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CYCLE XXXII

PREPARATION OF FUNCTIONAL FOODS FROM SELECTED PLANT MATERIALS AND THEIR BY-PRODUCTS

SSD CHIM/10

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ABSTRACT

The aim of this project was to prepare innovative functional foods with potential health-promoting components from selected plant materials and their by-products. For this purpose, *Arbutus unedo* L. fruits, *Myrtus communis* L. purple and white berries, *Acca sellowiana* (O. Berg) flowers, *Crocus sativus* L. flower juice, *Malus domestica* L. Borkh. var. Shampion fruits and *Diospyros kaki* L. var. Rojo Brillante fruits were used to prepare juices and smoothies (thick beverages made mainly from pureed raw fruit). The final products (smoothies and juices) were created from 3 different bases: B1 - apple juice, B2 - persimmon fruit purée and apple juice, and B3 - strawberry tree dry fruits and apple juice, respectively, with an addition of 0.1 % or 0.5 % saffron flower juice or 0.5 % purple myrtle berry extract or 0.5 % feijoa flowers or 0.5 % strawberry tree fruits or persimmon fruit purée. Moreover, the stability of the final products was assessed during a 6 month period of storage.

Potential health-promoting components were investigated in both the plant materials and the final food products. The total polyphenol content was estimated spectrophotometrically (Folin-Ciocalteu's assay) and phenolic compounds were determined in the examined samples using ultra-performance liquid chromatography photodiode detector-quadrupole time of flight/mass spectrometry (UPLC-PDA-QTof/MS) and quantified by UPLC-PDA. Furthermore, proanthocyanidins analysis, determination of colour, total soluble solids (TSS), titratable acidity (TA), dry matter, ashes, pH, and total sugars content by HPLC-ELSD, organic acids and vitamin C content was performed. Moreover, biological activities like antioxidant activity and inhibition on targeted digestive enzymes were investigated with different assays. Antioxidant activity was determined by evaluating total reducing power (FRAP and CUPRAC assays), free radical scavenging activity ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays), and oxygen radical absorption capacity (ORAC

assay). On the most interesting products, *in vitro* analysis on Caco-2 cells (cytotoxic activity and determination of intracellular ROS production) was also performed. Estimation of inhibition on targeted digestive enzymes (α-amylase, α-glucosidase and pancreatic lipase) was performed to evaluate the potential benefits for consumers with health problems such as diabetes and weight management. Finally, consumer evaluations of the new functional foods were evaluated using 5° hedonic scale.

Products with the base B3 (strawberry tree fruits + apple juice) and 0.5 % addition of purple myrtle berry extract, feijoa flowers and persimmon fruit purée, as well as with 0.1 % of saffron flower juice, were shown to be the most interesting to consumers thanks to the large amount of bioactive compounds, the best antioxidant activity and digestive enzyme inhibitory activity, as well as the highest quantity of organic acids and simple sugars. Positive evaluation of the sensory properties and ageing stability at 6-month timespan support the potential commercial use of this new functional food in smoothie form.

The study has been performed in collaboration with Prof. Aneta Wojdyło and Paulina Nowicka from the Department of Fruit, Vegetables and Plant Nutraceutical Technology (Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, Poland).

1. INTRODUCTION

The concept of "functional foods" was created in the European Union in 1999, when a working definition was established by the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE) (*Nutrition Society, 1999*). A food can be regarded as functional if it has been satisfactorily demonstrated to have beneficial effects on one or more target functions in the body, beyond the adequate effects in a way that is relevant to either an improved state of health and well-being or a reduction in the risk of disease. Functional foods must remain foodstuffs and demonstrate their effects when consumed in their recommended daily amounts (*Howlett, 2008*). The concept of functional foods often overlaps with nutraceuticals and novel food terms (*Ioannis and Van Houwelingen-Koukaliaroglou, 2005*).

In past years, there have been significant changes in the food industry, because of the relationship between human health and nutritional food components. It has created a trend for healthy eating. Worldwide, consumers have started to choose products not just because of their sensory attributes (appearance, taste, smell), but also due to their nutritional properties (content of vitamins, minerals, polyphenols). As a consequence, the food industry has started to develop and create new, healthy, safe, and attractive (in sensory terms) products, in order to satisfy customer' demand. Lately, an increased curiosity in ready-to-drink beverages has been noticed. Among them appeared to be smoothies - semi-liquid, smooth consistency blended beverages, prepared by mixing fruits and vegetables or other ingredients (fruit juices, yogurt, milk or honey) in appropriate proportions. Smoothies are regarded as so-called superfoods, defined as natural foods with beneficial and health-protecting qualities, which derive from the nutrients of the fruit components (*Nowicka et al., 2016a*).

This thesis deals with the design and creation of a product considered a “functional food” with antioxidant and/or antidiabetic properties suitable for daily use by both healthy individuals and those suffering from a wide range of chronic diseases such as diabetes and cardiovascular diseases. These disorders can be caused by inappropriate diet or lifestyle, as well as the action of free radicals and reactive oxygen species. In fact, there are many ways of preventing and treating these diseases including the consumption of products that naturally contain significant amounts of antioxidant compounds, such as polyphenols and reducing vitamins (*Tarko et al., 2015*). However, currently food stores offer mainly convenient food, ready-to-eat foodstuffs or highly processed products that during technological treatments have been deprived of many valuable compounds occurring in fresh products. Therefore, an important element of food production technology is to ensure a proper composition of valuable human health-promoting compounds, mostly vitamins, minerals and polyphenols in final food products.

Phenolic compounds are an important group of natural antioxidants that are found in a variety of plants like fruits or vegetables. Moreover, they have a significant impact on our health, thanks to their biological functions such as establishment of microbial symbioses, UV protection, pollen fertility and pollinator attraction (*Quideau et al., 2011*). Polyphenols play an important role in preventing and reducing the progression of diabetes, cancer, neurodegenerative and cardiovascular diseases. Furthermore, they have an important role as a prebiotic, increasing the ratio of beneficial bacteria in gut, which is significant for health, weight management, and disease prevention. One of the best-known groups of phenolic compounds are anthocyanins, which are the reason for the plants’ red colour. Their property is strong antioxidant activity in metabolic reactions, based on their ability to scavenge oxygen free radicals and other reactive species (*Kirakosyan et al., 2009; Šarić et al., 2009*). This quality makes anthocyanins a tool for use in studies on oxidative stress and its related pathologies, such as diabetes, cancer, inflammation, stroke,

Alzheimer's disease and apoptosis (*Chavez-Santoscoy et al., 2009; Kirakosyan et al., 2009, Šarić et al., 2009; Devalaraja et al., 2011*).

Furthermore, bioactive compounds derived from fruits stimulate insulin secretion and reduce serum cholesterol and triglycerides, as well as a blood pressure. The consumption of pectin-rich fruits, such as apples and pears, signal a low glycemic response that determines the ability of carbohydrates to increase blood glucose in the human body (*Nowicka et al., 2016a*). Dietary carbohydrates are hydrolysed by pancreatic α-amylase and intestinal α-glucosidase enzymes responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption (*De Sales et al., 2012*). The inhibition of these enzymes is specifically useful for the treatment of non-insulin-dependent diabetes because it slows down the release of glucose in the blood.

The idea of this PhD project was to prepare a type of functional food in liquid (juice) and semi-liquid form (smoothie). For this purpose, *Arbutus unedo* L. fruits, *Myrtus communis* L. purple and white berries, *Acca sellowiana* (O. Berg) flowers, *Crocus sativus* L. flower juice, *Malus domestica* L. Borkh. var. Shampion fruits and *Diospyros kaki* L. var. Rojo Brillante fruits were chosen as raw plant material for preparation. At the beginning, each matrix was investigated separately, to understand the composition and quantity of bioactive compounds, as well as the physico-chemical properties. Next, three fruits (apple, persimmon and strawberry tree) were chosen for beverage base preparation; instead of one to obtain the pleasant consistence of the smoothie appreciate by consumers. Furthermore, having the correct base (**B1** - 100 % apple juice, **B2** - 75 % apple juice + 25 % persimmon fruit purée, and **B3** - 75 % apple juice + 25 % strawberry tree fruits) 20 final functional foods were prepared through the addition of 0.1 or 0.5 % saffron flower juice or 0.5 % purple myrtle berry extract or 0.5 % feijoa flowers or 0.5 % strawberry tree fruits or 0.5 % persimmon fruit purée. All final products were stored (0, 3 and 6 months) and analysed.

1.1. Functional foods

Functional food is a relatively recent concept that originated in Japan in the mid-1980s that describes foods that contain ingredients with beneficial effects on health (Food for Specified Health Use - FOSHU) (*Corbo et al., 2014*). Functional foods were developed and defined in the United States as "foods and food components that provide a health benefit beyond basic nutrition" (*Serafini et al., 2012*). Finally, in Europe the concept of functional foods arrived in the latter half of the 1990s, and was explored by Functional Food Science in Europe (FuFoSE) (*Corbo et al., 2014*). Conceptually, it implies that foods and food components have the ability to beneficially influence body functions to help improve well-being, health and reduce the risk of diseases (*Ashwell, 2002*).

Functional foods are part of a normal dietary intake for optimized nutrition, and are defined by Functional Food Centre (*2019)* as "Natural or processed foods that contain biologically-active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, for the prevention, management, or treatment of chronic disease or its symptoms". Functional foods must remain foods, and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet. They are not pills or capsules, but part of a normal food pattern. From a practical point of view, a functional food is a natural food in which one of the components has been naturally enhanced through special growing conditions; a food from which a component has been removed so that the food has less adverse health effects (the reduction of saturated fatty acids); a food to which a component has been added to provide benefits (the addition of selected probiotic bacteria with proven health benefit characteristics to improve gut health); a food in which the bioavailability of one or more components has been increased to stimulate greater absorption of a beneficial component; a food in which the nature of one or more components has been chemically

modified to improve health (the hydrolysed protein in infant formulas to reduce the likelihood of allergenicity) or any combination of the above (*Ashwell, 2002*).

Nowadays, foods and beverages play a key role in disease prevention and treatment. For this reason, these products are requested more and more by consumers and they are becoming very attractive from a marketing point of view. Therefore, the production and consumption of functional foods has become a very important part in human nutrition, because of its beneficial influence on human health and well-being (*Corbo et al., 2014*). Epidemiological studies show that if consumption of fruits increases and is regular, general health may be improved. This lead to creation of fruit-derived products, with a wide range of health benefits (*Sun-Waterhouse, 2011*). In fact, functional food might be characterised by the health claims that underline enhanced function or reduction of disease risk. The importance of this claim is demonstrated by strict regulation by under the EU (Regulation EC No 1924/2006 and Regulation EU No 432/2012). Claims must be scientifically valid and clear to the customer (*Ashwell, 2002*), although the substantiation of their health influencing properties remains a scientific challenge (*European Comission, 2019*). Furthermore, according to *Howlett (2008)*, a food product must always be safe for its intended use.

1.2. Antioxidants

Oxygen is the basis of human life. Thanks to it, we are able to exist. However, oxygen is also involved in toxic reactions. Thereby, it threatens the well-being of the human body. Common biochemical reactions, increased exposure to unusual higher levels of dietary xenobiotics that result in the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive species are responsible for oxidative stress (*Nimse and Pal, 2015*) and are the cause of ageing and many chronic diseases, like age-related decline in the immune system, cancer, degenerative diseases of the nervous system

(Parkinson's and Alzheimer's diseases), cardiovascular diseases (CVD), and cataracts (*Ashwell, 2002*).

Antioxidants are molecules that inhibit radical reactions and delay or inhibit cellular damage. The presence of antioxidant defence is universal, because antioxidant defences are different from species to species. Moreover, antioxidants exist both in non-enzymatic and enzymatic forms in the extracellular and intracellular environment. Non-enzymatic antioxidants work by interrupting free-radical chain reactions, while enzymatic antioxidants work by breaking down and removing free radicals (*Nimse and Pal, 2015*).

The human body can defend itself against ROS and RNS, using several mechanisms. All of these defences complement one another because they act on different cellular compartments or on different oxidants. One of the significant defences is a system of antioxidant enzymes, where nutrition plays a principal role. In the catalytic activity and structure of these enzymes several essential minerals and trace elements are involved, like copper (Cu), manganese (Mg), zinc (Zn) and selenium (Se), which in insufficient quantity can impair enzymatic defences. Another example of defence is the group of small-molecular weight compounds that work as non-enzymatic antioxidants. In this group belong some vitamins (E and C), plant polyphenols, carotenoids and glutathione, and they are responsible for the regeneration of the buffer capacity of the body's antioxidant systems. If exposure to external sources of oxidants is high due to contact with heavy metals, atmospheric pollution, tobacco smoke or too much physical effort (for example), the body's antioxidant defences may be put under pressure to match this. The result of all these mechanisms is a state called oxidative stress, an imbalance between antioxidants and pro-oxidants. In normal situations, antioxidant defences adequately counterbalance pro-oxidant factors. An increase either in the production of oxidants or a deficiency in the defence system could disturb this balance, causing oxidative stress (*Ashwell, 2002*).

There are many ways of preventing and treating diseases caused by free radicals and reactive oxygen species. One of them is our daily diet, which contains products rich in antioxidant compounds, such as polyphenolic compounds and reducing vitamins, and is the starting point of our well-being. Polyphenols, such as flavonoids, phenolic acids and anthocyanins, are important constituents in many plants, and their identification and quantification can give vital information relating to potential health benefits, food quality, and antioxidant function. Moreover, fruits and vegetables are a very important part of the human diet, not only because they are rich vitamin and phenolic compound sources but also for medicinal purposes (*Sindhi et al., 2013*).

1.3. Obesity overweight and *diabetes mellitus* disease

Nowadays, it is known that body mass index (BMI) has a strong relationship to diabetes and insulin resistance, which is increasing worldwide, because of influence of particular factors, like the amount of glycerol, hormones, cytokines, proinflammatory markers and nonestrified fatty acids. Moreover, body mass and weight gain are the primary causes of rising incidences of type 1 and 2 diabetes (*Al-Goblan et al., 2014*).

Overweight and obesity are global epidemics, which can lead to morbidity and mortality in adults, and health problems in children (*Tucci et al., 2010*). The World Health Organisation (WHO) defines overweight and obesity as excessive or abnormal fat accumulation that may harm health. These disorders are classified in adults by body mass index (BMI), defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2). Moreover, overweight is a BMI higher or equal to 25, while obesity is a BMI higher than or equal to 30. It is the same for both genders and all ages of adults. However, according to WHO (*2018*), it should be considered a rough guide because it may not correspond to the same degree of fatness in different adult individuals, while in children age needs to be considered when defining overweight and obesity**.**

The basic cause of overweight and obesity is an energy imbalance between consumed and expended energy. Although fat metabolism is balanced to maintain homeostasis, high-fat diets tend to induce overconsumption and as a consequence, weight gain (*Fabroni et al., 2016*), that causes a major threat to world health (*Tucci et al., 2010*). An unhealthy life-style, connected with physical inactivity and high-fat diet, associated with development and lack of supportive policies in sectors such as transport, health, environment, food processing, and education leads to overweight and obesity (*WHO, 2018*). Furthermore, adiposity is related to genetic as well as to the environmental factors and increases the risk of hyperlipidaemia, hypertension, heart diseases and finally type 2 diabetes (*Fabroni et al., 2016*).

Dietary fats are one of the most important components of all living organisms which represent a fundamental constituent of human nutrition (25-35 % of daily energy intake) (*Cleveland Clinic, 2019*). The absorption of lipids takes place in the intestine and their key role is energy storage and supply, thermal regulation, membrane constituent, as well as some of them have an important function as fat-soluble vitamins and as essential fatty acids. The intestine of a human being is capable of absorbing around 95 % of ingested fat. Therefore, the intake of a high fat diet over a long period, may lead to adipose tissue, especially when combined with a lack of physical activity (*Tucci et al., 2010*).

Pancreatic lipase is an enzyme that has important role in the digestion of triglycerides (TG) (*Jamous et al., 2018*). TG are molecules which cannot be absorbed in the intestine, and therefore must be hydrolysed to fatty acids and 2-monoglycerides, which are more easily absorbed by the duodenum. The hydrolysis is catalysed by several human lipases, like the pre-duodenal (lingual and human gastric lipase) and the extra-duodenal (hepatic, lipoprotein, endothelial and pancreatic) lipases (*Tucci et al., 2010*). The inhibition of digestive enzymes is viewed as one approach to the treatment of overweight and obesity (*Jamous et al., 2018*).

As stated by the WHO, 422 million people worldwide suffer from diabetes. Furthermore, it is the seventh most common cause of death. *Diabetes mellitus* is a chronic disease characterised by increased plasma glucose concentrations (hyperglycaemia), caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. Insulin is the hormone that usually controls glucose levels, and diabetes results from impaired insulin secretion or reduced insulin action on its target tissues (insulin resistance). Moreover, it leads to damage to many of the body's organs and systems, particularly the eyes, kidneys, heart, blood vessels and nerves (*American Diabetes Association, 2009*).

Two main forms of diabetes are defined by clinical manifestations and causes. Type 1 diabetes (T1D), formerly known as insulin-dependent diabetes usually develops in young, lean individuals (children and adolescents) but is also being increasingly noted later in life. It is the result of an almost complete destruction of the pancreatic β cells, which produce insulin, usually as a consequence of an autoimmune process. Type 1 diabetes is characterised by plasma insulin levels that are very low. However, type 2 diabetes (T2D), formerly known as non-insulin-dependent diabetes, usually develops in overweight and/or older individuals. It has a very slow onset (the subject may be without clinical symptoms for several years) and is characterised by insulin resistance, resulting in chronically elevated plasma insulin and glucose levels. In addition, it results from the body's inability to respond properly to the action of insulin produced by the pancreas. Type 2 diabetes is much more common than type 1 and accounts for around 90 % of all diabetes cases worldwide and is being noted increasingly in adolescents. Both types of diabetes are complex diseases caused by mutations in more than one gene, as well as by environmental factors (*WHO global report on diabetes, 2008*).

Additionally, diabetes in pregnancy may give rise to several adverse outcomes, including congenital malformations, increased birth weight and an elevated risk of

perinatal mortality. Strict metabolic control may reduce these risks to the level of those of non-diabetic expectant mothers (*WHO global report on diabetes, 2008*).

The symptoms of diabetes may be pronounced, subdued or even absent. In the case of type 1 diabetes, the classic symptoms are excessive secretion of urine (polyuria), thirst (polydipsia), weight loss and tiredness, while in a case of type 2 diabetes the symptoms may be less noticed and the disease is only diagnosed several years after its onset, when complications are already present (*WHO global report on diabetes, 2008*). Long term complications of *diabetes mellitus* include retinopathy, nephropathy, neuropathy, microangiopathy and increased risk of cardiovascular disease (*De Sales et al., 2012*).

The mainstay of non-pharmacological diabetes treatment is diet and physical activity. One of the therapeutic approaches for treating type 2 *Diabetes mellitus* is to decrease the post-prandial glucose levels. This could be done by retarding the absorption of glucose through the inhibition of carbohydrate-hydrolysing enzymes, α-amylase and α-glucosidase, present in the small intestinal brush border that are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption. Inhibitors of these enzymes, like acarbose, delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise (*Van de Laar et al., 2005; Göke and Herrmann-Rinke, 1998*).

1.4. Plant material

The selected plants from Sardinia, Italy (IT) and Poland (PL) investigated in this thesis were chosen from the most common or the most promising for their agronomic and marketing exploitation. Specifically:

- Apples (*Malus domestica* Borkh. var. Shampion) (PL)
- Feijoa flowers (*Acca sellowiana* (O. Berg) Burret) (IT)
- Myrtle berries (purple and white) (*Myrtus communis* L.) (IT)
- Persimmon fruits (*Diospyros kaki* L. var. Rojo Brillante) (IT)
- Saffron flowers (*Crocus sativus* L.) (IT)
- Strawberry tree fruits (*Arbutus unedo* L.) (IT)

1.4.1. Apple (*Malus domestica* L. Borkh.)

Cultivated apple (*Malus domestica* L. Borkh) is a sweet, edible fruit growing on apple trees, belonging to the family Rosaceae. Apple trees are cultivated worldwide as fruit trees, and are the most widely grown species in the genus *Malus* (*Troggio et al., 2012*). The origins of this plant are in Central Asia, where it is possible to still find its wild ancestor (*Malus sieversii*). For thousands of years apple trees have been grown in Europe and Asia and were spread to North America by European colonists. Nowadays, apples are the most important fruits in Poland and several other countries in Europe and America (*Collett, 2011; Cornille et al., 2014*).

The cultivated plant is a deciduous tree, reaching heights of between 1.8 to 9 meters. The shape, size and branch density are determined by rootstock selection. The leaves are dark green, elliptical with serrated margins and slightly pubescent on the underside, while seeds are relatively small and black. The plant flowers in early summer. The apple flowers are epigenous and hermaphroditic with a variable number of stamens, while the five petals

are white with red-pink undersides. The fruits' maturation depends on the variety and takes place between late summer and autumn (*Eccher et al., 2014*).

Figure 1. Apple (*M. domestica* var. Shampion).

Apple fruit consumption is widespread around the world and it is on the market year-round. Apple fruit is a major source of phenol compounds and it represents a good source of dietary antioxidants. According to Kschonsek et al. (*2018*), generally, five major polyphenolic groups are found in various apple varieties: flavonols (quercetin and isorhamnetin derivatives), hydroxycinnamic acids, flavan-3-ols (catechin, epicatechin and procyanidins), anthocyanins (mainly cyanidin derivatives) and dihydrochalcones (phloretin glucosides). The flavonols are often associated with sugar moieties (the predominant sugars are glucose, galactose, rhamnose, xylose and arabinose), whereas dihydrochalcones are mainly associated with glucose and xyloglucose. The flavan-3-ols can be found in their monomers (catechin and epicatechin), oligomers, and polymers according to the data obtained by Wojdyło et al. (*2008*) and Oszmiański et al. (*2018*).

The majority of apples are consumed fresh, while small parts are processed into purées, concentrates and juices. A lot of research points to the beneficial sides of a diet rich in apple (*Roupas and Noakes, 2010*), which can reduce the risk of cancer (*Gerhauser, 2008*), cardiovascular diseases (*Hyson et al., 2000*), overweight and obesity (*Nagasako-Akazome et al., 2007*).

For the purpose of this study a particular variety of apple was chosen, due to its physico-chemical properties and geographical location (Poland). *M. domestica* var. Shampion (**Figure 1.**) is a hybrid cultivar of domesticated apple developed from crossing a Golden Delicious and a Cox Orange Pippin. Its fruits were used to prepare a cloudy juice. Apple juices, especially cloudy ones, are a rich source of natural antioxidants (polyphenols) according to (*Will et al., 2008*), that may be used in pharmaceutical or functional food preparation. Moreover, cloudy apple juice contains natural colloidal suspensions (e.g. proteins, pectins and free amino acids), which have a positive influence on stabilization for the colloidal system (*Siebert and Lynn, 1997*).

1.4.2. Feijoa (*Acca sellowiana* (O. Berg) Burret)

Feijoa (*A. sellowiana* (O. Berg) Burret) belongs to the family Myrtaceae and is known for its edible fruits and flowers. The plant (**Figure 2.**) is a small, evergreen tree or shrub, native to South America (Paraguay, Uruguay, Brazil and Argentina) and widely cultivated in the Mediterranean area and Oceania (*Mosbah et al., 2018; Aoyama et al., 2018*). The feijoa is ranked highly in decorative gardening for its beautiful silvery leaves and unusual bright crimson-red flowers with white-pink fleshy petals. Usually, it grows as a bush of 4-6 trunks, approximately 2.5 meters high (*Belous et al., 2014*). It is characterized by hermaphroditic flowers and fruit which ripens to the size of a small apple, with soft, white flesh and smooth, green skin. The fruit of the feijoa is characterized by a very aromatic flavour and juicy pulp around the seeds (*Mosbah et al., 2018*). The flowers are formed of four to six fleshy petals, rose-coloured inside and white outside, with red stamens reaching up to 2 cm above the flower, and slightly thickened stigma (*Belous et al., 2014*).

