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USING NOVEL EXTRACTS FROM CARROT RESIDUES AND BY-PRODUCTS IN THE CONSTRUCTION OF VALUABLE FUNCTIONAL FOODS

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Abstract

Even if there are certain EU development programs, agri-food resources are used today without an adequate strategy in terms of food safety and regional/state food security.
Significant volumes of edible parts of agri-food products are lost - increasing food waste, in conditions where certain climate changes and possible economic crises may block the recovery of some production systems.
Moreover, promoting a circular economy, recovering valuable bio-compounds from certain agri-food products and using them in the development of functional foods, could contribute to sustainable development and increased life expectancy.
The paper presents a case study that highlights how a series of valuable bio-compounds (less used today) can be recovered from agri-food products and used within innovative eco-friendly technologies - in the development of functional foods beneficial to the consumer's health.
Using non-polluting and efficient methods, innovative technologies were obtained that led to the production of food supplements with real effects in certain metabolic disorders (metabolic syndrome). Technologies that used electro-chemical, bio-chemical and spectral analysis methods (SFE, AAS, UV-VIS, analysis of the activity of some oxidoreductases, bio-membrane activation techniques) produced outstanding results reaching a validated TRL5 level.
As a result of the application of these technologies, safe and innocuous food supplements and functional foods were obtained from previously unused by-products

Key words**: **functional foods, technology, carrots, by-products

INTRODUCTION

Carrots are vegetables rich in antioxidants, vitamins, and dietary fiber while containing very few calories (only 41 Kcal per 100 grams of carrots) and little vegetable fat [**1**]. Besides the fact that only 100 grams of carrots cover the recommended daily amount of vitamin A and β-carotenes, carrots are a fair source of B vitamins, vitamin K, folic acid, and potassium [**2**]. 100 g fresh carrot contains 8285 mcg of beta-carotene and 16706 I.U. of vitamin A. Beta-carotene is one of the most powerful natural antioxidants, protecting the body against harmful free radicals. In the liver, carotenoids are transformed into vitamin A – so necessary for visual acuity, reproduction, maintenance of epithelial (skin) integrity, growth, and development. Fresh carrots are rich in vitamin C (there are 5.9 mg of vitamin C in 100 grams of carrots), which is almost 10% of the Recommended Dietary Allowance (RDA). The role of vitamin C in the body is very well known. It acts as both an antioxidant and a protector for healthy connective tissue and in many metabolic processes[**3**]. Fresh carrots contain large amounts of B vitamins: folic acid, vitamin B6 (pyridoxine - 100 g represent 10% of the Recommended Dietary Allowance (RDA), vitamin B1 (thiamine - 6% of the Recommended

Dietary Allowance), vitamin B5 (pantothenic acid - 5.5% of the RDA), vitamin B3 (niacin - 6% of the RDA), vitamin B2 (riboflavin - 4% of the RDA). All of these are bioactive compounds that work as enzyme co-factors in catalyzed biochemical processes in the body. It is known from the relevant published literature that the flavonoids in carrots act as protective factors against the oral cavity, lungs, and skin cancer [**3**]. Vitamin K, which is involved in certain redox processes beneficial for the body, is also contained in carrots, as there is 13.2 mcg of vitamin K in 100 g of carrots (stronger antioxidant -almost 11% of the RDA) [**4**]. In addition to these vitamins, carrots also contain important amounts of minerals: potassium, manganese, phosphorus, calcium, and copper. 100 g of carrots contain 320 mg of potassium (6.5% of the RDA)[5]. This mineral is important in maintaining a good heart rate and blood pressure and in minimizing the effects of sodium in food [**6**]. Manganese is found in an amount of 0.143 mg/100 g of carrots (7.2% of the RDA). This element acts as a co-factor for a very important redox enzyme („Superoxide Dismutase - SOD") [**7**].

