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Novel Carrot Snacks with Desired Health Benefits

Doctoral dissertation

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Publication 2: Yusuf E., Tkacz K., Turkiewicz I.P. Wojdyło A., Nowicka, P. 2021. Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC. *European Food Research and Technology*, 247, 3053–3062. https://doi.org/10.1007/s00217-021-03857-0

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Publication 4: Yusuf E.H., Wojdyło A., Bourbon A.I., Nowicka, P. 2023. Fruit-carrot-based smoothies as innovative products with a complex matrix of bioactive compounds effected on activities of selected digestive enzymes and cholinesterases *in vitro*. *Antioxidants*, 12(4), 917.
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ABSTRACT

Global dietary guidelines support boosting fruit and vegetable consumption and recommend five portions of fruit and vegetables per day. It results from the fact that fruit and vegetable consumption prevents cardiovascular disorders, obesity, diabetes, and cancer. Orange carrot is one of the top 10 consumable vegetables worldwide, famous for its high carotenoid and nutritional contents. However, different colored carrot varieties show other bioactive compounds and biological activities, as well. Yellow carrots are abundant with xanthophylls, purple carrots are rich in anthocyanins and white carrots contain colorless polyphenolic compounds. Therefore, the orange carrot is the most used carrot variety for food production, but for novel foods, other colored carrot varieties may apply for the production with different nutritional contents and sensorial characteristics, and may also bring a different perspective to the consumers.

Therefore, the study aimed to investigate 12 carrot varieties with different sizes and colors as appealing raw materials for the development of innovative snack products. These snacks were intended to possess enhanced health benefits and sensorial characteristics. For this purpose:

(i) the chemical composition, and health-promoting properties of different variants of carrots were analyzed;

(ii) carrot juices were produced and analysed for physicochemical features, nutritional contents, biological activities and sensorial characteristics;

(iii) carrot-based smoothies were prepared and evaluated for physicochemical features, nutritional contents, biological activities and sensorial characteristics;

(iv) purple, yellow, orange and white carrots were used with fruit solutions by osmotic dehydration, convective drying and microwave vacuum drying technologies to prepare dried carrot snacks. Dried carrot snacks were evaluated for physicochemical features, nutritional contents, biological activities and sensorial characteristics as well.

According to the results of raw carrot materials with different colors and sizes, the purple carrot samples had the highest values for the content of polyphenolics and carotenoids, with the highest activities against cholinesterase. Normal purple carrots showed the highest health-promoting activities in all tests and followed by mini purple carrots. In turn, the yellow carrot showed the lowest values for the content of polyphenols and antioxidant activities, while the white carrot demonstrated the lowest results for total carotenoid and chlorophyll contents. Thus, and create features of functional products. Therefore, the conducted study had shown that different-sized (normal, mini and micro) purple carrot varieties can provide high contents of polyphenolic compounds to combat oxidative stress-related diseases, and may increase the effectivity of the sensorial characteristics of carrot-based novel, functional foods.

Following the results of carrot juices, normal purple carrot juice demonstrated the best results for total phenolic acid, anthocyanin, and carotenoid contents, which had a direct impact on its pro-health potential. This juice was characterized by the highest biological and antioxidant potential, except for the α-glucosidase, and acetylcholinesterase inhibition. Normal yellow carrot juice showed the highest acetylcholinesterase inhibition activity with the lowest α-amylase and lipase enzyme inhibitions. For the results of carrot juice sensory evaluations, the best variety was the normal orange carrot, while the worst juice was voted as the base of white carrots. Hence, purple carrot juices can be used by beverage industries for the production of smoothies and/or blended juices for increasing the health-promoting characteristics of liquid products, but for creating the most preferable taste, the orange carrot variety is the most popular.

The results of carrot-based smoothies showed that the sour cherry juice–purple carrot smoothie had the highest total phenolic acid, anthocyanin and polymeric procyanidin contents. The raspberry juice–purple carrot smoothie showed the highest activities against lipase and butyrylcholinesterase enzyme inhibitions. Moreover, these characteristics are not only important for the nutritional perspective but also provide opportunities for beverage processing. Although, the apple juice–white carrot smoothie was voted for the highest product acceptance, the smoothie did not show potent nutritional content and biological activities.

Finally, combined methods of osmotic dehydration in fruit juices, convective drying and microwave vacuum drying made it possible to obtain stunning novel-colored dried carrot snacks. The osmotic

dehydration process increased the polyphenolic contents in the dried carrot samples. Moreover, the highest phenolic acid, anthocyanin, flavan-3-ol, polymeric procyanidin and flavonol contents were determined in samples dehydrated with sour cherry and chokeberry solutions. In turn, the lowest total phenolic content was observed in the orange carrot osmotically dehydrated in apple solution. Sensorial evaluations of each carrot snack indicated significant differences, the highest color acceptance was in the purple carrot-apple juice samples, the highest flavor score was observed in the orange carrot-sour cherry samples, while the highest overall taste score was voted for the white carrot-apple solution samples. Thus, the applied process provided novel and sensorily acceptable functional dried carrot snacks.

To conclude, normal-sized carrot varieties (purple, yellow and white) are suggested for novel food applications. Moreover, combinations of carrot materials with fruit solutions enrich bioactive compounds, as well as sensorial characteristics and boost the health-promoting features of final carrot-based food products.

Keywords: colorful carrot varieties, carrot juices, dried carrot snacks, carrot-blended smoothies, polyphenolic compounds, carotenoids, anti-aging, anti-obesity, anti-diabetes.

STRESZCZENIE

Rekomendacje światowych ekspertów w dziedzinie żywności i żywienia wskazują jednoznacznie na konieczność zwiększenia spożycia owoców i warzyw, w ilości minimum pięciu porcji dziennie. Wynika to z faktu, że spożywanie owoców i warzyw zapobiega przewlekłym chorobom niezakaźnym m.in.: układu krążenia, otyłości, cukrzycy i nowotworom.

Marchewka pomarańczowa to jedno z 10 najpopularniejszych jadalnych warzyw na świecie. Cechuje się ona wysoką zawartością karotenoidów i składników odżywczych. Jednakże należy podkreślić, iż odmiany marchwi o innych kolorach, mogą również być donorem związków biologicznie aktywnych i wykazywać właściwości prozdrowotne. Żółta marchew jest bogata w ksantofile, fioletowa marchew jest źródłem antocyjanów, a biała marchew zawiera bezbarwne związki polifenolowe. Stąd też, chociaż marchew pomarańczowa jest najczęściej używaną odmianą marchwi w produkcji żywności, to w przypadku projektowania nowej żywności, warto rozważyć wykorzystanie innych wariantów kolorowej marchwi, które pozwolą na opracowanie zupełnie nowych koncepcji, zarówno w kontekście cech fizykochemicznych, prozdrowotnych jak i sensorycznych, dając tym samym alternatywę konsumentom.

Dlatego też, celem pracy doktorskiej była ocena 12 odmian marchwi o różnych rozmiarach i barwie, jako atrakcyjnych surowców do opracowania innowacyjnych przekąsek o zaprogramowanych właściwościach prozdrowotnych i sensorycznych. W tym celu:
(i) przeanalizowano skład chemiczny i właściwości prozdrowotne różnych wariantów marchewek;
(ii) opracowano i przeanalizowano soki marchwiowe pod kątem cech fizykochemicznych, wartości odżywczych, aktywności biologicznej i cech sensorycznych;
(iii) opracowano i przeanalizowano produkty typu smoothie na bazie marchwi pod kątem cech fizykochemicznych, wartości odżywczych, aktywności biologicznej i cech sensorycznych
(iv) wykorzystano fioletowe, żółte, pomarańczowe i białe odmiany marchwi wraz z roztworami wybranych soków owocowych do produkcji przekąsek za pomocą kombinacji metod – odwadniania osmotycznego, suszenia konwekcyjnego oraz suszenia mikrofalowo-próżniowego. Przekąski z suszonej marchwi oceniano pod kątem właściwości fizykochemicznych, zawartości składników odżywczych, aktywności biologicznej oraz cech sensorycznych.

Interpretacja otrzymanych wyników badań w zakresie analizy marchwi o różnym zabarwieniu i rozmiarach, pozwoliła wskazać, iż próbki marchwi fioletowej charakteryzowały się najwyższą koncentracją związków polifenolowych i karotenoidów oraz najwyższą zdolnością do inhibicji cholinoesteraz. Fioletowe marchewki w normalnym rozmiarze wykazywały największy potencjał prozdrowotny we wszystkich testach, a następnie fioletowe marchewki w rozmiarze mini. Z kolei marchew żółta charakteryzowała się najniższą zawartością związków polifenolowych i aktywnością przeciwutleniającą, a marchew biała najniższym stężeniem karotenoidów i chlorofili. Generalnie można więc stwierdzić, iż marchewki fioletowe o różnych wielkościach (normalna, mini i mikro) mogą zapewnić wysoką zawartość związków polifenolowych w produktach, a tym samym kreować ich funkcjonalność w zakresie profilaktyki chorób związanych ze stresem oksydacyjnym.

Analiza soków na bazie marchwi o różnych kolorach i rozmiarach, pozwoliła wskazać, iż sok z marchewki fioletowej w rozmiarze normalnych cechował się najwyższą zawartością kwasów fenolowych, antocyjanów i karotenoidów, co miało bezpośredni wpływ na jego potencjał prozdrowotny. Sok ten cechował się najlepszą aktywnością we wszystkich testach biologicznych i przeciwutleniających, z wyjątkiem hamowania enzymów α-glukozydazy i acetylocholinoesterazy. Sok z żółtej marchwi o normalnym rozmiarze, wykazywał najwyższą aktywność hamowania acetylocholinoesterazy przy najniższej zdolności do inhibicji α-amylazy i lipazy. Z kolei analiza sensoryczna wykazała, iż najbardziej atrakcyjnym sokiem był ten opracowany na bazie marchewki pomarańczowej, a najmniej pożądany był sok z marchwi białej. Finalnie można więc stwierdzić, iż soki na baize fioletowej marchwi mogą być wykorzystywane przez przemysł do produkcji koktajli i/lub soków mieszanych w celu zwiększenia właściwości prozdrowotnych produktów płynnych, jednak w celu uzyskania najkorzystniejszego smaku najbardziej rekomendowana jest odmiana marchwi pomarańczowej.

Z kolei ocena produktów typu smoothie na bazie różnych kolorów marchwi pozwoliła stwierdzić, iż produkt zawierający w swoim składzie sok wiśniowy i marchew fioletową cechował się najwyższą zawartością kwasów fenolowych, antocyjanów i polimerów procyjanidyn. Z kolei smoothie z sokiem malinowym i marchewką fioletową wykazywało najwyższą zdolność do inhibicji lipazy i butyrylocholinoesterazy, co może wskazywać zarówno na jego potencjał przeciwotyłości jak i przeciwstarzeniu. Cechy te są nie tylko ważne z perspektywy żywieniowej, ale także stwarzają możliwości opracowywania nowych koncepcji napojów. Chociaż koktajl z soku jabłkowego i białej marchwi został oceniony przez panel sensoryczny jako ten najatrakcyjniejszy, nie cechował się cennym profilem związków bioaktywnych oraz nie wykazywał potencjału prozdrowotnego.

Różne kolory marchwi posłużyły także do opracowania produktów przekąskowych, z wykorzystaniem łączonych technik odwadniania osmotycznego, suszenia konwekcyjnego i mikrofalowo-próżniowego. Proces ten pozwolił na otrzymanie atrakcyjnych sensorycznie suszy o nowatorskiej kolorystyce. Proces odwadniania osmotycznego modulował zwiększenie zawartości związków polifenolowych w finalnych produktach. Najwyższą zawartość kwasu fenolowego, antocyjanów, flawan-3-oli, polimerów procyjanidyn i flawonoli oznaczono w próbkach odwodnionych w roztworach z wiśni i aronii. Z kolei najniższą całkowitą zawartością polifenoli cechowała się marchew pomarańczowa odwadniana w soku jabłkowym. Ocena sensoryczna wykazała, iż otrzymane przekąski różniły się istotnie od siebie, najwyższą akceptację barwy uzyskały próbki marchwi fioletowej odwodnione w soku jabłkowym, najwyższą ocenę pod względem smaku otrzymano w próbkach pomarańczowej marchwi odwadnianej w soku wiśniowym, natomiast najwyższą pożądalnością cechowała się marchew biała odwadniana w soku jabłkowym. Tym samym zastosowany proces dostarczył nowatorskich i akceptowalnych sensorycznie funkcjonalnych przekąsek z suszonej marchwi.

Finalnie wykazano, że do projektowania nowym produktów spożywczych warto wykorzystać marchew normalnej wielkości (fioletową, żółtą lub białą). Ponadto połączenia surowców marchwiowych z owocami pozwala na znaczne wzbogacenie finalnych produktów w związki bioaktywne, a tym samym kształtuje właściwości prozdrowotne i sensoryczne finalnych formulacji.

Słowa kluczowe: kolorowe odmiany marchwi, soki marchewkowe, przekąski z suszonej marchewki, smoothie na bazie marchewki, związki polifenolowe, karotenoidy, właściwości przeciwstarzeniowe, właściwości przeciwotłości, właściwości przeciwcukrzycowe.

ABBREVIATIONS

a* - Redness-greenness
ABTS - The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation-based assay
AChE – Acetylcholinesterase
AJ - Apple juice
b* - Yellowness-blueness
BuChE - Butyrylcholinesterase
Ca - Calcium
CASS - Chelating agent soluble solids
CD - Convective drying
DASS - Dilute alkaline soluble solids
DP - Degree of polymerisation
ELSD - The evaporative light scattering detector
Fe - Iron
FL - Fluorescence detection
FRAP - Fe^{3+} ion reducing ability
HPLC - High-performance liquid chromatography
K - Potassium
L* - Lightness
Mg - Magnesium
MiOC - Mini orange carrot
MiOCJ - Mini orange carrot juice
MiPC - Mini purple carrot
MiPCJ - Mini purple carrot juice
MiWC - Mini white carrot
MiWCJ - Mini white carrot juice
MiYC - Mini yellow carrot
MiYCJ - Mini yellow carrot juice
MOC - Micro orange carrot
MOCJ - Micro orange carrot juice
MPC - Micro purple carrot
MPCJ - Micro purple carrot juice
MWC - Micro white carrot
MWCJ - Micro white carrot juice
MYC - Micro yellow carrot
MYCJ - Micro yellow carrot juice
Na - Sodium
NOC - Normal orange carrot
NOCJ - Normal orange carrot juice
NPC - Normal purple carrot
NPCJ - Normal purple carrot juice
NTU - Nephelometric Turbidity Unit
NWC - Normal white carrot
NWCJ - Normal white carrot juice
NYC – Normal yellow carrot
NYCJ - Normal yellow carrot juice
OC - Orange carrot
OCAS - Orange carrot & Apple solution
OCCS - Orange carrot & Chokeberry solution
OCSCS - Orange carrot & Sour cherry solution
OCSS - Orange carrot & Sucrose solution
OD - Osmotic dehydration
ORAC - Oxygen radical absorbance capacity
PC - Purple carrot
PCAS - Purple carrot & Apple solution
PCCS - Purple carrot & Chokeberry solution
PCSCS - Purple carrot & Sour cherry solution
PCSS - Purple carrot & Sucrose solution
PDA - Photodiode array detection
PJ - Pear juice
PP - Polymeric procyanidins
PPC - Polyphenolic compounds
RJ – Raspberry juice
ROS - Reactive oxygen species
SCJ - Sour cherry juice
SJ - Strawberry juice
TA - Titratable acidity
TPC – Total phenolic content
UPLC - Ultraperformance liquid chromatography
UPLC-PDA-ESI-Q/TOF-MS - Ultraperformance liquid chromatography coupled with a photodiode-array detector and electrospray ionization quadrupole time-of-flight mass spectrometry
VMD – Microwave vacuum drying
WC - White carrot
WCAS - White carrot & Apple solution
WCCS - White carrot & Chokeberry solution
WCSCS - White carrot & Sour cherry solution
WCSS - White carrot & Sucrose solution
YC - Yellow carrot
YCAS - Yellow carrot & Apple solution
YCCS - Yellow carrot & Chokeberry solution
YCSCS - Yellow carrot & Sour cherry solution
YCSS - Yellow carrot & Sucrose solution

1. INTRODUCTION

Daucus carota L. is a member of theApiaceae family and grows in Europe, Asia, Africa, and Macaronesia. The cultivated carrot has evolved through the crossing of two subspecies, namely *D. carota* ssp. *carota* and *D. carota* ssp. *maximus* (Maxia et al., 2009). Initially, cultivated carrots displayed purple and yellow hues, later followed by white carrots, but presently, orange carrots have gained the highest popularity worldwide. Carrot is one of the top 10 consumable vegetables around the world, with high market share and nutrient values. Approximately 100 g of carrot provides around 41 kcal of energy, 0.93 g of protein, 9.58 g of carbohydrates, and 2.80 g of fiber (Simon, 2019; Xu et al., 2020).

Cultivated carrots are classified based on their root color, sugar-carotenoid content, and root shape, all of which are affected by development, temperature conditions during growth, and the use of fertilizers (Heinonen, 1990; Stein and Nothnagel, 1995). Additionally, the color transformation in carrots is attributed to carotenoids, which start to accumulate in young carrots after their first month of growth and are maintained until the secondary growth is concluded (Fuentes et al., 2012).

Carrot varieties exhibit a range of root lengths, spanning from 5 cm to 50 cm, which are important parameters for the marketing different cultivars. Additionally, cultivated carrots can be categorized into Eastern and Western types. Eastern carrots are characterized by their purple and yellow colors and branched roots, while Western carrots come in orange, red, and white shades with unbranched roots. Eastern carrot cultivars are rich in anthocyanins and western carrots are abundant in carotenes (Que et al., 2019). Nevertheless, purple carrots contain twice the amount of α-carotene and β-carotene as orange; moreover, purple carrot possesses a sweet flavor with lower overall sugar content. These stunning features of purple carrots make them a good alternative to their orange counterparts. The color variations in carrots arise from the presence of pigmented compounds, such as carotenoids (α-carotene and β-carotene), which give rise to the orange color, xanthophylls that produce the yellow color, and anthocyanins responsible for the purple hue. White carrots contain colorless polyphenolic compounds (PPCs) (Klein and Rodriguez-Concepcion, 2015; Akhtar et al., 2017).

Carrots contain highly valuable carotenoids, which vary in their chemical structure and types. Carotenes, such as α-carotene and β-carotene, consist of hydrocarbons, while xanthophylls like β-cryptoxanthin, lutein, and zeaxanthin have oxygen-containing functional groups (Nabi et al., 2020). More importantly, carotenoids are vitamin A precursors, and vitamin A is crucial for eyesight and cell regulation (Britton, 2009). Furthermore, carotenoids play a vital role in preventing various health conditions, including cancer, bone-related diseases, cellular oxidation, diabetes, obesity, and cardiovascular diseases (**Publication 1**; Mohd Hassan et al., 2019).

The other compounds in carrots, especially in purple carrots, are anthocyanins, which are a subgroup of polyphenols and are well known for their free-radical scavenging activities (**Publication 1**; Liu et al., 2019). Anthocyanins are synthesized by plants in response to various factors such as pathogens, UV radiation, pollination, and environmental stress during their growth (Meng et al., 2020). Additionally, PPCs are useful for healthy human body functions, supporting protection against diabetes, cardiovascular diseases, osteoporosis, asthma, cancer, aging, and neuroprotection (Ravindran et al., 2019).

In addition to color pigments, carrot is rich in vitamins. Vitamins in carrot varieties are another important content for product quality. One important vitamin found in carrots is β-carotene, which converts into vitamin A—an essential nutrient that helps combat night blindness and increases immune system function (Handelman, 2001). Another significant vitamin present in carrots is α-tocopherol, a precursor to vitamin E, which plays a crucial role in cell signaling, gene expression, and maintaining cell membrane stability within the human body (Luby et al., 2014). Moreover, carrots are abundant in vitamin C, which is necessary for blood pressure regulation, preventing iron deficiency, and boosting immune system function (Naidu, 2003; Ergun and Süslüoglu, 2018). Carrots also contain various vitamin B derivatives, including thiamine, riboflavin, cobalamin, and pyridoxine, which are in carrot varieties (Hsieh and Ko, 2008); and vitamin B is important for the functions of cell growth, brain, and digestion system.

The mineral content determines product quality which is an important parameter for raw food products (Szczepanek et al., 2015). Carrot roots are abundant in K, Mn, P, Ca, Na, Fe, and Mg (**Publication**

2; Ergun and Süslüoglu, 2018; Olzsyk et al., 2020). These minerals are essential for human healthy body functions. For instance, K is necessary for the proper functioning of muscles, nerves, and cells; Mn is required in trace amounts for various biosynthetic pathways; P shows functions for blood vessels and bones; Ca is essential for bones, teeth, and blood cells; Na is necessary for functions of muscles and nerves; Fe is important for the transportation of oxygen in the blood and muscle cells; besides, Mg adjusts blood sugar levels and blood pressure.

Carrots also serve as a source of carbohydrates, including simple sugars like fructose, glucose, and sucrose, along with a low amount of starch (**Publication 2;** Alasalvar et al., 2001). The sugar content of carrot varieties may vary depending on environmental and storage conditions (Sistrunk, 1967). Carrots are also rich in dietary fibers, which provide healthy bowel function, decreasing cholesterol levels, and heart diseases. In the presented dissertation, an assessment was conducted on the sugar profile, dietary fiber content, organic acid profile, and mineral content in various types and sizes of carrots, which has not been assessed in the scientific literature. These compounds have not been analyzed especially in the context of their impact on health-promoting properties, as presented in the present study.

Before the present study, two black carrot cultivars from Spain, and their anthocyanin contents were analyzed (Algarra et al., 2014). Additionally, fifteen black carrot cultivars were analyzed for their anthocyanin content (Kammerer et al., 2004), while total polyphenolic contents (Smeriglio et al., 2018) were assessed in the same study. Furthermore, the polyphenolic and carotenoid contents of four different colored carrots (orange, purple, white, and yellow) have been analyzed in separate studies (Alasalvar et al., 2001; Grassmann et al., 2007; Singh et al., 2018). However, until now, there has been no investigation into different variants of carrots in terms of size (normal, mini, and micro) and color (orange, white, yellow, purple). A comprehensive examination of the detailed profile of bioactive compounds, physicochemical properties, and health-promoting properties across various variants of carrots has not been presented in the existing literature.

The lack of recognition for different types of carrots has led to a market dominated by products derived solely from orange carrots, which are primarily processed into liquid forms such as juices or semiliquid products like mousses. Carrot juice, in particular, is preferred as a nonalcoholic beverage. Various techniques are applied to achieve a high yield of carrot juice, including the use of depolymerizing enzymes, mash heating, decanter technology, and underwater shockwave pretreatment (Sharma et al., 2012; Yasuda et al., 2017).

Fresh carrot juice exhibits a cloudy appearance and can be divided into two phases: a dissolved solid phase (cloud) and a liquid phase (serum). The serum contains water-soluble compounds, while the cloud contains insoluble vegetable tissues that are rich in the protein–carbohydrate complex (Schultz et al., 2014). Carrot juice has a neutral pH of ∼6 and is more alkaline compared to apple and orange juices. A 240 mL serving of carrot juice provides significant nutritional value, including 250% of the recommended daily intake of vitamin A, 33% of vitamin C, 20.70% of vitamin E, 40% of vitamin K, 14% of potassium and phosphorus, and around 10% of protein, calcium, magnesium, fiber, iron, thiamine, zinc, and riboflavin (Santana-Gálvez et al., 2019). These characteristics of carrot juice align well with the current trend of avoiding sugar-added beverages and opting for sugar-free, non-alcoholic fresh juices. Consequently, exploring the use of different types of carrots as a product with an interesting profile of nutraceutical ingredients and favorable sensory properties is worth considering, as disscussed in **Publication 3**.

Carrot has been extensively used in the production of blended beverages with high nutritional value, often in combination with other fruits. For instance, carrot juice has been blended with pineapple and orange to create nutritious beverages. Additionally, carrot juice has been fortified with pomegranate peel extract, which enhances its antimicrobial and antioxidant activities without compromising its sensory properties (Trigo et al., 2020). Other combinations include carrot-grape juice (Nadeem et al., 2018), carrot with mixed berry juice (Manu et al., 2017), apple-carrot juice (Kahraman et al., 2017), carrot juice with orange peel and pulp extracts (Adiamo et al., 2018), and carrot juice incorporated into yoghurts (Fan and Cliff, 2017).

In the market, the most commonly used carrot type for juicing is the orange carrot. However, the purple carrot, for instance, can be an appealing ingredient for novel fruit and vegetable juices due to its high content of anthocyanins, phenolic acids, and rich carotenoids.

An intriguing possibility is to utilize carrots in the preparation of smoothies, as demonstrated in **Publication 4.** Up until now, the blending of purple, yellow, orange, and white carrot varieties with raspberry (*Rubus idaeus*), apple (*Malus domestica*), pear (*Pyrus communis*), strawberry (*Fragaria* ^{x} *ananassa*), and sour cherry (*Prunus cerasus*) to create smoothies has not been explored. These fruits, belonging to the Rosaceae family, are widely favored in Poland. Consequenlty, the bioactive profiles of smoothies based on carrots have not yet been investigated. These aspects of the study hold potential value for future food research and the food industry, particularly those seeking novel ingredients with abundant nutritional content, appealing sensory characteristics, and health-promoting features.

The food industry continues to pursue innovative food products with extended shelf life while avoiding the use of preservatives (Prosapio and Norton, 2017). To achieve this, new technologies are being employed to preserve processed food products. Additionally, health-conscious consumers prioritize the equilibrium between the nutritional value and taste of food items. Currently, a range of plant-based snacks has been prepared using different methods offering diverse health benefits (Huang and Zhang, 2012) advantages. Nevertheless, snacking preferences constantly evolve in response to changing consumer needs.

Drying has been utilized in the food industry since ancient times, and today it is employed to create innovative products with extended shelf-life (Bochnak and Świeca, 2020). The specific methods and equipment employed in drying vary depending on the desired outcomes. For instance, OD of vegetables with fruit juices enhances the nutritional value, color, and flavor of the dried food products before the primary drying techniques are applied. In OD, water migrates from the fruits or vegetables to a hypertonic solution (Rastogi et al., 2005). Sugar and salt solutions have traditionally been used for OD, but recently, fruit solutions have also emerged as novel materials (Nowicka et al., 2015a). Importantly, OD-treated food products offer high nutritional content, appealing color, and pleasant aroma, making them suitable for consumers of all age groups. In addition, from the technical point of view, OD requires low energy and causes low heating damage to food products, which is extremely important (**Publication 5;** Jayaraman and Das Gupta, 1992).

Following OD, primary drying technologies such as convective and microwave vacuum drying methods are employed. CD involves the movement of water from the food product to the drying medium through a gas-solid interaction, driven by the difference in water vapor pressure between the food surface and the surrounding air (Herman-Lara et al., 2009). On the other hand, microwave vacuum drying offers a rapid alternative by utilizing microwaves to heat food products and a vacuum environment to reduce the dehydration temperature, resulting in production of high-quality foods (Lagnika et al., 2018). The dried food products obtained through these mothods are effectively preserved against microbial activities and oxidation reactions, allowing for long storage periods (Hernández-Santos et al., 2016).

Plant-based dried food products offer comparable benefits to fresh fruits and vegetables, as they are rich in health-promoting phytochemicals (Chang et al., 2016) and are gluten-free (Wojtowicz et al., 2019). Carrots, in particular, can serve as a model for vegetable snacks. Previously, carrots have been utilized to create fiber-rich, nutritious, whole-grain carrot chips (Norazmir et al., 2014). Research has demonstrated that techniques such as ultrasound drying and enzymatic treatment positively impact the carotenoid content of carrots (Konopacka et al., 2017). Furthermore, snacks incorporating barley flour and carrot pomace are abundant in soluble dietary fibers and possess a high nutritional content (Lotfi Shirazi et al., 2020). Consequently, dried carrot products boast a significant carotenoid content, along with essential micronutrients and macronutrients, which increase human immunity (Chen et al., 2016; Cao et al., 2019).

Importantly, different colors of carrot varieties with different fruit juices have not been explored in the production of dried carrot snacks that offer enhanced health benefits and sensory qualities. Therefore, in **Publication 5**, this topic was undertaken in the context of the possibility of designing attractive, high-residue, and healthy snacks using the previously discussed techniques.

Until now, the development of carrot-based products has primarily centered around the use of the widely popular orange carrot. However, a comprehensive review of the existing literature clearly shows that there is a lack of literature data on different colors and sizes of carrots, both in the context of physicochemical and biological characteristics, as well as the possibility of their potential applications in food processing. Therefore, conducting a thorough analysis of these raw materials and evaluating their potential applications become crucial for the advancement of food technology.

2. AIM AND HYPOTHESIS OF THE RESEARCH

The Ph.D. study aimed to investigate 12 carrot varieties with different sizes and colors as appealing raw materials for the development of innovative snack products. These snacks were intended to possess enhanced health benefits and sensorial characteristics. The main objective of the study was derived from the specific objectives outlined in the publications included within the dissertation:

P1. The study aimed to compare 12 carrot varieties with different colors and sizes in terms of their bioactive contents and health-promoting properties. The goal was to find the carrot variety with the highest health benefits for food processing. This was achieved by analyzing the polyphenolic and carotenoid contents and assessing the *in vitro* biological activities against enzymes related to diabetes (α-amylase and α-glucosidase), obesity (lipase), and age-related (AChE and BuChE) disorders (**Publication 1**).

P2. The study aimed to compare the chemical properties and antioxidant activities of the 12 colored carrot varieties. Parameters such as dry matter, ash, pectin, TA, pH, and various antioxidant activity tests (ABTS, FRAP, and ORAC) were measured. Additionally, the total PPCs, vitamin C, total tetraterpenoid contents, and profiles of sugar, minerals, and organic acids were determined (**Publication 2**).

P3. The study aimed at comparing and identifying the best carrot varieties for juice processing. The 12 colored carrot juices were evaluated for their bioactive compound contents (polyphenols and carotenoids), health benefits (antioxidant and *in vitro* biological activities), physicochemical characteristics of fresh carrot juices, namely pH, pectins, L-ascorbic acid, soluble solids, dry matter, ash, viscosity, turbidity, osmolality, sugar, organic acids, mineral content, and color, and their sensorial characteristics (**Publication 3**).

P4. The study aimed to utilize purple, yellow, orange, and white carrot varieties in combination with Polish fruit juices (raspberry, apple, pear, strawberry, and sour cherry) to produce smoothies. The resulting smoothies were analyzed for their physicochemical characteristics, sensorial qualities, bioactive compounds, and *in vitro* prohealth properties (**Publication 4**).

P5. The study aimed the feasibility of using combined OD-CD-VMD techniques to produce carrot snacks with health-promoting properties. Dried carrot snacks were prepared using these techniques, incorporating fruit juices to enhance the L-ascorbic acid and bioactive contents of the colored carrot varieties. The dried carrot materials were analyzed for water activity, color characteristics, L-ascorbic acid, and polyphenolic and carotenoid contents. Furthermore, the antioxidant activities, α-amylase, α-glucosidase, lipase, AChE, and BuChE inhibition activities, and sensorial acceptance of the products were evaluated (**Publication 5**).

The research objectives formulated in this study facilitated a comprehensive examination of the research hypothesis, which posits that carrots can be serve as a functional foundation for the production of liquid, semiliquid, and dry products dedicated to children in the form of a snack or a second breakfast. The research presented in this manuscript enabled the identification of the most valuable carrot varieties and determined the optimal directions for incorporating and utilizing carrots in dietary practices, food industry applications, and food service settings.

3. ORGANISATION OF THE RESEARCH

3.1. Material and scope of the study

The objectives of the study were conducted based on the four main phases presented in Figure 1.

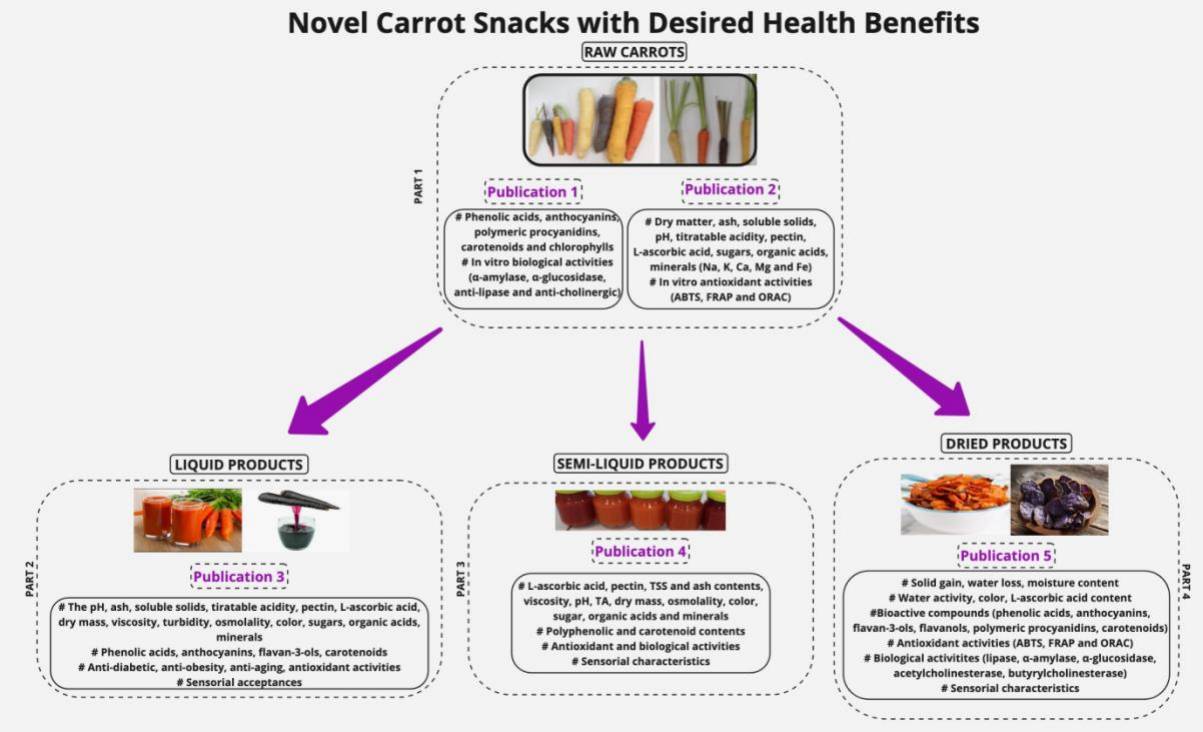

Figure 1. The structure and aim/objectives of the PhD dissertation

Twelve carrot varieties were investigated in the present study: MYC, MiYC, NYC, MPC, MiPC, NPC, MOC, MiOC, NOC, MWC, MiWC, and NWC. In the first stage of the PhD dissertation, 12 raw carrot materials were analyzed for physicochemical, bioactive compounds; *in vitro* antioxidant and biological activitites (**Publications 1, and 2**).

In the second stage of the study, 12 carrot juices from 12 different colored and sized carrot raw materials were used to produce the juices. For the scope, the carrot juices were investigated for bioactive compounds, biological activities and physicochemical characteristics (**Publication 3**).

In the third stage of the PhD dissertation, carrot-based smoothies which were prepared from purple, yellow, orange, and white carrots with raspberry (*Rubus idaeus*), apple (*Malus domestica*), pear (*Pyrus communis*), strawberry (*Fragaria* ^{x} *ananassa*), and sour cherry (*Prunus cerasus*) juices were investigated. In the study, carrot-blended smoothies were analyzed for physico-chemical features, bioactive compounds, sensorial characteristics and biological activities (**Publication 4**).

In the fourth stage of the study, dried carrots which were enriched with fruit juices (apple, sour cherry and chokeberry) by OD and dried by CD, and VMD technologies were investigated. For the study, product characteristics, bioactive compounds, antioxidant activities, biological activitites and sensorial characteristics were analyzed (**Publication 5**).

3.2. Technological processes

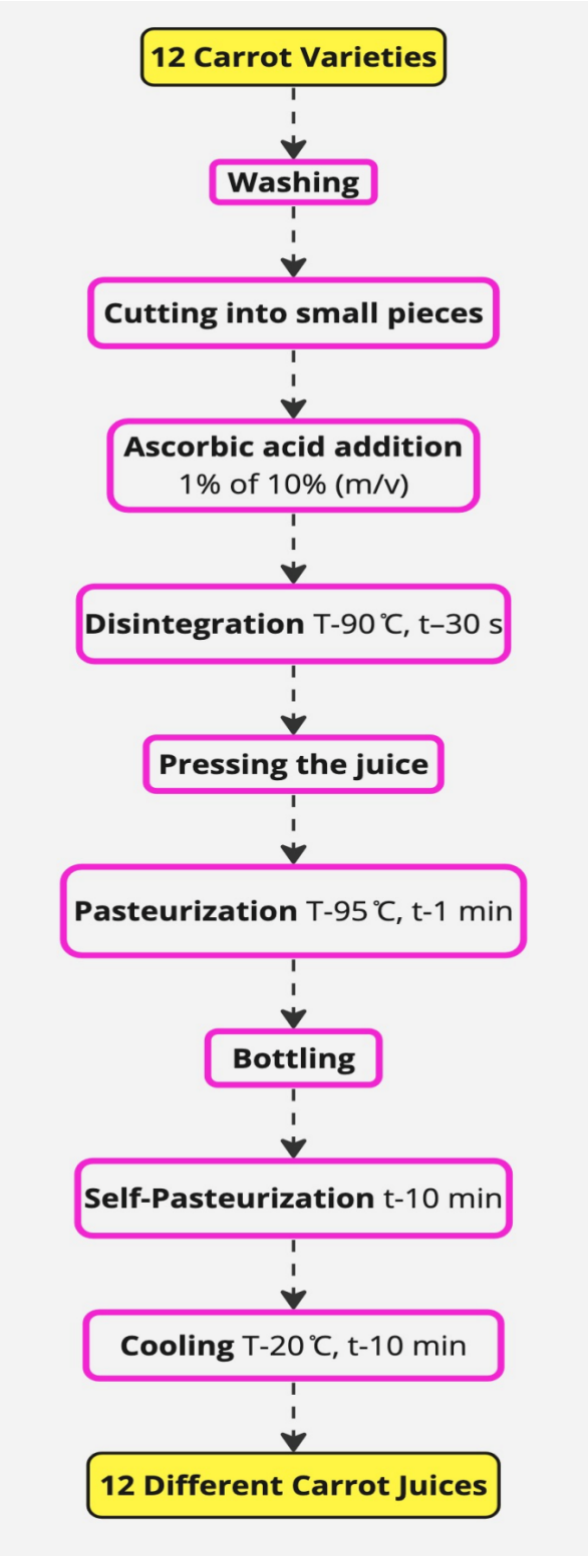

Figure 2. Carrot juice processing

In the second stage of the study, 12 carrot juices from 12 different colored and sized carrot raw materials were used to produce the juices. Technological process of this step was presented in Figure 2. For pressing the juice a laboratory hydraulic press (SRSE, Warsaw, Poland) and then for pasteurization - Thermomix (Vorwerk, Wuppertal, Germany) were used (**Publication 3**).

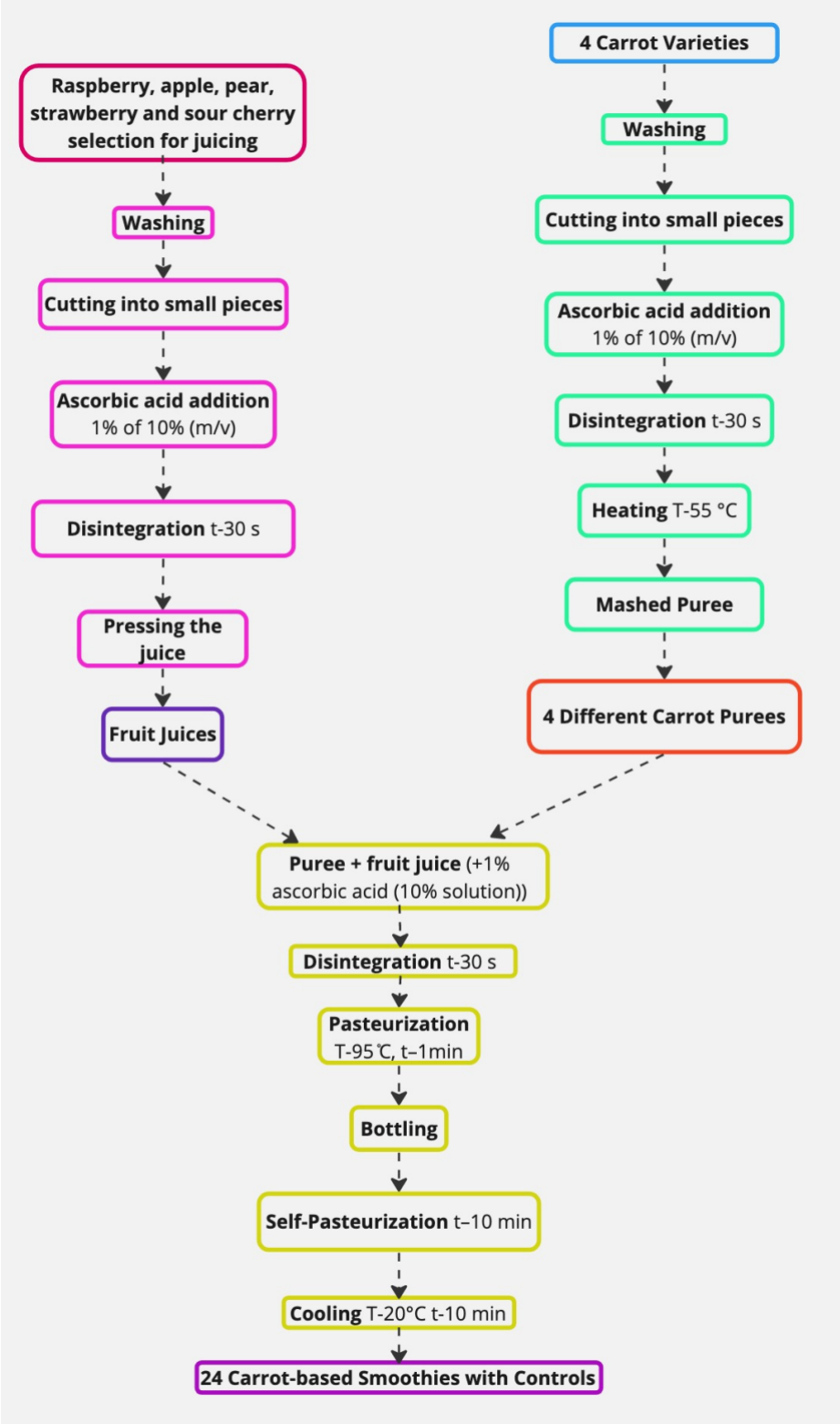

Figure 3. Carrot-based smoothie technology

The production of carrot-based smoothies (**Publication 4**) consisted of four steps:
(1) preparation of raspberry, apple, pear, strawberry, and sour cherry juices;
(2) preparation of purees from purple, yellow, orange, and white carrots;
(3) combining the ingredients into smoothies;
(4) thermal processing.

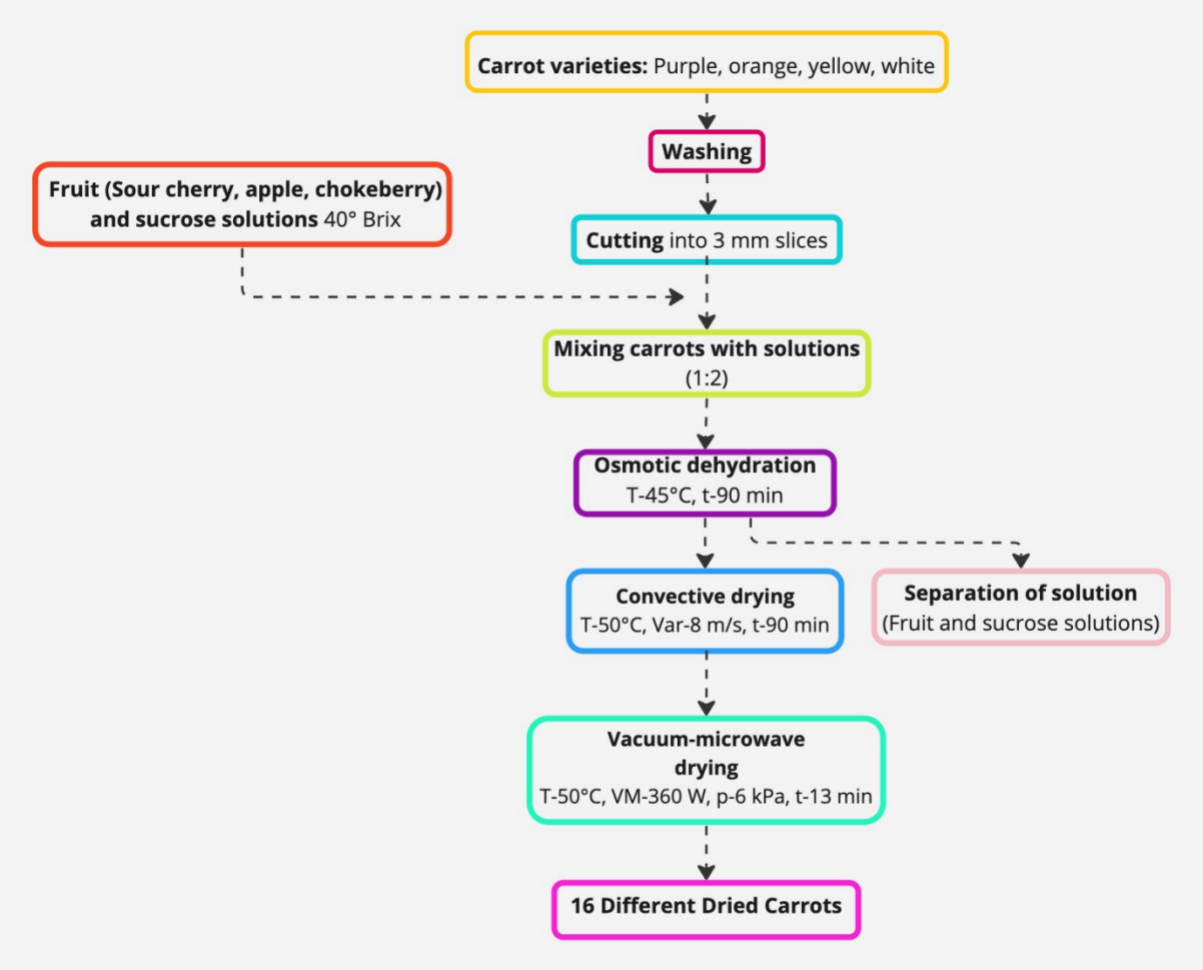

Figure 4. Dried carrot technology

Yellow, orange, purple and white carrots were dehydrated with concentrates of sour cherry, chokeberry, apple (Płońsk, Poland) and sucrose solution as control groups by OD-CD-VMD (**Publication 5**).

3.3. Research Methods

Chemical composition analyses

The research materials (raw carrot varieties and carrot-based food products were subjected to the following chemical composition analyses, depending on the stage of the study (**Publications 1-5**):
- quantification and identification of phenolic compounds by liquid chromatography with a UPLC-PDA-ESI-Q/TOF-MS according to Wojdyło et al. (2016);
- quantification of PP by direct phloroglucinolysis using UPLC coupled to FL according to Kennedy and Jones (2001);
- quantification and identification of carotenoids and chlorophylls by UPLC-PDA-ESI-Q/TOF-MS according to Wojdyło et al. (2018);
- quantification and identification of organic acids by UPLC coupled to PDA according to Wojdyło et al. (2018);
- quantification and identification of sugars by HPLC coupled to ELSD according to Wojdyło et al. (2018);
- basic chemical analyses:
    - total extract content by refractometric method according to PN-90/A-75101/02,
    - dry matter content according to PN-90/A-75101/03,
    - TA by the titrimetric method according to PN-90/A-75101/04,
    - pH by potentiometric method according to PN-90/A-75101/06,
    - total ash content according to PN-90/A-75101/08;
    - vitamin C content by the titration method according to PN-90/A-75101/11;
    - macroelement and microelement analyses by ASA;
    - pectin content by the Morris method according to Wojdyło et al. (2017).

Analyses of biological activity

Analyses of antioxidant activity were performed using the following methods:
- determination of antioxidant activity with the cation radical $ABTS^{\bullet+}$ according to Re et al. (1999);
- determination FRAP according to Benzie and Strain (1996);
- ORAC according to Ou et al. (2002).

Biological activities related to the regulation of enzyme activity were determined based on the following *in vitro* methods:
- determination of anti-ageing activity as the ability to inhibit the enzymes AChE and BuChE according to Gironés-Vilaplana et al. (2015);
- determination of antidiabetic activity as the ability to inhibit the enzymes α-amylase and α-glucosidase according to Nowicka et al. (2018);
- determination of anti-obesity activity as the ability to inhibit the enzyme pancreatic lipase according to Podsędek et al. (2014).

Sensory analysis

Sensory evalution tests were applied for carrot juices, carrot-based smoothies, and dried carrot foods (**Publication 3-4-5**). Sensory tests were conducted in a sensory analysis laboratory equipped with individual booths (at a controlled temperature (~20 °C), and combined natural/artificial light) designed according to ISO 8589:2009 standards. The laboratory was located at the Faculty of Biotechnology and Food Sciences, Wrocław University of Environmental and Life

Sciences (Poland). The sensory evaluation sessions were conducted from 10 a.m. to 1 p.m. by 9 fully trained panellists in the age between 25 and 43 years.

The sensory evaluations were performed using the 9-point hedonic scale (like extremely-9, like very much-8, like moderately-7, like slightly-6, neither like nor dislike-5, dislike slightly-4, dislike moderately-3, dislike very much-2 and dislike extremely-1). Carrot juices, dried carrot foods and smoothies were labeled with codes, served in transparent, small plastic glasses. After each sample, panelists drank water to neutralize their mouths for the next sample.

No ethical approval was required for this study, because of national laws. Participants were informed about their participation was entirely voluntary so that they could stop the analysis at any point and the responses would be anonymous.

3.4. Statistical Analysis

The chemical content, physical characteristics and biological activity results of raw carrot and carrot juice samples were shown with statistical information. The two-way analysis of variance (ANOVA, $p \leq 0.05$) and Duncan's test were performed by Statistica version 13.3 (Stat-Soft, Cracow, Poland). The results are demonstrated as the mean value (n = 3) (**Publication 1, 2-3**).

For the dried carrots and carrot-based smoothies, the statistical results were subjected to analysis of variance ($p < 0.05$), and Tukey's honestly significant difference test was performed using the R software (version 4.0.2, R Core Team, Austria). The results were expressed as mean values (n = 3 ± 5) ± standard deviation (**Publication 4-5**).

4. RESULTS AND DISCUSSION

4.1. Nutritional characteristics and prohealth properties of different carrot varieties

In the first part of the study, the chemical composition, bioactive compounds, and biological activities of raw carrot materials were analyzed. The study aimed to compare carrot varieties with different colors and sizes in terms of their chemical composition, including bioactive compounds and health-promoting properties. Such comprehensive studies on sugars, organic acids, pectins, minerals, vitamins, polyphenolic, and carotenoid contents, as well as *in vitro* biological activities against enzymes associated with diabetes (α-amylase and α-glucosidase), obesity (lipase), and age-related disorders (AChE and BuChE), and antioxidant activities (ABTS, ORAC, and FRAP) have not been conducted thus far on different carrot varieties exhibiting diverse colors and sizes.

The results are presented in **Publication 1 and Publication 2**:

Publication 1 - Yusuf, E., Wojdyło, A., Oszmiański, J., Nowicka, P., 2021. Nutritional, phytochemical characteristics and *in vitro* effect on α-amylase, α-glucosidase, lipase, and cholinesterase activities of 12 coloured carrot varieties. Foods 10, 808. https://doi.org/10.3390/foods10040808.

Publication 2 - Yusuf E., Tkacz K., Turkiewicz I.P. Wojdyło A., Nowicka, P. 2021. Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC. European Food Research and Technology, 247, 3053–3062. https://doi.org/10.1007/s00217-021-03857-0

In the present study, the polyphenolic compositions were analyzed using UPLC-PDA-Q/TOF-MS, and the results are presented in Table 1 (**Publication 1**). A total of 24 phenolic compounds (were identified, including 15 phenolic acids and 9 anthocyanins were detected. In the present study, 3-O-caffeoylquinic acid, 3-O-feruloylquinic acid, O-q-coumaroylquinic acid, dicaffeoylquinic acid derivative, and di-ferulic acid derivative were detected in white and orange carrots for the first time. Yellow carrots contained compounds like 4-O-caffeoylquinic acid, 3-O-feruloylquinic acid, di-ferulic acid derivative, and diferuoylquinic acid derivative. Purple carrots exhibited the presence of 4-O-caffeoylquinic acid, 3-O-feruloylquinic acid, O-q-coumaroylquinic acid, dicaffeoylquinic acid derivative, di-ferulic acid derivative, and diferuoylquinic acid derivative, with the highest contents observed in normal purple carrots.

The identified phenolic acids were derivatives of caffeic acid, caffeoylquinic acid, coumaroylquinic acid, ferulic acid, and feruloylquinic acid, all of which possess potent free radical scavenging properties.

In the analysis of purple carrots, nine anthocyanins were identified, but only five of them were quantified and included in Table 1 (**Publication 1**). In the present study, NPC and MiPC showed the presence of five anthocyanins were detected, namely cyanidin-3-O-xylosyl-glucosylgalactoside, cyanidin-3-O-xylosyl-galactoside, cyanidin-3-O-xylosyl-cinnamoyl-glucosylgalactoside, cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside, and cyanidin-3-O-xylosyl-p-coumaroylglucosyl-galactoside. On the other hand, MPC contained only two anthocyanins, cyanidin-3-O-xylosyl-galactoside, and cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside.

Comparing the total anthocyanin contents of the purple carrot varieties, NPC exhibited the highest anthocyanin content at 378.48 mg/100 g dm (dry matter), followed by MiPC with 255.08

mg/100 g dm, and MPC with 8.21 mg/100 g dm. These findings demonstrate the variation in anthocyanin composition and content among different purple carrot varieties.

To date, the quantification of polymeric procyanidins (PPs) in carrot varieties has not been carried out. This present study is the first to determine and quantify the procyanidin contents in carrots. The analysis revealed that the highest procyanidin contents were found in MiWC (78.92 mg/100 g dm), NOC (69.62 mg/100 g dm), and NWC (53.80 mg/100 g dm). Thus, normal-sized and white carrot samples were rich in PP.

The structure of procyanidins is characterized by their stereochemistry, hydroxylation pattern, flavan-3-ol constitutive units, and DP. Among the analyzed carrot varieties, the highest DP values were detected in MiWC (2.06), NYC (1.99), and MiYC (1.72). Based on the DP values, it can be concluded that the minisized and yellow carrot samples are particularly rich in flavanol units. Therefore, MiWC stands out not only for its high procyanidin contents but also for its high DP values.

Table 3 (**Publication 1**) provides the content of carotenoids and chlorophylls in the 12 carrot varieties, revealing significant differences ($p \leq 0.05$) among them. The present study identified several carotenoids in the carrot samples, including violaxanthin, astaxanthin, lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, (6R)-δ-carotene, α-carotene, γ-carotene, ε-carotene, β-carotene, and trans-apo-carotenal.

Among the analyzed carrot varieties, NPC demonstrated the highest ratio of different carotenoid types, specifically lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, γ-carotene, and trans-apo-carotenal. Notably, trans-apo-carotenal was exclusively detected in NPC.

According to Alasalvar et al. (2001), carrots have a high concentration of β-carotene and α-carotene, with β-carotene being the most abundant. In this study, the presence of β-carotene was detected in the following order: NYC > MYC > MiYC > MPC > NWC. Similarly, α-carotene was observed in the following order: NYC > MiOC > NOC > MiYC = NWC > MiWC. The highest levels of β-carotene were found in NYC and MYC, while the highest levels of α-carotene were observed in NYC and MiOC. Therefore, NYC exhibited the highest presence of both α and β-carotenes. However, Reif et al. (2013) found a different trend in their study, reporting a low amount of β-carotene in yellow carrots. It is worth noting that the contents of β-carotene and α-carotene can vary with temperature, which may explain the discrepancies in the observed results.

Carotenoids, being unstable pigments, can pose challenges when working with them. For example, orange carrots are recognized as a rich source of β-carotene and α-carotene (Reif et al., 2013). However, in this study, α-carotene was not detected in MOC due to the applied method, and there were no noticeable differences in the retention time of β-carotene in NOC, MiOC, or MOC. This unexpected finding was verified using the β-carotene standard. It is possible that the content of α-carotene was very low, resulting in the absence of a detectable peak for this compound. Furthermore, chlorophylls and xanthophylls can interfere with the measurement of α-carotene and β-carotene (Dietz et al., 1988). Additionally, the duration and temperature of storage are crucial factors that affect pigmentation. Surprisingly, high levels of δ-carotene and ε-carotenes were found in orange carrot samples of various sizes, which had not been previously reported.

Table 3 in **Publication 1** provided the identification and quantification of six xanthophyll pigments in carrot samples. In the present study, lutein was found solely in NPC and NYC, which is consistent with previous findings reported in the literature (Reif et al., 2013; Dietz et al., 1988).

Chlorophyll pigments play a crucial role in preventing chronic diseases (Kaszás et al., 2018). In the present study, pheophorbide a chlorophylls a, and b were identified as the chlorophyll pigments present in the carrot samples. Pheophorbide a was specifically found in NOC and NPC, while chlorophyll a was identified in NOC, NYC, NWC, MiYC, MWC, and MYC. Except for MiPC, all carrot varieties showed the content of chlorophyll b. Notably, the highest contents of both chlorophyll a and b were observed in NYC.

