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Extraction of Apple Pomace Using Supercritical CO_2 Extraction

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Abstract – Apple pomace, a by-product of apple juice and cider production, is a sustainable raw material from which valuable products such as nutritional supplements and pectin can be obtained. It contains significant amounts of antioxidant compounds that have been linked to several health benefits. Both traditional and new technologies can be used to extract valuable components from apple pomace, with an emphasis on new and environmentally friendly methods. One such technique is the use of supercritical CO_2 extraction. This method is considered environmentally friendly and can be used to extract valuable compounds such as antioxidants and pectin from apple pomace. This article examines the extraction parameters of apple pomace and analyzes the valuable substances in the extract samples. Apple pomace is a promising source of carbohydrates, proteins, amino acids, fatty acids, phenolic compounds, vitamins, and other compounds with a vast range of food applications.

Keywords – Added value products; apple pomace; agriculture; by-products; extraction.

1. INTRODUCTION

According to the Food and Agriculture Organization of the United Nations, the production of various vegetables and fruits worldwide exceeds two billion tons, which, depending on the cultivation technique, geographical regions and consumption, creates an unavoidably large amount of waste by-products [1]. In Europe alone, approximately 100 million tons of by-products and waste are generated every year in the beverage (26 %) and fruit (14.8 %) production sectors. It is the management of the by-products of fruit processing that is one of the main problems of the agri-food industry. A large amount of waste containing a rich composition of biological compounds can be considered dangerous because it can cause phytotoxicity phenomena such as plant growth disorders, drinking water quality deterioration, water pollution, inhibition of seed germination and many other problems in the environment and living organisms [2]. Properly treated, these by-products and wastes can form low-cost raw materials rich in potentially valuable components that can be used in other industries [3]. Using various purification and extraction methods, as well as analytical methods, it is possible to recover valuable bioactive components from fruit by-products and waste and further transform them into value-added products. Landfilling, improper disposal and handling of bio-waste can cause problems in bio-economic value chains, however, by using these products

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correctly, it is possible to achieve the potential to reduce the serious environmental problems associated with food waste [4].

Due to the wide distribution of apples, both geographically and seasonally, in recent years they are considered one of the most consumed fruits in the world. It should be noted that apple consumers cover a wide age range, from babies who eat them as purees, to teenagers who mainly drink apple juice and adults who eat them as fresh fruit [5]. In 2020, global apple production amounted to a total of 86.44 million metric tons [6]. Apples are most commonly processed into products such as juice, dried or frozen, and preserved as fresh slices, baby food, apple jelly, and vinegar. The pomace is the solid residue of any fruit, such as an apple or any other fruit, after the juice or oil has been squeezed out, mainly containing the pulp, seeds, peel and stem of the fruit. Apple pomace is the main by-product of apple juice production, accounting for 25 %, while about 75 % of the weight of the fruit is obtained as juice [7]. Reducing the use of food by-products and waste is an urgent issue in food processing, because the amount of food waste generated in the industrial process causes economic losses and creates difficulties in their management [8]. Considering the disproportionately large amount of materials produced during fruit processing, such as seeds, peels and their mixtures, a large amount of by-products are generated that are not used but are disposed of as waste or landfilled [9]. Studies conducted over the past decades have revealed that many food wastes can serve as a source of potentially valuable bioactive compounds [10] such as antioxidants, fiber and vitamins, which are of increasing scientific interest due to their beneficial effects on human health. In particular, industrial by-products obtained from fresh berries or fruits, as they are rich in antioxidants such as carotenoids, polyphenols, tocopherols and ascorbic acid [11]. Research also shows that the biological activity in the skins of fruits is higher than in the pulp, such as the skins of citrus fruits, apples, grapes and berries, the skin of which is the main source of natural antioxidants [12]. In addition to the valuable bioactive substances, the peel is an underrated and also a very valuable source of fiber. Therefore, it would be very useful to use whole fruits in processing, for example for jams, juices and other products [13].