Feijoa fruits contain significant concentrations of polyphenols, carotenoids and vitamins (*Sun-Waterhouse et al., 2013*). Feijoa flowers (buds) were studied for the first

time regarding their content in bioactive compounds and health properties by Aoyama et al. (*2018*). Polyphenols investigated and present in feijoa flower bud extract are ellagic and gallic acid, representative of tannins - pedunculagin, flavone and an anthocyanin constituent, cyanidin glucoside. Furthermore, new gossypetin glycoside (gossypetin-3-*O*-*α*-L-arabinofuranoside) was investigated in feijoa flower buds. Polyphenolic characterisation and the profile of feijoa flowers extracts is substantially different from feijoa fruits polyphenolic extracts (*Monforte et al., 2014*) and feijoa leaves polyphenolic extracts, which have been more intensively investigated in past years (*El-Shenawy et al., 2008; Ruberto and Tringali, 2004*).

Figure 2. Feijoa (*A. sellowiana* (O. Berg) Burret).

Feijoa fruits have high antioxidant activity, antibacterial and antifungal properties. Furthermore, they have significant antimicrobial effects on *Helicobacter pyroli* (*Basile, 1997; Basile et al., 2010*). While the feijoa fruits are well investigated and their use is widespread, knowledge of the petals is still scarce. Nowadays petals of the feijoa flowers can be eaten, usually in salads, sweets and as dressings (*De Souza et al., 2016*), since they possess a pleasant taste and intense colour.

1.4.3. Myrtle (*Myrtus communis* L.)

Myrtle (*M. communis* L.) belongs to family Myrtaceae. It is an aromatic, broadleaf evergreen shrub or small tree that is mainly native to the Mediterranean region (**Figure 3.**), and Western Asia. The plant grows to 2.4-3 m high and its branches form a close to full head. It is drought tolerant and needs very little water. It is covered with glossy, aromatic, evergreen, glabrous, and ovate to lanceolate leaves. It blooms in summer. The flowers are white or tinged with pink, with five petals and many stems. They give off a sweet, fragrant smell. The berries are pea-sized, ovoid-ellipsoid or orbicular, blue-black or white with seeds. The fruit develops from pale green to deep red and finally when it is fully mature it becomes dark indigo. The taste of the berries is bitter when unripe and sweet when ripe (*Sambul et al., 2011*).

Figure 3. White and purple myrtle shrub (*M. communis* L.).
[https://antropocene.it/en/2017/07/22/myrtus-communis/]

Purple myrtle berries have been widely investigated for their chemical composition and they are rich in tannins, phenols, fatty acids and essential oil. The phenolic compounds fraction is characterized by gallic acid, flavonoids and anthocyanins that represent the most abundant phytochemicals in myrtle berries. The anthocyanin profile shows five anthocyanin glucosides and four anthocyanin arabinosides with malvidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and petunidin-3-*O*-glucoside as major peaks. Flavonoid profile

is characterized by myricetin-3-*O*-galactoside, myricetin-3-*O*-rhamnoside, myricetin and quercetin (*Montoro et al., 2006; Tuberoso and Orrú, 2012*). Besides original composition in phenolic compounds, the type of solvent can greatly affect final composition of the extract and consequently, the biological activities (*Tuberoso et al., 2010*).

M. communis white berries has been less investigated and some information is reported by Şan et al. (*2015*) and Serreli et al. (*2017*), in which predominant phenolic compounds are flavonols (naringin, myricetin, myricetin-3-*O*-rhamnoside and myricetin-3-*O*-galactocside), hydroxybenzoic acids (gallic and ellagic acid) and caffeic acid ester (chlorogenic acid). Moreover, caffeic acid, vitexin, apigenin-7-*O*-glucoside and resveratrol are present in smaller amounts, as well as anthocyanins such as malvidin-3-*O*-glucoside.

M. communis has been used since ancient times for food and spice purposes, but it is also used in folk medicine for the treatment of several diseases (*Messaoud and Boussaid, 2011*). Different parts of the plant and essential oils from fruits, leaves and flowers are widely used in food, liqueurs and cosmetics and for flavouring sauces and meat (*Şan et al., 2015; Tuberoso and Orrú, 2012*). In addition, its leaves and fruits are used for hypoglycemic, antioxidant, antimicrobic, antifungal, constipation, antihemorrhagic, appetizing, wound healing purposes, and the treatment of coughs and oral diseases (*Taamalli et al., 2014; Tuberoso and Orrú, 2012*).

1.4.4. Persimmon (*Diospyros kaki* L.)

Persimmon (*Diospyros kaki* L.) belongs to the Ebenaceae family, and is a widely cultivated fruit in China, Japan and Korea (*Chen et al., 2016*), as *D. lotus* and *D. oleifera*, while *D. virginiana* is native to the eastern United States and *D. rhombifolia*, is known as an ornamental tree. Furthermore, consists of around 400 species and is widely distributed through tropical and temperate regions of Asia, Africa, Central-South America and Mediterranean countries. *Diospyros kaki* is classified into four types depending on the

effect of pollination on the flesh colour, the presence of seeds and their pattern of astringency loss: pollination-constant non-astringent, pollination-variant non-astringent, pollination-variant astringent, and pollination-constant astringent (*Celik and Ercisli, 2009; Bibi et al., 2007*). Moreover, persimmon is a sweet, delicious, tropical, fleshy-fibrous fruit with broad, stiff leaves, which, when ripe, contains thick, pulpy jelly encased in waxy thin-skinned shell (*Daood et al., 1992*).

Figure 4. Persimmon (*Diospyros kaki* Thunb. var. Rojo Brillante).

Persimmon fruits are source of many bioactive compounds, like polyphenols, vitamins (vitamin B1, B2, B3, A, E, K and C), minerals (calcium and potassium), sugars (sucrose and its glucose and fructose monomers), carotenoids, tocopherols (*Butt et al., 2015*) and dietary fibres (*Akter et al., 2010*). Generally, the main polyphenols in these fruits are flavonols (quercetin and rutin), hydroxycinnamic acids (caffeic, *p*-coumaric and ferulic acid), hydrocybenzoic acids (gallic, vanillic and syringic acid) and proanthocyanidin (catechin) (*Pu et al., 2013*).

The persimmon fruit is mainly eaten fresh but can be frozen, canned or dried, and is sometimes used in Oriental cooking or dried fruits and provides natural defence against free radicals (*Lee et al., 2008; Matsumura et al., 2016*), and has a cholesterol-lowering effect (*Hwang et al., 2017*). Usually whole fruits and slices are dried to obtain dried persimmon products. Moreover, the ripe, non-astringent and sweet fruits can be used as a

sweetening ingredient in fruity ice creams and baked products, as well as material for the preparation of other products such as nectars, jams and jellies (*Daood et al., 1992*). Although, large quantities of available by-products (persimmon peel) are mainly discarded as waste to avoid its bitter taste (which is connected with its astringency) the peels of various fruits are rich in bioactive compounds that variously benefit human health. If the peel is not processed further, it becomes waste and it need to be treated before disposal. This problem can be resolved by utilising its high value-added food products, including fraction of the dietary fibre that has a great importance in the preparation of functional foods. Moreover, persimmon peels may potentially contain more antioxidants qualitatively and quantitatively than the pulp. Persimmon pulp devoid of peel can be used to make purée, juice and sherbets, while peel polyphenol content contributes to the prevention of oxidative stress-related diseases, like diabetes (*Lee et al., 2006*).

For the purposes of this study a particular variety of persimmon was chosen, due to its physico-chemical purposes and geographical location (Sardinia, Italy). *Diospyros kaki* Thunb. var. Rojo Brillante (**Figure 4.**) is an astringent persimmon which has excellent nutritional and sensorial qualities. According to Mir-Marqués et al., (*2015*), the chemical composition of this fruit, particularly the content of minerals, depending on the soil type and the growing environment, may offer a route for authenticating fruit produced inside the protected designation of origin. In addition, the mineral composition of foods has a vital role in human health and adequate intakes of many elements are the key to a healthy diet.

1.4.5. Saffron (*Crocus sativus* L.)

Saffron *(Crocus sativus* L.) belongs to the family Iridaceae. The flowers are made up of, three red stigmas, three stamens and six light purple petals (**Figure 5.**). The cultivation of saffron is wide spread throughout the Eastern Mediterranean region, Europe, India and Western Asia, with Iran as the world's main producer. Commercial *C. sativus* comprises

the dried red stigma with a small portion of the yellowish style attached. It is in leaf from October to May. The flowers are hermaphrodite, have three stigmas, which are often dried and used in cooking as a seasoning and colouring agent. Saffron blooms only once a year and should be harvested within a very short window. It is picked during 3-4 weeks in October-November (*Srivastava et al., 2010; Zeka et al., 2015*).

Figure 5. Saffron (*Crocus sativus* L.). [https://www.etsy.com/hken/listing/527710330/saffron-bulbs-crocus-sativus-8pcs-rare]

Characteristic components of saffron stigma are safranal responsible for odour and aroma, crocin responsible for the red or reddish-brown colour of stigmas together with carotenes, crocetin and picrocrocin, which is a glycosidic precursor of safranal, and is responsible for the bitter taste of stigmas (*Srivastava et al., 2010*). In recent years, saffron floral by-products obtained after stigma separation have been investigated as potential sources of bioactive compounds (*Montoro et al., 2012; Tuberoso et al., 2016*). The major phenolic compounds investigated in *C. sativus* flower juice are flavonols, such as kaempferol-3-*O*-sophoroside and other kaempferol derivatives, quercetin and isorhamnetin glycosides. Anthocyanins present in saffron flower juice are mainly delphinidin-3,5-di-*O*-glucoside followed by delphinidin 3-*O*-glucoside, malvidin 3,5-di-*O*-glucoside, petunidin 3-*O*-glucoside and petunidin-3,7-di-*O*-glucoside**.**

The plant has a wide range of medical uses, thanks to its variety of biologically active ingredients, such as antioxidant, antidiabetic, antihypertensive, antidepressant, anti-

inflammatory, anxiolytic, aphrodisiac, anticonvulsant, santinociceptive, antitussive, antigenototoxic and cytotoxic effect and relaxant activity. Moreover, it improves learning and memory skills, and increases blood flow in choroid and retina (*Razak et al., 2017; Tuberoso et al., 2016; Srivastava et al., 2010*).

1.4.6. Strawberry tree (*Arbutus unedo* L.)

The strawberry tree *(A. unedo* L.) belongs to the family of Ericaceae and is an ever-green shrub (**Figure 6.**), native to the Mediterranean region, especially its islands (among which is Sardinia). It has also been able to adapt to the conditions on the south-western coast of Ireland. The leaves are simple, alternate, with a dark green colour and oblanceolate form, leathery, short-stalked and toothed. The flower is a clump of little cream-pink-coloured petals. The fruits are conspicuous, globular, orange-red when ripe, and grow up to 2 cm in diameter with a rough surface. There are two maturity phases of the fruits. The first phase is from the middle of October to the beginning of December. The second is around New Year's Eve. The plant has an important role from an ecological point of view. It helps to maintain the diversity of fauna, prevents soil erosion, regenerates rapidly after fires, grows in poor soils and may be used for phytoremediation, mainly against arsenic contamination. Strawberry tree honey is popular for its strong and distinctly bitter taste (*Oliveira et al., 2011*).

The fruits are rich in phenolic compounds, vitamins, minerals, sugars, fatty acids and sterols (*Vidrih et al., 2013*). Chemical composition of the fruit showed: sugars (from 42 % to 52 % of total sugar content, mainly sucrose, fructose), proteins, sterols (β-sitosterol, 5-α-cholestane, cholestan-3-cholesterol, stigmasterol, stigmast-4-en-3-one), fatty acids (15 fatty acids in total, mainly linoleic, linolenic and α-linolenic acid), minerals (K, Ca, P), vitamins (vitamin C, carotenoids, vitamin E, α-tocopherol and γ-tocopherol) (*Delgado-Pelayo et al., 2016*), organic and phenolic acids (fumaric, ursolic, oleanolic, lactic, malic,

suberic, citric, quinic, gallic, gentisic, protocatechuic, *p*-hydroxybenzoic, vanillic, *m*-anisic acid) (*Ayaz et al., 2000*) and phenolic compounds: anthocyanins (cyanidin-3-*O*-*β*-D-galactopyranoside, delphinidin-3-*O*-*β*-D-glucopyranoside, cyanidin-3-*O*-*β*-D-arabinoside) (*Pawlowska et al., 2006*), proanthocyanidins (> 80 % of the total flavonoid content), lupeol, *α*-amyrin, ursolic aldehyde, lupenone, amyrone, *α*-amyrenone, uvaol, quercetin, kaempherol, naringin, catechin, apigenin, fisetin, taxifalin, silybin (*Pallauf et al., 2008*).

Figure 6. Strawberry tree (*Arbutus unedo* L.).

Strawberry tree fruits have several medicinal effects, such as antioxidant activity and antitumor potential (*Mendes et al., 2011; Fortalezas et al., 2010*). Moreover, the fruits are used in folk medicine as antiseptic, diuretic and laxative agents, while in traditional medicine they are used to treat gastrointestinal disorders and dermatologic problems (*Oliveira et al., 2011*). Strawberry tree fruits are generally used for preparing jams, jellies, marmalades and alcoholic drinks (liqueurs, wines and brandies) (*Miguel et al., 2014*).

2. MATERIALS AND METHODS

2.1. Reagents and standards

All the chemicals were used of analytical grade. Acetonitrile for ultra-phase liquid chromatography (UPLC, gradient grade) analysis, neocuproin (2,9-dimethyl-1,10-phenanthroline) and ammonium acetate were purchased from Merck (Darmstadt, Germany), while cupric chloride and sodium acetate were purchased from Carlo Erba (Milan, Italy). Methanol, rhamnose, fructose, sorbitol, glucose, sucrose, ascorbic, oxalic, citric, isocitric, tartaric, malic, quinic, acetic, hydrochloric, formic, phosphoric and malic acid, sodium hydroxide, sodium chloride, calcium chloride, phloroglucinol, sodium acetate, Folin-Ciocalteau' reagent, sodium carbonate decahydrate, iron (II) sulfate heptahydrate, 2,4,6-tripyridyl-1,3,5-triazine (TPTZ), iron (III) chloride hexahydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein disodium (FL), 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH), disodium and dipotassium phosphate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,20-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, starch from potato, *α*-amylase from porcine pancreas (type VI-8), 3,5-dinitrosalicylic acid (DNS), potassium sodium tartrate tetrahydrate, sodium phosphate monobasic, dipotassium hydrogen orthophosphate dihydrogen, *p*-nitrophenyl-*α*-D-glucopyranoside (*p*NPG), *α*-glucosidase from *Saccharomyces cerevisiae* (type I), lipase (EC 3.1.1.3) from porcine pancreas (type II), *p*-nitrophenyl acetate and 4-methylumbelliferyl oleate (4-MUO) were purchased from Sigma-Aldrich (Steinheim, Germany). The following standards were used for the identification and quantification of phenolic compounds: cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, phenolic acids (gallic, neochlorogenic, chlorogenic, caffeic, *p*-coumaric, ellagic), phloretin, (-)-epigallate, (-)-

epicatechin, (+)-catechin, procyanidin B1 and B2, apigenin, isorhamnetin-3-*O*-rutinoside, kaempferol-7-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol, myricetin-3-*O*-galactoside, myricetin-3-*O*-rhamnoside, myricetin, quercetin-4`-*O*-glucoside, quercetin-3-*O*-rhamnoside, and quercetin-3-*O*-rutinoside were from Extrasynthese (Lyon, France). UPLC grade water, prepared by using an HLP SMART 1000 s system (Hydrolab, Gdańsk, Poland), was filtered through a 0.22 μm membrane filter immediately before use.

2.2. Plant material and sample preparation

All fresh plant materials were manually collected by professional pickers and identified by an expert botanist. These plant materials were used for both fine chemical characterization (e.g. preparing concentrated extracts) and final product preparation.

Apple. *Malus domestica* (var. Shampion) fruits (**Af**) (30 kg) were collected at commercial maturity in December 2016 from the LA-SAD SP. Z.O.O (Borzęcin, Błędów). Whole fruits were washed with distilled water, and around 5 g of whole fruit was immediately used to measure the dry matter (dm). All remaining plant material was divided into two parts. The first part was cut into slices, frozen, freeze-dried and homogenized into powder (**Ad**), by crushing the dried tissues using a closed laboratory mill to avoid hydration (IKA 11A; BIOSAN, Vilno, Lithuania). Obtained powder was kept in a refrigerator (−70 °C, Frilabo, Lyon, France) until extract preparation (**Paragraph 2.4.**). It was used later for the colour, sugars, organic acids and phenolic compounds measurements, as well as antioxidant and digestive eznymes inhibition activity analyses. The second part (**Figure 7.**) was cut in halves and arils and ground in Thermomix appliance (Vorwerk, Wuppertal, Germany). Pectinex Smash XXL enzyme (0.2 mL/kg of fruits) was added to the mash in order to break down the pectin compounds, which improves the process of fruit pressing and further processing of juice. The obtained pulps were pressed in a hydraulic press to

obtain juice (**Aj**). Collected cloudy apple juice was frozen and used later for the base (**B1, B2** and **B3**) of final product preparation.

Figure 7. Apple fruits during process of juice preparation.

Feijoa flowers. *Acca sellowiana* (feijoa) flowers (**Ff**) (5 kg) were collected in Uta (Sardinia, Italy) in May 2017 (**Figure 8.**). The dry matter (dm) was measured on fresh flowers soon after harvest and all remaining plant material was divided into three parts. The first part was frozen, freeze-dried, homogenized into powder (**Fd**), and stored as described above for apple fruits. Obtained powder was used for the analytical measurements as described above for apple fruits. Moreover, the freeze-dried sample (**Fd**), was purified for obtaining polyphenols fraction: dry plant material was extracted with a water/ethanol/acetone mixture (7/2/1, v/v), put on a chromatographic column filed with polymeric resin (Amberlite XAD 16, Brenntag Polska Sp.z.o.o., Kędzierzyn-Koźle, Poland) activated with 96 % ethanol in 2 % NaOH) and cleaned of all other fractions, beside the polyphenolic compounds. Concentrated dry sample of polyphenols (**Fc)** were obtained, and then distilled in a vacuum (Büchi Rotavapor R-114, Switzerland) until complete alcohol elimination. Furthermore, the freeze-dried sample (**Fd**), was used for final product preparation. The second part of the flowers was extracted with a mixture ethanol-water (80:20 v/v, solvent:plant ratio 9:1 v/w) (**Fe)**, while the third part of the

flowers was used to obtain petal juice fraction (**Fpj**) using manual pression, and ethanol:water extract (**Fpe)**, obtained in the same way as (**Fe**).

Figure 8. Feijoa flowers used for investigation and final products preparation.

Myrtle berries. *Myrtus communis* berries (purple **MPf**, and white **MWf**) (10kg and 2 kg, respectively) were randomly collected in Monte Arcosu (Sardinia, Italy) in December 2016 (**Figure 9.**). All berries were gently cleaned, and around 5 g of each type of berries (**MPd** and **MWd**), was immediately used to measure the dry matter (dm). All remaining plant material of (**MPf)** and (**MWf)** was divided into two parts, for both types of berries. The first part of both types of berries were immediately frozen, freeze-dried, homogenized into powders (**MPd** and **MWd**), and stored as described above for apple fruits. Obtained powder was used for the analitycal measurements as described above for apple fruits. Moreover, a part of the freeze-dried berries (**MPd** and **MWd**) was purified using the procedure described above for feijoa flowers. Concentrated, dry samples of polyphenols (**MPc** and **MWc)** were obtained. The second part of the fresh purple myrtle purple berries (**MPf**) was extracted with an ethanol 96 % (1:1, w/v). Extraction procedure: 5 kg of fruits were ground in a mortar, macerated with 5 L of ethanol 96 % and incubated twice for 30 min under sonication. The supernatant was filtrated using a strainer and then concentrated by distillation in a vacuum (Büchi Rotavapor R-114, Switzerland) until complete alcohol

elimination. Obtained extract (**MPe**) was stored as (**MPd**) and used later for final product preparation. The second part of the fresh white myrtle berries (**MWf**) were extracted with a mixture ethanol-water (80:20 v/v, solvent:plant ratio 1:1, v/w) and left for four months. Next, macerates were separated from the berries, and the liqueurs were produced by adding sucrose and water to obtain a final percentage of 28 % v/v (alcohol) and 32 % w/v (sugar) (**MWe**). Before bottling, the liqueur was filtered through IF350 cellulose acetate cardboard filter (Industrialfiltro srl, Cologno Monzese, MI, Italy).

Figure 9. Myrtle purple berries used for investigation and final products preparation.

Persimmon fruits. *Diospyros kaki* L. (var. Rojo Brillante) (**Kf**) fruits (15 kg) were collected at commercial maturity from the plantation "Melotto" near Villacidro (Sardinia, Italy) in October 2016 (**Figure 10.**). The dry matter (dm) was measured on the fresh fruits soon after harvest. All remaining plant material was divided into two parts. The first part was cut into pieces, frozen, freeze dried, homogenized into powders (**Kd**), and stored as described above for apple fruits. Obtained powder was used later for the analitycal measurements as described above for apple fruits. The second part of the whole fruits (**Kf**) was ground, heated at 80 °C in a Thermomix device (Vorwerk, Wuppertal, Germany), and mashed in a blender (Symbio, Zelmer, Rzeszów, Poland). Afterwards, the particle size of the mixture was further reduced in blender (Symbio, Zelmer, Rzeszów, Poland) down to

thin purée (**Kp**). Then, the purée was cooled to room temperature, frozen and used later for final products preparation.

Figure 10. Persimmon fruits used for investigation and final products preparation.

Saffron flowers. *Crocus sativus* flowers (**Sf**) (7kg) were collected in San Gavino Monreale (Sardinia, Italy) in November 2016 (**Figure 11.**). Flowers without stigma (the by-product after saffron spice collection) were squeezed (manual press) to obtain juice. The juice was evaluated for its dry matter, centrifuged, filtrated using 0.45 μm cellulose acetate filter, frozen, freeze-dried (**Sd**), and stored as described above for apple fruits. Obtained freeze-dried sample (**Sd**) was used later for the analytical measurements as described above for apple fruits, as well as for final products preparation.

Figure 11. Saffron flowers used for investigation and final products preparation.

Strawberry tree fruits. *Arbutus unedo* fruits (**Cf**) (10 kg) were randomly collected in Sinnai (Sardinia, Italy) in December 2016 at full ripening (**Figure 12.**). Whole fruits were gently cleaned and around 5 g were immediately used to measure the dry matter (dm). All remaining plant material was immediately frozen, freeze-dried, homogenized into powder (**Cd**), and stored as described above for apple fruits. Obtained powder was used later for the analytical measurements as described above for apple fruits. Moreover, freeze-dried fruits (**Cd**) were divided into two parts. The first part was used for obtaining polyphenols purified fraction (**Cc**), as described for feijoa flowers, while the second part was used for final product preparation.

Figure 12. Strawberry tree fruits used for investigation and final products preparation.

2.3. Final products preparation

Annex 1b. reports the list and the composition of 20 final products (**Figure 13.**) obtained from studied plant materials. The production process of the final products included 4 main technological stages:

- semi-product preparation;
- base preparation;
- 3 sets of final products preparation;
- thermal stabilization.

Semi-product preparation

For the preparation of raw plant products, the apple juice was prepared according to the procedure described above for the *M. domestica* L. var. Shampion. The persimmon purée was prepared according to the procedure described above for the *D. kaki* L. var. Rojo Brillante. The strawberry tree mousse was prepared mixing dry *A. unedo* fruits (**Cd**) with the apple juice, in proportions 33.25:67.75 (w/w), respectively leading to the initial water content in (**C**).