The juice yield is up to 60-70%, but waste represents a high percentage (30-40%) of the volume of processed carrots [8]. This waste must be properly processed to reduce losses, using techniques and technologies from the bio-economy („cascade processing" or „multi-stage processing") [**9**] In these types of processing, the wastes resulting from certain operations constitute raw materials for others [**10**]. From the relevant published literature, about 80% of the beta-carotene content of processed carrots remains in the waste resulting from processing [**11**]. In processed carrot waste there are also important amounts of dietary fiber, other carotenoids, vitamins, and minerals [**12**]. They can be extracted from waste and used as antioxidants in anti-carcinogenic and immune-stimulating processes, such as another seeds. Initially, carrots were used only for medicinal purposes. Later on, their use as „food" was also discovered. Today there are many types of carrots of different colors (white, yellow, orange, red, purple, dark purple). The best known are the orange ones, which have been cultivated in Central Europe since the 15th and 16th centuries. In „red" or „purple" and „very dark purple" carrots, in addition to carotenoids, there is also an important content of anthocyanins. Yellow carrots contain a lot of the compound lutein (lutein), which generates their color (215 mcg of „lutein" and „zeaxanthin" per 100 g of carrots).

MATERIALS AND METHODS

The importance of recovering valuable bio-compounds from carrot juice and waste processing

The recovery and use of valuable bio-compounds from carrots and processed residues is an essential resource for improving consumers' health. Maintaining a high level of activity of these valuable compounds is *primary* for technologists and processors [**11**]. All processes must be designed and conducted so that there is no loss in volume and activity of these important compounds (antioxidants, anticarcinogens, immunostimulatory „immune system enhancers") [**13**].

Objectives and strategy in scientific research

The main objective of the research is to identify the best options for processing carrots – to preserve the concentrations and activity of bio-compounds in carrots. A series of studies were necessary to achieve this objective. Through the proposed innovative technology there can be obtained ***5 Final products*** that are very useful, both for consumer nutrition and for the bioeconomy. ***The First Product*** obtained from untreated raw material is Carrot Juice (*P1*) made by pressing orange carrots - raw materials. ***The first study*** is about making the best product recipe for added carrot juice. ***The second study*** aims at identifying an innovative technology, through which to use the active principles from the residues left during processing (residues containing up to 80% carotenoids). Relevant published literature proved that only 60-70% juice yield is obtained after extraction of juice from carrots and the remaining carrot pomace may lose up to 20% (which is composed of 80% carotenoids). To obtain ***Product P1*** (natural carrot juice), carrots from a Romanian farm were used (as raw material) (orange carrots, cylindrical, medium length, and diameter). These raw materials have been checked against traces of pesticides, heavy metals, hormones, or synthetic treatments and were not genetically modified. Carrot juice (**P1**) was extracted by pressing the carrots (**figure 1**). To obtain ***Product P2*** (additive carrot juice) and the best Product Recipe there were a series of laboratory chemical analyses. The research strategy for obtaining Product **P2** is described in the „Material and Method” chapter. The waste resulting from the processing of carrots (with 80% carotenes) was subjected to further technological operations for higher utilization (2nd and 3rd Processing Paths) (**figure 2**). ***The second way of processing*** included the use of a Supercritical Fluid Extraction (SFE) Technique in which carotenoids were extracted from „pomaces” (carrot leftovers resulting from pressing) under the action of an extraction fluid in a supercritical state (CO2 at 32 degrees Celsius and pressure of 220-370 bar). These extraction products, rich in carotenoids, represented the ***P3 products*** and have been used in making functional foods. The residues from the extraction were then used to obtain pellets (***product P5***) – used in the bio-economy (for heat and electricity). ***The third way of processing*** residues met a series of technological operations aimed at recovering valuable bio-compounds from the pressing residues. Thus, a counter-current diffusion operation was used through microbiologically pure and ion free diffusion water, preheated to 45-55 degrees C. The resulting diffusion juice followed a series of successive treatments with CaO and CO_2 in a calcium-carbonic purification operation. The dietary fibers in the diffusion juice were thus separated and removed by filtration. After filtering, they were pressed and put into pellets shape (the ***P5 product*** - used in the bioeconomy). The juice left after separating the fibers was collected and enriched with enzyme activators, thus obtaining ***Product P4.*** It would also have been an important way of processing the residues left over from pressing to recover the valuable compounds. This pathway has already been studied by other researchers. Thus, functional ingredients could be obtained from carrot waste, both by hot air dehydration at a temperature of 50 degrees C and by lyophilization at -55 degrees C.