'Apple pomace' is the solid residue left over (25–30 % of all processed fruit) after the production of apple juice or wine. In the world, several million tons of apple pulp are formed during production processes. It has a high moisture content (70–75 %) [14] and biodegradable organic loads (high biological oxygen demand and chemical oxygen demand values). High moisture content makes apple pulp bulky and susceptible to microbial decay. Large amounts of apple pomace create industrial safety issues and violate pollution control norms. Dumping these creates unpleasant smelling by-products during the biodegradation process and creates high transportation costs. In the past, solar energy was used to dry apple cores in an open field to reduce their size. This method makes the apple pomace darker (enzymatic or oxidative browning) and unsuitable for value addition, especially human food fortification [15].

Many technologies have been developed for the separation of various food compounds in the food industry. Traditional processes such as crystallization, filtration, distillation or precipitation are being replaced by new processes using membranes or supercritical fluids [16]. Supercritical fluid extraction was used in the study and this method is a separation process in which substances are dissolved in a liquid capable of changing its solubility under certain conditions above the critical temperature and pressure (supercritical region). The properties of a supercritical fluid are used to selectively extract a specific compound or to fractionate mixtures by changing temperature and pressure without changing phase [17]. Due to the practical advantages of CO_2 (it is non-toxic and non-flammable, environmentally safe because the process is closed, it has low cost with high purity and suitable for extraction of

natural compounds) (P_c = 7.28 MPa; T_c = 304.1 K), it is the most commonly used solvent in Supercritical fluid extraction. When the extract is recovered in separators, the CO_2 is easily separated [18]. Many publications have shown the extraction of apple pomace and the results obtained, however, the results obtained lead to the conclusion that each machine produces different results and amounts of extract in the process itself [19]. In this paper, experiments were carried out on the determination of the oil content of apple pomace and its extraction using supercritical CO_2 extraction. The study used apple pomace from a juice plant to find the use of these by-products in the production of a value-added product. Analysis of the obtained oil was also carried out.

2. Material, Methods and Results

2.1. Material

Apple pomace, which consists of peels, seeds, stems and pulp, obtained in a juice factory, which is currently sold for biogas production, but by extracting apple pomace, it is possible to find valuable substances in them that can be used in the production of products with added value. The apple pomace was sublimated.

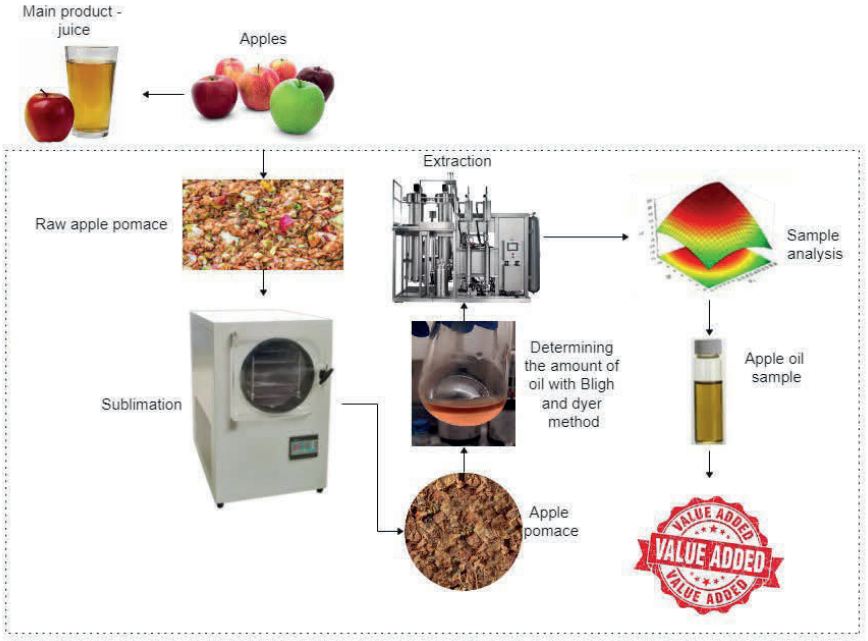

Fig. 1. Pathway of apple by-products to apple oil using supercritical CO_2 extraction method).

