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Unlocking the potential of carrot pomace: Enzymatic and impact-disintegrator-activator processing for elevated beta-carotene concentration in carrot powder

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ABSTRACT

Backgrounds: Carrot pomace is a by-product of the juice industry, which quickly spoils due to its high moisture content and is usually considered waste. However, it is a valuable product containing a significant amount of bioactive compounds, particularly carotenoids.

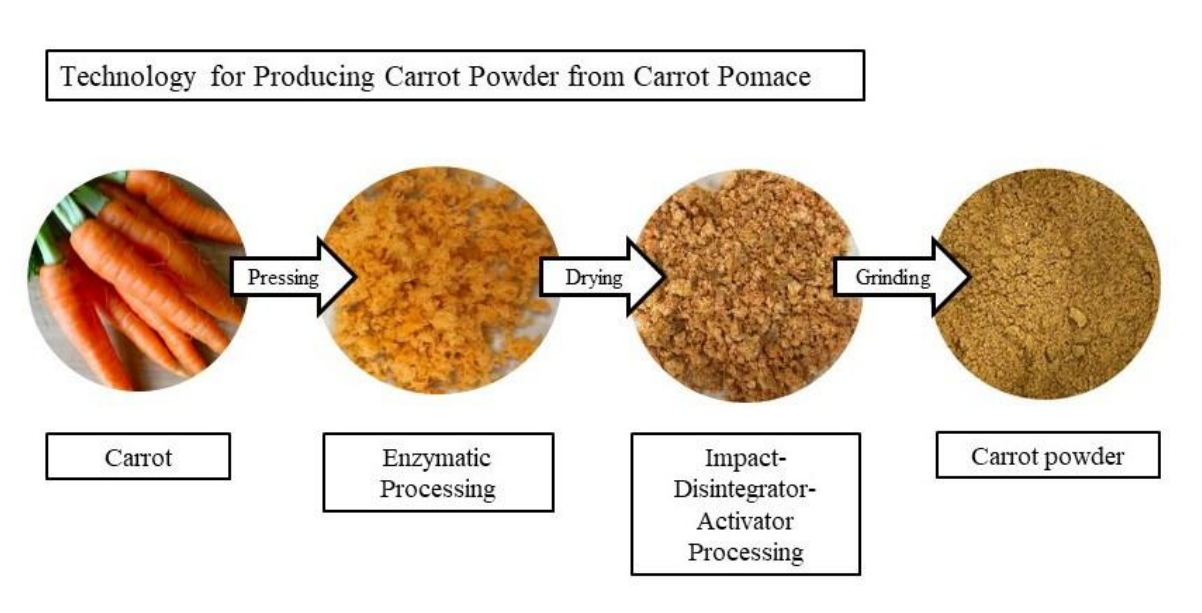
Context and purpose of this study: In this study, we aimed to develop a technology for obtaining carrot powder from carrot pomace with an increased availability of beta-carotene. The unique aspect of the technology involved the use of enzymatic and impact-disintegration-activation processing (IDAP) of the raw material to enhance the release of beta-carotene from plant cells.

Objective of this study: To achieve the goal, we have set objectives to compare various dosages of enzyme preparation and to evaluate devices for grinding carrots that can be used to make powder. At all stages, it was necessary to measure the amount of beta-carotene in the samples.

Results: It has been found that the yield of carotenoids from carrot pomace is 185% higher compared to whole carrots. Moreover, the application of enzymatic and impact-disintegration-activation processing significantly increases the carotenoid yield in the powder by 17 times compared to carrots (200% in terms of dry matter). This remarkable result was achieved through the described processing method and the selection of the enzyme preparation. Additionally, the mechanoactivation obtained by using the Desi-15 disintegrator played a crucial role. It was also observed that the use of impact-disintegration-activation processing for further grinding of dried carrots can increase the beta-carotene yield by 8 times.

Conclusions: The carrot pomace was processed, resulting in a fine-dispersed carrot powder that shows promising applications in the food or agricultural industries.

Keywords: Carrot pomace, beta-carotene, impact-disintegrator-activator processing (IDAP), enzymatic processing, pectolytic enzyme preparations



INTRODUCTION

Fruits and vegetables are an integral part of a healthy lifestyle, and their regular consumption reduces the risk of cardiovascular, metabolic, and degenerative diseases, as well as certain types of cancer [1-3]. This is primarily achieved due to their high content of vitamins, minerals, and phytochemicals, such as polyphenols, flavonoids, carotenoids, anthocyanins, etc., which exhibit potent antioxidant activity [4].

However, fresh fruits and vegetables are perishable products due to their high moisture content (often exceeding 80%). Moisture leads to microbial growth, which, in turn, causes product spoilage [4-5]. Drying fruits and vegetables is a widely used method, where water

removal halts the growth of microorganisms and enzymatic and non-enzymatic browning reactions in plant tissue. This helps preserve the structure, sensory characteristics, and nutritional value of the product. Additionally, drying saves storage space [6-7].

Recently, there has been a rapid increase in demand for fruit and vegetable powders due to their appealing taste, color, texture, and high energy content. Additionally, consumers are seeking compact and lightweight products [8]. In the food industry, these powders find various applications, including use as flavor enhancers and colorants, or as functional food additives to enhance the nutritional value of products [9].

