

The development and industrial implementation of technology for producing biologically active supplements

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ABSTRACT

This study presents the development and industrial implementation of technology for producing supplements rich in biologically active substances from grape and pumpkin seeds. The process involves drying seeds to optimal moisture levels, crushing to fine particle size, and processing via supercritical CO₂ extraction. Eight types of fat-soluble extracts were obtained and encapsulated in gelatin capsules. Quantitative and qualitative analyses determined the content of key bioactive compounds: linoleic and linolenic acids, α -tocopherol, β -carotene, and phenolic substances.

Keywords: gelatin encapsulation, grape and pumpkin seeds, liposoluble extracts, supercritical CO₂-extraction.

INTRODUCTION

The Republic of Moldova's agricultural sector offers diverse vegetable raw materials valued in domestic and international markets for their biologically active substances with beneficial health effects. Oil extraction from seeds of pumpkin, flax, milk thistle and grapes represents the most commonly used method for obtaining essential bioactive compounds (Batool *et al.*, 2022; Zhou *et al.*, 2022; Aziz *et al.*, 2020).

The bio-climatic and socio-economic conditions of the Republic of Moldova enable industrial utilization of grape and pumpkin seeds, as sufficient quantities accumulate annually at fruit and vegetable processing enterprises.

Pumpkin seeds contain 10.9-30.9% oil, with total unsaturated fatty acids comprising 73.1-80.5% of the lipid fraction. The predominant fatty acids are linoleic, oleic, palmitic, and stearic acids (Batool *et al.*, 2022). Pumpkin seed oil contains numerous biologically active substances including vitamins B₁, B₂, C, P, flavonoids, and various fatty acids (palmitic, stearic, oleic, linoleic). These compounds normalize metabolism, facilitate toxin elimination, and exhibit antiulcer, antisclerotic, and antiseptic properties.

Grape seeds prepared for industrial processing contain approximately 10-18% high-quality oil. The biological activity of grape oil stems from its complex of bioactive substances, notably resveratrol and bioflavonoids—oligomeric proanthocyanidins—which function as antioxidants preventing cellular degeneration. Grape oil also contains vitamin E, which acts synergistically with P-group vitamins to modify membrane phospholipids (Zueva, 2004). Bioactive substances from plant materials can be extracted using two main categories of techniques: conventional and innovative methods.

Conventional techniques, long-established and based on solvent extraction power and heat treatment, include Soxhlet extraction, hydro distillation, and maceration. Recently developed innovative techniques involve assisted extraction processes: microwave, enzymatic, ultrasonic, and supercritical fluid extraction.

Supercritical fluid extraction offers distinct advantages: lower viscosity and higher diffusion coefficient compared to liquid solvent extraction, providing superior mass transfer; reduced processing time; environmental friendliness due to minimal sample and solvent requirements; minimal losses through supercritical fluid reuse and recycling (Liviu, 2017). The bioavailability of bioactive substances can be optimized through encapsulation of lipid extracts obtained with supercritical fluids (CO₂ or N₂) using gelatin. This innovative process implementation offers comprehensive benefits. The purpose of this study is to research and implement technology for producing supplements rich in biologically active substances from native raw materials.

MATERIALS AND METHODS

Research was conducted at the Department of Food Technology and Microbiology of the PI National Institute for Applied Research in Agriculture and Veterinary Medicine, Republic of Moldova (IP NIARAVM).

Research materials included: Chardonnay (white cultivar) grape seeds; Cabernet Sauvignon (red cultivar) grape seeds; Pumpkin seeds.

Seeds were dried, crushed, and subjected to supercritical CO₂ extraction to obtain lipid extracts and pomace for bioactive substance analysis

Fatty Acid Analysis: Saturated, monounsaturated, and polyunsaturated fatty acids in lipid extracts were determined using gas chromatography with molecularly imprinted polymers for volatile compound capture. Analysis was performed at "Agro.Biolab Laboratory" LLC, Italy.

Mineral Analysis: Mineral substances in pomace were determined by flame atomic absorption spectroscopy at "Agro.Biolab Laboratory" LLC, Italy.

Bioactive Compound Analysis: Linoleic and linolenic acids, α -tocopherol were determined by UV spectrophotometry following lipid extract saponification.