2.1.1. Sample preparation

Drying is one of the most important thermal processes, in which food is preserved. The aim of drying is to reduce water content to a level that prevents enzymatic reactions and the development of microorganisms, which have a negative effect on the quality of the material being dried [20].

Freeze drying (sublimation) is a low temperature dehydration process that involves freezing the product, releasing the pressure, and then removing the ice by freeze drying (or liofilisation, sublimation). Unlike dehydration using most traditional methods, in which water is evaporated using heat, the lyophilized product is of excellent quality, its original vitamins, proteins, other beneficial substances and the shape of the product are preserved as if it were the original product [21]. The dried samples were ground to a fine powder using a food grade mixer. All samples were packaged and stored at −20 to −40 °C until further use.

2.1.2. Characterization of apple waste

Oven, freeze-dried and fresh apple pomace were characterized by moisture content. The moisture content of the fresh residue was 88.6±2.4 % and the water activity was 0.93±0.01. Drying of apple waste resulted in a water reduction of about 70 %, thus the freeze-dried and oven-dried samples reached a moisture content of 19.2±1.1 % and 20.5±1.7 %, respectively. Both dried samples had a water activity of 0.15±0.02.

2.1.3. Bligh and Dyer method

The method of Bligh and Dyer was used to determine the amount of oil. Six samples of apple pomace were tested (no water was added to three samples and 5 mL of distilled water was added to the other three samples). Sublimated apple pomace (1.5 g) was weighed into 20 mL screw cap tubes and 4 mL of methanol, 4 mL of chloroform and glass beads were added. The mixture was shaken for 4 minutes in a homogenizer, followed by the addition of 2 mL of distilled water and repeated shaking for 1 minute. After shaking and adding all necessary ingredients, the samples were centrifuged at 8000 rpm for 15 minutes at 5 °C. Clean beakers (10 mL) were weighed while the samples were in the centrifuge. After centrifugation, the prepared samples were filtered with a glass filter in a 100 mL Bunsen flask and filter paper with a thickness of 3 μm was used. After filtering the liquid fraction, the biomass was carefully left in the tube and chloroform (2 mL) was added again, shaken and continued to separate the liquid fraction from the biomass. As the filtrate stratified, the lower layer was separated with a 1 mL dispenser pipette so that the upper layer did not enter the sample. It was necessary to tilt the flask at a 45° angle and by dipping the pipette tip to the bottom layer, the oil in the sample was obtained. Then the samples were placed for a day to evaporate in a fume hood and then weighed to obtain data on the amount of oil in the apples. The table 1 shows the oil quantity results obtained.

TABLE. OIL AMOUNT IN APPLE POMACE OBTAINED WITH BLIGH AND DYER METHOD

	Nr.	Sample weight, g	Oil amount, g	Oil amount, %	Oil in 1 g sample, g
	1	1.5	0.0353	2.35	0.024
	2	1.45	0.0359	2.48	0.025
	3	1.47	0.0438	2.98	0.030
+5 ml H_2O	4	1.55	0.0234	1.51	0.015
	5	1.53	0.0275	1.80	0.018
	6	1.57	0.0268	1.71	0.017

The results show that more oil can be obtained without adding additional water and the highest amount of oil obtained was almost 3 % of the oil sample. In the course of extraction, at the highest usable pressure, 3 % of apple pomace oil was also obtained.

2.1.4. Supercritical CO_2 Extraction

A green extraction method was chosen - supercritical CO_2 extraction. The extraction was initially performed for 3 hours and 170 bars. It was concluded that it is impossible to obtain oil at such a low pressure. Therefore, the pressure was raised in the subsequent extraction steps. In the process, the solute or CO_2 was used at a constant flow (5.0 cm^3 min^{-1}) at 280 bar pressure, 30 °C and 3 hours. After 3 h, the extracts were collected at 30–50 °C with multiple runs and 10–15 min between each run. During the extraction at 280 bar pressure, the largest amount of oil was obtained and 3 % or 6 milligrams of apple pomace oil was obtained. The figure shows the supercritical CO_2 extraction process, showing that after extraction, the oil sample was collected as it flows through separators 1 and 2. In separator 1, the pressure was constant around 70 bar and in separator 2–60 bar in all experiments. The supercritical CO_2 extraction process is shown in Fig. 2.