Use the UV-Vis Molecular Spectroscopy in the Management of food additives on carrots juice

According to the proposed technological schemes (**Figures 1 and 2**), the **product P1** is obtained by pressing carrots (Variant V0) and following its addition, several variants of juice with additives are obtained, cataloged in a series of P2 products.

To observe the influence of some food additives on the raw pressed juice, from variant V0 (P1 on the Scheme in **Figure 1**), the following experimental variants were obtained:

-V0 – Variant of no additives juice (Control 1);

-V1 – Variant of juice V0 with preservative (acetic acid 1%);

-V2 – Variant of juice V0 with preservative (salicylic acid, 1%);

-V3 – Variant of juice V0 with carmine dye (E120, 1%);

-V4 – Variant of juice V0 with β-carotene dye (E 160 a, 1%);

-V5 – Variant of juice V0 with natural sweetener (sugar 3g/100mL);

-V6 – Variant of juice V0 with natural sweetener (stevioside 3 g/100 mL);

-V7 – Variant of juice V0 with synthetic sweetener (fructose 4%);

-V8 – Variant of juice V0 with sugar (3g/100mL) and carmine dye (1%);

-V9 – Variant of juice V0 with carmine dye (1%) and stevioside (3g/100mL);

-V10 – Variant of juice V0 with additives, according to a commercial juice recipe.

From the carrot residues left after pressing, through diffusion and a customized purification scheme (which includes Liming and Carbonation stages), a juice is obtained. This juice is then activated in the plasma field (P4 – same as V0 Activates or V0A). This juice is added with the same lines of food additives as in the case of the V0 variant.

On the same principles, from version V0A (juice obtained via method C and activated) the additive variants are obtained:

-V0A – Variant of juice without additives (Control 2);

-V1A – Variant of juice V0A with preservative (acetic acid 1%);

-V2A – Variant of juice V0A with preservative (salicylic acid, 1%);

-V3A – Variant of juice V0A with carmine dye (E120, 1%);

-V4A – Variant of juice V0A with a β-carotene pigment (E 160 a, 1%);

-V5A – Variant of juice V0A with natural sweetener (sugar 3g/100mL);

-V6A – Variant of juice V0A with natural sweetener (stevioside 3 g/100 mL);

-V7A – Variant of juice V0A with synthetic sweetener (fructose 4%);

-V8A – Variant of juice V0A with sugar (3g/100mL) and carmine dye (1%);

-V9A – Variant of juice V0A with carmine dye (1%) and stevioside (3g/100mL);

In review, -V10A – Variant of juice V0A with additives, according to a commercial juice recipe. By analyzing the data obtained, the influence of these food additives on the main bio-compounds in carrots can be highlighted. These valuable bio compounds are the ones that participate in the main redox processes that occur during carrot processing. Molecular absorption spectra curves were generated using a UV-VIS spectrophotometer type T92 Plus - manufactured by PG Instruments, U.K. The spectrophotometer was set to work at a 1cm bandwidth and record nanometer-by-nanometer molecular absorption values in both the UV (190-400 nm) and visible (400-

700 nm) ranges. Thus, the curves of molecular absorption spectra were obtained 207 for each experimental variant (series V0-V10 and V0A-V10A), in the two wavelength ranges UV and Vis.

The equipment automatically recorded the spectral curves, with the change of deuterium and tungsten lamps at 361 nm, by automatic programming. To recheck the obtained values, at each measurement, the T92 Plus spectrophotometer was set to develop an automatic retracking. To measure the molecular absorption, special parallelepipedal UV quartz cells with a square side in a section of 1cm were used. At each wavelength, it can be recorded an absorption maximum of a compound in the solution. To know the value at which the maximum of the molecular absorption spectra is recorded for certain compounds in the analyzed juices, reference substances, respectively Pure Analysis substances purchased from Merck GmbH, were used - in the calibration. Several calibration scales were made with these substances, which were then used in *The Single Addition Method* – a method that allowed the determination of the wavelength locations at which the maximum of the molecular absorption spectra of the analyzed bio compounds is recorded. The UV-WIN software runs on many types of Windows operating systems, providing full control over the instrument and accessories, data collection, and processing.