Base preparation

Base 1 (**B1**) was apple juice 100 %, while Base 2 (**B2**) was obtained by mixing persimmon fruits purée with apple juice (25:75, w/w) and Base 3 (**B3**) by mixing strawberry tree fruits mousse with apple juice (25:75, w/w). This proportion was chosen after preliminary tests, varying the amount of apple juice from 50 to 80 %, w/w. An amount of 75 % of apple juice was considered optimal due to the best semi-liquid consistency of the obtained mixtures. Base K (**BK**), pure persimmon fruits into purée, was used only for analytical purpose.

3 sets of final products preparation

Bases **(B1**, **B2,** and **B3)** were enriched with raw plant semi-products in appropriate proportions (**Annex 1b**), obtaining final products. The raw plant semi-products (**Sd**, **Fd**, and **Cd)** or persimmon purée (**Kp**) or the extract (**MPe**), were obtained with the procedure described above. Overall, Base **B1** sets consisted of 7 samples, while base **B2** and **B3** set was composed of 6 samples. For each set, one of the samples was a blank (**B1**, **B2,** and **B3**) and the other 4 were fortified with **S** 0.1 % (**S01**), **S** 0.5 % (**S05**), **M** 5 % (**M5**) and **F** 5 % (**F5**). Moreover, additional components: **C** 5 % (**C5**) and **K** 5 % (**K5**) were added to the base **B2** and **B3**, respectively, and both of these to the base **B1**.

Thermal stabilization

All products were heated to 100 °C, put into glass jars (130 mL), and pasteurized (for 10 minutes at 90 °C).

Figure 13. All final products immediately after preparation (0 months).

2.4. Ultrasound-assisted extraction (UAE) procedure

Powdered samples obtained from plant material (Ad, Fd, Kd, MPd, MWd, Sd, Cd) and fresh final product samples (before and during storage time) were extracted with two different procedures:

- 30 % methanol acidified with 1 % ascorbic acid and 1 % acetic acid. The extraction was performed twice by incubation for 20 min under sonication (Sonic 6D; Polsonic, Warsaw, Poland). Next, the slurry was centrifuged, and the supernatant was filtered through a hydrophilic PTFE 0.20 μm membrane (Millex Samplicity Filter, Merck). The

content of polyphenols in individual extracts was determined by means of LC-PDA-QTof/MS and UPLC-PDA method.

- 80 % methanol acidified with 1 % hydrochloric acid. The extraction was performed twice by incubation for 20 min under sonication (Sonic 6D). Next, the slurry was centrifuged. In individual extracts the total polyphenols content, antioxidant activity (FRAP, CUPRAC, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, ORAC assays) and digestive enzymes (α-amylase, α-glucosidase, pancreatic lipase) inhibition activity were determined.

2.5. Consumer evaluation of the final products

The sensory assessment of final products was carried out using a 5° hedonic scale with boundary indications: 'I do not like it very much' (1) - 'I like it very much' (5) according to Nowicka et al. (*2016a*). The assessment which included the following quality attributes, colour, aroma, taste, consistency, desirability and aroma type - and was conducted by a group of 10 trained Polish panellists (1 man and 9 women in the age group from 20 to > 60) (**Annex 2.**). Coded samples were provided to the panellists for evaluation at a temperature of ca. 20-25 °C in transparent, uniform, 50-mL plastic containers.

2.6. Physico-chemical analyses

All chemical analyses were performed for plant materials and 20 final products before and after storage time (3 and 6 months at 20±2°C), according to Wojdyło et al. (*2017*), and all determinations were done in triplicate in each replicate.

The dry matter was evaluated according to the PN-90/A-75101/03, by drying 2 g of the fresh plant material or final product for 2 h in a thermostatic oven at 105 ± 1 °C, and weighing until a constant weight value was attained. Results were expressed as g per 100 g of dry matter or fresh weight, for plant materials and final products respectively.

The total soluble solids (TSS) content was determined by refractometer PAL-88S (Atago Rx 5000, Atago Co. Ltd., Japan) and expressed as °Brix. Titratable acidity (TA) was determined by titration aliquots of homogenate of fresh final products by 0.1 N NaOH to an end point of pH 8.1 using an automatic pH titration system (pH-metr typ IQ 150; Warsaw, Polska) and expressed as g of malic acid per 100 g of fresh weight.

Total content of L-ascorbic acid (vitamin C) and ash content of fresh final products were determined by the PN norms - PN-90/A-75101/11 and PN-90/A-75101/08, respectively. Results of vitamin C and ash content were expressed as mg or g, respectively, per 100 g of fresh weight.

2.7. Colour measurement

The colour evaluation (**Figure 14.**) was assessed according to the Commission Internationale de l'Eclairage (CIE) parameters L^* (colour lightness, $L^* = 0$ black, $L^* = 100$ white), a^* (green colour $a^* < 0$, or red colour $a^* > 0$), b^* (blue colour $b^* < 0$, or yellow colour $b^* > 0$), and ΔE^* (total colour difference). For the dry plant material an A5 Chroma-Meter (Minolta CR300, Osaka, Japan) was used (*Wojdyło et al., 2014a*), and for the final products a Colour Quest XE Hunter Lab colorimeter (*Nowicka et al., 2016a*) was used. The colour coordinates of the final product samples were determined using Illuminant D65 and 10° observer angle. The samples were filled in a 1-cm cell, and $L^*a^*b^*$ values were measured against a white ceramic reference plate ($L^* = 93.92$, $a^* = -1.03$, $b^* = 0.52$). The total change in colour (ΔE^*) was calculated according to the following equation:

$$\boldsymbol{\Delta E} = \sqrt{(\boldsymbol{L_0}^* - \boldsymbol{L}^*)^2 + (\boldsymbol{a_0}^* - \boldsymbol{a}^*)^2 + (\boldsymbol{b_0}^* - \boldsymbol{b}^*)^2}$$

where: L_0^*, a_0^* and b_0^* denote to the value of lightness, redness and yellowness of the samples, respectively. Data were the mean of three measurements.

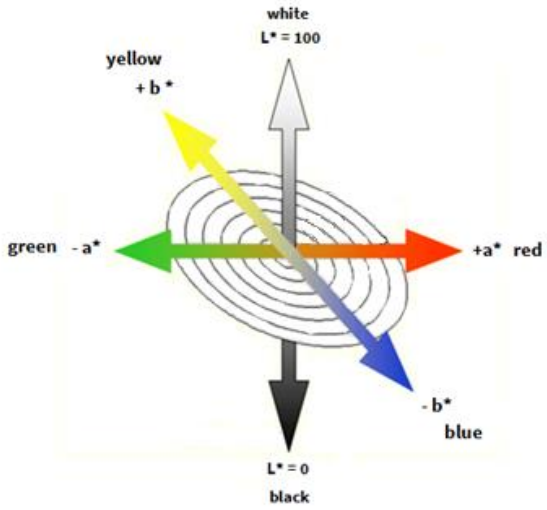


Figure 14. Graphical representation of the $L^*a^*b^*$ colour measurement system.

2.8. Determination of sugar and organic acid content

Sugars and organic acids were determined as described previously by Nowicka al. (*2016b*) and Nowicka et al. (*2019*), respectively. Dry plant material of *A. unedo*, *M. domestica* and *D. kaki* fruits, *M. communis* purple and white berries, *A. sellowiana* flowers and *C. sativus* flower juice (0.5 g), and 20 final product samples (6 g) were mixed with 20 or 50 mL of redistilled water, respectively. Next, all samples were ultrasonicated for 15 min, boiled for 30 min and after that centrifuged at 20.000g for 10 min. The supernatant was applied onto the Sep-Pak C-18 (containing 1 g of the carrier, Waters, Milford, USA) and eluted by water to give sample solution to estimation of sugar or organic acid content. 1 mL of the centrifuged liquid was filtered using a 0.45-μm Millipore filter.

Sugars: The HPLC-ELSD (HPLC with evaporative light scattering detector) was used for sugar analysis. A 40 μL sample was injected by autosampler (L-7200) into a Unison UK Amino 3 μL column (3 mm × 250 mm) (Imtakt, Kyoto, Japan) by liquid chromatography. Detection was carried out using evaporative light-scattering detector (PLELS 1000 Evaporative Light Scattering Detector, Polymer Laboratories, Varian, Darmstadt, Germany) with the following input parameters: temperature of the evaporator −80 °C;

temperature of the nebulizer −80 °C; nitrogen flow −1.2 SLM. The elution was carried out at 30 °C under an isocratic flow using 85 % acetonitrile solution at the flow rate of 0.7 mL/min. Sugar components were identified by comparison with the standards (rhamnose, fructose, sorbitol, glucose, sucrose). The calibration curves were prepared by plotting different concentrations ranging from 0.5 to 5 mg/mL ($R^2 \geq 0.9998$) of the standards versus the area measurements in HPLC. The results were expressed as g of total sugar content per 100 g product dry matter or fresh weight, according to the sample - plant materials and final products, respectively. Sugar content was determined in triplicate.

Organic Acids: The UPLC-PDA (UPLC with photodiode array detector) method was used for organic acids analysis. A 20 µL sample was injected by autosampler (Aquity, Waters, Milford, USA) into the Supelcogel TM C-610H column (30 cm x 7.8 mm; Supelco, Bellefonte, PA, USA). The elution was carried out at 30 °C under isocratic flow using 0.1 % phosphoric acid solution with a flow rate of 0.5 mL/min. Organic acids were separated and detected using a diode-array detector set up at 210 nm. Standard curves for pure standards of organic acids oxalic, citric, isocitric, tartaric, malic, quinic, ascorbic, shikimic, succinic and fumaric acids were used for quantification. The calibration curves were prepared in the range 0.5-10 mg/mL ($R^2 \geq 0.9998$) of standards versus the area measurements in UPLC. Results for organic acids were expressed as g of total organic acid content per 100 g dry matter or fresh weight, according to the sample - plant materials and final products, respectively. Organic acid content was determined in triplicate.

2.9. Idtification and quantification of polyphenols by LC-PDA-MS method

The extract of polyphenols for analysis was prepared as described in **Paragraph 2.4.**. Before the analysis the supernatant was filtered through a Hydropilic PTFE 0.20 mm membrane (Millex Samplicity Filter, Merck) and analysed with UPLC-MS technique.

Identification of phenolic compounds of the plant materials and the final products extracts was carried out using an ACQUITY Ultra Performance Liquid Chromatography system (UPLC) equipped with an autosampler, a photodiode detector (PDA) and binary solvent manager (Waters Corporation, Milford, MA, USA) series coupled to mass detector G2 QTof Micro mass spectrometer (Waters, Manchester, UK) equipped with electrospray ionization (ESI) as a source operating in negative and positive modes with spectra acquired over a mass range *m/z* 100 to 1800, as described previously by Wojdyło et al. (*2014b*).

For instrument control, data acquisition and processing, MassLynx software 4.0 ChromaLynx Application Manager Software (Waters Corporation, Milford, USA) was used. MS experiments were performed, one in negative mode (phenolic acids, flavan-3-ols, dihydrochalcones, flavonols) and one using positive ionization (anthocyanins) before and after fragmentation. Characterisation of the single components was carried out via the retention time and accurate molecular masses. Analyses were carried out with voltage ramping cycles from 0.3 to 2 V, using full scan mode, collision induced fragmentation experiments parameters were performed using argon as the collision gas. The optimum values of LC-MS parameters were: capillary and cone voltages were 2500 V and 30 V, respectively. The capillary temperature was set to 300 °C, while the source heater temperature was 100 °C, drying gas (nitrogen) flow rate of 300 L/h. Leucine encephalin was used as the mass reference compound at flow rate 2 µL/min, a concentration of 500 pg/µL and *m/z* at 554.2615 and 556.2771 Da were detected for negative and positive ionisation, respectively. Chromatographic separation was performed on UPLC BEH C18 column (1.7 µm, 2.1 x 100 mm, Waters Corporation, Milford, USA) at 30 °C. Samples (10 µL) were injected and elution completed in 15 min with sequence of linear gradients and isocratic flow rates of 0.42 mL/min. The mobile phase was composed of Solvent A (0.1 % formic acid, v/v) and Solvent B (100 % of acetonitrile). The program began with isocratic elution with 99 % A (0 to 1 min); a linear gradient was used until 12 min, lowering A to 25

%; from 13.5 to 15.0 min, it was returned to the initial composition (99 % A), and then held constant to re-equilibrate the column. The PDA spectra were recorded from 200 to 600 nm in steps of 2 nm, and runs were monitored at the following wavelengths: 280, 320, 360 and 520 nm for flavan-3-ols, phenolic acids, flavonols and anthocyanins, respectively. The characterization of the single components was carried out via retention time (R_t), spectra, accurate molecular masses, literature data and pure standards, if available. Each compound was optimized to its estimated molecular mass $[M-H]^-$ or $[M+H]^+$ in the negative and positive (for anthocyanins) mode before and after fragmentation. Calibration curves at concentrations ranging from 0.05 to 0.5 mg/mL ($r^2 \leq 0.9998$) were made for standards of phenolic compounds.

The quantitative analysis (UPLC-PDA) of polyphenols (anthocyanin, phenolic acid, flavan-3-ol, dihydrohalcone, flavonol) was performed according to Wojdyło et al. (*2014b*). Empower 3 software was used for chromatographic data collection and integration of chromatograms. The UPLC analyses were performed on a BEH Shield C18 analytical column (2.1 mm x 50 mm, 1.7 μm). The flow rate was 0.45 mL/min with the flow rate at 0.45 mL/min. A partial loop injection mode with a needle overfill was set up, enabling 5 μL injection volumes when a 10 μL injection loop was used. Acetonitrile (100 %) was used as a strong wash solvent and acetonitrile in water (10 %, v/v) as a weak wash solvent. Samples were analysed in triplicate and the results from UPLC-PDA were expressed as milligrams per 100 g of dry matter or fresh weight, according to the sample - plant materials and final products, respectively.

2.10. Analysis of proanthocyanidins by phloroglucinol method

The analysis of polymeric procyanidins by phloroglucinol method of freeze-dried plant materials and final products was performed according to Kennedy and Jones (*2001*). Samples were precisely weighed into 2 mL Eppendorf vials, then 0.8 mL of the methanolic

solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L) was added. After the addition of 0.4 mL of methanolic HCl (0.3 mol/L), the vials were closed and incubated for 30 min at 50 °C with continuous vortexing using a thermos shaker (TS-100; BIOSAN, Lithuania). The reaction was stopped by placing the vials in an ice bath, with drawing 0.5 mL of the reaction medium and diluting with 0.5 mL of 0.2 mol/L sodium acetate buffer. Next the vials were cooled in ice water and centrifuged immediately at 20,000 g for 10 min at 4 °C. The analytical column was kept at 15 °C by use of a column oven, whereas the samples were kept at 4 °C. The mobile phase was composed of solvent A (2.5 % acetic acid) and solvent B (acetonitrile). Elution was as follows: 0-0.6 min, isocratic 2 % B; 0.6-2.17 min, linear gradient from 2 % to 3 % B; 2.17-3.22 min, linear gradient from 3 % to 10 % B; 3.22-5.00 min, linear gradient from 10 % to 15 % B; 5.00-6.00 min, column washing; and reconditioning for 1.50 min. The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 369 nm. The calibration curves, which were based on peak area, were established using (+)-catechin, (-)-epicatechin and procyanidin B1 after phloroglucinol reaction as (+)-catechin and (-)-epicatechin-phloroglucinol adduct standards. The results were expressed as mg per 100 g of dry matter or fresh weight, according to the sample - plant materials and final products, respectively.

2.11. Determination of total phenolic content (Folin-Ciocalteu's assay), total reducing power (FRAP and CUPRAC assays) and free radical scavenging activity ($DPPH^{\bullet}$, $ABTS^{\bullet+}$ and ORAC assays)

All the assays were measured spectrophotometrically in triplicate.

2.11.1. Total Polyphenolic (TP) content

The total polyphenolic content (TP) was determined with a modified Folin-Ciocalteu's method (*Tuberoso et al., 2013*). Briefly, to 100 µL of methanol (blank), standard or the sample (proper dilution in methanol:water 80:20, v/v) was added 500 µL of Folin-Ciocalteau's phenol reagent. After 5 min, 3 mL of 10 % Na_2CO_3 (w/v) was added, the mixture was shaken, and then diluted with water to a final volume of 10 mL. After a 90 min incubation period at room temperature, 3 mL of each sample was transferred to 10 mm polystyrene cuvettes. The absorbance was measured at 725 nm, using a Varian Cary 50 Scan spectrophotometer, against a blank. A standard curve was plotted using different concentrations of gallic acid standard solutions (10-200 mg/kg) and the results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dry matter or fresh weight for plant material and final product, respectively. All solutions were used on the day they were prepared.

2.11.2. Cupric Reducing Antioxidant Capacity (CUPRAC) assay

The cupric ion reducing antioxidant activity (CUPRAC) assay was based on the redox reaction, producing a chromogen of Cu(I)-neocuproin according to Bektaşŏglu et al. (*2006*) procedure with slightly modifications. Briefly, in 10 mm polystyrene cuvettes were added and mixed in the following order: 1 mL of water, 500 µL of coper (II) chloride, 500 µL of neocuproine, 500 µL of ammonium acetate and 100 µL of methanol (blank), standard or the sample (proper dilution in methanol:water 80:20, v/v). The reaction was measured after a 30 min incubation period at room temperature, by spectrophotometric measurements of absorbance at 450 nm, using a Varian Cary 50 Scan spectrophotometer, against a blank. A standard curve was plotted using different concentrations of ferrous sulphate (0.1 - 2.0 mg/kg) and results were expressed as millimoles of Fe^{2+} per 100 g of dry

matter or fresh weight for plant material and final product, respectively. All solutions were used on the day they were prepared.

2.11.3. Ferric-Reducing Ability of Plasma (FRAP) assay

The FRAP assay was based on the reducing power of a compound (antioxidant) and assessed preparing a ferric complex of 2,4,6-tris(pyridin-2-yl)-1,3,5-triazine (TPTZ) and Fe^{3+}. A mechanism of antioxidant potential was to reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) and latter form a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. According to the procedure of Benzie and Strain (*1996*) with some modification (*Tuberoso et al., 2013*), the FRAP reagent was prepared by mixing acetate buffer (pH 3.6), with 10 mmol TPTZ and 20 mmol $FeCl_3$ at 10:1:1 (v/v/v). After, in 10 mm polystyrene cuvettes were mixed 100 µL of methanol (blank), standard or the sample (proper dilution in methanol:water 80:20, v/v) and 3 mL of the reagent. The absorbance was measured at 593 nm after 10 min incubation, using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan), against blank. A standard curve was plotted using different concentrations of Trolox (0.05-1.00 mmol) and results were expressed as millimoles of Trolox per 100 g of dry matter or fresh weight for plant material and final product, respectively. All solutions were used on the day they were prepared.

2.11.4. Oxygen Radical Absorption Capacity (ORAC) assay

The free radical scavenging activity was based on oxygen radical absorbance capacity (ORAC) and evaluated according Ou et al. (*2002*). Briefly, in 10 mm polystyrene cuvettes were mixed 100 µL of sample (proper dilution in methanol:water 80:20, v/v) and 900 µL of phosphate (Na_2HPO_4 + K_2HPO_4) buffer solution (75 mmol, pH 7.4). Next, 2.25 mL of fluorescein (42 nM) was added in cuvettes. Buffer solution was used as blank and

Trolox solution (25 µM) as calibration solution. Fluorescence readings were taken at 5 s and then every minute thereafter. Finally, 375 µL of freshly prepared AAPH reagent [2,2′-azobis(2-amidinopropane) dihydrochloride] (153 mmol) was added in cuvettes every 10 s. The fluorescence spectrophotometer was set up at an excitation wavelength of 493 nm and an emission wavelength of 515 nm and readings were recorded every 10 min for 40 min after the addition of AAPH. During the analysis all the cuvettes were incubated at 37 °C. The final ORAC values were calculated, using a regression equation between the net area under the fluorescence decay curve and the different Trolox concentration (0.05-0.8 mmol) and data were expressed as millimoles of Trolox per 100 g of dry matter or fresh weight for plant material and final product, respectively. All solutions were used on the day they were prepared.

2.11.5. Free radical scavenging ability assay using a DPPH antiradical anion (DPPH•)

The free radical scavenging ability assay, using DPPH• was based on the ability of the antioxidant to scavenge the radical anion of 1,1-diphenyl-2-picrylhydrazyl (DPPH). Following Tuberoso et al. (*2013*), in 10 mm polystyrene cuvettes were mixed 50 µL of methanol (blank), standard or the sample solution (proper dilution in methanol:water 80:20, v/v) and 2 mL of 0.06 mmol/L DPPH solution in methanol. After an incubation period of 60 min in the dark, spectrophotometric readings were carried out, using a Varian Cary 50 Scan spectrophotometer, against a blank at 517 nm. A standard curve was plotted using different concentrations of Trolox (0.05-0.80 mmol) and data were expressed as millimoles of Trolox per 100 g of dry matter or fresh weight for plant material and final product, respectively. The DPPH solution (0.06 mmol) was prepared from the concentrated solution (0.60 mmol), which was made the previous day. All other solutions were prepared on the day they were used.

2.11.6. Free radical scavenging ability assay using a stable ABTS radical cation ($ABTS^{\bullet+}$)

The free radical scavenging activity was based on the ability of the antioxidant to scavenge the radical cation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS). Following Re et al. (*1999*), ABTS was dissolved in water to a 2 mmol concentration. The ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with (70 mmol) potassium persulfate and kept in the dark at room temperature for 12-16 h before use. The ABTS solution (2 mmol) was diluted with redistilled water, obtaining solution (0.08 mmol) with an absorbance of 0.700 (±0.02) at 734 nm. After, in 10 mm polystyrene cuvettes were mixed 3 mL of diluted ABTS solution (0.08 mmol) and 30 µL of methanol (blank), standard or the sample (proper dilution in methanol:water 80:20, v/v). The absorbance was measured at 734 nm after 6 min incubation, using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan), against blank. A standard curve was plotted using different concentrations of Trolox (0.05 - 0.80 mmol) and data were expressed as millimoles of Trolox per 100 g of dry matter or fresh weight for plant material and final product, respectively. All solutions except the ABTS (2 mmol), were prepared on the day they were used.

2.12. *In-vitro* analysis on Caco-2 cell lines

2.12.1. Maintenance of intestinal cell culture

Caco-2 cells (ECACC, Salisbury, Wiltshire, UK) were cultured in monolayers at 37°C in a humidified atmosphere at 5 % of CO_2 (*Incani et al., 2016*). Caco-2 cells, at passage 45-60, were plated at a density of about 5 × 104/mL and used when fully differentiated (14-21 days post seeding), replacing the medium twice a week.

2.12.2. Cytotoxic activity of the extracts

The MTT assay was assessed on Caco-2 cells (*Schiller et al., 1992*) in order to evaluate any toxic activity of the tested extracts. Cells were seeded in 96-well plates (5×10^4 viable cells/mL in 100 μL), and exposed after differentiation to various concentrations of the extracts (at 0.1-20 μL extract/mL, in serum free medium) or an equivalent volume of MeOH:H_2O 80:20 (vehicle of all the compounds) for the controls, and incubated for 24 h. After incubation, the medium was removed and 100 μL of MTT solution (5 mg/mL of fresh medium) was added and left for 6 h at 37 °C. The medium was then aspirated, 100 μL of dimethyl sulfoxide (DMSO) was added in each well and the absorbance was read at 570 nm by using a micro plate reader (Infinite 200, Tecan, Salzburg, Austria). Viability of cells was expressed as percentage of cell controls viability.

2.12.3. Determination of intracellular ROS production

Intracellular ROS production was evaluated in differentiated Caco-2 cells seeded in 96-well plates as done for the MTT assay. The old medium was removed, cells were incubated for 20 min with 2′,7′ dichlorodihydrofluorescein diacetate (H2-DCF-DA) 10 μM, as reported by Deiana et al. (*2019*). Then, H2-DCF-DA was removed and cells were treated with 1-20 μL extract/mL concentration of the extracts for 30 min prior to incubation with the oxidizing agent TBH 2.5 mmol for 1 h. ROS production was monitored by reading the fluorescence emitted, taking readings at intervals of 5 min for 60 min, using a micro plate reader (Infinite 200, Tecan, Salzburg, Austria). The reading was performed using an excitation of 490 nm and an emission of 520 nm.

