison of different raw material extract efficiency is illustrated in Figure 1. Data are collected from batch experiments for 2 hours with hexane as a solvent under mild conditions.

**Conclusions**

The great interest which lutein and all carotenoids have aroused during the last decades is conditioned not only by their interesting structure but by their biological and physiological importance. It is evident that their application in agriculture, food and pharmaceutical industry has a great potential for the future. Thus study of their isolation from natural resources is very important nowadays.

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**Keywords**: extraction, *Calendula, Tagetes, Scenedesmus*, carotenoids, lutein.

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