Use the plasmatic field in activated the bio-compounds from carrots

An innovative cold plasma technology was used (together, with specific enzymatic activators) in the concentration separation of valuable bio-compounds from carrots. As activators we used acetyl-CoA (obligatory activator of NADH-reductase activity) and the rate of activity was monitored by the ratio NA D/NADH+H+. In conventional research, cold plasma results from using a Tesla coil string. Cold plasma is produced together with static electricity, which can also release hot plasma (which can affect less thermally stable biological compounds). Therefore, we used a novel Tesla T-wire Tesla coil string system ("T-coil in T-coil" - the inner coil is directly powered, the outer coil has a reversed power circuit, and a nano-rated CO2 gas circulates between them). This innovative system eliminates any static electricity and any heating from the mixture. This results in the separation and activation of biological compounds in a stable plasma field. This technology has been used both to activate bio-membranes (to maintain a product at a certain level of plasma induction) and to improve the yield of the supercritical extraction technique - in a Natural Products Helix Extractor [**10**].

The plasma field generated in this way can be used both for obtaining the P3 series of extraction products (on the B processing Pathway) and for activating some bio-compounds from the purified P4 juice (V0A) obtained on the C Pathway.

In review In these extractions, we used Helix Natural Product SFE equipment - delivered to the Research Infrastructure of the Bioengineering and Biotechnologies Laboratories, Research HUB INCESA, University of Craiova, Romania – By the world leader in this field, Applied Separations, U.S.A.

RESULTS AND DISCUSSIONS

The nutritional importance of the products obtained in these processing schemes (Pathways A,B, and C - **Figures 3 and 4**) is also generated by the concentrations of the active - reduced and oxidized - forms of vitamins A and provitamins A (alphacarotenes, beta-carotenes, lycopenes) as shown in the graphs in **Figures 1, 2.**

In carrot juice, the activity of some compounds that participate in some redox processes is very important. The main pairs of compounds that appear are:

-NAD and $NADH_2$ (very important for anaerobic oxidoreductase enzymes, in the environment);

-FMN and $FMNH_2$ (specific to aerobic oxidoreductases that influence oxidation or reduction at the liquid-juice/air interface and the shelf life of products);

-Reduced and oxidized hemoproteins;

-Natural pigments - provitamin A (reduced and oxidized lycopene, oxidized and reduced alpha-carotene forms, oxidized and reduced beta-carotene forms);

-Vitamins of type A (retinol) – oxidized and reduced forms.

The concentration ratio of oxidized and reduced forms of these bio compounds is extremely important for the health of consumers.

The variations in their concentrations following the addition of some food additives are presented in the graphs in Figure 1 (juices P1 and P2 - from V0 variants) and Figure 2 (juices P4 and P5 made from V_0A variants).

The best juices with additives P2, respectively P5 are considered to be those that induce the smallest changes in the curves of the molecular absorption spectra compared to the similar ones of the reference variants (no additives), from which they originate (P1, respectively P4).

From the analysis of the graphs in **Figures 1** and **2**, several aspects emerge:

- Preservatives, dyes, and sweeteners used alter quite a bit the concentrations of the oxidized and reduced active forms of the main compounds in carrots;
- Preservatives affect the color of carrot juices and therefore it is necessary to add dyes (beta-carotene or carmine). Carmine proved a protective activity, increased, against the attack of sugar on some valuable compounds in carrot juice;
- From the analysis of V0, V1, and V2 variants, preservatives are observed to retain higher concentrations of active forms of $FMNH_2$ and FMN, showing a synergistic effect when used;
- Sugar uses a good portion of the concentrations of the active forms of $FMNH_2$ and FMN, (a fact resulting from the comparative analysis of V0 with V5). Even the natural stevioside (a natural sweetener from *Stevia rebaudiana*) influences the concentrations of the active forms of $FMNH_2$ and FMN;
- From the analysis of the fructose-sweetened variant (synthetic sweetener used), it is observed that fructose (V7) greatly reduced the concentrations of active forms of bio-compounds involved in redox processes;
- Very important and interesting is the coloring effect and preservation of the active forms, even under the action of sweeteners, of the carmine dye (fact resulting from the comparative analysis, on pairs of data, from V5 with V8 and V6 with V9);
- The combinations of additives in V9 are superior, in manifestation, to those in V8, with stevioside consuming less active forms of redox agents than beet sugar;
- Concentrations of active forms of NAD and $NADH_2$ are protected by the activity of the red-carmine dye, which greatly influences the redox balances in the juice;
- In the case of variants obtained using method C (from V_0A to V_{10}A) carmine dye

protects most concentrations of active principles involved in redox processes;