Furthermore, in **Publication 2**, the study measured the content of L-ascorbic acid. The L-ascorbic acid content ranged from 1.0 to 5.3 mg/100 g FW. The highest values were found in MYC (5.3 mg/100 g FW), followed by MPC (5.0 mg/100 g FW) and MOC (3.6 mg/100 g FW). Conversely, the lowest results were observed in NYC (1.2 mg/100 g FW) and NWC (1.0 mg/100 g FW). Alasalvar et al. (2001) reported that white carrot had the lowest content of ascorbic acid (1.3 mg/100 g FW), while orange carrot exhibited the highest amount (5.3 mg/100 g FW). However, the authors were unable to quantify the ascorbic acid content in purple carrots. In our study, dark-colored carrot samples demonstrated significantly higher levels of L-ascorbic acid compared to other carrots, which aligns with similar findings in the literature (Nicolle et al., 2004). Additionally, microsized and minisized carrot samples exhibited better L-ascorbic acid content compared to normal-sized carrot samples. Another study by Matějková and Petříková (2010) compared the L-ascorbic acid contents of six carrot varieties during storage. The results indicated that after harvesting, L-ascorbic acid levels in carrot roots ranged from 54 to 132 mg kg^{-1}. However, after 30 days of storage, the levels decreased by nearly 50%. Therefore, the content of L-ascorbic acid is influenced by various factors such as variety, carbon dioxide levels, temperature, storage conditions, and age (Ahmad et al., 2019).

Sugars and organic acids play a crucial role in determining the quality and sensory appeal of raw materials (Wei et al., 2014). These natural compounds contribute to approximately 60% of the dry matter, soluble solid content, and flavor of fruits and vegetables (Zhao et al., 2016). In the present study, sugars and organic acids were found to contribute to the taste and sweetness of the different carrot varieties. Table 1 in **Publication 2** displayed significant differences ($p \leq 0.05$) in the contents of sugars and organic acids in carrot roots.

Fructose, sorbitol, glucose, and sucrose were identified as the sugar components in the studied carrot varieties. Fructose and glucose were present in all carrot samples, while sorbitol was detected only in NOC, MiOC, MiYC, MiPC, MOC, MYC, and MPC. Moreover, sucrose was not identified in MiPC. The highest levels of fructose were observed in NOC (3.8 g/100 g FW), MWC (1.9 g/100 g FW), and MiYC (1.9 g/100 g FW). The highest sorbitol levels were found in MiYC (0.5 g/100 g FW) and MYC (0.4 g/100 g FW). NWC (4.1 g/100 g FW) and MiPC (3.8 g/100 g FW) exhibited the highest glucose levels. NPC (9.7 g/100 g FW) and MiWC (7.5 g/100 g FW) displayed the highest sucrose contents. It is worth noting that sucrose is the principal storage sugar and its levels increase with maturity (Suojala, 2000). Alasalvar et al. (2001) reported that purple carrots predominantly contain sucrose, followed by lower contents of fructose and glucose, which is consistent with our findings. Additionally, mini-sized and normal-sized carrot samples showed higher sugar contents, while white carrot samples of all sizes were rich in sugars. Sugar accumulation during the maturation of carrot varieties is closely associated with their flavors (Wei et al., 2014), and the total sugar content of carrot, turnip, and radish roots increases with higher CO_2. Therefore, this characteristic is important in the context of climate change.

Organic acids play a significant role in plant biology and contribute to human well-being due to their moderate antibacterial activities (Adamczak et al., 2019). In the colored carrot varieties, eight organic acids were identified: oxalic, maleic, citric, isocitric, malic, lactic, fumaric, and adipic acids. Among these, oxalic, isocitric, malic, and fumaric acids were found in all carrot varieties. Bryant and Overell (1953) also reported the presence of malic, fumaric, and isocitric acids in carrot roots. The present study revealed that micro-sized carrots and purple samples exhibited higher contents of organic acids. Therefore, the combination of flavor compounds, sugars, organic acids, and pH levels determines the distinct aromas found in different carrot varieties.

The content of organic acids affects the pH of raw materials, as shown in Table 2 of **Publication 2**. The colored carrot samples showed high pH values (>5.3), which aligns with similar findings in the literature (Gajewski et al., 2007).

In **Publication 2** (Table 2), the study also presented the content of other chemical compounds, including pectins. Pectins are a subgroup of carbohydrates that provide functionality to plant cell walls and protect them against pathogens (Liu et al., 2018). However, pectins cannot be digested by enzymes in the human body, and their reduction by gut bacteria is important for preventing colon-related diseases (Fukunaga et al., 2003).

In our study, the pectin levels in carrot samples ranged from 0 to 2.19/100 g FM. The highest content of pectin was observed in MYC (2.19/100 g FM) and MOC (1.90/100 g FM). MiOC showed no presence of pectin, while MiPC had a low amount of pectin content (0.14/100 g FM). Among the different carrot sizes, microsized and normal-sized carrot samples exhibited higher amounts of pectins (ranging from 1.38 to 2.19/100 g FM and from 0.94 to 1.59/100 g FM, respectively). On the other hand, minisized carrot samples had the lowest levels of pectin (ranging from 0.00 to 1.31/100 g FM).

According to Müller-Maatsch et al. (2016), total pectin can be divided into CASS and DASS, with parsley containing 47 mg/g of CASS. Furthermore, pectins in carrots, cabbage, and onion are soluble in sodium carbonate, and the solubility of pectin can be influenced by postharvest conditions of fruits and vegetables (Sila et al., 2009). However, the maturation stage of plants is the primary period in which their water-soluble pectin content increases, as pectin fractions may help eliminate ROS, which are oxygen-free radicals or nonfree radicals.

The study also analyzed the mineral content of the carrots, which are essential for maintaining healthy body functions, and plants serve as good sources of minerals. Table 3 in **Publication 2** presents the mineral contents in different carrot varieties, revealing significant differences.

In our study, it was observed that NYC and NOC had high Na content, while NPC and MYC showed the highest K content. Ca was abundant in NWC and NYC, while MYC and MPC exhibited high Fe content. The highest Mg content was observed in MiYC and MYC.

Normal-sized carrot samples showed higher mineral contents than microsized and minisized carrots; moreover, yellow carrots exhibited better results for mineral contents than carrots of other colors for each size. According to Nicolle et al. (2004), K is the most abundant element in carrots, and a similar result was also observed in the present study.

All the above-mentioned ingredients have biological potential and can affect the selected properties of carrots. Therefore, the study also evaluated the health-promoting properties of the analyzed raw materials. Table 4 (**Publication 2**) presents the antioxidant activities of the 12 carrot varieties. Significant differences in antioxidant activities ($p \leq 0.05$) were observed in the carrot varieties.

Antioxidants inhibit ROS that trigger the development of cancers, cardiovascular disorders, aging-related disorders, and other diseases. antioxidant properties of plants are commonly used in the food industry. Carrots, in particular, are known to be rich in phytochemicals such as phenolic compounds, carotenoids, and ascorbic acid, which serve as important nutritional antioxidants in the human diet (Gil et al., 2002).

In the present study, *in vitro* antioxidant activity of the carrot samples was quantified using the ABTS, FRAP, and ORAC methods. Significantly different antioxidant activities were observed among the carrot varieties, depending on factors such as color, size, and the applied antioxidant activity measurement technique employed.

In the ABTS method, the antioxidant activities of the carrot samples ranged from 0.5 to 7.9 mmol TE/100 g dm. The highest antioxidant activity was observed in MiPC (7.9 mmol TE/100 g dm) > NPC (7.4 mmol TE/100 g dm) > MPC (4.0 mmol TE/100 g dm). On the other hand, NWC (0.5 mmol TE/100 g dm) and NYC (0.5 mmol TE/100 g dm) displayed the lowest antioxidant activities.

The findings align with Singh et al. (2018), who reported that the ABTS test showed the highest antioxidant activity in purple carrots. This relationship was also observed in the present study,

indicating a positive correlation between antioxidant activity and anthocyanin content. Sun et al. (2009) similarly reported the same relationship between antioxidant activity and anthocyanins.

In the FRAP test, the antioxidant activities of the carrot samples ranged from 0.3 to 5.8 mmol TE/100 g dm. MiPC displayed the highest antioxidant activity (5.8 mmol TE/100 g dm), followed by NPC (4.6 mmol TE/100 g dm) and MiOC (2.7 mmol TE/100 g dm). On the other hand, NWC (0.3 mmol TE/100 g dm) and NYC (0.3 mmol TE/100 g dm) exhibited the lowest antioxidant activities. These results indicate that minisized and purple carrots have higher antioxidant activities as measured by the FRAP assay. These findings are consistent with the study conducted by Singh et al. (2018), which also showed higher antioxidant activity for purple carrots in the FRAP assay.

The ORAC assay also yielded similar results, with the highest antioxidant activities observed in MiPC (17.1 mmol TE/100 g dm), followed by NPC (16.2 mmol TE/100 g dm) and MPC (15.4 mmol TE/100 g dm). According to Nicolle et al. (2004), the dark-colored carrot demonstrated the highest antioxidant activity, and the white carrot showed the lowest activity. This further supports the notion that dark-colored carrots, rich in anthocyanins, possess strong antioxidant activities.

In the present study, the total carotenoid content did not correlate with the total antioxidant activity. Mech-Nowak et al. (2012) also reported similar results. According to Smeriglio et al. (2018), the antioxidant activity of purple carrots results from anthocyanins and phenolic acids. Similar results were observed in the present study. Algarra et al. (2014) compared the antioxidant activities of purple carrots and orange carrots growing in the same region and showed that purple carrots exhibited higher antioxidant activities than orange carrots depending on the content of anthocyanins.

The enzyme inhibitory activities of 12 carrot varieties against α-amylase, α-glucosidase, pancreatic lipase, AChE, and BuChE were evaluated through *in vitro* assays and quantified as IC_{50}, representing the amount of sample required to reduce enzyme activity by 50%. The findings are presented in Table 4 (**Publication 1**). Notably, the carrot varieties exhibited noteworthy variations in all five inhibition activities ($p \leq 0.05$). It is important to highlight that investigations into the enzyme inhibition activities of carrot varieties have not been conducted previously.

The antidiabetic properties of 12 carrot varieties were evaluated through the assessment of their inhibition of α-amylase and α-glucosidase enzymes. These enzymes play a crucial role in carbohydrate digestion, and the inhibition of these enzymes may decrease postprandial blood glucose levels by impeding the breakdown of polysaccharides into glucose. Previous studies have demonstrated that various plant species possess bioactive compounds capable of diminishing the activity of enzymes associated with early-stage diabetes (Hanhineva et al., 2010).

In the present study, the IC_{50} values of the carrot samples for α-amylase inhibition ranged from 107.85 to 807.92 mg/mL. Notably, MiWC and MOC showed the highest activities against α-amylase, while NOC demonstrated the lowest activity. Compared to other fruits such as sour cherry, red grapefruit, pineapple, orange, and kiwi (Podsędek et al., 2014), most carrot extracts displayed higher IC_{50} values for α-amylase. It should be emphasized that the inhibitory activity of α-amylase is attributable to bioactive compounds present in plants, including glycosides, polysaccharides, steroids, and terpenoids (Mentreddy, 2007).

Overall, mini carrots showed the most pronounced inhibitory effect on α-amylase in our study.

The IC_{50} values of the carrot samples for inhibiting α-glucosidase ranged from 97.02 to 897.79 mg/mL. Notably, MiPC and MYC showed the highest inhibitory activities against α-glucosidase, while MiWC showed the lowest result. When compared to a group of fruits known for their significance in diabetes prevention, including chokeberry, apple, pear, and blackberry (Podsędek et al., 2014), the IC_{50} values for α-glucosidase inhibition in carrot extracts were higher.

Previous research has indicated that the potent antidiabetic activity of onions can be attributed to their phenolic acid, flavonoid, and anthocyanin content (Papoutsis et al., 2021). Additionally, the

antidiabetic activities of fruits and vegetables have been linked to their polyphenol and carotenoid contents (Nowicka and Wojdyło, 2019; Alam et al., 2020).

Comparing the α-amylase and α-glucosidase inhibitory activities of the carrot samples, it was found that MiWC exhibited the highest α-amylase inhibitory activity but the lowest α-glucosidase inhibitory activity. Moreover, positive correlations were observed between α-amylase inhibitory activity and phenolic acids (R^2 = 0.12), total phenolic (R^2 = 0.12), total organic acid (R^2 = 0.06), and total vitamin C contents (R^2 = 0.22). Conversely, negative correlations were observed with anthocyanins (R^2 = −0.35), PPs (R^2 = −0.11), total carotenoid and chlorophyll (R^2 = −0.35), total sugar (R^2 = −0.05), total pectin (R^2 = −0.25), and total mineral contents (R^2 = −0.30).

However, the α-glucosidase inhibitory activity displayed different correlation patterns compared to α-amylase inhibitory activity. Positive correlations were found between α-glucosidase inhibitory activity and total chlorophyll and carotenoid contents (R^2 = 0.11), total pectin (R^2 = 0.09), and total vitamin C (R^2 = 0.32). Conversely, negative correlations were observed with each analyzed fraction of polyphenols, total sugar, organic acid, and mineral contents.

Pancreatic lipase plays a role in the hydrolysis of triacylglycerols into free fatty acids, bile salts, and fat-soluble vitamins (Lunagariya et al., 2014). In our study, the IC_{50} values for pancreatic lipase inhibitory activities ranged from 5.29 to 12.25 mg/mL. Notably, the highest IC_{50} values were observed for NOC (5.29), MiYC (5.69), MYC (6.05), and NPC (6.12). Conversely, NWC (12.25) and MWC (11.36) exhibited the lowest activities against pancreatic lipase.

Consistent with the findings of Fabroni et al. (2016), this study also revealed that the total anthocyanin content correlated with pancreatic lipase activity.

Furthermore, it is noteworthy that the carrot samples exhibited higher inhibitory activities against pancreatic lipase compared to the diabetes-related enzymes. The pancreatic lipase inhibitory activity displayed positive correlations with the phenolic acid (R^2 = 0.42), anthocyanin (R^2 = 0.27), total phenolic (R^2 = 0.36), total carotenoid and chlorophyll (R^2 = 0.54), total organic acid (R^2 = 0.19), total pectin (R^2 = 0.06), and total vitamin C (R^2 = 0.50) contents. However, negative correlations were observed with PP content (R^2 = −0.24), total sugar (R^2 = −0.17), and total mineral (R^2 = −0.03) contents.

The inhibition of AChE and BuChE is considered important in the diagnosis and treatment of various diseases, including bladder distention, glaucoma, myasthenia gravis, and Alzheimer's disease (Colovic et al., 2013). In the present study, we investigated the potential inhibitory activities of carrot samples against AChE and BuChE.

The IC_{50} values of the carrot samples for AChE inhibition ranged from 10.14 to 18.96 mg/mL. Remarkably, MPC (10.14 mg/mL), MWC (12.05 mg/mL), and MiWC (12.31 mg/mL) exhibited the highest AChE inhibitory activities, while NPC (18.96 mg/mL) showed the lowest result. Previous studies have indicated that the content of polyphenols and carotenoids in carrots correlates with the activities of cholinesterase inhibitors (Mohammadzadeh Honarvar et al., 2017; Turkiewicz et al., 2019). In the present study, microsized and white carrot varieties showed elevated activities against AChE.

Furthermore, the IC_{50} values of the carrot samples for BuChE inhibition ranged from 7.83 to 19.02 mg/mL. MPC (7.83 mg/mL), NPC (7.85 mg/mL), and NYC (8.01 mg/mL) exhibited the highest BuChE inhibitory activities, while NWC (19.02 mg/mL) demonstrated the lowest activity. Therefore, normal-sized and purple carrot samples demonstrated superior activities against BuChE.

Regarding the correlations, AChE inhibitory activity was positively correlated with the contents of PP (R^2 = 0.39), total organic acid (R^2 = 0.45), total pectin (R^2 = 0.45), and total vitamin C (R^2 = 0.29). However, negative correlations were observed between AChE inhibitory activity and phenolic acids (R^2 = −0.75), anthocyanins (R^2 = −1.00), total phenolic (R^2 = −0.73), total carotenoid and chlorophyll (R^2 = −0.15), total sugar (R^2 = −0.47), and total mineral (R^2 = −0.19) contents.

On the other hand, BuChE inhibitory activity exhibited a strong positive correlation with the content of total carotenoid and chlorophyll (R^2 = 0.45), total organic acid (R^2 = 0.68), total pectin (R^2 = 0.57), total vitamin C (R^2 = 0.20), and total mineral (R^2 = 0.28). However, it showed a weaker positive correlation with phenolic acids (R^2 = 0.08) and total phenolic (R^2 = 0.07). Negative correlations were observed between BuChE inhibitory activity and anthocyanin (R^2 = −0.19), PPs (R^2 = −0.19), and total sugar (R^2 = −0.07) contents.

The study results showed that purple carrot samples exhibited the highest levels of polyphenolics and carotenoids, along with the highest activities against cholinesterase. Conversely, normal yellow carrot samples had the lowest values for polyphenol content, while micro white carrot samples demonstrated the lowest results for total phenolic acid, total carotenoid, and chlorophyll content.

In summary, this study aimed to find the best carrot variety with high nutrients for food processing. The results indicate that purple carrots are particularly intriguing due to their sensory appeal, with their sweetness, and vibrant color. Moreover, they are rich in bioactive compounds such as pectins, vitamin C, and PPs, which contribute to their significant potential for promoting health. These findings are crucial in the development of functional food for various social groups.

4.2. Analyses of carrot juices

In the second phase of the study, the focus shifted toward analyzing the bioactive compounds, physicochemical characteristics, biological activities, and sensory properties of carrot juices.

The study aimed to compare the content of bioactive compounds and health benefits among 12 different colored carrot juices. By identifying the most appealing carrot juice with high health-promoting activity and superior product quality, this research aims to provide valuable data for the fruit and vegetable industry. This data can be utilized for evaluating food materials as ingredients and developing innovative food products that are appealing to children. The results are presented in **Publication 3**:

Yusuf E.H., Wojdyło A., Nowicka, P. 2023. Possibility to use the different sizes and colors of carrots for the production of juices – comparison of bioactive compounds, nutritional quality, pro-health properties, and sensory evaluation. Journal of the Science of Food and Agriculture, 103(2), 933-943. https://doi.org/10.1002/jsfa.12206

In the present phase of the study, various parameters such as color, viscosity, and turbidity were evaluated for fresh carrot juices (refer to Table 1 in **Publication 3**). It is worth noting that the viscosity and molecular weight of hydrocolloids have been found to exhibit a positive correlation (Saha and Bhattacharya, 2010), with viscosity being a crucial parameter in determining the characteristics of liquid foods (Krokida et al., 2001).

In the present study, the viscosity of fresh carrot juices ranged from 5.6 to 20.1 mPas. The highest viscosity values were observed for NWCJ (20.1 mPas) and MiPCJ (18.3 mPas), while the lowest viscosities were found in NYCJ (5.6 mPas), MOCJ (6.8 mPas), and MiWCJ (6.8 mPas). Consequently, normal-sized and purple carrot juices exhibited superior viscosity results. It is important to measure viscosity to determine the heating and energy consumption rates of juices when there are changes in concentration (Nindo et al., 2005). Additionally, viscosity plays a significant role in the rheological properties of liquid food products, particularly influenced by pectin content. It should be noted that the pasteurization process can also increase the viscosity of juices (Vandresen et al., 2009). Therefore, carrot juices with higher viscosity are ideal for smoothie production when blended with different fruit or vegetable juices, while those with lower viscosity values are more suitable for manufacturing beverages.

In the context of fruit and vegetable juices, turbidity levels can vary between clear and cloudy juices, with different thresholds for each category. Cloudy juice products are expected to have a turbidity value of more than 250 NTU before centrifugation (Wojdyło et al., 2014). In the present study, significant differences in turbidity levels of carrot juices were observed, and all juices exhibited turbidity levels higher than 250 NTU before centrifugation.

Turbidity in fruit and vegetable juices is influenced by factors such as shape, size, color, and relative refractive indexes (Vaillant et al., 2008). Pectin content has also been identified as a key factor contributing to turbidity in juices (Markowski et al., 2009). Interestingly, in the present study, samples with the highest turbidity did not contain pectin. The turbidity of carrot juices ranged from 5.1% NTU to 94.1% NTU.

For cloudy juices, it is desirable to have stable turbidity, with final NTU results after calculations higher than 50% NTU (Dietrich et al., 1996). In the present study, the highest final turbidity levels were observed in MYCJ (94.1% NTU), MOCJ (73.4% NTU), MPCJ (70.8% NTU), and MWCJ (68.6% NTU). On the other hand, the lowest turbidity results were found in NOCJ (5.1%

NTU) and NWCJ (5.4% NTU). Therefore, it appears that all microsized carrots are particularly useful for producing cloudy juices with higher turbidity levels.

In the present study, the osmolality of fresh carrot juices was measured. Osmolality is a method used to assess the electrolyte–water balance in the body and determines the bioavailability of beverages for body hydration (Sadowska et al., 2017). It differentiates between effective osmoles (such as glucose, Na^+, K^+, Cl^-, and HCO_3-) that influence water osmosis across cell membranes, and ineffective osmoles (like urea and ethanol) that do not affect water osmosis (Rasouli, 2016).

The mean osmolality of the carrot juices in the study ranged from 446 to 520 mOsm/L. The highest osmolality values were observed in MiPCJ (520 mOsm/L) and NPCJ (512 mOsm/L), while the lowest osmolality values were recorded in MiYCJ (446 mOsm/L) and NOCJ (447 mOsm/L). It is noteworthy that all carrot juices in the study were found to be hypertonic solutions (>300 mOsm/L) with high levels of electrolytes. Therefore, minisized and purple carrot juices exhibited elevated levels of osmolality.

Color is the first parameter for consumers to purchase fruit or vegetable juice. Anthocyanins and carotenoids are associated with the color of fresh carrot juices (Pokhrel et al., 2017). In the present study, the color parameters of juices are presented in Table 1 (**Publication 3**). The mean L* of fresh carrot juices ranged from 31.2 to 49.1. The highest L* values were observed in yellow carrot juices, while the lowest values were observed in purple carrot juices. The reduced lightness observed in the juices can be attributed to the high anthocyanin content in purple carrots. Therefore, in the present study, the same results were observed for all purple carrot juice samples.

The parameters a* and b* represent different colors. In this study, the range of a* values was from 0.1 to 22.3. The highest a* values were observed in MiOCJ (22.3) and NOCJ (19.1), while the lowest a* values were detected in NWCJ (0.1) and MiWCJ (1.5). The mean b* values ranged from 1.6 to 32.7. The highest b* values were observed in NYCJ (32.7) and MiYCJ (29.8), whereas the lowest b* values were found in MiPCJ (1.6) and NPCJ (4.3). The darkest blue colors, which are indicative of anthocyanins imparting dark colors to purple carrot juices, were associated with the lowest b* values. Consequently, purple carrot juices displayed the lowest L* and b* values.
Furthermore, Table 2 (**Publication 3**) provides information on the sugar and organic acid content of the various colorful carrot juices. The current study revealed significant levels of fructose, glucose, and sucrose in all carrot juices. The total sugar content in fresh carrot juices ranged from 2.0 to 10.5 g/100 mL. Particularly, the minisized and orange carrot juices exhibited notably higher levels of total sugar content.

Organic acids play a crucial role in imparting distinct smells and aromas to fresh fruit and vegetable juices (Nishiyama et al., 2008). Carrot juices primarily contain oxalic, malonic, and D-malic acids as their main organic acid components. Oxalic acid forms strong associations with minerals such as potassium, sodium, calcium, and magnesium, resulting in oxalate salts. While sodium and potassium oxalate salts are soluble in water, calcium oxalate is insoluble and can form crystals in the kidneys and urinary tract. However, carrots contain approximately 35.6 mg/100 g of total oxalate (Savage et al., 2000), and the boiling treatment reduces the insoluble oxalate content in carrots (Chai and Liebman, 2005). Thus, in this study, the highest oxalic acid content was found in MYCJ (3.0 g/100 mL), followed by NWCJ (2.1 g/100 mL), whereas the lowest oxalic acid contents were observed in MiOCJ (0.1 g/100 mL) and NOCJ (0.1 g/100 mL).

Furthermore, a derivative of malic acid has been detected in orange and purple carrot juices (Tanriseven et al., 2020). Malic acid naturally occurs in fruits and vegetables and is known to stimulate human metabolism (Xie et al., 2011). In this study, the highest content of D-malic acid was observed in MYCJ (0.4 g/100 mL) and NPCJ (0.2 g/100 mL), while D-malic acid was not detected in NYCJ, NWCJ, and MiPCJ. The total organic acid content was highest in MYCJ and MPCJ, while it was lowest in NOCJ and MiPCJ. The pH of the 12 carrot juices was approximately 5, with the highest

pH values observed in NYCJ (5.9), NWCJ (5.9), and NPCJ (5.9), and the lowest pH value detected in MPCJ (5.5). Alkaline pH levels are undesirable for natural ingredients during food processing because inadequate pasteurization in the absence of additives may lead to microbial growth in the food products.

In the conducted research, the analysis of bioactive compounds was also performed. The phenolic content of the fresh carrot juices exhibited notable differences ($p \leq 0.05$) as indicated in Table 4 (**Publication 3**). Within this study, a total of 14 phenolic acids, four anthocyanins, and five flavan-3-ols were identified and quantified in the carrot juices.

The phenolic compounds identified in the present study were found to be consistent with those reported in the existing literature. However, some of these compounds were identified for the first time in carrot juices. For instance, compounds such as 5-O-trans-caffeoylquinic acid, dicaffeoylquinic acid, and O-q-coumaroylquinic acid have previously been detected in carrot juice (Ma et al., 2013), whereas 4-caffeoylquinic acid, 5-caffeoylquinic acid, caffeic acid, and ferulic acid were identified in a traditional purple carrot beverage (Tanriseven et al., 2020). Moreover, several compounds, including 3-O-caffeoylquinic acid, ferulic acid-hexoside, ferulic acid di-hexoside, 3-O-feruloylquinic acid, 5-O-feruloylquinic acid, di-ferulic acid derivative, diferuoylquinic acid derivative, and 4-O-feruloylquinic acid, were detected for the first time in the carrot juices during this study.

The quantitative analysis of phenolic acids revealed that chlorogenic acid was present in all fresh carrot juices, except for MiOCJ. The mean level of chlorogenic acid varied from 1.3 mg/100 mL (MWCJ) to 303.2 mg/100 mL (MiPCJ). Ferulic acid di-hexoside was detected in NPCJ (23.6 mg/100 mL) and MPCJ (1.7 mg/100 mL), while caffeic acid-hexoside was observed in NYCJ (0.9 mg/100 mL) and NOCJ (0.5 mg/100 mL). Diferuoylquinic acid derivative was quantified in NPCJ (4.1 mg/100 mL) and MiYCJ (3.6 mg/100 mL), and ferulic acid was detected solely in NPCJ (1.5 mg/100 mL). Moreover, various other phenolic acids were present in different amounts across the fresh carrot juices analyzed in this study.

The total phenolic acid content of the carrot juices ranged from 9.5 to 440.5 mg/100 mL. NPCJ (440.5 mg/100 mL) and MiPCJ (411.2 mg/100 mL) exhibited the highest total phenolic acid content, while MWCJ (9.5 mg/100 mL) and NYCJ (32.6 mg/100 mL) displayed the lowest content among the juices analyzed.

In the purple carrot juices, a total of four anthocyanins (cyanidin derivatives) were quantified. These identified anthocyanins were found to be similar to those reported in **Publication 1**. Specifically, cyanidin-3-O-xylosyl-glucosylgalactoside and cyanidin-3-O-xylosyl-cinpoyl-glucosylgalactoside were detected in two purple carrot juices (normal and mini). Cyanidin-3-O-xylosyl-galactoside was only found in MiPCJ (31.7 mg/100 mL), while cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside was observed exclusively in NPCJ (109.1 mg/100 mL).

In the present study, the flavan-3-ols identified in carrot juices were procyanidin B1, procyanidin B2, (−)-epicatechin, (−)-epicatechin-gallate, and procyanidin B4. Procyanidin B2 was found in all carrot juices analyzed. The highest content of procyanidin B2 was observed in MiPCJ (589.4 mg/100 mL), NPCJ (155.8 mg/100 mL), and NYCJ (81.6 mg/100 mL). However, (−)-epicatechin-gallate and procyanidin B4 were quantified only in MiYCJ (3.7 mg/100 mL) and NOCJ (2.4 mg/100 mL), respectively. The total catechin content was notably high in MiOCJ (691.3 mg/100 mL), MiPCJ (619.7 mg/100 mL), and NPCJ (181.2 mg/100 mL).

In summary, when considering the polyphenolic contents of the 12 carrot juices, MiPCJ (1087.6 mg/100 mL), MiOCJ (831.6 mg/100 mL), and NPCJ (793.7 mg/100 mL) exhibited the highest total polyphenolic content. On the other hand, MWCJ (45.4 mg/100 mL), MYCJ (86.0 mg/100 mL), and NOCJ (90.0 mg/100 mL) had the lowest total polyphenolic content. Notably, minisized and

purple carrot juices displayed higher levels of total phenolic content, indicating their richness in polyphenols.

The carotenoid content of the carrot juices was analyzed and presented in Table 4 of **Publication 3**. In the present study, a total of six carotenoid compounds were identified. Among them, γ-carotene was found in all 12 fresh carrot juices. NPCJ (8.3 mg/100 mL) and MiPCJ (5.1 mg/100 mL) exhibited the highest content of γ-carotene, while MiWCJ (0.2 mg/100 mL), MWCJ (0.2 mg/100 mL), and NWCJ (0.3 mg/100 mL) displayed the lowest γ-carotene content among the juices analyzed.

In a previous study reported by Stinco et al. (2019), the presence of β-carotene and lutein in fresh carrot juice was reported. In the present study, the same amount of lutein (0.1 mg/100 mL) was detected in NYCJ, NPCJ, and MiYCJ. Additionally, β-carotene was found in NYCJ (1.8 mg/100 mL), MiYCJ (1.5 mg/100 mL), and MYCJ (0.9 mg/100 mL). β-Cryptoxanthin, on the other hand, was only detected in NPCJ (0.4 mg/100 mL).

The total carotenoid content of the fresh carrot juices analyzed in this study ranged from 0.2 to 14.8 mg/100 mL. The highest levels of total carotenoids were found in NPCJ (14.8 mg/100 mL) and MiPCJ (5.8 mg/100 mL), while the lowest levels were observed in MiWCJ (0.2 mg/100 mL) and MWCJ (0.3 mg/100 mL). Notably, the total carotenoid contents of NPCJ (14.8 mg/100 g), NOCJ (5.1 mg/100 g), and MiPCJ (5.8 mg/100 g) did not undergo significant changes after food processing when compared to the total carotenoid contents reported in **Publication 1**. This could be attributed to the high antioxidant content, such as phenolic acids, present in these juices. In conclusion, normal-sized and purple carrot juices exhibited the highest total carotenoid content among the tested samples.

The PPCs present in the analyzed carrot juices have been associated with various health-promoting properties, including antioxidant and antidiabetic effects. The antioxidant activities of the colored fresh carrot juices were determined using the ABTS and FRAP assays, as shown in Table 5 (**Publication 3**). The antioxidant capacity of food products is influenced by the presence of carotenoids, phenolic compounds, and ascorbic acid (Pérez-Jiménez et al., 2008). In the present study, the ABTS assay results indicated antioxidant activities ranging from 0.2 to 1.7 mmol TE/100 mL. The highest ABTS results were observed in MiYCJ and MPCJ, while the lowest antioxidant activities were found in NWCJ and NOCJ. These findings were consistent with the results obtained from the FRAP assay. Overall, the minisized and purple carrot juices exhibited the highest antioxidant activities, which can be attributed to their high content of phenolic acids, anthocyanins, and carotenoids.

The biological activities of carrot juices against α-amylase, α-glucosidase, pancreatic lipase, AChE, and BuChE were evaluated using *in vitro* assays, and the results are shown in Table 5 (**Publication 3**).

The inhibition of AChE and BuChE is of particular interest due to its potential impact on central nervous system disorders such as Alzheimer's disease. In the present study, the percentage inhibition of AChE ranged from 6.7 to 50.3% across the different carrot juices. The highest AChE inhibition activities were observed in NYCJ (50.3%) and NPCJ (46.8%). These findings are consistent with the study by Poudyal et al. (2010), which reported that purple carrot juice exhibits potent inhibitory activity against AChE, mainly attributed to its high anthocyanin content. Similarly, in the present study, the highest AChE inhibition activity in NYCJ could be attributed to the presence of minerals, L-ascorbic acid, caffeic acid-hexoside, lutein, and/or β-carotene, which may contribute to the observed bioactivity.

The percentage inhibition of BuChE ranged from 2.5 to 14.1%, with NPCJ displaying the highest inhibition and MWCJ exhibiting the lowest inhibitory activity. Therefore, both normal-sized and colored carrot juices demonstrated heightened inhibitory activities against BuChE and AChE.

Following this, MiWCJ showed the most significant antiBuChE activity, while MOCJ exhibited the highest antiAChE activity.

In the present study, the IC_{50} value for α-amylase inhibition ranged from 7.7 to 1.6 mg/mL, with NPCJ displaying the highest α-amylase inhibition activity.

The IC_{50} value for α-glucosidase inhibition ranged from 0.6 to 0.2 mg/mL, with the highest α-glucosidase inhibition observed in MiOCJ and MYCJ, while NPCJ and MiYCJ exhibited the lowest inhibitory activity. Consequently, microsized and orange carrot juices showed the most significant α-glucosidase inhibition.

It is worth noting that fresh carrot juices displayed more potent antiα-glucosidase activity compared to antiα-amylase activity. Interestingly, NPCJ yielded the best results for antiα-amylase activity; however, it exhibited the lowest results for antiα-glucosidase activity. Furthermore, NPCJ demonstrated favorable results in antiaging tests. Therefore, based on the findings of the present study and in comparison with the raw carrot samples described in **Publication 1**, carrot juices exhibited enhanced antiα-amylase and antiα-glucosidase activities through the implementation of specific technologies. This improvement could be attributed to the conversion of sugar and pectin during juice processing.

In the present study, the antiobesity activity of colored carrot juices ranged from 0.7 to 0.1 mg/mL. The highest inhibition of pancreatic lipase was observed in NPCJ, MiOCJ, MiWCJ, and MPCJ, while NYCJ, NWCJ, and MiYCJ exhibited the lowest antiobesity activity. Remarkably, MOCJ demonstrated the most favorable results across all three tests. NPCJ displayed elevated results for both antiα-amylase and antiobesity activities, whereas MiOCJ and MPCJ showed satisfactory results for both antiα-glucosidase and antiobesity activities. Hence, akin to the observed antiα-amylase and antiα-glucosidase activities, the antiobesity activities of carrot samples exhibit impressive values following fresh juice processing.

Finally, Fig. 3 (**Publication 3**) presents the sensorial characteristics of the prepared colored carrot juices. The evaluation focused on the appearance, color, consistency, smell, and taste of the fresh carrot juices. However, it should be noted that micro-sized yellow carrot juices were excluded from the sensory test due to inadequate product quality for consumption, potentially resulting from high pH and unexpected oxidation.

As per the panelists' feedback, MiOCJ (7.7), NOCJ (7.6), and MOCJ (7.4) received satisfactory ratings for appearance. This preference could be attributed to the familiar orange color associated with carrots. Additionally, NYCJ (7.5) was ranked as the third-best among the colored products.

Consistency is indeed an important parameter in determining food choice. In the present study, four carrot juices (NYCJ, MOCJ, MiOCJ, and MiPCJ) exhibited identical consistency ratings of 7.1. On the other hand, NPCJ had the lowest consistency rating of 6.2.

The smell of food products plays a crucial role in attracting consumers and influencing their preferences. In this study, NYCJ (7.2) and NOCJ (7.1) were found to have the most appealing smells.

Moving on to flavor evaluation, the most attractive tastes were associated with NOCJ (7.7) and MOCJ (7.4). This preference could be attributed to the familiar taste of orange carrots, which aligns with their appearance and color. Conversely, MWCJ (5.3), MiWCJ (5.4), and MiYCJ (5.4) received the lowest taste acceptance ratings.

In summary, MiOCJ was found to have the highest overall acceptance from the panelists, particularly in terms of appearance, consistency, and color. NYCJ stood out for its appealing consistency and smell. When it comes to taste, NOCJ and MOCJs were the most preferred among the fresh carrot juices. Therefore, the traditional orange color and taste were found to be the most attractive features by the panelists. Furthermore, the favorable aroma of yellow carrot juice suggests its potential for use in various food applications.

Normal purple carrot juice exhibited superior performance in all biological tests, except for α-glucosidase inhibition. It displayed the highest levels of total phenolic acid, anthocyanin, and carotenoid contents among the tested samples. Therefore, purple carrot juices can be used in beverage industries for producing smoothies and/or blended juices for increasing the health-promoting properties of liquid or semiliquid food products. The sensorial acceptance of carrot juices relied mainly on orange carrot varieties for all traits. Based on the results of this study, it is recommended to explore further applications of purple carrot juices in the development of novel processed food products that offer significant health benefits.

4.3. Analyses of carrot-based smoothies

In the third part of the study, four colored carrot purees were blended with Poland's most popular fruit juices to analyze physicochemical properties, bioactive compounds, biological activities, and sensory characteristicsThe study aimed to use PC, YC, OC, and WC varieties to produce smoothies with RJ, AJ, PJ, SJ, and SCJ juices (the most popular fruit in Poland from the *Rosaceae* family).

The objective of the study was to thoroughly investigate these smoothies in terms of their physicochemical properties, sensory characteristics, bioactive compounds, and *in vitro* prohealth properties. By formulating this research objective, the researchers aimed to fully examine their hypothesis, which posits that carrots can serve as a functional base for producing smoothies specifically targeted towards children as snacks or second breakfast options. Furthermore, the addition of fruits from the Rosaceae family to the carrot base was expected to enhance the sensory appeal and health-promoting value of the final products, making them more desirable for the intended target group. The results are presented in **Publication 4**:

Yusuf E.H., Wojdyło A., Bourbon A.I., Nowicka, P. 2023. Fruit-carrot-based smoothies as innovative products with a complex matrix of bioactive compounds effected on activities of selected digestive enzymes and cholinesterases *in vitro*. *Antioxidants*, 12(4), 917.

Table 1 (**Publication 4**) presents the physicochemical parameters of the carrot-blended smoothies, including viscosity, pH, osmolality, and color characteristics. Significant differences ($p \leq 0.05$) were observed among the samples.

Viscosity plays a crucial role in determining the quality of smoothie products. In this study, the viscosities of the carrot-blended smoothies ranged from 3.30 to 54.08 mPas. The control groups of PC100% and YC100% had viscosities exceeding 100 mPas, while WC puree had a viscosity of 49 mPas, and OC puree had a viscosity of 15 mPas. The highest viscosity was observed in the PJ–PC (54.08 mPas) and SJ–YC (49.05 mPas) smoothies, while the AJ–OC smoothie had the lowest viscosity (3.30 mPas). These differences in viscosity are directly related to the characteristics of the individual purees used. Adding purees with higher viscosity, such as PC and YC, resulted in denser products compared to those containing OC puree.

To ensure that smoothie products are semifluid and drinkable, it is important to consider the proportion of juice to puree when designing a smoothie based on PC or YC. It is recommended to use a larger proportion of juice than puree to avoid exceeding a viscosity of 45 mPas, which was considered acceptable by the sensory panel.

The color of food products plays a significant role in influencing consumer purchase decisions. In the present study, the mean L* values of the carrot-based smoothies ranged from 32.21 to 55.58. The smoothies made with WC and YC purees blended with PJ and AJ had the highest lightness values, indicating a lighter color. On the other hand, the smoothies made with SCJ–WC (31.79), RJ–PC (32.21), and SCJ–YC (32.55) had the lowest lightness values, indicating a darker color.

Consistent with expectations, the addition of SCJ and RJ resulted in smoothies with darker colors, while AJ and PJ contributed to smoother ones with lighter colors.

Additionally, other color parameters such as a* and b* were measured in the present study. The highest redness (indicated by the highest a* values) was observed in the OC puree, as well as in the RJ, AJ, and PJ smoothies. On the other hand, the WC puree and PJ and AJ smoothies exhibited the highest greenness (represented by the highest negative a* values). The OC puree, along with the

AJ and PJ smoothies, showed the highest yellowness (highest b* values). Furthermore, the SCJ–WC (6.50), SCJ–PC (7.98), and RJ–WC (8.21) smoothies demonstrated the highest blueness (indicated by the highest negative b* values). Consequently, consistent with the lightness results, SCJ and RJ contributed to the development of a dark blue color in the smoothies.

The osmolality of the smoothies was also analyzed in this study. The smoothies that contained SCJ, such as SCJ–PC (804 mOsm/L), SCJ–YC (750 mOsm/L), and SCJ–WC (690 mOsm/L), exhibited the highest osmolality values. On the other hand, the smoothies containing SJ, such as SJ–OC (419 mOsm/L), SJ–PC (444 mOsm/L), and SJ–YC (446 mOsm/L), showed the lowest osmolality values.

These results indicate that SCJ-based smoothies have a higher osmolality, suggesting that they are rich in bioactive compounds and are easily absorbed by the digestive system. On the contrary, SJ-based smoothies demonstrated the lowest osmolality values, indicating a lower concentration of solutes.

The sugar and organic acid contents of the carrot-based smoothies were measured in this study, and the results are presented in Figs. 2 and 3 (**Publication 4**), indicating significant differences ($p \leq 0.05$). The smoothies contained fructose, sorbitol, glucose, and sucrose. The fructose content ranged from 0.87 to 5.74 g/100 mL. The highest fructose content was observed in the AJ–WC (5.74 g/100 mL), AJ–OC (5.37 g/100 mL), and PJ–OC (4.04 g/100 mL) smoothies, while the lowest fructose content was observed in the SJ–YC (0.87 g/100 mL), SJ–PC (0.97 g/100 mL), and RJ–PC (1.07 g/100 mL) smoothies. Therefore, the OC puree and AJ smoothies had the highest fructose content. Sorbitol was not detected in any of the samples, except for the PJ–OC (0.55 g/100 mL), PJ–PC (0.33 g/100 mL), and SCJ–OC (0.32 g/100 mL) smoothies. The sorbitol content was not observed in the RJ–PC, SJ–PC, RJ–WC, SJ–WC, RJ–YC, SJ–YC, RJ–OC, and SJ–OC smoothies. Hence, the sorbitol content was high in the OC puree and PJ smoothies. Glucose content varied from 0.16 to 5.07 g/100 mL, with the highest levels found in the SCJ–OC (5.07 g/100 mL), SCJ–WC (3.54 g/100 mL), and RJ–WC (3.18 g/100 mL) smoothies, while the lowest levels were observed in the PJ–PC (0.16 g/100 mL), RJ–PC (0.47 g/100 mL), and AJ–PC (0.68 g/100 mL) smoothies. Consequently, the WC puree and SCJ samples had higher glucose content. The highest sucrose content was observed in the AJ–PC (2.22 g/100 mL), PJ–PC (1.87 g/100 mL), and AJ–OC (1.65 g/100 mL) smoothies, while it was not detected in the SCJ–WC smoothie, and the lowest sucrose content was observed in the SCJ–YC (0.02 g/100 mL) and SCJ–OC (0.04 g/100 mL) smoothies. Hence, the PC puree and AJ samples were rich in sucrose. In summary, the AJ–OC (9.47 g/100 mL), AJ–WC (9.22 g/100 mL), and SCJ–OC (8.35 g/100 mL) smoothies and lowest in the RJ–PC (1.97 g/100 mL), SJ–YC (2.16 g/100 mL), and RJ–YC (2.22 g/100 mL) smoothies had the highest total sugar content.

In the present study, various organic acids were identified in the carrot-blended smoothies, including oxalic acid, isocitric acid, citric acid, maleic acid, tartaric acid, malic acid, malonic acid, quinic acid, succinic acid, shikimic acid, and fumaric acid. Oxalic acid and fumaric acid were present in all the smoothies, while the other organic acids were present in varying amounts in each sample. The WC100% (0.85 g/100 mL), RJ–WC smoothie (0.64 g/100 mL), YC100% (0.64 g/100 mL), and AJ–WC smoothie (0.56 g/100 mL) had the highest oxalic acid content, with WC samples being particularly rich in oxalic acid. The PC100% (0.004g/100 mL) and RJ–PC (0.003 g/100 mL) smoothies had the highest fumaric acid content, with PC samples being rich in fumaric acid. The RJ–PC smoothie was the only sample that showed the presence of all the organic acids studied. In terms of pH values, the control groups had the highest pH values, with YC > OC > PC > WC. Among the other samples, the PJ–PC (4.77), PJ–OC (4.70), and PJ–YC (4.69) smoothies had the highest pH values, while the RJ–WC (3.62) and RJ–OC (3.67) smoothies had the lowest pH values. These findings indicated that carrot purees have high pH values, but RJ exhibits an acidic pH. The acidic conditions in RJ help prevent microbial activities and provide beverage stability.

In the presented research, the pectin content of the carrot-blended smoothies was determined (Table 1, **Publication 4**). Pectin is considered a bioactive compound that plays a role as an immunomodulator in allergies and provides protection against cardiovascular diseases. It is also a soluble dietary fiber that contributes to increased gastrointestinal activities and decreased serum cholesterol levels. In the study, the highest pectin content was observed in the following order: PC100% > RJ–YC (1.10%) > WC100% > SCJ–PC (0.96%) > SCJ–OC (0.94%). On the other hand, the AJ–OC (0.26%) and SJ–OC (0.27%) smoothies had the lowest pectin content. These findings indicate that the SCJ–PC smoothie had a higher pectin content compared to the SCJ–OC smoothie. Therefore, purple carrot-included smoothies exhibited high pectin content, which can contribute to their potential health benefits.

In the presented study, the mineral contents of the carrot-blended smoothies Table 2 (**Publication 4**) were analyzed, and significant differences ($p \leq 0.05$) were observed. Minerals are essential for maintaining homeostasis in the body, and their deficiencies can lead to various diseases. The minerals Na, K, Ca, Fe, and Mg were identified in the carrot-based smoothies.

The highest Na content was observed in all YC puree samples. The highest K content was observed in the following order: SCJ–YC > RJ–YC > SCJ–PC. Regarding Ca content, the highest levels were found in the following order: RJ–YC > SCJ–PC > AJ–PC. The highest Fe content was observed in the following order: RJ–YC > AJ–PC > SCJ–PC > RJ–PC. Lastly, the highest Mg content was observed in the following order: SCJ–PC > RJ–YC > AJ–PC.

Smoothie production allows mixing products from different types of raw materials with each other, which not only shapes the sensory effect but also allows fortifying the basic ingredient (base) with other ingredients, which can freely shape the physicochemical and functional characteristics of the final product. In the case of this study, fruit juice blending increases the bioactive compounds of carrot-based smoothies because raw carrot materials do not contain flavonols and flavan-3-ols; however, due to the addition of fruit juices, the carrot-blended smoothies were rich in flavan-3-ols, phenolic acids, flavonols, anthocyanins, and PPs. The phenolic content of the carrot-based smoothies is presented qualitatively in Table 3 (**Publication 4**), while the quantitative analysis can be found in Supplementary S1 (**Publication 4**), revealing significant differences ($p \leq 0.05$).

Flavan-3-ols possess beneficial properties against oxidation, carcinogens, microbes, and neurological diseases. In the present study, we identified procyanidin B2 ([M–H]− at m/z = 577), procyanidin B4 ([M–H]− at m/z = 577), and epicatechin ([M–H]− at m/z = 289) as the flavan-3-ols present, and we quantified their levels in carrot-blended smoothies. Procyanidin B2 was identified in only RJ, AJ, SJ, and SCJ blended with the PC puree, and procyanidin B4 in RJ, PJ, and SJ blended with carrot. Therefore, flavan-3-ols were not observed in raw carrot materials.; However, through processing and the addition of different fruit juices, the flavan-3-ol content in the smoothies increased significantly, ranging from 12.14 to 127.93 mg/100 mL. The smoothies with the highest total flavan-3-ol content were SCJ–OC (127.93 mg/100 mL), SCJ–YC (113.22 mg/100 mL), and RJ–PC (84.08 mg/100 mL). Conversely, the smoothies with the lowest total flavan-3-ol content were PJ–OC (12.14 mg/100 mL), PJ–WC (19.89 mg/100 mL), and PJ–YC (25.07 mg/100 mL). Therefore, while sour cherry and raspberry juices increased the flavan-3-ol content in carrot-based smoothies, pear juice contributed to a lower level of flavan-3-ols in the blends.

In the original publication (**Publication 1**), 14 phenolic acids were identified and quantified in raw carrot materials. However, in the present study, we observed thirteen different phenolic acids in carrot-blended smoothies. Among all samples, 5-o-caffeoylquinic acid ([M–H]− at m/z = 353), 4-o-feruloylquinic acid ([M–H]− at m/z = 367), and di-ferulic acid derivatives ([M–H]− at m/z = 527) were consistently identified. The smoothies that contained SCJ followed by PJ exhibited the highest phenolic acid content. Additionally, we discovered two newly identified phenolic acids in the smoothies: cis-5-p-coumaroylquinic acid ([M–H]− at m/z = 337) and p-coumaric acid ([M–H]− at m/z

= 325). These two compounds were exclusively found in the SCJ–PC smoothie. Notably, smoothies containing PC demonstrated the highest number of distinct phenolic acids and the highest total phenolic acid content. On the other hand, the smoothies RJ–WC < RJ–YC < SJ–WC exhibited the lowest phenolic acid content. Therefore, PC puree and SCJ samples displayed the highest phenolic acid content, while WC puree and RJ samples exhibited the lowest phenolic acid content.

Flavonols have been found to enhance blood flow to the brain and heart, lower blood pressure, and protect cells from damage (Martin and Ramos, 2021). In our current study, we identified and quantified only two flavonols in the carrot-blended smoothies: quercetin-3-galactoside ([M–H]− at *m*/*z* = 609) and genistin ([M–H]− at *m*/*z* = 269). Moreover, genistin resulted from SCJ, and quercetin-3-galactoside resulted from RJ, SJ, and SCJ. The highest total flavonol content was observed in the following order: SCJ–WC > SCJ–YC > SCJ–OC. Hence, sour cherry juice is rich in flavonol content and antioxidant features. The planned study is to find the best ingredients for carrot-based smoothies to suggest for novel food production. Moreover, the high flavonol content of sour cherry juice made it a stunning ingredient for carrot blended smoothies.

In the present study, seven anthocyanins were quantified; however, from raw PC, only five different anthocyanins were quantified (**Publication 1**). In the present study, RJ, SJ, and SCJ increased the anthocyanin content of carrot-blended smoothies. Specifically, cyanidin-3-o-xylosyl-galactoside ([M–H]− at *m*/*z* = 581) and cyanidin-3-o-glucosyl-rutinoside were identified in smoothies containing RJ and SCJ. Cyanidin-3-arabinoside was exclusively observed in RJ-blended smoothies. SJ contributed to the presence of cyanidin-3-o-xylosyl-p-coumaroylglucosyl-galactoside, while SCJ resulted in cyanidin-3-o-xylosyl-cinpoyl-glucosylgalactoside. Among the different combinations, the smoothies with SCJ combined with PC, OC, and WC exhibited the highest total anthocyanin content observed in the following order: SCJ–PC > SCJ–OC > SCJ–WC. It is worth noting that similar to the high flavonol content, sour cherry juice displayed the highest anthocyanin content compared to raspberry, apple, pear, and strawberry juices.

PPs are known for their anticancer, anti-inflammatory, antioxidant, and antiallergenic properties (Dasiman et al., 2021). In the present study, the procyanidin content in the smoothies ranged from 4.26 to 25.56 mg/100 mL. The highest PP content was observed in the following order: SCJ–PC > SCJ–YC > SCJ–WC, whereas the lowest polymeric content was observed as follows: PJ–PC < PJ–WC < PJ–YC. Similar to flavan-3-ol content, sour cherry juice is abundant with the PPs and pear juice had a low amount of PPs. Therefore, sour cherry juice including carrot-based smoothies is rich in PPs and shows positive results for biological activities as well.

Carrots are widely recognized for their popularity and high carotenoid content. Carotenoids are bioactive compounds that serve as precursors to vitamin A and possess various beneficial properties, including anticancer, antidiabetic, antibacterial, and neuroprotective effects (Nabi et al., 2020). In this study, the carotenoid content of the carrot-based smoothies was analyzed and presented in Table 3 (**Publication 4**) and Supplementary S1 (**Publication 4**), revealing significant differences ($p \leq 0.05$) among the samples. However, it is worth noting that the smoothie manufacturing processes led to a reduction in both the types and quantities of carotenoids present. This decline can be attributed to factors such as heating, and exposure to air, light, and water, which can affect the stability and retention of carotenoids (Zakynthinos and Varzakas, 2016). In our analysis, we identified four carotenoids: α-cryptoxanthin (zeinoxanthin), β-carotene, pheophytin a, and lutein.

To explore the potential health benefits of the developed formulations, *in vitro* assessments were conducted in this study to evaluate their antioxidant, antiaging, and antidiabetic properties. The antioxidant activities of the carrot-blended smoothies were determined using ABTS, FRAP, and ORAC assays, and the results are presented in Table 4 (**Publication 4**) with significant differences ($p \leq 0.05$).

Bioactive compounds present in fruit-based and vegetable-based food products contribute to their antioxidant properties (Jideani et al., 2021). In the present study, the ABTS antioxidant activity ranged from 0.42 to 1.78 mmol Trolox/100 mL. The highest activity was observed in PC puree samples in the following order: RJ > SJ > SCJ. However, the lowest ABTS activity was observed in PJ samples. These findings indicate that dark-colored fruits and vegetables enhance the antioxidant potential of food and beverages. Similar trends were observed for FRAP and ABTS activities.

Furthermore, the ORAC activity ranged from 0.03 to 0.42 mmol Trolox/100 mL. The smoothies with RJ–PC > SCJ–OC > SCJ–PC combinations exhibited the lowest ORAC activity, while the lowest activity was observed in PJ smoothies. Therefore, the inclusion of PC, RJ, and SCJ contributed to the overall antioxidant characteristics of the smoothies. These samples were rich in flavan-3-ols, phenolic acids, flavonols, anthocyanins, and procyanidins, which likely contributed to their enhanced antioxidant potential.

It is worth highlighting that in this study, the quality rather than the quantity of bioactive compounds had a greater influence on the antioxidant potential of the final products. Similar observations have been made by other researchers (Wojdyło et al., 2018; Tkacz et al., 2021), who reported the high antioxidant activity of polymerized compounds and anthocyanins, which aligns with our findings. The potential of PPs has long been recognized, and our research further confirms their role in shaping the health-promoting properties of food products. Importantly, fortifying carrot puree with fruit juices, which serve as sources of secondary plant metabolites, allows for the development of final products with enhanced health benefits.

The results of the in vitro biological activities of the carrot-blended smoothies are presented in Table 4 (**Publication 4**), with significant differences ($p \leq 0.05$) observed for α-amylase [IC_{50}], α-glucosidase [IC_{50}], lipase [IC_{50}], AChE [% inhibition], and BuChE [% inhibition].

Previous studies have reported that the inhibition of α-amylase and α-glucosidase enzymes can help in managing diabetes (Tundis et al., 2010). In the present study, the smoothies with SJ–PC, SCJ–YC, and AJ–PC combinations exhibited the highest α-amylase inhibition activity was observed in the following order: SJ–PC > SCJ–YC > AJ–PC. However, the lowest α-amylase inhibition activity was observed as follows: PJ–WC < SCJ–WC < AJ–OC < SJ–YC. Thus, smoothies containing PC demonstrated the highest α-amylase inhibition activity. Additionally, the highest α-glucosidase inhibition activity was observed in the following order: RJ–OC > SJ–WC > SCJ–YC, whereas the lowest α-glucosidase inhibition activity was observed as follows: AJ–PC < PJ–YC < PJ–PC. Therefore, SJ, SCJ, and AJ) exhibited the highest α-amylase inhibition activity, while those with RJ, SJ, and SCJ showed the highest α-glucosidase inhibition activity. The PC puree showed the lowest α-glucosidase inhibition activity, and the YC puree showed the lowest α-glucosidase and α-amylase inhibition activities. These findings suggest that the α-amylase inhibition activity may be influenced by the content of anthocyanins and pectins, while the α-glucosidase inhibition activity is associated with the interaction of PPs, flavonols, and organic acids.

The inhibition of pancreatic lipase has been associated with a potential decrease in obesity (Lunagariya et al., 2014). In this study, the highest lipase inhibition activity was observed in the following order: RJ–PC > SCJ–PC > RJ–WC, whereas the lowest lipase inhibition activity was observed as follows: PJ–OC < PJ–YC < PJ–WC. These results indicate that the RJ–PC smoothie had the highest lipase inhibition activity. Similar to the α-amylase inhibition activity, it is likely that anthocyanins and pectins are responsible for the lipase inhibition activities observed.

The inhibition of AChE and BuChE has been associated with potential reductions in nervous system disorders related to aging (Patočka et al., 2004). In the present study, the smoothies with RJ and SCJ combinations exhibited the highest AChE inhibition activity. Furthermore, the smoothie with RJ–PC combination showed the highest BuChE inhibition activity, similar to the lipase inhibition activity. Moreover, the lipase, AChE, and BuChE inhibition activities were consistent with the

antioxidant activity results, identifying the RJ–PC smoothie as the most promising product. These findings suggest that the increased anthocyanin content in the carrot-blended smoothies contributed to the inhibition of these enzymes and antioxidant activities. Furthermore, the RJ–PC smoothie showed the highest PP content, and these bioactive compounds might also contribute to the observed activities.

Finally, Fig. 4 (**Publication 4**) and Table 5 (**Publication 4**) showcase the sensorial attributes of carrot-blended smoothies. These include the distinct carrot taste and aroma, visual appeal, sweetness levels, and the predominant flavors of raspberry, apple, pear, strawberry, or sour cherry. The smoothies' overall acceptance was also evaluated by the panelists.

