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Supercritical fluid CO_2-extraction of carotenoids from the Kazakhstan carrots

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1. Introduction

The most important task of the economic policy of the Republic of Kazakhstan is the formation of a national "green" economy of innovative type on the basis of supporting priority areas of technological breakthrough. In this regard, to ensure the needs of domestic medicine and food industry the study of the local source of new drugs is relevant.

Carotenoids play an important role in the metabolic processes of the body. They have a fairly large list of important pharmacological properties. The main properties of carotenoids are antioxidant[1], radioprotective[2], photoprotective[3], anticarcinogenic[4], and immunomodulatory[5] activities. Massive sources of carotenoids are various types of plant materials, where these compounds are in the form of complexes. Natural carotenoid complexes have higher stability, biological activity, and digestibility than individual products of chemical and microbiological synthesis.

Existing methods for isolating carotenoids from plant materials are few, and most of them are based on the direct extraction of the target component with oils or organic solvents. One of the challenges in the development of drugs and dietary supplements containing carotenoids is to develop a unified way to isolate carotenoids from natural objects.

Supercritical fluid extraction (SCF) technology is in use from the late 19th century as a tool to understand the natural mineralization, the commercial exploitation of SCF technology has begun in the 1970s[6]. Supercritical fluid CO_2 extraction of bioactive compounds has received increasing attention in the last decades. Supercritical extraction is used for the extraction of caffeine from coffee beans, vegetable oils, and biologically active complexes from some plant objects (for example, chamomile flowers, hops, etc.).

The leading role of SCF technology in solving social and economic problems is obvious since it makes it possible to create in the territory of Kazakhstan new production facilities and modernize outdated ecologically dangerous enterprises for the environment; ecological cleanliness is inherent in SCF technology.

Therefore, development of a method for isolating carotenoids from a plant material by using supercritical fluid CO_2-extraction was initiated.

2. Results and discussion

The authors for the first time conducted a study of the carotenoid composition of carrots of various colors using modern methods of analysis, which allowed to obtain reliable results. It was revealed that the content of carotenoids in carrot roots varies greatly depending on the color: β-carotene is most common in orange root crops, lycopene is red-colored. On the basis of the carried research, it was concluded that the carotenoid composition of root crops is due to several factors and, first of all, the ecological and geographical conditions of the region where the root crops are grown.

The objects of the study are orange-red carrots "Karotel" variety (*Daucus carota* L.), collected from Turkestan, Almaty and Pavlodar regions, Kazakhstan. Fresh, thoroughly washed and powdered (particle size in the range 5.0-7.0 mm) raw materials were dried at 22-25 °C to the storable moisture content.

According to the accepted procedures of the State Pharmacopoeia of the Republic of Kazakhstan[7], 1st edition, "Karotel" carrots contained 10-15 % moisture, 13-14 % ash, and 51-53 % extractives content. For most types of plant materials, the permissible limit of moisture is usually 12-15%.

In order to isolate carotenoids from "Karotel" carrots, technological parameters were selected. Extracting solvent, a ratio between solvent and solid plant material and process temperature are the most important optimum extraction parameters. A quantitative measure for this is the valuable compounds extraction yield from plant material. The most suitable solvents of all the tested were dichloromethane, acetone, and chloroform. The process of extracting the number of carotenoids from carrots was carried out by hot maceration. The obtained extract is concentrated and subjected to thin-layer chromatography (TLC). Selection of TLC systems for separation and identification the sum of carotenoids was carried out. The following solvents were used to prepare the systems: hexane, chloroform, dichloromethane, acetone, ethanol, *n*-butanol. Currently, we have studied about 40 systems for TLC; the mixture of hexane-acetone 10:0.5 was the mobile phase that achieved a good separation of the lycopene from the carrot, obtaining the separation factor R_f= 0.62 when using the silica gel plates.

The carotenoid concentration was determined by UV visible spectrophotometry. The results of analysis showed β-carotene content ranged from 11.2 to 15.9 mg% at λ = 450 nm and lycopene ranged from 10.5 to 12.3 mg% at λ = 474 nm.

One possible environmentally friendly alternative is supercritical fluid extraction (SFE), in particular using supercritical CO_2 "green technology" because it is physiologically harmless, environmentally safe, non-explosive, exhibits high selectivity as a result of low viscosity, high diffusivity, and liquid-like density, the possibility to work at moderate temperatures avoiding thermal degradation of carotenoids, obtaining a solvent free extract, and in some cases higher yields[8, 9].

The carotenoids were extracted from carrots by using a Supercritical Carbon Dioxide Extractor (Thar 1000 F, Thar Technologies, Inc., Pittsburgh, PA, USA). The effects of moisture content, particle size, solvent flow-rate, pressure and temperature on the extraction yield were evaluated. Various temperatures (40, 60, 70 °C) and pressures (150-200 bars), addition of co-solvent (5, 10, and 15 % ethanol), extraction time (40 min), and CO_2 flow rate (10 g/min) for the modified extraction processes were compared.

The statistical analysis of the experiment indicated that individual factors such as pressure and temperature, and interactive factors such as pressure with temperature and time had significant effects on carotenoid yield. An increase in the degree of extraction of carotenoids (up to 25 % of their content in the raw materials) with increasing pressure at 60 °C during the extraction process is shown in "Karotel" carrots, which was collected from the Pavlodar region of Kazakhstan.

3. Conclusions

For the first time in research work to obtain carotenoids from Kazakhstan "Karotel" carrots and to optimize the technology used maceration and supercritical fluid extraction (SCF). The raw materials were successively extracted with hexane, dichloromethane, chloroform, ethyl alcohol, a mixture of acetone-ethyl alcohol at a ratio of (5: 1). The most saturated extract is received by dichloromethane. TLC method allows the qualitative detection of carotenoids in carrots. Quantitative analysis of carotenoids and lycopene was determined by UV spectrophotometry. The effects of various parameters of supercritical carbon dioxide (SC-CO_2) fluid extractions of "Karotel" carrots on the extraction yields of carotenoid-rich extracts were investigated. Increasing the operational conditions of temperature and pressure indicated that the supercritical CO_2 selectively extracted different carotenoids. Work on optimizing the method of obtaining carotenoid continues.

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