The creation of carrot powders is motivated by the wide consumption of carrots globally, with an annual production of approximately 36 million tons [10]. Carrots serve as a valuable source of compounds, including vitamins, carotenoids, minerals, and dietary fibers, imparting highly beneficial properties. Notably, carrots contain beta-carotene, a provitamin A not synthesized by the human body [11-12]. Carotenoids and vitamin A offer numerous health benefits, demonstrating anticancer, antimutagenic, immunomodulatory, and antioxidant properties, while vitamin A is essential for vision and skin health [12-15].

However, around 25-30% of harvested carrots are discarded by producers due to non-compliance with market quality standards [16]. Companies dealing with fruits and vegetables sometimes lack storage space or equipment for processing unused carrots, leading to further disposal in supermarkets, restaurants, and households. This results in a substantial amount of waste, posing economic and environmental challenges. Despite ongoing efforts by scientists to develop various technologies for processing food waste, including carrots [17-20] 15-20% of discarded carrots are utilized as animal feed or soil fertilizer. Most of the waste ends up in landfills, contributing to unpleasant odors, the release of greenhouse gases during decomposition, and the proliferation of insects and other pests [10].

Addressing this issue could involve the utilization of carrot waste to enhance the value of its primary components and potentially produce high-value-added products. In addition to fresh carrots, carrot pomace, which remains after carrot juice production, can serve as a raw material for creating carrot powder. This raw material already has a well-disrupted cell wall due to grinding, heating, and the presence of pectolytic enzymes commonly used in juice production, and carotenoids are effectively extracted and absorbed by the body [21].

The potential applications of such powder are extensive. It can be used as a colorant in confectionery and culinary applications. Additionally, as a functional component, it can be incorporated into the production of dairy products, cheeses, and oils. It is important to note that natural carotenoids are fat-soluble compounds that are sensitive to sunlight and oxygen but more resistant to the temperatures used in technological processes [22]. There are studies on how carrot powder was used in recipes for butter, tortillas, noodles, and even sausages to enrich them with beta-carotene [23-25].

The primary operations in the technology of creating food powders are drying and grinding, which significantly impact the quality of the final product and the quantitative content of beneficial substances [26]. There is a considerable amount of research focused on selecting the optimal drying equipment for food powder production [27-29]. However, the grinding process also plays a crucial role. Grinding, similar to heat treatment, can influence the bioavailability of bioactive substances, i.e., their assimilation by the body. This is because these processes break down the thick and sturdy cell walls of plants, releasing the accumulated nutrients within the cells [30]. Typically, grinding on an industrial scale is

carried out using various types of mills and crushers, with disintegrators being of particular interest.

The disintegrator is a multifunctional impact mill designed for fine grinding, during which the mechanical activation of components in the raw material takes place [31]. Material grinding in the disintegrator occurs through a series of rapid high-speed impacts on the rotor's working elements. The impact speeds increase as the material particles move from the center of the rotors toward the periphery. The working components consist of two counter-rotating rotors with several concentrically arranged rows of impact elements of different shapes, such as blades and fingers. The thickness of the ground products can range from a few microns to hundreds of microns, depending on the material properties, disintegrator's design, and operating conditions.

One of the distinctive features and advantages of disintegrators compared to other grinding units is the high-speed nature of the grinding process. Within a short time, interval, typically about 0.1 seconds, the material processed in these units receives 2 to 7 high-intensity impacts. Therefore, the advantages of using a disintegrator include obtaining fine-dispersed powder, high productivity, and preserving the original purity of the material. Consequently, the powder produced from plant raw materials exhibits enhanced biological accessibility and assimilation [32-33].

Previous studies by the authors demonstrate that the processing of rye grain on an impact-disintegrator-activator type installation increases the content of the albumin-water-soluble protein fraction of rye grain, allowing for enhanced bioavailability and digestibility of the protein [34]. Another study shows that processing carrots and beets on the disintegrator-convective thermolabile installation DKC-1TL allows for obtaining fine-dispersed vegetable powders. The incorporation of these powders into bakery products enhances their nutritional and biological value as well as the quality indicators of the final products [35]. In the work of other Russian scientists on the production of functional food products with enhanced antioxidant activity, the raw material was processed using the Dezi-11 disintegrator [36-37]. Despite the existence of studies that use disintegrator grinding, the authors did not find similar technologies for processing carrot pomace involving enzymatic and impact-disintegrator-activator processing (IDAP).

MATERIALS AND METHODS

Materials: For this research work, fresh samples of the Nantes carrot variety (Daucus carota subsp. sativus “Nantes”) were taken and cleaned before use. Part of the carrots were finely grated using a fine electric grater, while another part was processed to obtain carrot juice using the Bork S701 centrifugal juicer. The initially grated samples were then treated with the enzyme preparations Fructozym MA and Fructozym BE. After the enzymatic treatment, the samples were dried using the convective drying method with a dehydrator at a temperature of 50°C for 12 hours. To obtain carrot powders, the samples were additionally ground using the Bosch TSM6A01 rotary-blade mill and the Desi-15 disintegrator. The obtained carrot powders were stored in a sealed container in a dry and dark place to preserve their quality.