The panelists assessed various aspects of the smoothies, such as their appearance, sweetness, perception of the carrot taste, identification of the dominant flavor (raspberry, apple, pear, strawberry, or sour cherry), detection of the carrot scent, and their desire for the smoothies.

The appearance of food plays a crucial role in consumer decision-making, as it is determined by factors such as surface color, shape, and size, influencing whether it is chosen or rejected (Hutchings, 1977). In the present study, the smoothies with the highest acceptance for their appearance were PC and WC variants, while the following ranked lowest in terms of appearance: SJ–YC < SJ–WC < PJ–PC = SCJ–OC.

Carrot varieties are rich in nutritional content; however, the flavor and aroma of carrots are often not favored, particularly among children. Consequently, the evaluation of carrot-blended smoothies focused on both the taste and smell of carrots. The findings revealed that the strongest carrot taste was observed in the following order: PJ–PC > AJ–PC > PJ–OC, while the weakest carrot taste was found in RJ–WC < SCJ–OC < SCJ–PC. This suggests that PJ is unable to mask the carrot taste effectively, whereas SCJ can easily suppress it. On the other hand, the smoothie based on PC exhibited the most pronounced carrot aroma, while the weakest carrot aroma was detected in SCJ–YC < SJ–YC = SJ–PC. Consequently, PC puree samples showcased the most distinct carrot aroma, although the sweet smell of strawberry changed the overall aroma of the carrot-blended smoothies.

This study demonstrates that by incorporating different fruit juices, the taste, and smell of carrots can be suppressed to appeal to individuals of all age groups. Consequently, the prepared carrot-blended smoothies were subjected to evaluation regarding their perceived taste, including raspberry, apple, pear, strawberry, or sour cherry flavors. As anticipated, the RJ, AJ, PJ, SJ, and SCJ smoothies garnered the highest scores for taste, but some notable exceptions were observed. For example, the PJ–YC smoothie was deemed to have an apple taste, while AJ smoothies were noted for their pear flavor. In conclusion, many carrot-blended smoothies were evaluated as lacking the distinct flavor and/or aroma of carrots.

Lastly, the acceptance of carrot-based smoothies was assessed. Among the evaluated options, AJ–WC received the highest level of acceptance, while SJ–YC obtained the lowest level of acceptance.

Among the tested smoothies, the raspberry juice–purple carrot combination exhibited the highest antioxidant activity across all assays and demonstrated inhibition effects against lipase and BuChE enzymes. These attributes are not only important from a nutrition perspective but also provide opportunities for beverage processing.

The sour cherry juice–purple carrot smoothie demonstrated the most favorable outcomes in terms of total soluble solids, dry mass, osmolality, as well as total phenolic acid, anthocyanin, and procyanidin contents. On the other hand, the apple juice–purple carrot smoothie exhibited the highest carotenoid content. Interestingly, despite its relatively modest nutritional content and biological activities, the apple juice–white carrot smoothie received the highest acceptance rating from consumers.

The smoothies crafted from purple carrots, raspberry, and sour cherry juice boast abundant bioactive compounds with notable biological activities. These fruits and vegetables can be effectively utilized in the development of functional and novel food products aimed at enhancing their nutritional properties.

4.4. Evaluation of dried carrot product analyses

In the fourth part of the study, OD–CD–VMD technologies applied carrot snacks which were prepared using four colored carrot varieties (purple, orange, yellow, and white carrots), three fruit concentrates (apple, chokeberry, and sour cherry), and sucrose solution (control group) were analyzed.

The study aimed to prepare dried carrot snacks, using combined processes of OD–CD–VMD in which fruit juices were used to increase the bioactive compounds content of colored carrot varieties and enhance their prohealth properties. The water activities, color characteristics, L-ascorbic acid, polyphenolic, and carotenoid contents of the dried carrot materials were analyzed. In addition, antioxidant activities, α-amylase, α-glucosidase, lipase, AChE, and BuChE inhibition activities, and sensorial acceptance of the products were evaluated. The results are presented in **Publication 5.**

Yusuf E.H., Wojdyło A., Krzysztof L., Masztalerz K., Nowicka, P. 2023. The effect of combined drying process (OD-CD-VMD) on nutritional, phytochemical, and sensory profiles, and biological activities of colored dried carrot. *LWT*, 173, 114231.

In the present study, the changes in moisture content of the dried carrot samples before and after OD are presented in Table 2 (**Publication 5**). Among the fresh carrot materials, the white carrot exhibited the highest moisture content (91.89 g/100 mL), followed by the orange carrot (91.03 g/100 mL), yellow carrot (90.72 g/100 mL), and purple carrot (89.11 g/100 mL) samples. The dry weights of all the raw carrot materials in the different solutions were statistically similar and exceeded 92.00/100 g.

During the OD process of the carrot samples in sucrose, sour cherry, apple, and chokeberry solutions, noticeable reductions in moisture content were observed, ranging from 67.31 to 73.49 g/100 mL after OD. The carrot samples dehydrated in the sucrose solution and subjected to WCSCS, WCCS, and YCAS exhibited the highest moisture contents, measuring 69.82, 69.57, and 69.14 g/100 mL, respectively. Conversely, the PCAS, WCAS, and PCSCS samples demonstrated the lowest moisture contents at 67.31/100, 68.00/100, and 68.18 g/100 mL, respectively.

Table 2 (**Publication 5**) presents findings regarding solid gains and water losses of the carrot samples during the OD process. The solid gain followed the order: YCAS > YCSCS = PCSCS. On the other hand, the control groups OCSS and WCSS demonstrated the lowest solid gains.

Water losses were observed to be significantly higher than solid gains due to the permeability of cell membranes, which allows the transfer of small molecules like water but prevents the absorption of larger molecules (Lagnika et al., 2018). In this study, the highest water losses were noted in the WCSCS samples, followed by the OCSCS samples. However, it is important to note that the WCSCS samples, which exhibited the third-lowest solid gain, preceded the OCSS and WCSS samples, respectively.

The water losses of the samples followed the order: PCAS < PCSCS < PCCS. Consequently, the PCSCS samples, which had the second-highest solid gain and the second-lowest water loss, displayed this pattern. These outcomes can be attributed to the concentration gradient, which facilitated the transfer of solids from the solutions to the carrot samples while allowing water to move from the carrot samples to the solutions. However, excessive solid gains, which are undesirable in OD, can have an adverse effect on product quality (Maleki et al., 2020).

The drying process consists of three distinct stages: heating, constant, and falling periods (Ihns et al., 2011). During the heating period, the product's temperature gradually rises to match that of the dry air. The constant period, which is not always present in all drying processes, is relatively

short. In this phase, the thermal energy from the heated air causes free moisture to evaporate from the food products, leaving the product's surface wet with liquid. In the falling period, the moisture content decreases as it migrates out of the food products (Ek et al., 2018).

In the present study, the drying process was illustrated through drying curves, depicting the three steps of the process, for the colored carrot samples that underwent osmotic dehydration in various solutions using CD and VMD. Figure 1 (**Publication 5)** showcases these drying curves. It is worth noting that the application of microwave drying in conjunction with CD can significantly reduce the drying periods of food products (Szadzińska et al., 2019).

During the CD and VMD processes, the MR of the carrot materials (white, orange, purple, and yellow) varied depending on the solution used (Fig. 1, **Publication 5**). White carrot showed the lowest moisture content when combined with each solution (sucrose, sour cherry, apple, and chokeberry solutions), whereas purple carrot showed the highest moisture content. Yellow and orange carrots demonstrated minimal changes when exposed to different solutions. Within each solution, white carrot dried in a shorter time compared to the other varieties due to its higher water loss. Purple carrot samples displayed the shortest drying periods in sour cherry, apple, and chokeberry solutions, except the sucrose solution. In the case of the sucrose solution, the yellow carrot exhibited the shortest drying period. Among the solutions, the apple solution required the longest drying time for each carrot sample.

In processed foods, water activity (a_w) plays a crucial role in preventing microbial activity and maintaining stability (Su et al., 2018). In the present study, the a_w values of the dried carrot snacks are presented in Table 3 (**Publication 5**). These values were found to be significantly different ($p \leq 0.05$) and ranged from 0.24 to 0.34. The control groups exhibited the highest a_w values, followed by the PCSCS and OCSCS samples. On the other hand, the OCCS samples displayed the lowest aw value, followed by the PCCS and YCCS samples. These results indicate that chokeberry juice served as the most effective medium in controlling water activity.

Furthermore, all the carrot snacks exhibited aw values below 0.6, indicating the inhibition of microbial growth. It is worth noting that aw values below 0.4 have been reported to decrease the activities of Maillard reactions, lipid oxidation, hydrolysis, and enzymatic reactions (Labuza et al., 1970).

The color of food products plays a significant role in consumers' decisions to purchase and consume them. The color values of the dried carrot snacks are presented in Table 3 (**Publication 5**) and exhibit significant differences ($p \leq 0.05$). Among the samples showed the second highest L* values (60.31, preceded by the control groups), followed by the YCAS (57.84) and the OCAS (51.12) samples, which showed the lightest colors. However, the WCCS samples showed the darkest color (19.51), followed by the PCCS (20.01) and the PCSCS (21.22) samples. As reported in a previous study, high anthocyanin contents increase the darkness in food products (Liu et al., 2014). Thus, L* values are related to the chemical content of food products, and the dark colors of the chokeberry carrot samples indicate that these dried food products have high polyphenolic contents, which are essential for healthy body functions.

In terms of a* values (redness), the PCAS followed by the OCAS and PCSCS samples. Conversely, the WCSS samples displayed the highest values for b* (greenness), followed by the YCSS and WCAS samples. Consequently, the WCAS samples appeared light-green in color, while the OCAS samples had a light-red hue. The YCAS samples demonstrated the highest values for b* (yellowness), followed by the OCAS, OCSS, and YCSS samples. Additionally, the PCSS samples exhibited the highest values for b* (blueness), followed by the WCCS and PCCS samples, resulting in a dark-blue coloration.

In conclusion, the L* values of the dried carrot samples increased during the drying process due to heating and reduced moisture content of the carrot slices (Wu et al., 2014). Moreover, it is

important to note that the color of food products can be influenced by various factors such as light, temperature, pH, oxidation, and metal ions (Szadzińska et al., 2017).

The OD process increases the bioactive profile of dried carrot products because raw carrot materials include only phenolic acid and anthocyanins (**Publication 1**), but with the fruit solutions dried carrots contain flavan-3-ols and flavonols as well. The phenolic compounds of the dried carrot samples are presented in Table 4 (**Publication 5**) with significant differences ($p \leq 0.05$). All samples were rich in phenolic acids, anthocyanins, flavan-3-ols, flavonols, and PP. A total of nine phenolic acids, six anthocyanins, nine flavan-3-ols, and three flavonols were identified and quantified from all 16 dried carrot snacks with different amounts. This study is the first to identify and quantify these polyphenolic contents in dried carrot snacks prepared from four different carrot varieties (white, yellow, orange, and purple) and three fruit juices (sour cherry, chokeberry, and apple juices). Raw carrot varieties are rich in phenolic acids and anthocyanins (**Publication 1**). However, dried carrot food products are also rich in flavan-3-ols and flavonols due to OD.

In comparison with the control groups, the highest phenolic acid content was observed in snacks dehydrated using chokeberry solution. Similar results are observed in the literature as well (Nowicka et al., 2015b). The highest anthocyanin content was observed in the PCSS-control group (86.52 mg/100 g p), followed by the PCCS samples (64.23 mg/100 g p). It is worth noting that in a previous study (**Publication 1**), only five anthocyanins were quantified from fresh purple carrot samples. However, in the present study, six anthocyanins were identified and quantified in the dried carrot snacks due to the incorporation of sour cherry and chokeberry solutions. These solutions enriched the dried carrot snacks with cyanidin-3-glucoside, pelargonidin-3-rutinoside, cyanidin-3-sambubioside, and pelargonidin-3-glucosides.

Although the dried purple carrot products showed higher anthocyanin contents compared to the control groups, it is important to note that the total anthocyanin contents in the dried carrot snacks were lower than in the raw materials. This suggests that anthocyanins may have undergone degradation due to factors such as temperature, heat, and enzymatic reactions during the drying processes (McSweeney and Seetharaman, 2015).

Flavan-3-ols have been recognized for their role in protecting against cardio-metabolic risks (Raman et al., 2019). The amount of flavan-3-ols in food products can be affected by various factors such as food processing, storage, and the environment (Aron and Kennedy, 2008). In this study, flavan-3-ols were incorporated into dried carrot snacks using the OD process and applied solutions, as these compounds were not naturally present in the raw carrot varieties (**Publication 1**). The flavan-3-ols identified and quantified in the dried carrot food products included arbutin, (+)-catechin, procyanidin B4, LC, resveratrol, procyanidin B2, (-)-epigallocatechin-gallate, (-)-epigallate, and procyanidin A2. These flavan-3-ols existed in the dried carrot snacks as monomers and dimers. The WCSCS samples exhibited the highest flavan-3-ol content (293.84 mg/100 g p), followed by the OCSCS (271.28 mg/100 g p) and YCSCS samples (243.30 mg/100 g p).

However, it is important to note that PPs differ from tannins and consist of flavan-3-ol formations. PPs have been found to exhibit stronger antioxidant activities compared to vitamins C and E, making them useful in combating pneumonitis, lung cancer, and neurodegenerative diseases (Hou and Wang, 2022). In the raw carrot materials, the orange and white carrot samples displayed the highest PP content (**Publication 1**); however, after the drying processes, adding fruit juice solutions changed the PP contents of dried carrot snacks. The highest PP contents were observed in the sour cherry solution included samples and in terms of varieties of carrot–purple carrot had the highest content of PP and followed by yellow and white carrots included in dried carrot samples. Furthermore, polymerization degrees (DPs) create alterations in PPs. As reported in a previous study, the number of hydroxyl groups and molecular weights of PPs increase with high DPs. In the present

study, the highest DPs were observed in the OCAS samples (13.77), followed by the YCSCS (3.76) and PCSCS samples (3.19).

Flavonols have been shown to possess antiobesity, neuroprotective, cardioprotective, and antioxidant activities (Liu et al., 2021). In the present study, the OD process was found to increase the flavonol contents only in the samples dehydrated using sour cherry and chokeberry solutions. The identified and quantified flavonols in the dried carrot samples were myricetin-3-galactoside, quercetin-3-galactoside, and kaempferol-7-glucuronide. As mentioned earlier, raw carrot materials do not naturally contain flavonols, and during the OD process, all the flavonols were derived from the fruit juice solutions that were added to the dried carrots. Among the dried carrot snacks, the chokeberry juice samples exhibited the highest flavonol content. It is worth noting that according to the literature, the chokeberry solution experiences less degradation during the OD process (Turkiewicz et al., 2019).

Carotenoids, which are abundant in raw fruits and vegetables, exhibit antioxidant, antitumor, antidiabetic, antiaging, and anti-inflammatory activities (Crupi et al., 2023). However, it should be noted that the content of carotenoids tends to decrease with applied food processes. In the present study, the carotenoid contents of the dried carrot snacks were found to be significantly different ($p \leq 0.05$), and the specific values can be found in Table 4 (**Publication 5**). Among the carotenoids, only α-cryptoxanthin was observed in the dried carrot snacks due to the thermal food processing involved. Existing literature suggests that dehydration and heating processes result in the breakdown of carrot cells, leading to the loss of turgor pressure (Nieto et al., 2013), and carotenoids tend to degrade under the influence of heat and light exposure (Saini et al., 2015). The control groups, PCSS and OCSS, exhibited the highest α-cryptoxanthin contents, followed by the OCCS and PCCS samples. Additionally, the WCSS, YCSS, WCSCS, OCSCS, WCAS, and WCCS samples did not demonstrate any detectable presence of carotenoids. Therefore, it can be inferred that during the OD process, carotenoids from carrots may migrate into the solutions used.

Novel food products that exhibit free radical prevention activities have garnered significant interest. In this study, antioxidant activities were also assessed and are presented in Table 5 (**Publication 5**) for ABTS, FRAP, and ORAC assays. The dried carrot samples that included the chokeberry solution displayed the highest ABTS values, primarily attributed to the high TPC of the chokeberry solution. However, when excluding the control groups, the WCAS samples exhibited the lowest ABTS values, followed by the OCAS samples. It is noteworthy that in the current study, the combination of technologies employed led to a decrease in the overall antioxidant activities compared to raw carrot materials (**Publication 2**). Similar findings have been reported in the literature regarding dried pomegranate seeds (Bchir et al., 2012). Thus, the reduction in antioxidant activities in dried products can be attributed to the decline in PPs and vitamins, as well as the influence of increased temperature (Turkiewicz et al., 2020).

The IC_{50} values (mg/mL) for the α-amylase, α-glucosidase, and pancreatic lipase inhibition activities of the dried carrot snacks are presented in Table 6 (**Publication 5**). In this study, the α-amylase inhibition activities ranged from 259.68 to 62.69 mg/mL (for the PCAS and the YCAS samples, respectively), while the α-glucosidase inhibition activities ranged from 258.29 to 18.44 mg/mL (for the WCAS and the OCSCS samples, respectively). A comparison between raw carrot materials and dried carrot food products revealed both significant increases and decreases in the α-amylase and α-glucosidase inhibition activities, depending on the carrot varieties and the type of solution used. For example, the dried yellow carrot–apple juice samples exhibited superior α-amylase inhibition activity compared to raw yellow carrot, while the dried orange carrot-sour cherry juice samples demonstrated higher α-glucosidase inhibition activity than the raw orange carrot (**Publication 1**). Furthermore, the dried carrot samples that included sour cherry solution displayed the highest activities against α-glucosidase. Hence, the incorporation of fruit juices into dried carrot

snacks enhances their biological activities against α-amylase and α-glucosidase enzyme inhibition activities.

In the present study, pancreatic lipase activity was also assessed as it is responsible for the hydrolysis of triacylglycerol, and inhibiting this enzyme reduces the breakdown of lipids into fatty acids, potentially aiding in lipid management (Lunagariya et al., 2014). The pancreatic lipase activities of the dried carrot snacks ranged from 4.99 to −20.31 mg/mL (for the OCCS and the OCSCS samples, respectively). Interestingly, the sample that included orange carrot and sour cherry exhibited the highest lipase activity, while the samples containing orange carrot and chokeberry solution showed the lowest pancreatic lipase inhibition effect. In terms of raw materials, white carrot showed a lipase inhibition value of 12.25, while orange carrot had a value of 5.29 (IC_{50}, mg/mL) (**Publication 1**). Therefore, incorporating sour cherry solution, with its specific chemical properties, increases the lipase enzyme inhibition activities of the dried carrot snacks. This can be advantageous in combating childhood obesity while also enhancing the appealing color and taste characteristics of the products.

AChE and BuChE inhibition activities have been associated with a reduced risk of age-related diseases, including Alzheimer's disease (Colovic et al., 2013). In the present study, the AChE and BuChE inhibition activities were expressed as percentages of inhibition. The highest AChE inhibition activities were observed in the YCSCS and YCAS samples, with values of 30.55/100 and 23.58/100 g, respectively. Conversely, the lowest AChE inhibition activities were observed in the OCSCS and PCSCS samples, with values of 6.12/100 and 10.18/100 g, respectively. It is worth noting that the raw carrot materials exhibited the following order of AChE inhibition activities: orange > yellow > white > purple carrot samples (**Publication 1**). However, after undergoing the OD and drying processes, the yellow carrot–sour cherry and yellow carrot–apple juice samples exhibited the highest AChE inhibition effects, while the orange carrot-sour cherry samples displayed the lowest AChE inhibition. Notably, higher AChE inhibition values have been reported in the literature for Japanese quince, ranging from 60.20/100 to 32.54/100 g (Turkiewicz et al., 2020).

Moving on to BuChE inhibition, the activity of dried carrot snacks ranged from 14.84/100 to 55.77/100 g. The highest BuChE inhibition activities were observed in the YCSCS and YCCS samples, with values of 55.77/100 and 41.43/100 g, respectively. Conversely, the lowest BuChE inhibition activities were observed in the OCAS and OCCS samples, with values of 14.84/100 and 17.80/100 g, respectively. The order of BuChE inhibition activities in the raw carrot materials was as follows: purple > yellow > orange > white carrot (**Publication 1**). Similar to the AChE enzyme inhibition activity, the YCSCS samples displayed the highest BuChE inhibition activity.

The appeal of a processed food product relies on various factors such as its color, appearance, texture, scent, and flavor. The sensory evaluation results of the dried carrot snacks are depicted in Fig. 2 (**Publication 5**), displaying mean scores for each evaluation characteristic. The processes of OD, CD, and VMD contributed to an increase in the color acceptance of the carrot snacks. The color scores ranged from 7.11 to 4.11, with the OCSS samples receiving the highest score for color, followed by the PCAS and OCAS samples. On the other hand, the OCSCS, PCCS, YCCS, and PCSS samples received the lowest color acceptances. Regarding the smell scores of the carrot snacks, they ranged from 7.11 to 5.11. The highest smell acceptances were observed in the OCSCS, WCAS, and YCAS samples, while the WCCS, YCSCS, and YCSS samples had the lowest smell acceptances.

To assess the flavor of the food products, the panelists were asked to identify the fruits whose taste they detected in the snacks. The WCSS, OCSS, YCSS, PCSS, PCSCS, WCAS, and YCAS samples were recognized as having a carrot taste. Furthermore, the WCCS, OCCS, and PCCS samples were identified as having a chokeberry taste, while the OCAS samples were associated with an apple flavor. Surprisingly, the PCAS samples were perceived to have a sour cherry taste, and the WCSCS,

OCSCS, YCSCS, and YCCS samples were identified as having a black currant taste. It is worth noting that, interestingly, black currant solution was not used in the OD processes of the carrot snacks in the present study.

The taste scores of the dried carrot snacks ranged from 6.77 to 3.33. The samples were ranked in terms of taste as follows: OCSS > WCSS = WCAS > OCAS, with the lowest taste scores observed for the PCCS, OCCS, and YCCS samples, respectively. It is noteworthy that while the OCCS samples exhibited an increased L-ascorbic acid content, their low taste acceptance may not make them preferable to consumers. On the other hand, OCAS snacks, which have high L-ascorbic acid content and favorable color and taste acceptance, can be recommended for consumption.

Crispiness is an important characteristic to consider for dried food products, and the OD process has been found to slightly increase the hardness of such products (Lagnika et al., 2018). In the present study, the WCSCS, OCSCS, PCSCS, WCAS, and WCCS samples were rated as neither hard nor soft. However, the PCCS samples were perceived as very hard, while the OCCS, YCAS, YCSCS, PCSS, and OCSS samples were rated as hard. It is worth noting that high hardness is generally considered an unattractive feature of food products, as mentioned in a previous study (Zou et al., 2013).

The application of novel colored carrot snacks using OD in fruit juices has yielded impressive results, particularly with the utilization of CD and VDM technologies. The OD process has led to an increase in polyphenolic contents in the dried carrot samples; however, the thermal processes have resulted in a decrease in carotenoid contents. The polyphenolic contents varied among the dried carrot snacks, with the highest levels of phenolic acids, flavonols, and anthocyanins observed in samples dehydrated in chokeberry solution. Additionally, samples dehydrated in sour cherry solution exhibited high flavan-3-ol content, along with the highest PP contents. The dried carrot osmotic dehydrated in chokeberry solution displayed the highest antioxidant activities. Moreover, the dried carrot snacks dehydrated in sour cherry solutions showed the highest activities in terms of α-glucosidase, pancreatic lipase, AChE, and BuChE enzyme inhibition. Sensory evaluations revealed significant differences among the carrot snacks. For instance, the orange carrot–sour cherry samples obtained high scores in smell, while the white carrot–apple solution samples, despite having lower levels of bioactive compounds and biological activities, received high overall taste scores. These findings hold promise for future applications of carrot-based foods.

5. CONCLUSIONS

The results presented in this dissertation confirm the research hypothesis that colored carrot varieties are rich in bioactive compounds and possess health-promoting properties, making them suitable as novel food ingredients in various applications.

Based on the conducted study, the following conclusions can be drawn:

1. Among the analyzed colors of carrots (white, orange, yellow, and purple), the purple carrot samples exhibited the highest content of PPs and carotenoids, along with superior activities against cholinesterase. Among the purple carrot varieties, normal purple carrots displayed the highest health-promoting activities, followed by mini purple carrots. The different-sized purple carrot varieties, including normal, mini, and micro, can provide high contents of PPs to combat oxidative stress-related diseases. Purple carrots also demonstrated the highest levels of total sugar, organic acids, polyphenolic contents, and antioxidant activities (mini > normal > micro). In turn, the yellow carrots showed the lowest values for the content of polyphenols and antioxidant activities, while the white carrots demonstrated the lowest results for total phenolic acid, total carotenoid, and chlorophyll contents. Therefore, the conducted study had shown that purple carrots are the richest source of bioactive compounds (pectins, vitamin C, and PPs) and may increase the effective sensorial characteristics of carrot-based novel foods.

2. Normal purple carrot juice demonstrated the best activities for all biological and antioxidant tests, except for the α-glucosidase inhibition effect. However, normal yellow carrot juice displayed the highest AChE inhibition activity but had the lowest α-amylase and lipase inhibition effects. Normal purple carrot juice showed the best results in terms of total phenolic acid, anthocyanin, and carotenoid contents. From a sensory evaluation perspective, normal orange carrot juice received the highest acceptance ratings, while juices based on white carrots performed poorly. Therefore, purple carrot juices can be employed in the beverage industry to produce smoothies and blended juices, enhancing the health-promoting properties of liquid products, while orange varieties are favored for their taste.

3. The sour cherry juice–purple carrot smoothie showed the highest levels of total phenolic acid, anthocyanins, and procyanidin contents. The raspberry juice–purple carrot smoothie showed the highest antioxidant activities against lipase and BuChE inhibitions. These characteristics not only hold nutritional significance but also offer opportunities for beverage processing. Nevertheless, the apple juice–white carrot smoothie garnered the highest product acceptance, despite not exhibiting potent nutritional content and biological activities.

4. The combined methods of OD in fruit juices, convective drying (CD), and microwave vacuum drying (VMD) enabled the production of intriguing novel colored carrot snacks. The OD process increased the polyphenolic contents in the dried carrot samples. Samples dehydrated in sour cherry and chokeberry solutions showcased the highest phenolic acid, anthocyanin, flavan-3-ol, polymeric procyanidin, and flavonol contents. Conversely, orange carrot OD in apple solution exhibited the lowest total phenolic compounds. Sensory evaluations of each carrot snack indicated significant differences, with the purple carrot–apple juice samples receiving the highest color acceptance, the orange carrot–sour cherry samples

achieving the highest flavor score, and the white carrot–apple solution samples earning the highest overall taste score. Finally, it should be concluded that the proposed process is extremely interesting in the context of the possibility of designing a functional carrot snack.

In conclusion, normal-sized carrot varieties, including purple, yellow, and white, are suggested for novel food applications. Purple carrots, in different sizes as raw materials and juices, offer the highest health-promoting features. Normal yellow carrot juice showed the highest AChE inhibition activity, while the apple juice–white carrot smoothie garners high consumer acceptance. Combining carrot materials with fruit solutions enhances bioactive compound content, sensory characteristics, and overall health-promoting features of the final food products. Therefore, implementing such approaches in the fruit and vegetable industry can promote healthy eating and the utilization of nutritious food options for consumers.

6. LITERATURE

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PN-90/A-75101/03 Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie zawartości suchej masy metodą wagową.

PN-90/A-75101/04 Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie kwasowości ogólnej.

PN-90/A-75101/06 Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie pH metodą potencjometryczną.

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ANNEXES

Publications included in the doctoral dissertation entitled " **Novel Carrot Snacks with Desired Health Benefits**" together with statements.

1.1.*Publication 1:* **Yusuf E.,** Wojdyło A., Oszmiański J., Nowicka, P. 2021. Nutritional, phytochemical characteristics and in vitro effect on α-amylase, α-glucosidase, lipase, and cholinesterase activities of 12 coloured carrot varieties. Foods, 10(4), 808. https://doi.org/10.3390/foods10040808

1.2.*Publication 2:* **Yusuf E.,** Tkacz K., Turkiewicz I.P. Wojdyło A., Nowicka, P. 2021. Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC. European Food Research and Technology, 247, 3053–3062. https://doi.org/10.1007/s00217-021-03857-0

1.3.*Publication 3:* **Yusuf E.H.,** Wojdyło A., Nowicka, P. 2023. Possibility to use the different sizes and colors of carrots for the production of juices – comparison of bioactive compounds, nutritional quality, pro-health properties, and sensory evaluation. Journal of the Science of Food and Agriculture, 103(2), 933-943. https://doi.org/10.1002/jsfa.12206

1.4.*Publication 4:* **Yusuf E.H.,** Wojdyło A., Bourbon A.I., Nowicka, P. 2023. Fruit-carrot-based smoothies as innovative products with a complex matrix of bioactive compounds effected on activities of selected digestive enzymes and cholinesterases in vitro. Antioxidants, 12(4), 917. https://doi.org/10.3390/antiox12040917

1.5.*Publication 5:* **Yusuf E.H.,** Wojdyło A., Krzysztof L., Masztalerz K., Nowicka, P. 2023. The effect of combined drying process (OD-CD-VMD) on nutritional, phytochemical, and sensory profiles, and biological activities of colored dried carrot. LWT, 173, 114231. https://doi.org/10.1016/j.lwt.2022.114231

Publication 1

Yusuf E., Wojdyło A., Oszmiański J., Nowicka, P. 2021. Nutritional, phytochemical characteristics and in vitro effect on α-amylase, α-glucosidase, lipase, and cholinesterase activities of 12 coloured carrot varieties. Foods, 10(4), 808.

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Nutritional, Phytochemical Characteristics and In Vitro Effect on α-Amylase, α-Glucosidase, Lipase, and Cholinesterase Activities of 12 Coloured Carrot Varieties

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Abstract: Twelve carrot varieties with different colours (purple, orange, yellow, and white) and sizes (normal, mini, and micro) were analysed for prospective health benefits (activities against diabetes-, obesity-, and aging- related enzymes—α-amylase, α-glucosidase, lipase, acetylocholinesterase, and butyrylocholinesterase, respectively) and nutritional contents (polyphenols, carotenoids, and chlorophylls). The conducted studies showed that the highest content of total polyphenols was observed in different sizes of purple carrots. The normal yellow and mini orange carrots demonstrated the highest content of carotenoids. According to the study results, the mini purple carrot showed the highest activities against diabetes-related enzyme (α-glucosidase); furthermore, the highest activities of cholinesterase inhibitors were observed for micro purple carrot. Nevertheless, normal orange carrot exhibited the highest activity against lipase. The results of the present study showed that purple-coloured carrot samples of different sizes (normal, mini, and micro) exhibited attractive nutritional contents. However, their pro-health effects (anti-diabetic, anti-obesity, anti-aging) should not be seen in the inhibition of amylase, glucosidase, lipase, and cholinesterase. Probably the mechanisms of their action are more complex, and the possible health-promoting effect results from the synergy of many compounds, including fibre, phytochemicals, vitamins, and minerals. Therefore, it would be worth continuing research on different varieties of carrots.

Keywords: coloured carrots; phenolic acids; procyanidins; anthocyanins; carotenoids; enzyme inhibition effect

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1. Introduction

Daucus carota L. is an *Apiaceae* member and grows in Europe, Asia, Africa, and Macaronesia. The cultivated carrot has evolved from the crossing of *D. carota* ssp. *carota* and *D. carota* ssp. *maximus* [1]. Interestingly, the first cultivated carrots were purple and yellow, followed by white carrots, and currently, orange carrots are the most popular in the world. Carrot is one of the top 10 consumable vegetables around the world, with high market share and nutrient values. One hundred grams of carrot provides approximately 41 kcal energy, 0.93 g protein, 9.58 g carbohydrates, and 2.8 g fibre [2,3].

Cultivated carrots are grouped by root colour, sugar-carotenoid content, and root shape, which are affected by development period, temperature during this time, and fertilizers [4,5]. Another reason for colour change in carrots is carotenoids, which in young carrots begin to accumulate after their first month of growth and is maintained about until the secondary growth is concluded [6].

Carrot varieties range from 5 cm to 50 cm for their root lengths, which are an important parameter for the marketing of carrot cultivars. In addition, cultivated carrots are classified as eastern and western carrots. Eastern carrots are purple and yellow coloured with branched roots; western carrots are orange, red, and white with unbranched roots. In this

case, eastern carrot cultivars are rich in anthocyanins and western carrots are abundant in carotenes [7]. Nevertheless, purple carrot is twice as rich in α- and β-carotene contents as orange carrot; moreover, purple carrot possesses a sweet flavour with low total sugar content. These stunning features of purple carrot make it a good alternative to orange carrot. The colour of carrots results from the presence of pigmented compounds, for instance, carotenoids (α-carotene and β-carotene) in carrots produce orange colour, xanthophylls produce yellow colour, and anthocyanins produce purple colour; white carrots do not contain any colour pigments [8,9].

Carotenoids are highly valuable components of carrots. Moreover, their types alter with the chemical structure. For instance, carotenes such as α- and β-carotenes contain hydrocarbons, and xanthophylls such as β-cryptoxanthin, lutein, and zeaxanthin have an oxygen-containing functional group [10]. More importantly, carotenoids are vitamin A precursors, and vitamin A is crucial for eyesight and cell regulation [11]. Carotenoids also help to prevent cancer, bone-related diseases, cell oxidation, diabetes, obesity, and cardiovascular diseases [12,13].

The other compounds in carrot, especially in purple carrot, are anthocyanins, which are a subgroup of polyphenols and are well known for their free-radical scavenging activities [14]. Anthocyanins are produced against pathogens, UV radiation, pollination, and environmental stress during plant growth [15]. Additionally, polyphenolic compounds are useful for healthy human body functions, supporting protection against diabetes, cardiovascular diseases, osteoporosis, asthma, cancer, aging, and neuroprotection [16]. Anthocyanins are used as a food colourant to manufacture various food products such as canned strawberries, pasta, and rice muffins without egg or gluten [17,18].

In addition to colour pigments, carrot is a good source of vitamins B, C, E, and H; folic acid; pantothenic acid; and minerals such as K, Na, Ca, Mg, P, S, Mn, Fe, Cu, and Zn [19]. Moreover, carrot is rich in trace mineral molybdenum, which is crucial for the metabolism of carbohydrates and fats and iron absorption [20]. In addition, purple carrot contains 0 g fat, 31 g of carbohydrates with 1 g of fibre, and 1 g of protein; orange carrot includes 0.2 g of total fat, 6.9 g of carbohydrates with 2 g of fibre, and 0.7 g of protein.

Carrot contains simple sugars such as glucose, sucrose, and fructose and fibres such as cellulose and hemicelluloses. Moreover, carrot contains polyacetylenes, which might be able to destroy malignant cells such as leukaemia, myeloma, and lymphoma cells. Additionally, carrot contains luteolin that could protect against age-related symptoms in the brain, but the mechanisms are far from being elucidated [21,22].

Despite these interesting nutritional properties, many people do not consume carrot. Thus, to attract consumers, manufacturers prepare mixed carrot bags of yellow, purple, white, and orange carrots and call them "rainbow carrots"; moreover, mini (baby) carrots that are approximately up to 5 cm in size were created for consumption by young generations.

Therefore, the present study aimed to compare carrot varieties (different colours and sizes) in terms of bioactive contents and health-promoting properties. The present study also attempted to find the best carrot variety with high health benefits and elevated product quality for food processing. Thus far, such studies have not been conducted, especially in terms of determining polyphenolic and carotenoid contents and in vitro biological activities against enzymes related to diabetes (α-amylase and α-glucosidase), obesity (lipase), and age-related (acetylcholinesterase and butyrylcholinesterase) disorders.

2. Materials and Methods

2.1. Chemicals

Standards of carotenoids, chlorophylls, and polyphenolics were purchased from Extrasynthese (Lyon, France). Acetonitrile, methanol, and formic acid for analyses of ultra-performance liquid chromatography (UPLC; gradient grade) and ascorbic acid were purchased from Merck (Darmstadt, Germany). Dipotassium hydrogen, orthophosphate dihydrogen, sodium phosphate monobasic, starch from potato, α-amylase from porcine

pancreas (type VI-8; the European Community number (EC number) 3.2.1.1.; *p*-nitrophenyl-α-D-glucopyranoside, α-glucosidase from *Saccharomyces cerevisiae* (type I, EC number 3.2.1.20), lipase from porcine pancreas type II (EC number 3.1.1.3), *p*-nitrophenyl acetate, acetylcholinesterase from *Electrophorus electricus* (electric eel) (type VI-S; EC number 3.1.1.7), butyrylcholinesterase from equine serum (EC number 3.1.1.8), acetylthiocholine iodide, S-butyrylthiocholine chloride, and DTNB (5,5-dinitrobis-(2-nitrobenzoic acid)) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Plant Material and Sample Preparation

Normal and mini-sized carrots were purchased from Fusion Gusto (Dąbrowa, Poland). Micro carrots were purchased from Cato Produce (Johannesburg, South Africa) in June 2020.

Carrot samples were grouped according to their sizes and colours. "Normal size" carrots in diameter (d) were between 20 mm and 45 mm and they weighed (m) from 50 g to 150 g; "mini size" carrots—20 mm > d > 10 mm and 50 g > m > 8 g; "micro size" d < 10 mm and m < 8 g.

Therefore, the following varieties of carrot were investigated: yellow carrot (micro (MYC), mini (MiYC), and normal (NYC), purple carrot (micro (MPC), mini (MiPC), and normal (NPC), orange carrot (micro (MOC), mini (MiOC), and normal (NOC), and white carrot (micro (MWC), mini (MiWC), and normal (NWC) (Figure 1). The carrot roots were washed, dried, cut into slices, and then frozen at −80 °C. The sliced carrots were then freeze-dried (24 h; Christ Alpha 1–4 LSC, Melsungen, Germany) and crushed by a laboratory mill (IKA A 11, Staufen, Germany) to obtain the homogeneous dry material for analysis.

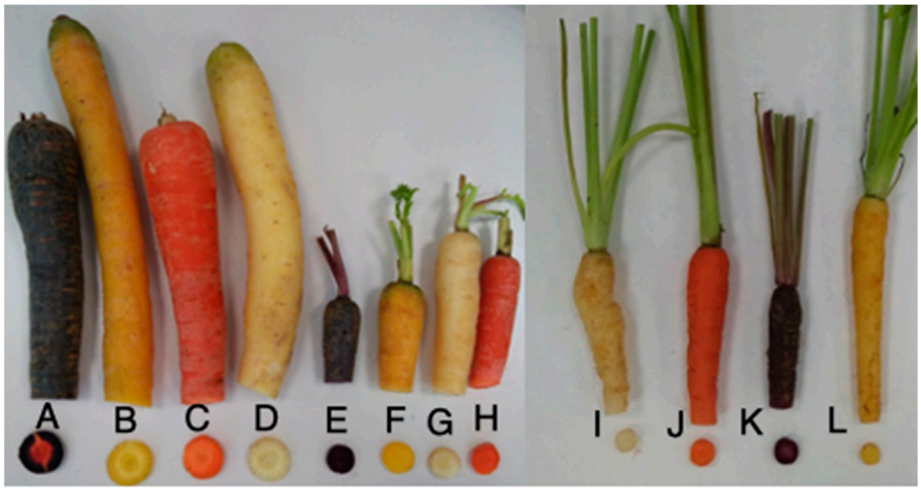

Figure 1. Types of carrots and varieties (A—normal purple carrot (NPC), B—normal yellow carrot (NYC), C—normal orange carrot (NOC), D—normal white carrot (NWC), E—mini purple carrot (MiPC), F—mini yellow carrot (MiYC), G—mini white carrot (MiWC), H—mini orange carrot (MiOC), I—micro white carrot (MWC), J—micro orange carrot (MOC), K—micro purple carrot (MPC), L—micro yellow carrot (MYC)).

2.3. Identification and Quantification of Polyphenols

The powder of roots (~1 g) was mixed with 9 mL of the mixture containing HPLC-grade methanol: H_2O (30:70%, *v*/*v*), ascorbic acid (2%), and acetic acid (1%) of the reagent. The extraction was performed twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with periodic shaking [23]. Following, the slurry was centrifuged at 19,000× *g* for 10 min. The supernatant was filtered through a hydrophilic PTFE (polytetrafluoroethylene) 0.20 µm membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for analysis. The extraction was performed in triplicate. Qualitative (liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-MS-Q/TOF)) and quantitative (ultra performance liquid chromatography with photodiode array detec-

tor (UPLC-PDA)) analyses of polyphenols (phenolic acids at 320 nm, and anthocyanins at 520 nm) were performed according to Wojdyło et al. [24]. Polyphenols were separated by ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 × 100 mm, Waters Corporation, Milford, USA) at 30 °C. The injection and elution of the samples (5 µL) were concluded in 15 min with a sequence of linear gradients and a flow rate of 0.42 mL/min. The solvent A (2.0% formic acid, *v*/*v*) and solvent B (100% acetonitrile) comprised the mobile phase. The procedure operated through gradient elution with 99–65% solvent A (0–12 min), solvent A was later lowered to 0% for condition column (12.5–13.5 min), and the gradient returned to the initial composition (99% A) for 15 min to re-equilibrate the column. In addition, in different varieties of carrot, the content of polymeric procyanidins was determined and performed according to Kennedy and Jones [25]. All estimations were performed in triplicate. The results were expressed as mg per kg of dry matter (dm).

2.4. Identification and Quantification of Carotenoids and Chlorophylls

To obtain the samples for the determination of carotenoids and chlorophylls, the lyophilized carrot powders (~0.20 g) containing 10% $MgCO_3$ and 1% butylhydroxytoluene (BHT) were shaken with 5 mL of a ternary mixture of methanol/acetone/hexane (1:1:2, by vol.) at 300 rpm (DOS-10L Digital Orbital Shaker, Elmi Ltd., Riga, Latvia) for 30 min in the dark to prevent oxidation. The samples were centrifugated (4 °C, 7 min at 19,000× *g*; MPW- 350, Warsaw, Poland), and recovered supernatants were acquired after the 4 times re-extracted from solid residue. Combined fractions were evaporated. The pellet was solubilized using methanol and filtered through a hydrophilic polytetrafluoroethylene (PTFE) 0.20 µm membrane (Millex Samplicity® Filter, Merck, Darmstadt, Germany). Then carotenoids and chlorophylls were analysed by LC-MS-Q/TOF (identification) and UPLC-PDA (quantification) on an ACQUITY UPLC BEH RP C18 column protected by a guard column of the same materials (1.7 mm, 2.1 mm × 100 mm, Waters Corp., Milford, MA, USA) was performed at 30 °C. The elution solvents were a linear gradient of acetonitrile:methanol (70:30%, *v*/*v*) (A) and 0.1% formic acid (B) at flow rates of 0.42 mL/min. The analysis was performed at 450 nm (carotenoids) and 660 nm (chlorophylls). The obtained spectra and retention times were compared with the authentic standards and in this way the bioactive compounds were determined. The tests were implemented in triplicate, and the results are presented as mg per kg of dm.

2.5. Determination of Biological Activities of Carrot Varieties

Roots were investigated for α-amylase, α-glucosidase [26], and lipase inhibitory effects [27]. The acarbose was used as a positive control in the case of α-amylase and α-glucosidase, whereas orlistat was used as a positive control for pancreatic lipase. Additionally, the activities of cholinesterase inhibitors were evaluated with the acetylcholinesterase (AChE) and butyrylcholinesterase (BuChe) methods according to Ferreres et al. [28].

The results were presented as IC_{50} (mg/mL) (the amount of sample can reduce enzyme activity by 50%). All tests: α-amylase, α-glucosidase, anti-lipase, and anti-cholinergic activity were performed in triplicate using a SynergyTM H1 microplate reader (BioTek, Winooski, VT, USA).

2.6. Statistical Analysis

The two-way analysis of variance (ANOVA, $p \leq 0.05$) and Duncan's test were performed by Statistica version 13.3 (Stat-Soft, Cracow, Poland). The results are demonstrated as the mean value (n = 3).

3. Results and Discussion

3.1. Identification and Quantification of Phenolic Compounds in Coloured Carrot Varieties

Polyphenolic compounds are the secondary plant metabolites and are crucial for the colour, nutritional, antioxidant, and sensory features of foods. In the present study, the polyphenolic compositions were determined by UPLC-PDA-Q/TOF-MS, and the results

are shown in Table 1. Twenty-four phenolic compounds (15 phenolic acids and nine anthocyanins) were detected. The identified phenolic acids showed similarities for purple, yellow, orange, and white carrot varieties to those reported in the literature [29]. The determined phenolic acids were derivatives of caffeic, caffeoylquinic, coumaroylquinic, ferulic, and feruloylquinic acid. In the present study, 3-O-caffeoylquinic acid, 3-O-feruloylquinic acid, O-q-coumaroylquinic acid, dicaffeoylquinic acid derivative, and di-ferulic acid derivative were detected in white and orange carrots for the first time. 4-O-caffeoylquinic acid, 3-O-feruloylquinic acid, di-ferulic acid derivative, and diferuoylquinic acid derivative were found in yellow carrots. 4-O-caffeoylquinic acid, 3-O-feruloylquinic acid, O-q-coumaroylquinic acid, dicaffeoylquinic acid derivative, di-ferulic acid derivative, and diferuoylquinic acid derivative were detected in purple carrots for the first time.

Table 1. Overall phenolic compounds identified in 12 carrots varieties.

Compound	R_t (min)	Λmax (nm)	MS [M-H] (*m*/*z*) *	MS/MS (*m*/*z*)
Phenolic acids				
3-O-caffeoylquinic acid	5.90	324	353	135/179/191
Caffeic acid-hexoside	6.31–6.82–7.45	324	341	179/135
5-O-caffeoylquinic acid	7.54	325	353	179/191
4-O-caffeoylquinic acid	8.03	325	353	179/191
Ferulic acid-hexoside	8.69–9.22–9.80	324–325	355	193/175
Ferulic acid di-hexoside	9.41	324	517	355/193/175
3-O-feruloylquinic acid	10.10	322	367	173/193
O-q-coumaroylquinic acid	10.70	312	337	191
4-O-feruloylquinic acid	10.84	323	367	173/193
Caffeic acid-hexoside	10.90	324	341	179/135
5-O-feruloylquinic acid	11.23	325	367	191/193
Dicaffeoylquinic acid derivative	13.68–14.58–15.11	327	515	185/353
Di-ferulic acid derivative	14.27	327	527	203/365/366
Ferulic acid	14.88	324	193	
Diferuoylquinic acid derivative	16.20	324	543	
Anthocyanins				
Cyanidin-3-O-xylosyl-glucosylgalactoside	5.58	517	743	287
Delphinidin-3-O-rutinoside	5.86		611	303
Cyanidin-3-O-xylosyl-galactoside	6.14	518	581	287
Delphinidin-3-O-sambubioside	6.90		597	303
Cyanidin-3-O-xylosyl-sinapoyl-glucosylgalactoside	7.22	530	949	287
Cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside	7.57	528	919	287
Cyanidin-3-O-xylosyl-p-coumaroylglucosyl-galactoside	7.68	527	889	287
Ferulic acid derivative of pelargonidin 3-xylosylglucosylgalactoside	8.22	527	903	271
Ferulic acid derivative of peonidin 3-xylosylglucosylgalactoside	8.34	530	933	301

* MS $[M + H]^+$—for anthocyanins.

Table 2 presents the contents of phenolic compounds in carrot samples. The carrot samples showed significant differences in polyphenolic compositions ($p \leq 0.05$).

Table 2. Quantifications of phenolic compounds in 12 carrot varieties.

Compounds	Normal Orange	Normal Purple	Normal Yellow	Normal White	Mini Orange	Mini White	Mini Yellow	Mini Purple	Micro White	Micro Yellow	Micro Orange	Micro Purple
3-O-caffeoylquinic acid	5.06 ‡ f	46.55 a	nd k	3.24 g	12.51 c	7.84 e	nd k	15.52 b	1.05 j	nd k	12.08 d	1.94 h
5-O-caffeoylquinic acid	32.48 g	534.58 a	16.37 l	17.09 k	322.54 b	19.35 j	32.73 f	289.94 c	3.98 m	72.75 d	34.70 e	21.71 h
4-O-caffeoylquinic acid	nd f	55.24 a	0.40 e	nd f	nd f	nd f	4.22 d	10.56 c	nd f	20.04 b	nd f	nd f
Ferulic acid-hexoside	7.92 e	94.73 a	nd h	nd h	9.65 d	3.89 f	nd h	67.18 b	0.87 g	nd h	nd h	12.04 c
Ferulic acid di-hexoside	nd e	54.16 a	nd e	nd e	nd e	nd e	nd e	8.63 b	nd e	nd e	6.64 c	3.11 d
3-O-feruloylquinic acid	2.79 e	52.87 a	2.08 f	3.33 d	5.33 b	4.39 c	1.87 g	nd k	0.72 j	nd k	nd k	1.62 h
O-q-coumaroylquinic acid	0.83 g	22.20 b	nd h	1.45 f	1.63 e	2.51 d	nd h	27.64 a	nd h	nd h	nd h	5.95 c
5-O-feruloylquinic acid	nd h	7.80 a	0.96 f	nd h	nd h	0.93 g	1.70 d	4.53 c	nd h	5.77 b	nd h	1.13 e
Dicaffeoylquinic acid derivative	8.21 c	29.27 a	nd h	3.10 f	nd h	10.14 b	nd h	nd h	2.65 g	nd h	3.57 d	3.51 e
Di-ferulic acid derivative	80.49 b	7.08 k	9.53 j	11.78 h	75.13 d	27.54 g	31.46 e	27.85 f	0.92 m	204.36 a	79.42 c	6.46 l
Ferulic acid	30.41 e	33.73 d	1.04 k	nd m	42.69 b	16.83 g	12.18 h	27.50 f	0.92 l	62.14 a	34.24 c	5.31 j
Diferuoylquinic acid derivative	nd f	6.79 b	0.77 e	nd f	nd f	nd f	2.31 d	nd f	nd f	10.42 a	nd f	2.98 c
4-O-feruloylquinic acid	13.35 f	nd k	7.74 g	5.78 h	34.45 c	14.25 e	18.31 d	nd k	0.87 j	76.72 a	36.94 b	nd k
Caffeic acid-hexoside	1.00 b	nd c	nd c	nd c	nd c	nd c	nd c	nd c	nd c	5.71 a	nd c	nd c
Total phenolic acids	182.54 f	945.00 a	38.90 l	45.77 k	503.93 b	107.68 g	104.78 h	479.33 c	11.98 m	457.92 d	207.59 e	65.76 j
Cyanidin-3-O-xylosyl-glucosylgalactoside		45.04 a						22.14 b				nd c
Cyanidin-3-O-xylosyl-galactoside		16.01 b						51.88 a				0.75 c
Cyanidin-3-O-xylosyl-cinpoyl-glucosylgalactoside		43.09 a						8.83 b				nd c
Cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside		257.47 a						168.65 b				7.46 c
Cyanidin-3-O-xylosyl-p-coumaroylglucosyl-galactoside		16.87 a						3.60 b				nd c
Total anthocyanins		378.48 a						255.08 b				8.21 c
Polymeric procyanidins	69.62 b	44.44 j	20.64 m	53.80 c	38.05 l	78.92 a	51.19 f	46.50 h	52.93 e	49.26 g	44.09 k	53.05 d
DP	1.16 j	1.31 h	1.99 b	1.44 f	1.00 k	2.06 a	1.72 c	1.62 d	1.49 e	1.48 e	1.35 g	1.32 gh
Total Polyphenolic Content	253.32 e	1369.23 a	61.53 m	101.01 k	542.98 c	188.66 g	157.69 h	782.53 b	66.40 l	508.66 d	253.03 f	128.34 j

nd—not detected; polyphenols, polymeric procyanidins—mg/100 g dm; DP—degree of polymerisation; significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Duncan's test.

In the present study, di-ferulic acid derivative and 5-O-caffeoylquinic acid (5-CQA) were found in all coloured carrot samples. In the literature, 5-CQA (chlorogenic acid) was also detected in sea fennel, bean, and spinach, and it is important because of health benefits such as anti-carcinogenic, anti-inflammatory, anti-diabetic, and anti-obesity properties [30,31]. Other phenolic acids were found in different amounts in all carrot varieties. The highest total phenolic acid contents were observed in NPC (945.00 mg/100 g dm), MiOC (503.93 mg/100 g dm), and MiPC (479.33 mg/100 g dm). According to the obtained results, mini-sized and purple carrot samples had abundant phenolic acid content. Thus, the differences in phenolic acid profiles of carrots might be related to the root size, colour, and chemical compositions of the carrot varieties. Orange, purple, yellow, and white carrots are results of phenolic and carotenoid contents during the maturation period as well as storage temperature [32,33].

Nine anthocyanins were also identified in purple carrot samples of each size (Table 1). The identified anthocyanins showed similar compounds as those reported in the literature [34–37]. Five of the anthocyanidins were quantified in purple carrot varieties. In the present study, NPC and MiPC showed the presence of five anthocyanins (cyanidin-3-O-xylosyl-glucosylgalactoside, cyanidin-3-O-xylosyl-galactoside, cyanidin-3-O-xylosyl-cinpoyl-glucosylgalactoside, cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside, and cyanidin-3-O-xylosyl-p-coumaroylglucosyl-galactoside). However, MPC showed only two anthocyanins (cyanidin-3-O-xylosyl-galactoside and cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside). Comparison of the total anthocyanin contents of purple carrots showed that NPC had the highest anthocyanin content (378.48 mg/100 g dm), followed by MiPC (255.08 mg/100 g dm) and MPC (8.21 mg/100 g dm).

Procyanidins are subclasses of proanthocyanidins and are known for their antihypertensive, anti-allergic, antioxidant, and anti-microbial activities. Procyanidins have not been quantified in carrot varieties thus far. The present study is the first to quantify procyanidin contents in carrots. In the present study, the highest procyanidin contents were found in MiWC, NOC, and NWC. Thus, normal-sized and white carrot samples were rich in polymeric procyanidins. The structures of procyanidins consist of stereochemistry, hydroxylation pattern, flavan-3-ol constitutive units, and degree of polymerisation (DP, number of flavanol units) [38]. In the present study, the highest DP values were detected in MiWC (2.06), NYC (1.99), and MiYC (1.72). According to the DP values noted in the present study, mini-sized and yellow carrot samples were rich in flavanol units. Therefore, MiWC showed high procyanidin contents and DP values.

To summarise the polyphenolic contents of coloured carrot varieties, the highest total phenolic contents were found in NPC, MiPC, and MiOC. Thus, purple carrot has the highest total content of polyphenols, which was also confirmed by other authors [29,36,39–41]. Moreover, phenolic contents may change depending on genes, environment, and climate factors [42].

3.2. Identification and Quantification of Carotenoids in Carrot Varieties

Table 3 shows the content of carotenoids and chlorophylls of the 12 carrot varieties with significant differences ($p \leq 0.05$). In the present study, violaxanthin, astaxanthin, lutein, zeaxathin, α-cryptoxanthin, β-cryptoxanthin, (6R)-δ-carotene, α-carotene, γ-carotene, ε-carotene, β-carotene, and trans-apo-carotenal were identified as carotenoids. NPC exhibited the highest ratio of different carotenoid types (lutein, zeaxathin, α-cryptoxanthin, β-cryptoxanthin, γ-carotene, and trans-apo-carotenal). Trans-apo-carotenal was detected only in NPC.

Table 3. Quantifications of carotenoids and chlorophylls of 12 carrot varieties.

Compounds	Normal Orange	Normal Purple	Normal Yellow	Normal White	Mini Orange	Mini White	Mini Yellow	Mini Purple	Micro White	Micro Yellow	Micro Orange	Micro Purple
Violaxanthin	0.03 ‡ abc	0.04 abc	0.05 ab	0.01 c	0.02 bc	0.01 c	0.06 a	0.04 abc	0.01 c	0.04 abc	0.02 bc	0.03 abc
Astaxanthin	2.58 h	3.43 f	5.08 c	0.32 k	2.61 h	0.32 k	8.09 a	4.65 d	0.41 j	6.78 b	3.01 g	4.47 e
Lutein	nd c	0.19 a	0.14 b	nd c	nd c	nd c	nd c	nd c	nd c	nd c	nd c	nd c
Zeaxanthin	0.17 b	0.70 a	nd c	nd c	nd c	nd c	nd c	nd c	nd c	nd c	nd c	nd c
α-cryptoxanthin	0.02 b	0.05 a	nd b	nd b	0.01 b	nd b	nd b	nd b	nd b	nd b	0.01 b	0.02 b
Beta-cryptoxanthin	nd f	1.65 a	0.27 b	0.08 e	nd f	0.10 e	0.20 c	nd f	0.09 e	0.27 b	nd f	0.13 d
(6R)-δ-carotene	0.47 a	0.24 d	0.05 fgh	0.08 f	0.03 h	0.06 fgh	0.47 a	0.07 fg	0.17 e	0.41 b	0.04 gh	0.30 c
α-carotene	6.91 c	nd f	14.43 a	2.88 d	13.28 b	1.40 e	2.89 d	nd f	nd f	nd f	nd f	nd f
γ-carotene	4.02 d	12.22 a	0.61 g	0.60 g	5.25 c	0.41 h	nd k	nd k	0.26 j	2.09 f	2.63 e	11.79 b
ε-carotene	0.16 f	0.54 b	1.02 a	0.16 f	0.30 e	nd h	0.40 d	nd h	0.10 g	0.46 c	nd h	0.46 c
β-carotene	nd f	nd f	14.49 a	0.85 e	nd f	nd f	3.22 c	nd f	nd f	4.14 b	nd f	1.71 d
Trans-apo-carotenal	nd a	0.01 a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a
Total carotenoids	14.36 f	19.07 c	36.14 a	4.98 j	21.50 b	2.30 l	15.33 e	4.76 k	1.04 m	14.19 g	5.71 h	18.91 d
Pheophorbide a	0.01 ab	0.02 a	nd b	nd b	nd b	nd b	nd b	nd b	nd b	nd b	nd b	nd b
Chlorophyll a	0.03 c	nd d	0.15 a	0.01 cd	nd d	nd d	0.06 b	nd d	0.01 cd	0.06 b	nd d	nd d
Chlorophyll b	0.01 d	0.08 b	0.16 a	0.05 bc	0.01 d	0.02 cd	0.07 b	nd d	0.01 d	0.01 d	0.03 cd	0.01 d
Total chlorophylls	0.05 def	0.10 c	0.31 a	0.06 de	0.01 g	0.02 fg	0.13 b	0.00 g	0.02 fg	0.07 d	0.03 efg	0.01 g
Total carotenoid and chlorophyll content	14.42 f	19.16 c	36.46 a	5.04 j	21.50 b	2.33 l	15.45 e	4.76 k	1.05 m	14.25 g	5.75 h	18.91 d

nd—not detected; carotenoids and chlorophylls—mg/100 g dm; significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Duncan's test.