Total phenolic substances and carotenoids were determined by visible spectrophotometry using Folin-Ciocalteu reagent in hexane solutions and hexane:acetone: ethanol extraction mixture, respectively.

Analyses were conducted at the IP NIARAVM, Food Quality Control Laboratory.

For each parameter analyzed, the determinations were performed in n independent repetitions (n =3).

The results were statistically processed and presented as mean $\pm$ standard deviation (mean $\pm$ SD), to highlight the analytical and biological variability of the samples.

RESULTS AND DISCUSSIONS

The technological process for obtaining supplements with fat-soluble extracts from white and red grape seeds and pumpkin seeds was developed using supercritical CO₂ extraction and gelatin encapsulation equipment.

The manufacturing technology for vegetable lipid extract supplements involves complex procedures outlined in the following flowchart (Figure 1).

Process operations

Raw material reception. Raw materials included 'Chardonnay' grape seeds, 'Cabernet-Sauvignon' grape seeds and pumpkin seeds supplied by "Oil Eco Grup" LLC. Fresh seeds exhibited moisture content above 14%, necessitating drying prior to processing.

Seed drying. Drying was performed in hot air installations at 70°C until optimal moisture content of 8-10% was achieved. Proper drying is essential for storage and processing; excessive moisture intensifies hydrolysis, increases seed acidity, and triggers biological processes leading to seed degradation. Optimal moisture produces powdery, friable

grinding with high oil absorption capacity. Higher moisture levels impair shredding and result in sticky grinding, complicating subsequent processing.

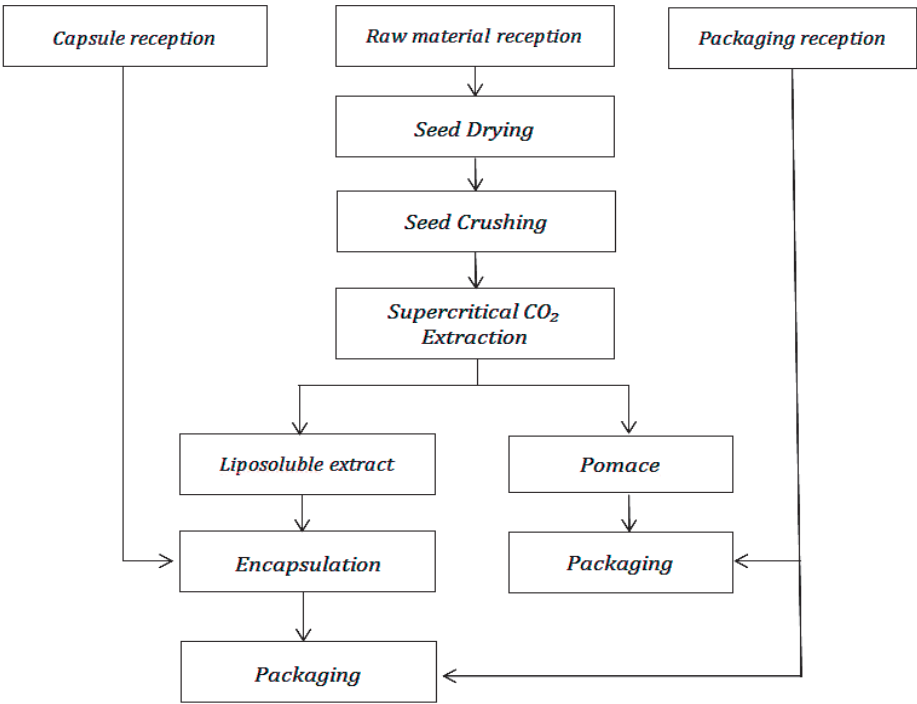


Figure 1. Manufacturing technology flowchart for supplements

Seed crushing. Grape and pumpkin seeds were crushed to fine particle size (Figures 2-4), breaking oleoplasm cell membranes and releasing oil as fine droplets from disrupted intercellular structures. Technical and technological limitations restrict grinding efficiency to 70-80% cell breakage, leaving oil trapped in intact cells. The specific morphological structure of seeds (hard shell, soft kernel) produces non-homogeneous grinding, with variety and species origin playing determining roles.



Figure 2. Chrushed white grape seeds



Figure 3. Chrushed red grape seeds



Figure 4. Chrushed pumpkin seeds

Grinding induces structural changes, creating conditions for oil oxidation and intensifying respiration and fermentation processes through protective shell removal. These factors reduce grinding stability, limiting retention time to very short intervals.