The research utilized liquid high concentration pectolytic enzyme preparations from the company ERBSLÖH, namely Fructozym MA and Fructozym BE. These preparations contain the following enzymes: α-amylase, exo-β-glucanase, pectinase, endo-polygalacturonase, xylanase, and cellulase. Both preparations are predominantly composed of endo-polygalacturonase, which is a pectolytic enzyme of endo-action, present in quantities of 212 PGU/ml and 87 PGU/ml, respectively. This enzyme catalyzes the hydrolysis of pectic acid.

Indicators of carrot, carrot pomace, and carrot powder: To determine the quality indicators of carrot powder, its moisture content and beta-carotene content (μg/g) were measured, and the particle size distribution of the powder was analyzed.

Moisture content was determined using a moisture analyzer (Shimadzu MOC63u, Japan). Particle size analysis of carrot powder was performed using a laser analyzer (Malvern Instruments Mastersizer 2000, UK).

The method for determining the beta-carotene content in the plant material is based on extracting beta-carotene from the product with a mixture of petroleum ether and acetone, followed by solvent evaporation, separation of beta-carotene from other carotenoids in the obtained extract using column chromatography, and spectrophotometric determination of carotene content [38].

Sample for testing and preparation of the sample solution: Three grams of the prepared sample were weighed and transferred quantitatively to a mortar. Then, 20 cm3 of the extraction mixture (a solution of 0.2 g of hydroquinone in 40 cm3 of acetone with the addition of 160 cm3 of petroleum ether) was added. Next, 20 g of anhydrous sodium sulfate and 30 g of quartz sand were added, and the mixture was thoroughly triturated. The obtained extract was decanted onto a filter in a 500 cm3 conical flask. The extraction was repeated until a clear solution was obtained. The remaining residue was quantitatively transferred to the filter and rinsed with a mixture, collecting the filtrate in a conical flask. The extracts were combined, transferred to a 1000 cm3 separating funnel, and washed several times with 300 cm3 of water to remove acetone residues. The extract was shaken gently, avoiding the formation of an emulsion. The extraction was repeated with another sample, following the same procedures, but using a solution of sodium chloride with a mass concentration of 300 g/dm3 instead of water. To the washed extract, 15 g of anhydrous sodium sulfate was added, mixed, and left for 15 minutes. The solution was filtered through a filter, half-filled with anhydrous sodium sulfate, into a 500 cm3 round-bottom flask. The sodium sulfate layer was washed with three portions of 10 cm3 of petroleum ether, collecting the washings together with the filtrate. The obtained filtrate was evaporated on a vacuum evaporator at 30°C water bath temperature and quantitatively transferred to a 50 cm3 volumetric flask, and the volume was brought to the mark with petroleum ether (V1).

Chromatographic separation: A cotton wool plug was placed in the wide, lower part of the chromatographic column. The column was connected to Vitt's filtration assembly, and the mixture of aluminum oxide and anhydrous sodium sulfate was carefully added to the column to a height of 15-20 cm. A round-bottom flask with a capacity of 100 cm3 was used to collect the eluate from the column. Vitt's filtration assembly was connected to a water pump. Next, the column was filled with petroleum ether. When the level of petroleum ether was about 1 cm above the surface of the adsorbent, a precise volume of the sample solution (V2) ranging from 5 to 20 cm3, depending on the intensity of the color, was added. When the level of the sample solution dropped to about 1 cm above the surface of the adsorbent, 30 cm3 of petroleum ether was added. This addition was repeated until a clear eluate was obtained (usually after adding 20 cm3 of petroleum ether). Depending on the intensity of the color of the obtained eluate, its volume was adjusted (concentrated or diluted with petroleum ether, if necessary) to ensure that 0.4-3.0 μg of carotene was present in 1 cm3 of the eluate. The final volume of the eluate (V3) was recorded.

Spectrophotometric measurement: The measurements of the optical density of the eluate were carried out at a wavelength of 450 nm, using cuvettes with an optical path length of 10 mm, and using petroleum ether as the comparison solution.

The molar absorption coefficient $A_{1\,cm}^{1\%}$ for pure beta-carotene solution in petroleum ether at 450 nm is 2530.

Data processing: The beta-carotene content in the product is calculated using the following formula:

$$W(C_{40}H_{56}) = \frac{A \cdot V_1 \cdot V_3}{0.25 \cdot V_2 \cdot m}$$

Where: $W(C_{40}H_{56})$ is the content of beta-carotene in the product, micrograms per gram of the product;A is the optical density of the eluate obtained (2530); V_1 is the volume of the sample solution used, cm^3 (50 cm^3); V_2 is the volume of the sample solution used for chromatographic separation, cm^3; V_3 is the final volume of the eluate, cm^3;

0.25 is the correction factor for beta-carotene;

m is the mass of the product sample, grams.

The final result is the arithmetic mean value of three parallel determinations. The statistical significance of the experiment was determined using the student's t-test.

RESULTS

The effect of enzyme preparations on the yield of beta-carotene: For this experiment, the influence of two enzyme preparations, Fructozym MA and Fructozym BE, produced by ERBSLÖH Geisenheim (Germany), on the beta-carotene yield from finely grated carrots was compared (Figure 1). The enzyme preparations were incubated in a water bath at 50°C for two hours, and the enzyme dosage used was 0.03% of the raw material's mass (RMM).