Carotenoids, which cannot be synthesised by animals and humans, have to be obtained from the diet as crucial precursors for healthy body functions [43]. According to Alasalvar et al. [39], β-carotene and α-carotene are the most abundant carotenoids in carrots. In the present study, β-carotene was detected in NYC > MYC > MiYC > MPC > NWC, whereas α-carotene was observed in NYC > MiOC > NOC > MiYC = NWC > MiWC. The highest content of β-carotene was found in NYC and MYC, whereas the highest α-carotene content was observed in NYC and MiOC. Thus, NYC showed the highest presence of both α and β-carotenes. Reif et al. [44] showed a different trend in their study and indicated a low amount of β-carotene in yellow carrots. The contents of β-carotene and α-carotene change with temperature, which may have caused discrepancies in the observed results.

Carotenoids are unstable pigments, which can create some problems while working with them. For instance, orange carrot is known to be a rich source of β-carotene and α-carotene [44]. However, in the present study, α-carotene was not detected in MOC; moreover, according to the applied method, and retention time differences in β-carotene were not found in NOC, MiOC, or MOC. This was a surprising observation but was confirmed by the standard of β-carotene. Maybe the content was very low, and therefore no peak of this compound was observed. Moreover, α- and β-carotene can be pressed by chlorophylls and xanthophylls as well [45]. In addition, storage period and temperature during the storage are other important factors for pigmentation.

High levels of δ-carotene and ε-carotenes were found in orange carrot samples of various sizes and have not been reported before.

Six xanthophyll pigments were identified and quantified in carrot samples (Table 3). In the present study, lutein was found solely in NPC and NYC. Similar results have been reported in the literature [44,46].

Chlorophyll pigments are essential to prevent chronic diseases [47]. In the present study, pheophorbide a, chlorophylls a, and b were found as chlorophylls pigments in the carrot samples. Pheophorbide a was solely determined in NOC and NPC, in turn chlorophyll a was identified in NOC, NYC, NWC, MiYC, MWC, and MYC. Except for MiPC, all carrot varieties showed the content of chlorophyll b. Moreover, chlorophyll a and b contents were high in NYC. Thus, chlorophyll contents were found to change within cultivars [48].

To summarise, the total carotenoid contents were the highest in NYC, MiOC, NPC, and MPC. Normal-sized and purple carrot samples showed greater total carotenoid contents. The total chlorophyll contents were the highest in NYC, MiYC, NPC, and MYC. Therefore, normal-sized and yellow carrot samples were rich in chlorophyll contents. The highest total carotenoid and chlorophyll results were observed in NYC, MiOC, NPC, and MPC. Normal-sized and purple carrot samples were rich in carotenoid and chlorophyll contents. The carotenoid contents of carrots may change depending on variety, maturity, growth conditions, growing season, soil, and genetic factors [49].

3.3. Enzyme Inhibitory Activities by Coloured Carrot Roots

The enzyme inhibitory activities of 12 carrot varieties against α-amylase, α-glucosidase, pancreatic lipase, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE) were evaluated by in vitro assays and expressed as IC_{50}—the amount of sample can reduce enzyme activity by 50%. The results are shown in Table 4. The carrots showed significant differences in all five inhibition activities ($p \leq 0.05$). It should be noted that enzyme inhibition activities of carrot varieties have not been investigated thus far.

Table 4. In vitro inhibition activity (α-amylase, α-glucosidase, pancreatic lipase, acetylcholinesterase, butyrylcholinesterase (IC_{50}, mg/mL) of different carrot varieties.

Type of Carrot	α-Amylase *	α-Glucosidase **	Lipase ***	AChE [1]	BuChe [2]
Normal orange	807.92 ‡ a	125.93 h	5.29 l	13.13 h	9.36 h
Normal yellow	441.45 b	151.08 f	6.83 e	14.14 g	8.01 k
Normal purple	239.49 c	643.91 b	6.12 h	18.96 a	7.85 l
Normal white	219.21 d	116.53 j	12.25 a	16.05 c	19.02 a
Mini orange	128.96 h	307.09 d	6.81 e	16.45 b	15.77 c
Mini yellow	122.58 k	356.68 c	5.69 k	15.08 e	13.94 e
Mini purple	127.06 j	97.02 m	6.94 d	15.74 d	16.8 b
Mini white	107.85 m	897.79 a	9.58 c	12.31 j	14.03 d
Micro orange	111.03 l	251.83 e	6.29 g	14.61 f	9.03 j
Micro yellow	218.66 e	104.18 l	6.05 j	14.61 f	10.75 g
Micro purple	181.37 g	148.96 g	6.49 f	10.14 l	7.83 l
Micro white	199.79 f	106.06 k	11.36 b	12.05 k	11.05 f

Significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Duncan's test, * inhibition activity of acarbose < 5 mg/mL; ** inhibition activity of acarbose < 5 mg/mL; *** inhibition activity of Orlistat < 1 mg/mL; [1] AChE–acetylcholinesterase; [2] BuChe –butyrylcholinesterase.

The 12 carrot varieties were assayed for their anti-diabetic properties (inhibition of α-amylase and α-glucosidase enzymes). The enzymes α-amylase and α-glucosidase are responsible for carbohydrate digestion, and the inhibition of these enzymes may decrease postprandial blood glucose levels by reducing the breakdown of polysaccharides into glucose. The bioactivities of many plant species have been shown to decrease early-stage diabetes-related enzyme activities [50]. In the present study, the IC_{50} values of the carrot samples for inhibiting α-amylase ranged from 107.85 to 807.92 mg/mL. MiWC and MOC showed the highest activities against α-amylase, whereas the lowest activity was shown by NOC. Most carrot extracts had higher IC_{50} for α-amylase than sour cherry, red grapefruit, pineapple, orange, and kiwi [27]. To clarify, α-amylase inhibitory activity is a result of bioactive compounds of plants such as glycosides, polysaccharides, steroids, and terpenoids [51]. Overall, mini carrots showed the highest inhibitory effect against α-amylase.

The IC_{50} values of the carrot samples for inhibiting α-glucosidase ranged from 97.02 to 897.79 mg/mL. MiPC and MYC showed the highest inhibitory activities against α-glucosidase, whereas the lowest result was shown by MiWC. The IC_{50} values for α-glucosidase were higher than chokeberry, apple, pear, and blackberry, a group of fruits recognized as important in the prevention of diabetes [27]. A previous study reported that the high anti-diabetic activity of onion is associated with its content of phenolic acids, flavonoids, and anthocyanins [52]. Polyphenol and carotenoid contents were shown to increase the anti-diabetic activities of fruits and vegetables [53,54]. A comparison of α-amylase and α-glucosidase inhibitory activities of carrot samples revealed that MiWC had the highest α-amylase inhibitory activity but the lowest α-glucosidase inhibitory activity. Micro-sized samples showed the highest α-glucosidase activities. Moreover, α-amylase inhibitory activity demonstrated non-significant correlations with total carotenoid and chlorophyll contents ($R^2 = 0.05$), phenolic acid content ($R^2 = 0.04$), and procyanidin content ($R^2 = 0.01$). However, chemical contents of carrot varieties showed correlations with the α-glucosidase inhibitory activity that were different from those observed for α-amylase inhibitory activity; the following R^2 values were noted for procyanidin, and total carotenoid and chlorophyll contents: 0.50 and 0.01, respectively, and no correlation was observed with phenolic acid content ($R^2 = 0.00$). Therefore, phenolics are correlated with diabetes-related enzymes [55]; however, in the present study high positive correlation in carrot varieties was not determined.

Pancreatic lipase hydrolyses triacylglycerols into free fatty acids, bile salts, and fat-soluble vitamins [56]. In the present study, the IC_{50} values for pancreatic lipase inhibitory activities ranged from 5.29 to 12.25 mg/mL. The highest IC_{50} values were observed for

NOC (5.29), MiYC (5.69), MYC (6.05), and NPC (6.12); however, NWC (12.25) and MWC (11.36) showed the lowest activities against pancreatic lipase. According to Fabroni [57], total anthocyanin content correlates with pancreatic lipase activity; however, in the present study, similar results were not observed, because normal-sized orange carrot exhibited elevated activities against pancreatic lipase. Moreover, in the literature lentil cultivars have shown potent activity (IC_{50} from 6.26 to 9.26 mg/mL) against pancreatic lipase [58]. Hence, phenolics, ascorbic acid, and carotenoids are responsible for pancreatic lipase inhibition [59].

More importantly, the carrot samples exhibited higher inhibitory activities against pancreatic lipase than against diabetes-related enzymes. The pancreatic lipase inhibitory activity showed a positive correlation with the total carotenoid and chlorophyll contents (R^2 = 0.29), phenolic acid content (R^2 = 0.17), and to a low extent with procyanidin content (R^2 = 0.06).

The inhibitions of AChE and BuChE are thought to be important for the diagnosis and treatment of diseases such as bladder distention, glaucoma, myasthenia gravis, and Alzheimer's disease [60]. In the present study, carrot samples were investigated for potential inhibitor activities against AChE and BuChE. The IC_{50} values of the carrot samples for AChE inhibition ranged from 10.14 to 18.96 mg/mL. The highest AChE inhibitory activities were observed for MPC (10.14 mg/mL), MWC (12.05 mg/mL), and MiWC (12.31 mg/mL), whereas the lowest result was observed for NPC (18.96 mg/mL). Moreover, in the literature white ginger exhibited more potent activities for AChE inhibition than red ginger variety [61]. In addition, a previous study showed that the IC_{50} value of Hippophaë cultivars for inhibiting AChE ranged from 20.16 to 40.60 mg/mL [62]. *Dipsacus* root showed higher activity against AChE than the other parts of the plant [63]. The content of polyphenols and carotenoids in carrots was shown to correlate with activities of cholinesterase inhibitors [64,65]. In the present study, micro-sized and white carrot varieties showed elevated activities against AChE. Furthermore, the IC_{50} values of the carrot samples for inhibiting BuChE ranged from 7.83 to 19.02 mg/mL. MPC (7.83 mg/mL), NPC (7.85 mg/mL), and NYC (8.01 mg/mL) showed the highest BuChE inhibitory activities, whereas the lowest activity was shown by NWC (19.02 mg/mL). Thus, normal-sized and purple carrot samples showed superior activities against BuChE. On the other hand, AChE inhibitory activity was correlated with the content of total carotenoid and chlorophyll (R^2 = 0.27), procyanidins (R^2 = 0.17), and phenolic acids (R^2 = 0.02), whereas BuChE inhibitory activity was correlated with the content of procyanidins (R^2 = 0.17), total carotenoid and chlorophyll (R^2 = 0.07), and phenolic acids (R^2 = 0.03). Therefore, phenolics are thought to be related to cholinesterase inhibition [66].

Overall, MiPC showed the highest activities against α-glucosidase, whereas MPC exhibited the highest activity against AChE and BuChE. NOC showed the highest inhibition activity against pancreatic lipase. Thus, purple carrot samples were more functional for enzyme inhibition than other coloured carrots of different sizes.

4. Conclusions

The present study evaluated 12 carrot varieties of different sizes and colours for bioactive compounds and their biological activities. The study results showed that purple carrot samples had the highest values for the content of polyphenolics and carotenoids, with the highest activities against cholinesterase. Normal yellow carrot showed the lowest values for the content of polyphenols. Micro white carrot demonstrated the lowest results for total phenolic acid, total carotenoid, and chlorophyll content. Moreover, the activities against diabetes-, obesity- and aging-related enzymes of the carrot varieties were found to be correlated with the content of phenolics and carotenoids.

Overall, the present study attempted to find the best carrot variety with high nutrients for food processing. Normal purple carrot showed the highest health-promoting activities in all tests, followed by mini purple carrot. Thus, different-sized purple carrot varieties can provide high contents of bioactive compounds to combat oxidative stress-related diseases.

However, the mechanisms of the pro-health action of carrots were not confirmed in this study, and their activities as α-amylase, α-glucosidase, lipase, acetylocholinesterase, and butyrylocholinesterase inhibitors was rather poor. Probably the mechanisms of their action are more complex, and the possible health-promoting effect results from the synergy of many compounds, including fibre, phytochemicals, vitamins, and minerals. Therefore, it would be worth continuing research on different varieties of carrots.

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OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Yusuf E., Wojdyło A., Oszmiański J., Nowicka, P. 2021. Nutritional, phytochemical characteristics and *in vitro* effect on α-amylase, α-glucosidase, lipase, and cholinesterase activities of 12 coloured carrot varieties. *Foods*, *10*(4), 808. https://doi.org/10.3390/foods10040808

mój udział polegał na tworzeniu planu badań, przygotowaniu materiału badawczego, przeprowadzeniu doświadczeń i analiz, z uwzględnieniem analizy zawartości związków bioaktywnych z wykorzystaniem technik UPLC oraz właściwości prozdrowotnych *in vitro*. Dodatkowo otrzymane wyniki opracowałam pod kątem statystycznym i merytorycznym, przygotowując manuskrypt, a także brałam udział w współredagowaniu tekstu w procesie recenzji.

potwierdzam powyższe deklaracje Emel Hasan Yusuf
Pauline Nowicka
02.06.2023

02.06.2023 .....
data i podpis

DECLARATION

I declare that in the publication entitled:

Yusuf E., Wojdyło A., Oszmiański J., Nowicka, P. 2021. Nutritional, phytochemical characteristics and *in vitro* effect on α-amylase, α-glucosidase, lipase, and cholinesterase activities of 12 coloured carrot varieties. *Foods*, *10*(4), 808. https://doi.org/10.3390/foods10040808

my participation consisted in creating a research plan, preparing research material, conducting experiments and analyses, including the analysis of the content of bioactive compounds using UPLC techniques and health-promoting properties by *in vitro*. In addition, I analyzed the obtained results in statistical and substantive terms, preparing the manuscript, and I also participated in the co-editing of the text in the review process.

I confirm the indicated commitment of Emel Hasan Yusuf

Pauline Nowicka
02.06.2023

02.06.2023 .....
the date and sign

Publication 2

Yusuf E., Tkacz K., Turkiewicz I.P. Wojdyło A., Nowicka, P. 2021. Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC. European Food Research and Technology, 247, 3053–3062.

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ORIGINAL PAPER

Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC

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Abstract
Twelve carrot varieties in different colours and sizes were investigated for chemical properties (dry matter, ash, pectins, titratable acidity, and pH), contents of vitamin C, sugar, organic acids, mineral (sodium, potassium, calcium, iron, and magnesium), and anti-oxidant activities (ABTS, FRAP, and ORAC). Moreover, total polyphenolics and total tetraterpenoids of colourful carrot varieties were presented. According to the study, sucrose was the dominant sugar and isocitric acid was the most common organic acid in carrot samples. In the case of mineral content, potassium, sodium, calcium, magnesium, and iron were identified, while copper was not identified in carrots. Additionally, most of the analyzed carrots were a good source of pectins (average—1.3%), except for mini-orange carrot. Purple-coloured carrot samples demonstrated the highest results for total sugar (11.2 g/100 g fm), total organic acid (2.8 g/100 g fm), total polyphenolic contents (224.4 mg/100 g fm), and anti-oxidant activities (17.1 mmol Trolox equivalents/100 g dm). In turn, the lowest results were observed in normal yellow carrot for total polyphenols (7.3 mg/100 g fm), and anti-oxidant activities (2.5 mmol Trolox equivalents/100 g dm); besides, the lowest total tetraterpenoids were determined in micro-white carrot—0.2 mg/100 g fm.

Keywords Carrot roots · Chemical properties · Polyphenolic compounds · Carotenoids · Antioxidant activity

Introduction

Global dietary guidelines support boosting fruit and vegetables consumption and recommend five portions of fruit and vegetables per day [1]. It results from the fact that fruit and vegetables consumption prevent non-communicable diseases such as cardiovascular disorders, obesity, diabetes, and cancer [2]. However, many people do not consume fruit and vegetables for varied reasons such as price, eating habits, time, and concerns about fruit and vegetables production [3]. Thus, poor diet is the one reason to cause 80% of death from non-communicable diseases [4].

Childhood is a period when people can gain healthy eating habits from their parents [5]. Parental nutritional knowledge increases the consumption of more fruit, vegetables, and fiber intakes of children [6]. Besides, the food choices of parents influence the eating habits of children as well [7]. For instance, to increase the fruit and vegetables consumption of children, parents may purchase more colourful fruit and vegetables. Moreover, family members can consume these raw materials together to gain more healthy eating habits. Hence, rainbow-healthy raw foods promote people to enhance their diet [8].

To clarify, colourful carrot varieties may apply with their elevated health benefits. Carrot breeding provides purple, orange, red, yellow, and white colours [9]. Besides, the chemical contents of carrots change depending on colours; carotenoids create orange and yellow colours [10, 11], purple colour is the result of anthocyanins [12], and white carrot contains no colour pigments [13]. Except for colour features, bioactive compounds of carrots are precious with activities against diabetes, cardiovascular diseases, obesity, cancers, and aging [14, 15].

Like other fruit and vegetables, nutrients in carrots change with genes, environmental factors, fertilizer, and storage periods [16]. Carrot roots are rich in different carotenoid types as well as polyphenols [17]. However, carrot

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varieties are not restricted to those compounds. Hence, carrots are valuable with vitamin and mineral contents as well.

The mineral content determines product quality which is an important parameter for raw food products [18]. Carrot roots are abundant in potassium (K), manganese (Mn), phosphorus (P), calcium (Ca), sodium (Na), iron (Fe), and magnesium (Mg) [19, 20]. These elements are essential minerals for human healthy body functions. Proof of these, K is necessary for functions of muscles, nerves, and cells; Mn is required in trace amounts for biosynthetic pathways; P shows functions for blood vessels and bones; Ca is essential for bones, teeth and blood cells; Na is necessary for functions of muscles and nerves; Fe is important for oxygen transportation in blood and muscle cells; besides, Mg adjusts blood sugar levels and blood pressure [21].

Vitamins in carrot varieties are another important content for product quality. Moreover, the main vitamin types in carrot varieties are the results of carotenoids. For instance, β-carotene is converted to vitamin A which is important against night blindness and increases immune system functions [22]. Second, α-tocopherol is the precursor of vitamin E that is crucial for cell signalling, gene expression, and cell membrane stabilities in the human body [23]. Except for these two vitamins, carrots are abundant with vitamin C which is necessary for controlling blood pressure, preventing iron deficiency and boosting immune system functions [20, 24]. Moreover, vitamin B derivatives (thiamine, riboflavin, cobalamin, and pyridoxine) are rich in carrot varieties as well [25]; and vitamin B is important for the functions of cell growth, brain, and digestion system.

Besides, carrots are good sources of carbohydrates such as simple sugars like fructose, glucose, and sucrose, a small amount of starch and fibers [26]. Sugar contents and quantities of carrot varieties may change with the impact of environmental and storage conditions [27]. Dietary fibers, which provide healthy bowel function, decreasing cholesterol level, and heart diseases, are grouped as soluble and insoluble. For instance, pectin and hemicellulose are soluble; cellulose is insoluble fiber [28]. Moreover, mono-, di-, or oligo-saccharides of fruit and vegetables are categorized as prebiotics by CODEX Alimentarius [29].

On the other hand, fruit and vegetables are rich in organic acids. The most known acid of raw materials is ascorbic acid (vitamin C) with its high anti-oxidant activities. However, many others boost healthy body functions, as well. For instance, benzoic acid demonstrates antibacterial activity; hydroxycinnamic acid is an anti-inflammatory agent; gallic acid fights with mutagenic factors. Moreover, acetic, succinic, citric, lactic, malic acids, and their salts help to iron absorption [30].

Therefore, the study aims to compare 12 coloured carrot varieties with their chemical properties (dry matter, ash, pectin, titratable acidity, and pH), and anti-oxidant activities (ABTS, FRAP, and ORAC). Moreover, carrot varieties have been contrasted for total polyphenolic compounds, vitamin C, and total tetraterpenoid contents. Additionally, sugar, mineral, and organic acid profiles of carrot varieties have been presented. The study is the first to evaluate the chemical contents and anti-oxidant activities of colourful carrot varieties together.

Materials and methods

Chemicals

Standards of sugars, organic acids, carotenoids, chlorophylls, and polyphenolics were purchased from extrasynthese (Lyon, France). Acetonitrile, methanol, and formic acid for analyses of ultra-performance liquid chromatography (UPLC; Gradient grade) and ascorbic acid were purchased from Merck (Darmstadt, Germany). The additional reagents were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Plant material and sample preparation

Normal and mini-sized carrots were purchased from Fusion Gusto (Dąbrowa, Poland). Micro carrots were purchased from Cato Produce (Johannesburg, South Africa) in June 2020. Normal-sized carrots were between 20 and 45 mm and weights from 50 to 150 g; mini-sized carrots were between 10 and 20 mm and weights from 8 to 50 g; micro-sized carrots were smaller than 10 mm and weights were smaller than 8 g.

The following varieties of carrot were investigated: micro-yellow carrot (MYC), mini-yellow carrot (MiYC), normal yellow carrot (NYC); micro-purple carrot (MPC), mini-purple carrot (MiPC), normal purple carrot (NPC); micro-orange carrot (MOC), mini-orange carrot (MiOC), normal orange carrot (NOC); micro-white carrot (MWC), mini-white carrot (MiWC), and normal-white carrot (NWC).

Approximately 2 kg of carrot roots were separated into two parts. One part of carrot varieties was analyzed for the contents of dry matter, ash, soluble solids, pH, titratable acidity, pectin, L-ascorbic acid, sugars, and organic acids. The second part of carrot roots was washed, dried, cut into slices, and then frozen at − 80 °C. The sliced carrots were then freeze-dried (24 h; Christ Alpha 1–4 LSC, Melsungen, Germany) and crushed by a laboratory mill (IKA A 11, Staufen, Germany) to obtain the homogeneous dry material for analysis.

Physicochemical analyses

Titratable acidity (TA) was evaluated by 0.1 N NaOH to an endpoint of pH 8.1 using an automatic pH titration system (pH-meter type IQ 150; Warsaw, Polska) and expressed as g malic acid/100 g FW (fresh weight). The pH of carrots was measured using the same equipment as that used for TA. The dry matter was estimated by mixing the sample with diatomaceous earth, pre-dried, and final drying under reduced pressure. The dry matter and TA were determined using the following PN norms: PN-EN 12,145:2001 and PN-EN 12,145:2000, respectively. Pectin content (mg/100 g FW) was evaluated according to Pijanowski et al. [31]. The contents of ash (%), L-ascorbic acid (mg/100 g FW), sugars, and organic acids (g/100 g FW) were determined according to Wojdyło et al. [32]. The content of sugars was estimated by HPLC-ELSD (Merck, Hitachi, Japan). The content of organic acids in carrots was quantified by Ultra Performance Liquid Chromatography with Photodiode Array Detector (Acquity UPLC System, Waters Corp., Milford, MA, USA). Organic acids were identified using reference standards (Merck KGaA, Darmstadt, Germany). The content of minerals in carrots was determined using Atomic Absorption Spectrophotometers (AA-7000F/AAC SHIMADZU, Shimadzu Corporation). All measurements were performed in triplicate.

Quantification of bioactive compounds

The quantification (UPLC/PDA/FL—ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 × 100 mm, Waters Corporation, Milford, USA) at 30 °C. The injection and elution of the samples (5 µL) were concluded in 15 min with a sequence of linear gradients and a flow rate of 0.42 mL/min. The solvent A (2.0% formic acid, v/v) and solvent B (100% acetonitrile) comprised the mobile phase. The procedure operated through gradient elution with 99–65% solvent A (0–12 min), solvent A was later lowered to 0% for condition column (12.5–13.5 min), and the gradient returned to the initial composition (99% A) for 15 min to re-equilibrate the column) of polyphenolics was performed according to Wojdyło et al. [33]. The polyphenols' quantification was performed by external calibration curves and reference standards.

The quantifications (UPLC-PDA) of tetraterpenoids were performed according to Wojdyło et al. [34] by retention times and spectra to compare with authentic standards. The determination of tetraterpenoids was performed according to Kolniak-Ostek [35]. The total values of polyphenols and carotenoids were given as fresh weights (FW) after the identification and quantification of each bioactive compound. The results are expressed as mg/100 g FW.

Determination of anti-oxidant activity of carrot varieties

The ORAC (oxygen radical absorbance capacity), ABTS + (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), and FRAP (the ferric reducing ability of plasma) anti-oxidant activity assays were performed as described, respectively [36–38]. The results of anti-oxidant activity tests are shown as mmol of Trolox equivalents (TE) per 100 g of dry matter (dm). All tests were performed in triplicate using a microplate reader SynergyTM H1 (BioTek, Winooski, VT, USA).

Statistical analysis

The two-way analysis of variance (ANOVA, $p \leq 0.05$) and Duncan's test were performed by Statistica version 13.3 (Stat-Soft, Cracow, Poland). The results are shown as the mean value ($n = 3$) ± standard deviation (SD).

Results and discussion

Quantification of bioactive compounds in different varieties of carrot

The total L-ascorbic acid, total polyphenolic, and total tetraterpenoid contents of carrot roots are shown in Fig. 1.

Humans cannot synthesise L-ascorbic acid and should obtain the vitamin from other sources such as fruits and vegetables [39]. Besides, the recommended daily intake of ascorbic acid is 75 mg/day for an adult woman and 90 mg/day for an adult man [40]. In the present study, the content of L-ascorbic acid ranged from 1.0 to 5.3 mg/100 g FW. The highest values were observed for MYC (5.3 ± 0.0 a mg/100 g FW), followed by MPC (5.0 ± 0.0 b mg/100 g FW) and MOC (3.6 ± 0.0 c mg/100 g FW). Conversely, the lowest results were detected for NYC (1.2 ± 0.4 l mg/100 g FW) and NWC (1.0 ± 0.1 m mg/100 g FW). According to Alasalvar et al. [26], white carrot (1.3 mg/100 g FW) had the lowest ascorbic acid content, while orange carrot (5.3 mg/100 g FW) showed the highest amount of L-ascorbic acid. However, the authors could not quantify the ascorbic acid content in the purple carrot. In the present study, dark-coloured carrot samples exhibited much higher L-ascorbic acid content than other carrots, and similar results have been reported in the literature [41]. Moreover, micro- and mini-sized carrot samples showed better results for L-ascorbic acid content than normal-sized carrot samples. In another study [42], the contents of L-ascorbic acid of six carrot varieties were compared during the storage periods. The result of the study demonstrated that after harvesting, L-ascorbic acid levels of carrot roots ranged from 54 to 132 mg kg^{-1}. However,

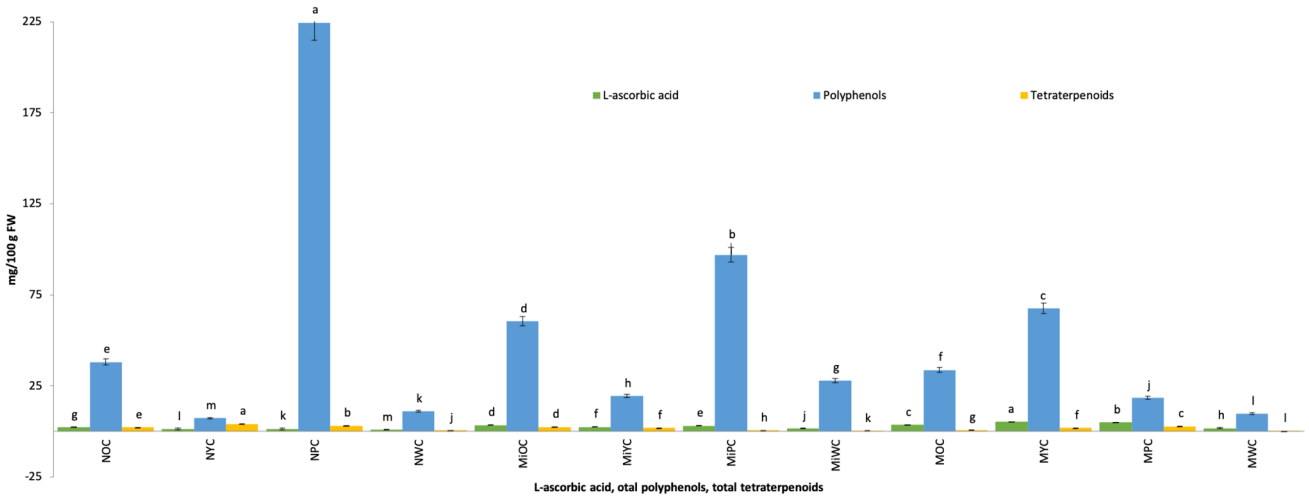

Fig. 1 Total L-ascorbic acid, total polyphenol, and total tetraterpenoid contents in colourful carrot varieties. The L-ascorbic acid, total polyphenol, and total tetraterpenoid contents (mg/100 g FW) in 12 carrot varieties; significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter were not significantly different ($p > 0.05$) according to Duncan's test

after 30 days of storage, L-ascorbic acid levels decreased by almost 50%. Therefore, the content of L-ascorbic acid changes depending on variety, carbon dioxide, temperature, storage, and age [43].

The total phenolic contents of carrot roots ranged from to 224.4 mg/100 g FW. The NPC (224.4 ± 9.5 a mg/100 g FW) and MiPC (97.0 ± 4.1 b mg/100 g FW) had the highest total polyphenolic contents. However, NYC (7.3 ± 0.3 m mg/100 g FW) and MWC (9.8 ± 0.4 l mg/100 g FW) demonstrated the lowest contents of total phenolics. In the literature, the total phenolic content of beetroot is 257.2 mg/100 g FW, turnip 127.0 mg/100 g FW, and yam is 92.0 mg/100 g FW [44, 45]. High total phenolic contents of plants are related to antimicrobial and anti-oxidant activities of species, as well [46, 47]. Moreover, some phenolics activate anti-oxidant molecules in the cell [48]. Furthermore, phenolics are a part of chemicals that fight against diabetes-related diseases [49]. Thus, the activities of phenolics can be influenced by many factors such as plant species, varieties, climate, storage conditions, and biotic and abiotic stress factors [50].

On the other hand, carotenoids and chlorophylls are a part of tetraterpenoids and support healthy body functions against aging, cataract, and cancer [51, 52]. In the present study, total tetraterpenoids of 12 carrot varieties ranged from 0.2 to 4.1 mg/100 g FW. The highest tetraterpenoid contents were observed in NYC (4.1 ± 0.2 a mg/100 g FW) and NPC (3.1 ± 0.1 b mg/100 g FW); but the lowest tetraterpenoid contents were determined in MWC (0.2 ± 0.0 l mg/100 g FW) and MiWC (0.4 ± 0.0 k mg/100 g FW). Normal-sized and purple carrots were rich with total tetraterpenoid contents. In the literature, sweet potato and potato had 2.4 mg/100 g and 0.0 mg/100 g of total tetraterpenoid contents, respectively [53]. Therefore, tetraterpenoid contents can be affected for varied reasons such as plant types, harvesting conditions, climate, and soil factors of plants [54, 55]. Moreover, tetraterpenoid contents are closely related to the colours of plant parts or vice versa.

To summarise, when L-ascorbic acid, total polyphenolic, and total tetraterpenoid contents were evaluated together, any correlation was not found. However, MYC had the highest L-ascorbic acid content with the third place of the highest total polyphenols (after the NPC and MiPC). Besides, NPC had the highest total polyphenolics and the second-highest value of tetraterpenoids, as well.

Sugar and organic acid profile of different carrot varieties

Sugars and organic acids are crucial for the quality and sensory attractiveness of raw materials [56]. These natural compounds comprise approximately 60% of dry matter, soluble solid content, and flavour of fruits and vegetables [57]. In the present study, sugars and organic acids were found to provide the taste and sweetness to the carrot varieties. As shown in Table 1, significant differences ($p \leq 0.05$) in sugar and organic acid contents were observed in carrot roots. Fructose, sorbitol, glucose, and sucrose were determined as sugar components in the studied carrot varieties. Fructose and glucose were observed in all carrot samples; however, sorbitol was detected only in NOC, MiOC, MiYC, MiPC, MOC, MYC, and MPC. Moreover, sucrose was not identified in MiPC. The highest fructose levels were observed in NOC (3.8 g/100 g FW), MWC (1.9 g/100 g FW), and MiYC (1.9 g/100 g FW); the highest sorbitol levels were found in MiYC (0.5 g/100 g FW) and MYC (0.4 g/100 g FW); the

Table 1 Sugar and organic acid contents of carrot varieties

Compounds	NOC	NYC	NPC	NWC	MiOC	MiYC	MiPC	MiWC	MOC	MYC	MPC	MWC
Fructose	3.78*^{a}	0.25^{g}	0.64^{f}	1.42^{c}	1.21^{d}	1.85^{b}	1.40^{c}	0.80^{e}	1.20^{d}	0.65^{f}	0.79^{e}	1.86^{b}
Sorbitol	0.01^{f}	0.00^{f}	0.00^{f}	0.00^{f}	0.23^{c}	0.53^{a}	0.23^{c}	0.00^{f}	0.10^{e}	0.39^{b}	0.18^{d}	0.00^{f}
Glucose	1.31^{h}	0.79^{m}	0.91^{l}	4.09^{a}	1.65^{f}	2.81^{c}	3.83^{b}	1.10^{j}	1.80^{e}	1.91^{d}	0.95^{k}	1.45^{g}
Sucrose	0.53^{g}	4.56^{c}	9.68^{a}	1.57^{d}	0.40^{h}	0.31^{j}	0.00^{k}	7.45^{b}	0.56^{f}	0.84^{e}	0.82^{e}	0.81^{e}
Total sugars	5.63^{d}	5.60^{d}	11.24^{a}	7.09^{c}	3.49^{k}	5.50^{e}	5.46^{f}	9.35^{b}	3.66^{j}	3.79^{h}	2.73^{l}	4.11^{g}
Oxalic acid	0.26^{f}	0.20^{k}	0.17^{l}	0.23^{h}	0.22^{j}	0.52^{b}	0.26^{g}	0.22^{j}	0.44^{c}	0.58^{a}	0.32^{e}	0.43^{d}
Maleic acid	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.01^{a}	0.01^{a}	0.00^{a}
Citric acid	0.10^{d}	0.04^{f}	0.06^{e}	0.00^{g}	0.00^{g}	0.00^{g}	0.00^{g}	0.00^{g}	0.00^{g}	0.14^{a}	0.11^{c}	0.12^{b}
Isocitric acid	0.63^{h}	0.70^{e}	0.68^{g}	0.48^{j}	0.30^{k}	0.49^{j}	0.28^{l}	1.78^{a}	0.70^{f}	1.14^{b}	0.89^{c}	0.78^{d}
Malic acid	0.34^{k}	0.60^{g}	0.86^{a}	0.67^{d}	0.52^{h}	0.69^{c}	0.66^{e}	0.22^{m}	0.64^{f}	0.50^{j}	0.70^{b}	0.26^{l}
Lactic acid	0.31^{b}	0.18^{f}	0.21^{e}	0.00^{g}	0.00^{g}	0.00^{g}	0.00^{g}	0.00^{g}	0.30^{d}	0.00^{g}	0.40^{a}	0.31^{c}
Fumaric acid	0.00^{h}	0.06^{c}	0.02^{f}	0.02^{ef}	0.03^{d}	0.02^{e}	0.02^{ef}	0.01^{g}	0.08^{a}	0.09^{a}	0.08^{b}	0.00^{h}
Adipic acid	0.05^{g}	0.14^{d}	0.18^{c}	0.05^{g}	0.00^{k}	0.04^{h}	0.01^{j}	0.00^{k}	0.10^{e}	0.33^{a}	0.26^{b}	0.06^{f}
Total organic acids	1.70^{f}	1.93^{d}	2.18^{c}	1.45^{g}	1.07^{j}	1.76^{e}	1.23^{h}	2.24^{b}	2.25^{b}	2.79^{a}	2.78^{a}	1.95^{d}

The sugar and organic acid contents (g/100 g FW) in 12 carrot varieties

Significant at $p \leq 0.05$

*Values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Duncan's test

highest glucose levels were detected in NWC (4.1 g/100 g FW) and MiPC (3.8 g/100 g FW), and the highest sucrose contents were found in NPC (9.7 g/100 g FW) and MiWC (7.5 g/100 g FW). Besides, sucrose is the principal storage sugar and the level increases with maturity [58]. According to Alasalvar et al. [26], sucrose is the main sugar of purple carrot and is followed by lower contents of fructose and glucose. Similar results were also observed in the present study. Mini- and normal-sized carrot samples had the highest sugar contents, while white carrot samples of each size were rich in sugars. Sugar accumulation during the maturation of carrot varieties is related to the flavours of carrots [56, 59], and total sugar contents of carrot, turnip, and radish roots increase with high CO_2. Thus, this feature is important in terms of climate change.

Organic acids are significant compounds of plants for biological pathways as well as human well-being, because organic acids support moderate antibacterial activities [60]. Eight organic acids, namely oxalic, maleic, citric, isocitric, malic, lactic, fumaric, and adipic acids, were observed in the coloured carrot varieties. Among these organic acids, only oxalic, isocitric, malic, and fumaric acids were observed in all carrot varieties. Additionally, Bryant and Overell [61] identified malic, fumaric, and isocitric acids in carrot roots, as well. The present study showed that micro-sized carrots and purple samples were rich in organic acid contents. Thus, flavour compounds, sugars, organic acids, and pH are the factors that determine the specific aromas of carrot varieties. Moreover, organic acid composition changes with climate, growth conditions, and varieties [62].

Nutritional and chemical components of carrot varieties

Table 2 presents the basic chemical compositions of the 12 carrot varieties. The carrot root samples significantly differed in their chemical compositions ($p \leq 0.05$).

Mean dry matter contents of the carrots ranged from 10.9 to 16.4%. Dry matter contents were the highest in NPC (16.4%) and NOC (15.1%), followed by MiWC (14.8%), MWC (14.7%), and MPC (14.4%). Gajewski et al. [63] studied different carrot varieties as well and confirmed the highest content of dry matter in purple carrots. Nevertheless, MiWC and MWC showed higher dry matter content than NWC. Thus, normal-sized white carrot holds more water than micro- and mini-white carrot samples.

In the present study, titratable acidity (TA) was determined as well. TA values ranged from 0.2 to 0.40 g/100 g FW. The highest TA was observed in MYC (0.4 g/100 g FW) and MPC (0.4 g/100 g FW), while the lowest results were observed for MiWC (0.2 g/100 g FW) and MiOC (0.2 g/100 g FW) samples. In terms of size and colour features of carrots, the micro-sized and purple-coloured carrot roots showed higher TA values. Moreover, TA influences flavour features of food products, and this parameter determines the pH and durability of carrot products. Therefore, high TA values in micro carrots make them much attractive for sensory characteristics and the food industry.

TA affects the pH of raw materials. The coloured carrot samples showed high pH values (> 5.3). Similar results were also noted in the literature [63]. In the present study, the pH

Table 2 Chemical results of carrot varieties

Type of carrot	Dry matter	Ash	Pectin	TA	pH
NOC	$15.06 \pm 0.34^{\ddagger b}$	0.78 ± 0.00^{hi}	1585.00 ± 0.18^{c}	0.27 ± 0.01^{f}	5.79 ± 0.04^{bcd}
NYC	11.81 ± 0.07^{de}	1.17 ± 0.04^{b}	1080.00 ± 0.01^{e}	0.25 ± 0.01^{g}	5.54 ± 0.01^{f}
NPC	16.39 ± 0.09^{a}	1.42 ± 0.03^{a}	935.00 ± 0.0^{ef}	0.35 ± 0.01^{c}	5.35 ± 0.02^{h}
NWC	10.92 ± 0.14^{f}	1.07 ± 0.01^{c}	995.00 ± 0.06^{ef}	0.24 ± 0.0^{gh}	5.42 ± 0.0^{gh}
MiOC	11.14 ± 0.72^{ef}	0.74 ± 0.06^{i}	0.00 ± 0.00^{g}	0.23 ± 0.0^{hi}	5.82 ± 0.0^{bc}
MiYC	12.27 ± 0.25^{d}	0.82 ± 0.06^{gh}	830.00 ± 0.00^{f}	0.27 ± 0.00^{f}	5.48 ± 0.0^{fg}
MiPC	12.39 ± 0.24^{d}	0.99 ± 0.05^{d}	140.00 ± 0.00^{g}	0.25 ± 0.00^{g}	5.69 ± 0.00^{e}
MiWC	14.82 ± 0.14^{b}	1.12 ± 0.02^{bc}	1310.00 ± 0.10^{d}	0.22 ± 0.01^{i}	6.04 ± 0.08^{a}
MOC	13.33 ± 0.46^{c}	0.87 ± 0.01^{fg}	1900.00 ± 0.00^{b}	0.31 ± 0.00^{d}	5.83 ± 0.00^{b}
MYC	13.27 ± 0.40^{c}	0.90 ± 0.00^{ef}	2190.00 ± 0.00^{a}	0.40 ± 0.00^{a}	5.99 ± 0.00^{a}
MPC	14.42 ± 0.59^{b}	0.95 ± 0.00^{de}	1430.00 ± 0.00^{cd}	0.38 ± 0.00^{b}	5.75 ± 0.0^{cde}
MWC	14.68 ± 0.04^{b}	0.80 ± 0.00^{ghi}	1380.00 ± 0.23^{d}	$0.29 \pm .0.00^{e}$	5.74 ± 0.05^{de}

Dry matter [%]; ash [%]; pectin (mg/100 g FW)

TA titratable acidity (g malic acid/100 g FW)

†Significant at $p \leq 0.05$

‡Values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Duncan's test

values of the carrots ranged from 5.4 to 6.0. The highest pH values were observed in MiWC (6.0), MYC (6.0), MOC (5.8), and MiOC (5.8). Moreover, the orange samples of different sizes showed much higher pH values (MOC: 5.8, MiOC: 5.8 and NOC: 5.8). According to Podsedek [64], the differences between samples are due to differences in varieties, soil pH, climate, growth, and storage conditions.

Pectins are subgroups of carbohydrates, provide functionalities to plant cell walls, and protect them against pathogens [65, 66]. However, enzymes in the human body cannot digest pectins, and gut bacteria help to reduce pectins which are important to prevent colon-related diseases [67]. In the present study, pectin levels ranged from 0 to 2190 mg/100 g FM in carrot samples. The highest content of pectin was seen in MYC (2190 mg/100 g FM) and MOC (1900 mg/100 g FM). In turn, MiOC showed no presence of pectin, and MiPC had a low amount of pectin content (140 mg/100 g FM). In the study, micro- and normal-carrot samples showed a high amount of pectins (from 1380 to 2190 mg/100 g FM and from 935 to 1585 mg/100 g FM, respectively); however, pectin contents were the lowest levels (from 0 to 1310 mg/100 g FM) in mini-carrot samples. According to Müller-Maatsch et al. [68], total pectin is divided into chelating agent soluble solids (CASS) and dilute alkaline soluble solids (DASS), and parsley has 47 mg/g of CASS. Besides, pectins of carrot, cabbage, and onion are sodium-carbonate soluble [69], and the solubility of pectin can alter with postharvest conditions of fruit and vegetables [70]. However, maturation is the main period of plants rising their water-soluble pectin content [71], because pectin fractions may eliminate reactive oxygen species (ROS) which are (non) free radicals of oxygen [72].

The other parameters analyzed in the study were minerals. They are essential for healthy body functions, and plants are good sources of minerals. Table 3 shows the contents of minerals in different varieties of carrots with significant differences. In the present study, it was observed that NYC and NOC were rich in Na; NPC and MYC showed the highest content of K; Ca was abundant in NWC and NYC; Fe was rich in MYC and MPC; and finally, the highest content of Mg was observed in MiYC and MYC. Normal-sized carrot samples showed higher mineral contents than micro- and mini-sized carrots; moreover, yellow carrots exhibited better results for mineral contents than carrots of other colours for each size. According to Nicolle et al. [41], K is the most abundant element in carrots, and a similar result was also observed in the present study. Additionally, obvious differences were noted when mineral values were compared with the ash contents of the carrots. NPC and NYC had the highest ash contents, while the lowest results were observed for MiOC and NOC. Thus, normal-sized carrots showed the highest results for ash content, and purple carrots exhibited the highest results for ash content in each size.

Antioxidant activity in carrot varieties

Table 4 presents the anti-oxidant activities of the 12 carrot varieties. Significant differences in anti-oxidant activities ($p \leq 0.05$) were observed in the carrot varieties.

Antioxidants inhibit reactive oxygen species that trigger the development of cancers, cardiovascular disorders, aging-related disorders, and other diseases [72]. Therefore, the anti-oxidant properties of plants make them a common food material for use in the food industry. Carrot is rich in

Table 3 Minerals of carrot varieties

Type of carrot	Macroelements Na	Macroelements K	Macroelements Ca	Macroelements Mg	Microelements Fe
NOC	378.0*^{b}	1773^{j}	23.3^{d}	24.4^{h}	1.5^{l}
NYC	494.0^{a}	1954^{c}	32.7^{b}	11.7^{k}	1.9^{k}
NPC	95.9^{k}	2400^{a}	27.3^{c}	3.8^{m}	2.2^{j}
NWC	372.0^{c}	1718^{l}	37.6^{a}	61.4^{e}	2.3^{h}
MiOC	102.0^{j}	1479^{m}	22.2^{e}	37.9^{g}	2.7^{f}
MiYC	355.0^{d}	1805^{g}	17.6^{l}	79.9^{a}	3.1^{e}
MiPC	274.0^{f}	1745^{k}	20.3^{j}	40.5^{f}	5.1^{d}
MiWC	301.0^{e}	1922^{d}	18.6^{k}	72.1^{c}	2.5^{g}
MOC	136.0^{h}	1782^{h}	20.3^{h}	3.9^{l}	5.3^{c}
MYC	86.1^{l}	2007^{b}	22.0^{f}	74.0^{b}	13.4^{a}
MPC	55.8^{m}	1820^{f}	20.3^{h}	14.2^{j}	12.9^{b}
MWC	169.0^{g}	1869^{e}	20.7^{g}	69.5^{d}	5.4^{c}

Minerals (mg/100 g dm)

Significant at $p \leq 0.05$

*Values (mean of three replications) followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Duncan's test

Table 4 The anti-oxidant activities [mmol TE/100 g dm] of carrot varieties

Type of carrot	ABTS	FRAP	ORAC
NOC	0.82 ± 0.03*^{fg}	0.71 ± 0.21^{e}	3.13 ± 0.19^{j}
NYC	0.48 ± 0.01^{g}	0.28 ± 0.01^{e}	2.52 ± 0.03^{l}
NPC	7.38 ± 0.39^{b}	4.60 ± 0.2^{b}	16.17 ± 1.03^{b}
NWC	0.46 ± 0.02^{g}	0.27 ± 0.03^{e}	2.84 ± 0.12^{k}
MiOC	2.42 ± 0.08^{d}	2.66 ± 0.76^{c}	4.28 ± 0.30^{g}
MiYC	0.96 ± 0.01^{f}	0.64 ± 0.02^{e}	3.86 ± 0.30^{h}
MiPC	7.93 ± 0.51^{a}	5.77 ± 0.17^{a}	17.06 ± 0.84^{a}
MiWC	0.81 ± 0.04^{fg}	0.58 ± 0.04^{e}	5.09 ± 0.20^{e}
MOC	1.02 ± 0.04^{f}	0.73 ± 0.02^{e}	5.93 ± 1.22^{d}
MYC	2.00 ± 0.14^{e}	1.62 ± 0.07^{d}	4.92 ± 0.11^{f}
MPC	3.98 ± 0.17^{c}	2.21 ± 0.63^{c}	15.42 ± 1.14^{c}
MWC	1.06 ± 0.03^{f}	0.82 ± 0.06^{e}	3.42 ± 0.13^{i}

TE Trolox equivalents

Significant at $p \leq 0.05$

*Values (mean of three replications) followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Duncan's test

phytochemicals such as phenolic compounds, carotenoids, and ascorbic acid that are essential as nutritional antioxidants in the human diet [73]. In the present study, in vitro anti-oxidant activity assays of all the carrot samples were quantified by ABTS, FRAP, and ORAC methods. Remarkable differences were observed in carrot varieties depending on colour, size, and/or the applied anti-oxidant activity techniques.

In the ABTS method, activities of carrots ranged from 0.5 to 7.9 mmol TE/100 g dm. The highest results were observed for MiPC (7.9 mmol TE/100 g dm) > > NPC (7.4 mmol TE/100 g dm) > > MPC (4.0 mmol TE/100 g dm). The lowest results were observed in NWC (0.5 mmol TE/100 g dm) and NYC (0.5 mmol TE/100 g dm). According to Singh et al. [74], the ABTS test showed the highest anti-oxidant activity in purple carrot. A similar result was observed in the present study, as well. Therefore, anti-oxidant activity showed a positive correlation with anthocyanins. Sun et al. [75] reported the same relationship.

In the FRAP test, anti-oxidant activities ranged from 0.3 to 5.8 mmol TE/100 g dm. The MiPC (5.8 mmol TE/100 g dm) showed the highest results, followed by NPC (4.6 mmol TE/100 g dm) and MiOC (2.7 mmol TE/100 g dm). The lowest result was observed for NWC (0.3 mmol TE/100 g dm) and NYC (0.3 mmol TE/100 g dm). Hence, mini-sized and purple carrots exhibit the highest anti-oxidant activities by FRAP. Singh et al. [74] also showed the highest results for purple carrot in the FRAP assay.

The ORAC assay also showed similar results. The highest results were again observed for MiPC (17.1 mmol TE/100 g dm), followed by NPC (16.2 mmol TE/100 g dm) and MPC (15.4 mmol TE/100 g dm). Thus, purple carrots showed the highest anti-oxidant activity results in the ORAC assay for each size. The lowest results were observed for NYC (2.5 mmol TE/100 g dm) and NWC (2.8 mmol TE/100 g dm). According to Nicolle et al. [41], the dark-coloured carrot demonstrated the highest anti-oxidant activity, and the white carrot showed the lowest activity in the ORAC assay. Therefore, dark-coloured carrots are rich in anthocyanins and thus exhibit strong anti-oxidant activities.

In the present study, the total carotenoid content did not correlate with the total anti-oxidant activity. Mech-Nowak

et al. [76] also reported similar results. According to Smeriglio et al. [77], the anti-oxidant activity of purple carrot results from anthocyanins and phenolic acids. Similar results were observed in the present study. Algarra et al. [78] compared the anti-oxidant activities of purple carrot and orange carrot growing in the same region and showed that purple carrot exhibited higher anti-oxidant activities than orange carrot depending on the content of anthocyanins.

Conclusions

The study evaluates the carrot varieties with different size and colour features for vitamin C as well as chemical contents (dry matter, ash, pectin, titratable acidity, and pH), sugar, organic acids, minerals (sodium, potassium, calcium, iron, and magnesium), and anti-oxidant activities. Besides, total polyphenolics and total tetraterpenoids of colourful carrot varieties were presented. According to study results, carrot samples were rich in fructose, sorbitol, glucose, and sucrose as sugars; citric, isocitric, maleic, malic, lactic, fumaric, oxalic and adipic acids were the organic acid compounds in coloured carrot samples. Moreover, sucrose was the dominant sugar and isocitric acid was the most common organic acid in carrot samples. Purple-coloured carrot samples demonstrated the highest results for total sugar, total organic acid, total polyphenolic contents, and anti-oxidant activities (Mini > Normal > Micro). In turn, the lowest results were observed in normal yellow carrot for total polyphenols, and anti-oxidant activities; besides, the lowest total tetraterpenoids were determined in micro-white carrot. However, normal-white carrot exhibited low anti-oxidant results for all three tests (ABTS, FRAP, and ORAC) and the lowest L-ascorbic acid content as well. The conducted study had shown that purple carrots are particularly interesting roots, they are sweet and colorful (they will arouse sensory interest), and additionally, they are rich in bioactive compounds (pectins, vitamin C, and polyphenolic compounds). Therefore, it is worth considering their use in the food industry, especially in the design of children's products.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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OŚWIADCZENIE

Oświadczam, że w pracy pt.:
Yusuf E., Tkacz K., Turkiewicz I.P. Wojdyło A., Nowicka, P. 2021. Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC. *European Food Research and Technology*, 247, 3053–3062. https://doi.org/10.1007/s00217-021-03857-0
mój udział polegał na tworzeniu planu badań, przygotowaniu materiału badawczego, przeprowadzeniu doświadczeń i analiz, z uwzględnieniem analizy zawartości cukrów z wykorzystaniem techniki HPLC-ELSD oraz kwasów organicznych z użyciem UPLC. Dodatkowo prowadziłam analizy podstawowych wyróżników składu chemicznego i właściwości przeciwutleniających metodami ABTS, FRAP i ORAC. Ponadto otrzymane wyniki opracowałam pod kątem statystycznym i merytorycznym, przygotowując manuskrypt, a także brałam udział w współredagowaniu tekstu w procesie recenzji.
Jestem także członkiem Interdyscyplinarnej Międzynarodowej Szkoły Doktorskiej na UPWr, współfinansowanej ze środków Europejskiego Funduszu Społecznego w ramach Programu Operacyjnego Wiedza Edukacja Rozwój, na podstawie umowy nr POWR.03.05.00-00-Z062/18 z dnia 4 czerwca 2019 r., która wsparła finansowo prezentowane wyniki.

potwierdzam zadeklarowane zaangażowanie
Emel Hasan Yusuf

Paulina Nowicka
02.06.2023

02.06.2023 ..... Emel Yusuf
data i podpis

DECLARATION

I declare that in the publication entitled:
Yusuf E., Tkacz K., Turkiewicz I.P. Wojdyło A., Nowicka, P. 2021. Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC. *European Food Research and Technology*, 247, 3053–3062. https://doi.org/10.1007/s00217-021-03857-0
my participation consisted in creating a research plan, preparing research material, conducting experiments and analyses, including the analysis of sugar content by using HPLC-ELSD technique, and the content of organic acids by using UPLC. In addition, I measured basic chemical parameters in carrot, and antioxidant activities by ABTS, FRAP, and ORAC methods. Moreover, I analyzed the obtained results in statistical and substantive terms, preparing the manuscript, and I also participated in the co-editing of the text in the review process.
I am also a member of Interdisciplinary International Doctoral School at UPWr, co-financed by the European Social Fund under the Operational Program Knowledge Education Development, under contract No. POWR.03.05.00-00-Z062/18 of June 4, 2019, which financially supported the presented results.

I confirm the indicated commitment
of Emel Hasan Yusuf

Paulina Nowicka
02.06.2023

02.06.2023 ..... Emel Yusuf
the date and sign

Publication 3

Yusuf E.H., Wojdyło A., Nowicka, P. 2023. Possibility to use the different sizes and colors of carrots for the production of juices – comparison of bioactive compounds, nutritional quality, pro-health properties, and sensory evaluation. Journal of the Science of Food and Agriculture, 103(2), 933-943.

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Possibility to use the different sizes and colours of carrots for the production of juices - comparison of bioactive compounds, nutritional quality, pro-health properties, and sensory evaluation

The carrot juices are as nutridrinks

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Abstract

BACKGROUND

Carrot is a popular vegetable consumed by people of all age groups and is used in various food products because of its high nutritional content, especially vitamin A.

RESULTS

In the present study, colourful fresh carrot juices of 12 carrot varieties were investigated for *in-vitro* antidiabetic, antiaging, and anti-obesity activities with antioxidant potential by ABTS and FRAP assays. The studied juices were also compared for physicochemical characteristics: titratable acidity, pH, pectin content, total soluble solids, dry mass, ash, viscosity, turbidity, osmolality and colour. The study results showed that normal purple carrot juice exhibited the best activities in all biological and antioxidant tests, except for anti-α-glucosidase activity. Normal purple carrot juice also had the highest total mineral content with elevated results for titratable acidity, pH, total soluble solids, dry mass, ash, viscosity, and osmolality.

CONCLUSION

Purple carrot juices demonstrated elevated health-promoting activities and could be used in blended beverage recipes to attract children's attention. The results of sensorial characteristics (appearance, colour and taste) of juices, however, showed that people are more familiar with orange carrot products.

Keywords: vegetable juices, carotenoids, polyphenolic compounds, LC/MS-QTof, UPLC-PDA, pro-health properties

INTRODUCTION

The history of carrots goes back to ancient times. Wild carrots originated from Asia, Persia and Afghanistan.[1] However, the history of cultivated carrots is uncertain, and Asian regions are thought to be the origin of cultivated carrots.[2] Carrot is used in various food products and/or beverages because of its high health benefits. The consumption of fresh carrot juice increases total antioxidant levels in adults; this may help to protect the cardiovascular system.[3] Additionally, clinical trials have shown that carrot juice provides various human health benefits in terms of protection against cancer and cardiovascular diseases, together with enhancement of immune system function.[4]

Fresh carrot juice is cloudy and has two phases: a dissolved solid phase (cloud) and a liquid phase (serum). The serum contains water-soluble compounds, and the cloud contains insoluble vegetable tissues that are rich in the protein-carbohydrate complex.[5] Carrot juice has neutral pH (~6), and it is more alkaline than apple and orange juices. A 240 mL of carrot juice provides 250% of vitamin A, 33% of vitamin C, 20.70% of vitamin E, 40% of vitamin K, 14% of potassium and phosphorus, and about 10% of protein, calcium, magnesium, fibre, iron, thiamine, zinc and riboflavin of recommended daily intake.[4] These characteristics of carrot juice perfectly fit into the new trend, where people are avoiding the consumption of sugar-added beverages and are giving more preference to consume sugar-free non-alcoholic fresh juice.