Supercritical CO₂ Extraction

Using pilot-scale supercritical extraction equipment, fat-soluble extracts (Figure 5) and pomace (Figure 6) were obtained according to extraction regimes (Table 1). The CO₂

extraction process involves setting pressure and temperature parameters, fixing extraction duration, and monitoring solvent flow rate. Supercritical CO₂ fluid functions as a lipophilic solvent, extracting volatile molecules (sesquiterpenes, monoterpenes, terpenols), tocopherols (vitamin E), glycerides, tocotrienols, phytosterols, carotenoids, and squalenes.



Figure 5. Extracts of seeds: white grape, red grape, white and red grape, pumpkin



Figure 6. Pomace of seeds: white grape, red grape, pumpkin

For the eight supercritical extractions, extractor cell loading varied from 366-386 g for pumpkin seeds and 506-576 g for grape seeds. Extract masses obtained in separator I ranged from 117.0-120.0 g for pumpkin seeds and 51.0-59.0 g for grape seeds. Higher extract yields from pumpkin seeds reflect their greater lipid content compared to grape seeds.

Encapsulation. Seamless soft gelatin capsules were produced using AC-powered equipment (220V) with a 3,600-capsule/hour capacity. Production was based on gelatinous mass physical properties. Capsules formed at the exit of the encapsulator cell, where a fat-soluble CO₂ extract and a gelatin mass were heated to 60-70°C under air pressure.

Table 1. Extraction regimes from grape and pumpkin seeds

Seed sample	Extraction regimes	Pressure, MPa	Temperature, °C	Time, min	Flow rate, L/h	Raw material, g	Extract Mass Separator I, g	Extract Mass Separator II, g	Pomace, g	Yield, %
White grape 'Chardonnay'	1	22.6	52.0	60	53	552	59	5	488	11.59
	2	36.2	48.7	120	54- 56	506	56	32	418	17.39
	3	26.0	49.3	190	58	576	57	5	514	10.76
Red grape 'Cabernet – Sauvignon'	1	25.6	48.8	120	54- 57	563	57	12	494	12.26
	2	35.6	49.1	120	53	561	56	14	491	12.48
Mixed White/Red	1	35.0	49.3	120	54	560	51	17	492	12.14
Pumpkin seeds	1	25.9	53.3	120	50	386	117	11	258	33.16
	2	30.2	59.6	150	50-52	366	120	4	242	33.88

Oil pulsation in cells created jet separation, with surface tension forces producing spherical shapes. Capsules (0.4g fill weight) solidified gradually in cooled vegetable oil flow (10-12°C). Post-formation capsules were transferred to oil-filled trays with regulated CO₂ extract and gelatin mass consumption. Visual inspection on illuminated tables confirmed proper parameters: a rounded shape without stripes, mechanical damage, or air bubbles (Figure 7).

**Figure 7.** Seamless soft gelatin capsules

Packaging. Inspected capsules are packaged in blisters or glass/plastic containers with lids in rooms equipped with general ventilation and air purification systems.

Bioactive Substance Content Analysis

Gas chromatography analysis at "Agro.Biolab Laboratory" LLC, Italy, determined fatty acids content in fat-soluble extracts (Table 2). Linoleic acid predominated: 71.7-72.2% in grape seed extracts and 43.9-44.0% in pumpkin seed extracts. Linolenic acid content was 0.51-0.52% in grape extracts and 0.33% in pumpkin extracts. Grape extracts demonstrated 37-40% higher linoleic (ω -6) and linolenic (ω -3) acid content than pumpkin extracts, establishing these CO₂-extracted lipid fractions as valuable bioactive substance sources with significant oxidoreductive potential.