2.13. Digestive enzymes inhibition assays

The *α*-amylase and *α*-glucosidase inhibitory effect of the plant extracts and final products was based on the Von Worthington (*1993*), assayed according to the procedure described previously by Nowicka et al. (*2016b*), while the inhibition of pancreatic lipase activity was determined according to Podsędek et al. (*2014*), with slight modifications. The inhibition of these three enzymes activity was determined in triplicate using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan) and the results were expressed as IC_{50}.

2.13.1. *α*-Amylase inhibitory assay

Briefly, a total of 50 µL of each sample extract and 50 µL of 0.02 M sodium phosphate buffer (pH 6.8 with 6 mmol NaCl) containing *α*-amylase solution 4 mg/mL was incubated at 25 °C for 10 min. After preincubation, 50 µL of a 0.5 % starch solution in 0.02 M sodium phosphate buffer (pH 6.8 with 6 mmol NaCl) was added to each cuvette at timed intervals. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 200 µL of dinitrosalicylic acid colour reagent (DNS). The test cuvettes were then incubated in a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was than diluted after adding 3 mL of distilled water, and absorbance was measured at 540 nm. The absorbance of sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sample extracts) were recorded as well. The *α*-amylase inhibitory activity was calculated according to the equation below:

$$\textbf{Inhibition activity (\%)} = \left(1 - \frac{A_B - A_A}{A_D - A_C}\right) \textbf{x } 100$$

where, A_A and A_B were the absorbance of the incubated mixtures consisting of sample extract and starch with or without enzyme, respectively, while A_C and A_D were the absorbance of incubated mixtures consisting of starch and enzyme or only starch, respectively.

2.13.2. *α*-Glucosidase inhibitory assay

The inhibition of *α*-glucosidase activity was determined by measuring the amount of glucose hydrolysed from *p*-nitrophenyl-*α*-D-glucopyranoside. An *α*-glucosidase enzyme solution was prepared by dissolving 0.5 g of intestinal acetone powder from rat in 10 mL of saline (0.9 %, w/v) and sonicated 12 times at regular intervals (30 s sonications and 30 s off) in an ice bath. After centrifugation at 3000 rpm for 30 min at 4 °C, the resulting supernatant was diluted twice with 0.1 M potassium phosphate buffer (pH 6.9) and was used as the enzyme solution. For the *α*-glucosidase assay, in cuvettes with reduction 50 µL of the sample extract was combined with 50 µL of a fresh prepared enzyme solution (*α*-glucosidase 4U/mL) and incubated for 10 min. The enzyme reaction was initiated by adding 20 µL of 5 mmol *p*-nitrophenyl-*α*-D-glucopyranoside solution in the above buffer (pH 6.9). The mixtures were incubated at 37 °C for 20 min. Finally, 1 mL of 0.1 M sodium carbonate solution was added and the absorbance was read at 405 nm. The rate of *α*-glucosidase inhibition activity was calculated as a percentage of the control by the following equation:

$$\textbf{Inhibition activity (\%)} = \left(1 - \frac{A_A - A_B}{A_C - A_D}\right) \textbf{x } 100$$

where, A_A and A_C were the absorbance of sample and control, containing substrate, enzyme and sample extract or buffer, respectively, while absorbance of blank sample (A_B) containing the sample extract and enzyme, and blank control (A_D) consisted of the buffer, substrate and enzyme added stopping of the reaction with 0.1 M Na_2CO_3 solution.

2.13.3. Pancreatic lipase inhibitory assay

Briefly, the 50 mmol stock solution of *p*-nitrophenyl acetate in dimethylsulfoxide (DMSO) was prepared. A final concentration of 20 mmol *p*-nitrophenyl acetate was reached by adding pure water. Lipase from porcine pancreas type II was dissolved in water

at 10 mg/mL, and next centrifuged at 13 000 rpm for 5 min. The reaction mixture was combined with 0.2 mL of sample extract, 0.2 mL of lipase solution, 1.4 mL of 0.1 M buffer (20 mmol Tris-base, 150 mmol NaCl, 1.3 mmol $CaCl_2$) (pH 7.4), and 0.2 mL of 20 mmol *p*-nitrophenyl acetate solution. In the control sample, the extract was replaced with 0.2 mL or water, while a blank was without the *p*-nitrophenyl acetate. The absorbance at 400 nm was read against water immediately after incubation at 37°C for 10 min. The pancreatic lipase inhibitory activity was calculated according to the equation below:

$$\textbf{Inhibitory activity (\%)} = \left(\frac{(\mathbf{A_{control} - A_{blank\ control}}) - (\mathbf{A_{sample} - A_{sample\ blanc}})}{\mathbf{A_{control} - A_{control\ blank}}}\right) \textbf{x 100}$$

where, A_{sample} was absorbance of the sample, containing sample extract, buffer, enzyme and *p*-NA; $A_{sample\ blank}$ containing sample extract, buffer, enzyme and water; $A_{control}$ containing buffer in place of sample extract, buffer, enzyme and *p*-NA; A_{blank} containing buffer in place of sample extracts, buffer, enzyme and water.

2.14. Statistical analysis

All data included in this study were presented as the mean value (n = 3) ± standard deviation. All statistical analyses were performed with Statistica version 7.0 (StatSoft, Krakow, Poland). Significant differences ($p \leq 0.05$) between means were evaluated by one-way ANOVA and Duncan's multiple range test. For biological activities one-way analysis of variance (ANOVA) followed by Tukey's test was performed in order to ascertain possible significant differences between groups using the Graph Pad Prism 5 software (GraphPad software, San Diego, CA, USA).

3. RESULTS AND DISCUSSION

Data obtained in this thesis project are discussed separately for the investigation of the raw plant materials (**CHAPTER 3.1.**) and the final products (**CHAPTER 3.2.**).

Investigation of the raw plant extracts was performed to verify similarities or differences with data in the literature and to evaluate potentially interesting peculiarities of the selected material. Furthermore, some plant materials (like white myrtle berries and feijoa flowers) have not, or have only partially been investigated before. Final products were then obtained using some of the most interesting plant materials selected after their investigation. Three sets of final products were developed by adding selected plant materials: the first based on apple juice, the second based on persimmon fruit and apple juice and the third based on strawberry tree fruits and apple juice.

CHAPTER 3.1. - Evaluation of selected plant materials and their by-products

Investigation of the raw plant material and by-product extracts of *A. unedo* berries, purple and white *M. communis* berries, *A sellowiana* flowers, *C. sativus* flower juice, *M. domestica* (var. Shampion) fruits and *D. kaki* (var. Rojo Brillante) fruits was performed using different analytical approaches useful for characterizing both content of bioactive compounds and technological properties.

3.1.1. Dry matter and colour measurement

Table 1. reports dry matter (g/100 g fw) and the colour parameters results in the fresh and dry plant materials, respectively. Dry matter is an index of substances, such as sugars, fibres and other organic and inorganic compounds that can have a nutritional importance. It can be seen that plants containing seeds or harder parts like *M. communis* berries, *A. unedo* fruits and *A. sellowiana* flowers have higher amounts of dry matter.

Table 1. Dry matter (g /100 g fw) and colour parameters of selected plant materials.

Sample code	Dry matter (g/100 g fw)	Colour parameters		
		L^*	a^*	b^*
Cd	33.25 ± 2.67b	54.62 ± 0.10d	18.71 ± 0.11a	27.25 ± 0.07b
MWd	32.15 ± 1.47c	68.18 ± 0.16b	-3.10 ± 0.06f	20.76 ± 0.12d
MPd	35.78 ± 1.99a	47.41 ± 0.19e	0.46 ± 0.07e	10.19 ± 0.12f
Fd	21.36 ± 1.54d	45.62 ± 0.05f	5.72 ± 0.04c	11.90 ± 0.02e
Sd	15.45 ± 1.16f	NP	NP	NP
Ad	14.08 ± 0.26g	57.08 ± 0.87c	2.24 ± 0.09d	22.56 ± 0.21c
Kd	17.22 ± 1.18e	74.17 ± 0.15a	10.72 ± 0.05b	51.58 ± 0.02a

Data are given as mean ± standard deviation (n=3). Mean values within a column followed by different letters (a-g) are significantly different (homogenous groups) at $p \leq 0.05$; NP= no possible to perform analysis, because of small amount of the sample (saffron samples). Sample codes have their references in **Annex 1a.**.

In our study, contents of dry matter differed significantly ($p \leq 0.05$) between the analysed plant materials (**Table 1.**). The lowest dry matter was determined in apple fruits (14.08 g/100 g fw), saffron flower juice (15.45 g/100 g fw) and persimmon fruits (17.22 g/100 g fw). The highest dry matter was observed in purple and white myrtle berries (35.78 and 32.15 g/100 g fw) and strawberry tree fruits (33.25 g/100 g fw). Moreover, the dry matter of feijoa flowers was 21.36 g/100 g fw. Compared to other scientific data, dry matter of apple fruits was similar to that detected by Oszmiański et al. (*2018*), where the average dry matter concentration in 22 old apple cultivars analysed was 14.62 g/100 g fw and varied from 12.30 to 17.12 g/100 g fw. However, the dry matter of *A. unedo* fruits was lower than that in findings of Özcan and Hacıseferoğulları (*2007*), Orak et al. (*2011*) and Barros et al. (*2010*), ranging from 40.30 to 46.28 %, while dry matter of *M. communis* white and purple berries was higher than that detected by Hacıseferoğulları et al. (*2012*); (26.09 and 24.28 %, respectively) and slightly lower than dry matter content detected by Tuberoso et al. (*2010*); (38.30 g/100 g fw). Comparing investigated plant by-products (*C. sativus* flowers and *A. sellowiana* flowers) with the results of other studies, the dry matter of saffron flower obtained in the present study was slightly lower than findings of Tuberoso et al. (*2016*); (17.6 %), while dry matter of feijoa flowers has not been investigated so far according to our best knowledge. Finally, dry matter of persimmon fruits (compared with other scientific findings) was slightly lower than that detected by Chen et al. (*2016*), where dry matter content in five cultivars of persimmon fruit ranged from 23.66 to 18.90 %.

Colour parameters of analysed dry plant materials and their by-products were evaluated according to the colour space CIE system (*International Commission on Illumination, 2008*) by determination of colour lightness (L^*), redness (a^*), and yellowness (b^*). These parameters are very important feature for determining product quality as they contribute to the first impression of the consumer of a food product (*Nowicka et al.,*

2016a). Obtained results (**Table 1.**) showed that the colour of the analysed plant materials and their by-products differed significantly ($p \leq 0.05$).

The value of the parameter L^* in analysed plant materials and their by-products ranged from 45.62 (Fd) to 74.17 (Kd). In general, the brightest samples were persimmon fruits, followed by white myrtle berries ($L^* = 68.18$), while the darkest were feijoa flowers, followed by purple myrtle berries ($L^* = 47.41$). The value of parameter a^*, which is responsible for the red colour of samples, ranged from -3.10 (MWd) to 18.71 (Cd). Moreover, the value of parameter b^*, which is responsible for the yellow colour of samples, ranged from 10.19 (MPd) to 51.58 (Kd).

Regarding colour parameters of myrtle berries, several data can be found in the literature, but they are referred to liquid extracts, like macerate and liqueur (*Serreli et al., 2017; Tuberoso et al., 2007*) and to our best knowledge, no other data regarding colour parameters (L^*, a^* and b^*) of white myrtle berries has been published. Also colour measurement of feijoa flowers was investigated for the first time in the present study.

Strawberry tree and persimmon fruits have been evaluated by some authors. For example, Orak et al. (*2011*) focused on L^*, a^* and b^* parameters evaluation in the external and internal parts of *A. unedo* dried fruits. The differences between the lightness (L^*) of both parts were found to be significant ($p < 0.05$), and ranged from 32.19 (external part) to 53.75 (internal part). Moreover, a^* and b^* parameters were significantly different between both parts of the fruit. The value of a^* parameter was higher for the external part of the strawberry tree fruit (31.99), than for the internal part (11.92), while b^* was lower (23.57 and 47.52), respectively. Comparing these scientific data with the results of our study, the L^* value was slightly higher (54.62), while a^* and b^* values were mean values (18.71 and 27.25, respectively) of two *A. unedo* samples investigated by Orak et al. (*2011*). Other authors (*Celik and Ercisli, 2009*) were evaluating colour parameters in the skin and flesh of *D. kaki* Thunb. They detected significant differences between both studied samples. The

parameters values ranged from 63.39, 32.29 and 62.04 to 64.59, 10.42 and 55.87 for L^*, a^* and b^*, respectively. Our sample was brighter (74.17), and less yellow (51.58), than both investigated samples (*Celik and Ercisli, 2009*). Moreover, our sample was less reddish (10.72), than the skin sample and more reddish than the flesh sample.

Apples are a very common matrix investigated by researchers. The colour parameters of different varieties of *M. domestica* fruits have been investigated by many authors. For example, Dobrzański and Rybczyński (*2002*) determined colour parameters of two apple cultivars (Champion and Jonagold) skin in display conditions. According to their findings, brightness, redness and yellowness parameters ranged from 40 to 72, -10 to 53 and -5 to 43, respectively. The L^* and a^* values of our sample were similar to those detected by Dobrzański and Rybczyński (*2002*), while b^* values were slightly higher.

Finally, in the present study the colour of dry saffron flower juice was not possible to detect, because of small amounts of the sample. Although, Tuberoso et al. (*2016*) detected colour parameters in saffron floral by-products juices extracts after 28 and 48 hours of petal storage at room temperature. The values ranged for L^* from 72.9 to 73.7, a^* from -5.2 to 6.1, and b^* from 22.5 to 22.8, respectively. For a future study, it could be interesting to compare the scientific data with the colour parameters of dry saffron flower juice.

3.1.2. Chemical investigation

Several classes of chemical compounds were evaluated for each plant material in this study. Significant differences ($p \leq 0.05$) in sugars and organic acids were found among the analysed plant materials, which can be useful for their sensory characteristics, while phenolic compounds are important bioactive compounds.

3.1.2.1. Sugar content

The analysed plant materials were determined for sugar content. Sugars are carbohydrates, being one of three basic macronutrients, which are necessary to sustain life. The total content and individual profiles of these compounds seems to be decisive in shaping the taste and degree of sweetness of raw plant materials (*Nowicka et al., 2019*). In this study, a standard mixture of selected sugars (different concentration) was prepared and the HPLC-ELSD chromatogram is presented in **Figure 15.**. In this way, the exact profile of sugars in different plant materials was analysed, and the results of these analyses were presented in **Table 2.**. Thus, this detailed analysis permits, at the first stage of this study, to identify their sugar profile, which is important in terms of sensory properties for final product preparation.

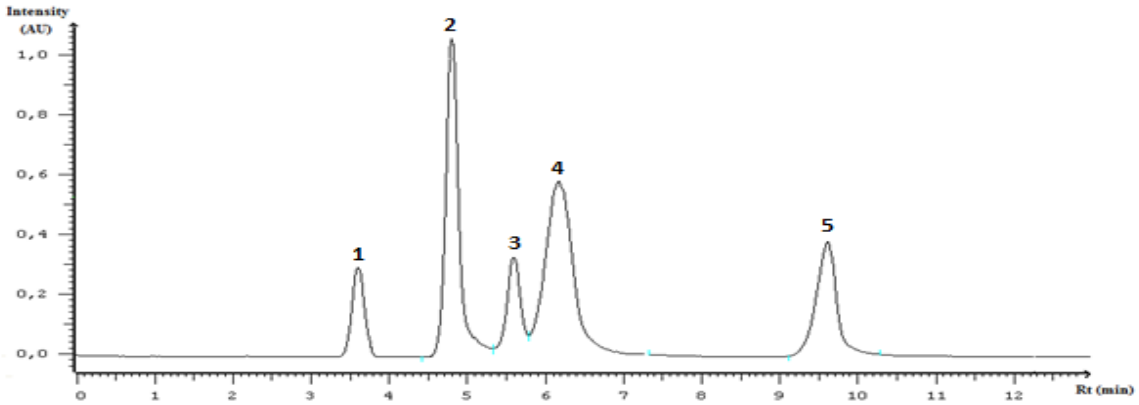


Figure 15. The HPLC-ELSD chromatogram of the standard mixture of sugars.
1- rhamnose; 2- fructose; 3- sorbitol; 4- glucose; 5- sucrose.

The highest total sugar content was detected in apple fruits (60.62 g/100 g dm). This result was comparable to those obtained in different apple cultivars by Oszmiański et al. (*2018*), where the total sugar content of all old apple cultivars grown in Poland ranged from 7.41 to 11.99 g/100 g of fresh matter. Additionally, in purple myrtle berries a high total sugar content (49.20 g/100 g dm) was detected (*Mulas et al., 2013*), while the lowest was observed in white myrtle berries (23.84 g/100 g dm).

Table 2. Sugar content (g /100 g dm) of selected plant materials.

Sample code	Sugar content (g/100 g dm)					
	Rhamnose	**Fructose**	**Sorbitol**	**Glucose**	**Sucrose**	**Total**
Cd	nd	18.28 ± 0.08d	nd	10.31 ± 0.02e	0.74 ± 0.03b	29.33 ± 0.02f
MWd	nd	11.80 ± 0.04g	nd	12.04 ± 0.08e	nd	23.84 ± 0.04g
MPd	nd	22.29 ± 0.03c	nd	26.91 ± 0.05a	nd	49.20 ± 0.08b
Fd	nd	15.58 ± 0.07e	nd	18.90 ± 0.13b	nd	34.48 ± 0.05d
Sd	4.18 ± 0.25a	13.65 ± 0.02f	nd	13.22 ± 0.09d	nd	31.05 ± 0.06e
Ad	nd	49.84 ± 0.03a	0.30 ± 0.01a	2.72 ± 0.00f	7.76 ± 0.02a	60.62 ± 0.03a
Kd	nd	28.05 ± 0.01b	nd	15.63 ± 0.13c	nd	43.68 ± 0.01c

Data are given as mean ± standard deviation (n=3). Mean values within a column followed by different letters (a-g) are significantly different (homogenous groups) at $p \leq 0.05$; nd = not detected (< LOD). Sample codes have their references in **Annex 1a.**.

Concerning sugar composition, fructose (44-82 % of total sugar) and glucose (4-55 % of total sugar) were the main sugars occurring in all investigated plant materials. The highest concentrations of fructose were found in apple fruits (49.84 g/100 g dm, 82 % of total sugar) (*Oszmiański et al., 2018*), and persimmon fruits (28.05 g/100 g dm, 64 % of total sugar), comparable to results obtained by Senica et al. (*2016*) (288.8 mg/g dw), while the lowest was found in white myrtle berries (11.80 g/100 g dm) and saffron flower juice (13.65 g/100 g dm) (*Righi et al., 2015*). Furthermore, the highest concentration of glucose was detected in purple myrtle berries (26.91 g/100 g dm) and the lowest in apple fruits (2.72 g/100 g dm, 5 % of total sugars), comparable to results obtained by Aprea et al. (*2017*).

Sucrose was only detected in low amounts in strawberry tree fruits (0.74 g/100 g dm, 3 % of total sugar) (*Miguel et al., 2014*) and apple fruits (7.76 g/100 g dm, 13 % of total sugar) (*Oszmiański et al., 2018*). In addition, a peculiarity of saffron flower juice and apple fruits was noticed: in these plant materials 4.18 g/100 g dm (13 % of total sugar) of rhamnose was detected, the presence of which (in tepals) was also reported by Moraga et al. (*2013*) and 0.30 g/100 g dm (0.5 % of total sugar) of sorbitol (*Aprea et al., 2017*),

respectively. The presence of sorbitol can have a positive influence in final product preparation, because it is non-cariogenic. Therfore it helps with protection against tooth decay. Moreover, it slows the rise of blood glucose and the insulin response connected to the ingestion of glucose. Hence, it can be used as a sugar alternative for people with diabetes (*Diabetes.co.uk, 2019*).

Dietary sugars (mono- and disaccharides) naturally occur in fruits and vegetables, and are absorbed into the bloodstream mainly as glucose and fructose. Fructose is changed into glucose, lactate and fatty acids in splanchnic organs. The principal nutritional function of carbohydrates is to provide convenient energy to all cells in the human body. The capacity for profitable energy transfer is very high for glucose and lower for fructose. Fruit and vegetable consumption significantly protects against cardiovascular and metabolic diseases (*Tappy, 2018*).

To sum up, the presence of fructose and glucose in analysed plant materials will guarantee the sweetness of beverages. These results demonstrate that the final product (functional food) preparation will not require any additional sweeteners. Moreover, fructose was generally to be more abundant than glucose in investigated plant materials. Hence, fructose, which is one of the most important dietary monosaccharides, is known to be the sweetest of all naturally occurring carbohydrates (*Hanover and White, 1993*). Moreover, taking into account that fructose has a lower glycemic index and higher sweetness index than glucose (*Nutrients Review, 2019*), this has positive aspects. It does not lead to a rapid rise of blood glucose levels. Obviously, to avoid any problems for human health due to the consumption of simple sugars, the total amount introduced with the food product and the possible interference with other factors that can reduce their metabolism (e.g. presence of fibres, organic acids, inhibition of digestive enzymes, etc.) should be evaluated.

3.1.2.2. Organic acid content

The analysed plant materials were tested for organic acid content. Organic acids are organic compounds with acidic properties being used in food preservation, because of their effects on bacteria (*Hirshfield et al., 2003*). In this study, a standard mixture of selected organic acids (different concentrations) was prepared and the UPLC-PDA chromatogram is presented in **Figure 16.**. In this way, the exact profile of organic acids in different plant materials was analysed, and the results of these analyses were presented in **Table 3.**. This detailed analysis permits, at the first stage of this study, the identification of their organic acid profile, being important in the terms of sensory properties and stability of final products. The content of organic acids may be also interesting given that certain acids encourage a decrease of the postprandial blood glucose and insulin responses (*Nour et al., 2010a*).

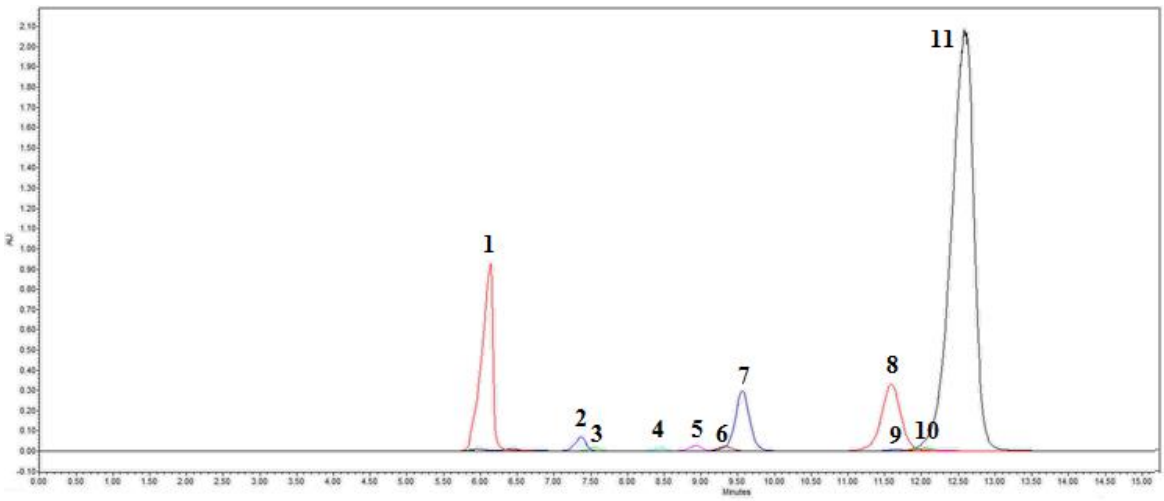


Figure 16. The UPLC chromatogram of a standard mixture of organic acids.