Thus far, carrot has been used to produce blended beverages with high nutritional values, such as pineapple, orange and carrot juice. Carrot juice has also been fortified with pomegranate peel extract, which increases the antimicrobial and antioxidant activity of the juice without altering its sensory features.[49] Carrot juice has also been combined with different fruit juices, for example, carrot-grape juice,[6] carrot with mixed berry juice,[7] apple-carrot juice,[8] carrot juice with orange peel and pulp extracts[9] and carrot juice with yoghurts.[10]

In the present study, 12 coloured carrot juices obtained from different carrot varieties were compared in terms of their content of bioactive compounds and health benefits; moreover, finding the most attractive carrot juice with high health-promoting activity and elevated product quality will help the fruit and vegetable industry to use the data for evaluating food materials as food ingredients and for creating novel food products to attract the interest of children. The present study is the first to estimate the content of polyphenols and carotenoids and to determine antioxidant and *in-vitro* biological activities of fresh carrot juices against noncommunicable diseases. The physicochemical characteristics of fresh carrot juices, namely pH, pectins, L-ascorbic acid, soluble solids, dry matter, ash, viscosity, turbidity, osmolality, sugar, organic acids, mineral content and colour, and their sensorial characteristics were also studied.

MATERIALS AND METHODS

Reagents and chemicals

Reagents for antioxidant and biological activity tests were purchased from Sigma-Aldrich (Steinheim, Germany). Standards for sugar, organic acids, carotenoids, chlorophylls and polyphenols were acquired from Extrasynthese (Lyon Nord, France). Acetonitrile, methanol and formic acid for ultra-performance liquid chromatography (UPLC; gradient grade) were purchased from Merck (Darmstadt, Germany).

Materials

Purchased commercial carrot varieties, namely 'yellow carrot (micro (MYCJ), mini (MiYCJ) and normal (NYCJ))', 'purple carrot (micro (MPCJ), mini (MiPCJ) and normal (NPCJ))', 'orange carrot (micro (MOCJ), mini (MiOCJ) and normal (NOCJ))', and 'white carrot (micro (MWCJ), mini (MiWCJ) and normal (NWCJ))', were used to produce fresh colourful carrot juices. Normal-sized carrots were between 20 mm and 45 mm in length and weighed 50 g to 150 g; mini-sized carrots were between 10 mm to 20 mm in length and weighed 8 g to 50 g;

micro-sized carrots were smaller than 10 mm in length and weighed less than 8 g. Thus, 12 fresh carrot juices were obtained.

Juicing technology

The 12 carrot varieties were washed and cut into small pieces and then 1% of ascorbic acid solution as 10% of the total weight of the carrot varieties was added; the carrot pieces were then disintegrated at 90 °C for 30 s in Thermomix (Vorwerk, Wuppertal, Germany). Juice was prepared using a laboratory hydraulic press (SRSE, Warsaw, Poland). The prepared carrot juices were pasteurised at 95 °C in Thermomix for 1 min. The juices were then bottled in sterilised glass jars, self-pasteurised for 10 min, cooled to room temperature (22 °C), and analysed (Fig. 1). Obtained carrot juices are shown in Figure 2.

Measurements of quality indices

Titratable acidity (TA) was determined by titration against 0.1 N NaOH to an endpoint of pH 8.1 by using an automatic pH titration system (pH meter type IQ 150; Warsaw, Poland), and the pH was measured with the same equipment as that used for TA. Pectin content was determined according to the method of Pijanowski et al.[11] The dry mass was obtained by mixing the sample with diatomaceous earth, pre-dried, and then final drying under reduced pressure. Total soluble solids (TSS) were measured using a portable refractometer (Atago RX-5000, Atago Co. Ltd., Saitama, Japan). One drop of juice was placed on the refractometer glass prism, and TSS was measured as 'Brix'. TA, dry matter and L-ascorbic acid were determined according to the following PN norms: PN-EN 12145:2001, PN-EN 12145:2000 and PN-A-04019, respectively. Ash (%), sugar, and organic acid content (g/100 mL) were evaluated according to Wojdyło et al.[12] Ash was measured by furnace method, individual sugars were determined by HPLC-ELSD and organic acids were determined by HPLC-PDA. The viscosity was measured with a rotation viscometer MC1 (DV-II+ PRO VISCOMETER, Brookfield, England) at 20 °C and expressed as mPas. The turbidity of carrot juices was determined by a

turbidimeter (Turbiquant 3000T, Merck, Darmstadt, Germany) by using 2.5 cm round cuvettes. Turbidity was expressed as a nephelometric turbidity unit (NTU).

%NTU = (Tc/To) x 100

To and **Tc** are the juice turbidities before and after centrifugation at 4200 x g for 15 min at 20 °C.

The osmotic strength of juices was evaluated by an osmometer. The mineral content of carrot juices was determined using an atomic absorption spectrophotometer AA-7000F/AAC SHIMADZU (Shimadzu Corporation). All measurements were performed in triplicate.

Instrumental colour measurement

Colour parameters (CIEL*a*b*) of carrot juices were quantified with a Color Quest XE Hunter Lab colourimeter (Reston, USA). The L* (lightness), a* (redness – greenness), and b* (yellowness – blueness) values were determined using CIE standard Illuminant D65 at 10° observer angle.

Qualitative and quantitative determination of phenolic compounds

Phenolic compounds of carrot juices were identified by LC/MS-Q-Tof (Waters, Manchester, UK). The retention times and spectra were compared with standards. The polyphenol content was quantified using external calibration curves by comparing with standards. Carrot juices were prepared for polyphenol analysis by LC/MS and UPLC according to Wojdyło et al.[12] Juice samples (2 mL) were mixed with 6 mL of HPLC-grade methanol:H_2O (30:70%, v/v), ascorbic acid (2%), and acetic acid (1%) of the reagent. After sonication (Sonic 6D, Polsonic, Warsaw, Poland) for 15 min, carrot juices were put into the fridge for 24 hours, then centrifugated at 19,000×g for 10 min. The supernatant was filtered through a hydrophilic PTFE (polytetrafluoroethylene) 0.20 μm membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for analysis. All samples were measured in triplicate and expressed as mg per 100 mL of juice.

Qualitative and quantitative determination of carotenoids

Carrot juices containing 10% $MgCO_3$ and 1% butylhydroxytoluene (BHT) were shaken with 5 mL of a ternary mixture of methanol/acetone/hexane (1:1:2, by vol.) at 300 rpm for 30 min in dark to prevent oxidation. Recovered supernatants were acquired after 4 times re-extraction from solid residue. Combined fractions were obtained after centrifugation (4 °C, 7 min at 19,000 *g*; MPW-350, Warsaw, Poland) and evaporated. The pellet was subtilised using 2 mL of 100% methanol, filtered through a 0.20-μm hydrophilic polytetrafluoroethylene (PTFE) membrane, and used for analysis. Carotenoids were analysed by LC-MS-Q/TOF (identification) and UPLC-PDA (quantification) on an ACQUITY UPLC BEH RP C18 column protected by a guard column of the same material at 30 °C. The elution solvents were a linear gradient of acetonitrile:methanol (70:30%, v/v) (A) and 0.1% formic acid (B) at the flow rate of 0.42 mL/min. The operation was monitored at 450 nm. All measurements were performed in triplicate and expressed as mg per 100 mL of carrot juices from different carrot varieties.

Determination of biological activities

Antioxidant activity of carrot juices was determined by ABTS and FRAP assays according to Re et al.[13] and Benzie & Strain,[14] respectively. Antioxidant powers of carrot juices were measured by reduction of ABTS+• radicals, and absorbances were read at 734 nm. The ferric reduction of juices was determined by FRAP, at low pH, colourless ferric complex (Fe3+-tripyridyltriazine) changes to a blue-coloured ferrous complex (Fe2+-tripyridyltriazine) by the action of electron-donating antioxidants and the absorbance was measured at 593 nm.

Anti-obesity and antidiabetic activities of carrot juices were analysed for the α-amylase, α-glucosidase,[15] and lipase inhibitory effects.[16] The α-amylase enzyme inhibition activity was performed for reactions of iodine with starch after incubation at 37 °C and absorbances were read at 600 nm. The α- glucosidase enzyme inhibition activity was performed for the reaction of the enzyme with β-D-glucosidase substrate and activity was evaluated at 405 nm. The

pancreatic lipase enzyme inhibition activity was performed with p-nitrophenol formed from p-nitrophenyl acetate after incubation at 37 °C, the activities were read at 400 nm.

The antiaging activity was evaluated with acetylcholinesterase and butyrylcholinesterase methods according to Gironés-Vilaplana et al.[17] The thiocholine reacts with 5,5′-dithiobis-(2-nitrobenzoic acid) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate. The substrates of acetylcholine iodine and butylcholine chloride were evaluated at 405 nm.

All tests, namely antioxidant (ABTS and FRAP), α-amylase, α-glucosidase, lipase inhibition effects, and anticholinergic activity, were performed in triplicate using a microplate reader (Synergy™ H1; BioTek, Winooski, VT, USA).

Sensory analysis of carrot juices

Sensory evaluation of carrot juices was performed using a 9 points hedonic scale (Like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely). Panellists voted the carrot juices for appearance, colour, consistency, smell and taste. The sensory analysis was performed on 11 of the 12 juice samples due to spoilling of MYCJ. Carrot juices were presented with codes for sensory evaluation, and the test was performed at room temperature. Juice samples were served in transparent, small plastic glasses. After each sample, panellists drank water to neutralise their mouths for the next sample.

Statistical analysis

Two-way analysis of variance (ANOVA, $p \leq 0.05$) and Duncan's test were performed using Statistica version 13.3 (Stat-Soft, Kraków, Poland). The results were expressed as mean value ($n = 3$) ± standard deviation (SD).

RESULTS AND DISCUSSION

Physicochemical parameters of carrot juices

The basic physicochemical compositions of the 12 carrot juices are presented in Table 1. The carrots showed significant differences ($p \leq 0.05$) in physicochemical compositions.

TA and pH are evaluated together for food analyses to determine food quality.[18] In the present study, the mean TA values ranged from 0.1 to 0.2 g/100 mL. The highest TA was noted for NPCJ (0.2 g/100 mL) and MPCJ (0.2 g/100 mL). The lowest TAs were noted for all other carrot juices. Moreover, there was a decrease in TA for fresh carrot juices as compared to that for raw carrot materials after juice processing.[19] The pH of the 12 carrot juices was approximately 5; the highest pH values were observed in NYCJ (5.9), NWCJ (5.9), and NPCJ (5.9), while the lowest pH value was detected in MPCJ (5.5). Alkaline pH is undesirable for natural ingredients during food processing because if the pasteurisation is not adequate in the absence of additives, microbial growth might occur in the food products.

Pectin is a significant component of fruits and vegetables as it decreases the fat content in the blood and protects against cardiovascular diseases.[20] In the present study, pectin content was determined in juices obtained from normal-sized carrots, MiOCJ and MiPCJs. The highest pectin content was observed in MiPCJ (0.4%) and NOCJ (0.2%). In the literature, the average pectin content of untreated carrot juice was reported as 0.51%, and after pre-treatment (hydraulic press), the pectin content reduced from 0.2% to 0.8%.[21] The small-scale changes in the pectin content of carrot juice after treatment are linked with pectin methylesterase, which is deactivated at approximately 80 °C.

Fruits and vegetables are the main sources of L-ascorbic acid and thus offer protection against cancer and cardiovascular diseases.[22] During the processing of fruits and vegetables, the content of L-ascorbic acid decreases with temperature.[23] In the present study, the same amount of L-ascorbic acid was added to all carrot juices. The content of L-ascorbic acid ranged from 4.8 to 59.2 mg/100 mL. The highest L-ascorbic acid content was observed in NYCJ (59.2 mg/100 mL) and MiYCJ (43.5 mg/100 mL), but the lowest content was detected in NPCJ (4.8

mg/100 mL) and MWCJ (9.9 mg/100 mL). Hence, the lowest L-ascorbic acid content in normal purple carrot juice might be related to carrot age. Mini-sized and yellow carrot juices showed elevated levels of L-ascorbic acid content.

TSS is another remarkable characteristic to determine the quality and stability of fresh juices in addition to TA and pH.[25] In the present study, TSS ranged from 7.2 to 9.4 Brix. The highest values were observed in NPCJ (9.4 Brix) and MiPCJ (9.1 Brix), while the lowest values were detected in NYCJ (7.2 Brix) and NWCJ (7.2 Brix). The highest dry matter and ash content was observed in NPCJ (9.4% and 1.0%, respectively) similar to TSS. The lowest dry mass and ash content was determined in NYCJ, NWCJ (7.3%) and MiOCJ (0.6%) respectively. Thus, mini-sized and purple carrot juices showed elevated levels of dry mass; while normal-sized yellow and purple fresh carrot juices showed attractive results for ash content, indicating that these juices had a high content of minerals.[26] Elevated ash and dry matter contents increase the nutritional value of carrot juices. However, the high content of dry matter causes a high level of turbidity in carrot juices, which is an unattractive feature for consumers. The appearance and colour stability of a product are important characteristics for consumers to purchase food products. Thus, the acceptance of carrot juice is inversely related to its high dry mass and turbidity levels.

In the present study, the colour, viscosity and turbidity of fresh carrot juices were also evaluated (Table 1). The molecular weight of hydrocolloid and viscosity exhibit a positive correlation,[27] and viscosity is a crucial parameter to determine the characteristics of liquid foods.[28] In the present study, the viscosity of fresh carrot juices ranged from 5.6 to 20.1 mPas. The highest results were observed for NWCJ (20.1 mPas) and MiPCJ (18.3 mPas), and the lowest viscosities were detected for NYCJ (5.6 mPas), MOCJ (6.8 mPas), and MiWCJ (6.8 mPas). Thus, normal-sized and purple carrot juices showed better results for viscosity. Viscosity measurement is required to determine heating and energy consumption rates of juices with

changes in concentration.[29] Moreover, viscosity influences the rheological properties of liquid food products depending on pectin content. The pasteurisation process is another factor that increases the viscosity of juices.[30] Thus, the high viscosity of carrot juices indicates that these juices are best for smoothie production when blended with different fruit or vegetable juices. Carrot juices with low viscosity values are best for manufacturing beverages.

Clear and cloudy fruit and vegetable juices show different turbidity levels, and cloudy juice products should have a turbidity value of more than 250 NTU before centrifugation.[31] In the present study, turbidity levels of carrot juices showed significant differences, and all juices exhibited turbidity levels higher than 250 NTU before centrifugation. Turbidity changes with shape, size, colour, and relative refractive indexes of fruit and vegetable juices.[32] According to Markowski et al.,[33] pectin content is the main reason for turbidity in fruit and vegetable juices. In the present study, samples with the highest turbidity had no pectin content, and the turbidity of carrot juices ranged from 5.1%NTU to 94.1%NTU. For stable turbidity in cloudy juices, the final NTU results after calculations should be higher than 50%NTU.[34] In the present study, the highest final turbidity levels were observed in MYCJ (94.1%NTU), MOCJ (73.4%NTU), MPCJ (70.8%NTU) and MWCJ (68.6%NTU). The lowest results were observed in NOCJ (5.1%NTU) and NWCJ (5.4%NTU). Thus, all micro-sized carrots seem to be most useful for producing cloudy juices.

The osmolality of fresh carrot juices was also measured in the present study. Osmolality is a method to measure the electrolyte-water balance of the body, [50] and determines the bioavailability of beverages for body hydration.[35] Besides, urea and ethanol like solutions are called ineffective osmoles which do not change water osmosis. However, glucose, Na^+, K^+, Cl^-, and HCO_3^- like solutes are called effective osmoles and they change the water osmosis through the cell membrane.[51] In the present study, the mean osmolality ranged from 446 to 520 mOsm/l. The highest osmolality was observed in MiPCJ (520 mOsm/l) and NPCJ (512

mOsm/l), while the lowest osmolality was recorded in MiYCJ (446 mOsm/l) and NOCJ (447 mOsm/l). All carrot juices were hypertonic solutions (>300 mOsm/l) with high amounts of electrolytes. Therefore, mini-sized and purple carrot juices had elevated levels of osmolality.

Colour is the first parameter for consumers to purchase fruit or vegetable juice. Anthocyanins and carotenoids are associated with the colour of fresh carrot juices.[25] In the present study, the colour parameters of juices are presented in Table 1. The mean lightness (L*) of fresh carrot juices ranged from 31.2 to 49.1. The highest L* values were observed in yellow carrot juices, while the lowest values were observed in purple carrot juices. Reduced lightness in juices results from high anthocyanin content in purple carrots.[36] Therefore, in the present study, the same results were observed for all purple carrot juice samples.

Redness-greenness (a*) and yellowness-blueness (b*) are the other colour parameters. In the present study, a* values ranged from 0.1 to 22.3. The highest a* values were observed in MiOCJ (22.3) and NOCJ (19.1), while the lowest a* values were detected in NWCJ (0.1) and MiWCJ (1.5). The mean b* values ranged from 1.6 to 32.7. The highest b* values were observed in NYCJ (32.7) and MiYCJ (29.8), while the lowest b* values were found in MiPCJ (1.6) and NPCJ (4.3). The lowest b* values are related to dark blue colours, as anthocyanins impart dark colours to purple carrot juices. Thus, purple carrot juices showed the lowest lightness (L*) and the lowest yellowness (b*).

Sugar, organic acid and mineral contents of carrot juices

The sugar and organic acids content of colourful carrot juices are shown in Table 2.

In the present study, high amounts of fructose, glucose and sucrose were determined in all carrot juices. The total sugar content of fresh carrot juices ranged from 2.0 to 10.5 g/100 mL. The mini-sized and orange carrot juices showed elevated results for total sugar content.

Organic acids are the other significant components that provide a specific smell and aroma to fresh fruit and vegetable juices.[37] Oxalic, malonic, and d-malic acids are the main organic acids of carrot juices. Oxalic acid demonstrates vigorous association with minerals like potassium, sodium, calcium and magnesium which are called oxalate salts. Sodium and potassium oxalate salts are water-soluble but, calcium oxalate is insoluble and forms kidney and urinary tract calcium oxalate crystals.[52] However, carrot has 35.6 mg/100 g of total oxalate,[53] and insoluble oxalate content in carrot reduces with boiling treatment.[24] Therefore, in the present study, the highest oxalic acid content was determined in MYCJ (3.0 g/100 mL) and followed by NWCJ (2.1 g/100 mL), however, the lowest oxalic acid contents were in MiOCJ (0.1 g/100 mL) and NOCJ (0.1 g/100 mL). On the other hand, a malic acid derivative has been detected in orange carrot juice[38] and purple carrot juice.[39] Malic acid naturally occurs in fruits and vegetables and has the characteristic of stimulating human metabolism.[40] In the present study, the highest content of d-malic acid was observed in MYCJ (0.4 g/100 mL) and NPCJ (0.2 g/100 mL); however, d-malic acid was not detected in NYCJ, NWCJ and MiPCJ. The total organic acid content was the highest in MYCJ, and MPCJ, in turn, the lowest in NOCJ, and MiPCJ. Thus, purple carrot juice exhibited the highest results for both TA and pH.

The mineral contents of carrot juices are given in Table 3. Mineral content may decrease with heating.[41] In the present study, the same trends were observed when the results were compared for the mineral content of raw carrot materials.[19] For instance, a normal orange carrot demonstrates 378.0 mg/100 g of Na, however, after juicing process the amount of Na in NOCJ was 33.0 mg/100 g. The highest total mineral content was observed in NPCJ (273.8 mg/100 mL) and NYCJ (259.00 mg/100 mL), while the lowest mineral content was observed in MiOCJ (196.8 mg/100 mL) and NOCJ (200.4 mg/100 mL). Thus, the present study showed that juices from different carrot varieties, especially normal-sized and purple carrot juices, had abundant content of minerals.

The polyphenol content of coloured carrot juices

The phenolic content of fresh carrot juices showed significant differences ($p \leq 0.05$) (Table 4). In the present study, 14 phenolic acids, 4 anthocyanins, and 5 flavan-3-ols were identified and quantified from the carrot juices.

The phenolic compounds identified in the present study were similar to those reported in the literature; however, some of them were identified for the first time in carrot juices. For example, 5-O-trans-caffeoylquinic acid, dicaffeoylquinic acid and O-q-coumaroylquinic acid have been detected in carrot juice,[42] while 4-caffeoylquinic acid, 5-caffeoylquinic acid, caffeic acid and ferulic acid were identified in a traditional beverage of purple carrot.[39] 3-O-caffeoylquinic acid, ferulic acid-hexoside, ferulic acid di-hexoside, 3-O-feruloylquinic acid, 5-O-feruloylquinic acid, di-ferulic acid derivative, diferuoylquinic acid derivative and 4-O-feruloylquinic acid were detected for the first time in the carrot juices.

In quantitative measurements of phenolic acids, chlorogenic acid was measured in all fresh carrot juices, except MiOCJ. The mean chlorogenic acid level ranged from 1.3 mg/100 mL (MWCJ) to 303.2 mg/100 mL (MiPCJ).

Ferulic acid di-hexoside was detected in NPCJ (23.6 mg/100 mL) and MPCJ (1.7 mg/100 mL); caffeic acid-hexoside was observed in NYCJ (0.9 mg/100 mL) and NOCJ (0.5 mg/100 mL); diferuoylquinic acid derivative was quantified in NPCJ (4.1 mg/100 mL) and MiYCJ (3.6 mg/100 mL); and ferulic acid was detected solely in NPCJ (1.5 mg/100 mL). In the present study, other phenolic acids were found in different amounts in fresh carrot juices.

The total phenolic acid content of carrot juices ranged from 9.5 to 440.5 mg/100 mL. The highest total phenolic acid content was observed in NPCJ (440.5 mg/100 mL) and MiPCJ (411.2 mg/100 mL), while the lowest content was detected in MWCJ (9.5 mg/100 mL) and NYCJ (32.6 mg/100 mL).

Four anthocyanins (cyanidin derivatives) were quantified in purple carrot juices. The identified anthocyanins were similar to those reported in the literature.[36] Cyanidin-3-O-xylosyl-glucosylgalactoside and cyanidin-3-O-xylosyl-cinpoyl-glucosylgalactoside were determined in two purple carrot juices (normal and mini). Cyanidin-3-O-xylosyl-galactoside was detected only in MiPCJ (31.7 mg/100 mL), while cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside was observed only in NPCJ (109.1 mg/100 mL).

In the present study, flavan-3-ols identified in carrot juices were procyanidin B1, procyanidin B2, (-)-epicatechin, (-)-epicatechin-gallate and procyanidin B4. Procyanidin B2 was identified in all carrot juices. The highest procyanidin B2 content was observed in MiPCJ (589.4 mg/100 mL), NPCJ (155.8 mg/100 mL) and NYCJ (81.6 mg/100 mL). However, (-)-epicatechin-gallate and procyanidin B4 were quantified only in MiYCJ (3.7 mg/100 mL) and NOCJ (2.4 mg/100 mL) respectively. The total catechin content was high in MiOCJ (691.3 mg/100 mL), MiPCJ (619.7 mg/100 mL) and NPCJ (181.2 mg/100 mL).

To summarize the polyphenolic contents of the 12 carrot juices, the highest total polyphenolic content was observed in MiPCJ (1087.6 mg/100 mL), MiOCJ (831.6 mg/100 mL) and NPCJ (793.7 mg/100 mL). However, the total polyphenolic content was the lowest in MWCJ (45.4 mg/100 mL), MYCJ (86.0 mg/100 mL) and NOCJ (90.0 mg/100 mL). Regarding the trends in carrot juices, mini-sized and purple carrot juices were rich in total phenolic content.

Carotenoid content of coloured carrot juices

The carotenoid content of carrot juices is shown in Table 4.

In the present study, six carotenoid compounds were identified. γ-Carotene was determined in all 12 fresh carrot juices. The highest content of γ-carotene was observed in NPCJ (8.3 mg/100 mL) and MiPCJ (5.1 mg/100 mL), while the lowest γ-carotene content was observed in MiWCJ (0.2 mg/100 mL), MWCJ (0.2 mg/100 mL) and NWCJ (0.3 mg/100 mL).

A previous study reported the presence of β-carotene and lutein in fresh carrot juice.[43] In the present study, the same amount of lutein was detected in NYCJ (0.1 mg/100 mL), NPCJ (0.1 mg/100 mL) and MiYCJ (0.1 mg/100 mL), while β-carotene was determined in NYCJ (1.8 mg/100 mL), MiYCJ (1.5 mg/100 mL) and MYCJ (0.9 mg/100 mL). β-Crytoxanthin was detected only in NPCJ (0.4 mg/100 mL).

The total carotenoid content of fresh carrot juices ranged from 0.2 to 14.8 mg/100 mL. The highest levels were determined in NPCJ (14.8 mg/100 mL) and MiPCJ (5.8 mg/100 mL), while the lowest results were observed in MiWCJ (0.2 mg/100 mL) and MWCJ (0.3 mg/100 mL). The total carotenoid contents of NPCJ (14.8 mg/100 g), NOCJ (5.1 mg/100 g) and MiPCJ (5.8 mg/100 g) did not change a lot after food processing when compared with the total carotenoid contents of NPC (19.07 mg/100 g), NOC (14.36 mg/100 g) and MiPC (4.76 mg/100 g) raw materials.[36] This might be attributed to the high content of antioxidants such as phenolic acids. Thus, normal-sized and purple carrot juices showed the highest total carotenoid content.

Antioxidant and biological activities of carrot juices

The results of antioxidant activity tests (ABTS and FRAP) of coloured fresh carrot juices are shown in Table 5.

The antioxidant activities of food products are based on the content of carotenoids, phenolic compounds, and ascorbic acid.[44] In the present study, according to the ABTS assay, the antioxidant activities ranged from 0.2 to 1.7 mmol Trolox/100 mL. The highest ABTS results were observed in MiYCJ, and MPCJ, in turn, the lowest antioxidant activities were observed in NWCJ and NOCJ.

The FRAP results ranged from 0.1 to 1.0 mmol Trolox/100 mL. The highest results were observed in NPCJ (1.0 mmol Trolox/100 mL) and MiPCJ (0.7 mmol Trolox/100 mL), while the lowest results (0.1 mmol Trolox/100 mL) were detected in NWCJ, MWCJ, MYCJ and MOCJ. Thus, both ABTS and FRAP methods showed similar results: mini-sized and purple

carrot juices revealed the highest antioxidant activities due to high phenolic acid, anthocyanins and carotenoid content.

Biological activities of carrot juices against α-amylase, α-glucosidase, and pancreatic lipase were evaluated by *in vitro* assays, and the results were expressed as IC_{50}, and the results of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were expressed as % inhibition. As shown in Table 5.

The inhibition of AChE and BuChE is reported to affect some central nervous system disorders such as Alzheimer's disease.[45] In the present study, the % inhibition of AChE ranged from 6.7% to 50.3%. The best AChE inhibition activities were observed in NYCJ (50.3%) and NPCJ (46.8%). As reported by Poudyal et al.,[46] the most effective inhibitory activity of purple carrot juice results from its high anthocyanins content; this finding was also observed in the present study. Thus, the highest AChE inhibition activity detected in NYCJ might be related to the high amounts of minerals, L-ascorbic acid, caffeic acid-hexoside, lutein and/or β-carotene.

The % inhibition of BuChE ranged from 2.5% to 14.1%. The highest inhibition of BuChE was observed in NPCJ, while MWCJ demonstrated the lowest inhibitory activity against BuChE. Thus, normal-sized and all coloured carrot juices showed elevated inhibitory activities against BuChE and AChE. This was followed by MiWCJ for anti-BuChE activity and by MOCJ for anti-AChE activity.

α-Amylase and α-glucosidase are responsible for carbohydrate digestion, and inhibition of these enzymes is known to control diabetes.[47] In the present study, the IC_{50} value for α-amylase inhibition ranged from 7.7 mg/mL to 1.6 mg/mL. The highest α-amylase inhibition activity was observed in NPCJ.

The IC_{50} value for α-glucosidase inhibition ranged from 0.6 mg/mL to 0.2 mg/mL. The highest α-glucosidase inhibition was observed in MiOCJ, and MYCJ, while the lowest inhibitory

activity was shown by NPCJ and MiYCJ. Thus, micro-sized and orange carrot juices showed the highest α-glucosidase inhibition.

Fresh carrot juices showed more potent anti-α-glucosidase activity than anti-α-amylase activity. Interestingly, NPCJ exhibited the best results for anti-α-amylase activity; however, the sample showed the lowest results for anti-α-glucosidase activity. NPCJ also showed attractive results in anti-aging tests. Thus, based on the results of the present study and when compared with the raw carrot samples, [36] carrot juices were found to exhibit increased anti-α-amylase and anti-α-glucosidase activities with applied technologies. This might be attributed to the conversion of sugar and pectin after juice processing.

Pancreatic lipase catalyses the conversion of fat molecules into fatty acids, bile salts, and fat-soluble vitamins.[48] In the present study, the anti-obesity activity of coloured carrot juices ranged from 0.7 mg/mL to 0.1 mg/mL. The highest inhibition of pancreatic lipase was detected in NPCJ, MiOCJ, MiWCJ and MPCJ. Purple carrot juice has been shown to affect lipid metabolism in rats.[46] The lowest anti-obesity activity was shown by NYCJ, NWCJ, and MiYCJ. The conducted studies showed that MOCJ exhibited the best results in all three tests; NPCJ showed elevated results for both anti-α-amylase and anti-obesity activities, but MiOCJ and MPCJ showed satisfactory results for both anti-α-glucosidase and anti-obesity activities. Thus, similar to anti-α-amylase and anti-α-glucosidase activities, the anti-obesity activities of carrot samples provide stunning values to fresh juice processing.

Sensorial features of carrot juices

The sensorial characteristics of the prepared coloured carrot juices are presented in Figure 3.

The fresh carrot juices were evaluated for their appearance, colour, consistency, smell and taste. In the present study, micro-sized yellow carrot juices could not be evaluated for the sensory test because the product quality was not good enough for consumption, and the possible reasons might be high pH and unexpected oxidation process. According to panellists,

MiOCJ (7.7), NOCJ (7.6) and MOCJ (7.4) exhibited satisfactory results for appearance. This might be attributed to the familiar orange colour of carrot. Moreover, NYCJ (7.5) ordered as the third-best coloured product.

Consistency is another important parameter for food choice. In the present study, four carrot juices (NYCJ, MOCJ, MiOCJ and MiPCJs) showed the same values for consistency (7.1). The lowest consistency was shown by NPCJ (6.2). The smell of food products attracts consumers and also influences their purchase preferences. In the present study, the highest attractive smell was observed for NYCJ (7.2) and NOCJ (7.1). Finally, the flavour was evaluated. The most attractive tastes were observed for NOCJ (7.7) and MOCJ (7.4). The reason might be the familiar taste of orange carrot, similar to its appearance and colour. The lowest taste acceptance was noted for MWCJ (5.3), MiWCJ (5.4) and MiYCJ (5.4).

To summarize, the appearance, consistency and colour of MiOCJ received the highest acceptance from all panellists. NYCJ showed the highest attractiveness for consistency and smell features. The most preferred tastes of fresh carrot juices were shown by NOCJ and MOCJs. Thus, traditional orange colour and taste were found to be the most attractive features to the panellists. Yellow carrot juice might be used for various food applications because of its favourable aroma.

CONCLUSIONS

The study compared the physicochemical characteristics and biological activities of 12 carrot juices according to size and colour. Normal purple carrot juice demonstrated the best activities for all biological and antioxidant tests, except for the α-glucosidase inhibition effect. The normal purple carrot juice showed the best results for total phenolic acid, anthocyanin, and carotenoid contents. Purple carrot juices can be used in beverage industries for producing smoothies and/or blended juices for increasing the health-promoting properties of liquid or semi-liquid food products. The sensorial acceptance of carrot juices relied mainly on orange

carrot varieties for all traits. Thus, the study recommends more applications of purple carrot juices for novel processed food products with high health benefits.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to influence the study.

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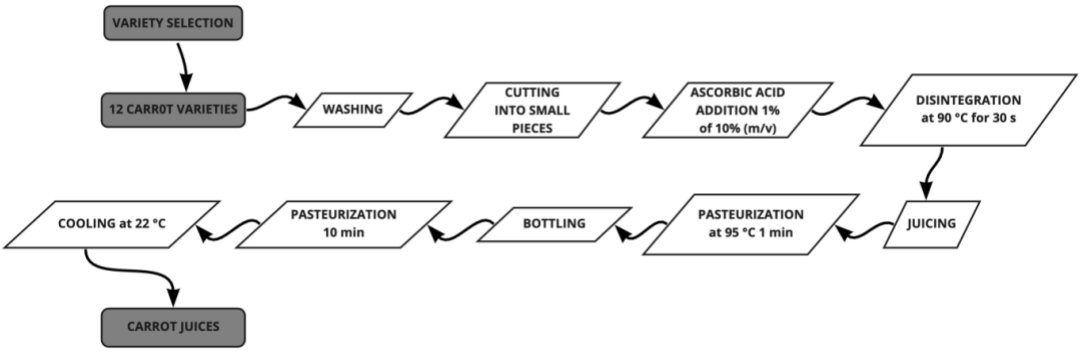

FIGURE LEGENDS

Figure 1. Applied technology to obtain coloured carrot juices

Figure 2. Carrot juices, 1-Micro yellow carrot juice (MYCJ); 2-Micro white carrot juice (MWCJ); 3- Micro orange carrot juice (MOCJ); 4-Normal white carrot juice (NWCJ); 5-Normal yellow carrot juice (NYCJ); 6-Mini yellow carrot juice (MiYCJ); 7- Mini white carrot juice (MiWCJ); 8-Micro purple carrot juice MPCJ; 9-Mini purple carrot juice (MiPCJ); 10-Normal orange carrot juice (NOCJ); 11-Normal purple carrot juice (NPCJ); 12-Mini orange carrot juice (MiOCJ)

Figure 3. Sensory results, 1-NYCJ, 2-NPCJ, 3-NWCJ, 4-NOCJ, 5-MOCJ, 6-MWCJ, 7-MPCJ, 8-MiWCJ, 9-MiOCJ, 10-MiPCJ, 11-MiYCJ

Figure 1.

Figure 2.

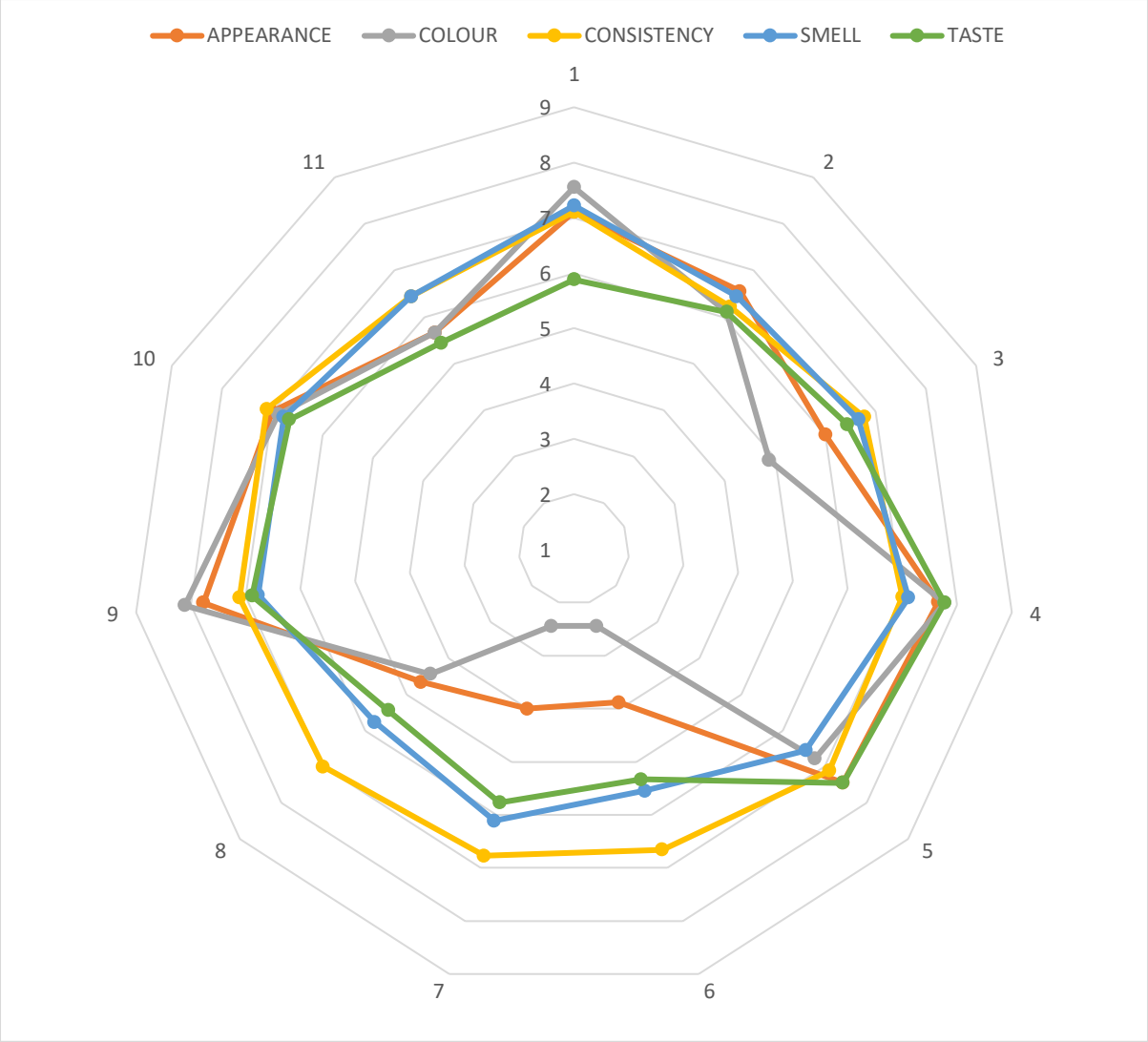
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Figure 3.

Table 1. Physicochemical parameters of 12 carrot juices

	TA	pH	Pectin	L-ascorbic acid	TSS	Dry mass	Ash	Viscosity	Turbidity	Osmolality	Colour Results		
											L *	a *	b *
MYCJ	0.1±0.0b	5.7±0.0c	nd	10.1±0.0e	7.7±0.3d	8.4±0.1c	0.7±0.1b	8.4±0.9c	94.1±2.1a	479±20.3d	42.4±0.0d	5.5±0.0de	19.4±0.0f
MiYCJ	0.1±0.0b	5.7±0.0c	nd	43.5±1.0b	7.6±0.3d	8.2±0.0d	0.7±0.0b	7.8±0.0c	9.5±0.1i	446±18.9g	48.4±0.0a	5.9±0.0d	29.8± 0.2b
NYCJ	0.1±0.0b	5.9±0.0a	nd	59.2±0.6a	7.2±0.3e	7.3±0.0e	0.9±0.0a	5.6±0.6c	9.1±0.6i	460±19.5f	49.1±0.2a	4.0±0.1fg	32.7±0.2a
MPCJ	0.2±0.0a	5.5±0.0e	nd	12.1±2.8e	8.2±0.3c	9.2±0.1a	0.7±0.1b	7.4±0.6c	70.8±0.1c	498±21.1c	36.6± 0.1e	5.4±0.0de	10.7±0.1i
MiPCJ	0.1±0.0b	5.7±0.0c	0.4±0.0a	17.6±3.6d	9.1±0.4ab	9.3±0.1a	0.7±0.1b	18.3±2.6ab	14.9±0.1g	520±22.1a	31.2±1.6f	2.1±1.3hi	1.6±0.3k
NPCJ	0.2±0.0a	5.9±0.1a	0.1±0.0c	4.8±0.1f	9.4±0.4a	9.4±0.1a	1.0±0.1a	16.4±0.6b	20.1±0.1f	512±21.7b	32.6±1.8f	3.0±1.4gh	4.3±0.2j
MOCJ	0.1±0.0b	5.7±0.0c	nd	10.1±3.6e	7.8±0.3d	8.4±0.1c	0.7±0.0b	6.8±0.6c	73.4±0.8b	471±20.0e	45.5±0.3c	17.3±0.1c	26.9±0.3d
MiOCJ	0.1±0.0b	5.8±0.0b	0.1±0.0c	31.3±2.2c	8.9±0.4b	8.9±0.1b	0.6±0.0b	7.5±1.7c	30.5±0.2e	499±21.2c	45.9±0.0c	22.3±0.1a	28.3±0.1c
NOCJ	0.1±0.0b	5.6±0.0d	0.2±0.0b	13.3±0.5e	7.6±0.3d	8.1±0.0d	0.7±0.0b	6.9±1.3c	5.1±0.2j	447±19.0g	46.5±0.1bc	19.1±0.1b	28.1±0.0c
MWCJ	0.1±0.0b	5.6±0.0d	nd	9.9±0.1e	7.8±0.3d	8.8±0.2b	0.8±0.1b	7.1±0.2c	68.6±1.1d	478±20.3d	41.7±0.2d	4.4±0.0ef	15.1±0.1h
MiWCJ	0.1±0.0b	5.7±0.0c	nd	30.4±0.4c	7.8±0.3d	8.0±0.1d	0.7±0.1b	6.8±0.2c	12.4±0.1h	497±21.1c	48.2±0.0a	1.5±0.0i	20.4±0.1e
NWCJ	0.1±0.0b	5.9±0.0a	0.1±0.1c	10.7±0.2e	7.2±0.3e	7.3±0.0e	0.8±0.0b	20.1±3.8a	5.4±0.1j	473±20.1e	47.8±0.4ab	0.1±0.4j	16.2±0.5g

TA (g oxalic acid/100 mL); Pectin [%]; L-ascorbic acid (mg/100 mL); TSS-Total Soluble Solids [Brix]; Dry mass [%]; Ash [%]; Viscosity [mPas]; Turbidity [%NTU]; Osmolality [mOsm/liter];
† significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Duncan's test. nd-Not detected.

Table 2. The sugar and organic acid contents of 12 carrot juices

	MYCJ	MiYCJ	NYCJ	MPCJ	MiPCJ	NPCJ	MOCJ	MiOCJ	NOCJ	MWCJ	MiWCJ	NWCJ
Fructose	1.6±0.1e	1.1±0.1g	0.8±0.0h	1.3±0.1f	2.4±0.1a	0.6±0.0j	1.8±0.1d	2.1±0.1c	2.2±0.1b	2.4±0.1a	0.4±0.0k	2.1±0.1c
Glucose	1.7±0.1f	1.4±0.1h	1.7±0.1f	1.2±0.1j	4.6±0.2b	0.9±0.0 l	1.6±0.1g	3.7±0.2c	3.5±0.2d	2.2±0.1e	1.0±0.0k	5.0±0.2a
Sucrose	1.0±0.0k	0.9±0.0 l	3.2±0.1d	1.6±0.1g	3.5±0.2c	5.2±0.2a	1.5±0.1h	4.0±0.2b	2.9±0.1e	1.9±0.1f	0.6±0.0m	1.4±0.1j
Σ Sugar	**4.2±0.2h**	**3.4±0.1k**	**5.8±0.3f**	**4.1±0.2j**	**10.5±0.4a**	**6.8±0.3d**	**4.8±0.2g**	**9.9±0.4b**	**8.5±0.4c**	**6.5±0.3e**	**2.0±0.1 l**	**8.5±0.4c**
Oxalic acid	3.0±0.1a	0.5±0.0h	0.6±0.0g	1.9±0.1c	0.3±0.0j	0.2±0.0k	0.8±0.0f	0.1±0.0 l	0.1±0.0 l	1.6±0.1d	1.1±0.0e	2.1±0.1b
Malonic acid	0.9±0.0a	0.5±0.0b	0.1±0.0f	0.5±0.0b	0.2±0.0e	0.2±0.0e	0.4±0.0c	0.4±0.0c	0.2±0.0e	0.3±0.0d	0.5±0.0b	0.1±0.0f
d-Malic acid	0.4±0.0a	0.1±0.0c	nd	0.1±0.0c	nd	0.2±0.0b	0.1±0.0c	0.1±0.0c	0.1±0.0c	0.1±0.0c	0.1±0.0c	nd
Σ Organic acid	**4.2±0.2a**	**1.1±0.0g**	**0.7±0.0h**	**2.5±0.1b**	**0.5±0.0k**	**0.6±0.0j**	**1.3±0.1f**	**0.6±0.0j**	**0.5±0.0k**	**2.0±0.1d**	**1.7±0.1e**	**2.2±0.1c**

The sugar (g/100 mL), and organic acid contents (g/100 mL) in 12 carrot juices; significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Duncan's test. nd-Not detected.

Table 3. The mineral contents of 12 carrot juices

	Na	K	Ca	Mg	Fe	Σ Mineral content
MYCJ	20.78±0.88g	190.77±8.09bc	4.61±0.20a	2.00±0.08a	1.25±0.05b	219.41±9.31 g
MiYCJ	45.58±1.93c	160.56±6.81d	1.02±0.04g	0.93±0.04cd	0.60±0.03e	208.68±8.85 k
NYCJ	80.46±3.41a	174.87±7.42cd	2.28±0.10cd	1.05±0.04b	0.33±0.01f	258.99±10.99 b
MPCJ	18.46±0.78g	222.29±9.43a	2.31±0.10cd	0.89±0.04c	1.77±0.07a	245.72±10.43 c
MiPCJ	36.25±1.54d	174.85±7.42cd	2.52±0.11c	0.97±0.04bc	0.95±0.04d	215.55±9.14 h
NPCJ	34.84±1.48d	234.50±9.95a	3.06±0.13b	0.88±0.04c	0.54±0.02e	273.82±11.62 a
MOCJ	19.86±0.84g	189.41±8.04bc	1.25±0.05f	0.72±0.03e	0.61±0.03e	211.85±8.99 j
MiOCJ	29.63±1.26e	163.84±6.95d	1.64±0.07e	1.05±0.04b	0.60±0.03e	196.77±8.35 m
NOCJ	32.99±1.40de	163.92±6.95d	2.41±0.10c	0.87±0.04c	0.22±0.01g	200.40±8.50 l
MWCJ	24.75±1.05f	196.01±8.32b	1.89±0.08d	0.84±0.04d	1.04±0.04c	224.53±9.53 f
MiWCJ	51.43±2.18b	187.67±7.96bc	1.29±0.05f	0.87±0.04c	0.58±0.02e	241.85±10.26 d
NWCJ	35.94±1.52d	188.14±7.98bc	2.11±0.09d	0.86±0.04d	0.26±0.01fg	227.32±9.64 e

The content of minerals (mg/100 mL) in 12 carrot juices; significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Duncan's test

Table 4. Phenolic, and carotenoid, contents of carrot juices

	MYCJ	MiYCJ	NYCJ	MPCJ	MiPCJ	NPCJ	MOCJ	MiOCJ	NOCJ	MWCJ	MiWCJ	NWCJ
3-O-caffeoylquinic acid	nd	nd	1.6±0.1h	25.1±1.1b	21.5±0.9c	34.7±1.5a	9.0±0.4e	14.5±0.6d	2.2±0.1h	0.7±0.0h	7.5±0.3f	3.6±0.2g
5-O-caffeoylquinic acid	25.3±1.1e	77.0±3.3c	19.1±0.8e	82.2±3.5c	303.2±12.9a	233.9±9.9b	52.7±2.2d	nd	26.2±1.1e	1.3±0.1f	59.7±2.5d	22.8±1.0e
4-O-caffeoylquinic acid	4.3±0.2d	17.5±0.7c	nd	nd	25.4±1.1b	46.7±2.0a	nd	nd	nd	nd	nd	nd
Ferulic acid-hexoside	nd	nd	2.7±0.1g	18.5±0.8a	10.7±0.5e	14.0±0.6c	12.1±0.5d	16.1±0.7b	4.8±0.2f	0.3±0.0h	14.5±0.6c	nd
Ferulic acid di-hexoside	nd	nd	nd	1.7±0.1b	nd	23.6±1.0a	nd	nd	nd	nd	nd	nd
3-O-Feruloylquinic acid	nd	3.4±0.1b	1.0±0.0cd	1.1±0.0cd	nd	19.9±0.8a	nd	nd	0.6±0.0d	0.7±0.0d	nd	1.5±0.1c
O-q-coumaroylquinic acid	nd	nd	nd	24.9±1.1a	22.1±0.9b	7.5±0.3c	nd	2.2±0.1e	nd	nd	3.2±0.1d	nd
5-O-Feruloylquinic acid	0.9±0.0d	1.2±0.0c	nd	1.3±0.1c	3.0±0.1b	nd	nd	nd	nd	nd	3.5±0.1a	nd
Dicaffeoylquinic acid derivative	nd	nd	nd	nd	nd	10.5±0.4a	nd	nd	0.9±0.0d	1.8±0.1c	6.4±0.3b	0.9±0.0d
Di-ferulic acid derivative	5.0±0.2g	49.9±2.1b	2.0±0.1h	17.1±0.7f	20.0±0.8e	5.1±0.2g	32.4±1.4d	56.2±2.4a	14.9±0.6f	0.3±0.0h	41.3±1.8c	6.4±0.3g
Ferulic acid	nd	nd	nd	nd	nd	1.5±0.1a	nd	nd	nd	nd	nd	nd
Diferuoylquinic acid derivative	nd	3.6±0.2b	nd	nd	nd	4.1±0.2a	nd	nd	nd	nd	nd	nd
4-O-Feruloylquinic acid	6.6±0.3d	16.5±0.7a	2.1±0.1g	nd	nd	nd	12.7±0.5b	16.0±0.7a	3.3±0.1f	0.5±0.0h	9.0±0.4c	5.0±0.2e
Caffeic acid-hexoside	nd	nd	0.9±0.0a	nd	nd	nd	nd	nd	0.5±0.0b	nd	nd	nd
Others	11.5±0.5e	27.5±1.2c	3.1±0.1h	19.6±0.8d	5.3±0.2g	39.0±1.7a	7.4±0.3f	35.3±1.5b	1.1±0.0j	3.8±0.2gh	11.2±0.5e	10.1±0.4e
Σ Phenolic acids	**53.7±2.3f**	**196.5±8.3c**	**32.6±1.4g**	**191.6±8.1c**	**411.2±17.4b**	**440.5±18.7a**	**126.3±5.4e**	**140.3±6.0de**	**54.5±2.3f**	**9.5±0.4h**	**156.3±6.6d**	**50.3±2.1fg**
Cyanidin-3-O-xylosyl-glucosylgalactoside	nd	nd	nd	nd	14.9±0.6b	18.6±0.8a	nd	nd	nd	nd	nd	nd
Cyanidin-3-O-xylosyl-galactoside	nd	nd	nd	nd	31.7±1.3a	nd	nd	nd	nd	nd	nd	nd
Cyanidin-3-O-xylosyl-cinpoyl-glucosylgalactoside	nd	nd	nd	nd	10.0±0.4b	44.3±1.9a	nd	nd	nd	nd	nd	nd
Cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside	nd	nd	nd	nd	nd	109.1±4.6a	nd	nd	nd	nd	nd	nd

Σ Anthocyanins					**56.7±2.4b**	**172.0±7.3a**						
Procyanidin B1	nd	nd	7.7±0.3b	nd	nd	nd	nd	656.1±27.8a	4.4±0.2b	7.5±0.3b	14.6±0.6b	6.6±0.3b
Procyanidin B2	26.1±1.1ef	60.5±2.6d	81.6±3.5c	27.8±1.2ef	589.4±25.0a	155.8±6.6b	22.3±0.9ef	35.2±1.5e	25.7±1.1ef	21.6±0.9ef	14.4±0.6f	68.9±2.9cd
(-)-Epicatechin	6.2±0.3cd	5.7±0.2d	nd	8.4±0.4b	30.3±1.3a	nd	6.8±0.3c	nd	3.1±0.1f	6.8±0.3c	nd	4.6±0.2e
(-)-Epicatechin-gallate	nd	3.7±0.2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Procyanidin B4	nd	nd	nd	nd	nd	nd	nd	nd	2.4±0.1a	nd	nd	nd
Others	nd	nd	nd	nd	nd	25.4±1.1a	nd	nd	nd	nd	nd	nd
Σ Flavan-3-ols	**32.3±1.4e**	**69.9±3.0d**	**89.3±3.8d**	**36.2±1.5e**	**619.7±26.3b**	**181.2±7.7c**	**29.1±1.2e**	**691.3±29.3a**	**35.5±1.5e**	**35.9±1.5e**	**29.0±1.2e**	**80.1±3.4d**
Σ Polyphenolic Content	**86.0±3.6fg**	**266.4±11.3c**	**121.9±5.2efg**	**227.8±9.7cd**	**1087.6±46.1a**	**793.7±33.7b**	**155.4±6.6def**	**831.6±35.3b**	**90±3.8fg**	**45.4±1.9g**	**185.3±7.9de**	**130.4±5.5ef**
Lutein	nd	0.1±0.0a	0.1±0.0a	nd	nd	0.1±0.0a	nd	nd	nd	nd	nd	nd
β-cryptoxanthin	nd	nd	nd	nd	nd	0.4±0.0a	nd	nd	nd	nd	nd	nd
(6R)-δ-carotene	nd	nd	nd	0.2±0.0d	0.3±0.0c	0.8±0.0a	0.2±0.0d	0.2±0.0d	0.5±0.0b	nd	nd	nd
γ-carotene	0.9±0.0j	1.7±0.1g	1.5±0.1h	4.2±0.2c	5.1±0.2b	8.3±0.4a	1.9±0.1f	2.2±0.1e	4.0±0.2d	0.3±0.0k	0.2±0.0 l	0.3±0.0k
ε-carotene	nd	0.2±0.0e	0.2±0.0e	0.4±0.0c	0.4±0.0c	5.2±0.2a	0.3±0.0d	0.4±0.0c	0.6±0.0b	nd	nd	0.1±0.0f
β-carotene	0.9±0.0c	1.5±0.1b	1.8±0.1a	nd	nd	nd	nd	nd	nd	nd	nd	nd
Σ Carotenoids	**1.8±0.1f**	**3.5±0.1d**	**3.6±0.2d**	**4.8±0.2c**	**5.8±0.2b**	**14.8±0.6a**	**2.4±0.1ef**	**2.8±0.1de**	**5.1±0.2bc**	**0.3±0.0g**	**0.2±0.0g**	**0.4±0.0g**

Polyphenols, and carotenoids-mg/100 mL; significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Duncan's test. nd-Not detected.

Table 5. The antioxidant [mmol TE/100 mL] and *in vitro* inhibition activities (Acetylcholinesterase-AChE, butyrylcholinesterase-BuChE, α-amylase, α-glucosidase, pancreatic lipase) of coloured carrot juices

	AChE[% of inhibition]	BuChE [% of inhibition]	α-amylase inhibition effect [IC_{50}]	α-glucosidase inhibition effect [IC_{50}]	Lipase inhibition effect [IC_{50}]	Antioxidant activity ABTS	Antioxidant activity FRAP
MYCJ	10.5±0.4j	5.4±0.2g	2.1±0.1j	0.2±0.0j	0.4±0.0b	0.8±0.0e	0.1±0.0f
MiYCJ	11.5±0.5h	4.0±0.2h	2.2±0.1j	0.6±0.0a	0.3±0.0c	1.7±0.1a	0.4±0.0c
NYCJ	50.3±2.1a	9.9±0.4b	7.7±0.3a	0.4±0.0f	0.7±0.0a	0.5±0.1f	0.3±0.0d
MPCJ	8.6±0.4 l	6.2±0.3f	7.3±0.3b	0.3±0.0h	0.1±0.0e	1.6±0.1a	0.4±0.0c
MiPCJ	18.9±0.8g	5.4±0.2g	2.9±0.1h	0.5±0.0c	0.2±0.0d	1.0±0.0d	0.7±0.1b
NPCJ	46.8±2.0b	14.1±0.6a	1.6±0.1 l	0.6±0.0a	0.1±0.0e	1.4± 0.1b	1.0±0.0a
MOCJ	26.2±1.1e	9.0±0.4c	2.1±0.1k	0.3±0.0h	0.2±0.0d	0.8±0.2e	0.1±0.0f
MiOCJ	9.6±0.4k	3.2±0.1j	4.9±0.2e	0.2±0.0j	0.1±0.0e	0.6±0.0f	0.4±0.0c
NOCJ	40.8±1.7c	9.0±0.4c	4.3±0.2f	0.4±0.0e	0.3±0.0c	0.2±0.0g	0.2±0.0e
MWCJ	23.7±1.0f	2.5±0.1k	3.9±0.2g	0.4±0.0d	0.2±0.0d	0.6±0.0f	0.1±0.0f
MiWCJ	6.7±0.3m	7.2±0.3e	6.7±0.3c	0.3±0.0g	0.1±0.0e	1.2±0.1c	0.3±0.0d
NWCJ	34.6±1.5d	7.8±0.3d	5.8±0.2d	0.5±0.0b	0.4±0.0b	0.2±0.0g	0.1±0.0f

TE-Trolox equivalents, significant at $p \leq 0.05$; ‡ values (mean of three replications) followed by the same letter within the same column was not significantly different ($p \leq 0.05$) according to Duncan's test.

Wrocław, 31.05.2023r.