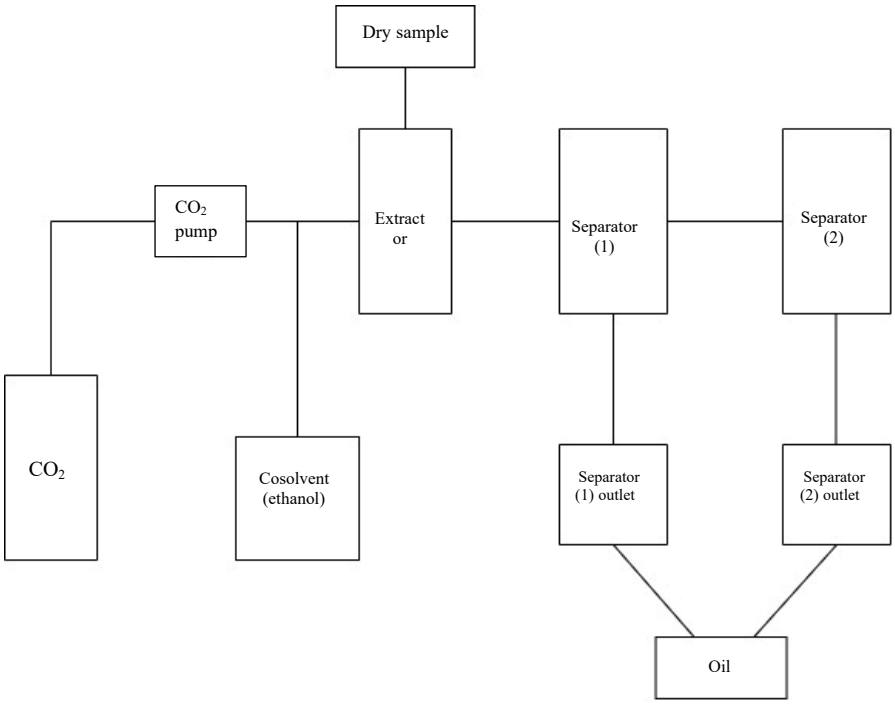

Fig. 2. Supercritical CO_2 extraction process.

3. CHROMATOGRAPHIC ANALYSIS OF THE EXTRACTS

Gas chromatography was used to analyze the results. Gas chromatography is a common chromatographic technique used in analytical chemistry for the separation and analysis of compounds that evaporate without decomposition. This process involves vaporizing and injecting the sample onto the head of the chromatography column. The sample is transported through the column with the aid of an inert gaseous mobile phase. The column itself contains a liquid stationary phase that is absorbed on the surface of an inert solid. Non-polar gas chromatography columns separate compounds primarily by their boiling point, while polar columns offer additional separation depending on the polarity of the compounds [22]. The procedure of sample preparation and chromatography conditions: 20 mg of the sample were

placed into the bootle and triglicerides were saponified by 0.5 N KOH in MeOH for 1 hour at 75° C, then the methylation was done by addition of sulfuric acid for 1 hour at 75° C. Extraction of fatty acid methyl esters (FAMEs) was performed by addition 10 ml of Hexane with methyl behenate (C22:0) as an internal standard. The chromatography was performed on Shimadzu GC-20 with FID detector on Restek SP-2560 column (100 m × 0.25 mm I.D., 0.20 um) in the following conditions: injector temp. –250° C; detector temp. –250 °C; oven - 180 °C isothermal for 32 min, column flow – 2 mL/min, ramped to 215 °C at 20 °C/min, hold 22.75 min; carrier gas He, flow rate – 45.0 mL/min; injection 1 μL in split mode 20:1. The results are shown in Table 2.