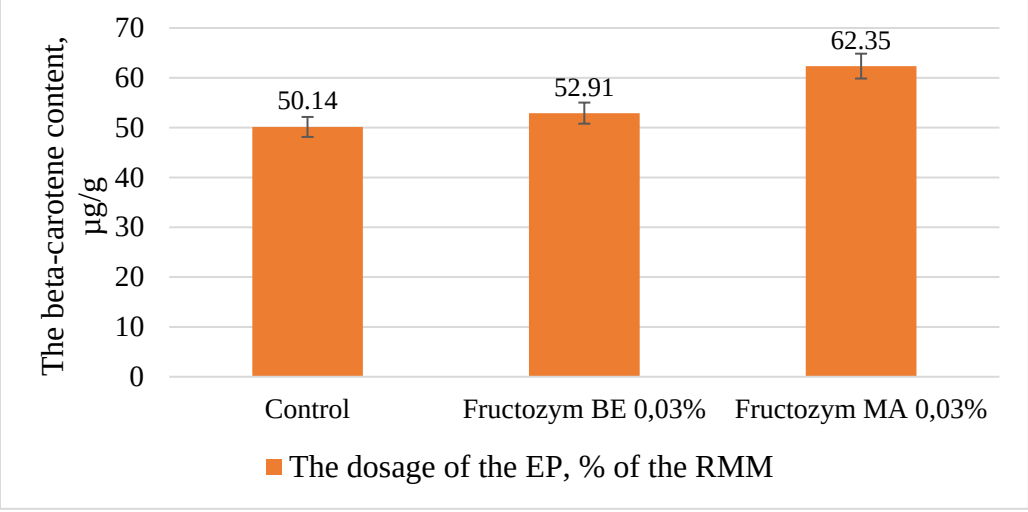


Figure 1. The effect of enzyme preparations (EP) Fructozym BE and Fructozym MA on the yield of beta-carotene from carrots.

This experiment demonstrated that both enzyme preparations increase the yield of beta-carotene, but Fructozym MA showed a higher result, which may be attributed to the presence of the enzyme exo-β-glucanase, which is absent in the Fructozym BE preparation, and the higher content of endo-polygalacturonase [39].

The next experiment (Figure 2) was conducted to determine the optimal dosage of the Fructozym MA preparation. The enzyme preparation was also incubated in a water bath at 50°C for two hours.

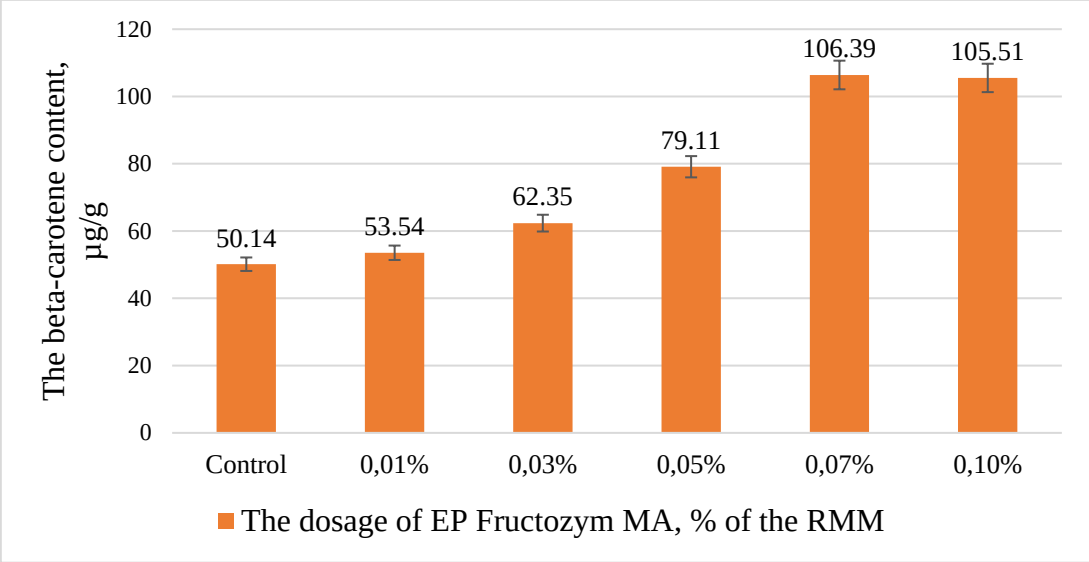


Figure 2. Changes in the amount of beta-carotene depend on the dosage of Fructozym MA.

The results demonstrate that in this experiment, the highest yield of beta-carotene was obtained from carrots treated with the enzyme preparation at a dosage of 0.07% of the raw material's mass. At this dosage, the carotenoid yield increased by more than 2 times compared to the control sample. However, with an increase in the dosage of the enzyme preparation to 0.1%, the beta-carotene yield gradually starts to decrease. Additionally, an experiment was conducted to determine the optimal incubation time of the enzyme preparation Fructozym MA at a dosage of 0.07%, as shown in Figure 3.