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OŚWIADCZENIE

Oświadczam, że w pracy pt.:
Yusuf E.H., Wojdyło A., Nowicka, P. 2023. Possibility to use the different sizes and colors of carrots for the production of juices – comparison of bioactive compounds, nutritional quality, pro-health properties, and sensory evaluation. *Journal of the Science of Food and Agriculture,* 103(2), 933-943.https://doi.org/10.1002/jsfa.12206
mój udział polegał na tworzeniu planu badań, przygotowaniu materiału badawczego i opracowaniu technologii produkcji soków na bazie różnych wariantów marchewki, przeprowadzeniu doświadczeń i analiz, z uwzględnieniem analizy zawartości cukrów z wykorzystaniem techniki HPLC-ELSD, kwasów organicznych z użyciem UPLC, a także zawartości związków bioaktywnych (LC/MS i UPLC) oraz właściwości prozdrowotnych *in vitro*. Dodatkowo prowadziłam analizy podstawowych wyróżników składu chemicznego i właściwości sensorycznych otrzymanych soków. Ponadto otrzymane wyniki opracowałam pod kątem statystycznym i merytorycznym, przygotowując manuskrypt, a także brałam udział w współredagowaniu tekstu w procesie recenzji.
Jestem także członkiem Interdyscyplinarnej Międzynarodowej Szkoły Doktorskiej na UPWr, współfinansowanej ze środków Europejskiego Funduszu Społecznego w ramach Programu Operacyjnego Wiedza Edukacja Rozwój, na podstawie umowy nr POWR.03.05.00-00-Z062/18 z dnia 4 czerwca 2019 r., która wsparła finansowo prezentowane wyniki.

potwierdzam zadeklarowane zaangażowanie
Emel Hasan Yusuf
Pauline Nowicka
02.06.2023

02.06.2023 .....
data i podpis

DECLARATION

I declare that in the publication entitled:
Yusuf E.H., Wojdyło A., Nowicka, P. 2023. Possibility to use the different sizes and colors of carrots for the production of juices – comparison of bioactive compounds, nutritional quality, pro-health properties, and sensory evaluation. *Journal of the Science of Food and Agriculture,* 103(2), 933-943.https://doi.org/10.1002/jsfa.12206
my participation consisted in creating a research plan, preparing research material, and juice processing, conducting experiments and analyses, including the analysis of sugar content by using HPLC-ELSD technique, the content of organic acids by using UPLC, the content of bioactive compounds (by LC/MS and UPLC), and also pro-health properties by *in vitro* methods. In addition, I measured basic chemical parameters in carrot juices, and them sensory evaluation. Moreover, I analyzed the obtained results in statistical and substantive terms, preparing the manuscript, and I also participated in the co-editing of the text in the review process.
I am also a member of Interdisciplinary International Doctoral School at UPWr, co-financed by the European Social Fund under the Operational Program Knowledge Education Development, under contract No. POWR.03.05.00-00-Z062/18 of June 4, 2019, which financially supported the presented results.

I confirm the indicated commitment
of Emel Hasan Yusuf
Pauline Nowicka
02.06.2023

02.06.2023 .....
the date and sign

Publication 4

Yusuf E.H., Wojdyło A., Bourbon A.I., Nowicka, P. 2023. Fruit-carrot-based smoothies as innovative products with a complex matrix of bioactive compounds effected on activities of selected digestive enzymes and cholinesterases in vitro. Antioxidants, 12(4), 917.

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antioxidants

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Article

Fruit–Carrot-Based Smoothies as Innovative Products with a Complex Matrix of Bioactive Compounds Effected on Activities of Selected Digestive Enzymes and Cholinesterases In Vitro

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Abstract: In this study, four different carrot varieties (purple, yellow, white, and orange) were used in the production of smoothies with raspberry, apple, pear, strawberry, and sour cherry juices. The in vitro inhibition effects against α- amylase, α- glucosidase, pancreatic lipase, acetylcholinesterase, and butyrylcholinesterase were measured, bioactive compounds, physicochemical characteristics, including sensorial features were described. The antioxidant activities of the studied samples were analyzed using the ORAC, ABTS, and FRAP methods. The raspberry–purple carrot smoothie showed the highest antioxidant activity against lipase and butyrylcholinesterase enzyme activity. The sour cherry–purple carrot smoothie showed the highest total soluble solids, total phenolic acid, total anthocyanins, and procyanidin contents; dry mass; and osmolality. Although the apple–white carrot smoothie achieved the highest acceptance after sensorial evaluation, it did not exhibit any potent biological activities. Thus, food products with purple carrot, raspberry, and sour cherry ingredients are suggested as functional and/or novel matrix compositions with high antioxidant potential.

Keywords: polyphenolic compounds; carotenoids; pro-health properties; sensory evaluation; fruit

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1. Introduction

In this era of high-speed technology and fast-food preferences, people do not have enough time to download an application on their PC and/or wait for the food or beverages ordered. Furthermore, the amount of fruit or vegetables consumed is insufficient to acquire essential vitamins and minerals that boost the immune system against viruses and/or microorganisms.

To boost the immune system and healthy body functions, fruit–vegetable juices, nectars, or smoothies, which can be easily consumed, are preferred instead of raw fruit and vegetables. A wide range of beverages with different ingredients is available in the market. Many of them have been investigated as functional beverages, such as sea buckthorn [1], pumpkin, and purple carrot (PC) blended smoothies [2]. In a research study, a carrot-blended tomato smoothie was applied with pumpkin, lemon juice, mineral water, and marine salt [3], which was found to increase the lycopene and carotene contents, with increased bioavailability. Thus, carrot-based smoothies can be a good choice for healthy beverage alternatives.

Carrot is a popular vegetable with high nutritional contents such as carotenoids, which are vitamin A precursors and essential for cell regulation and eyesight [4]; polyphenolics—phenolic acids and flavan-3-ols (orange, white, yellow, and purple carrots); and in the case of purple carrot, also anthocyanins, which are effective against aging, diabetes, cardiovascular diseases, cancers, and neurological disorders [5]. Moreover, the high vitamin and mineral contents of carrot might help reduce food-related deficiencies

and are responsible for the proper conduct of biochemical reactions and functioning of the human body. Carrots are also a good source of dietary fiber (both soluble and insoluble fraction), which influences the inhibition of the absorption of dietary fats and their collection in the tissues of the liver. In addition, it influences lower blood glucose levels and improves peristalsis.

Nevertheless, children may not desire to consume healthy carrot beverages for several reasons, such as taste, smell, and/or appearance. As role models, parents should support their children in consuming more healthy beverages. Fruit and vegetable snacks are not only healthy but also have additional benefits as they do not contain gluten, which is harmful to people with celiac disease [6]. Hence, plant-based ingredients might be a good alternative for all age groups for snacking.

Therefore, the aim of this study was to use purple (PC), yellow (YC), orange (OC), and white carrot (WC) varieties to produce smoothies with raspberry (RJ), apple (AJ), pear (PJ), strawberry (SJ), and sour cherry (SCJ) juices (the most popular fruit in Poland from the *Rosaceae* family), and to investigate the resulting smoothies with respect to the physicochemical characteristics, sensorial characteristics, bioactive compounds, and in vitro pro-health properties. Thus, the formulated research objective allowed for complete verification of the research hypothesis which assumes that the carrot can be a functional base in the production of smoothies dedicated to children in the form of a snack or a second breakfast, and that the addition of fruits from the *Rosaceae* family to the carrot base can shape the sensory and health-promoting value of the finished products, making them more desirable by the indicated target group. Furthermore, the research presented in this manuscript allows for the identification of the optimal directions for the use and application of carrots in the diet, industry, and food service. Consequently, the presented results may in the future bring measurable health-related and economic benefits worldwide.

2. Materials and Methods

2.1. The Technology of Smoothie Preparation

The PC, OC, YC, and WC varieties were purchased from Fusion Gusto (Dabrowa, Poland). Raspberry (*Rubus idaeus*), apple (*Malus domestica*), pear (*Pyrus communis*), strawberry (*Fragaria* ^{x} *ananassa*), and sour cherry (*Prunus cerasus*) were purchased from retail markets in Poland. These fruits were chosen because (i) all of them belong to the *Rosaceae* family; (ii) they are very popular and liked across the world; (iii) they mask undesirable flavors and appearance well; and (iv) they are readily available; therefore, all recipes can be reproduced easily.

Fruit juices were produced freshly using a laboratory hydraulic press (SRSE, Warsaw, Poland) and stored in a freezer until the production of carrot-based smoothies.

Raw carrot materials were washed and sliced, and 1% L-ascorbic acid (10% *w*/*v*) was added to the carrot slices and disintegrated for 30 s in a Thermomix (Vorwerk, Wuppertal, Germany). The carrot purees thus prepared were pasteurized at 95 °C in the Thermomix for 1 min and then mixed with fruit juices in a 1:1 ratio. The mixtures were mixed at 90 °C for 30 s in the Thermomix and pasteurized at 95 °C for 1 min. The resulting smoothies were transferred to sterilized glass jars, self-pasteurized for 10 min, and cooled to room temperature (20 °C) until further analysis. The 20 carrot-blended smoothies obtained and the four carrot purees (control groups) are shown in Figure 1.

Figure 1. Prepared carrot blended smoothies (1, raspberry juice with purple carrot puree (RJ–PC); 2, apple juice with purple carrot puree (AJ–PC); 3, pear juice with purple carrot puree (PJ–PC); 4, strawberry juice with purple carrot puree (SJ–PC); 5, sour cherry juice with purple carrot puree (SCJ–PC); 6, purple carrot puree %100 (PC%100); 7, raspberry juice with white carrot puree (RJ–WC); 8, apple juice with white carrot puree (AJ–WC); 9, pear juice with white carrot puree (PJ–WC); 10, strawberry juice with white carrot puree (SJ–WC); 11, sour cherry juice with white carrot puree (SCJ–WC); 12, white carrot puree %100 (WC%100); 13, raspberry juice with yellow carrot puree (RJ–YC); 14, apple juice with yellow carrot puree (AJ–YC); 15, pear juice with yellow carrot puree (PJ–YC); 16, strawberry juice with yellow carrot puree (SJ–YC); 17, sour cherry juice with yellow carrot puree (SCJ–YC); 18, yellow carrot puree %100 (YC%100); 19, raspberry juice with orange carrot puree (RJ–OC); 20, apple juice with orange carrot puree (AJ–OC); 21, pear juice with orange carrot puree (PJ–OC); 22, strawberry juice with orange carrot puree (SJ–OC); 23, sour cherry juice with orange carrot puree (SCJ–OC); 24, orange carrot puree %100 (OC%100)).

2.2. Physicochemical Characteristics

The L-ascorbic acid content of the samples was determined according to the PN-A-04019. The TSS content was measured using a portable refractometer (Atago RX-5000, Atago Co. Ltd., Saitama, Japan) and expressed in "°Brix." The dry mass was evaluated as follows: the samples were mixed with diatomaceous earth, pre-dried, and then subjected to final drying under reduced pressure. The TA was determined by titration against 0.1 N NaOH to an endpoint of pH 8.1 using an automatic pH titration system (pH meter type IQ 150; Warsaw, Poland), and the pH was measured using the same equipment. The TA and dry mass were determined according to the PN norms PN-EN 12145:2001 and PN-EN12145:2000, respectively. The viscosity was measured using a rotation viscometer MC1 (DV-II+ PRO VISCOMETER, Brookfield, England) at 20 °C and expressed as mPas. The pectin content was determined according to Pijanowski et al. [7]. The osmotic strength was evaluated using an osmometer (Marcel OS 3000). The ash (%), sugar, and organic acid contents (g/100 mL) were evaluated according to Wojdyło et al. [8]. The ash content was measured using the furnace method, individual sugars were determined using HPLC-ELSD (High-Performance Liquid Chromatography-Evaporative Light Scattering Detector), and organic acids were determined using UPLC-PDA (Ultra-Performance Liquid Chromatography-Photodiode Array Detection). The mineral content was determined using

an atomic absorption spectrophotometer (AA-7000F/AAC SHIMADZU, Shimadzu Corporation). Color parameters (CIEL*a*b*) of the samples were quantified using a Color Quest XE Hunter Lab colorimeter (Reston, VA, USA). The L* (lightness), a* (redness–greenness), and b* (yellowness–blueness) values were determined using the CIE standard Illuminant D65 at a 10° observer angle. All measurements were performed in triplicate.

2.3. Identification and Quantification of Polyphenolics and Carotenoids

Phenolic compounds were identified using LC/MS-Q-Tof (Liquid Chromatography/Mass Spectrometry-Quadrupole Time-of-flight) (Waters, Manchester, UK). The observed retention times and spectra were compared with the standards. The polyphenolic content was quantified using external calibration curves compared with the standards and investigated using LC/MS and UPLC, according to Wojdyło et al. [8]. About 2 g of the carrot-blended smoothies was mixed with 8 mL of HPLC-grade methanol–H_2O mixture (30:70%, *v*/*v*), ascorbic acid (2%), and acetic acid (1%), and was then sonicated (Sonic 6D, Polsonic, Warsaw, Poland) for 15 min. Then, the samples were stored in a refrigerator for 24 h and centrifuged at 19,000× *g* for 10 min. The supernatant was filtered through a 0.20-µm hydrophilic polytetrafluoroethylene (PTFE) membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for further analysis. All samples were measured in triplicate and expressed as mg per 100 mL.

The carrot-based smoothies, which contained 10% $MgCO_3$ and 1% butylhydroxytoluene, were shaken with 5 mL of a ternary mixture of methanol, acetone, and hexane (1:1:2, by vol.) at 300 rpm for 30 min in the dark to prevent oxidation. The supernatants were acquired after four re-extractions from solid residues. The combined fractions were obtained after centrifugation (4 °C, 7 min at 19,000× *g*; MPW-350, Warsaw, Poland) and evaporated. The pellet was subtilized using 2 mL of 100% methanol, filtered through a 0.20-µm hydrophilic PTFE membrane, and used for further analysis. The carotenoid content was analyzed at 30 °C using LC-MS-Q/TOF (identification) and UPLC-PDA (quantification) on an ACQUITY UPLC BEH RP C18 column protected by a guard column of the same material. The elution solvents were a linear gradient of acetonitrile–methanol mixture (70:30%, *v*/*v*) (A) and 0.1% formic acid (B) at a flow rate of 0.42 mL/min. This procedure was monitored at 450 nm. All measurements were performed in triplicate and expressed as mg per 100 mL.

2.4. Analyses of In Vitro Antioxidant and Biological Activities

For all antioxidant and biological assays, about 5 gr of the supernatant of carrot-based smoothies was used. The antioxidant activity of the carrot-blended smoothies was determined using ABTS, FRAP, and ORAC assays according to Re et al. [9], Benzie and Strain [10], and Ou et al. [11], respectively. It was measured by the reduction of ABTS+• radicals, and the absorbance was read at 734 nm. The ferric reduction of the samples was determined using the FRAP assay. At low pH, the colorless ferric complex (Fe3+-tripyridyltriazine) changed to a blue ferrous complex (Fe^{2+}-tripyridyltriazine) by the action of electron-donating antioxidants, and the absorbance was read at 593 nm. The ORAC of the smoothies was determined using a spectrofluorometric method, and fluorescence decreased with the oxidation of free radicals in the presence of antioxidants.

Antidiabetic and antiobesity activities of the carrot-blended smoothies were investigated to understand α-amylase, α-glucosidase [12], and lipase inhibitory effects [13]. The α-amylase enzyme inhibition activity was analyzed using the reaction of iodine with starch after incubation at 37 °C, and the absorbance was read at 600 nm. The α-glucosidase enzyme inhibition activity was evaluated using the reaction of the enzyme with the β-D-glucosidase substrate, and the absorbance was read at 405 nm. The pancreatic lipase enzyme inhibition activity was analyzed using *p*-nitrophenol formed from *p*-nitrophenyl acetate after incubation at 37 °C, and the absorbance was read at 400 nm. The antiaging activity was determined using AChE and BuChE, according to Gironés-Vilaplana et al. [14]. The reaction of thiocholine with 5,5′-dithiobis-(2-nitrobenzoic

acid) produced 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate. The absorbance of the substrates of acetylcholine iodine and butylcholine chloride was read at 405 nm.

All tests to evaluate the antioxidant activity (ABTS, FRAP, and ORAC); α-amylase, α-glucosidase, and lipase inhibition effects; and anticholinergic activity were performed in triplicate using a microplate reader (Synergy TM H1; BioTek, Winooski, VT, USA).

2.5. Sensorial Evaluation

Sensory tests were carried out in a sensory analysis laboratory equipped with individual booths (at a controlled temperature of ~20 °C under combined natural/artificial light) designed according to the ISO 8589:2009 standards. The sensory laboratory was located at the Faculty of Biotechnology and Food Sciences of the Wrocław University of Environmental and Life Sciences (Poland). Nine fully trained panelists aged 25–43 years conducted the sensory evaluation sessions from 10 a.m. to 1 p.m. All panelists were provided with the same training to make them accustomed to the sensory attributes of smoothies and to understand the descriptors being used because they had to be able to identify differences between products, describe different product attributes (qualitatively), and scale the intensity of the attributes (quantitatively). In addition, all panelists were nonsmokers.

Sensory evaluation of the carrot-based smoothies was carried out using a 9-point hedonic scale (like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike lightly, dislike moderately, dislike very much, and dislike extremely). The panelists voted for the smoothies based on their appearance, sweetness, carrot taste, which fruit they sense most (raspberry, apple, pear, strawberry, and sour cherry), carrot smell, and delight. The smoothies were assigned codes for sensory evaluation and evaluated at room temperature. The smoothie samples were served in small white plastic glasses. After each sample, the panelists drank water to neutralize the taste in their mouths for the next sample.

According to the national laws, no ethical approval was required for this study. The panelists were informed about the study's aim and that their participation was entirely voluntary so they could stop the evaluation at any point and the responses would be anonymous.

2.6. Statistical Analyses

Results were subjected to analysis of variance ($p < 0.05$), and Tukey's honestly significant difference tests (Tukey's multiple comparisons of means 95% familywise confidence level) were performed using the R software (version 4.1.2, R Core Team, Austria). The results were demonstrated as mean values ($n = 3$) ± standard deviation.

3. Results and Discussion

3.1. Physicochemical Characteristics of the Carrot-Blended Smoothies

The L-ascorbic acid, pectin, TSS, and ash contents; viscosity; pH; TA; dry mass; osmolality; and color characteristics of the carrot-blended smoothies are presented in Table 1, with significant differences ($p \leq 0.05$).

Table 1. Physicochemical parameters of carrot-blended smoothies.

										Color		
Sample	**L-Ascorbic Acid**	**Viscosity**	**pH**	**TA**	**Pectin**	**TSS**	**Dry mass**	**Ash**	**Osmolality**	**a**	**b**	**L**
RJ–PC	155.08 ± 1.16 ^{c}	22.75 ± 0.49 ^{g}	3.75 ± 0.02 ^{ö}	1.21 ± 0.01 ^{a}	0.81 ± 0.04 ^{defg}	10.20 ± 0.22 ^{b}	10.95 ± 0.12 ^{j}	0.48 ± 0.12 ^{jkl}	534.00 ± 11.33 ^{gh}	21.67 ± 0.15 ^{e}	11.37 ± 0.26 ^{n}	32.21 ± 0.18 ^{pr}
AJ–PC	102.21 ± 0.83 ^{ghj}	28.37 ± 0.61 ^{f}	4.49 ± 0.01 ^{f}	0.34 ± 0.00 ^{h}	0.58 ± 0.11 ^{kl}	12.20 ± 0.26 ^{a}	12.80 ± 0.02 ^{b}	0.53 ± 0.08 ^{j}	683.00 ± 14.49 ^{c}	18.80 ± 0.24 ^{g}	10.67 ± 0.09 ^{ö}	33.83 ± 0.13 ^{n}
PJ–PC	109.52 ± 0.80 ^{f}	54.08 ± 1.16 ^{b}	4.77 ± 0.05 ^{d}	0.27 ± 0.01 ^{k}	0.80 ± 0.16 ^{efgh}	10.90 ± 0.23 ^{b}	12.17 ± 0.08 ^{d}	0.55 ± 0.02 ^{hj}	652.00 ± 13.83 ^{cde}	17.19 ± 0.51 ^{h}	10.09 ± 0.38 ^{p}	34.32 ± 0.40 ^{n}
SJ–PC	142.33 ± 2.14 ^{d}	47.28 ± 1.02 ^{cd}	4.12 ± 0.02 ^{j}	0.58 ± 0.02 ^{f}	0.88 ± 0.07 ^{cde}	8.80 ± 0.19 ^{c}	9.52 ± 0.06 ^{n}	0.55 ± 0.06 ^{hj}	444.00 ± 9.42 ^{ij}	19.59 ± 0.50 ^{fg}	10.93 ± 0.30 ^{ö}	35.04 ± 0.43 ^{m}
SCJ–PC	104.67 ± 0.27 ^{fghj}	46.98 ± 1.01 ^{cd}	4.06 ± 0.03 ^{k}	0.82 ± 0.04 ^{d}	0.96 ± 0.03 ^{cd}	12.40 ± 0.26 ^{a}	13.11 ± 0.05 ^{a}	0.72 ± 0.09 ^{cde}	804.00 ± 17.06 ^{a}	16.64 ± 0.62 ^{h}	7.98 ± 0.29 ^{r}	32.79 ± 0.25 ^{ö}
PC%100	91.27 ± 2.18 ^{k}	>100.00 ± 0.00 ^{a}	5.29 ± 0.02 ^{b}	0.23 ± 0.01 ^{l}	1.26 ± 0.03 ^{a}	10.10 ± 0.21 ^{b}	12.60 ± 0.01 ^{c}	1.06 ± 0.06 ^{a}	663.00 ± 14.06 ^{cd}	7.90 ± 0.57 ^{l}	5.89 ± 0.18 ^{t}	32.74 ± 0.03 ^{ö}
RJ–WC	154.09 ± 0.70 ^{c}	11.52 ± 0.25 ^{jk}	3.62 ± 0.01 ^{r}	1.12 ± 0.01 ^{b}	0.88 ± 0.05 ^{cde}	8.80 ± 0.19 ^{c}	9.75 ± 0.01 ^{m}	0.49 ± 0.16 ^{jk}	548.00 ± 11.62 ^{gh}	21.03 ± 0.04 ^{e}	8.21 ± 0.01 ^{r}	36.77 ± 0.01 ^{l}
AJ–WC	101.40 ± 0.91 ^{j}	4.43 ± 0.10 ^{mn}	4.28 ± 0.03 ^{h}	0.34 ± 0.03 ^{h}	0.75 ± 0.04 ^{ghj}	10.70 ± 0.23 ^{b}	11.81 ± 0.07 ^{ef}	0.45 ± 0.01 ^{kl}	611.00 ± 12.96 ^{def}	3.19 ± 0.07 ^{p}	19.67 ± 0.07 ^{j}	51.78 ± 0.06 ^{d}
PJ-WC	173.65 ± 2.98 ^{b}	8.85 ± 1.06 ^{klm}	4.51 ± 0.01 ^{f}	0.31 ± 0.01 ^{j}	0.83 ± 0.11 ^{def}	10.70 ± 0.23 ^{b}	11.97 ± 0.08 ^{e}	0.59 ± 0.06 ^{fghj}	661.00 ± 14.02 ^{cd}	0.57 ± 0.14 ^{r}	21.10 ± 0.11 ^{h}	55.58 ± 0.47 ^{a}
SJ-WC	155.28 ± 0.10 ^{c}	30.75 ± 0.64 ^{f}	4.05 ± 0.01 ^{kl}	0.53 ± 0.04 ^{g}	0.50 ± 0.00 ^{l}	7.60 ± 0.16 ^{de}	8.71 ± 0.17 ^{p}	0.61 ± 0.01 ^{efghj}	462.00 ± 9.80 ^{i}	17.13 ± 0.12 ^{h}	16.18 ± 0.08 ^{l}	45.19 ± 0.41 ^{h}
SCJ-WC	101.51 ± 1.72 ^{hj}	6.45 ± 0.64 ^{lmn}	3.89 ± 0.01 ^{n}	0.83 ± 0.01 ^{cd}	0.79 ± 0.13 ^{fgh}	10.60 ± 0.22 ^{b}	11.89 ± 0.21 ^{ef}	0.55 ± 0.12 ^{ghj}	690.00 ± 14.64 ^{c}	19.98 ± 0.76 ^{f}	6.50 ± 0.11 ^{s}	31.79 ± 0.59 ^{r}
WC%100	91.54 ± 0.54 ^{k}	49.05 ± 6.47 ^{d}	5.23 ± 0.01 ^{c}	0.15 ± 0.01 ^{n}	1.03 ± 0.14 ^{bc}	7.50 ± 0.16 ^{de}	9.19 ± 0.04 ^{ö}	0.72 ± 0.00 ^{def}	534.00 ± 11.33 ^{gh}	4.17 ± 0.04 ^{ö}	24.17 ± 0.01 ^{f}	53.78 ± 0.03 ^{b}
RJ–YC	141.11 ± 0.63 ^{d}	36.60 ± 0.85 ^{e}	3.74 ± 0.02 ^{ö}	1.17 ± 0.02 ^{a}	1.10 ± 0.21 ^{b}	8.90 ± 0.19 ^{c}	10.06 ± 0.04 ^{kl}	0.72 ± 0.15 ^{defg}	533.00 ± 11.31 ^{gh}	21.44 ± 0.25 ^{e}	15.22 ± 0.18 ^{m}	38.38 ± 0.12 ^{k}
AJ–YC	196.76 ± 0.16 ^{a}	12.60 ± 1.70 ^{jkl}	4.34 ± 0.00 ^{g}	0.37 ± 0.02 ^{h}	0.76 ± 0.04 ^{fgh}	10.20 ± 0.22 ^{b}	11.56 ± 0.06 ^{g}	0.57 ± 0.03 ^{fghj}	625.00 ± 13.26 ^{def}	6.51 ± 0.08 ^{m}	34.49 ± 0.76 ^{d}	51.60 ± 0.21 ^{d}
PJ–YC	117.13 ± 0.85 ^{e}	17.55 ± 2.23 ^{hi}	4.69 ± 0.02 ^{e}	0.25 ± 0.01 ^{l}	0.73 ± 0.08 ^{hjk}	9.80 ± 0.21 ^{b}	11.13 ± 0.06 ^{hj}	0.58 ± 0.02 ^{fghj}	572.00 ± 12.13 ^{fgh}	5.49 ± 0.06 ^{n}	35.76 ± 0.41 ^{c}	53.92 ± 0.16 ^{b}
SJ–YC	152.37 ± 3.18 ^{c}	49.05 ± 0.21 ^{bc}	4.06 ± 0.00 ^{k}	0.55 ± 0.00 ^{fg}	0.72 ± 0.10 ^{hjk}	7.80 ± 0.17 ^{de}	8.53 ± 0.11 ^{p}	0.74 ± 0.04 ^{cd}	446.00 ± 9.46 ^{ij}	14.59 ± 0.21 ^{j}	23.86 ± 0.30 ^{f}	47.28 ± 0.20 ^{g}
SCJ–YC	105.35 ± 6.22 ^{fgh}	8.85 ± 0.64 ^{klm}	3.99 ± 0.00 ^{m}	0.85 ± 0.04 ^{c}	0.77 ± 0.14 ^{fgh}	10.80 ± 0.23 ^{b}	12.12 ± 0.09 ^{d}	0.84 ± 0.27 ^{c}	750.00 ± 15.91 ^{b}	19.91 ± 0.46 ^{f}	11.20 ± 0.27 ^{n}	32.55 ± 0.09 ^{öp}
YC%100	76.98 ± 1.68 ^{l}	>100.00 ± 0.00 ^{a}	5.35 ± 0.00 ^{a}	0.24 ± 0.01 ^{l}	0.59 ± 0.01 ^{jkl}	6.70 ± 0.14 ^{f}	9.44 ± 0.16 ^{n}	0.94 ± 0.01 ^{b}	463.00 ± 9.82 ^{i}	8.93 ± 0.02 ^{k}	40.47 ± 0.22 ^{a}	52.76 ± 0.69 ^{c}
RJ–OC	140.22 ± 0.25 ^{d}	8.10 ± 0.85 ^{klmn}	3.67 ± 0.00 ^{p}	1.17 ± 0.00 ^{a}	0.92 ± 0.06 ^{cde}	8.90 ± 0.19 ^{c}	10.14 ± 0.16 ^{k}	0.42 ± 0.01 ^{klm}	523.00 ± 11.09 ^{h}	26.58 ± 0.74 ^{b}	22.66 ± 1.15 ^{g}	39.63 ± 0.62 ^{j}
AJ–OC	171.27 ± 0.04 ^{b}	3.30 ± 0.85 ^{n}	4.32 ± 0.02 ^{g}	0.35 ± 0.01 ^{h}	0.26 ± 0.02 ^{m}	10.30 ± 0.22 ^{b}	11.73 ± 0.04 ^{fg}	0.29 ± 0.06 ^{mn}	606.00 ± 12.86 ^{ef}	25.79 ± 0.17 ^{b}	37.11 ± 0.54 ^{b}	48.84 ± 0.27 ^{f}
PJ–OC	107.35 ± 1.25 ^{fg}	4.35 ± 1.06 ^{mn}	4.70 ± 0.03 ^{de}	0.24 ± 0.01 ^{l}	0.75 ± 0.17 ^{hj}	9.80 ± 0.21 ^{b}	11.14 ± 0.02 ^{hj}	0.27 ± 0.01 ^{n}	577.00 ± 12.24 ^{fg}	26.21 ± 0.23 ^{b}	37.54 ± 0.67 ^{b}	50.48 ± 0.16 ^{e}
SJ–OC	137.34 ± 1.90 ^{d}	18.45 ± 0.64 ^{gh}	4.04 ± 0.01 ^{l}	0.55 ± 0.01 ^{f}	0.27 ± 0.03 ^{m}	7.40 ± 0.16 ^{ef}	8.27 ± 0.04 ^{r}	0.30 ± 0.16 ^{lmn}	419.00 ± 8.89 ^{j}	24.55 ± 0.07 ^{c}	29.42 ± 0.19 ^{e}	45.15 ± 0.89 ^{h}
SCJ–OC	89.13 ± 3.17 ^{k}	3.75 ± 0.21 ^{mn}	3.91 ± 0.01 ^{n}	0.76 ± 0.01 ^{e}	0.94 ± 0.03 ^{cd}	10.10 ± 0.21 ^{b}	11.27 ± 0.21 ^{h}	0.40 ± 0.01 ^{klm}	662.00 ± 14.04 ^{cd}	23.36 ± 0.36 ^{d}	17.37 ± 0.43 ^{k}	37.18 ± 0.08 ^{l}
OC%100	80.05 ± 0.62 ^{l}	15.15 ± 1.48 ^{hi}	5.33 ± 0.00 ^{ab}	0.20 ± 0.02 ^{m}	nd	8.20 ± 0.17 ^{cd}	9.97 ± 0.04 ^{lm}	0.63 ± 0.07 ^{defgh}	459.00 ± 9.74 ^{i}	28.70 ± 0.09 ^{a}	36.67 ± 0.34 ^{b}	50.47 ± 0.10 ^{e}

L-ascorbic acid (mg/100 mL); Viscosity [mPas]; TA (g malic acid/100 mL); Pectin [%]; TSS, Total Soluble Solids [°Brix]; Dry mass [%]; Ash [%]; Osmolality [mOsm/liter]; significant at $p \leq 0.05$; values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Tukey's test; not detected (nd).

The L-ascorbic acid content is essential for normal body functions such as the synthesis of folic acid, tyrosine, and tryptophan; hydroxylation of proline, glycine, carnitine, lysine, and catecholamine; and iron absorption, besides acting as an antioxidant agent [15]. Moreover, although fruit and vegetables are rich in vitamin C, the amount of vitamin C decreases during food processing due to heating and air exposure [16]. In the present study, L-ascorbic acid was added to the smoothies to prevent enzyme activity and color changes. The L-ascorbic acid content in the smoothies ranged from 76.98 mg/100 mL to 196.76 mg/100 mL. The highest L-ascorbic acid content was observed in the AJ–YC (196.76 mg/100 mL), PJ–WC (173.65 mg/100 mL), and AJ–OC (171.27 mg/100 mL) smoothies, whereas the lowest L-ascorbic acid content was observed in the SCJ–OC smoothie (89.13 mg/100 mL). Thus, AJ provides the highest amount of vitamin C to YC and OC purees.

Viscosity is one of the characteristics of liquid food products [17]. In the present study, the viscosities of the carrot-blended smoothies ranged from 3.30 to 54.08 mPas. The viscosities of the control groups of PC100% and YC100% were more than 100 mPas; in the case of WC, viscosity was 49 mPas, whereas in OC puree, it was 15 mPas. Nevertheless, the highest viscosity was observed in the PJ–PC (54.08 mPas) and SJ–YC (49.05 mPas) smoothies, whereas the lowest viscosity was observed in the AJ–OC smoothie (3.30 mPas). This results directly from the characteristics of the individual purees. The addition of those that were characterized by the highest viscosity (PC and YC puree) resulted in the fact that products based on them were more dense than those with the addition of OC puree. In the case of smoothie development, viscosity is an extremely important factor that shapes the quality of the final product. By design, this product must be semifluid and drinkable. Therefore, when designing a smoothie based on PC or YC, it is worth considering using a larger proportion of juice than puree, so that the final viscosity does not exceed 45 mPa (intrinsic viscosity in the opinion of the sensory panel).

In a previous study, TA and pH were evaluated together to determine food quality [18]. In the present study, TA ranged from 0.15 to 1.21 g/100 mL. The highest TA was observed in the RJ–PC (1.21 g/100 mL), RJ–OC (1.17 g/100 mL), and RJ–YC (1.17 g/100 mL) smoothies, whereas the lowest TA was observed in the PJ–OC (0.24 g/100 mL) and PJ–YC (0.25 g/100 mL) smoothies, except for the control groups, which included only carrot purees. Hence, RJ smoothies showed the highest TA, whereas PJ smoothies showed the lowest TA. Regarding pH values, the control groups showed the highest pH values in the following order: YC > OC > PC > WC. Besides the control groups, the highest pH values were observed in the PJ–PC (4.77), PJ–OC (4.70), and PJ–YC (4.69) smoothies, whereas the lowest pH values were observed in the RJ–WC (3.62) and RJ–OC (3.67) smoothies. These results demonstrated that carrot purees show high pH, but RJ shows an acidic pH. High acidic conditions prevent microbial activities and provide beverage stability [19]. Hence, TA and pH showed a contrasting finding that RJ–PC showed the highest TA value but with a low pH value.

Pectin plays the role of an immunomodulator in allergies [20] and protects against cardiovascular diseases [21]. Moreover, soluble dietary fibers (e.g., pectins) play a vital role in increasing gastrointestinal activities [22] and decreasing serum cholesterol [23]. Insoluble dietary fibers such as pectic polysaccharides, hemicellulose, and cellulose of carrot pomace also provide important health benefits such as reducing lipid and cholesterol levels [24]. In the present study, the highest pectin content was observed in the following order: PC100% > RJ–YC (1.10%) > WC100% > SCJ–PC (0.96%) > SCJ–OC (0.94%). However, the lowest pectin content was observed in the AJ–OC (0.26%) and SJ–OC (0.27%) smoothies. Based on these results, the SCJ–PC smoothie showed a higher pectin content than the SCJ–OC smoothie.

The TSS content influences the sweetness of food products due to the presence of soluble proteins and organic materials [25]. In the present study, the TSS content of carrot-blended smoothies ranged from 7.40 to 12.40 °Brix. The highest TSS content was observed in the SCJ–PC (12.40 °Brix) and AJ–PC (12.20 °Brix) smoothies, whereas the lowest TSS

content was observed in the SJ–OC (7.40 °Brix), SJ–WC (7.60 °Brix), and SJ–YC (7.80 °Brix) smoothies. Therefore, the PC puree provided a higher sugar content, as shown by the high TSS content, but SJ reduced the sugar content and TSS.

The dry mass of the carrot-blended smoothies showed the same trend as the TSS content. The highest dry mass was observed in the SCJ–PC (13.11%) and AJ–PC (12.80%) smoothies, whereas the lowest dry mass was observed in the SJ–OC (8.27%), SJ–YC (8.53%), and SJ–WC (8.71%) smoothies. Nevertheless, the ash content of the carrot-based smoothies showed a different trend from that of the TSS and dry mass.

Osmolality determines the bioavailability of beverages in body hydration [26]. In the present study, the highest osmolality was observed in the SCJ–PC (804 mOsm/L), SCJ–YC (750 mOsm/L), and SCJ–WC (690 mOsm/L) smoothies, whereas the lowest osmolality was observed in the SJ–OC (419 mOsm/L), SJ–PC (444 mOsm/L), and SJ–YC (446 mOsm/L) smoothies. Hence, SCJ smoothies showed the highest osmolality, which shows that SCJ–carrot smoothies are not only rich in bioactive contents but are also easily absorbed by the digestive system. However, SJ–carrot smoothies showed the lowest osmolality.

The color of food products is an important parameter that increases consumer purchase rates. In the present study, the mean lightness (L*) of the carrot-based smoothies ranged from 32.21 to 55.58. The highest lightness was observed in PJ and AJ with WC and YC purees, whereas the lowest lightness (i.e., with the darkest color) was observed in the SCJ–WC (31.79), RJ–PC (32.21), and SCJ–YC (32.55) smoothies. Thus, as expected, SCJ and RJ provided the darkest colors, whereas AJ and PJ provided the lightest colors.

Moreover, other parameters such as a* (redness–greenness) and b* (yellowness–blueness) were also measured in the present study. The highest redness (the highest a* results) was observed in the OC puree and RJ, AJ, and PJ smoothies, whereas the highest greenness was observed in the WC puree and PJ and AJ smoothies. The highest yellowness was observed in the OC puree and AJ and PJ smoothies, whereas the highest blueness was observed in the SCJ–WC (6.50), SCJ–PC (7.98), and RJ–WC (8.21) smoothies. Hence, similar to lightness results, SCJ and RJ provided the dark blue color.

Sugar and organic acid contents of the carrot-blended smoothies are shown in Figures 2 and 3, with significant differences ($p \leq 0.05$).

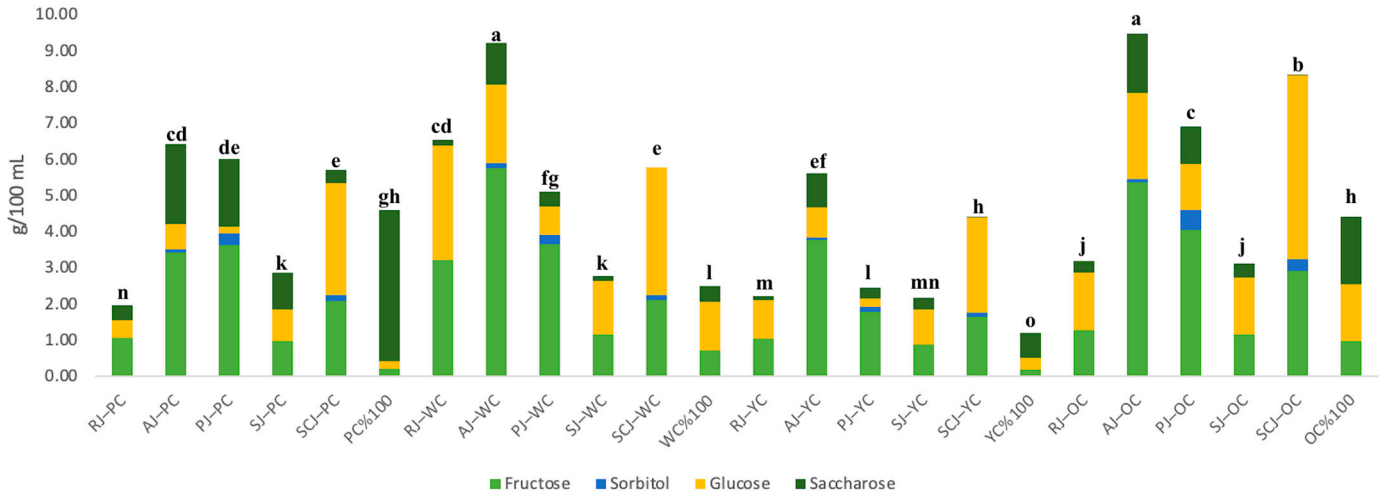

Figure 2. The total sugars (g/100 mL) in carrot-blended smoothies, letters above columns indicate statistical significance at $p \leq 0.05$ according to Tukey's test (the same letter = not significantly different). Total sugar content: RJ–PC, 1.97n. AJ–PC, 6.41cd. PJ–PC, 6.00de. SJ–PC, 2.87k. SCJ–PC, 5.72e. PC%100, 4.59gh. RJ–WC, 6.53cd. AJ–WC, 9.22a. PJ–WC, 5.11fg. SJ–WC, 2.78k. SCJ–WC, 5.77e. WC%100, 2.50l. RJ–YC, 2.22m. AJ–YC, 5.61ef. PJ–YC, 2.45l. SJ–YC, 2.16mn. SCJ–YC, 4.42h. YC%100, 1.21o. RJ–OC, 3.19j. AJ–OC, 9.47a. PJ–OC, 6.91c. SJ–OC, 3.11j. SCJ–OC, 8.35b. OC%100, 4.40h.

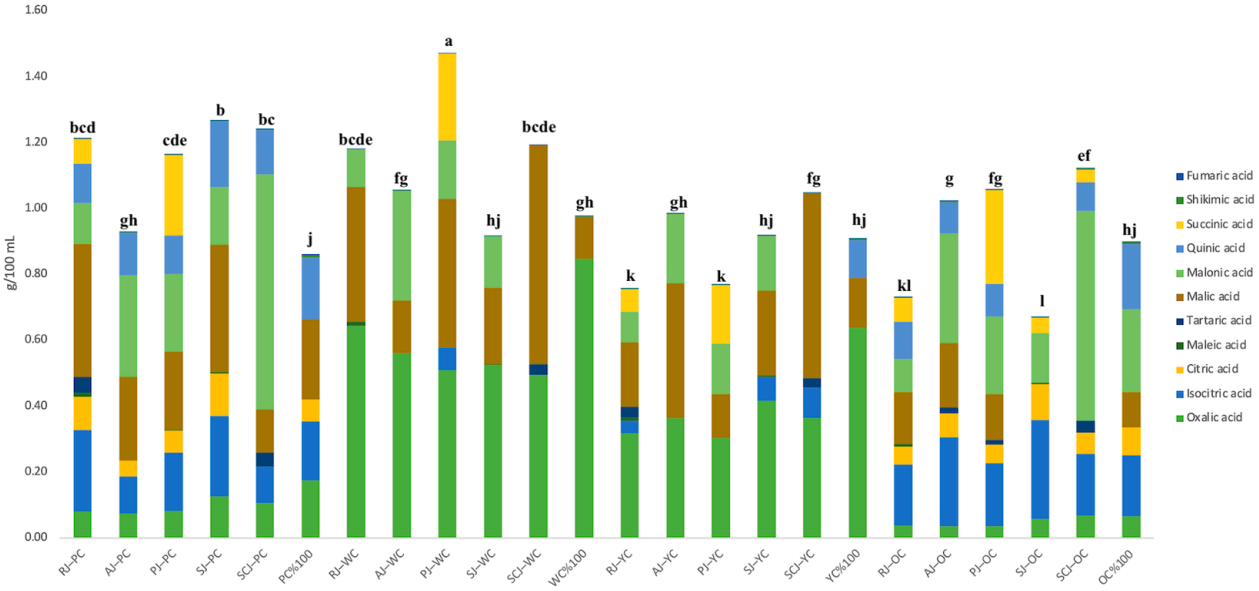

Figure 3. The organic acids (g/100 mL) in carrot-blended smoothies, letters above columns indicate statistical significance at $p \leq 0.05$ according to Tukey's test (the same letter = not significantly different). Total organic acid content: RJ–PC, 1.21bcd. AJ–PC, 0.93gh. PJ–PC, 1.17cde. SJ–PC, 1.27b. SCJ–PC, 1.24bc. PC%100, 0.86j. RJ–WC, 1.18bcde. AJ–WC, 1.06fg. PJ–WC, 1.47a. SJ–WC, 0.92hj. SCJ–WC, 1.19bcde. WC%100, 0.98gh. RJ–YC, 0.76k. AJ–YC, 0.99gh. PJ–YC, 0.77k. SJ–YC, 0.92hj. SCJ–YC, 1.05fg. YC%100, 0.91hj. RJ–OC, 0.73kl. AJ–OC, 1.02g. PJ–OC, 1.06fg. SJ–OC, 0.67 l. SCJ–OC, 1.12ef. OC%100, 0.90hj.

In the present study, the presence of fructose, sorbitol, glucose, and saccharose was observed in carrot-based smoothies. Fructose levels ranged from 0.87 to 5.74 g/100 mL. The highest fructose content was observed in the AJ–WC (5.74 g/100 mL), AJ–OC (5.37 g/100 mL), and PJ–OC (4.04 g/100 mL) smoothies, whereas the lowest fructose content was observed in the SJ–YC (0.87 g/100 mL), SJ–PC (0.97 g/100 mL), and RJ–PC (1.07 g/100 mL) smoothies. Thus, the OC puree and AJ smoothies showed the highest fructose content. However, sorbitol was not determined in all samples. The highest sorbitol content was observed in the PJ–OC (0.55 g/100 mL), PJ–PC (0.33 g/100 mL), and SCJ–OC (0.32 g/100 mL) smoothies. The sorbitol content was not observed in the RJ–PC, SJ–PC, RJ–WC, SJ–WC, RJ–YC, SJ–YC, RJ–OC, and SJ–OC smoothies. Hence, the sorbitol content was high in the OC puree and PJ smoothies. The highest glucose content was observed in the SCJ–OC (5.07 g/100 mL), SCJ–WC (3.54 g/100 mL), and RJ–WC (3.18 g/100 mL) smoothies. The lowest glucose content was observed in the PJ–PC (0.16 g/100 mL), RJ–PC (0.47 g/100 mL), and AJ–PC (0.68 g/100 mL) smoothies. Therefore, the WC puree and SCJ samples were rich in glucose. The highest saccharose content was observed in the AJ–PC (2.22 g/100 mL), PJ–PC (1.87 g/100 mL), and AJ–OC (1.65 g/100 mL) smoothies, whereas saccharose was not observed in the SCJ–WC smoothie, and the lowest saccharose content was observed in the SCJ–YC (0.02 g/100 mL) and SCJ–OC (0.04 g/100 mL) smoothies. Thus, the PC puree and AJ samples were rich in saccharose. To summarize, the total sugar content was highest in the AJ–OC (9.47 g/100 mL), AJ–WC (9.22 g/100 mL), and SCJ–OC (8.35 g/100 mL) smoothies and lowest in the RJ–PC (1.97 g/100 mL), SJ–YC (2.16 g/100 mL), and RJ–YC (2.22 g/100 mL) smoothies. The OC puree and AJ samples showed the highest total sugar content.

Organic acids provide a specific smell and taste to fruit- and vegetable-based food [27]. In the present study, the presence of oxalic acid, isocitric acid, citric acid, maleic acid, tartaric acid, malic acid, malonic acid, quinic acid, succinic acid, shikimic acid, and fumaric acid was identified from the carrot-blended smoothies. Oxalic acid and fumaric acid were present in all smoothies, whereas other organic acids were observed in different

amounts in each sample. The highest oxalic acid content was observed in the WC100% (0.85 g/100 mL), RJ–WC smoothie (0.64 g/100 mL), YC100% (0.64 g/100 mL), and AJ–WC smoothie (0.56 g/100 mL), and the highest fumaric acid content was observed in the PC100% (0.004 g/100 mL) and RJ–PC smoothie (0.003 g/100 mL). Thus, WC samples were rich in oxalic acid [19], and PC samples were rich in fumaric acid. The RJ–PC smoothie was the only sample that showed the presence of all organic acids studied. In summary, the PC puree samples were rich in total organic acids, whereas the OC puree samples showed a low total organic acid content.

Mineral contents of the carrot-blended smoothies are shown in Table 2, with significant differences ($p \leq 0.05$).

Table 2. The mineral contents of carrot-based smoothies.

Sample	Na	K	Ca	Fe	Mg
RJ–PC	20.65 ± 0.44^{k}	172.72 ± 3.66^{def}	2.86 ± 0.06^{f}	0.41 ± 0.01^{c}	0.47 ± 0.01^{cd}
AJ–PC	29.81 ± 0.63^{fg}	159.00 ± 3.37^{fg}	4.79 ± 0.10^{c}	0.47 ± 0.01^{b}	1.14 ± 0.02^{b}
PJ–PC	24.70 ± 0.52^{hj}	163.02 ± 3.46^{efg}	2.66 ± 0.06^{fgh}	0.26 ± 0.01^{f}	0.45 ± 0.01^{cd}
SJ–PC	23.84 ± 0.51^{j}	161.53 ± 3.43^{fg}	2.73 ± 0.06^{fg}	0.26 ± 0.01^{f}	0.45 ± 0.01^{cd}
SCJ–PC	33.22 ± 0.70^{f}	174.54 ± 3.70^{cdef}	5.33 ± 0.11^{b}	0.42 ± 0.01^{c}	1.27 ± 0.03^{a}
PC%100	59.25 ± 1.26^{b}	326.20 ± 6.92^{a}	8.18 ± 0.17^{a}	0.63 ± 0.01^{a}	1.28 ± 0.03^{a}
RJ–WC	27.22 ± 0.58^{gh}	145.19 ± 3.08^{g}	2.38 ± 0.05^{hj}	0.24 ± 0.01^{f}	0.42 ± 0.01^{d}
AJ–WC	21.70 ± 0.46^{k}	115.33 ± 2.45^{kl}	2.18 ± 0.05^{j}	0.19 ± 0.00^{j}	0.46 ± 0.01^{cd}
PJ–WC	25.96 ± 0.55^{h}	129.30 ± 2.74^{hj}	2.43 ± 0.05^{ghj}	0.23 ± 0.00^{fg}	0.42 ± 0.01^{d}
SJ–WC	27.25 ± 0.58^{gh}	131.92 ± 2.80^{hj}	2.40 ± 0.05^{ghj}	0.20 ± 0.00^{j}	0.39 ± 0.01^{def}
SCJ–WC	25.65 ± 0.54^{hj}	172.04 ± 3.65^{def}	2.19 ± 0.05^{j}	0.19 ± 0.00^{j}	0.44 ± 0.01^{cd}
WC%100	41.50 ± 0.88^{d}	190.73 ± 4.05^{c}	3.66 ± 0.08^{e}	0.26 ± 0.01^{f}	0.44 ± 0.01^{cd}
RJ–YC	52.49 ± 1.11^{c}	177.98 ± 3.78^{cde}	5.44 ± 0.12^{b}	0.49 ± 0.01^{b}	1.16 ± 0.02^{b}
AJ–YC	38.78 ± 0.82^{de}	137.59 ± 2.92^{gh}	2.72 ± 0.06^{fg}	0.20 ± 0.00^{hj}	0.41 ± 0.01^{de}
PJ–YC	37.76 ± 0.80^{e}	139.22 ± 2.95^{gh}	2.69 ± 0.06^{fgh}	0.23 ± 0.00^{fg}	0.42 ± 0.01^{d}
SJ–YC	36.80 ± 0.78^{e}	148.53 ± 3.15^{g}	2.86 ± 0.06^{f}	0.25 ± 0.01^{f}	0.42 ± 0.01^{d}
SCJ–YC	37.07 ± 0.79^{e}	209.42 ± 4.44^{b}	2.45 ± 0.05^{ghj}	0.35 ± 0.01^{d}	0.42 ± 0.01^{d}
YC%100	68.33 ± 1.45^{a}	222.04 ± 4.71^{b}	4.24 ± 0.09^{d}	0.31 ± 0.01^{e}	0.37 ± 0.01^{f}
RJ–OC	25.59 ± 0.54^{hj}	158.22 ± 3.36^{fg}	2.10 ± 0.04^{j}	0.25 ± 0.01^{f}	0.40 ± 0.01^{de}
AJ–OC	23.74 ± 0.50^{j}	125.23 ± 2.66^{jk}	1.68 ± 0.04^{l}	0.20 ± 0.00^{hj}	0.38 ± 0.01^{ef}
PJ–OC	25.59 ± 0.54^{hj}	114.40 ± 2.43^{kl}	1.59 ± 0.03^{l}	0.22 ± 0.00^{gh}	0.42 ± 0.01^{d}
SJ–OC	21.36 ± 0.45^{k}	108.20 ± 2.30^{l}	2.11 ± 0.04^{j}	0.26 ± 0.01^{f}	0.41 ± 0.01^{de}
SCJ–OC	24.26 ± 0.51^{hj}	165.65 ± 3.51^{def}	1.90 ± 0.04^{k}	0.26 ± 0.01^{f}	0.42 ± 0.01^{d}
OC%100	39.46 ± 0.84^{de}	180.58 ± 3.83^{cd}	3.53 ± 0.07^{e}	0.32 ± 0.01^{e}	0.49 ± 0.01^{c}

The content of minerals (mg/100 mL) in carrot-blended smoothies, significant at $p \leq 0.05$; values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Tukey's test.

Minerals are essential for homeostasis, and their deficiencies may cause diseases [28]. In the present study, sodium (Na), potassium (K), calcium (Ca), iron (Fe), and magnesium (Mg) were identified in carrot-based smoothies. The highest Na content was observed in all YC puree samples, whereas the lowest Na content was observed in the RJ–PC (20.65 mg/100 mL) and AJ–WC (21.70 mg/100 mL) smoothies. The highest K content was observed in the following order: SCJ–YC > RJ–YC > SCJ–PC, whereas the lowest K content was observed in the following order: SJ–OC < PJ–OC < AJ–WC. Thus, the YC puree and SCJ smoothies were rich in K. The highest Ca content was observed in the following order: RJ–YC > SCJ–PC > AJ–PC, whereas the lowest Ca content was observed in the following order: PJ–OC < AJ–OC < SCJ–OC. Therefore, PC puree samples were rich in Ca. The highest Fe content was observed in the following order: RJ–YC > AJ–PC > SCJ–PC > RJ–PC, whereas the lowest Fe content was observed in the following order: AJ–WC = SCJ–WC < SJ–WC. Thus, the PC puree and RJ smoothies were rich in Fe. Moreover, the highest Mg content was observed in the following order: SCJ–PC > RJ–YC > AJ–PC, whereas the lowest Mg content was observed in the following order: AJ–OC < SJ–WC. Thus, the PC puree samples were rich in Mg. According to the literature, mineral contents and ash contents are related to each other. In the present study, the highest ash content was observed in the SCJ–YC (0.84%), SJ–YC (0.74%), and SCJ–PC (0.72%) smoothies, whereas the lowest ash content

was observed in the PJ–OC (0.27%), AJ–OC (0.29%), and SJ–OC (0.30%) smoothies. Thus, YC puree samples showed the highest ash content, whereas OC puree samples showed a lower ash content.

3.2. Identification and Quantification of Polyphenolics and Carotenoids

Raw carrot materials do not contain flavanols and flavan-3-ols [29]; however, due to the addition of fruit juices in the present study, the carrot-blended smoothies were rich in flavan-3-ols, phenolic acids, flavanols, anthocyanins, and polymeric procyanidins. The phenolic content of the carrot-based smoothies is shown in Table 3 (qualitatively), and Supplementary S1 (quantitatively) with significant differences ($p \leq 0.05$).

Flavan-3-ols exhibit activities against oxidation, carcinogens, microbes, and neurological diseases [30]. In the present study, procyanidin B2 ([M–H]− at m/z = 577), procyanidin B4 ([M–H]− at m/z = 577), and epicatechin ([M–H]− at m/z = 289) were identified, and flavan-3-ols were quantified in carrot-blended smoothies. Procyanidin B2 was identified in only RJ, AJ, SJ, and SCJ blended with the PC puree, procyanidin B4 in RJ, PJ, and SJ blended with carrot. Therefore, flavan-3-ols were not observed in raw carrot materials [29]; however, after processing and the addition of different fruit juices, the flavan-3-ol content increased and ranged from 12.14 to 127.93 mg/100 mL in the smoothies. The highest total flavan-3-ol content was observed in the SCJ–OC (127.93 mg/100 mL), SCJ–YC (113.22 mg/100 mL), and RJ–PC (84.08 mg/100 mL) smoothies, whereas the lowest total flavan-3-ol content was observed in the PJ–OC (12.14 mg/100 mL), PJ–WC (19.89 mg/100 mL), and PJ–YC (25.07 mg/100 mL) smoothies. Thus, SCJ samples were rich in flavan-3-ols.

Table 3. Identified polyphenolics by LC/MS in carrot-based smoothies.