- Without the support of the polysaccharides in the juice (lignin, cellulose, hemicellulose being removed in the processing method C), the concentrations of oxidized and reduced forms of NAD and $NADH_2$ are strongly reduced, under the action of acetic acid (V1A);

Following the application of the proposed innovative processing scheme (**figure 3**), at least 3 main ways of processing carrots can be obtained (among which methods B and C start from "carrots wastes"). Thus, 5 types of juices are obtained: *P1 (Raw Juice)* and *P2 (Additivated Juice)* -made with method A and having pressed carrots as raw materials; *P3 (Extracted Juice with Carotenes and Lycopene)* – made with method B, through Supercritical Fluid Extraction; *P4 (Purified Juice)* and P5 *(Final Juice from recovering carrots wastes)* – the last two are made with processing method C. P3 juice can be used to build *Functional Food*. *Animal Feed* is obtained from residues processed with methods B and C (carrots pomaces, wastes). The most important 4 juices (P1, P2, P4, and P5) were analyzed in the paper in order to recommend the best recipe for carrot juice undergoing industrialization. P3 juice is highly nutritious and can be considered a premium product, with a significantly higher cost.

- The introduction of calcium-carbonic purification processes (processing method C) to remove some fibers (lignin, cellulose, and hemicellulose) that would disturb certain categories of consumers and that would block the synergy of certain additive processes. This idea is also a novelty and is adapted to these processing technologies, to obtain hypocaloric products;

Methods B and C - of processing carrot residues are proposed to make better use of all existing resources, taking into account that the processing yield is 60-70%, and in the 30-40% lost there are about 80% carotenes.

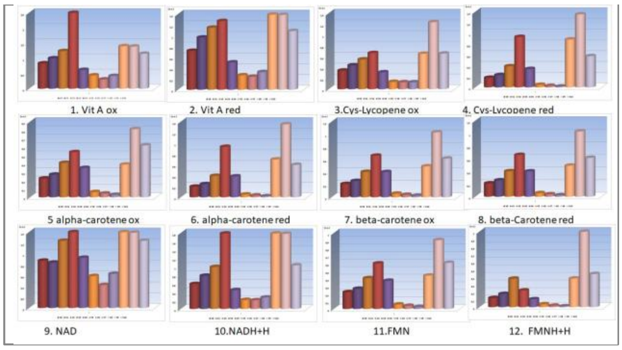

Figure 1.- The registered values for main bio-compounds from normal Experimental Variants

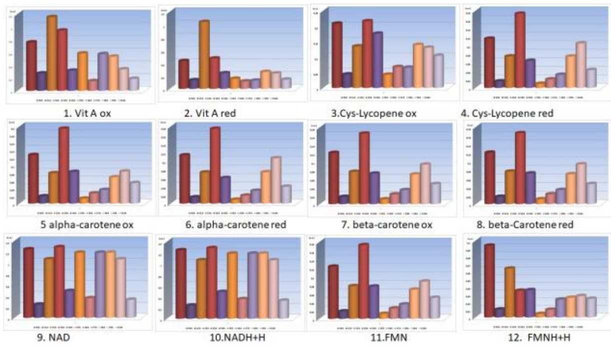

Figure 2- The registered values for main bio-compounds from activated Experimental Variants