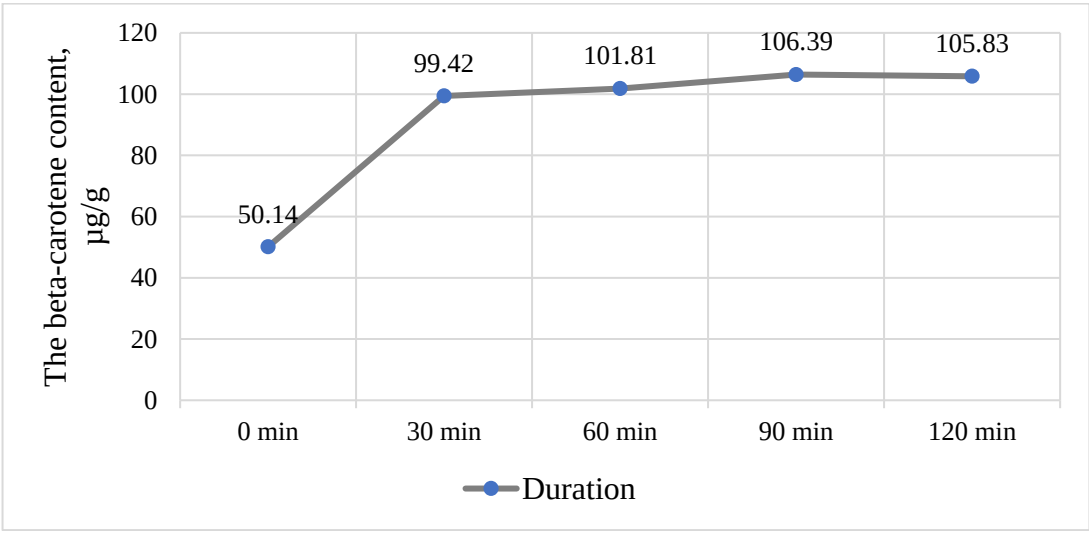


Figure 3. Changes in the amount of beta-carotene depend on the duration of EP treatment. Fructozym MA, dose 0.07%.

Thus, the optimal duration of enzyme preparation treatment is 90 minutes, longer exposure does not yield significant benefits.

The effect of the degree of destruction on the yield of beta-carotene: The experiment was conducted with carrots grated using an electric grater and dried using convective air drying at 50°C for 12 hours. Subsequently, the carrots were further pulverized using a rotary-blade mill (Bosch TSM6A01) and a Desi-15 disintegrator (IDAP). The appearance of the samples and the results are shown in Figures 4 and 5.

(C) (1) (2)

Figure 4. The appearance of the dried carrot samples
C – control sample without additional grinding;
1 – carrot powder obtained using a rotary-blade mill.
2 – carrot powder obtained using a disintegrator (IDAP).

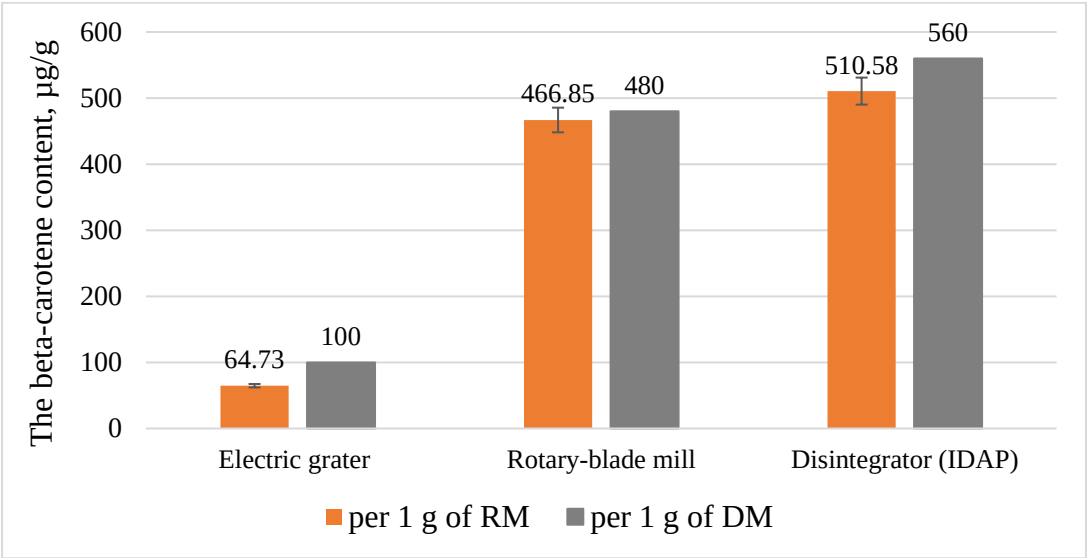
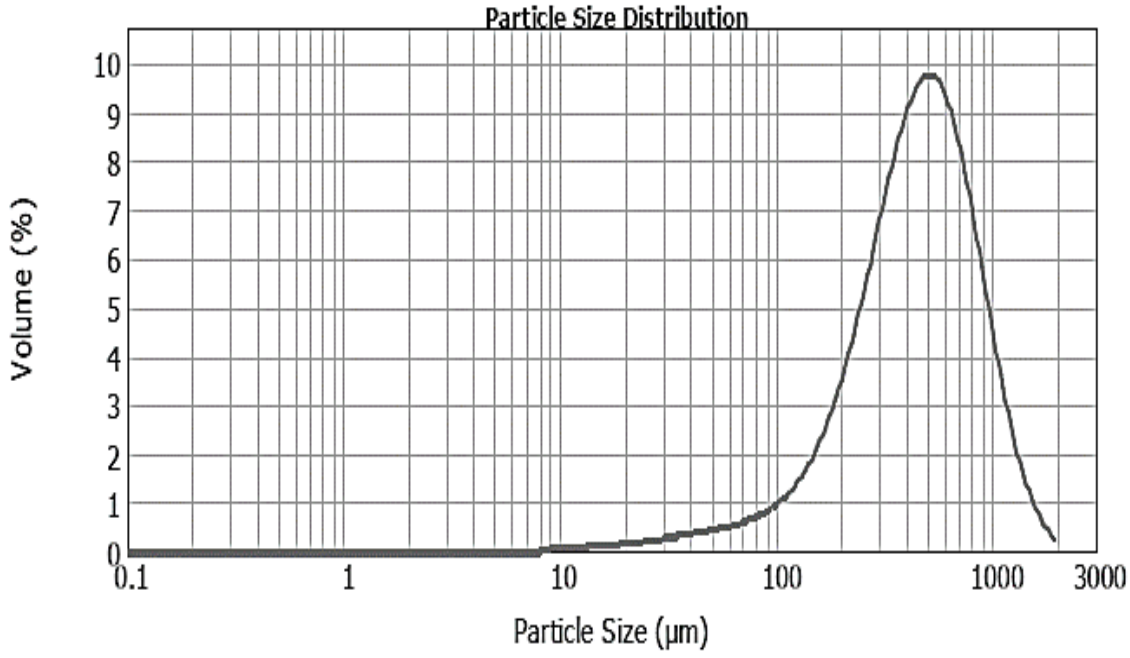