	Compound	Rt (min)	λmax (nm)	MS [M–H]− (m/z)	MS/MS (m/z)	Purple Carrot						White Carrot						Yellow Carrot						Orange Carrot				
						PC	RJ	AJ	PJ	SJ	SCJ	WC	RJ	AJ	PJ	SJ	SCJ	YC	RJ	AJ	PJ	SJ	SCJ	OC	RJ	AJ	PJ	SJ	SCJ
Flavan-3-ols	procyanidin B2	4.26	280	577	289	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	nd	+	nd	nd	nd	+	+
	procyanidin B4	3.92	279	577	289	+	+	nd	+	+	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	+	+	nd	+	nd	nd	+	+
	epicatechin	4.74	278	289	289	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Phenolic acids	3-O-caffeoylquinic acid	5.90	324	353	135/179 /191	+	+	+	+	+	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+
	chlorogenic acid	3.86	326	353	191	+	+	nd	+	+	nd	+	+	+	+	nd	+	+	nd	+	nd	nd	nd	+	+	+	nd	nd	+
	5-O-caffeoylquinic acid	7.54	325	353	179/191	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4-O-caffeoylquinic acid	8.03	325	353	179/191	+	+	+	+	+	+	nd	+	+	nd	+	nd	nd	nd	nd	nd	+	+	nd	nd	nd	nd	+	+
	ferulic acid-hexoside	8.89	325	355	193/175	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	nd	nd	nd	nd	+	+	+	+	+	+
	ferulic acid di-hexoside	9.41	324	517	355/193 /175	+	+	+	+	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	3-O-feruloylquinic acid	10.10	322	367	173/193	nd	+	+	+	+	+	+	+	+	+	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	4-O-feruloylquinic acid	10.84	323	367	173/193	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	caffeic acid -hexoside	10.90	324	341	179/135	+	+	+	+	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	di-ferulic acid derivative	14.27	327	527	203/365 /366	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	dicaffeoylquinic acid	7.01	320	515	353/191	+	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	cis-5-p-coumaroylquinic acid	6.32	320	337	163/191	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	p-coumaric acid	4.87	312	325	163/119	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Flavanols	quercetin-3-galactoside	7.71	354	609	447/301	nd	+	nd	nd	+	+	nd	+	nd	nd	+	+	nd	+	nd	nd	+	+	nd	+	nd	nd	+	+
	genistin	6.86	326	269	133	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+
Anthocyanins	cyanidin-3-O-xylosyl -glucosylgalactoside	5.58	517	743 $^{+}$	287	+	+	+	+	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	cyanidin-3-O -xylosyl-galactoside	6.14	518	581 $^{+}$	287	nd	+	nd	nd	nd	+	nd	+	nd	nd	nd	+	nd	+	nd	nd	nd	+	nd	+	nd	nd	nd	+
	cyanidin-3-O-xylosyl -cinpoyl-glucosylgalactoside	7.22	530	949 $^{+}$	287	+	+	+	+	+	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	cyanidin-3-O-xylosyl -feruloyl-glucosylgalactoside	7.57	528	919 $^{+}$	287	+	nd	+	+	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 3. *Cont.*

Compound	Rt (min)	λmax (nm)	MS [M–H]− (m/z)	MS/MS (m/z)	Purple Carrot						White Carrot						Yellow Carrot						Orange Carrot					
					PC	RJ	AJ	PJ	SJ	SCJ	WC	RJ	AJ	PJ	SJ	SCJ	YC	RJ	AJ	PJ	SJ	SCJ	OC	RJ	AJ	PJ	SJ	SCJ
cyanidin-3-O-xylosyl -p-coumaroylglucosyl-galactoside	7.68	527	889 $^{+}$	287	+	+	+	+	+	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd
cyanidin-3-O -glucosyl-rutinoside	5.98	520	757 $^{+}$	611/297	nd	nd	nd	nd	nd	+	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	+	nd	+	nd	nd	nd	+
cyanidin-3-arabinoside	5.11	520	419 $^{+}$	287	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd

not detected (nd); +—for anthocyanins positive mode (MS $[M–H]^{+}$).

As reported in a previous study, phenolic acids show antioxidant and anti-inflammatory activities [31]. In our previous work, fourteen phenolic acids were identified and quantified from raw carrot materials [29]. However, in the present study, 13 different phenolic acids were observed in the carrot-blended smoothies. In all samples, 5-o-caffeoylquinic acid ([M–H]− at m/z = 353), 4-o-feruloylquinic acid ([M–H]− at m/z = 367), and diferulic acid derivatives ([M–H]− at m/z = 527) were identified. The highest phenolic acid content was observed in all samples after the addition of SCJ, followed by PJ. In the smoothies, cis-5-*p*-coumaroylquinic acid ([M–H]− at m/z = 337) and *p*-coumaric acid ([M–H]− at m/z = 325) were newly identified phenolic acids. The cis-5-*p*-coumaroylquinic acid and *p*-coumaric acid were only identified in the SCJ–PC smoothie. Moreover, PC puree smoothies showed the highest number of different types of phenolic acids and total phenolic acid content. The lowest phenolic acid content was observed in the following order: RJ–WC < RJ–YC < SJ–WC. Hence, the PC puree and SCJ samples showed the highest phenolic acid content, whereas the WC puree and RJ samples showed the lowest phenolic acid content.

Flavanols promote blood flow to the brain and heart, decrease blood pressure, and prevent cell damage [32]. In the present study, only quercetin-3-galactoside ([M–H]− at m/z = 609) and genistin ([M–H]− at m/z = 269) were identified and quantified from the carrot-blended smoothies. Moreover, genistin resulted from SCJ, and quercetin-3-galactoside resulted from RJ, SJ, and SCJ. The highest total flavanol content was observed in the following order: SCJ–WC > SCJ–YC > SCJ–OC. Thus, sour cherry was rich in flavanols.

Anthocyanins protect against type 2 diabetes, cardiovascular diseases, and cancer [33]. In the present study, seven anthocyanins were quantified; however, from raw PC, only five different anthocyanins were quantified [29]. In the present study, RJ, SJ, and SCJ increased the anthocyanin content of carrot-blended smoothies. Cyanidin-3-o-xylosyl-galactoside ([M–H]− at m/z = 581) and cyanidin-3-o-glucosyl-rutinoside resulted from RJ and SCJ, whereas cyanidin-3-arabinoside resulted only from RJ; cyanidin-3-o-xylosyl-*p*-coumaroylglucosyl-galactoside resulted from SJ, and cyanidin-3-o-xylosyl-cinpoyl-glucosylgalactoside resulted from SCJ. The highest total anthocyanin content was observed in the following order: SCJ–PC > SCJ–OC > SCJ–WC. Therefore, similar to the flavanol content, sour cherry was also rich in anthocyanins

Polymeric procyanidins exhibit anticancer, anti-inflammatory, antioxidant, and antiallergenic characteristics [34]. In the present study, the procyanidin content ranged from 4.26 to 25.56 mg/100 mL in the smoothies. The highest polymeric procyanidin content was observed in the following order: SCJ–PC > SCJ–YC > SCJ–WC, whereas the lowest polymeric content was observed as follows: PJ–PC < PJ–WC < PJ–YC. Thus, SCJ increased the polymeric procyanidin content of the carrot-based smoothies, whereas PJ decreased it. Moreover, in the present study, the DP (the number of flavanol units) of polymeric procyanidins was explored. The highest DP values were observed in the following order: RJ–PC > AJ–PC > PJ–PC. However, DP was not observed in the SJ–PC, SJ–WC, SJ–YC, and SJ–OC smoothies. Hence, these results show that PC changes the DP values, but SJ demonstrates the antagonistic characteristic with carrot, and the DP was not determined.

Carrot is a popular vegetable with a high carotenoid content; carotenoids are essential bioactive chemicals, vitamin A precursors, and anticancer, antidiabetic, antibacterial, and neuroprotective agents [35]. The carotenoid content of the carrot-based smoothies is shown in Table 3 and Supplementary S1, with significant differences ($p \leq 0.05$).

In the present study, only four carotenoids were identified: α-cryptoxanthin (zeinoxanthin), β-carotene, pheophytin a, and lutein. However, raw carrots showed 12 carotenoid types [29]. Thus, the smoothie manufacturing processes followed decreased the carotenoid types and contents. The primary reasons might be heating, air, light, and water exposures of carotenoids [36].

As PC is rich in α-cryptoxanthin, α-cryptoxanthin and β-carotene were quantified only in the AJ–PC smoothie [29]. However, PC did not contain β-carotene [29], and it resulted from AJ. Therefore, the highest total carotenoid content was observed in the following

order: AJ–PC > SJ–PC > PJ–PC, but carotenoids were absent in the RJ–PC smoothie and all WC-based smoothies.

3.3. Analyses of In Vitro Antioxidant and Biological Activities

The antioxidant activities (ABTS, FRAP, and ORAC) of the carrot-blended smoothies are shown in Table 4, with significant differences ($p \leq 0.05$).

Bioactive compounds in fruit- and vegetable-based food products increase their antioxidant characteristics [37]. In the present study, the ABTS antioxidant activity ranged from 0.42 to 1.78 mmol Trolox/100 mL. The highest activity was observed in PC puree samples in the following order: RJ > SJ > SCJ. However, the lowest ABTS activity was observed in PJ samples. Thus, these results showed that dark-colored fruit and vegetables increase the antioxidant activities of food and beverages. Similar results were observed for the FRAP and ABTS activities. However, unlike the ABTS activity, the SCJ–YC smoothie showed the lowest FRAP activity. Moreover, the ORAC activity ranged from 0.03 to 0.42 mmol Trolox/100 mL. The highest ORAC activity was observed in the following order: RJ–PC > SCJ–OC > SCJ–PC, whereas the lowest ORAC activity was observed in PJ smoothies. Thus, PC, RJ, and SCJ increased the antioxidant characteristics of smoothies. These samples were rich in flavon-3-ols, phenolic acids, flavanols, anthocyanins, and procyanidins. It should be emphasized that in the case of the developed products, not the quantity, but the quality of bioactive compounds had a greater impact on the shape of antioxidant potential of the final products. This was also indicated by other authors [1; 8; 12], who showed the high antioxidant activity of polymerized compounds and anthocyanins, which was confirmed in this study. The potential of polyphenolic compounds has been known for a long time, and the conducted research confirms this fact, indicating that the fortification of carrot puree with fruit juices, which are donors of secondary metabolites of plants, allows for the shaping of the health-promoting properties in the final products.

Table 4. The antioxidant [mmol TE/100 mL] and in vitro inhibition activities (acetylcholinesterase, butyrylcholinesterase, α-amylase, α-glucosidase, pancreatic lipase) of carrot-based smoothies.

Sample	ABTS	FRAP	ORAC	α-Amylase [IC50]	α-Glucosidase [IC50]	Lipase [IC50]	AChE [% inh]	BuChE [% inh]
RJ–PC	1.78 ± 0.31 ^{a}	1.47 ± 0.03 ^{a}	0.42 ± 0.02 ^{a}	543.86 ± 11.54 ^{ghj}	278.78 ± 5.91 ^{hj}	3.18 ± 0.07 ^{r}	9.77 ± 0.21 ^{b}	21.13 ± 0.45 ^{a}
AJ–PC	1.03 ± 0.05 ^{hjk}	0.87 ± 0.01 ^{jk}	0.12 ± 0.00 ^{f}	339.98 ± 7.21 ^{mn}	2966.52 ± 62.93 ^{a}	11.14 ± 0.24 ^{h}	6.53 ± 0.14 ^{gh}	14.41 ± 0.31 ^{bc}
PJ–PC	1.06 ± 0.06 ^{gh}	0.96 ± 0.02 ^{gh}	0.10 ± 0.01 ^{fg}	472.06 ± 10.01 ^{hj}	834.76 ± 17.71 ^{c}	10.85 ± 0.23 ^{hj}	5.16 ± 0.11 ^{kl}	13.33 ± 0.28 ^{c}
SJ–PC	1.71 ± 0.02 ^{ab}	1.32 ± 0.04 ^{b}	0.13 ± 0.01 ^{e}	312.44 ± 6.63 ^{n}	729.38 ± 15.47 ^{d}	7.94 ± 0.17 ^{l}	8.01 ± 0.17 ^{cd}	15.23 ± 0.32 ^{b}
SCJ–PC	1.67 ± 0.21 ^{b}	1.25 ± 0.05 ^{c}	0.22 ± 0.02 ^{c}	350.34 ± 7.43 ^{lm}	312.01 ± 6.62 ^{ghj}	3.70 ± 0.08 ^{p}	4.84 ± 0.10 ^{lm}	11.01 ± 0.23 ^{d}
PC%100	1.51 ± 0.47 ^{c}	1.08 ± 0.04 ^{ef}	0.28 ± 0.04 ^{b}	374.94 ± 7.95 ^{kl}	3020.95 ± 64.08 ^{a}	15.17 ± 0.32 ^{efg}	4.88 ± 0.10 ^{lm}	5.17 ± 0.11 ^{h}
RJ–WC	0.98 ± 0.03 ^{jk}	0.99 ± 0.02 ^{g}	0.06 ± 0.01 ^{k}	445.34 ± 9.45 ^{hj}	182.99 ± 3.88 ^{k}	5.23 ± 0.11 ^{o}	7.22 ± 0.15 ^{ef}	14.37 ± 0.30 ^{bc}
AJ–WC	0.58 ± 0.06 ^{m}	0.55 ± 0.02 ^{nö}	0.05 ± 0.00 ^{l}	752.39 ± 15.96 ^{f}	680.19 ± 14.43 ^{de}	14.12 ± 0.30 ^{g}	5.83 ± 0.12 ^{j}	6.41 ± 0.14 ^{g}
PJ–WC	0.81 ± 0.05 ^{l}	0.80 ± 0.04 ^{lm}	0.03 ± 0.00 ^{n}	2378.99 ± 50.47 ^{b}	618.14 ± 13.11 ^{e}	16.18 ± 0.34 ^{cde}	4.50 ± 0.10 ^{m}	4.27 ± 0.09 ^{l}
SJ–WC	1.17 ± 0.17 ^{ef}	1.13 ± 0.02 ^{e}	0.09 ± 0.01 ^{ghj}	391.47 ± 8.30 ^{jk}	158.57 ± 3.36 ^{l}	5.93 ± 0.13 ^{m}	5.96 ± 0.13 ^{hj}	9.03 ± 0.19 ^{ef}
SCJ–WC	1.04 ± 0.06 ^{ghj}	0.84 ± 0.02 ^{kl}	0.16 ± 0.00 ^{d}	1907.24 ± 40.46 ^{c}	287.00 ± 6.09 ^{hj}	8.64 ± 0.18 ^{kl}	4.85 ± 0.10 ^{lm}	9.98 ± 0.21 ^{de}
WC%100	0.40 ± 0.02 ^{p}	0.41 ± 0.01 ^{r}	0.01 ± 0.00 ^{ö}	539.63 ± 11.45 ^{ghj}	353.09 ± 7.49 ^{gh}	20.76 ± 0.44 ^{a}	5.14 ± 0.11 ^{kl}	3.53 ± 0.07 ^{m}
RJ–YC	1.12 ± 0.01 ^{fg}	1.10 ± 0.04 ^{e}	0.06 ± 0.01 ^{k}	995.64 ± 21.12 ^{e}	290.58 ± 6.16 ^{hj}	5.32 ± 0.11 ^{no}	8.09 ± 0.17 ^{c}	21.03 ± 0.45 ^{a}
AJ–YC	1.03 ± 0.06 ^{hjk}	0.91 ± 0.03 ^{hj}	0.05 ± 0.00 ^{l}	548.30 ± 11.63 ^{gh}	495.43 ± 10.51 ^{f}	14.65 ± 0.31 ^{fg}	2.96 ± 0.06 ^{o}	4.44 ± 0.09 ^{kl}
PJ–YC	0.42 ± 0.12 ^{öp}	0.53 ± 0.02 ^{öp}	0.04 ± 0.00 ^{m}	663.66 ± 14.08 ^{fg}	867.60 ± 18.40 ^{c}	16.90 ± 0.36 ^{bc}	4.59 ± 0.10 ^{lm}	4.84 ± 0.10 ^{hj}
SJ–YC	1.21 ± 0.08 ^{e}	1.04 ± 0.01 ^{f}	0.06 ± 0.00 ^{k}	1759.15 ± 37.32 ^{c}	311.45 ± 6.61 ^{ghj}	9.78 ± 0.21 ^{jk}	7.54 ± 0.16 ^{cde}	6.46 ± 0.14 ^{g}
SCJ–YC	0.76 ± 0.09 ^{l}	0.38 ± 0.02 ^{r}	0.16 ± 0.01 ^{d}	338.59 ± 7.18 ^{mn}	172.95 ± 3.67 ^{kl}	5.44 ± 0.12 ^{no}	10.77 ± 0.23 ^{a}	20.72 ± 0.44 ^{a}
YC%100	0.32 ± 0.03 ^{r}	0.36 ± 0.00 ^{r}	0.04 ± 0.00 ^{m}	3772.45 ± 80.03 ^{a}	1060.69 ± 22.50 ^{b}	16.53 ± 0.35 ^{cd}	7.10 ± 0.15 ^{efg}	4.63 ± 0.10 ^{jkl}
RJ–OC	1.11 ± 0.08 ^{fgh}	1.12 ± 0.04 ^{e}	0.08 ± 0.01 ^{j}	717.65 ± 15.22 ^{f}	126.09 ± 2.67 ^{m}	5.71 ± 0.12 ^{mn}	6.94 ± 0.15 ^{fg}	14.39 ± 0.31 ^{bc}
AJ–OC	0.93 ± 0.08 ^{k}	0.87 ± 0.03 ^{k}	0.05 ± 0.00 ^{l}	1859.88 ± 39.45 ^{c}	188.08 ± 3.99 ^{k}	15.57 ± 0.33 ^{def}	5.48 ± 0.12 ^{jk}	4.69 ± 0.10 ^{jk}
PJ–OC	0.48 ± 0.05 ^{nö}	0.59 ± 0.02 ^{n}	0.03 ± 0.00 ^{n}	1198.85 ± 25.43 ^{d}	658.98 ± 13.98 ^{de}	17.93 ± 0.38 ^{b}	2.45 ± 0.05 ^{p}	4.94 ± 0.10 ^{hj}
SJ–OC	1.36 ± 0.11 ^{d}	1.19 ± 0.08 ^{d}	0.10 ± 0.03 ^{fg}	419.00 ± 8.89 ^{j}	247.10 ± 5.24 ^{j}	8.96 ± 0.19 ^{kl}	3.81 ± 0.08 ^{n}	7.22 ± 0.15 ^{g}
SCJ–OC	1.39 ± 0.06 ^{d}	0.74 ± 0.10 ^{m}	0.27 ± 0.03 ^{b}	1066.91 ± 22.63 ^{e}	282.21 ± 5.99 ^{hj}	7.75 ± 0.16 ^{l}	7.43 ± 0.16 ^{def}	8.67 ± 0.18 ^{f}
OC%100	0.53 ± 0.00 ^{mn}	0.49 ± 0.01 ^{p}	0.09 ± 0.00 ^{ghj}	1087.83 ± 23.08 ^{de}	401.47 ± 8.52 ^{g}	16.67 ± 0.35 ^{cd}	5.02 ± 0.11 ^{klm}	4.43 ± 0.09 ^{kl}

TE, Trolox equivalents. Significant at $p \leq 0.05$; values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Tukey's test.

The results of the in vitro biological activities of carrot-blended smoothies are shown in Table 4, with significant differences ($p \leq 0.05$) for α-amylase [IC50], α-glucosidase [IC50], lipase [IC50], AChE [% inhibition], and BuChE [% inhibition].

As reported in a previous study, the inhibition of α-amylase and α-glucosidase may control diabetes [38]. In the present study, the highest α-amylase inhibition activity was observed in the following order: SJ–PC > SCJ–YC > AJ–PC. However, the lowest α-amylase inhibition activity was observed as follows: PJ–WC < SCJ–WC < AJ–OC < SJ–YC. Thus, PC samples showed the highest α-amylase inhibition activity. Nevertheless, the highest α-glucosidase inhibition activity was observed in the following order: RJ–OC > SJ–WC > SCJ–YC, whereas the lowest α-glucosidase inhibition activity was observed as follows: AJ–PC < PJ–YC < PJ–PC. SJ, SCJ, and AJ smoothies showed the highest α-amylase inhibition activity, and RJ, SJ, and SCJ smoothies showed the highest α-glucosidase inhibition activity. The PC puree showed the lowest α-glucosidase inhibition activity, whereas the YC puree showed the lowest α-glucosidase and α-amylase inhibition activities. Hence, α-amylase inhibition activity might be related to the content of anthocyanins, but also to the content of pectins, whereas α-glucosidase inhibition activity is due to the interaction with polymeric procyanidins, flavanol, and also organic acids.

The inhibition of pancreatic lipase might decrease obesity [39]. In the present study, the highest lipase inhibition activity was observed in the following order: RJ–PC > SCJ–PC > RJ–WC, whereas the lowest lipase inhibition activity was observed as follows: PJ–OC < PJ–YC < PJ–WC. Therefore, the RJ–PC smoothie showed the highest lipase inhibition activity. Moreover, similar to α-amylase inhibition activities, anthocyanins and pectins might be responsible for lipase inhibition activities.

The inhibition of AChE and BuChE might reduce nervous system disorders related to aging [40]. In the present study, the highest AChE inhibition activity was observed in the following order: SCJ–YC > RJ–PC > RJ–YC, whereas the lowest AChE inhibition activity was observed as follows: PJ–OC < AJ–YC < SJ–OC. Thus, RJ and SCJ smoothies showed an increased AChE inhibition activity. Moreover, the highest BuChE inhibition activity was observed in the following order: RJ–PC > RJ–YC > SJ–PC, whereas the lowest BuChE inhibition activity was observed as follows: PJ–WC < AJ–YC < AJ–OC. Similar to the lipase inhibition activity, the RJ–PC smoothie showed the highest BuChE inhibition activity as well. Moreover, its lipase, AChE, and BuChE inhibition activities were similar to the results of the antioxidant activity assays, which identify it as the best product. Thus, the anthocyanin content increased the enzyme inhibition and antioxidant activities of the carrot-blended smoothies. Furthermore, the RJ–PC smoothie showed the highest polymeric procyanidin content, and these bioactive compounds might also shape the activities.

To sum up, the obtained products, in particular those developed on the basis of PC puree, show a pro-health effect at the level of in vitro research. On the one hand, processing processes (e.g., heating and oxidation) affect the degradation of polyphenolic compounds compared to fresh raw materials. On the other hand, creating recipes and combining several plant materials can cause a synergy effect, which can ultimately increase the health-promoting effect. However, in order to demonstrate the health-promoting effect with certainty in the future, selected formulations should be tested on a human model, taking into account physiological processes, digestive processes, and biotransformation that may change the activities in the body.

3.4. Sensorial Evaluation of Carrot-Blended Smoothies

The sensorial characteristics of the carrot-blended smoothies are presented in Figure 4 and Table 5, including carrot savor and aroma, appearance and sweetness characteristics, raspberry, apple, pear, strawberry or sour cherry flavor, and acceptance of the smoothies.

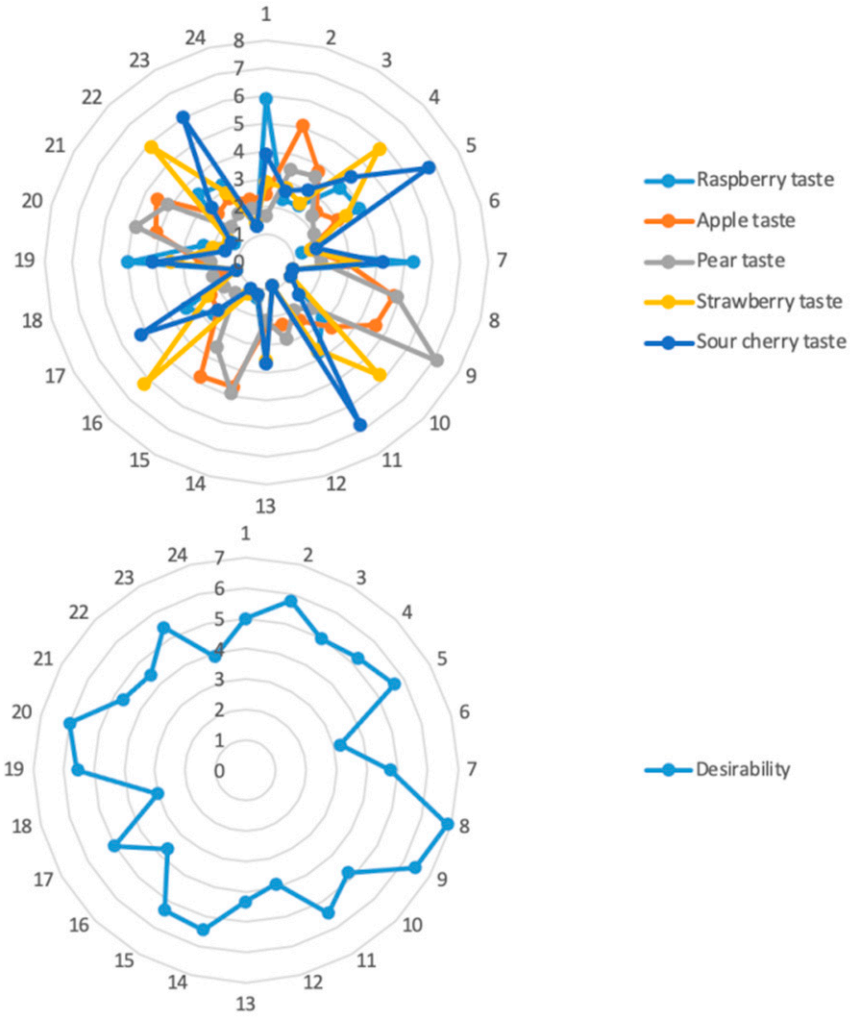

Figure 4. Sensorial characteristics of carrot blended smoothies according to the flavor of the final product and sensorial preferences (1, RJ–PC; 2, AJ–PC; 3, PJ–PC; 4, SJ–PC; 5, SCJ–PC; 6, PC%100; 7, RJ–WC; 8, AJ–WC; 9, PJ–WC; 10, SJ–WC; 11, SCJ–WC; 12, WC%100; 13, RJ–YC; 14, AJ–YC; 15, PJ–YC; 16, SJ–YC; 17, SCJ–YC; 18, YC%100; 19, RJ–OC; 20, AJ–OC; 21, PJ–OC; 22, SJ–OC; 23, SCJ–OC; 24, OC%100).

Table 5. Sensorial characteristics of carrot blended smoothies.

	Appearance	Sweetness	Carrot Taste	Carrot Smell
RJ–VC	7.77 ± 0.16^{ab}	2.77 ± 0.06^{h}	4.55 ± 0.10^{jk}	5.88 ± 0.12^{ef}
AJ–VC	6.77 ± 0.14^{efg}	6.88 ± 0.15^{a}	6.55 ± 0.14^{de}	5.55 ± 0.12^{efg}
PJ–VC	6.11 ± 0.13^{g}	6.00 ± 0.13^{b}	7.00 ± 0.15^{d}	6.00 ± 0.13^{de}
SJ–VC	7.55 ± 0.16^{bc}	3.22 ± 0.07^{fg}	3.33 ± 0.07^{no}	3.33 ± 0.07^{n}
SCJ–VC	6.88 ± 0.15^{defg}	3.55 ± 0.08^{de}	3.22 ± 0.07^{o}	4.11 ± 0.09^{kl}
VC%100	2.22 ± 0.05^{m}	3.66 ± 0.08^{d}	8.11 ± 0.17^{b}	9.00 ± 0.19^{a}
RJ–WC	7.44 ± 0.16^{bcd}	2.44 ± 0.05^{j}	2.66 ± 0.06^{p}	3.66 ± 0.08^{lm}
AJ–WC	7.55 ± 0.16^{bc}	6.66 ± 0.14^{a}	5.22 ± 0.11^{gh}	5.44 ± 0.12^{fg}
PJ–WC	7.33 ± 0.16^{bcde}	6.55 ± 0.14^{a}	4.33 ± 0.09^{kl}	3.77 ± 0.08^{lm}
SJ–WC	5.00 ± 0.11^{j}	3.55 ± 0.08^{de}	4.11 ± 0.09^{kl}	4.00 ± 0.08^{l}
SCJ–WC	8.22 ± 0.17^{a}	3.33 ± 0.07^{ef}	3.33 ± 0.07^{no}	3.55 ± 0.08^{mn}
WC%100	5.66 ± 0.12^{h}	5.00 ± 0.11^{c}	8.66 ± 0.18^{a}	7.77 ± 0.16^{b}
RJ–YC	6.33 ± 0.13^{g}	3.00 ± 0.06^{gh}	4.22 ± 0.09^{kl}	4.22 ± 0.09^{jkl}
AJ–YC	6.88 ± 0.15^{defg}	6.88 ± 0.15^{a}	5.00 ± 0.11^{hj}	3.88 ± 0.08^{l}

Table 5. *Cont.*

	Appearance	Sweetness	Carrot Taste	Carrot Smell
PJ–YC	6.55 ± 0.14^{fg}	6.88 ± 0.15^{a}	4.33 ± 0.09^{kl}	3.55 ± 0.08^{mn}
SJ–YC	4.33 ± 0.09^{k}	3.66 ± 0.08^{d}	3.66 ± 0.08^{m}	3.33 ± 0.07^{n}
SCJ–YC	7.33 ± 0.16^{bcde}	4.11 ± 0.09^{d}	4.00 ± 0.08^{l}	3.00 ± 0.06^{o}
YC%100	3.11 ± 0.07^{l}	4.77 ± 0.10^{c}	7.66 ± 0.16^{bc}	6.88 ± 0.15^{c}
RJ–OC	6.44 ± 0.14^{fg}	3.66 ± 0.08^{d}	3.55 ± 0.08^{mn}	3.66 ± 0.08^{lm}
AJ–OC	7.00 ± 0.15^{cdef}	6.88 ± 0.15^{a}	5.66 ± 0.12^{fg}	4.55 ± 0.10^{jk}
PJ–OC	6.88 ± 0.15^{defg}	6.77 ± 0.14^{a}	6.11 ± 0.13^{ef}	5.11 ± 0.11^{gh}
SJ–OC	6.33 ± 0.13^{g}	4.00 ± 0.08^{d}	3.66 ± 0.08^{m}	3.88 ± 0.08^{l}
SCJ–OC	6.11 ± 0.13^{g}	4.00 ± 0.08^{d}	2.77 ± 0.06^{p}	4.66 ± 0.10^{hj}
OC%100	5.11 ± 0.11^{j}	5.66 ± 0.12^{b}	7.55 ± 0.16^{c}	6.44 ± 0.14^{cd}

Significant at $p \leq 0.05$; values (mean of three replications) followed by the same letter within the same column were not significantly different ($p \leq 0.05$) according to Tukey's test.

The panelists evaluated the products for appearance, sweetness, whether they sense the carrot taste, what flavor they sense the most (raspberry, apple, pear, strawberry, or sour cherry flavor), whether they sense the carrot smell, and their desire for the smoothies.

Food appearance depends on the surface color, shape, and size of the food at first sight and determines whether consumers buy or reject it [41]. In the present study, the highest acceptance for the appearances of the smoothies was in the following order: SCJ–WC > RJ–PC > SJ–PC = AJ–WC, whereas the lowest acceptance for the appearance was as follows: SJ–YC < SJ–WC < PJ–PC = SCJ–OC. Thus, PC and WC smoothies showed the highest acceptance for appearance.

Sweet taste is one of the parameters for consumers to purchase a food product. In the present study, the highest sweetness was observed in the following order: AJ–PC = AJ–YC = PJ–YC = AJ–OC, whereas the lowest sweetness was observed as follows: RJ–WC < RJ–PC < RJ–YC. These results show that AJ increases the sugar content in the carrot-blended smoothies, whereas RJ decreases it.

Carrot varieties are rich in nutritional content; however, many people, especially children, do not prefer carrot flavor and aroma in beverages. Therefore, carrot-blended smoothies were evaluated for both carrot taste and smell. The results showed that the highest carrot taste was observed in the following order: PJ–PC > AJ–PC > PJ–OC, whereas the lowest carrot taste was observed as follows: RJ–WC < SCJ–OC < SCJ–PC. Thus, PJ cannot mask the carrot taste, whereas SCJ can easily suppress it. Nevertheless, the highest carrot aroma was observed in the following order: PJ–PC > RJ–PC > AJ–PC, whereas the lowest carrot aroma was observed as follows: SCJ–YC < SJ–YC = SJ–PC. Hence, PC puree samples showed the highest carrot aroma, but the sweet smell of strawberry changed the aroma of the carrot-blended smoothies.

This study showed that with different fruit juices, carrot taste and smell can be suppressed to attract consumption by all age groups. Therefore, the prepared carrot blended smoothies were evaluated for the presence of raspberry, apple, pear, strawberry, or sour cherry taste. As expected, RJ, AJ, PJ, SJ, and SCJ smoothies showed the highest results for taste; however, some extreme results were observed as well. For instance, the PJ–YC smoothie was evaluated as having the taste of apple, and AJ smoothies were evaluated as having the taste of pear. In conclusion, many carrot-blended smoothies were evaluated as not having the flavor and/or aroma of carrot.

Finally, carrot-based smoothies were evaluated for acceptance. The highest acceptance was observed in the following order: AJ–WC > PJ–WC > AJ–OC, whereas the lowest acceptance was observed in the following order: SJ–YC < RJ–YC < SJ–OC. Thus, the AJ–WC smoothie might be used in future beverage preparations.

4. Conclusions

This study compared the in vitro biological activities and physicochemical characteristics of different carrot–fruit juice smoothies (raspberry, apple, pear, strawberry, and sour cherry juices). The raspberry juice–purple carrot smoothie showed the highest TA and antioxidant activity (in all assays) against lipase and BuChE inhibitions. These characteristics are not only important from the nutrition perspective but also provide opportunities for beverage processing. The sour cherry juice–purple carrot smoothie showed the highest results for TSS, dry mass, osmolality, total phenolic acid, and total anthocyanin and procyanidin contents. The apple juice–purple carrot smoothie showed the highest carotenoid content. Interestingly, the apple juice–white carrot smoothie was voted for the highest product acceptance, although it did not show potent nutritional content and biological activities. Purple carrot, raspberry, and sour cherry juice smoothies are rich in bioactive compounds with biological activities. The fruit and vegetables studied might be applied together in functional and/or novel food products to improve their nutritional characteristics. On the other hand, bioavailability assays are essential for further analyses to see the biotransformation of the bioactive compounds in carrot-based smoothies during the digestion process. Additionally, shelf-life studies of the obtained smoothies are being conducted, which will answer a number of questions related to the dynamics of changes in the chemical composition and physicochemical properties of aging products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox12040917/s1, Supplementary S1: Polyphenolic and carotenoid contents of carrot-based smoothies.

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mój udział polegał na tworzeniu planu badań, opracowaniu technologii produkcji smoothies na bazie różnych wariantów marchewki, przeprowadzeniu doświadczeń i analiz, z uwzględnieniem analizy zawartości cukrów z wykorzystaniem techniki HPLC-ELSD, kwasów organicznych z użyciem UPLC, a także zawartości związków bioaktywnych (LC/MS i UPLC) oraz właściwości prozdrowotnych *in vitro*. Dodatkowo prowadziłam analizy podstawowych wyróżników składu chemicznego i właściwości sensorycznych otrzymanych produktów półpłynnych. Ponadto otrzymane wyniki opracowałam pod kątem statystycznym i merytorycznym, przygotowując manuskrypt, a także brałam udział w współredagowaniu tekstu w procesie recenzji.
Jestem także członkiem Interdyscyplinarnej Międzynarodowej Szkoły Doktorskiej na UPWr, współfinansowanej ze środków Europejskiego Funduszu Społecznego w ramach Programu Operacyjnego Wiedza Edukacja Rozwój, na podstawie umowy nr POWR.03.05.00-00-Z062/18 z dnia 4 czerwca 2019 r., która wsparła finansowo prezentowane wyniki.

Potwierdzam zadeklarowane zaangażowanie
Emel Hasan Yusuf
Paulina Nowicka
02.06.2023

02.06.2023 Emel Yusuf
data i podpis

DECLARATION

I declare that in the publication entitled:
Yusuf E.H., Wojdyło A., Bourbon A.I., Nowicka, P. 2023. Fruit-carrot-based smoothies as innovative products with a complex matrix of bioactive compounds effected on activities of selected digestive enzymes and cholinesterases *in vitro*. *Antioxidants*, 12(4), 917.
https://doi.org/10.3390/antiox12040917
my participation consisted in creating a research plan, developing a technology for the production of smoothies based on different variants of carrots, conducting experiments and analyses, including the analysis of sugar content by using HPLC-ELSD technique, the content of organic acids by using UPLC, the content of bioactive compounds (by LC/MS and UPLC), and also pro-health properties by *in vitro* methods. In addition, I measured basic chemical parameters in carrot semi-liquid products, and them sensory evaluation. Moreover, I analyzed the obtained results in statistical and substantive terms, preparing the manuscript, and I also participated in the co-editing of the text in the review process.
I am also a member of Interdisciplinary International Doctoral School at UPWr, co-financed by the European Social Fund under the Operational Program Knowledge Education Development, under contract No. POWR.03.05.00-00-Z062/18 of June 4, 2019, which financially supported the presented results.

I confirm the declared commitment
of Emel Hasan Yusuf
Paulina Nowicka
02.06.2023

02.06.2023 Emel Yusuf
the date and sign

Publication 5

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The effect of combined drying process (OD-CD-VMD) on nutritional, phytochemical, and sensory profiles, and biological activities of colored dried carrot

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ABSTRACT

The drying process is used in the food industry to increase the shelf-life of food products without adding preservatives. In this study, using a combination of osmotic dehydration (OD), convective, and microwave-vacuum drying technologies, carrot snacks were prepared using four colored carrot varieties (purple, orange, yellow, and white carrots), three fruit concentrates (apple, chokeberry, and sour cherry), and sucrose solution (control group). The results showed that the OD process increased the polyphenolic content of the dried carrot samples; however, their carotenoid content decreased due to heating processes, and α-cryptoxanthin was the only carotenoid found in all carrot snacks. In addition, yellow carrot dehydrated in the sour cherry solution exhibited the highest total polyphenolic content (TPC), with the highest acetylcholinesterase and butyrylcholinesterase enzyme inhibition activities. In general, the use of the chokeberry solution resulted in high antioxidant activities and, in the case of orange carrot, the highest α-glucosidase and pancreatic lipase activities. Furthermore, sensorial acceptance of carrot snacks changed with product characteristics. The purple carrot-apple juice snack was evaluated as the best-colored product, and the orange carrot-sour cherry snack was evaluated as the best-smelling product. Finally, the obtained products were found to be attractive snacks with pro-health properties.

1. Introduction

The search for novel food products with long storage periods is ongoing in the food industry. To achieve this, new technologies are applied to extend the shelf-life of processed food products without adding preservatives (Prosapio & Norton, 2017). Moreover, conscious consumers ensure the balance between the health aspects of food products and their taste. So far, various plant snacks have been prepared using different methods with diverse health benefits (Huang & Zhang, 2012). However, snacking trends change frequently depending on consumer needs.

Drying is used in the food industry since ancient times. Nowadays, drying technologies are used to manufacture novel products with long shelf-life (Bochnak & Świeca, 2020). The methods and devices used in drying change depending on the expected outcomes. For instance, osmotic dehydration (OD) of vegetables with fruit juices increases the nutritional content, and enhances the color and savor of the dried food products before the primary drying technologies are applied. In OD, water transports from the fruit or vegetable to the hypertonic solution (Rastogi et al., 2005, pp. 221–249). Sugar and salt solutions are the most used materials in OD; however, recently, fruit solutions have also been applied as novel materials (Nowicka et al., 2015a). More importantly, food products subjected to OD can be consumed by all age groups, because obtained products is characterized by high nutritional content, attractive color and smell features. In addition, from technical point of view OD requires low energy and causes low heating damage to food products, which is extremely important (Jayaraman & Das, 1992).

After OD, the primary drying technologies are applied, such as convective and microwave vacuum drying methods. Convective drying uses a gas-solid approach in which water in the food product moves to the drying medium due to the gradient between water vapor pressure on the food surface and air (Herman-Lara et al., 2010). On the other hand, microwave vacuum drying is expeditious and uses microwaves to heat the food products and vacuum domain to reduce dehydration

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temperature, resulting in high-quality foods (Lagnika et al., 2018). Dried food products are preserved from microbial activities and oxidation reactions, with long storage periods (Hernández-Santos et al., 2016).

Plant-based dried food products are as good as, fruits and vegetables are rich in health-promoting phytochemicals (Chang et al., 2016) and gluten free (Wójtowicz et al., 2019). Carrot can be used as a model for vegetable snacks. So far, carrot has been used in the production of fiber-rich, nutritious, whole-grain carrot chips (Norazmir et al., 2014). Studies have reported that ultrasound drying and enzymatic treatment have a positive effect on the carotenoid content of carrot (Konopacka et al., 2017) and that the barley flour-carrot pomace snack is rich in soluble dietary fibers, with the high nutritional content (Lotfi Shirazi et al., 2020). Thus, dried carrot has a high carotenoid content, micro and macro elements, all of which increase human immunity (Cao et al., 2019; Chen et al., 2016).

This study aimed to prepare dried carrot snacks using OD, convective drying, and vacuum microwave drying, in which fruit juices were used to increase the L-ascorbic acid and bioactive contents of colored carrot varieties and enhance their pro-health properties. The water activities, color characteristics, and L-ascorbic acid, polyphenolic and carotenoid contents of the dried carrot materials were analyzed. In addition, antioxidant activities, α-amylase, α-glucosidase, lipase, acetylcholinesterase, and butyrylcholinesterase inhibition activities, and sensorial acceptance of the products were evaluated. This study is the first to evaluate the physicochemical, bioactive compounds, biological activities and sensorial acceptances of dried colored carrot food products.

2. Materials and methods

2.1. Chemicals

Formic acid, acetonitrile and methanol for analyses of ultra-performance liquid chromatography (UPLC; gradient grade); orthophosphate dihydrogen, dipotassium hydrogen, sodium phosphate monobasic, starch from potato, alfa-amylase from porcine pancreas (type VI-8; the European Community number (EC number) 3.2.1.1.; p-nitrophenyl-alfa-D-glucopyranoside, alfa-glucosidase from *Saccharomyces cerevisiae* (type I, EC number 3.2.1.20), lipase from porcine pancreas type II (EC number 3.1.1.3), p-nitrophenyl acetate, acetylcholinesterase from *Electrophorus electricus* (electric eel) (type VI-S; EC number 3.1.1.7), butyrylcholinesterase from equine serum (EC number 3.1.1.8), acetylthiocholine iodide, S-butyrylthiocholine chloride, and DTNB (5,5-dinitrobis-(2-nitrobenzoic acid) were purchased from Merck (Darmstadt, Germany). Standards for polyphenolic (3-o-caffeoylquinic acid, *cis*-5-p-coumaroylquinic acid, pelargonidin-3-rutinoside, cyanidin-3-glucoside, arbutin, catechin, quercetin-3-galactoside, and keampferol-7-glucuronide) and carotenoid (α-cryptoxantin) compounds were acquired from Extrasynthese (Lyon, France). Samples were prepared for chromatography analysis by applying the HLP SMART 1000 s system (Hydrolab, Gdańsk, Poland), with filtration by 0.22 μm membrane filter before use.

2.2. Materials

Yellow, orange, purple and white carrots were purchased from Fusion Gusto (Dąbrowa, Poland). Before drying processes, the carrot samples were cut into slices of about 3 mm wide. Concentrates of sour cherry, chokeberry, and apple were acquired from Rauch (Płońsk, Poland). Sucrose solution was prepared in the laboratory of the Department of Fruit and Vegetable Technology and used as the control.

2.3. Osmotic dehydration and drying experiments

OD was carried out using beakers with carrot samples (100 g) and osmotic solutions (200 mL) that were kept in water baths at 45 °C for 90 min (Lech et al., 2015). As mentioned earlier, four solutions were used: (i) sucrose solution 40 g/100 mL, (ii) commercial concentrated chokeberry juice (40 g/100 mL), (iii) commercial concentrated sour cherry juice (40 g/100 mL), and (iv) commercial concentrated apple juice (40 g/100 mL).

Colored carrot samples purple, orange, yellow, and white were mixed separately in each of the solutions sour cherry, chokeberry, apple, and sucrose solutions and then dried by combining two different technologies: (i) convective pre-drying (CD) and (ii) vacuum-microwave finish drying (VMD). CD was carried out for 90 min at 50 °C using a dryer designed and made by the Institute of Agricultural Engineering (Wroclaw, Poland). VMD was carried out using an SM200 dryer (Plazmatronika, Poland) at 360 W, which was reduced to 120 W when the temperature was above 80 °C to avoid overheating of the samples. The temperature during VMD was measured using an infrared camera i50 (Flir Systems AB, Stockholm, Sweden). The dried carrot snacks are listed in Table 1.

2.4. Water activity, moisture content, and color parameters

Water activities of the dried carrot samples were determined in triplicate using a Novasina water activity meter (LabMas-terav., Lachen, Switzerland) at 20.0 ± 0.5 °C.

The moisture (M) content of the samples was determined (n = 3) using a vacuum-dryer (SPT-200; ZEAMiL Horyzont, Kraków, Poland) at 70 °C at the pressure of 100 Pa for 24 h.

The color of the 16 dried carrot snacks was determined using an A5 Chroma-Meter (Minolta CR300; Osaka, Japan), referring to color space CIE L*a*b*. The lightness (L*), redness-greenness (a*), and yellowness-blueness (b*) values were determined using an Illuminant D65 and 10° observer angle. Values were the mean of five replicates.

2.5. L-ascorbic acid, polyphenol, and carotenoid contents

The L-ascorbic acid contents of the dried carrot samples were determined according to the following PN-A-04019 procedure (Mazurek & Włodarczyk-Stasiak, 2013).

Polyphenols were analyzed according to the procedure of Wojdyło et al. (2018). The powder of dried carrot food products (~0.5 g) was mixed with 5 mL of the mixture containing HPLC-grade methanol: H_2O (30:70 fraction), ascorbic acid (2 g/100 mL), and acetic acid (1 g/100 mL). After sonication (Sonic 6D, Polsonic, Warsaw, Poland), the slurry was centrifuged at 19000×*g* for 10 min. The supernatant was filtered through a 0.20 μm hydrophilic polytetrafluoroethylene (PTFE) membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and subjected to liquid chromatography-mass spectrometry/quadrupole time-of-flight (LC-MS-Q/TOF) and ultra-performance liquid chromatography with a photodiode array detector (UPLC-PDA) to determine

Table 1
Dried carrot samples.

Dried carrot samples	Abbreviations
White carrot & Sucrose solution	WCSS
Orange carrot & Sucrose solution	OCSS
Yellow carrot & Sucrose solution	YCSS
Purple carrot & Sucrose solution	PCSS
White carrot & Sour cherry solution	WCSCS
Orange carrot & Sour cherry solution	OCSCS
Yellow carrot & Sour cherry solution	YCSCS
Purple carrot & Sour cherry solution	PCSCS
White carrot & Apple solution	WCAS
Orange carrot & Apple solution	OCAS
Yellow carrot & Apple solution	YCAS
Purple carrot & Apple solution	PCAS
White carrot & Chokeberry solution	WCCS
Orange carrot & Chokeberry solution	OCCS
Yellow carrot & Chokeberry solution	YCCS
Purple carrot & Chokeberry solution	PCCS

the polyphenol contents (flavon-3-ols at 280 nm, phenolic acids at 320 nm, flavanols at 360 nm, and anthocyanins at 520 nm). The polymeric procyanidin (PP) content was also determined (Kennedy & Jones, 2001).

The carotenoid contents of the dried carrot snacks were analyzed following the procedure of Yusuf et al. (2021). The dried carrot food products (~0.5 g) were shaken in a DOS 10L Digital Orbital Shaker (Elmi Ltd., Riga, Latvia) with 5 mL of a ternary mixture of methanol/acetone/hexane (1:1:2, by vol.) at 300 rpm for 30 min in the dark and to prevent oxidation 10 g/100 mL $MgCO_3$, 1 g/100 mL butylhydroxytoluene were also added. The samples were centrifuged (4 °C, 7 min at 19000×*g*; MPW-350, Warsaw, Poland), and the supernatants were obtained after four rounds of centrifugation of the re-extracted solid residue. The combined fractions were evaporated. The pellet was solubilized using methanol and filtered through a 0.20 μm hydrophilic PTFE membrane (Millex Samplicity® Filter, Merck, Darmstadt, Germany). The carotenoids and chlorophylls were analyzed using LC-MS-Q/TOF (identification) and UPLC-PDA (quantification).

All evaluations were performed in triplicate, and the results were expressed as mg per 100 g of product (p).

2.6. Antioxidant and biological activity assays

The antioxidant activities of the dried carrot food products were determined using ABTS, FRAP, and oxygen radical absorbance capacities (ORAC) assays following the procedures of Re et al. (1999); Benzie and Strain (1996); and Ou et al. (2002), respectively. The antioxidant activities of the dried food products were measured by the reduction of ABTS+• radicals, and the absorbance was read at 734 nm. The ferric reduction of the carrot snacks was evaluated using FRAP; at low pH, the colorless ferric complex (Fe^{3+}-tripyridyltriazine) transformed to a blue ferrous complex (Fe^{2+}-tripyridyltriazine) by the action of electron-donating antioxidants, and the absorbance was measured at 593 nm. The ORAC of the dried carrot snacks was determined using a spectrofluorometric method, which showed that fluorescence decreased by the oxidation of free radicals in the presence of antioxidants.

The obesity and hyperglycemic enzyme activities were determined according to the procedures of Podsędek et al. (2014), and Nowicka et al. (2016), respectively. Based on the amount of p-nitrophenol formed from p-nitrophenyl acetate, the pancreatic lipase enzyme inhibition activity was read at 400 nm using p-nitrophenol formed from p-nitrophenyl acetate after incubation of the basic samples with enzyme and substrate at 37 °C. The reference samples contained buffer instead of enzymes, and orlistat was used as a positive control. The α-amylase enzyme inhibition activity was determined for the reactions of iodine with starch after incubation at 37 °C at 600 nm. The α-glucosidase enzyme inhibition activity was determined for the reaction of the enzyme with the β-D-glucosidase substrate at 405 nm. The reference samples contained buffer instead of enzymes, and acarbose was used as a positive control. The results were expressed as IC50 in mg/mL.

The antiaging activity was evaluated using acetylcholinesterase and butyrylcholinesterase following the methods of Gironés-Vilaplana et al. (2015). The reactions of thiocholine with 5,5′-dithiobis-(2-nitrobenzoic acid) produced 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate. The substrates of acetylcholine iodine and butylcholine chloride were read at 405 nm, and the results were expressed as percentages of inhibition.

2.7. Sensory analyses of dried carrot snacks

Sensory tests were conducted in a sensory analysis laboratory equipped with individual booths (at a controlled temperature (~20 °C), and combined natural/artificial light) designed according to ISO 8589:2009 standards. The laboratory was located at the Faculty of Biotechnology and Food Sciences, Wrocław University of Environmental and Life Sciences (Poland). The sensory evaluation sessions were conducted from 10 a.m. to 1 p.m. by 9 fully trained panellists in the age between 25 and 43 years. All panelists received the same training to make them accustomed to the sensory attributes of dried products, and to understand the descriptors being used. It is important because they had to be able to discriminate differences between products, describe the different product attributes (qualitatively), and scale the intensity of the attribute (quantitatively).

The sensory evaluation of the carrot snacks was performed using the 9-point hedonic scale (like extremely-9, like very much-8, like moderately-7, like slightly-6, neither like nor dislike-5, dislike slightly-4, dislike moderately-3, dislike very much-2 and dislike extremely-1). Panelists voted the carrot snacks for color, smell, taste acceptance, consistency (very hard, hard, a little hard, neither hard nor soft, a little soft, soft, and very soft), and taste felt (black currant, carrot, apple, sour cherry, raspberry, and chokeberry), and the results have been given with standard deviations. For sensory evaluation, the carrot snacks were labeled with codes, and the test was performed at room temperature. The snacks were served in white, small plastic containers. After tasting each sample, the panelists neutralize their mouth.

No ethical approval was required for this study, because of national laws. Participants were informed about the their participation was entirely voluntary so that they could stop the analysis at any point and the responses would be anonymous.

2.8. Statistical analysis

The results were subjected to analysis of variance ($p < 0.05$), and Tukey's honestly significant difference test was performed using the R software (version 4.0.2, R Core Team, Austria). The results were expressed as mean values ($n = 3 \pm 5$) ± standard deviation.

3. Results and discussion

3.1. Osmotic dehydration

OD is used to produce novel food products with high sensory and nutritional features (Ahmed et al., 2016). In the present study, changes in the moisture contents of the dried carrot samples before and after OD are shown in Table 2. Among fresh carrot materials, the highest moisture content was observed in white carrot (91.89 g/100 mL), followed by orange carrot (91.03 g/100 mL), yellow carrot (90.72 g/100 mL) and purple carrot (89.11 g/100 mL) samples. Dry weights of all raw carrot materials in all solutions were statistically similar and were higher than 92.00 g/100 g. During the OD process of the carrot samples in sucrose, sour cherry, apple, and chokeberry solutions, distinct decreases were observed in the moisture contents, which ranged from 67.31 g/100 mL to 73.49 g/100 mL after OD. The highest moisture contents were observed in the carrot samples dehydrated in the sucrose solution and subjected to WCSCS (69.82 g/100 mL), WCCS (69.57 g/100 mL) and YCAS (69.14 g/100 mL). The lowest moisture contents were observed in the PCAS (67.31 g/100 mL), WCAS (68.00 g/100 mL), and PCSCS (68.18 g/100 mL) samples.

The results of solid gains and water losses of the carrot samples during the OD process are presented in Table 2. The solid gain was in the following order: YCAS > YCSCS = PCSCS. Nevertheless, control groups OCSS and WCSS demonstrated the lowest solid gains. Water losses were much higher than solid gains because of the cell membrane permeability, which allows transferring of small molecules such as water but prevents the absorption of large molecules (Lagnika et al., 2018). In the present study, the highest water losses were observed in the WCSCS samples, followed by the OCSCS samples. However, the WCSCS samples, the third lowest solid gain preceded by the OCSS and WCSS samples, respectively. The water losses of the samples were in the following order: PCAS < PCSCS < PCCS. Thus, the PCSCS samples, the second highest solid gain and the second lowest water loss. These results are attributable to the concentration gradient, which transferred solids from solutions to carrot samples and transferred water from carrot samples to

Table 2
Solid gain, water loss and moisture content during osmotic dehydration of four varieties of carrots in sucrose, sour cherry, apple and chokeberry osmotic solutions.

	Mc of fresh carrot, g/100 mL	Mc after OD, g/100 mL	Dry weight after OD-VMD, g/100 g	SG	WL
WCSS	91.890 ± 1.950 ^{a}	72.350 ± 1.530 ^{a}	97.230 ± 2.060 ^{a}	0.085 ± 0.001 ^{h}	0.458 ± 0.015 ^{bc}
OCSS	91.030 ± 1.930 ^{a}	73.490 ± 1.560 ^{a}	92.520 ± 1.960 ^{a}	0.084 ± 0.005 ^{h}	0.457 ± 0.010 ^{bc}
YCSS	90.720 ± 1.920 ^{a}	72.300 ± 1.530 ^{a}	95.580 ± 2.030 ^{a}	0.119 ± 0.003 ^{ef}	0.405 ± 0.009 ^{de}
PCSS	89.110 ± 1.890 ^{a}	70.340 ± 1.490 ^{a}	97.580 ± 2.070 ^{a}	0.113 ± 0.001 ^{f}	0.378 ± 0.007 ^{ef}
WCSCS	91.890 ± 1.950 ^{a}	69.820 ± 1.480 ^{a}	94.470 ± 2.000 ^{a}	0.097 ± 0.002 ^{g}	0.524 ± 0.021 ^{a}
OCSCS	91.030 ± 1.930 ^{a}	68.930 ± 1.460 ^{a}	93.920 ± 1.990 ^{a}	0.111 ± 0.002 ^{f}	0.476 ± 0.013 ^{b}
YCSCS	90.720 ± 1.920 ^{a}	68.410 ± 1.450 ^{a}	94.870 ± 2.010 ^{a}	0.155 ± 0.003 ^{b}	0.377 ± 0.028 ^{ef}
PCSCS	89.110 ± 1.890 ^{a}	68.180 ± 1.450 ^{a}	92.830 ± 1.970 ^{a}	0.155 ± 0.001 ^{b}	0.330 ± 0.012 ^{g}
WCAS	91.890 ± 1.950 ^{a}	68.000 ± 1.440 ^{a}	95.170 ± 2.020 ^{a}	0.147 ± 0.004 ^{bc}	0.428 ± 0.011 ^{cd}
OCAS	91.030 ± 1.930 ^{a}	68.220 ± 1.450 ^{a}	95.240 ± 2.020 ^{a}	0.125 ± 0.001 ^{e}	0.429 ± 0.001 ^{cd}
YCAS	90.720 ± 1.920 ^{a}	69.140 ± 1.470 ^{a}	93.190 ± 1.980 ^{a}	0.167 ± 0.004 ^{a}	0.372 ± 0.018 ^{ef}
PCAS	89.110 ± 1.890 ^{a}	67.310 ± 1.430 ^{b}	96.920 ± 2.060 ^{a}	0.141 ± 0.003 ^{c}	0.320 ± 0.018 ^{g}
WCCS	91.890 ± 1.950 ^{a}	69.570 ± 1.480 ^{a}	93.570 ± 1.980 ^{a}	0.137 ± 0.005 ^{cd}	0.442 ± 0.021 ^{bc}
OCCS	91.030 ± 1.930 ^{a}	68.650 ± 1.460 ^{a}	96.170 ± 2.040 ^{a}	0.120 ± 0.007 ^{ef}	0.444 ± 0.010 ^{bc}
YCCS	90.720 ± 1.920 ^{a}	68.980 ± 1.460 ^{a}	95.650 ± 2.030 ^{a}	0.140 ± 0.005 ^{c}	0.396 ± 0.016 ^{de}
PCCS	89.110 ± 1.890 ^{a}	68.670 ± 1.460 ^{a}	95.180 ± 2.020 ^{a}	0.126 ± 0.006 ^{de}	0.355 ± 0.014 ^{fg}

Mc-moisture content, C-content, SG-solid gain, WL-water loss; significant at $p \leq 0.05$; values followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Tukey's test.

solutions; however, the excessive solid gain, which is undesirable in OD, adversely affects the quality of the products (Maleki et al., 2020).

3.2. Drying time, drying kinetics, and moisture ratio (MR)

The drying process involves three steps: heating, constant, and falling periods (Ihns et al., 2011). The heating period is the first phase when the temperature of the product reaches the temperature of dry air. The constant period is short and not mandatory for all drying processes. In this phase, the thermal energy of the heated air evaporates free moisture from the food products, and the surface of the product is soaked liquid. During the falling period, the moisture content decreases and migrates from the food products (Ek et al., 2018). In the present study, the three steps of the drying process were presented in drying curves. The drying curves of the colored carrot samples that were osmotically dehydrated in different solutions using CD and VMD are presented in Fig. 1. The application of microwave drying in convective drying shortens the drying periods of food products (Szadzińska et al., 2018). During CD and VMD, the MR of the carrot materials (white, orange, purple, and yellow) changed with respect to the solution used (Fig. 1). White carrot showed the lowest moisture content when mixed with each solution (sucrose, sour cherry, apple, and chokeberry solutions) whereas purple carrot showed the highest moisture content. Yellow and orange carrots showed slight changes when mixed with different solutions. In each solution, white carrot dried in a shorter time than others due to the high water loss. The purple carrot samples showed the lowest drying periods in sour cherry, apple, and chokeberry solutions, except for the sucrose solution. In the sucrose solution, yellow carrot showed the lowest drying period. Among solutions, the apple solution required the highest time for drying each of the carrot samples.