1- oxalic acid; 2- citric acid; 3- isocitric acid; 4- tartaric acid; 5- malic acid; 6- quinic acid; 7- ascorbic acid; 8- shikimic acid; 9- succinic acid; 10- lactic acid; 11- fumaric acid.

Among eleven organic acids identified in analysed plant materials, the major ones were: malic (6-46 % of total organic acid), quinic (3-52 % of total organic acid) and citric acid (0-72 % of total organic acid). Moreover, oxalic and fumaric acid were detected in all

investigated plant materials, while isocitric, tartaric, ascorbic, lactic, shikimic and succinic in some of them.

The highest total organic acid content was detected in feijoa flowers (42.31 g/100 g dm). According to our best knowledge to date, there is no other report on the organic acid composition of feijoa flowers in the literature, while regarding feijoa fruits some organic acids (malic, citric, ascorbic and oxalic acid) were found by Romero Rodriguez et al. (*1992*) and Castellanos et al. (*2016*). Additionally in apple fruits and strawberry tree fruits high total organic acid content (28.99 and 23.21 g/100 g dm, respectively) was also detected, while the lowest one was observed in saffron flower juice (13.21 g/100 g dm), as well as in purple and white myrtle berries (14.11 and 15.34 g/100 g dm, respectively).

Malic acid is the predominant organic acid in apple fruits, the role of which is to maintain healthy liver condition and help in the digestion process (*Nour et al., 2010a*). The highest concentrations of malic acid were found in purple and white myrtle berries (3.85 and 4.45 g/100 g dm, 27 and 29 % of total organic acid, respectively), the presence of which was confirmed also by Mulas et al. (*2013*). In addition large amounts of malic acid were found in saffron flower juice (3.06 g/100 g dm, 23 % of total organic acid, respectively), while the lowest was found in apple and persimmon fruits (0.77 and 0.65 g/100 g dm, 3 and 4 % of total acid content, respectively).

Quinic acid is a principal biochemical intermediate in the shikimate pathway (of aromatic compounds occuring in plants, bacteria, funghi and algae) but not biosynthesized in animals and humans (*Pero and Lund, 2009*). The highest concentration of quinic acid was detected in strawberry tree fruits (12.10 g/100 g dm, 52 % of total organic acid) (*Oliviera et al., 2011*), while the lowest in feijoa flowers (1.29 g/100 g dm, 3 % of total organic acid). In addition high quinic acid content was observed in purple and white myrtle berries (4.76 and 5.80 g/100 g dm, 38 and 34 % of total organic acid, respectively); (*Mulas*

et al., 2013), as well as in saffron flower juice (5.32 g/100 g dm, 40 % of total organic acid).

Citric acid, aside from being a natural preservative, gives foods and beverages a sour taste. Moreover, it is metabolite and an intermediary in the oxidative metabolism of cells (*Abdel-Salam et al., 2014*). Citric acid was detected in all investigated plant materials, except in strawberry tree fruits; in which large amounts of isocitric acid were observed (8.72 g/100 g dm, 38 % of total organic acid). The highest content of citric acid was identified in feijoa flowers (30.50 g/100 g dm, 72 % of total organic acid). In other plant materials the amount of this acid was found in much lower quantities, and its presence was confirmed by Alipour et al. (*2014*), Serrano-Diaz et al. (*2013*), Nour et al. (*2010a*) and Chen et al. (*2016*). The lowest amount of citric acid among investigated plant materials was found in apple fruits (1.22 g/100 g dm, 10 % of total organic acid), while its isomer was found also in small amount in apple fruits (0.06 g/100 g dm, 1 % of total organic acid).

Oxalic acid was the next detected compound. This acid is a final product in the metabolism of some amino acids in mammals, which dietary intake is c.a. 50 mg, depending on the type of food (*EMEA, 2003*). The highest amounts of this acid were observed in feijoa flowers (7.31 g/100 g dm, 17 % of total organic acid), while in other plant materials its amount was detected in range from 0.03 (Ad) to 1.44 (MWd) g/100 g dm. The presence of oxalic acid in *C. sativus* flower juice, *D. kaki* fruits and *A. unedo* berries was confirmed in the findings of Serrano-Diaz et al. (*2013*), Lee et al. (*2012*) and Miguel et al. (*2014*).

According to the findings of Iqbal et al. (*2004*) ascorbic acid is an antioxidant that protects the human organism from oxidative stress damage. This compound is crucial in wound healing, bone formation and the preservation of healthy gums. Moreover, this acid has an important role in various metabolic functions (e.g. the activation of vitamins from group B and folic acid, alteration of cholesterol to bile acids, and alteration of amino acids

to serotonin - neurotransmitter). Ascorbic acid was identified only in strawberry tree and apple fruits (0.04 and 0.02 g/100 g dm; 0.17 and 0.19 % of total organic acid, respectively). The presence of this acid was confirmed by Miguel et al. (*2014*) and Aprea et al. (*2017*).

Furthermore, tartaric acid was detected in persimmon fruits (0.60 g/100 g dm, 3 % of total organic acid) and in very small amounts in apple fruits (0.41 g/100 g dm). Lee et al. (*2012*) detected this acid in lower amount (1396.91-2448.49 mg/kg) than in the persimmon fruits of our study, while Khosravi et al. (*2015*) detected tartaric acid in red and white apple juice. This acid is an antioxidant and can be used as an addative to foods to give a sour taste. Moreover, its salt or ester (tartrate) is considered an inhibitor of kidney stone creation (*TMIC, 2019*).

Lactic acid, characterised by a mild taste, is responsible for flavour enhancement, reduction of pH and microbal inhibition (*Dziezak, 2003*). Moreover, it is a major metabolic intermediary in living organisms (*Ramis-Ramos, 2003*). On the other hand, succinic acid has an important role in mitochondrial function (respiratory chain and Krebs cycle). Both these organic acids were detected in saffron flower juice (1.97 and 0.67 g/100 g dm, 15 and 5 % of total organic acid, respectively), the presence of which was also confirmed by Serrano-Diaz et al. (*2013*). In addition, in persimmon fruits small amounts (0.02 g/100 g dm; 1 % of total organic acid) of succinic acid were found, also detected by Novillo et al. (*2015*).

To sum up, the organic acids are important in terms of nutrition, health and well-being in humans, because of their wide range of beneficial functions.

Table 3. Organic acid content (g /100 g dm) in selected plant materials.

Sample code	Organic acid content (g/100 g dm)											
	Oxalic	**Citric**	**Isocitric**	**Tartaric**	**Malic**	**Quinic**	**Ascorbic**	**Shikimic**	**Succininc**	**Lactic**	**Fumaric**	**Total**
Cd	0.18±0.01e	nd	8.72±0.12a	nd	1.97±0.10e	12.10±0.02a	0.04±0.00a	0.12±0.01d	nd	nd	0.06±0.00b	23.21±0.18c
MWd	1.44±0.02b	3.30±0.03c	nd	nd	4.45±0.04a	5.80±0.04b	nd	0.32±0.02b	nd	nd	0.02±0.00d	15.34±0.25e
MPd	0.75±0.03c	4.61±0.12b	nd	nd	3.85±0.03b	4.76±0.04d	nd	0.15±0.02c	nd	nd	nd	14.11±0.13f
Fd	7.31±0.12a	30.50±0.18a	nd	nd	2.61±0.13d	1.29±0.01f	nd	0.45±0.01a	nd	nd	0.15±0.00a	42.31±0.12a
Sd	0.33±0.00d	1.85±0.03d	nd	nd	3.06±0.12c	5.32±0.04c	nd	nd	0.67±0.13a	1.95±0.14a	0.04±0.00c	13.21±0.04g
Ad	0.03±0.00f	1.22±0.02f	0.06±0.03b	0.04±0.00b	5.47±0.05a	4.85±0.01e	0.02±0.00b	0.17±0.00c	nd	nd	0.14±0.00a	12.00±0.30g
Kd	0.09±0.00f	3.90±0.04c	nd	0.61±0.05a	3.84±0.09c	9.05±0.09b	nd	nd	0.13±0.00b	nd	0.02±0.00d	17.63±0.18c

Data are given as mean ± standard deviation (n=3). Mean values within a column followed by different letters (a-g) are significantly different (homogenous gropus) at $p \leq 0.05$; nd = not detected (< LOD). Sample codes have their references in **Annex 1a.**.

3.1.2.3. Qualitative identification of phenolic compounds

The identification of polyphenolic compounds in extracts from the seven analysed plant materials was conducted by comparing the retention time, α_{max}, UV-vis spectra, as well as mass spectra (MS/MS experiments) in negative and positive ion mode, to those of available standards. Moreover, comparison with available data was used to tentatively identify the phenolic compounds without available commercial standards. Additionally, analysis was performed in purified fractions of selected analysed plant materials (*A. unedo* fruits, *M. communis* white and purple berries and *A. sellowiana* flowers). Obtained results were presented in **Table 4.** and **Figure 17.**.

The use of the LC-PDA-QTof/MS has enabled the confirmation of the presence of 102 different phenolic compounds, belonging to seven subclasses (anthocyanins, hydroxybenzoic and hydroxycinnamic acids, dihydrohalcones, flavan-3-ols, flavonols and flavones). Furthermore, analysis of polymeric procyanidins by UPLC-FL method was performed, and the results confirmed the presence of these compounds in all investigated plant materials. Regarding each analysed plant material, LC-MS metabolic profiles highlighted the presence of a large group of polyphenols, specifically: 36 in *A. unedo* fruits, 18 in *M. communis* white berries, 25 in *M. communis purple berries,* 26 in *A. sellowiana* flowers, 19 in *C. sativus* flower juice, 17 in *M. domestica* juice, and 18 in *D. kaki*. To our knowledge, there have been just a few studies on the chemical composition of feijoa *A. sellowiana* (O. Berg) Burret flowers (*Aoyama et al., 2018*). Consequently, one of the purposes of this study was to identify a broad range of phenolic compounds and their contents in *A. sellowiana* flowers. For this purpose, evaluation of bioactive compounds in feijoa flower extracts was performed (*Montoro et al., 2020*). This is one of the first studies of the phenolic compound composition of this part of the plant.

The positive LC-MS metabolic profiles of the seven selected plant materials highlighted the presence of 15 compounds in total (anthocyanins A1-A15). Among them, four were identified only in strawberry tree fruits. They were delphinidin-3-*O*-galactoside (A2; $[M+H]^+$ at *m/z* = 465.1909; MS/MS fragment at *m/z* = 303.1100, and R_t = 3.664)**,** cyanidin-3-*O*-galactoside (A6; $[M+H]^+$ at *m/z* = 449.1959; MS/MS fragment at *m/z* = 287.1116, and R_t = 4.135), cyanidin-3-*O*-arabinoside (A8; $[M+H]^+$ at *m/z* = 419.1797; MS/MS fragment at *m/z* = 287.1116, and R_t = 4.629), delphinidin (A12; $[M+H]^+$ at *m/z* = 303.1743, and R_t = 5.196), also confirmed by Pallauf et al. (*2008*). The other four, detected only in purple myrtle berries, were delphinidin-pentoside (A5; $[M+H]^+$ at *m/z* = 435.1818; MS/MS fragment at *m/z* = 303.1100, and R_t = 4.133), delphinidin-3-*O*-arabinoside (A10; $[M+H]^+$ at *m/z* = 435.1818; MS/MS fragment at *m/z* = 303.1100, and R_t = 4.906), peonidin-3-*O*-glucoside (A11; $[M+H]^+$ at *m/z* = 463.2164; MS/MS fragment at *m/z* = 301.1330, and R_t = 5.156), petunidin-3-*O*-arabinoside (A14; $[M+H]^+$ at *m/z* = 449.1959; MS/MS fragment at *m/z* = 317.1295, and R_t = 5.537) and malvidin-3-*O*-arabinoside (A15; $[M+H]^+$ at *m/z* = 463.2120; MS/MS fragment at *m/z* = 331.1458, and R_t = 6.300), also confirmed by Montoro et al. (*2006*) and Scorrano et al. (*2017*). Moreover, delphinidin-3,5-*O*-diglucoside (A1; $[M+H]^+$ at *m/z* = 627.2785; MS/MS fragments at *m/z* = 465.1909/303.1100, and R_t = 3.040), petunidin-3,5-*O*-diglucoside (A3; $[M+H]^+$ at *m/z* = 641.3018; MS/MS fragments at *m/z* = 479.1909/317.1295, and R_t = 3.716) were detected only in saffron flower juice (*Tuberoso et al., 2016; Goupy et al., 2013*). In addition, delphinidin-3-*O*-glucoside (A4; $[M+H]^+$ at *m/z* = 465.1953; MS/MS fragment at *m/z* = 303.1100, and R_t = 3.856) was detected in purple myrtle berries, feijoa flowers and saffron flower juice, cyanidin-3-*O*-glucoside (A7; $[M+H]^+$ at *m/z* = 449.1959; MS/MS fragment at *m/z* = 287.1116, and R_t = 4.397) was detected in strawberry tree fruits, purple myrtle berries and feijoa flowers, petunidin-3-*O*-glucoside (A9; $[M+H]^+$ at *m/z* = 479.2150; MS/MS fragment at *m/z* = 317.1295, and R_t = 4.648) was detected in purple myrtle berries

and saffron flower juice, and malvidin-3-*O*-glucoside (A13; $[M+H]^+$ at *m/z* = 493.2339; MS/MS fragment at *m/z* = 331.1495, and R_t = 5.391) was detected in purple and white myrtle berries, as well as in saffron flower juice (*Montoro et al., 2006; Tuberoso et al., 2016; Pallauf et al., 2008; Goupy et al., 2013*). Although, the findings of Butt et al. (*2015*) confirmed the presence of anthocyanins in *D. kaki* fruits, in our and other authors' studies these compounds were not detected. Additionally, some authors (*Kolniak-Ostek et al., 2013; Oszmiański et al., 2018*) confirmed the presence of this group of polyphenols in apple fruits, while in our plant material they were absent. It is worth noting that only in *A. unedo* fruits galactose derivatives of anthocyanins were found. In other analysed plant materials diglucose, glucose or arabinose derivatives of these compounds were present.

The negative LC-MS metabolic profiles highlighted the presence of a total of 36 hydroxybenzoic acids and their derivatives (B1-B36), 5 hydroxycinnamic acids (C1-C5), 2 dihydrochalcones (D1-D2), 6 flavan-3-ols (E1-E6), 36 flavonols and 1 flavone (F1-F37) in investigated plant materials.

Among detected hydroxybenzoic acids and their derivatives were 13 compounds: gallic acid glucoside I and II (B1 and B5; $[M+H]^-$ at *m/z* = 331.1266 and 331.1334; MS/MS fragments at *m/z* = 271.1605/169.1417 and 271.1990/169.1417, and R_t = 1.156 and 1.366, respectively), galloyl glucoside I (B2; [M+H]- at *m/z* = 331.1334; MS/MS fragment at *m/z* = 169.0417, and Rt = 1.274), 3-*O*-galloylquinic acid also known as theogallin (B6; $[M+H]^-$ at *m/z* = 343.0742; MS/MS fragment at *m/z* = 191.1410, and R_t = 1.551), gallic acid 4-O-*β*-D-glucopyranoside (B10; $[M+H]^-$ at *m/z* = 331.1334; MS/MS fragment at *m/z* = 169.0417, and R_t = 2.080), galloyl shikimic acid (B13; $[M+H]^-$ at *m/z* = 325.0878; MS/MS fragments at *m/z* = 169.0417/125.4180, and R_t = 2.314), digalloyl quinic acid I and II (B16 and B20; $[M+H]^-$ at *m/z* = 495.1837 and 495.0435; MS/MS fragments at *m/z* = 343.1255/191.3072 and 343.1158/191.3100, and R_t = 2.755 and 3.210, respectively), digalloyl shikimic acid I and II (B24 and B27; $[M+H]^-$ at *m/z* = 477.0493;

MS/MS fragments at *m/z* = 325.0808/169.0417, and R_t = 4.129 and 4.618, respectively), strictinin ellagitannin (B28; $[M+H]^-$ at *m/z* = 633.0900; MS/MS fragments at *m/z* = 463.1637/301.0667/275.1263, and R_t = 4.700), gallotannin derivative (B29; $[M+H]^-$ at *m/z* = 1109.1115; MS/MS fragments at *m/z* = 972.1873/635.1085/301.1021, and R_t = 4.869) and gallotannin (B34; $[M+H]^-$ at *m/z* = 939.1841; MS/MS fragments at *m/z* = 769.0749/301.1447, and R_t = 6.956), which were present only in strawberry tree fruits. These findings were confirmed also by Fortalezas et al. (*2010*) and Mendes et al. (*2011*). Moreover, castalagin (B11; $[M+H]^-$ at *m/z* = 933.1019; MS/MS fragments at *m/z* = 785.1813/481.0917/301.1057, and R_t = 2.084), casuarin (B14; $[M+H]^-$ at *m/z* = 783.1445; MS/MS fragments at *m/z* = 481.0421/301.0667, and R_t = 2.420), ellagitannin II (B17; $[M+H]^-$ at *m/z* = 933.1114; MS/MS fragments at *m/z* = 781.0445/633.0131/301.1057, and R_t = 2.829), ellagitannin IV (B22; $[M+H]^-$ at *m/z* = 783.0759; MS/MS fragments at *m/z* = 481.0917/301.0667, and R_t = 3.331), nilocitin (B23; $[M+H]^-$ at *m/z* = 481.0938; MS/MS fragments at *m/z* = 301.0667/257.1438, and R_t = 3.653), casuarinin (B26; $[M+H]^-$ at *m/z* = 935.0146; MS/MS fragments at *m/z* = 765.1799/545.1230, and R_t = 4.391), ellagic acid (B33; $[M+H]^-$ at *m/z* = 300.0631, and R_t = 6.028) and methyl ellagic acid I and II (B35 and B36; $[M+H]^-$ at *m/z* = 394.0062; MS/MS fragments at *m/z* = 315.1020/301.0631, and R_t = 7.336 and 8.128, respectively) were detected only in feijoa flowers, while ellagic acid arabinoside (B31; $[M+H]^-$ at *m/z* = 433.0224; MS/MS fragment at *m/z* = 301.0631, and R_t = 5.703) and xyloside (B32; $[M+H]^-$ at *m/z* = 433.0735; MS/MS fragment at *m/z* = 301.0631, and R_t = 5.885) were detected in *A. sellowiana* flowers and *A. unedo* fruits (*Montoro et al., 2020; Pallauf et al., 2008*). In addition, according to Pereira et al. (*2017*) seven hydroxybenzoic acid derivatives were detected only in purple and white myrtle berries. Among them were galloyl-HHDP-glucose I and II (B3 and B7; $[M+H]^-$ at *m/z* = 633.1283; MS/MS fragments at *m/z* = 481.0828/301.0667, and R_t = 1.325 and 1.591, respectively), digalloyl-HHDP-glucose I and II (B9 and B12; $[M+H]^-$ at *m/z* = 785.1292;

MS/MS fragments at *m/z* = 633.0180/481.0828/301.0631, and R_t = 1.892 and 2.124, respectively), ellagitannin I (B15; $[M+H]^-$ at *m/z* = 933.1457; MS/MS fragments at *m/z* = 633.0283/481.0783/301.0667, and R_t = 2.435), and III (B19; $[M+H]^-$ at *m/z* = 783.0645; MS/MS fragments at *m/z* = 481.0186/301.1021, and R_t = 3.035), as well as quinic acid 3,5-di-*O*-gallate (B18; $[M+H]^-$ at *m/z* = 495.1843; MS/MS fragments at *m/z* = 343.0666/325.0878/191.3072/169.3098, and R_t = 2.950). Finally, five hydroxybenzoic acid derivatives: galloyl glucoside II and III (B4 and B8; $[M+H]^-$ at *m/z* = 331.0639; MS/MS fragment at *m/z* = 169.0117, and R_t = 1.331 and 1.627), protocatechuic acid (B21; $[M+H]^-$ at *m/z* = 153.1245, and R_t = 3.316), syringic acid (B25; $[M+H]^-$ at *m/z* = 197.0458; MS/MS fragments at *m/z* = 179.1098/135.0354, and R_t = 4.181) and salicylic acid (B30; $[M+H]^-$ at *m/z* = 136.1212, and R_t = 5.037) were detected only in persimmon fruits, and their presence was confirmed by Jiménez-Sánchez et al. (*2015*), Ancillotti et al. (*2018*) and Pu et al. (*2013*). In apple fruits and saffron flower juice no hydroxycinnamic acid or derivatives were detected.

Hydroxycinnamic acids were the next group of polyphenols indentified in the analysed plant materials. Among them, was chlorogenic acid (C2; $[M+H]^-$ at *m/z* = 353.0838; MS/MS fragment at *m/z* = 191.0534, and R_t = 3.723), caffeic acid (C3; $[M+H]^-$ at *m/z* = 311.0807; MS/MS fragment at *m/z* = 179.1098, and R_t = 4.036) and *p*-coumaric acid (C4; $[M+H]^-$ at *m/z* = 163.0349 and R_t = 4.463), present in apple and persimmon fruits (*Schieber et al., 2001; Pu et al., 2013*). The other two, detected only in apple fruits were neochlorogenic acid (C1; $[M+H]^-$ at *m/z* = 353.1287; MS/MS fragments at *m/z* = 191.3100/136.0212, and R_t = 3.489) and *p*-coumaroyloquinic acid (C5; $[M+H]^-$ at *m/z* = 337.0912; MS/MS fragment at *m/z* = 163.3014, and R_t = 4.904), also confirmed by Oszmiański et al. (*2018*). In other plant materials, the presence of hydroxycinnamic acids was not detected.

The LC/MS analysis also allowed identification of 2 dihydrochalcone compounds, phloretin-2'-*O*-xyloglucoside (D1; $[M+H]^-$ at *m/z* = 567.1703; MS/MS fragment at *m/z* = 273.0757, and R_t = 7.433) and phloretin-2'-*O*-glucoside, also known as phloridzin (D2; $[M+H]^-$ at *m/z* = 435.1332; MS/MS fragment at *m/z* = 273.0733, and R_t = 8.186) only in apple fruits (*Oszmiański et al., 2018*).

In the present study, 2 monomers, 3 dimers, 1 trimer and polymeric procyanidins were the major components of flavan-3-ols constituents in analysed plant materials. Among them, two were identified in strawberry tree, apple and persimmon fruits. They were procyanidin B1 (E1; $[M+H]^-$ at *m/z* = 577.1293; MS/MS fragment at *m/z* = 289.0708, and R_t = 3.256), (+)-catechin (E3; $[M+H]^-$ at *m/z* = 289.0673; MS/MS fragment at *m/z* = 245.0780, and R_t = 3.669). In turn, the presence of procyanidin B3 (E2; $[M+H]^-$ at *m/z* = 577.1055; MS/MS fragments at *m/z* = 289.2014/245.2391, and R_t = 3.562) was unique to strawberry tree fruits, while (-)-epicatechin (E4; $[M+H]^-$ at *m/z* = 289.0673; MS/MS fragment at *m/z* = 245.0780, and R_t = 4.143), procyanidin B2 (E5; $[M+H]^-$ at *m/z* = 577.1293; MS/MS fragment at *m/z* = 289.0708, and R_t = 4.631), and procyanidin C1 (E6; $[M+H]^-$ at *m/z* = 866.1908; MS/MS fragments at *m/z* = 577.1188/289.0708, and R_t = 4.956) were detected only in *M. domestia* fruits. The presence of these compounds in *A. unedo, M. domestica* and *D. kaki* was confirmed by Pallauf et al. (*2008*), Kolniak-Ostek et al. (*2013*) and Butt et al. (*2015*), respectively. In other analysed plant materials (feijoa flowers, saffron flower juice and both types of myrtle berries) no flavan-3-ol was detected.