Figure 5. The content of beta-carotene in the dried carrot samples µg per 1 g of raw material (RM) and per 1 g of dry matter (DM).

Additionally, a granulometric analysis of these powders was performed using the laser analyzer Mastersizer 2000, and the results are presented in Figure 6 and Table 1, showing the composition analysis of powders obtained from the rotor-blade mill and the disintegrator.



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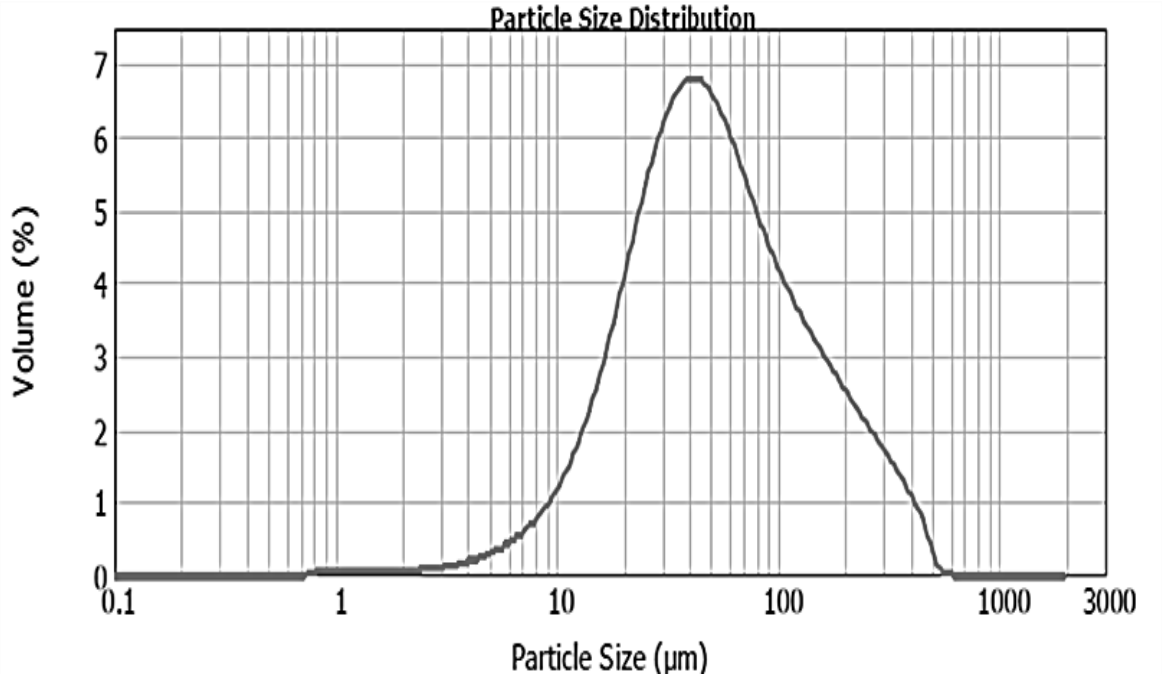

(2)

Figure 6. Granulometric composition of powders 1– Rotor-blade mill; 2 – Disintegrator (IDAP).

Table 1. Particle size distribution of powders. The percentage distribution of particle sizes in the powders was obtained using a rotor-blade mill and a disintegrator.

Particle size, µm	Rotor-blade mill, %	Disintegrator (IDAP), %
0-1	-	0,11
1-10	0,15	5,13
10-50	2,37	**47,58**
50-100	4,10	27,34
100-200	12,27	13,10
200-500	**43,23**	6,72
500-1000	32,24	0,02
1000-2000	5,63	-

By analyzing the obtained data, it can be confidently stated that the degree of grinding significantly influences the extraction of beta-carotene from carrots. There is a difference of 7 times or more in the amount of beta-carotene detected in the tested samples. As shown in Figure 4, the powder obtained from the same raw material using a disintegrator has a finer and more homogeneous structure and a more intense color compared to the powder obtained from the rotor-blade mill.