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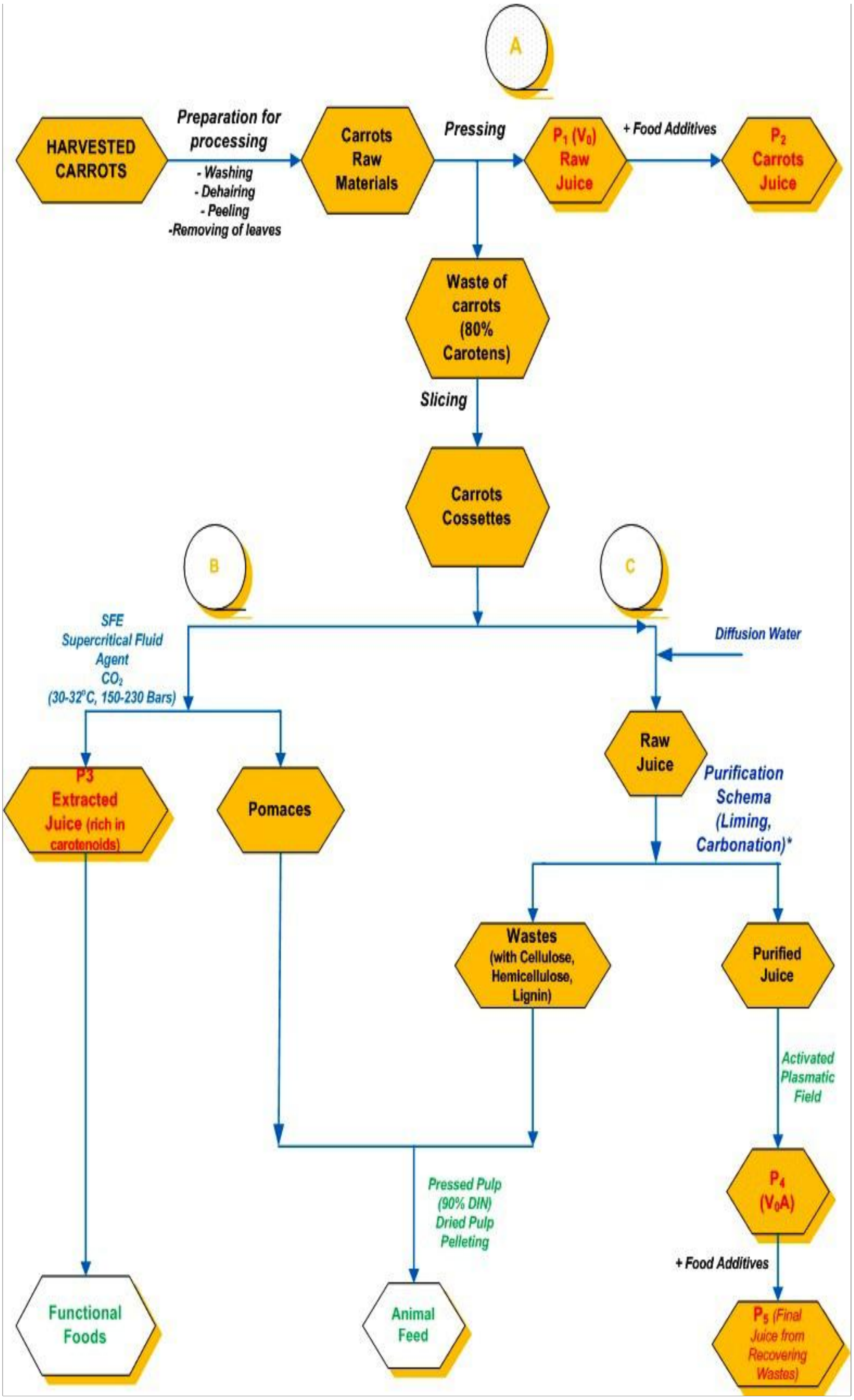