Flavonols were the last group of polyphenols identified in all plant materials tested. Examination of the chromatograms of analysed plant materials, obtained in full-scan mode in the triple quadrupole system revealed the presence of some peaks at *m/z* c.a. 315, 285, 317 and 301 corresponding to flavonol aglycones, such as isorhamnetin, kaempferol, myricetin and quercetin, derivatives, respectively. Moreover, in mass spectrometry, C-glycosyl flavones pass through cross-ring cleavages of sugar residues producing main

signals (ions created by losses of 120, 90 and 60 amu) that allows us to distinguish between *O*-glycosyl flavones (losses of 132 amu for pentose, 146 amu for rhamnose and 162 amu for hexose, moieties, respectively) (*Wojdyło et al., 2016*).

Flavonol-*O*-hexoside, such as quercetin-3-*O*-galactoside (F19; $[M+H]^-$ at *m/z* = 463.0861; MS/MS fragment at *m/z* = 301.0994, and R_t = 6.345) was detected in all analysed plant materials, while quercetin-3-*O*-glucoside (F20; $[M+H]^-$ at *m/z* = 463.1774; MS/MS fragment at *m/z* = 301.1447, and R_t = 6.494) was found in all of them except *C. sativus* flower juice (*Wojdyło et al., 2008; Montoro et al., 2020; Ancillotti et al., 2018; Babou et. al., 2016; Goupy et al., 2013*). Furthermore, the analysis in the QTof trap confirmed the presence of flavonol-*O*-pentosides, such as quercetin-3-*O*-arabinoside and -xyloside (F25 and F27; $[M+H]^-$ at *m/z* = 433.0160; MS/MS fragment at *m/z* = 301.1421, and R_t = 6.765 and 7.063, respectively), which were detected in *A. unedo*, *A. sellowiana* and *M. domestica* (*Pallauf et al., 2008; Montoro et al., 2020; Wojdyło et al., 2008*), while other quercetin pentoside (F26; $[M+H]^-$ at *m/z* = 433.0565; MS/MS fragment at *m/z* = 301.0959, and R_t = 6.957) was detected in fejioa flowers and persimmon fruits. Unique to saffron flower juice were other ten flavonols. The mass spectrometric characterization of compounds, provided evidence of the presence of four kaempferol derivatives: kaempferol-3-*O*-sophoroside-7-*O*-glucoside (F1; $[M+H]^-$ at *m/z* = 771.0181; MS/MS fragments at *m/z* = 609.0240/285.1257, and R_t = 3.546), kaempferol-3,7-*O*-diglucoside (F3; $[M+H]^-$ at *m/z* = 609.0341; MS/MS fragments at *m/z* = 447.0543/285.1292, and R_t = 4.636), kaempferol-3-*O*-sophoroside (F13; $[M+H]^-$ at *m/z* = 609.0240; MS/MS fragment at *m/z* = 285.1226, and R_t = 5.978), and kaempferol-3-*O*-rutinoside (F21; $[M+H]^-$ at *m/z* = 593.0418; MS/MS fragment at *m/z* = 285.1226, and R_t = 6.538). Five others were detected as isorhamnetin derivatives: isorhamnetin-3,7-*O*-digalactoside (F4; $[M+H]^-$ at *m/z* = 639.0883; MS/MS fragments at *m/z* = 477.0327/315.0605, and R_t = 4.747), isorhamnetin-3,7-*O*-diglucoside (F10 ; $[M+H]^-$ at *m/z* = 639.1035; MS/MS fragments at *m/z* =

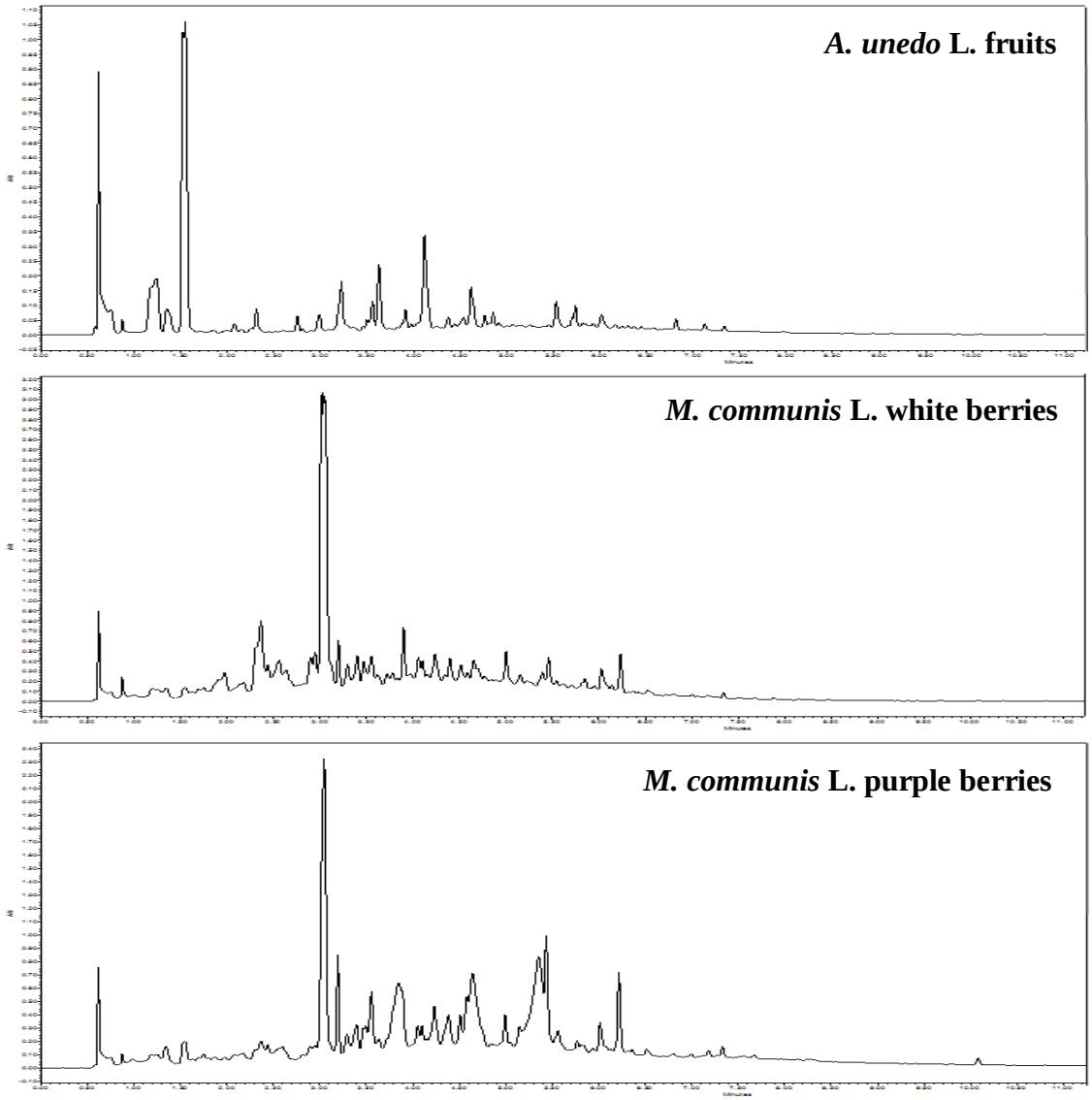
477.1314/315.0948, and R_t = 5.709), isorhamnetin-3-*O*-sophoroside (F16 ; $[M+H]^-$ at *m/z* = 639.0983; MS/MS fragment at *m/z* = 315.0948, and R_t = 6.158), isorhamnetin-3-*O*-rutinoside (F24; $[M+H]^-$ at *m/z* = 623.1223; MS/MS fragment at *m/z* = 315.0871, and R_t = 6.697) and isorhamnetin-3-*O*-glucoside (F31; $[M+H]^-$ at *m/z* = 477.1314; MS/MS fragment at *m/z* = 315.0871, and R_t = 7.539). The presence of these compounds in saffron was also confirmed by Tuberoso et al. (*2016*) and Goupy et al. (*2013*). In addition, according to our knowledge isorhamnetin-3,7-*O*-di-galactoside has not been found previously by other authors on *C. sativus*. Moreover, four other flavonols: kaempferol-3-*O*-galactoside (F22; $[M+H]^-$ at *m/z* = 447.0629; MS/MS fragment at *m/z* = 285.1326, and R_t = 6.640), two kaempferol-hexosides I and II (F32 and F34; $[M+H]^-$ at *m/z* = 447.1543; MS/MS fragment at *m/z* = 285.1326, and R_t = 7.568 and 7.764, respectively), and one flavone (apigenin; F37; $[M+H]^-$ at *m/z* = 269.2073, and R_t = 10.743), were detected only in fejoa flowers. Additionally, despite the findings of other authors (*M. domestica* - Kschonsek et al. (*2018*); *D. kaki* - Pu et al. (*2013*); *M. communis* - Barboni et al. (*2010*); *C. sativus* - Tuberoso et al. (*2016*)), regarding quercetin aglycone (F35; $[M+H]^-$ at *m/z* = 301.1065, and R_t = 8.774), in the present study this compound was found only in *A. sellowiana* flowers. The presence of these compounds was partialy confirmed by Montoro et al. (*2020*).

Particular to myrtle berries (purple and white) was the presence of myricetin (F33; $[M+H]^-$ at *m/z* = 317.1114, and R_t = 7.683), myricetin gallactoside-gallate (F6; $[M+H]^-$ at *m/z* = 631.1107; MS/MS fragments at *m/z* = 479.1073/317.1114, and R_t = 4.995), and myricetin-3-*O*-arabinoside (F14; $[M+H]^-$ at *m/z* = 449.0362; MS/MS fragment at *m/z* = 317.0678, and R_t = 6.027), while only in persimmon fruits quercetin-3-*O*-rutinoside (F12; $[M+H]^-$ at *m/z* = 609.1419; MS/MS fragment at *m/z* = 301.0319, and R_t = 5.957), quercetin derivative III (F23; $[M+H]^-$ at *m/z* = 477.0915; MS/MS fragment at *m/z* = 301.0355, and R_t = 6.665), and kaempferol-3-*O*-rhamnoside (F30; $[M+H]^-$ at *m/z* = 431.0875; MS/MS fragment at *m/z* = 285.0408, and R_t = 7.491) were detected. In turn, only in strawberry tree

fruits quercetin galloylhexose (F11; $[M+H]^-$ at *m/z* = 615.1291; MS/MS fragments at *m/z* = 463.0950/301.1092, and R_t = 5.816), quercetin derivative II (F15; $[M+H]^-$ at *m/z* = 633.1003; MS/MS fragments at *m/z* = 463.0861/301.1021, and R_t = 6.053), and myricetin-3-*O*-xyloside (F17; $[M+H]^-$ at *m/z* = 449.1883; MS/MS fragment at *m/z* = 317.1114, and R_t = 6.161) were found. The presence of these compounds in myrtle berries, persimmon and strawberry tree fruits was confirmed by Pereira et al. (*2017*), Serreli et al. (*2017*), Ancillotti et al. (*2018*), Luca-Gonzalez et al. (*2018*) and Fortalezas et al. (*2010*). Furthermore, myricetin-3-*O*-galactoside (F8; $[M+H]^-$ at *m/z* = 479.0610; MS/MS fragment at *m/z* = 317.1114, and R_t = 5.460), myricetin-3-*O*-glucoside (F9; $[M+H]^-$ at *m/z* = 479.0162; MS/MS fragment at *m/z* = 317.1114, and R_t = 5.534), myricetin-3-*O*-rhamnoside (F18; $[M+H]^-$ at *m/z* = 463.0861; MS/MS fragment at *m/z* = 317.1114, and R_t = 6.239), and quercetin-3-*O*-rhamnoside (F29; $[M+H]^-$ at *m/z* = 447.0148; MS/MS fragment at *m/z* = 301.1447, and R_t = 7.325), were all found in myrtle berries (purple and white) and strawberry tree fruits, while myricetin-3-*O*-glucoside and quercetin-3-*O*-rhamnoside were additionally found in persimmon and apple fruits, respectively. The presence of the evaluated compounds in these plant materials was confirmed in the findings of Pereira et al. (*2017*), Jiménez-Sánchez et al. (*2015*) and Wojdyło et al. (*2008*). In both saffron flower juice and feijoa flowers quercetin-3,7-*O*-diglucoside (F7; $[M+H]^-$ at *m/z* = 625.1044; MS/MS fragments at *m/z* = 463.0333/301.0924, and R_t = 5.356) were detected, while in the first flower by-product and in persimmon fruits kaempferol-3-*O*-glucoside (F28; $[M+H]^-$ at *m/z* = 447.0543; MS/MS fragment at *m/z* = 285.1226, and R_t = 7.320) was also found, and in the second one and strawberry tree fruits quercetin derivative I (F5; $[M+H]^-$ at *m/z* = 633.0900; MS/MS fragments at *m/z* = 463.0633/301.0667, and R_t = 4.766) was found. Regarding *C. sativus* flower juice, the presence of the above compounds was confirmed by Goupy et al. (*2013*), while their presence in *A. sellowiana* flowers was only partially confirmed by the data of Montoro et al. (*2020*), because to our knowledge these are the

first data reporting the presence of these compounds. Finally, kaempferol (F36; $[M+H]^-$ at *m/z* = 285.1706, and R_t = 9.435) was a compound detected in all plant materials instead of apple and persimmon fruits. It is noteworthy that, isorhamnetin derivatives and only one detected flavone (apigenin) were found only in *C. sativus* flower juice, while myricetin derivatives were unique to *M. communis* berries (purple and white) and *A. unedo* fruits.

To sum up, the detailed LC-PDA-QTof/MS analysis allowed us to highlight the different phenolic compounds in the seven analysed plant materials. These differences were useful in finding specific markers of the plants, and some of these phenolic compounds display particular biological activity.



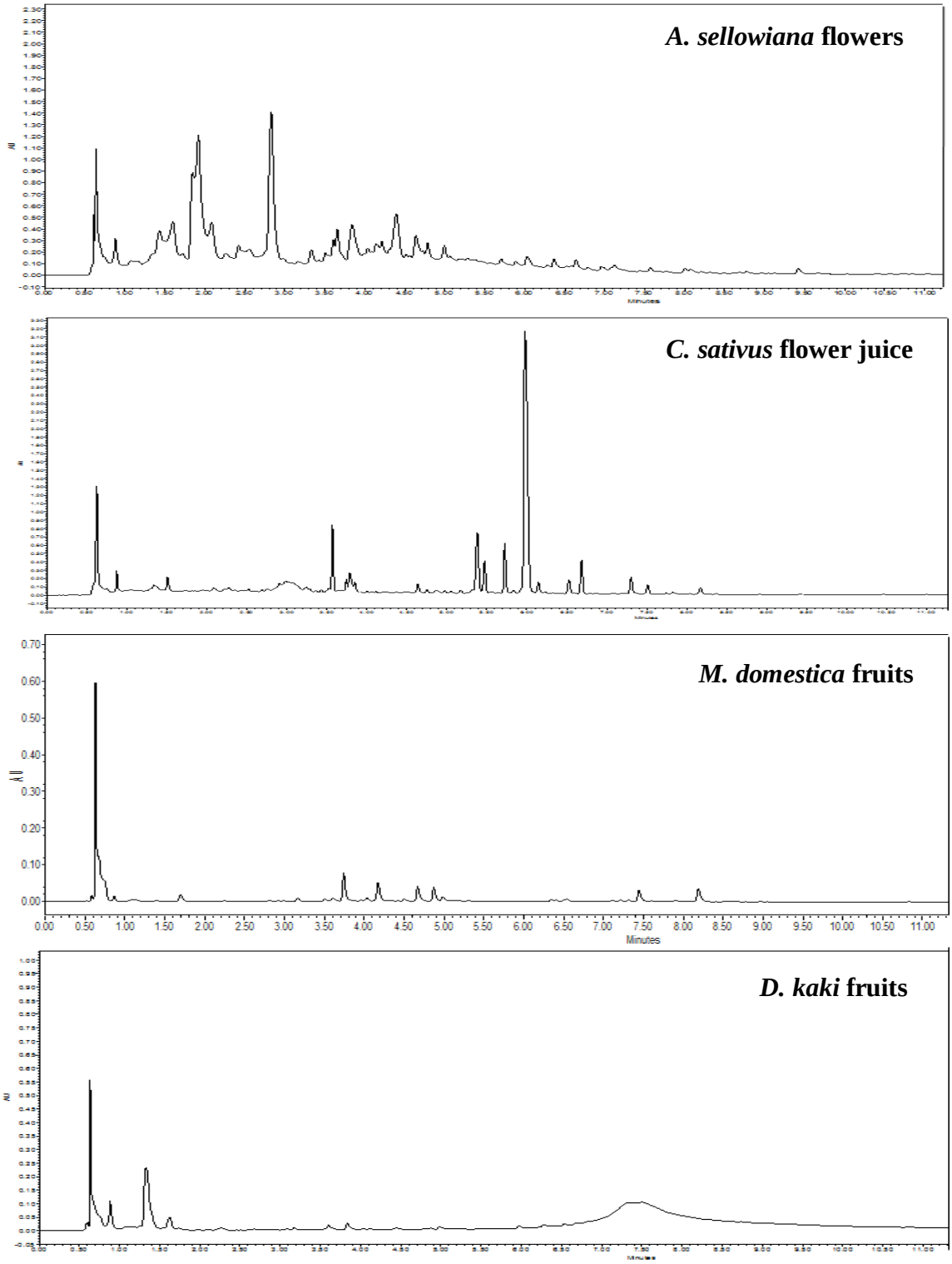


Figure 17. UPLC-PDA fingerprinting of selected plant materials detected at 280 nm.

Table 4. Identification of phenolic compounds by LC-PDA-QTof/MS method in selected plant materials.

Nr	Compound	R_t (min)	λ_{max} (nm)	MS $[M-H]^-$ (*m/z*)	MS/MS $[M-H]^-$ (*m/z*)	Plant material						
						Cd	MWd	MPd	Fd	Sd	Ad	Kd
Anthocyanins												
A1	Delphinidin-3,5-*O*-diglucoside	3.040	518	627.2785$^+$	465.1909/303.1100	-	-	-	-	**X**	-	-
A2	Delphinidin-3-*O*-galactoside	3.664	522	465.1909$^+$	303.1100	**X**	-	-	-	-	-	-
A3	Petunidin-3,5-*O*-diglucoside	3.716	516	641.3018$^+$	479.1909/317.1295	-	-	-	-	**X**	-	-
A4	Delphinidin-3-*O*-glucoside	3.856	515	465.1953$^+$	303.1100	-	-	**X**	**X**	**X**	-	-
A5	Delphinidin-pentoside	4.133	520	435.1818$^+$	303.1100	-	-	**X**	-	-	-	-
A6	Cyanidin-3-*O*-galactoside	4.135	515	449.1959$^+$	287.1116	**X**	-	-	-	-	-	-
A7	Cyanidin-3-*O*-glucoside	4.397	520	449.1959$^+$	287.1116	**X**	-	**X**	**X**	-	-	-
A8	Cyanidin-3-*O*-arabinoside	4.629	515	419.1797$^+$	287.1116	**X**	-	-	-	-	-	-
A9	Petunidin-3-*O*-glucoside	4.648	525	479.2150$^+$	317.1295	-	-	**X**	-	**X**	-	-
A10	Delphinidin-3-*O*-arabinoside	4.906	520	435.1818$^+$	303.1100	-	-	**X**	-	-	-	-
A11	Peonidin-3-*O*-glucoside	5.156	525	463.2164$^+$	301.1330	-	-	**X**	-	-	-	-
A12	Delphinidin	5.196	523	303.1743$^+$		**X**	-	-	-	-	-	-
A13	Malvidin-3-*O*-glucoside	5.391	519	493.2339$^+$	331.1495	-	**X**	**X**	-	**X**	-	-
A14	Petunidin-3-*O*-arabinoside	5.537	525	449.1959$^+$	317.1295	-	-	**X**	-	-	-	-
A15	Malvidin-3-*O*-arabinoside	6.300	525	463.2120$^+$	331.1458	-	-	**X**	-	-	-	-
Hydroxybenzoic acids												
B1	Gallic acid glucoside I	1.156	280	331.1266	271.1605/169.1417	**X**	-	-	-	-	-	-
B2	Galloyl glucoside I	1.274	277	331.1334	169.0417	**X**	-	-	-	-	-	-
B3	Galloyl HHDP-glucose I	1.325	271	633.1283	481.0828/301.0667	-	**X**	**X**	-	-	-	-

B4	Galloyl glucoside II	1.331	270	331.0639	169.0117	-	-	-	-	-	-	**X**
B5	Gallic acid glucoside II	1.366	270	331.1334	271.1990/169.1417	**X**	-	-	-	-	-	-
B6	3-*O*-Galloylquinic acid (Theogallin)	1.551	273	343.0742	191.1410	**X**	-	-	-	-	-	-
B7	Galloyl HHDP-glucose II	1.591	272	633.1283	481.0828/301.0667	-	**X**	**X**	-	-	-	-
B8	Galloyl glucoside III	1.627	273	331.0639	169.0117	-	-	-	-	-	-	**X**
B9	Digalloyl-HHDP-glucose I	1.892	280	785.1292	633.0180/481.0828/301.0631	-	**X**	**X**	-	-	-	-
B10	Gallic acid 4-*O*-*β*-D-glucopyranoside	2.080	320	331.1334	169.0417	**X**	-	-	-	-	-	-
B11	Castalagin	2.084	280	933.1019	785.1813/481.0917/301.1057	-	-	-	**X**	-	-	-
B12	Digalloyl-HHDP-glucose II	2.124	280	785.1292	633.0180/481.0828/301.0631	-	**X**	**X**	-	-	-	-
B13	Galloyl shikimic acid	2.314	272	325.0878	169.0417/125.4180	**X**	-	-	-	-	-	-
B14	Casuarin	2.420	374	783.1445	481.0421/301.0667	-	-	-	**X**	-	-	-
B15	Ellagitannin I	2.435	280	933.1457	633.0283/481.0783/301.0667	-	**X**	**X**	-	-	-	-
B16	Digalloylquinic acid I	2.755	273	495.1837	343.1255/191.3072	**X**	-	-	-	-	-	-
B17	Ellagitannin II	2.829	270	933.1114	781.0445/633.0131/301.1057	-	-	-	**X**	-	-	-
B18	Quinic acid 3,5-di-*O*-gallate	2.950	273	495.1843	343.0666/325.0878/191.3072/169.3098	-	**X**	**X**	-	-	-	-
B19	Ellagitannin III	3.035	275	783.0645	481.0186/301.1021	-	**X**	**X**	-	-	-	-
B20	Digalloylquinic acid II	3.210	276	495.0435	343.1158/191.3100	**X**	-	-	-	-	-	-
B21	Protocatechuic acid	3.316	328	153.1245		-	-	-	-	-	-	**X**
B22	Ellagitannin IV	3.331	280	783.0759	481.0917/301.0667	-	-	-	**X**	-	-	-
B23	Nilocitin	3.653	270	481.0938	301.0667/257.1438	-	-	-	**X**	-	-	-
B24	Digalloyl shikimic acid I	4.129	278	477.0493	325.0808/169.0417	**X**	-	-	-	-	-	-
B25	Syringic acid	4.181	320	197.0458	179.1098/135.0354	-	-	-	-	-	-	**X**