The particle size analysis vividly shows the composition of the powders, highlighting that the powder obtained through the disintegrator has a finer particle size. Most of the powder obtained with the disintegrator consists of particles sized 10-50 µm, whereas the powder from the rotor-blade mill has a main particle size range of 200-500 µm (10 times larger). Additionally, during the analysis of the latter powder, 30% of particularly large particles with a size over 3 mm were detected, which were beyond the measurement range of the instrument.

The complex effect of enzyme preparations and IDAP on the yield of beta-carotene: During carrot juice production, carrots are crushed, and the pomace is subjected to heat and pectolytic enzyme treatment. The resulting pomace contains fewer carotenoids since a portion of them is extracted into the juice. However, the disrupted cell walls release more substances trapped within the plant cell, which are better absorbed by the body [40]. Therefore, it was decided to determine the amount of extractable beta-carotene in carrot pomace and use it for obtaining powder processed using IDAP. For comparison, fresh carrot samples were taken, and grated, and carrot juice was extracted from a part of these carrots. A portion of the pomace obtained after grinding and juice extraction in the juicer was treated with the enzyme preparation Fructozym MA, which showed promising results in previous experiments with carrots. Three dosages were selected: 0.05%, 0.07%, and 0.10% of the raw material mass. The results of the experiment are presented in Figure 7.

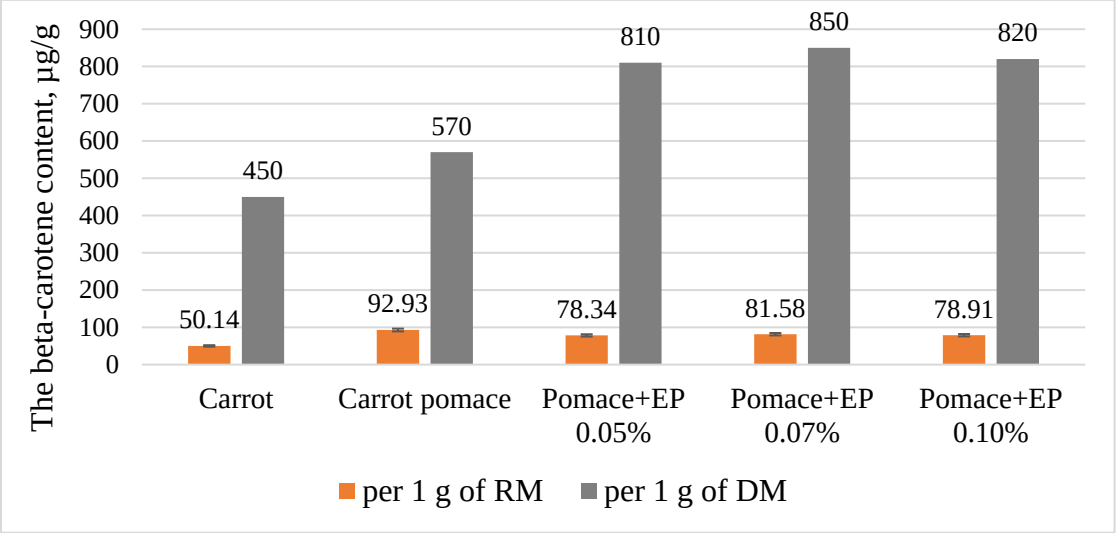

Figure 7. The beta-carotene content in samples of raw carrot and carrot pomace, µg per 1 g of raw material (RM) and 1 g of dry matter (DM). Enzyme preparation Fructozym MA (EP).

Thus, it can be concluded that the sample of carrot pomace with the addition of the enzyme preparation Fructozym MA at a dosage of 0.07% of the raw material mass contains the highest amount of beta-carotene per 1 g of dry matter.

In the next series of experiments, the carrot pomace was dried, further pulverized, and the amount of beta-carotene in the dried samples was measured. Drying was also conducted using the convective method, and the pulverization was done using the IDAP, which showed promising results in previous experiments. Additionally, the pomace samples treated with the enzyme preparation Fructozym MA at a dosage of 0.07% of the raw material mass were dried and further pulverized. The results are presented in Figure 8.