Figure 3. The innovative technology for these functional foods

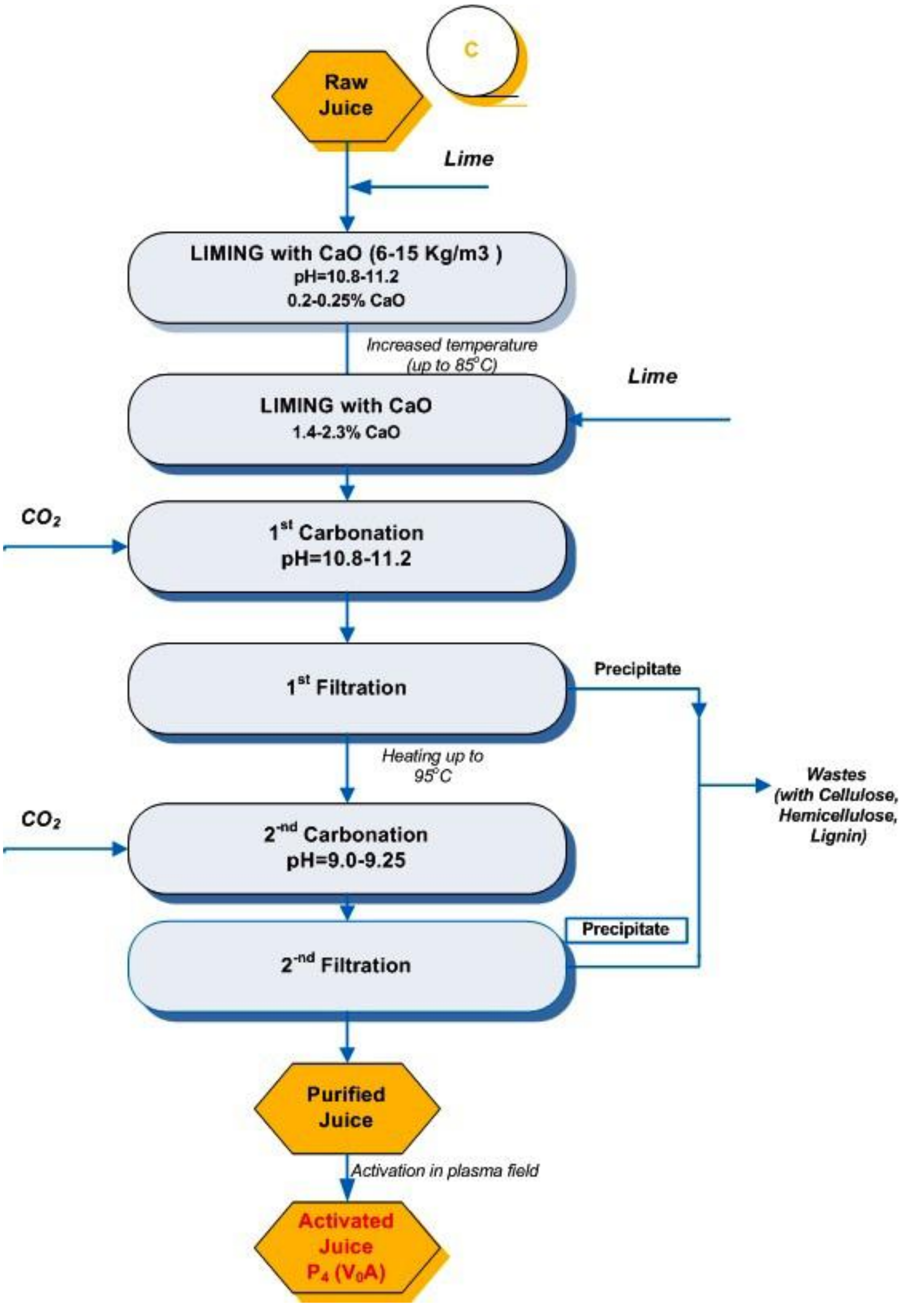

Figure 4 – The purposed innovative tasks for separation-concentration

CONCLUSIONS

To obtain products with high antioxidant potential from carrots, it is necessary to use innovative technologies, especially for products that come from the waste resulting from the processing of carrots (and which follow processing methods B and C);

- Knowing the change in the concentration ratios of active forms of NAD and NADH+H+
is very important for the study of the redox processes in carrot juices and their antioxidant capacity;
- Knowing the change in the concentration ratios of active forms of FMN and FMNH+H+ is very important for the study of redox processes at the interface of carrot juices/air and, especially, for the improvement of the storage period;
- From this complex study, of the variants of juices V1÷V10, reported to V0 (Witness Variant), the best experimental variants were V1 (with few additives) and V10 (better sensorial but high additive).
- From this complex study, of the variants of juices V1A÷V10A, reported to V0A (Witness Variant), the best experimental

variants were V1 (with few additives) and V9A(better sensorial but high additive).

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