3.3. Determination of water activity (a_w) and color measurements of dried carrot snacks

In processed foods, a_w is an important parameter in the prevention of microbial activity and maintenance of stability (Su et al., 2018). In the present study, the a_w values of the dried carrot snacks are shown in Table 3. They were significantly different ($p \leq 0.05$) and ranged from 0.24 to 0.34. The control groups showed the highest followed by the PCSCS and OCSCS samples. Nevertheless, the OCCS samples showed the lowest a_w value followed by the PCCS and YCCS samples, which

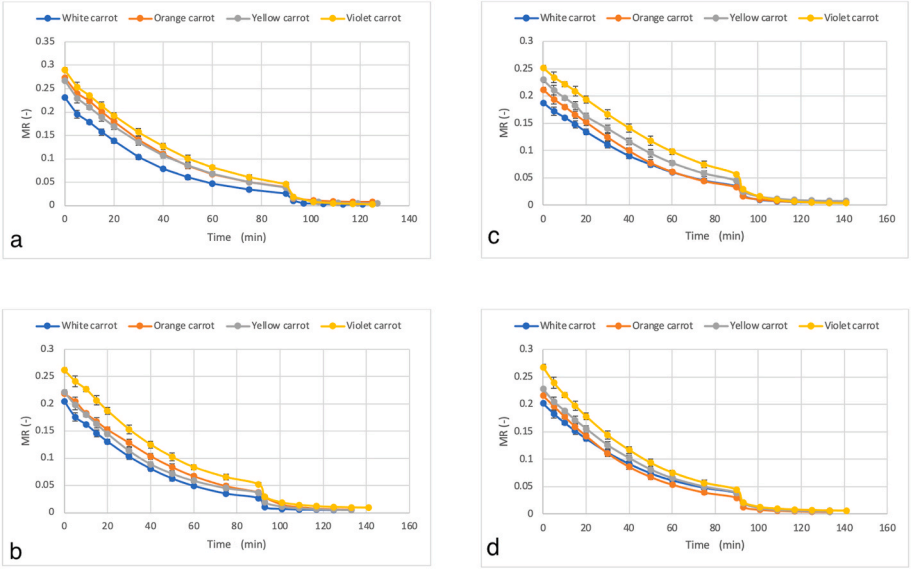

Fig. 1. Drying kinetics of carrots dried by convective pre-drying and vacuum-microwave finish drying osmotically dehydrated in a) sucrose solution, b) sour cherry solution, c) apple solution and d) chokeberry solution (MR-Moisture ratio).

Table 3
Water activities, color features and L-ascorbic acid contents of dried carrot snacks.

	Water activity	L*	Color a*	b*	L-ascorbic acid
WCSS	0.342 ± 0.010 ^{a}	71.560 ± 0.210 ^{b}	1.550 ± 0.010 ^{n}	24.760 ± 1.070 ^{d}	12.150 ± 0.520 ^{gh}
OCSS	0.341 ± 0.010 ^{a}	62.110 ± 1.820 ^{c}	23.760 ± 0.660 ^{c}	39.870 ± 1.520 ^{b}	24.040 ± 1.020 ^{c}
YCSS	0.332 ± 0.010 ^{ab}	76.300 ± 0.050 ^{a}	4.910 ± 0.130 ^{m}	39.580 ± 0.570 ^{b}	28.220 ± 1.200 ^{b}
PCSS	0.342 ± 0.010 ^{a}	39.240 ± 0.710 ^{f}	12.190 ± 0.230 ^{hjk}	- 2.060 ± 0.100 ^{l}	40.770 ± 1.730 ^{a}
WCSCS	0.286 ± 0.009 ^{cd}	30.260 ± 0.630 ^{g}	13.660 ± 0.110 ^{gh}	9.010 ± 0.090 ^{fgh}	5.740 ± 0.240 ^{m}
OCSCS	0.305 ± 0.009 ^{bc}	24.720 ± 2.110 ^{hj}	13.390 ± 1.050 ^{ghj}	11.390 ± 1.050 ^{ef}	8.390 ± 0.360 ^{kl}
YCSCS	0.278 ± 0.008 ^{de}	28.620 ± 0.040 ^{g}	15.470 ± 0.100 ^{ef}	12.990 ± 0.030 ^{e}	6.980 ± 0.300 ^{lm}
PCSCS	0.311 ± 0.009 ^{bc}	21.220 ± 0.560 ^{jk}	22.400 ± 0.240 ^{c}	7.130 ± 0.060 ^{hj}	15.380 ± 0.650 ^{de}
WCAS	0.257 ± 0.008 ^{efg}	60.310 ± 0.120 ^{cd}	8.470 ± 0.110 ^{l}	29.890 ± 0.080 ^{c}	9.500 ± 0.400 ^{jk}
OCAS	0.256 ± 0.008 ^{efg}	51.120 ± 0.360 ^{e}	26.420 ± 0.280 ^{b}	41.140 ± 0.310 ^{b}	14.420 ± 0.610 ^{ef}
YCAS	0.258 ± 0.008 ^{efg}	57.840 ± 0.370 ^{d}	11.710 ± 0.070 ^{k}	44.460 ± 0.780 ^{a}	12.920 ± 0.550 ^{g}
PCAS	0.269 ± 0.008 ^{def}	27.350 ± 0.210 ^{gh}	29.210 ± 0.090 ^{a}	8.490 ± 0.080 ^{gh}	13.150 ± 0.560 ^{fg}
WCCS	0.268 ± 0.08 ^{def}	19.510 ± 0.520 ^{k}	14.430 ± 0.300 ^{fg}	3.270 ± 0.010 ^{k}	10.940 ± 0.460 ^{hj}
OCCS	0.239 ± 0.007 ^{g}	22.970 ± 0.840 ^{jk}	16.290 ± 0.540 ^{e}	9.920 ± 0.640 ^{fg}	16.360 ± 0.690 ^{d}
YCCS	0.25 ± 0.008 ^{fg}	23.000 ± 1.390 ^{jk}	11.900 ± 0.460 ^{jk}	4.710 ± 0.260 ^{jk}	9.670 ± 0.410 ^{jk}
PCCS	0.246 ± 0.07 ^{fg}	20.010 ± 0.010 ^{k}	18.320 ± 0.210 ^{d}	3.300 ± 0.130 ^{k}	10.360 ± 0.440 ^{j}

L* (Lightness), a* (redness-greenness), b* (yellowness-blueness); water activity (a_w), L-ascorbic acid (mg/100 g p); significant at $p < 0.05$; values followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Tukey's test.

indicates that chokeberry juice was the most effective medium. The a_w values of all carrot snacks were lower than 0.6, which indicates the inhibition of microbial growth. Besides, it has been reported that a_w values below 0.4 decrease the activities of Maillard, lipid oxidation, hydrolysis, and enzymatic reactions (Labuza et al., 1970).

The color of food products is an important parameter in deciding to purchase and/or consume them. The color values of the dried carrot snacks are presented in Table 3 with significant differences ($p \leq 0.05$). The WCAS samples showed the second highest L* values (60.31, preceded by the control groups), followed by the YCAS (57.84) and the OCAS (51.12) samples, which showed the lightest colors. However, the WCCS samples showed the darkest color (19.51), followed by the PCCS (20.01) and the PCSCS (21.22) samples. As reported in a previous study, high anthocyanin contents increase the darkness in food products (Liu et al., 2014). Thus, L* values are related to the chemical content of food products, and the dark colors of the chokeberry carrot samples indicate that these dried food products have high polyphenolic contents, which are essential for healthy body functions. The PCAS samples showed the highest a* (redness) values, followed by the OCAS and the PCSCS samples. Moreover, the WCSS samples showed the highest (greenness) values, followed by the YCSS and the WCAS samples. Therefore, the WCAS samples were light-green colored, and the OCAS samples were light-red colored. The YCAS samples showed the highest b* (yellowness) values, followed by the OCAS, the OCSS and the YCSS samples. Nevertheless, the PCSS samples showed the highest b* (blueness) values, followed by the WCCS and the PCCS samples. Thus, the OCAS samples were light-red-yellow colored, the YCAS samples were light-yellow colored, and the WCCS and the PCCS samples were dark-blue colored. In conclusion, L* values of the dried carrot samples were increased during the drying process due to heating and reduced moisture of the carrot slices (Wu et al., 2014). Moreover, the color of food products may change due to light, temperature, pH, oxidation, and metal ions (Szadzińska et al., 2017).

3.4. Determination of L-ascorbic acid, polyphenols, and carotenoids in dried carrot snacks

L-Ascorbic acid functions as an antioxidant, anti-inflammatory agent, and cofactor for enzymes to boost immune system activities (Man et al., 2018). The L-ascorbic acid contents of the dried carrot snacks are presented in Table 3 with significant differences ($p \leq 0.05$), which ranged from 5.74 to 40.77 mg/100 g p. The highest L-ascorbic acid contents were observed in the control groups. As reported in a previous study, oxidation, changes in the moisture content, and temperature may influence the ascorbic acid content in dried food products (Cui et al., 2010). Not taking into account control groups, the OCCS, PCSCS, and OCAS (16.39, 15.38, and 14.42 mg/100 g p, respectively) samples showed the highest L-ascorbic acid contents. However, the lowest L-ascorbic acid contents were observed in the samples dehydrated with the sour cherry solution: WCSCS (5.74 mg/100 g p), YCSCS (6.98 mg/100 g p), and OCSCS (8.39 mg/100 g p). Thus, L-ascorbic acid content decreased due to the heating process and air, which is consistent with previous reports (Ek et al., 2018; Igwemmar et al., 2013).

The phenolic compounds of the dried carrot samples are presented in Table 4 with significant differences ($p \leq 0.05$). All samples were rich in phenolic acids, anthocyanins, flavan-3-ols, flavanols and PP. A total of nine phenolic acids, six anthocyanins, nine flavan-3-ols and three flavanols were identified and quantified from all 16 dried carrot snacks with different amounts. This is the first study to present the identification and quantification of the polyphenolic contents of the dried carrot snacks made from four carrot varieties (white, yellow, orange, and purple) and three fruit juices (sour cherry, chokeberry, and apple juices). Raw carrot varieties are rich in phenolic acids and anthocyanins (Yusuf et al., 2021). However, dried carrot food products are also rich in flavan-3-ols and flavanols due to OD.

In comparison with the control groups, the highest phenolic acid content was observed in snacks dehydrated using chokeberry solution. Similar results are observed in the literature as well (Nowicka et al., 2015b). The highest anthocyanin content was observed in the PCSS-control group (86.52 mg/100 g p), followed by the PCCS samples (64.23 mg/100 g p). In a previous study, five anthocyanins have been identified from fresh purple carrot samples (Yusuf et al., 2021). However, in the present study, six anthocyanins were identified and quantified from the dried carrot snacks. The total amounts of anthocyanins were observed in the PCSS, YCSCS, PCSCS, PCAS, and chokeberry juice samples. The anthocyanin contents were higher in the dried purple carrot products than in the control groups. Nevertheless, the total anthocyanin contents in the dried carrot snacks were lower than in raw materials. This showed that polyphenols were degraded by temperature, heat, and enzymes during the drying processes (McSweeney & Seetharaman, 2015).

Flavan-3-ols function as cardio-metabolic risk protectors (Raman et al., 2019). The flavan-3-ol content in food products is influenced by food processing, food storage, and environmental factors (Aron & Kennedy, 2008). In the present study, using the OD process, flavan-3-ols were added to the dried carrot snacks because they were not present in raw carrot varieties (Yusuf et al., 2021). The highest flavan-3-ol contents were observed in the WCSCS samples (293.84 mg/100 g p), followed by the OCSCS (271.28 mg/100 g p) and YCSCS samples (243.30 mg/100 g p).

Nevertheless, PPs are different from tannins and contain formations of flavan-3-ols. The antioxidant activities of PPs are higher than vitamins C and E, with applications against pneumonitis, lung cancer, and neurodegenerative diseases (Hou & Wang, 2021). In the raw carrot

Table 4
Quantification of polyphenols and carotenoids in dried carrots.

	WCSS	OCSS	YCSS	PCSS	WCSCS	OCSCS	YCSCS	PCSCS	WCAS	OCAS	YCAS	PCAS	WCCS	OCCS	YCCS	PCCS
2-O-caffeyl-L-malate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.670 ± 0.180 ^{a}	nd	nd	nd	nd
Other	2.870 ± 0.060 ^{j}	nd	nd	23.250 ± 0.490 ^{h}	9.250 ± 0.200 ^{j}	8.280 ± 0.180 ^{j}	67.490 ± 1.4300 ^{e}	57.500 ± 1.220 ^{f}	4.040 ± 0.090 ^{j}	1.890 ± 0.040 ^{j}	9.820 ± 0.210 ^{j}	32.470 ± 0.690 ^{g}	169.190 ± 3.590 ^{b}	151.640 ± 3.220 ^{c}	187.670 ± 3.980 ^{a}	100.200 ± 2.130 ^{d}
2,5-di-hydroxy-benzoic acid	nd	nd	nd	nd	nd	nd	17.800 ± 0.380 ^{a}	11.920 ± 0.250 ^{b}	nd	nd	nd	nd	nd	nd	nd	nd
cis-5-p-coumaroylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	2.940 ± 0.060 ^{ef}	1.820 ± 0.040 ^{f}	3.580 ± 0.080 ^{e}	5.480 ± 0.120 ^{d}	nd	14.600 ± 0.310 ^{b}	17.850 ± 0.380 ^{a}	11.410 ± 0.240 ^{c}
5-O-caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	2.090 ± 0.040 ^{e}	nd	2.130 ± 0.050 ^{e}	3.700 ± 0.080 ^{d}	9.670 ± 0.210 ^{a}	8.390 ± 0.180 ^{b}	nd	6.100 ± 0.130 ^{c}
p-coumaric acid	nd	nd	nd	nd	nd	nd	nd	nd	0.630 ± 0.010 ^{b}	nd	0.860 ± 0.020 ^{a}	nd	nd	nd	nd	nd
3-O-caffeoylquinic acid	nd	nd	nd	15.000 ± 0.320 ^{d}	nd	nd	7.630 ± 0.160 ^{f}	11.490 ± 0.240 ^{e}	3.220 ± 0.070 ^{g}	nd	3.280 ± 0.070 ^{g}	10.870 ± 0.230 ^{e}	42.200 ± 0.900 ^{a}	37.100 ± 0.790 ^{b}	32.110 ± 0.680 ^{c}	33.440 ± 0.710 ^{c}
4-O-caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.900 ± 0.080 ^{a}	nd	nd	nd	nd
Ferulic acid di-hexoside	nd	nd	nd	nd	nd	nd	2.800 ± 0.060 ^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd
4-O-feruloylquinic acid	nd	nd	0.820 ± 0.020 ^{b}	nd	nd	nd	nd	nd	nd	nd	nd	4.260 ± 0.090 ^{a}	nd	nd	nd	4.560 ± 0.100 ^{a}
Σ Phenolic acids [mg/100 g p]	**2.870 ± 0.060 ^{k}**	nd	**0.820 ± 0.030 ^{k}**	**38.250 ± 0.810 ^{g}**	**9.250 ± 0.200 ^{jk}**	**8.280 ± 0.180 ^{jk}**	**95.730 ± 2.030 ^{d}**	**80.900 ± 1.720 ^{e}**	**12.930 ± 0.270 ^{hj}**	**3.710 ± 0.080 ^{jk}**	**19.670 ± 0.420 ^{h}**	**69.370 ± 1.470 ^{f}**	**221.050 ± 4.690 ^{b}**	**211.730 ± 4.490 ^{b}**	**237.630 ± 5.040 ^{a}**	**155.720 ± 3.300 ^{c}**
Cyanidin-3-O-xylosyl-glucosylgalactoside	nd	nd	nd	3.590 ± 0.080 ^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cyanidin-3-glucoside	nd	nd	nd	1.860 ± 0.040 ^{b}	nd	nd	2.300 ± 0.050 ^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cyanidin-3-O-xylosyl-feruloyl-glucosylgalactoside	nd	nd	nd	3.830 ± 0.080 ^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pelargonidin-3-rutinoside	nd	nd	nd	77.240 ± 1.640 ^{a}	nd	nd	nd	57.030 ± 1.210 ^{b}	nd	nd	nd	nd	nd	nd	nd	53.610 ± 1.140 ^{b}
Cyanidin-3-sambubioside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	7.340 ± 0.160 ^{a}	nd
Pelarginidin-3-glucoside	nd	nd	nd	nd	nd	nd	nd	2.530 ± 0.050 ^{e}	nd	nd	nd	nd	4.340 ± 0.090 ^{c}	5.270 ± 0.110 ^{b}	5.880 ± 0.120 ^{a}	3.780 ± 0.080 ^{d}
Other	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.800 ± 0.060 ^{b}	nd	nd	nd	6.850 ± 0.150 ^{a}
Σ Anthocyanins [mg/100 g p]	nd	nd	nd	**86.520 ± 1.840 ^{a}**	nd	nd	**2.300 ± 0.050 ^{e}**	**59.560 ± 1.260 ^{c}**	nd	nd	nd	**2.800 ± 0.060 ^{e}**	**4.340 ± 0.090 ^{e}**	**5.270 ± 0.110 ^{e}**	**13.220 ± 0.280 ^{d}**	**64.230 ± 1.360 ^{b}**
Other	nd	nd	nd	13.650 ± 0.290 ^{e}	nd	109.200 ± 2.320 ^{a}	104.980 ± 2.230 ^{a}	46.820 ± 0.990 ^{b}	44.460 ± 0.940 ^{b}	34.340 ± 0.730 ^{c}	54.870 ± 1.160 ^{b}	nd	nd	20.410 ± 0.430 ^{d}	20.490 ± 0.430 ^{d}	11.160 ± 0.240 ^{e}
Arbutin	12.770 ± 0.270 ^{f}	13.610 ± 0.290 ^{f}	nd	nd	236.440 ± 5.020 ^{a}	162.080 ± 3.440 ^{b}	138.320 ± 2.930 ^{c}	65.100 ± 1.380 ^{d}	28.680 ± 0.610 ^{e}	nd	26.690 ± 0.570 ^{e}	nd	nd	28.570 ± 0.610 ^{e}	31.080 ± 0.660 ^{e}	14.120 ± 0.300 ^{f}
(+)-Catechin	17.530 ± 0.370 ^{c}	nd	8.350 ± 0.180 ^{d}	25.070 ± 0.530 ^{a}	nd	nd	nd	nd	nd	nd	7.430 ± 0.160 ^{d}	21.280 ± 0.450 ^{b}	nd	nd	nd	nd
Procyanidin B4		nd		nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

(*continued on next page*)

Table 4 (*continued*)

	WCSS	OCSS	YCSS	PCSS	WCSCS	OCSCS	YCSCS	PCSCS	WCAS	OCAS	YCAS	PCAS	WCCS	OCCS	YCCS	PCCS
	15.640 ± 0.330 [a]		8.130 ± 0.170 [b]													
LC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	123.740 ± 2.620 [a]	nd	nd	nd	nd
Resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	16.600 ± 0.350 [c]	12.210 ± 0.260 [d]	23.370 ± 0.500 [a]	18.830 ± 0.400 [b]	nd	nd	nd	nd
Procyanidin B2	nd	nd	nd	nd	57.400 ± 1.220 [b]	nd	nd	31.210 ± 0.660 [c]	nd	nd	nd	nd	153.740 ± 3.260 [a]	nd	nd	nd
(−)-Epigallocatechin-gallate	nd	nd	nd	5.760 ± 0.120 [b]	nd	nd	nd	nd	nd	nd	nd	40.320 ± 0.860 [a]	nd	nd	nd	nd
(−)-Epigallate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	26.100 ± 0.550 [a]	nd	nd
Procyanidin A2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.510 ± 0.050 [b]	2.540 ± 0.050 [b]	2.950 ± 0.060 [a]	nd
Σ Flavan-3-ols [mg/100 g p]	**45.940 ± 0.970 [j]**	**13.610 ± 0.290 [k]**	**16.480 ± 0.350 [k]**	**44.480 ± 0.940 [j]**	**293.840 ± 6.230 [a]**	**271.280 ± 5.750 [b]**	**243.300 ± 5.160 [c]**	**143.130 ± 3.040 [f]**	**89.740 ± 1.900 [h]**	**46.550 ± 0.990 [j]**	**112.350 ± 2.380 [g]**	**204.180 ± 4.330 [d]**	**156.240 ± 3.310 [e]**	**77.620 ± 1.650 [h]**	**54.510 ± 1.160 [j]**	**25.280 ± 0.540 [k]**
Myricetin-3-galactoside	nd	nd	nd	nd	6.430 ± 0.140 [a]	3.770 ± 0.080 [b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Quercetin-3-galactoside	nd	nd	nd	nd	11.020 ± 0.230 [e]	7.820 ± 0.170 [f]	36.860 ± 0.780 [b]	25.190 ± 0.530 [d]	nd	nd	nd	nd	38.990 ± 0.830 [b]	34.160 ± 0.720 [c]	43.130 ± 0.910 [a]	25.420 ± 0.540 [d]
Other	nd	nd	nd	nd	24.280 ± 0.520 [d]	19.210 ± 0.410 [e]	16.500 ± 0.350 [e]	6.030 ± 0.130 [f]	nd	nd	nd	nd	47.080 ± 1.000 [b]	44.290 ± 0.940 [bc]	50.960 ± 1.080 [a]	42.370 ± 0.900 [c]
Keampferol-7-glucuronide	nd	nd	nd	nd	nd	nd	12.860 ± 0.270 [a]	9.040 ± 0.190 [b]	nd	nd	nd	nd	nd	nd	nd	nd
Σ Flavanols [mg/100 g p]	nd	nd	nd	nd	**41.740 ± 0.890 [e]**	**30.810 ± 0.650 [f]**	**66.220 ± 1.400 [d]**	**40.250 ± 0.850 [e]**	nd	nd	nd	nd	**86.080 ± 1.830 [b]**	**78.450 ± 1.660 [c]**	**94.090 ± 2.000 [a]**	**67.790 ± 1.440 [d]**
Polymeric procyanidins	41.470 ± 0.880 [ef]	32.530 ± 0.690 [hj]	34.040 ± 0.720 [hj]	34.300 ± 0.730 [hj]	65.970 ± 1.400 [b]	51.250 ± 1.090 [d]	97.330 ± 2.060 [a]	99.900 ± 2.120 [a]	58.130 ± 1.230 [c]	40.420 ± 0.860 [f]	55.630 ± 1.180 [cd]	45.250 ± 0.960 [e]	30.390 ± 0.640 [j]	24.540 ± 0.520 [k]	39.890 ± 0.850 [fg]	35.380 ± 0.750 [gh]
DP	nd	12.550 ± 0.270 [b]	nd	nd	nd	nd	3.760 ± 0.080 [c]	3.190 ± 0.070 [cd]	nd	13.770 ± 0.290 [a]	nd	nd	2.990 ± 0.060 [d]	2.290 ± 0.050 [e]	3.170 ± 0.070 [cd]	2.970 ± 0.060 [d]
Σ Polyphenolic content [mg/100 g p]	**90.270 ± 1.910 [h]**	**58.690 ± 1.250 [j]**	**51.330 ± 1.090 [j]**	**203.550 ± 4.320[f]**	**410.800 ± 8.710 [c]**	**361.620 ± 7.670 [d]**	**508.630 ± 10.790[a]**	**426.940 ± 9.060[bc]**	**160.790 ± 3.410 [g]**	**104.450 ± 2.220 [h]**	**187.650 ± 3.980 [fg]**	**321.600 ± 6.820 [e]**	**501.100 ± 10.630 [a]**	**399.900 ± 8.480 [c]**	**442.520 ± 9.390 [b]**	**351.370 ± 7.450 [d]**
α-cryptoxanthin	nd	0.102 ± 0.004 [b]	nd	0.242 ± 0.010 [a]	nd	nd	0.029 ± 0.001 [f]	0.057 ± 0.002 [de]	nd	0.056 ± 0.002 [e]	0.037 ± 0.002 [f]	0.071 ± 0.003 [cd]	nd	0.081 ± 0.003 [c]	0.070 ± 0.003 [cd]	0.080 ± 0.003 [c]
Σ Carotenoids [mg/100 g p]	nd	**0.102 ± 0.004[b]**	nd	**0.242 ± 0.010 [a]**	nd	nd	**0.029 ± 0.001 [f]**	**0.057 ± 0.002 [de]**	nd	**0.056 ± 0.002 [e]**	**0.037 ± 0.002 [f]**	**0.071 ± 0.003 [cd]**	nd	**0.081 ± 0.003 [c]**	**0.070 ± 0.003 [cd]**	**0.080 ± 0.003 [c]**

nd—not detected; polyphenols and carotenoids—mg/100 g p; significant at $p \leq 0.05$; values followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Tukey's tes.

materials, the highest PP content is observed in orange and white carrot samples (Yusuf et al., 2021); however, after the drying processes, significant changes in the PP contents occurred in the dried carrot snacks. In the present study, the highest PP contents were observed in sour cherry solution samples, in the following order: PCSCS > YCSCS > WCSCS. Therefore, it can be concluded that sour cherry is rich in PPs. Furthermore, polymerization degrees (DPs) create alterations in PPs. As reported in a previous study, the number of hydroxyl groups and molecular weights of PPs increase with high DPs (Yang et al., 2021). In the present study, the highest DPs were observed in the OCAS samples (13.77), followed by the YCSCS (3.76) and PCSCS samples (3.19).

Flavanols have antiobesity, neuroprotective, cardioprotective, and antioxidant activities (Liu et al., 2021). In the present study, only the sour cherry solution–dried carrot samples and the chokeberry solution–dried carrot samples showed the flavanol content. Moreover, the highest flavanol contents were observed in the chokeberry juice samples as chokeberry shows less degradation during OD (Turkiewicz et al., 2019).

To sum up, the YCSCS samples showed the highest TPC (508.63 mg/100 g p), followed by the WCCS (501.10 mg/100 g p) and YCCS samples (442.52 mg/100 g p). All dried carrot samples treated with sour cherry, apple, and chokeberry solutions exhibited higher TPCs than the control groups.

Carotenoids produce color in food products from yellow to red and demonstrate health-promoting functions (Fratianni et al., 2013). In the present study, the carotenoid contents of the dried carrot snacks were significantly different ($p \leq 0.05$) and are presented in Table 4 α-Cryptoxanthin was the only carotenoid that was observed in the dried carrot snacks due to thermal food processing. According to the literature, dehydration and heating processes break down carrot cells, with the loss of turgor pressure (Nieto et al., 2013), and carotenoids degrade under heating and light exposure (Saini et al., 2015). The highest α-cryptoxanthin contents were observed in two control groups–PCSS and OCSS followed by the OCCS and PCCS samples. Moreover, the WCSS, YCSS, WCSCS, OCSCS, WCAS, and WCCS samples did not show any carotenoid existence.

3.5. Determination of in vitro biological activities of dried carrot snacks

The antioxidant activities of the dried carrot snacks are shown in Table 5 for ABTS, FRAP, and ORAC assays. The highest values for ABTS were observed in the chokeberry solution samples due to the high TPC of the chokeberry solution. However, without considering the control groups, the lowest ABTS values were observed in the WCAS samples, followed by the OCAS samples. Based on the FRAP assay, the highest ABTS values were observed in the WCCS samples, followed by the PCCS, and YCCS samples. Moreover, the lowest FRAP antioxidant activities were observed in the OCAS samples followed by the WCAS samples. Nevertheless, the highest ORAC activities were observed in the YCCS samples, followed by the OCCS and PCCS samples, whereas the lowest activities were observed in the WCAS samples, and the OCSCS, =PCSCS, and = WCCS samples showed similar activities. In our previous study of antioxidant activities in raw carrot, the highest antioxidant activities for these three assays have been observed for raw purple carrot (Yusuf et al., 2021). In the present study, with the combination of technologies used, different samples showed high antioxidant activities, but the total amount of antioxidant activities decreased. Similar results have also been reported in the literature for dried pomegranate seeds (Bchir et al., 2012). Thus, the decrease in the antioxidant activities of dried products is due to the reduction in polyphenolic compounds and vitamins and the increase in temperature (Turkiewicz et al., 2020).

The α-amylase, α-glucosidase, and pancreatic lipase inhibition activities of the dried carrot snacks are presented in Table 6 as IC_{50} values (mg/mL). The enzymes α-amylase and α-glucosidase are responsible for carbohydrate digestion, and inhibition of these enzymes reduces the breakdown of polysaccharides into glucose (Hanhineva et al., 2010). In the present study, the α-amylase inhibition activities ranged from 259.68 to 62.69 mg/mL (for the PCAS and the YCAS samples, respectively), and the α-glucosidase inhibition activities ranged from 258.29 to 18.44 mg/mL (for the WCAS and the OCSCS samples, respectively). However, the α-amylase inhibition activities of raw purple carrot and yellow carrot were 239.49 and 441.45 (IC_{50}, mg/mL). Besides, the α-glucosidase inhibition activities of raw white and orange carrot

Table 5
The antioxidant activities (ABTS, FRAP, ORAC) of dried carrot food products.

	ABTS [mmol Trolox/100g p]	FRAP [mmol Trolox/100 g p]	ORAC [mmol Trolox/100 g p]
WCSS	0.11 ± 0.02 ^{l}	0.05 ± 0.01 ^{j}	0.09 ± 0.02 ^{fg}
OCSS	0.12 ± 0.08 ^{l}	0.05 ± 0.00 ^{j}	0.05 ± 0.01 ^{h}
YCSS	0.21 ± 0.06 ^{kl}	0.13 ± 0.01 ^{hj}	0.08 ± 0.01 ^{g}
PCSS	1.27 ± 0.21 ^{f}	1.36 ± 0.08 ^{d}	0.18 ± 0.00 ^{e}
WCSCS	0.52 ± 0.04 ^{h}	0.90 ± 0.16 ^{ef}	0.10 ± 0.01 ^{fg}
OCSCS	0.49 ± 0.08 ^{hj}	0.82 ± 0.13 ^{f}	0.09 ± 0.01 ^{fg}
YCSCS	0.40 ± 0.04 ^{hj}	0.29 ± 0.03 ^{g}	0.11 ± 0.01 ^{f}
PCSCS	0.74 ± 0.31 ^{g}	1.34 ± 0.12 ^{d}	0.09 ± 0.00 ^{fg}
WCAS	0.32 ± 0.03 ^{jk}	0.23 ± 0.02 ^{gh}	0.08 ± 0.00 ^{g}
OCAS	0.33 ± 0.07 ^{jk}	0.22 ± 0.04 ^{gh}	0.11 ± 0.00 ^{f}
YCAS	0.45 ± 0.08 ^{hj}	0.34 ± 0.03 ^{g}	0.11 ± 0.00 ^{f}
PCAS	1.62 ± 0.65 ^{e}	1.29 ± 0.03 ^{d}	0.23 ± 0.03 ^{d}
WCCS	5.01 ± 0.37 ^{a}	3.64 ± 0.12 ^{a}	0.09 ± 0.01 ^{fg}
OCCS	2.39 ± 0.30 ^{d}	1.03 ± 0.05 ^{e}	0.58 ± 0.01 ^{b}
YCCS	4.28 ± 0.60 ^{c}	2.39 ± 0.05 ^{c}	0.76 ± 0.05 ^{a}
PCCS	4.53 ± 0.29 ^{b}	3.18 ± 0.12 ^{b}	0.55 ± 0.07 ^{c}

Significant at $p \leq 0.05$; values followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Tukey's test.

Table 6
The *in vitro* inhibition activities (α-amylase, α-glucosidase, pancreatic lipase, acetylcholinesterase-AChE, butyrylcholinesterase-BuChE) of dried carrot food products.

	α-amylase inhibition effect [IC_{50}]	α-glucosidase inhibition effect [IC_{50}]	Lipase inhibition effect [IC_{50}]	AChE[g/100 g of inhibition]	BuChE [g/100 g of inhibition]
WCSS	250.21 ± 5.31^{ab}	190.97 ± 4.05 ^{e}	8.09 ± 0.17 ^{a}	17.00 ± 0.36 ^{e}	11.56 ± 0.25 ^{j}
OCSS	185.52 ± 3.94 ^{d}	1099.16 ± 23.32 ^{a}	8.59 ± 0.18 ^{a}	21.36 ± 0.45 ^{c}	14.14 ± 0.30 ^{i}
YCSS	161.25 ± 3.42 ^{e}	414.10 ± 8.78 ^{b}	8.39 ± 0.18 ^{a}	15.32 ± 0.32 ^{f}	15.52 ± 0.33 ^{hi}
PCSS	237.27 ± 5.03 ^{bc}	361.05 ± 7.66 ^{c}	7.22 ± 0.15 ^{b}	17.82 ± 0.38 ^{de}	14.87 ± 0.32 ^{i}
WCSCS	67.27 ± 1.43 ^{j}	32.61 ± 0.69 ^{ij}	nd	nd	nd
OCSCS	90.13 ± 1.91 ^{f}	18.44 ± 0.39 ^{j}	nd	6.12 ± 0.13 ^{h}	39.40 ± 0.84 ^{bc}
YCSCS	73.70 ± 1.56 ^{hij}	29.66 ± 0.63 ^{j}	1.81 ± 0.04 ^{f}	30.55 ± 0.65 ^{a}	55.77 ± 1.18 ^{a}
PCSCS	82.15 ± 1.74 ^{fhi}	38.26 ± 0.81 ^{ij}	0.66 ± 0.01 ^{g}	10.18 ± 0.22 ^{g}	21.40 ± 0.45 ^{g}
WCAS	84.20 ± 1.79 ^{fh}	258.29 ± 5.48 ^{d}	5.13 ± 0.11 ^{c}	21.50 ± 0.46 ^{c}	24.47 ± 0.52 ^{f}
OCAS	69.90 ± 1.48 ^{ij}	57.53 ± 1.22 ^{ghi}	3.18 ± 0.07 ^{e}	17.08 ± 0.36 ^{e}	14.84 ± 0.31 ^{i}
YCAS	62.69 ± 1.33 ^{j}	79.73 ± 1.69 ^{g}	2.21 ± 0.05 ^{f}	23.58 ± 0.50 ^{b}	37.27 ± 0.79 ^{cd}
PCAS	259.68 ± 5.51 ^{a}	123.50 ± 2.62 ^{f}	3.99 ± 0.08 ^{d}	17.81 ± 0.38 ^{e}	26.46 ± 0.56 ^{ef}
WCCS	84.62 ± 1.80 ^{fh}	41.03 ± 0.87 ^{hij}	2.25 ± 0.05 ^{f}	16.97 ± 0.36 ^{e}	35.62 ± 0.76 ^{d}
OCCS	228.40 ± 4.85 ^{c}	66.38 ± 1.41 ^{gh}	4.99 ± 0.11 ^{c}	18.13 ± 0.38 ^{de}	17.80 ± 0.38 ^{h}
YCCS	75.56 ± 1.60 ^{ghij}	25.16 ± 0.53 ^{j}	3.13 ± 0.07 ^{e}	19.40 ± 0.41 ^{d}	41.43 ± 0.88 ^{b}
PCCS	154.54 ± 3.28 ^{e}	79.20 ± 1.68 ^{g}	3.10 ± 0.07 ^{e}	21.42 ± 0.45 ^{c}	27.47 ± 0.58 ^{e}

Significant at $p < 0.05$; values followed by the same letter within the same column were not significantly different ($p > 0.05$) according to Tukey's test.

samples are reported to be 116.53 and 125.93 (IC_{50}, mg/mL) (Yusuf et al., 2021). A comparison of raw carrot materials and dried carrot food products revealed, significant increases and decreases in the α-amylase and α-glucosidase inhibition activities. For instance, the α-amylase inhibition activity of the dried yellow carrot-apple juice samples was better than that of raw yellow carrot, and the dried orange carrot-sour cherry juice samples showed a higher α-glucosidase inhibition activity than raw orange carrot. According to the literature, dried pomegranate food products show α-amylase and α-glucosidase inhibition activities from 107 to 216 (IC_{50}, mg/mL) (Cano-Lamadrid et al., 2018). However, α-amylase and α-glucosidase inhibition activities of dried Japanese quince range from 7.1 to 21.8 (IC_{50}, mg/mL) for α-amylase and from 1.6 to 3.1 (IC_{50}, mg/mL) for α-glucosidase inhibition (Turkiewicz et al., 2021). Thus, combining dried carrot snacks with fruit juices increases the biological activities of the food products against α-amylase and α-glucosidase enzyme inhibition activities. Nevertheless, pancreatic lipase is responsible for triacylglycerol hydrolysis and its inhibition reduces the lipid breakdown into fatty acids (Lunagariya et al., 2014). The pancreatic lipase activities of the dried carrot snacks ranged from 4.99 to −20.31 mg/mL (for the OCCS and the OCSCS samples, respectively). The orange carrot samples showed both the lowest and the highest pancreatic lipase enzyme inhibition. Moreover, two samples-WCSCS and OCSCS showed negative results. Among raw materials, white carrot showed a lipase enzyme inhibition value of 12.25, and orange carrot showed 5.29 (IC_{50}, mg/mL) (Yusuf et al., 2021). Thus, sour cherry solution, due to its chemical properties, increases the lipase enzyme inhibition activities of the dried carrot snacks, which can be beneficial in preventing childhood obesity despite producing attractive color and taste features.

The AChE and BuChE inhibition activities have been reported to reduce the incidence of age-related diseases such as Alzheimer's disease (Colovic et al., 2013). In the present study, the AChE and BuChE inhibition activities were reported as percentages of inhibition. The highest AChE inhibition activities were observed in the YCSCS and YCAS samples (30.55 and 23.58 g/100 g, respectively), whereas the lowest were observed in the OCSCS and PCSCS samples (6.12 and 10.18 g/100 g, respectively). The AChE inhibition activities of the raw materials were in the following order: orange > yellow > white > purple carrot samples (Yusuf et al., 2021). Nevertheless, after OD and drying processes, the yellow carrot-sour cherry samples and the yellow carrot-apple juice samples showed the highest AChE inhibition effects. Interestingly, the orange carrot-sour cherry samples showed the lowest AChE inhibition. A higher AChE inhibition is found in the literature for Japanese quince, which ranged from 60.20 to 32.54 g/100 g (Turkiewicz et al., 2020). The BuChE inhibition activity of the dried carrot snacks ranged from 14.84 to 55.77 g/100 g. The highest BuChE inhibition activities were observed in the YCSCS and the YCCS samples (55.77 and 41.43 g/100 g, respectively), whereas the lowest were observed in the OCAS and OCCS samples (14.84 and 17.80 g/100 g, respectively). The BuChE inhibition activities in the raw carrot materials were in the following order: purple > yellow > orange > white carrot (Yusuf et al., 2021). Thus, similar to the AChE enzyme inhibition activity, the YCSCS samples showed the highest BuChE inhibition activity.

3.6. Sensorial characteristics of dried carrot snacks

The attractiveness of a processed food product depends on its color, appearance, texture, scent, and flavor. The sensory evaluation results of the dried carrot snacks are presented in Fig. 2. Mean scores were presented for each evaluation characteristic. Color, smell, consistency, flavor and overall taste of 16 carrot snacks were evaluated. OD, CD, and VMD increased the color acceptance of the carrot snacks. The color results ranged from 7.11 ± 0.15 to 4.11 ± 0.09, with the OCSS samples showing the highest color score, followed by the PCAS and OCAS samples. However, the OCSCS, PCCS, YCCS, and PCSS samples showed the lowest color acceptances. Smell scores of the carrot snacks ranged from

Fig. 2. Sensory characteristics of dried carrot snacks (1-WCSS, 2-OCSS, 3-YCSS, 4-PCSS, 5-WCSCS, 6-OCSCS, 7-YCSCS, 8-PCSCS, 9-WCAS, 10-OCAS, 11-YCAS,12-PCAS,13-WCCS, 14-OCCS, 15-YCCS, and 16-PCCS); a) Color and smell features, b) Taste and consistencies of dried carrot snacks. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

7.11 ± 0.15 to 5.11 ± 0.11. The highest smell acceptances were observed in the OCSCS, WCAS, and YCAS samples, whereas the lowest were observed in the WCCS, YCSCS, and YCSS samples.

To evaluate the flavour of the food products, the panelists were requested to vote for fruits whose taste they felt in the snacks (black currant, carrot, apple, sour cherry, raspberry, and chokeberry). The WCSS, OCSS, YCSS, PCSS, PCSCS, WCAS, and YCAS samples were voted as having the taste of carrot. Moreover, the WCCS, OCCS, and PCCS samples were voted as having a chokeberry taste, and the OCAS samples were voted as having the apple flavor. However, the PCAS samples were voted as having a sour cherry taste, and the WCSCS, OCSCS, YCSCS, and YCCS samples were voted as having a black currant taste. Interestingly, in the present study, black currant solution was not used for the OD processes of the carrot snacks.

Taste scores of the dried carrot snacks ranged from 6.77 ± 0.14 to 3.33 ± 0.07. The taste scores of the samples were in the following order: OCSS > WCSS = WCAS > OCAS, and the lowest taste scores were observed for the PCCS, OCCS, and YCCS samples, respectively. For instance, the OCCS samples showed an increased L-ascorbic acid content, but with a low taste acceptance, they may not be suggested to consumers. On the other hand, OCAS snacks can be suggested for consumption due to their high L-ascorbic acid content, color and taste acceptance.

Crispiness is another important parameter for dried food products. The OD process slightly increases the hardness of the dried products (Lagnika et al., 2018). In the present paper, the WCSCS, OCSCS, PCSCS, WCAS, and WCCS samples were voted as neither hard nor soft. However,

the PCCS samples were voted as very hard, and the OCCS, YCAS, YCSCS, PCSS, and OCSS samples were voted as hard. As reported in a previous study, high hardness is an unattractive feature for food products (Zou et al., 2013).

4. Conclusion

The novel colored carrot snacks with osmotically dehydrated fruit juices showed stunning results by convective and VDM technologies. The OD process increased the polyphenolic contents in the dried carrot samples; however, the thermal processes decreased the carotenoid contents. The α-cryptoxanthin was the only carotenoid observed in the dried carrot snacks. After the OD process, the highest solid gains were observed in the yellow carrot-apple solution, yellow carrot-sour cherry solution, and purple carrot-sour cherry solution samples. Moreover, the highest L-ascorbic acid contents were observed in the orange carrot-chokeberry and purple carrot-sour cherry solution samples, 16.36 and 15.38 mg/100 g dm, respectively. The polyphenolic contents of the dried carrot snacks differed between the products. The highest phenolic acid content was observed in the yellow carrot-chokeberry solution samples (237.63 mg/100 g dm); the highest anthocyanin content was observed in the purple carrot-chokeberry solution samples (64.23 mg/100 g dm); a high flavan-3-ol content was observed in the white carrot-sour cherry solution samples (156.24 mg/100 g dm); the highest polymeric proanthocyanidins content was observed in the purple carrot-sour cherry solution samples (99.90 mg/100 g dm); and a high flavanol content was observed in the yellow carrot-chokeberry solution samples (94.09 mg/100 g dm). Interestingly, the highest total phenolic compunds was observed in the yellow carrot-sour cherry solution samples (508.63 mg/100 g dm). Nevertheless, the lowest total phenolic compounds was observed in the orange carrot-apple solution samples (104.45 mg/100 g dm).

The highest ABTS and FRAP activities were observed in the white carrot-chokeberry solution samples, however, the highest ORAC result was observed in the yellow carrot-chokeberry solution samples. Moreover, the highest α-amylase activity was observed in the yellow carrot-apple solution samples; the highest α-glucosidase and pancreatic lipase activities were observed in the orange carrot-sour cherry solution samples; and the highest acetylcholinesterase and butyrylcholinesterase enzyme inhibition activities were observed in the yellow carrot-sour cherry solution.

Sensorial evaluations of each carrot snack indicated significant differences. For instance, the highest color acceptance was observed in the purple carrot-apple juice samples; a high smell score was observed in the orange carrot-sour cherry samples; and a high overall taste score was observed in the white carrot-apple solution samples. Thus, the results of this study can be useful for future carrot-based food applications.

CRediT authorship contribution statement

Emel Hasan Yusuf: Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Aneta Wojdyło:** Investigation, Methodology, Validation. **Krzysztof Lech:** Formal analysis, Investigation, Data curation, Visualization. **Klaudia Masztalerz:** Formal analysis, Investigation, Data curation, Visualization. **Paulina Nowicka:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing interest

The authors declare that there is no conflict of interest to influence the study.

Data availability

Data will be made available on request.

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Oświadczam, że w pracy pt.:
Yusuf E.H., Wojdyło A., Krzysztof L., Masztalerz K., Nowicka, P. 2023. The effect of combined drying process (OD-CD-VMD) on nutritional, phytochemical, and sensory profiles, and biological activities of colored dried carrot. *LWT*, 173, 114231. https://doi.org/10.1016/j.lwt.2022.114231

mój udział polegał na tworzeniu planu badań, przeprowadzeniu doświadczeń i analiz, z uwzględnieniem analizy zawartości związków bioaktywnych (LC/MS i UPLC) oraz właściwości prozdrowotnych *in vitro*. Dodatkowo prowadziłam analizy podstawowych wyróżników składu chemicznego i właściwości sensorycznych otrzymanych produktówprzekąskowych. Ponadto otrzymane wyniki opracowałam pod kątem statystycznym i merytorycznym, przygotowując manuskrypt, a także brałam udział w współredagowaniu tekstu w procesie recenzji.
Jestem także członkiem Interdyscyplinarnej Międzynarodowej Szkoły Doktorskiej na UPWr, współfinansowanej ze środków Europejskiego Funduszu Społecznego w ramach Programu Operacyjnego Wiedza Edukacja Rozwój, na podstawie umowy nr POWR.03.05.00-00-Z062/18 z dnia 4 czerwca 2019 r., a także byłam kierownikiem grantu finansowanego przez UPWR no. N020/0007/20, które wsparły finansowo prezentowane wyniki.

Potwierdzam zadeklarowane zaangażowanie
Emel Hasan Yusuf
Paulina Nowicka
02.06.2023

02.06.2023 ..... data i podpis

DECLARATION

I declare that in the publication entitled:
Yusuf E.H., Wojdyło A., Krzysztof L., Masztalerz K., Nowicka, P. 2023. The effect of combined drying process (OD-CD-VMD) on nutritional, phytochemical, and sensory profiles, and biological activities of colored dried carrot. *LWT*, 173, 114231. https://doi.org/10.1016/j.lwt.2022.114231

my participation consisted in creating a research plan, conducting experiments and analyses, including the analysis of bioactive compounds (by LC/MS and UPLC), and also pro-health properties by *in vitro* methods. In addition, I measured basic chemical parameters in carrot dried products, and them sensory evaluation. Moreover, I analyzed the obtained results in statistical and substantive terms, preparing the manuscript, and I also participated in the co-editing of the text in the review process.
I am also a member of Interdisciplinary International Doctoral School at UPWr, co-financed by the European Social Fund under the Operational Program Knowledge Education Development, under contract No. POWR.03.05.00-00-Z062/18 of June 4, 2019, and I was the grant manager of the project financed by the Doctoral School of UPWr no: N020/0007/20, which financially supported the presented results.

I confirm the indicated commitment
of Emel Hasan Yusuf
Paulina Nowicka
02.06.2023

02.06.2023 ..... the date and sign

SCIENTIFIC ACHIEVEMENTS

Education

UPWr, PhD candidate 2019-Present

Anadolu University, Master of Health Sciences 2013-2016

- I studied at the Faculty of Pharmacy / Department of Pharmaceutical Botany. I worked on *Ajuga postii* Briq. and *Ajuga relicta* P.H.Davis (Lamiaceae) species for their pharmaceutical uses.

Anadolu University, Master of Science 2010-2015

- I studied at the Faculty of Science / Department of Biology. I worked on Lichen Biodiversity.

Universita Degli Studi di Perugia, ERASMUS 2011-2012

- During my master studies, l went to Italy with the Erasmus Scholarship. l studied there one semester at Scienze della Natura e dell'Ambiente.

Anadolu University, English prep class & Bachelor Degree 2005-2010

- I studied at the Department of Biology.

Experience

2019-Present

PhD Candidate • The Wroclaw University of Environmental and Life Sciences **(**UPWr)

My dissertation topic is "Novel carrot products with desired health benefits"

07/2022-01/2023

Visiting Researcher • Max Rubner Institut (MRI). Karlsruhe-Germany.

On food processing UV-C & Heat treatments, mineral bioaccessibility, particle distributions with sensorial acceptance studies

11/2021-05/2022

Visiting Researcher • Instituto de la Grasa, CSIC, Sevilla-Spain.

The work was a part of my PhD, l worked on *in vitro* cytotoxic activities of raw carrot varieties and polysaccharide contents of dried carrot snacks with bioactive compound analysis and biological activity assays

10/2020-01/2021

Visiting Researcher • Institute of Food Science, Technology and Nutrition, Spanish Research Council (Madrid)-(ICTAN-CSIC)

I worked on chocolate labels with the funding of EIT Food.

2013–2018

Research & Teaching Assistant • Anadolu University (Turkey)

I was responsible for assisting students in teaching laboratory and researching for my Master of Health Science studies.

06/2009–09/2009

Summer Intern-Food Department • Six Flags, St. Louis-Missouri (USA)

Scientific Publications not Included in the Dissertation

1) **Yusuf E.H.** 2023. Comparison of Life Cycle Assessments and Nutritional Contents of Soy Protein and Wheat Protein (Seitan) Based Vegan Bacon Products for Human and Environmental Health. Journal of the Science of Food and Agriculture 103(7)-3315-3321

2) **Yusuf E.H.** 2023. Taste Masking in Vegan Food Processing with Natural Substitutes

Future of Food: Journal on Food, Agriculture and Society 11 (1)

3) **Yusuf E.H.,** Pérez-Jiménez J. 2021. Labels on bars of solid chocolate and chocolate bar sweets in the Polish market: a nutritional approach and implications for the consumer

Journal of Food Composition and Analysis 102
4) **Yusuf E.H.** 2021. An Overview of Biotransformation for the Sustainability of Sweet Tasting Proteins as Natural Sugar Replacers. Chemistry Proceedings
5) **Yusuf E.H.** 2021. Total Phenolic Content and Antioxidant Activities of Invasive *Erigeron annuus* Pers. (Asteraceae) from Different Localities. J. Agric. Environ. Food Sci. 5 (2), 173-178.
6) **Yusuf (Sönmez) E.**, Köse Y.B. 2017. Morpho-anatomical investigations on *Ajuga postii* Briq and *Ajuga relicta* P.H.Davis. Biyolojik Çeşitlilik ve Koruma, 10 (1), 39-48 .

7) **Yusuf (Sönmez) E.,** Köse Y.B. 2017. The total phenolic contents and antioxidant activities of endemic species *Ajuga postii* Briq. and *Ajuga relicta* P.H.Davis (Lamiaceae) from Turkey. Indian J of Pharmaceutical Education and Research, 51(4):700-5.

8) Köse Y.B., **Yusuf (Sönmez) E.,** Yücel E. 2016. Ecological Features of *Centaurea* L. Section *Phalolepis* (Cass.) DC. in Turkey. Applied Ecology and Environmental Research, 14(4):523-536

Attended Conferences

1) **Yusuf E.H.,** Wojdyło A., Nowicka P. 2023. Nutritional content and health benefits of orange and purple carrot-based smoothies enriched with sour cherry and apple juices during the 3- and 6-month storage periods.

Oral Presentation-The 3rd International Conference on Raw Materials to Processed Foods, 18-19.05.2023 Istanbul/Turkey

2) **Yusuf E.H.,** Wojdyło A., Nowicka P. 2023. Comparison of Sensorial Characteristics of UV-C treated and Thermally treated Carrot Juices with Selected Mineral Bioaccessibility.

Poster Presentation-11th E3S annual Symposium, 15-16.05.2023 Uppsala/Sweden

3) **Yusuf E.H.,** Wojdyło A., Lech K., Masztalerz K., Nowicka P. 2023. Biological content and biological activities of dried orange and purple carrots enriched with fruit juices after the 6-month storage period

Oral Presentation-Xth International Session of Young Scientific Staff "Food Science Development. Sustainable Future", 11-12.05.2023 Warsaw/Poland

4) **Yusuf E.H.,** Wojdyło A., Lech K., Masztalerz K., Nowicka P. 2021. Colourful Carrot Snacks Manufacturing by Applying Osmotic Dehydration, Convective Drying and Vacuum Microwave Drying

Poster Presentation-XXI EuroFoodChem, 22-24.11.2021Online

5) **Yusuf E.H.,** Wojdyło A., Nowicka P. 2021. Fancy, health promoted carrot mixed smoothies with high sensory features to attract children

Poster Presentation-EFFoST2021, 1-4.11.2021 Lausanne/Switzerland

6) **Yusuf E.H.** 2021. Encapsulation: A Promising Technology for Future Food Applications, but What Policies are Countries Following Today?

Poster Presentation-The 2nd International Electronic Conference on Foods - "Future Foods and Food Technologies for a Sustainable World", 15-30.10.2021 Online

7) **Yusuf E.H.,** Nowicka P., Wojdyło A., Lech K., Masztalerz K. 2021. Contents of Bioactive Compounds, Water activities and Colour Features of Dried Carrot Snacks

Poster Presentation-8th International Conference "Human – Nutrition – Environment", 13-14.10.2021 Rzeszow/Poland

8) **Yusuf E.H.,** Nowicka P., Wojdyło A., Tkacz K., Turkiewicz I. 2021. Physico-chemical Characteristics, Phenolic, Carotenoid Contents and Biological activities of Coloured Fresh Carrot Juices

Oral presentation-8th International Conference "Human – Nutrition – Environment", 13-14.10.2021 Rzeszow/Poland

9) **Yusuf E.H.** 2021. Food System Reforms Regarding Climate Change

Poster Presentation-Climate Policy and Energy System Transformation: New Opportunities and Challenges of the Consideration of Co- Benefits, 13-17.09.2021 Freiberg/Germany

10) **Yusuf E.H.,** Nowicka P., Wojdyło A., Lech K., Masztalerz K. 2021. A Different Approach for Healthy Snacks: Increased Vitamin C, Mineral Contents and Consuming Desire of Dried Carrots

Poster Presentation-The 6th International ISEKI-Food Conference, 23-25.06.2021 Online

11) **Yusuf, E.H.,** Pérez-Jiménez, J. 2021. Exploring Labels of Chocolate in Poland: Practical Implications for the Consumer

Poster Presentation-XXV Jornadas Internacionales de Nutrición Práctica, 20-22.04.2021 Online

12) **Yusuf (Sönmez) E.**, Goger F., Köse Y.B. 2018. The Total Phenolic Contents and Antioxidant Properties of Invasive Species *Erigeron annuus* Pers. (Asteraceae) grow at different locations in Turkey

Oral presentation-The 4th International Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP4), 18-22.04.2018 Antalya/Turkey

13) **Yusuf (Sönmez) E.**, Köse Y.B. 2017. Molecular and phytochemical studies on two endemic species: *Ajuga postii* Briq. and *Ajuga relicta* P.H.Davis (Lamiaceae) from Turkey

Oral presentation-International Symposium on Biodiversity and Edible Wild Species, 3-7.04.2017 Antalya/Turkey

14) **Yusuf (Sönmez) E.**, Köse Y.B., Demirci B., Karaca N., Demirci F. 2017. Biological Activities of the Essential Oils of *Ajuga postii* Briq. and *Ajuga relicta* P.H.Davis (Lamiaceae) from Turkey

Poster presentation-International Symposium on Advances in Lamiaceae Science, 26-29.04.2017 Antalya/Turkey

15) Ulgen A.C., **Yusuf (Sönmez) E.**, Köse Y.B. 2015. Endemic Plants and Their Therapeutic Uses in Turkey

Poster presentation-11th International Symposium on Pharmaceutical Sciences, 9-12.06.2015 Ankara/Turkey

16) **Yusuf (Sönmez) E.**, Köse Y.B. 2015. Morpho-Anatomical Investigations on Two Relict Endemic Species: Ajuga postii Briq and Ajuga relicta P. H. Davis (Lamiaceae) from Turkey

Poster presentation-Pharma Middle East Conference, 02-04.11.2015 Dubai/UAE

Attended Courses

1) LCMS/MS Analysis, Shim-pol 28-31.05.2023 Jachranka/Poland

2) Climate Policy and Energy System Transformation: New Opportunities and Challenges of the Consideration of Co-Benefits Climate Policy and Energy System Transformation: New Opportunities and Challenges of the Consideration of Co-Benefits, 13-17.09.2021 Freiberg/Germany

3) Innovating Food Value Chain Innovations: Novel Management Perspectives on New Product Development - From Simulation to Reality Innovating Food Value Chain Innovations: Novel Management Perspectives on New Product Development - From Simulation to Reality-EITFOOD 7-11.09.2020

4) The Northern Lights on Food Masterclass – Lund University 1-3.09.2020 Online

5) Food labelling - Food Standards Agency (food.gov.uk) 19.09.2020

6) Food allergy - Food Standards Agency (food.gov.uk) 06.06.2020

7) Summer School on New Product Development for the food industry Summer School on New Product Development for the food industry-EITFOOD 24-28.08.2020

8) An Introduction to Food Systems: Scientific, Technical and Socioeconomic Principles to Facilitate the Creation of Food Value Networks- EITFOOD Online

Awards

- STER Scholarship
- VolkswagenStiftung
- EIT Food RIS Talent Fellowship 2020
- Bon Scholarship
- NAWA (The Polish National Agency for Academic Exchange) Scholarship
- Power 3.5 Scholarship – European Union Funding

Erasmus Scholarship