B26	Casuarinin	4.391	278	935.0146	765.1799/545.1230	-	-	-	**X**	-	-	-
B27	Digalloyl shikimic acid II	4.618	275	477.0493	325.0808/169.0417	**X**	-	-	-	-	-	-
B28	Strictinin ellagitannin	4.700	275	633.0900	463.1637/301.0667/275.1263	**X**	-	-	-	-	-	-
B29	Gallotannin derivative	4.869	278	1109.1115	972.1873/635.1085/301.1021	**X**	-	-	-	-	-	-
B30	Salicylic acid	5.037	320	136.1212		-	-	-	-	-	-	**X**
B31	Ellagic acid arabinoside	5.703	359	433.0224	301.0631	**X**	-	-	**X**	-	-	-
B32	Ellagic acid xyloside	5.885	361	433.0735	301.0631	**X**	-	-	**X**	-	-	-
B33	Ellagic acid	6.028	366	300.0631		-	-	-	**X**	-	-	-
B34	Gallotannin	6.956	278	939.1841	769.0749/301.1447	**X**	-	-	-	-	-	-
B35	Methyl ellagic acid I	7.336	360	394.0062	315.1020/301.0631	-	-	-	**X**	-	-	-
B36	Methyl ellagic acid II	8.128	360	394.0062	315.1020/301.0631	-	-	-	**X**	-	-	-
Hydroxycinnamic acids												
C1	Neochlorogenic acid	3.489	317	353.1287	191.3100/136.0212	-	-	-	-	-	**X**	-
C2	Chlorogenic acid	3.723	323	353.0838	191.0534	-	-	-	-	-	**X**	**X**
C3	Caffeic acid	4.036	320	311.0807	179.1098	-	-	-	-	-	**X**	**X**
C4	*p*-Coumaric acid	4.463	323	163.0349		-	-	-	-	-	**X**	**X**
C5	*p*-Coumaroyloquinic acid	4.904	310	337.0912	163.1014	-	-	-	-	-	**X**	-
Dihydrochalcones												
D1	Phloretin-2'-*O*-xyloglucoside	7.433	280	567.1703	273.0757	-	-	-	-	-	**X**	-
D2	Phloretin-2'-*O*-glucoside (Phloridzin)	8.186	280	435.1332	273.0733	-	-	-	-	-	**X**	-
Flavan-3-ols												
E1	Procyanidin B1	3.256	280	577.1293	289.0708	**X**	-	-	-	-	**X**	**X**
E2	Procyanidin B3	3.562	277	577.1055	289.2014/245.2391	**X**	-	-	-	-	-	-

E3	(+)-Catechin	3.669	280	289.0673	245.0780	**X**	-	-	-	-	**X**	**X**
E4	(-)-Epiatechin	4.143	280	289.0673	245.0780	-	-	-	-	-	**X**	-
E5	Procyanidin B2	4.631	280	577.1293	289.0708	-	-	-	-	-	**X**	-
E6	Procyanidin C1	4.956	280	866.1908	577.1188/289.0708	-	-	-	-	-	**X**	-
Flavonols and Flavones												
F1	Kaempherol-3-*O*-sophoroside-7-*O*-glucoside	3.546	346	771.0181	609.0240/285.1257	-	-	-	-	**X**	-	-
F2	Quercetin-3,7-*O*-digalactoside	4.519	349	625.0223	463.0290/301.0951	-	-	-	-	**X**	-	-
F3	Kaempferol-3,7-*O*-diglucoside	4.636	345	609.0341	447.0543/285.1292	-	-	-	-	**X**	-	-
F4	Isorhamnetin-3,7-*O*-digalactoside	4.747	350	639.0883	447.0327/315.0605	-	-	-	-	**X**	-	-
F5	Quercetin derivative I	4.766	359	633.0900	463.0633/301.0667	**X**	-	-	**X**	-	-	-
F6	Myricetin galactoside-gallate	4.995	360	631.1107	479.1073/317.1114	-	**X**	**X**	-	-	-	-
F7	Quercetin-3,7-*O*-diglucoside	5.356	352	625.1044	463.0333/301.0924	-	-	-	**X**	**X**	-	-
F8	Myricetin-3-*O*-galactoside	5.460	356	479.0610	317.1114	**X**	**X**	**X**	-	-	-	-
F9	Myricetin-3-*O*-glucoside	5.534	356	479.0162	317.1114	**X**	**X**	**X**	-	-	-	**X**
F10	Isorhametin-3,7-*O*-diglucoside	5.709	343	639.1035	477.1314/315.0948	-	-	-	-	**X**	-	-
F11	Quercetin galloylhexose	5.816	360	615.1291	463.0950/301.1092	**X**	-	-	-	-	-	-
F12	Quercetin-3-*O*-rutinoside	5.957	359	609.1419	301.0319	-	-	-	-	-	-	**X**
F13	Kaempferol-3-*O*-sophoroside	5.978	358	609.0240	285.1226	-	-	-	-	**X**	-	-
F14	Myricetin -3-*O*-arabinoside	6.027	364	449.0362	317.0678	-	**X**	**X**	-	-	-	-
F15	Quercetin derivative II	6.053	366	633.1003	463.0861/301.1021	**X**	-	-	-	-	-	-
F16	Isorhamnetin-3-*O*-sophoroside	6.158	352	639.0983	315.0948	-	-	-	-	**X**	-	-
F17	Myricetin-3-*O*-xyloside	6.161	350	449.1883	317.1114	**X**	-	-	-	-	-	-
F18	Myricerin-3-*O*-rhamnoside	6.239	347	463.0861	317.1114	**X**	**X**	**X**	-	-	-	-

F19	Quercetin-3-*O*-galactoside	6.345	348	463.0861	301.0994	**X**	**X**	**X**	**X**	**X**	**X**	**X**
F20	Quercetin-3-*O*-glucoside	6.494	350	463.1774	301.1447	**X**	**X**	**X**	**X**	-	**X**	**X**
F21	Kaempferol-3-*O*-rutinoside	6.538	352	593.0418	285.1226	-	-	-	-	**X**	-	-
F22	Kaempherol-3-*O*-galactoside	6.640	347	447.0629	285.1326	-	-	-	**X**	-	-	-
F23	Quercetin derivative III	6.665	356	477.0915	301.0355	-	-	-	-	-	-	**X**
F24	Isorhamnetin-3-*O*-rutinoside	6.697	352	623.1223	315.0871	-	-	-	-	**X**	-	-
F25	Quercetin-3-*O*-arabinoside	6.765	350	433.0160	301.1421	**X**	-	-	**X**	-	**X**	-
F26	Quercetin-pentoside	6.957	360	433.0565	301.0959	-	-	-	**X**	-	-	**X**
F27	Quercetin-3-*O*-xyloside	7.063	350	433.0160	301.1421	**X**	-	-	**X**	-	**X**	-
F28	Kaempferol-3-*O*-glucoside	7.320	360	447.0543	285.1226	-	-	-	-	**X**	-	**X**
F29	Quercetin-3-*O*-rhamnoside	7.325	348	447.0148	301.1447	**X**	**X**	**X**	-	-	**X**	-
F30	Kaempferol-3-*O*-rhamnoside	7.491	360	431.0875	285.0408	-	-	-	-	-	-	**X**
F31	Isorhanetin-3-*O*-glucoside	7.539	352	477.1314	315.0871	-	-	-	-	**X**	-	-
F32	Kaempherol-hexoside I	7.568	350	447.1543	285.1326	-	-	-	**X**	-	-	-
F33	Myricetin	7.683	367	317.1114		-	**X**	**X**	-	-	-	-
F34	Kaempherol-hexoside II	7.764	350	447.1543	285.1326	-	-	-	**X**	-	-	-
F35	Quercetin	8.774	360	301.1065		-	-	-	**X**	-	-	-
F36	Kaempferol	9.435	360	285.1706		**X**	**X**	**X**	**X**	**X**	-	-
F37	Apigenin	10.743	360	269.2073		-	-	-	**X**	-	-	-

X = detected;- = not detected (< LOD); * = tentatively attribution; Sample codes have their references in **Annex 1a.**.

3.1.2.4. Quantification of phenolic compounds

Evaluation of the phenolic content in each plant material was performed according to UPLC-PDA analysis. The quantitative analysis revealed the presence of a wide range of polyphenolic compounds in all investigated matrixes (**Table 5.**). For a more sensitive identification of proanthocyanidins (PAC), the quantitative analysis was performed using the UPLC-FL method described in **Paragraph 2.10.**, based on the comparison of their retention times with those of standards (**Figure 18.**), if available, and published data.

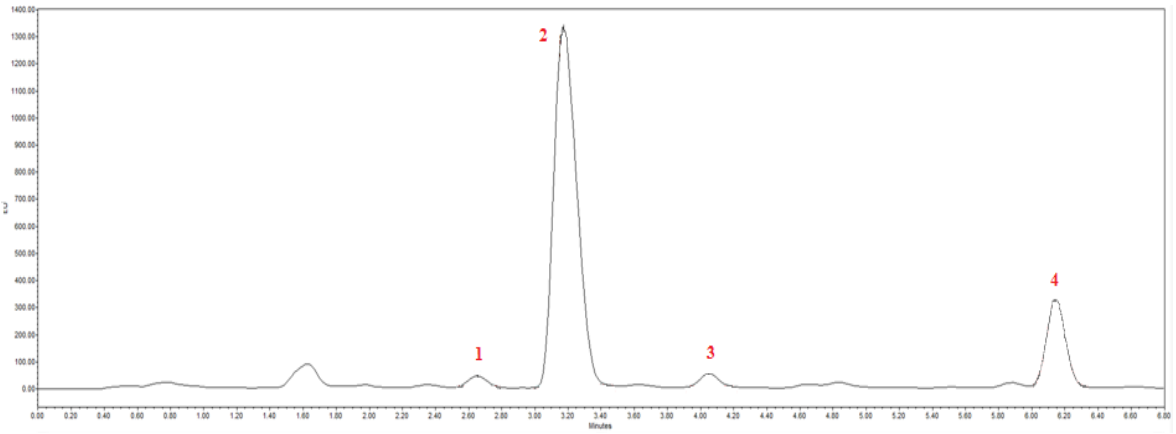


Figure 18. The UPLC-FL chromatogram of the standard mixture of proanthocyanidins.
1- catechin derivative; 2- epicatechin derivative; 3- catechin; 4-epicatechin.

The major polyphenolic groups in *A. unedo* fruits, *M. communis* purple and white berries, *A. sellowiana* flowers, *C. sativus* flower juice, *M. domestica* fruits, and *D. kaki* fruits were different anthocyanins (mainly delphinidin, cyanidin and malvidin derivatives), hydroxybenzoic acids and their derivatives (mainly gallic and ellagic acid derivatives), hydroxycinnamic acids, dihydrochalcones, flavan-3-ols, flavonols (mainly quercetin, kaempferol and myricetin derivatives), and single flavone (apigenin). The total phenolic content determined by UPLC ranged from 816.91 to 3208.33 mg/100 g dm for *M. communis* white berries < *C. sativus* flower juice < *M. domestica* fruits < *M. communis* purple berries < *D. kaki* fruits < *A. unedo* fruits < *A. sellowiana* flowers, respectively. Statistically significant differences ($p \leq 0.05$) were observed between all obtained results.

These obtained values were comparable to those reported by Wojdyło et al. (*2008*), Barboni et al. (*2010*), Serreli et al. (*2017*) and Tuberoso et al. (*2016*), for apples, purple and white myrtle berries and saffron flower juice, while higher than those reported by Guimarães et al. (*2013*) for strawberry tree fruits. In addition, Pu et al. (*2013*) found in fruits of six *D. kaki* genotypes total phenolic content much lower than that obtained in our study, while Ancillotti et al. (*2018*) detected in Rojo Brillante cultivars higher total phenolic content, excluding the values of polymeric procyanidins obtained and calculated in our study using phloroglucinol method. In turn, according to our best knowledge, no other data regarding total phenolic content of feijoa flowers has been published.

Anthocyanins were the subclass of polyphenols detected in all investigated plant materials, except apple and persimmon. In strawberry tree fruits delphinidin and cyanidin derivatives were detected, of which the most abundant was cyanidin-3-*O*-galactoside (57.59 mg/100 g dm), while four others (delphinine, cyanidin-3-*O*-glucoside, delphinidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside) were found in lower amounts (0.53, 1.03, 5.54 and 11.21 mg/100 g dm, respectively). The results obtained were much higher than those of Pawlowska et al. (*2006*). Considering the results of purple myrtle berries, delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and malvidin-3-*O*-glucoside were the three main compounds responsible for the red colour of the fruits (326.47, 292.90 and 771.10 mg/100 g dm, respectively). The values of other seven detected anthocyanins ranged from 0.24 (peonidin-3-*O*-glucoside) to 75.10 mg/100 g dm (cyanidin-3-*O*-glucoside). In turn, in white berries only malvidin-3-*O*-glucoside (2.67 mg/100 g dm) was detected. The above data were comparable with the findings of Tuberoso et al. (*2007*), Scorrano et al. (*2017*) and Messaoud and Boussaid (*2011*). Furthermore, in both floral by-products few different anthocyanins were found. Saffron flower juice was rich in delphinidin-3,5-*O*-diglucoside and delphinidin-3-*O*-glucoside (175.92 and 110.32 mg/100 g dm, respectively), as well as three others, that were present in much lower quantities

(0.37, 2.90 and 14.32 mg/100 g dm) for petunidin-3-*O*-glucoside, petunidin-3,5-*O*-diglucoside, and malvidin-3-*O*-glucoside, respectively. In turn, in feijoa flowers the major anthocyanin was cyanidin-3-*O*-glucosdie (124.66 mg/100 g dm), while delphinidin-3-*O*-glucoside was detected in low quantities (0.29 mg/100 g dm). The data obtained were comparable with the findings of Goupy et al. (*2013*), Tuberoso et al. (*2016*) and Montoro et al. (*2020*).

According to Ghosh and Konishi (*2007*) the anthocyanins have a wide range of biological activities (antioxidant, cardioprotective, anti-inflammatory, antitumor and eye function properties). Moreover, as reported by Zhang et al. (*2004*), anthocyanins present in berries, grapes and cherries, may be important for the avoidance of type-2 diabetes. Regarding the prevention of obesity and diabetes cyanidin-3-glucoside is able to diminish obesity connected insulin resistance through transcription factor, which is a principal mediator of insulin indicating in adipocyte and pancreatic β cells (*Jurgoński et al., 2013*). Although, the protective effect of anthocyanins starts from reduction of lipids, which leads to inhibition of pancreatic lipase. In addition, polyphenols cause changes in the structure of lipids mixture, which leads to the creation of an unsuitable environment for the activty pancreativc lipase (*Fraga et al., 2010*).

Significant differences in phenolic acid content (hydroxybenzoic acid and its derivatives, hydroxycinnamic acids) were found ($p \leq 0.05$). The highest concentration of the first subclass was detected in *A. unedo* fruits and *A. sellowiana* flowers (802.21 and 774.66 mg/100 g dm, respectively), while the second subclass was only present in *M. domestica* and *D. kaki* in total contents of 13.66 and 0.94 mg/100 g dm, respectively.

A wide range of hydroxybenzoic acid and its derivatives were found in all analysed plant materials except apples and saffron flower juice. In strawberry tree fruits the major one was theogallin (467.21 mg/100 g dm), while two digalloyl shikimic acids (I and II) and galloyl glucoside I were detected in lower amounts (105.03, 63.45 and 86.33 mg/100 g dm,

respectively). Additionally, two gallic acid glucosides (I and II), gallic acid 4-*O*-*β*-D-glucopyranoside, galloyl shikimic acid, two digalloylquinic acids (I and II), strictinin ellagitannin, ellagic acid arabinoside and gallotannin and its derivatives were detected in much lower quantities, ranging from 0.29 to 21.51 mg/100 g dm. In contrast feijoa flowers were rich in castalagin, casuarin, ellagitannin II, ellagitannin IV, nilocitin and casuarinin (110.03, 25.26, 357.35, 23.13, 56.74 and 127.63 mg/100 g dm, respectively), as well as in ellagic acid, its pentosides (arabinoside and xyloside) and methyl derivatives (I and II) in quantities ranging from 1.40 to 35.66 mg/100 g dm). According to our best knowledge, no other data regarding the exact quantity of each single hydroxycinnamic acid and its derivatives in *A. unedo* and *A. sellowiana* has been published so far. Moreover, both types of myrtle berries (white and purple) were rich in ellagitannin III (192.50 and 167.50 mg/100 g dm) and ellagitannin I (37.61 and 53.48 mg/100 g dm), respectively. Furthermore, galloyl HHDP-glucose I (41.05 and 61.41 mg/100 g dm), and II (26.23 and 70.13 mg/100 g dm), digalloyl-HHDP-glucose I (6.67 and 9.30 mg/100 g dm), and II (9.29 and 11.35 mg/100 g dm), and quinic acid 3,5-di-*O*-gallate (15.80 and 16.12 mg/100 g dm), were detected as well in the first and the second type of fruits, respectively. These obtained results were partially similar to the findings of Tuberoso et al. (*2010*). Finally, in persimmon fruits some hydroxybenzoic acids and their derivatives were detected. Among them, as the major representative was gallic acid glucoside II (100.66 mg/100 g dm). Furthermore, galloyl glucoside III (12.24 mg/100 g dm), protocatechuic acid (0.27 mg/100 g dm), syringic acid (0.17 mg/100 g dm), and salicylic acid (1.05 mg/100 g dm), were detected.

Dihydrochalcones, principally phloretin, have the power to increase the adhering action of bioactive components to the surface of lipids, changing bipolar potential of the lipid bilayer. Moreover, the presence of these compounds helps the inhibition of active glucose transporters into SGLT1 and SGLT2 cells, as well as various urea transporters.

Additionally, it has strong antioxidant activity (*Gromova, 2006*). Phloretin-2'-*O*-xyloglucoside and phloretin-2'-*O*-glucoside (phloridzin) were present in analysed apple samples in quantities of 31.71 and 35.93 mg/100 g dm, respectively. These values were comparable with the results of Wojdyło et al. (*2008*).

The *in vitro* and *in vivo* studies of Barbosa et al. (*2011*) confirmed that the presence of (-)-epicatechin allows insulin synthesis stimulation and increases the level of cAMP in β cells of pancreas, which increases the secretion of this hormone. Moreover, transformation of proinsulin into insulin is more effective; thus insulin levels in the blood are higher. Flavan-3-ols (monomers, dimers, trimer and polymeric proanthicyanidins) were the major subclass of, *A. sellowiana* flowers, *A. unedo*, *D. kaki*, and *M. domestica* fruits, polyphenols, representing from 68.51 to 95.67 % of the total phenolic content. In turn, the poorest in these compounds was *C. sativus* flower juice, with a quantity not exceeding 0.47 % of the total phenol content. In addition, both types of *M. communis* berries showed low amounts of flavan-3-ols (13.38 and 24.59 % of total phenolic content for purple and white berries, respectively). The characterisation of total flavan-3-ols subclass, besides monomers ((+)-catechin and (-)-epicatechin), dimmers (procyanidin B1, B2 and B3) and trimer (procyanidin C1), was carried out by acidic depolymerisation of analysed freeze-dried plant material powders in a presence of an excess of phloroglucinol method, followed by UPLC-FL analysis of the reaction medium. According to Wojdyło et al. (*2013a*) this method was used with success for the quantification of polymeric proanthocyanidins in fruits and other food products from plant materials. These results were shown as the sum of polymeric proanthocyanidins (PP). The study of Goh et al. (*2015*) reported that these compounds inhibit the activity of two key enzymes (α-amylase and α-glucosidase) for starch digestion in humans. According to these findings, the analysed plant materials flavan-3-ols could potentially influence the digestibility of starch, which is a future for low glycemic index development in foods.

Polymeric proanthocyanidins were the major compounds among the flavan-3-ols phenolic in the studied plant materials. The content of PP showed the highest concentration in persimmon fruits (2652.17 mg/100 g dm), followed by feijoa flowers (2333.88 mg/100 g dm), strawberry tree fruits (2101.59 mg/100 g dm) and apples (1698.17 mg/100 g dm). In contrast, the lowest concentration of PP was observed in saffron flower juice (8.13 mg/ 100 g dm) followed by white and purple myrtle berries (200.91 and 340.90 mg/100 g dm). Additionally, monomeric flavan-3-ol, such as (+)-catechin concentrations ranged from 8.92 to 90.69 mg/100 g dm (persimmon < apple < strawberry tree fruit, respectively), while 91.08 mg/100 g dm of (-)-epicatechin was detected in *M. domestica* fruits. The values of procyanidin B1 ranged from 5.11 to 81.86 mg/100 g dm (persimmon < apple < strawberry tree fruit, respectively), while 13.23 mg/100 g dm of procyanidin B3 was detected in *A. unedo* fruits. Moreover, 21.98 and 1.68 mg/100 g dm of proanthocyanidn B2 and C1 (respectively) was evaluated in apples. These values regarding persimmon and strawberry tree fruits were much higher than those in the findings of Ancillotti et al. (*2019*) and Pallauf et al. (*2008*), while comparable with results of Wojdyło et al. (*2008*), regarding apples.

Our analysis revealed statistically significant differences in flavonol concentrations in different analysed plant materials. The content of flavonoids ranged from 5.33 mg/100 g dm in persimmon to 1431.85 mg/100 g dm in saffron flower juice. Two main compounds of this subclass of polyphenols detected in strawberry tree fruits were quercetin-3-*O*-xyloside and quercetin-3-*O*-rhamnoside (10.78 and 13.07 mg/100 g dm, respectively). Furthermore, *A. unedo* fruits were rich in other quercetin derivatives (0.33-2.45 mg/100 g dm), myricetin derivatives (0.24-2.56 mg/100 g dm) and kaempferol (5.01 mg/100 g dm). These results were higher than those of Guimarães et al. (*2013*) and Pallauf et al. (*2013*). On the other hand the major flavonoids detected in both types of myrtle berries were myricetin derivatives. Myricetin-3-*O*-galactoside (212.52 and 66.12 mg/100 g dm) and

myricetin-3-*O*-rhamnoside (169.28 and 107.37 mg/100 g dm,) were the main flavonols present in purple and white myrtle berries, respectively. Other myricetin derivatives were detected in amounts ranging from 1.23 to 27.82 mg/100 dm (white berries) and form 12.39 to 27.54 mg/100 g dm (purple berries). In addition, quercetin derivatives (1.50-23.28 mg/100 g dm, and 6.00-30.92 mg/100 g dm) and kaempferol (15.37 and 10.90 mg/100 g dm) were found in white berries and purple berries, respectively). Obtained results were lower than these presented by Barboni et al. (*2010*) and higher than the findings of Tuberoso et al. (*2010*). Considering the contents of flavonols in feijoa flowers, the major ones were quercetin-3-*O*-galactoside, kaempferol-3-*O*-galactoside and kaempferol (15.02, 11.01 and 115.64 mg/100 g dm, respectively). Moreover, in *A. sellowiana* flowers other quercetin derivatives (1.07-8.75 mg/100 g dm), two kaempferol-hexosides I and II (2.75 and 0.59 mg/100 g dm) and apigenin (1.07 mg/100 g dm) were detected. According to our best knowledge this is the first study regarding quantitative analysis of phenolic compounds of feijoa flowers. Regarding the content of flavonols in saffron by-products, several quercetin, kaempferol, and isorhamnetin derivatives were detected. The major ones were kaempferol-3-*O*-sophoroside (565.57 mg/100 g dm), quercetin-3,7-*O*-diglucosdie (232.53 mg/100 g dm), isorhamnetin-3-*O*-rutinosdie (145.36 mg/100 g dm), isorhamnetin-3,7-*O*-diglucoside (128.40 mg/100 g dm) and kaempherol-3-*O*-sophoroside-7-*O*-glucoside (91.20 mg/100 g dm). Other values of compounds belonging to this subclass of polyphenols ranged from 15.52 to 44.82 mg/100 g dm for isorhamnetin derivatives, from 12.83 to 66.21 mg/100 g dm for kaempferol derivatives, and c.a. 2.00 mg/100 g dm for other two quercetin derivatives. The results obtained were comparable to those of Tuberoso et al. (*2016*). According to many authors (*Oszmiański et al., 2009; Malec et al., 2014; Górnaś et al., 2015*) apple was a matrix in which were found only quercetin derivatives (1.96-29.00 mg/100 g dm). Among detected flavonols the major one was quercetin-3-*O*-galactoside, while the minor was quercetin-3-*O*-glucoside. The last

investigated matrix was persimmon in which small amounts (0.15-2.17 mg/100 g dm) of quercetin and kaempferol derivatives were found. The data obtained were partially similar to the results obtained by Ancillotti et al. (*2019*) and Luca-Gonzalez et al. (*2018*). The presence of flavonols is very important for human health, because of their effective antioxidant activity and inhibition of digestive enzymes (α-amylase and α-glucosidase) (*Nowicka et al., 2016b*).