TABLE 2. APPLE POMACE OIL RESULTS

	Fatty acid methyl esters (wt.% of total fatty acid)	**Average**	**Deviation**
Methyl myristate C14:0	C14:0	0.26	0.00
Methyl palmitate C16:0	C16:0	8.59	0.12
Methyl heptadecanoate C17:0	C17:0	0.10	0.01
Methyl stearate C18:0	C18:0	3.20	0.04
Methyl arachidate C20:0	C20:0	1.73	0.03
Methyl heneicosanoate C21:0	C21:0	0.15	0.01
Methyl behenate C22:0	C22:0	0.70	0.03
Methyl lignocerate C24:0	C24:0	0.00	0.00
	Total saturated fatty acids	14.87	0.12
Methyl palmitoleate C16:1 (c9)	9c-C16:1	0.07	0.01
Methyl oleate C18:1 (c9), (Z)-octadec-9-enoic acid, Oleic acid (ω-9)	9c-C18:1	28.22	0.01
vaccenic acid - C18:1n11c	11c-C18:1	0.23	0.01
	Total monounsaturated fatty acids	29.07	0.05
Methyl linoleate C18:2 (c9, c12), Linoleic Acid (LA) (ω-6)	C18:2n-6	53.53	0.07
Methyl eicosadinoate C20:2 (c11, c14), Eicosadienoic acid (ω-6)	C20:2n-6	0.33	0.04
	Total n-6 long-chain polyunsaturated fatty acids	53.86	0.11
α-Methyl linolenate C18:3 (c9, c12, c15), a-Linolenic acid - ALA (ω-3)	C18:3n-3	1.27	0.03
Methyl eicosapentaenoate C20:5 (c5, c8, c11, c14, c17), Eicosapentaenoic acid-EPA (ω-3)	C20:5n-3	0.32	0.02
Methyl docosahexaenoate C22:6 (c4, c7, c10, c13, c16, c19), Cervonic acid - DHA (ω-3)	C22:6n-3	0.36	0.14
	Total n-3 long-chain polyunsaturated fatty acids	1.96	0.19
	Total cis- polyunsaturated fatty acid	55.82	0.08
$W_{FAx,}$ % (wt.% of total sample mass)		69.2	11.7

4. CONSLUSIONS

The resulting compounds can be used in the production of various products. Below are the possibilities of using the fatty acids obtained in various industries.

Methyl Myristate is used as a component in the preparation of a high-density biodiesel. Also used as a niosome medium in which an anti-cancer drug could be transported within [23].

Methyl palmitate is a fatty acid methyl ester. It has a role as a metabolite. Methyl palmitate is a natural product found in *Zanthoxylum beecheyanum*, *Lonicera japonica*, and other organisms with data available [24].

Methyl heptadecanoate is a fatty acid methyl ester. Methyl Heptanoate can be used as pharmaceutical intermediate chemical in pharmaceutical industries. Methyl Heptanoate can be used in as fragrance agent in the fragrance industries. Methyl Heptanoate can be used in cosmetics industries as surfactant, emulsifier, stabilizer and fragrance agent [25].

Methyl stearate is used as a nonionic surfactant, thereby enhancing the solubility of chemicals by dissociating aggregates and unfolding proteins. It is a fatty acid ester, which is used as an emulsifier and stabilizer [26].

Methyl arachidate is a fatty acid methyl ester resulting from the formal condensation of the carboxy group of arachidonic acid with methanol. Methyl arachidonate has activity of human blood serum metabolite [27].

Methyl heneicosanoate is intended to be used as a replacement for petroleum diesel or it can be blended with petroleum diesel fuel in any proportion, so called, “Biodiesel” and is generally regarded as being more environmentally friendly [28].

Methyl Palmitoleate is used as a component in the preparation of biodiesel fuels [29].

Methyl oleate, (Z)-octadec-9-enoic acid, Oleic acid (ω-9) can be used in the production of cosmetics, flavors and fragrances [30]

Linoleic Acid which is used as a flavouring ingredient in condiments and spices [31]. This fatty acid was the most abundant in the apple pomace sample. This fatty acid can be used not only in food, but also in the cosmetic industry, as research shows that it has skin regenerating properties, as well as soothing the skin and being used as an antioxidant [32]. In the medical industry, it can be used in the production of drugs against cholesterol and to improve metabolism [33].

As indicated above, the obtained apple pomace oil can be used in various industries, starting with food and ending with biofuel production.

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