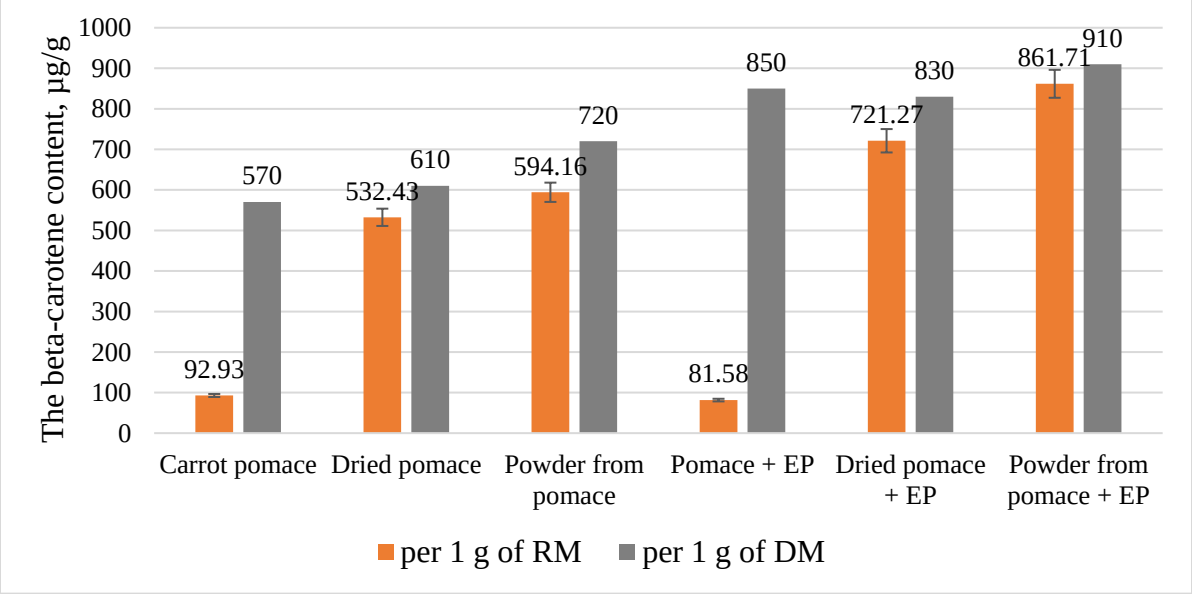

Figure 8. The beta-carotene content in carrot pomace and powder samples µg per 1 g of raw material (RM) and 1 g of dry matter (DM). Enzyme preparation Fructozym MA, dose 0.07% (EP).

Indeed, from the histogram, it is evident that the combined treatment of carrot pomace with the enzymatic preparation and the IDAP significantly increases the beta-carotene yield from the raw material.

DISCUSSION

The experiments resulted in the development of a technology for producing carrot and carrot pomace powder with high beta-carotene availability. The mathematical processing and analysis of the data demonstrated a positive effect of extensive disruption of carrots and pomace on beta-carotene content. Grinding the raw materials with a rotor-blade mill increased the beta-carotene yield by 7 times and using a disintegrator (IDAP) increased it by 8 times compared to the grating. The powder obtained through disintegration had a finer particle size distribution. The majority of particles in the disintegrated powder ranged from 10 to 50 µm, whereas in the rotor-blade mill powder, the main particle size was 200 to 500 µm (10 times larger).

Furthermore, a positive influence of enzymatic treatment on carrot's beta-carotene content was established. Through experimentation, a suitable enzyme preparation and its optimal dosage were identified. Both Fructozym MA and Fructozym BE enzymatic preparations enhanced beta-carotene yield, with Fructozym MA showing superior results, likely due to the presence of the enzyme exo-β-glucanase, which is absent in Fructozym BE, and a higher concentration of endo-polygalacturonase. The highest beta-carotene yield was achieved with carrots treated with a 0.07% dosage of the enzymatic preparation, resulting in a more than twofold increase compared to the control sample. The optimal duration for enzymatic treatment was found to be 90 minutes, after which the beta-carotene level plateaued.

Additionally, experiments were conducted to determine the beta-carotene content in various samples of carrot pomace. The best result in terms of beta-carotene quantity was observed in the sample of carrot powder treated with the enzymatic preparation Fructozym MA. Compared to the regular powder from carrot pomace, the treated powder contained 1.5 times more beta-carotene. Furthermore, in comparison to untreated carrot pomace, the treated powder had 9.3 times more beta-carotene.

It can be concluded that there is a technological possibility for producing carrot powder from carrot juice waste with high beta-carotene content and increased bioavailability, thanks to the utilization of IDAP. This technology shows promise and is suitable for processing various secondary plant raw materials. Such finely dispersed powder with high absorbability will be in demand in the food, agricultural, and medicine, industries particularly when the original raw material contains biologically active substances.

In addition, this technology of carrot pomace processing can become a valuable resource for the food industry, enabling the efficient utilization of carrot juice waste and other plant-based raw materials to produce a high-quality powder with high nutritional and biological potential. The application of this developed technology can reduce waste processing costs and promote sustainable development in the food sector.

An important direction for further research is the detailed investigation of the biological availability and absorption of beta-carotene from the developed powder in the human body through clinical studies. This will ensure its effectiveness and safety as a functional food product or supplement. Additionally, further research could explore the application of the developed powder in the food industry to create new functional products, such as beverage additives, cosmetic products, or products for skin health support. Studies on the economic efficiency of carrot powder production and the assessment of its market potential can also provide valuable information

for companies and entrepreneurs interested in implementing this technology on an industrial scale.

CONCLUSION

The obtained research results hold significant importance for the food industry and scientific community, particularly in the field of food products and healthy nutrition. The technology developed for producing carrot powder with increased beta-carotene availability, based on enzymatic treatment and high levels of carrot and pomace disruption, opens new prospects for creating beta-carotene-rich functional products.

In conclusion, the prospects for applying the developed technology of carrot powder with high beta-carotene content are vast and require further research to fully unleash its potential and contribute to the promotion of healthy nutrition and sustainable resource utilization.

Abbreviations: IDAP: impact-disintegrator-activator processing, EP: enzyme preparations, RMM: raw material's mass, RM: raw material, DM: dry matter.

Author's Contributions: All authors contributed to this study.

Conflict of Interest: The authors declare no conflict of interest.

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