Table 2. Fatty acid content in fat-soluble seed extracts

Fatty acid	Fatty acid content in fat-soluble seed extracts, %			
	White grape	Red grape	Mixed White/Red	Pumpkin
Lauric acid (C 12:0)	< *LOQ	< *LOQ	< *LOQ	< *LOQ
Myristic acid (C 14:0)	0.05±0.01	0.04±0.01	0.04±0.01	0.10±0.01
Palmitic acid (C 16:0)	7.30±0.05	7.70±0.06	7.47±0.04	11.75±0.07
Palmitoleic acid (C 16:1)	0.15±0.01	0.19±0.01	0.16±0.02	0.14±0.03
Stearic acid (C 18:0)	4.32±0.04	3.24±0.04	3.79±0.04	5.95±0.03
Oleic acid (C 18:1)	15.80±0.13	16.20±0.20	15.9±0.17	37.10±0.11
Linoleic acid (C 18:2)	71.80±0.26	72.20±0.30	72.0±0.29	44.00±0.23
Linolenic acid (C 18:3)	0.53±0.01	0.51±0.02	0.51±0.01	0.33±0.01
Arachidic acid (C 20:0)	< *LOQ	< *LOQ	0.12±0.01	0.53±0.08
* - LOQ: Limit of detection LOQ = 0.01				

Mineral substance analysis of three pomace samples revealed varying concentrations of Ca, K, Mg, Fe, and Zn (Table 3). Grape seed pomace showed higher calcium content, while pumpkin seed pomace contained higher levels of iron, magnesium, potassium, and zinc.

Table 3. Mineral Content in Post-Extraction Seed Pomace

Mineral	Mineral content in post-extraction seed pomace, mg/kg		
	White grape	Red grape	Pumpkin
Calcium (Ca)	5880±1.31	6104±1.90	1133±1.99
Iron (Fe)	60.2±0.67	75.5±0.59	183±0.72
Magnesium (Mg)	1139±1.31	1155±1.30	5737±2.61
Potassium (K)	20.4±0.14	22.8±0.26	140±0.91
Zinc (Zn)	623±1.30	599±1.31	1808±1.99
*Note- Values represent the mean ± standard error (SD), n=3			

Spectrophotometric analysis at IP NIARAVM, Food Quality Control Laboratory, determined bioactive substance content in grape and pumpkin seed extracts (Table 4).

Table 4. Bioactive substance content in seed extracts

Seeds extract	α-tocoferol, mg/100 g	β-caroten, mg/100g	Phenolic Substances, mg/100 g *	Linoleic acid, %	Linolenic acid, %
White grape	93.7±0.30	-	95.2±0.11	69.8±0.33	0.4±0.02
Red grape	45.8±0.20	-	45.4±0.13	68.6±0.20	0.3±0.01
White and Red grape	94.7±0.21	-	97.3±0.16	70.2±0.35	0.4±0.02
Pumpkin, variant I	97.4±0.40	0.72±0.02	-	41.7±0.20	0.3±0.01
Pumpkin, variant II	89.8±0.40	0.88±0.02	-	40.4±0.27	0.2±0.01
* - Total phenolic substances expressed as gallic acid equivalents					
Note - Values represent the mean ± standard error, n=3					

α -Tocopherol content in grape seed extracts ranged from 45.8-97.4 mg/100g, while pumpkin seed extracts contained 0.72-0.88 mg/100g β -carotene. Phenolic substance content in grape seed extracts was 45.4-97.3 mg gallic acid equiv/100g. The mixed white and red grape seed extract showed the highest linoleic acid content (70.2%), while pumpkin seed extracts contained 40.4-41.7% linoleic acid. Linolenic acid comprised 0.2-0.4% of total fatty acids.

Modern technology for manufacturing bioactive substance supplements from grape and pumpkin seeds in gelatin capsule form was implemented at "Oil Eco Grup" LLC (Figure 8).

The technological equipment operates on 220V AC power with encapsulator capacity up to 15,000 capsules/hour. The production cycle from encapsulation to packaging requires 2-3 days. The production facility, employing 5 personnel, can produce 1.5 million capsules monthly.



Figure 8. Technological equipment for food supplement production

CONCLUSIONS

The present study showed that technological regimes for supercritical CO₂ extraction of biologically active substances from selected native raw materials ('Chardonnay' grape seeds, 'Cabernet Sauvignon' grape seeds, and pumpkin seeds) were successfully developed. Production documentation under experimental conditions was established.

Also, comprehensive analysis determined the content of key bioactive substances in concentrated post-extraction extracts: linoleic and linolenic acids, α -tocopherol, and β -carotene.

Gelatin capsule samples were produced, and documentation for bioactive substance supplement production under experimental conditions was established through collaboration between IP NIARAVM and "Oil Eco Grup" LLC.

The technology demonstrates successful scale-up potential with an established production capacity of 1.5 million capsules monthly.

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