The purified fractions (Cc, MWc, MPc and Fc), prepared according to the procedure described in **Paragraph 2.2.**, were used as concentrated extracts for better characterization of compounds present in low amounts. In fact, they were c.a. 19, 36, 16 and 9 times more concentrated in bioactive compounds compared to the ones without treatment, respectively. The highest total polyphenol content in purified samples was detected in MPc sample (39496.14 mg/100 g dm), while the lowest one was in Fc sample (9503.89 mg/100 g dm). Moreover, significant differences were found between samples before and after treatment, regarding polymeric proanthocyanidins, detected using phloroglucinol method. The highest total content of PP was presented in purified samples of *A. unedo* fruits (13371.65 mg/100 g dm), while the lowest in non-treated *M. communis* white berries (313.01 mg/100 g dm).

Table 5. Quantification of phenolic compounds (mg/100 g of dm) in selected plant materials.

Code	Compound	Plant material						
		Cd	**MWd**	**MPd**	**Fd**	**Sd**	**Ad**	**Kd**
Anthocyanins								
A1	Delphinidin-3,5-*O*-diglucoside	nd	nd	nd	nd	175.92±0.14a	nd	nd
A2	Delphinidin-3-*O*-galactoside	5.54±0.06a	nd	nd	nd	nd	nd	nd
A3	Petunidin-3,5-*O*-diglucoside	nd	nd	nd	nd	2.90±0.03a	nd	nd
A4	Delphinidin-3-*O*-glucoside	nd	nd	326.47±1.12a	0.29±0.01c	110.32±0.55b	nd	nd
A5	Delphinidin-pentoside	nd	nd	17.10±0.10a	nd	nd	nd	nd
A6	Cyanidin-3-*O*-galactoside	57.59±0.10a	nd	nd	nd	nd	nd	nd
A7	Cyanidin-3-*O*-glucoside	1.03±0.00c	nd	75.10±0.16b	124.66±0.45a	nd	nd	nd
A8	Cyanidin-3-*O*-arabinoside	11.21±0.03a	nd	nd	nd	nd	nd	nd
A9	Petunidin-3-*O*-glucoside	nd	nd	292.90±0.11a	nd	0.37±0.00b	nd	nd
A10	Delphinidin-3-*O*-arabinoside	nd	nd	3.25±0.05a	nd	nd	nd	nd
A11	Peonidin-3-*O*-glucoside	nd	nd	0.24±0.01a	nd	nd	nd	nd
A12	Delphinidin	0.53±0.02a	nd	nd	nd	nd	nd	nd
A13	Malvidin-3-*O*-glucoside	nd	2.67±0.03c	771.10±1.34a	nd	14.32±0.23b	nd	nd
A14	Petunidin-3-*O*-arabinoside	nd	nd	1.32±0.04a	nd	nd	nd	nd
A15	Malvidin-3-*O*-arabinoside	nd	nd	26.38±0.21a	nd	nd	nd	nd
Hydroxybenzoic acids								
B1	Gallic acid glucoside I	18.87±0.23a	nd	nd	nd	nd	nd	nd
B2	Galloyl glucoside I	86.33±0.12a	nd	nd	nd	nd	nd	nd

B3	Galloyl HHDP-glucose I	nd	41.05±0.55b	61.41±0.10a	nd	nd	nd	nd
B4	Galloyl glucoside II	nd	nd	nd	nd	nd	nd	100.66±1.45a
B5	Gallic acid glucoside II	8.86±0.04a	nd	nd	nd	nd	nd	nd
B6	3-*O*-Galloylquinic acid (Theogallin)	467.21±1.44a	nd	nd	nd	nd	nd	nd
B7	Galloyl HHDP-glucose II	nd	26.23±0.14b	70.13±0.24a	nd	nd	nd	nd
B8	Galloyl glucoside III	nd	nd	nd	nd	nd	nd	12.24±0.08a
B9	Digalloyl-HHDP-glucose I	nd	6.67±0.05b	9.30±0.02a	nd	nd	nd	nd
B10	Gallic acid 4-O-*β*-D-glucopyranoside	2.76±0.03a	nd	nd	nd	nd	nd	nd
B11	Castalagin	nd	nd	nd	110.03±1.13a	nd	nd	nd
B12	Digalloyl-HHDP-glucose II	nd	9.29±0.07b	11.35±0.06a	nd	nd	nd	nd
B13	Galloyl shikimic acid	21.51±0.04a	nd	nd	nd	nd	nd	nd
B14	Casuarin	nd	nd	nd	25.26±0.45a	nd	nd	nd
B15	Ellagitannin I	nd	37.61±0.12b	53.48±0.12a	nd	nd	nd	nd
B16	Digalloylquinic acid I	11.38±0.10a	nd	nd	nd	nd	nd	nd
B17	Ellagitannin II	nd	nd	nd	357.35±1.28a	nd	nd	nd
B18	Quinic acid 3,5-di-*O*-gallate	nd	15.80±0.07b	16.12±0.11a	nd	nd	nd	nd
B19	Ellagitannin III	nd	192.50±0.08a	167.50±1.13b	nd	nd	nd	nd
B20	Digalloylquinic acid II	2.43±0.03a	nd	nd	nd	nd	nd	nd
B21	Protocatechuic acid	nd	nd	nd	nd	nd	nd	0.27±0.01a
B22	Ellagitannin IV	nd	nd	nd	23.13±0.14a	nd	nd	nd
B23	Nilocitin	nd	nd	nd	56.74±0.42a	nd	nd	nd
B24	Digalloyl shikimic acid I	105.03±1.22a	nd	nd	nd	nd	nd	nd
B25	Syringic acid	nd	nd	nd	nd	nd	nd	0.17±0.00a

B26	Casuarinin	nd	nd	nd	127.63±1.66a	nd	nd	nd
B27	Digalloyl shikimic acid II	63.45±0.74a	nd	nd	nd	nd	nd	nd
B28	Strictinin ellagitannin	0.29±0.00a	nd	nd	nd	nd	nd	nd
B29	Gallotannin derivative	1.62±0.01a	nd	nd	nd	nd	nd	nd
B30	Salicylic acid	nd	nd	nd	nd	nd	nd	1.05±0.03a
B31	Ellagic acid arabinoside	8.40±0.23b	nd	nd	14.45±0.12a	nd	nd	nd
B32	Ellagic acid pentoside	nd	nd	nd	10.51±0.15a	nd	nd	nd
B33	Ellagic acid	nd	nd	nd	35.66±0.44a	nd	nd	nd
B34	Gallotannin	0.98±0.00a	nd	nd	nd	nd	nd	nd
B35	Methyl ellagic acid I	nd	nd	nd	1.40±0.00a	nd	nd	nd
B36	Methyl ellagic acid II	nd	nd	nd	12.51±0.06a	nd	nd	nd
Hydroxycinnamic acids								
C1	Neochlorogenic acid	nd	nd	nd	nd	nd	5.66±0.04a	nd
C2	Chlorogenic acid	nd	nd	nd	nd	nd	1.03±0.00a	0.48±0.00b
C3	Caffeic acid	nd	nd	nd	nd	nd	1.80±0.02a	0.31±0.01b
C4	*p*-Coumaric acid	nd	nd	nd	nd	nd	1.40±0.03a	0.12±0.00b
C5	*p*-Coumaroyloquinic acid	nd	nd	nd	nd	nd	3.77±0.08a	nd
Dihydrochalcones								
D1	Phloretin-2'-*O*-xyloglucoside	nd	nd	nd	nd	nd	31.71±0.12a	nd
D2	Phloretin-2'-*O*-glucoside (Phloridzin)	nd	nd	nd	nd	nd	35.93±0.22a	nd
Flavan-3-ols								
E1	Procyanidin B1	81.86±0.22a	nd	nd	nd	nd	9.96±0.12b	5.11±0.02c
E2	Procyanidin B3	13.23±0.07a	nd	nd	nd	nd	nd	nd

E3	(+)-Catechin	90.69±0.41a	nd	nd	nd	nd	15.61±0.13b	8.92±0.03c
E4	(-)-Epiatechin	nd	nd	nd	nd	nd	91.08±0.88a	nd
E5	Procyanidin B2	nd	nd	nd	nd	nd	21.98±0.16a	nd
E6	Procyanidin C1	nd	nd	nd	nd	nd	1.68±0.04a	nd
PP	Other polymeric procyanidins	2101.59±96.79c	200.91±16.55f	340.90±26.49e	2333.88±75.44b	8.13±0.44g	1698.17±45.23d	2652.74±55.31a
DP	Degree of polymerization	4.79e	7.93b	7.24c	16.17a	1.00f	6.36d	1.09f
Flavonols and Flavones								
F1	Kaempherol-3-*O*-sophoroside-7-*O*-glucoside	nd	nd	nd	nd	91.20±0.55a	nd	nd
F2	Quercetin-3,7-*O*-digalactoside	nd	nd	nd	nd	1.92±0.12a	nd	nd
F3	Kaempferol-3,7-*O*-diglucoside	nd	nd	nd	nd	12.83±0.13a	nd	nd
F4	Isorhamnetin-3,7-*O*-digalactoside	nd	nd	nd	nd	15.52±0.22a	nd	nd
F5	Quercetin derivative I	2.45±0.11a	nd	nd	1.07±0.11b	nd	nd	nd
F6	Myricetin galactoside-gallate	nd	33.96±0.22a	22.47±0.08b	nd	nd	nd	nd
F7	Quercetin-3,7-*O*-diglucoside	nd	nd	nd	1.83±0.18b	232.53±4.16a	nd	nd
F8	Myricetin-3-*O*-galactoside	1.13±0.09c	66.12±0.41b	212.52±3.54a	nd	nd	nd	nd
F9	Myricetin-3-*O*-glucoside	0.24±0.02c	1.23±0.08b	34.06±0.11a	nd	nd	nd	0.31±0.00c
F10	Isorhametin-3,7-*O*-diglucoside	nd	nd	nd	nd	128.40±2.68a	nd	nd
F11	Quercetin galloylhexose	1.14±0.08a	nd	nd	nd	nd	nd	nd
F12	Quercetin-3-*O*-rutinoside	nd	nd	nd	nd	nd	nd	0.35±0.01a
F13	Kaempferol-3-*O*-sophoroside	nd	nd	nd	nd	565.57±3.54a	nd	nd
F14	Myricetin -3-*O*-arabinoside	nd	27.82±0.12a	27.54±0.31a	nd	nd	nd	nd
F15	Quercetin derivative II	1.29±0.06a	nd	nd	nd	nd	nd	nd
F16	Isorhamnetin-3-*O*-sophoroside	nd	nd	nd	nd	44.82±0.16a	nd	nd

F17	Myricetin-3-*O*-xyloside	2.56±0.03a	nd	nd	nd	nd	nd	nd
F18	Myricerin-3-*O*-rhamnoside	2.19±0.01c	107.37±1.28b	169.28±1.23a	nd	nd	nd	nd
F19	Quercetin-3-*O*-galactoside	1.97±0.09f	2.60±0.01d	7.56±0.18c	15.02±0.23a	2.01±0.06f	29.00±0.22b	0.74±0.05e
F20	Quercetin-3-*O*-glucoside	0.70±0.00f	1.50±0.03e	6.00±0.45a	3.11±0.04b	nd	1.96±0.03d	2.17±0.11c
F21	Kaempferol-3-*O*-rutinoside	nd	nd	nd	nd	49.96±0.11a	nd	nd
F22	Kaempherol-3-*O*-galactoside	nd	nd	nd	11.01±0.16a	nd	nd	nd
F23	Quercetin derivative III	nd	nd	nd	nd	nd	nd	0.57±0.04a
F24	Isorhamnetin-3-*O*-rutinoside	nd	nd	nd	nd	145.36±2.51a	nd	nd
F25	Quercetin-3-*O*-arabinoside	0.33±0.01c	nd	nd	2.93±0.02b	nd	5.27±0.13a	nd
F26	Quercetin-pentoside	nd	nd	nd	8.75±0.06a	nd	nd	0.36±0.01b
F27	Quercetin-3-*O*-xyloside	10.78±0.011b	nd	nd	7.46±0.11c	nd	13.75±0.07a	nd
F28	Kaempferol-3-*O*-glucoside	nd	nd	nd	nd	35.97±0.42a	nd	0.69±0.02b
F29	Quercetin-3-*O*-rhamnoside	13.07±0.22d	23.28±0.10c	30.92±0.22a	nd	nd	16.03±0.31b	nd
F30	Kaempferol-3-*O*-rhamnoside	nd	nd	nd	nd	nd	nd	0.15±0.00a
F31	Isorhanetin-3-*O*-glucoside	nd	nd	nd	nd	39.55±0.31a	nd	nd
F32	Kaempherol-hexoside I	nd	nd	nd	2.75±0.04a	nd	nd	nd
F33	Myricetin	nd	4.93±0.03b	12.39±0.02a	nd	nd	nd	nd
F34	Kaempherol-hexoside II	nd	nd	nd	0.59±0.01a	nd	nd	nd
F35	Quercetin	nd	nd	nd	1.89±0.04a	nd	nd	nd
F36	Kaempferol	5.01±0.12e	15.37±0.11c	10.90±0.08d	115.64±1.23a	66.21±0.15b	nd	nd
F37	Apigenin	nd	nd	nd	1.07±0.01a	nd	nd	nd
Total		**3208.33b**	**816.91f**	**2777.69c**	**3406.60a**	**1743.81e**	**1985.78d**	**2787.40c**

Data are given as mean ± standard deviation (n=3).For each of the studied compound, values with different letters (a-g) are significantly different (homogenous groups) at $p \leq 0.05$;.nd = not detected (< LOD).

3.1.3. Antioxidant activity

The use of different antioxidant assays is necessary to test activity in different mediums and understand chemical mechanisms. For this purpose, tested plant materials were evaluated for total antioxidant activity using two methods (CUPRAC and FRAP), as well as free radical scavenging activity by three different methods ($DPPH^{\bullet}$, $ABTS^{\bullet+}$ and ORAC). Moreover, the total phenolic content was estimated with the Folin-Ciocalteu's assay, and all obtained results were presented in **Table 6.**.

Table 6. Antioxidant activity of selected plant materials.

Sample code	Antioxidant activity					
	TP	**CUPRAC**	**FRAP**	**ORAC**	**$DPPH^{\bullet}$**	**$ABTS^{\bullet+}$**
	mg GAE/100 g dm	mmol Fe^{2+}/100 g dm	mmol Trolox/100 g dm			
Cd	2188.12±2.76e	65.63±0.14e	13.98±1.39e	22.32±0.16e	14.71±0.01e	26.10±0.65d
MWd	4545.03±3.90b	150.30±0.19c	42.04±2.76a	92.53±1.70a	41.98±0.05b	45.60±2.15a
MPd	5605.78±12.29a	212.00±0.16a	32.03±2.35c	38.19±2.72c	50.97±0.02a	33.78±1.23b
Fd	2165.83±6.68e	88.37±0.20d	25.16±0.72d	29.72±0.43d	20.98±0.03d	28.37±1.04c
Sd	3231.19±5.32d	43.09±0.05f	9.33±0.52e	32.85±2.85d	5.79±0.01f	9.68±0.11e
Ad	190.36±5.37f	5.50±0.05g	0.96±0.03f	2.18±0.33f	1.00±0.04g	1.06±0.01f
Kd	3808.60±0.41c	168.01±0.04b	36.87±5.91b	73.52±1.56b	29.47±0.00c	29.03±1.02c

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-g) are significantly different (homogenous groups) at $p \leq 0.05$. Sample codes have their references in **Annex 1a.**.

The antioxidant mechanisms exposed by different methods showed the same tendency in the final results. The *in vitro* antioxidant assays that were used showed significant differences ($p \leq 0.05$) between all analysed plant materials. The values obtained for investigated samples ranged from 5.50 to 212.00 mmol Fe^{2+}/100 g dm (CUPRAC), and 0.96-42.04, 2.18-92.53, 1.00-50.97, and 1.06-45.60 mmol Trolox/100 g dm for FRAP, ORAC, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively.

Generally, the highest values were observed in myrtle berries (purple and white). Intrestingly, the results obtained by CUPRAC and $DPPH^{\bullet}$ assay, were higher in purple

berries, while the results obtained using three other tests (FRAP, ORAC and $ABTS^{\bullet+}$) were higher in white berries. These data were comparable with the findings of Tuberoso et al. (*2010*), who investigated antioxidant activity in three different extracts (EtOH, water and EtOH-Aceton) from myrtle berries using $DPPH^{\bullet}$ and FRAP assays. Moreover, it was worth noting that persimmon fruits showed high antioxidant power, measured by all five methods. The results of $DPPH^{\bullet}$ assay for *D. kaki* fruits was similar (29.47 mmol Trolox/100 g dm) to the results of Matsumura et al. (*2016*), who measured antioxidant radical scavenging activities in edible portions of dried persimmon. Current free radical scavenging activity ($DPPH^{\bullet}$) results measured in strawberry tree fruits were slightly lower (14.71 mmol Trolox/100 g dm) than data reported by Oliveira et al. (*2016*) in different ripening stages. Finally, the FRAP and $DPPH^{\bullet}$ results of saffron flower juice were higher (9.33 and 5.79 mmol Trolox/100 g dm, respectively) than in findings of Tuberoso et al. (*2016*).

The lowest values of antioxidant activity measured by all methods were observed in apple fruits. These results were much lower than those measured by $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and FRAP in the findings of Wojdyło et al. (*2008*). Moreover, this work was one of the first studies about the antioxidant activity of feijoa flower extracts. According to our best knowledge, Montoro et al. (*2020*) was the first to investigate antioxidant activity (FRAP, CUPRAC, $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and total phenol content) of feijoa flower macerate, petal macerate and petal juice. The results obtained in the above mentioned study for *A. sellowiana* extracts were lower than those obtained in the present one.

The values of total polyphenol content ranged from 190.36 to 5605.78 mg GAE/100 g dm. Purple and white *M. communis* berries and *D. kaki* fruits showed the highest antioxidant potential, while *M. domestica* fruits showed the lowest antioxidant activity. Moreover, strong correlation between total phenol content and antioxidant activity (Pearson correlation: 0.8963, 0.7882, 0.6935, 0.8799 and 0.7398 for CUPRAC, FRAP,

ORAC, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively) was observed. These polyphenol contents in analysed plant materials partially confirmed the data obtained by the HPLC-PDA analysis. Also, different authors (*Girones-Vilaplana et al., 2014; Wojdyło et al., 2007*) confirmed that polyphenols have strong antioxidant properties, and there is a significant correlation between phenolic concentration and free radical scavenging activity. Differences in molecular structure, including the number or placement of binding hydroxyl groups, may cause differences in antioxidant activity.

Antioxidants generally scavenge free radicals, therefore it is crucial to measure the free radical scavenging activity using $DPPH^{\bullet}$. Moreover, there are specific radicals (hydrogen peroxide or superoxide) that are involved in oxidative stress, hence some specifical analysis of the scavenging properties of the crude extracts on these radicals should be done. Furthermore, reducing power assays are necessary to measure the ability of the antioxidant to produce reduction of e.g. ferric ions (Fe^{2+}) through tests such as FRAP. All these methods are *in vitro* models, which are not connected with a particular disease model. Some scientist claim (*Kasote et al., 2015*) that almost all plants or their phytochemicals have antioxidant activity *in vitro*. Nevertheless, *in vivo* antioxidants occurring in plants have to pass through varied physiopharmacological. As a result, the antioxidant activity of plant materials is affected by few factors *in vivo*, such as gut absorption, bioavailability, metabolism, as well as the appearance of co-antioxidants and transition metal ions.

To sum up, as reported in the study of Moharram and Youssef (*2014*), not all different antioxidants are connected, thus antioxidant activity should be determined by more than one method. Various antioxidant methods are able to offer a complete profile of the antioxidant content of plant materials. Moreover, in plant extracts, the antioxidant activity is generally performed by polyphenols and correlates positively with their concentration.

3.1.4. Inhibitory activity toward digestive enzymes

In the present study the anti-amylase, anti-glucosidase and anti-pancreatic lipase activity of all analysed plant materials was investigated using potato starch, *p*-nitrophenyl-*α*-D-glucopyranoside or *p*-nitrophenyl as substrates, respectively. The obtained results were reported in **Table 7.** as IC_{50} values (mg of dry plant material/mL).

Table 7. Digestive enzymes inhibitory activity of selected plant materials.

Sample code	Enzyme inhibition IC_{50} (mg of dried plant material/mL)		
	***α*-amylase**	***α*-glucosidase**	**pancreatic lipase**
Cd	13.63 ± 0.06f	3.83 ± 0.01f	1.29 ± 0.01g
MWd	14.71 ± 0.01e	4.12 ± 0.12e	1.79 ± 0.03e
MPd	19.21 ± 0.11b	5.38 ± 0.01c	2.39 ± 0.10c
Fd	16.24 ± 0.12c	4.48 ± 0.09d	2.11 ± 0.01d
Sd	19.19 ± 0.01b	8.63 ± 0.22b	1.41 ± 0.05f
Ad	81.84 ± 0.23a	34.08 ± 0.01a	17.59±0.15a
Kd	14.99 ± 0.01d	4.07 ± 0.05e	10.07 ± 0.02b

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-g) are significantly different (homogenous groups) at $p \leq 0.05$. Sample codes have their references in **Annex 1a.**.

Generally, significant differences ($p \leq 0.05$) were found among the analysed plant materials in inhibitory activities toward *α*-amylase, *α*-glucosidase and pancreatic lipase. The inhibition of these three tests, ranged from 13.63 to 81.84, 4.07 to 34.08, and 1.29-17.59 mg of dried plant material/mL, respectively.

It was noticed that plant materials examined in this study showed strong inhibitory activity towards *α*-glucosidase and pancreatic lipase, but only moderate inhibitory effects towards *α*-amylase. The order of plant materials being the most potent *α*-amylase inhibitors was as follows: Cd (IC_{50} = 13.63) > MWd (IC_{50} = 14.71) > Kd (IC_{50} = 14.99). Regarding *α*-glucosidase, the strongest inhibition was shown by the strawberry tree fruits (3.83 mg dm/mL). Furthermore, a similar tendency in digestive enzyme inhibition was observed as in the case of the *α*-amylase. It was noticed that inhibition potency of apple was much

weaker than the other plant materials. Taking into account the inhibition power against pancreatic lipase, it was observed that the most effective were strawberry tree fruits, white myrtle berries and saffron flower juice (1.29, 1.79 and 1.41 mg dm/mL, respectively). Similar tendencies were observed in the findings of Podsdęk et al. (*2014*). This author tested 40 different types of fruits for *α*-amylase and *α*-glucosidase activity. Among them were, blue honeysuckle and red gooseberry, which exhibited the highest inhibitory activity with respect to carbohydrate degrading enzymes, while a number of other fruits (example: peach or pear), did not show any activity at all.

Thanks to different mechanism, most of the detected phenolic compounds were reported to have antidiabetic effect. Big differences in structure among different subclasses of polyphenols and within the group effect their solubility stability, and bonding ability with the digestive enzymes (*Wojdyło et al., 2016*). Furthermore, some other studies showed that inhibition of *α*-glucosidase was connected with the content of hydroxycinnamic derivatives such as *p*-coumaric acid (*Wang et al., 2015*), but in this study and the study of Nowicka et al. (*2016a*), no correlation was found between this subclass of phenolic acids and *α*-amylase, *α*-glucosidase and pancreatic lipase. This could imply that other polyphenols may be involved in inhibition, i.e. anthocyanins, dihydrohalcones, flavan-3-ols, flavonols, or polymeric procyanidins. Hence, Akkarachiyasit et al. (*2010*), claimed that cyanidin-3-*O*-galactose was a very good inhibitor of rat intestinal sucreose activity (*α*-glucosidase inhibition). Furthermore, Boath et al. (*2012*) suggested that flavonols may interact with phenolic acids or anthocyanins, causing the inhibition of *α*-glucosidase.

To conclude, *α*-glucosidase, pancreatic *α*-amylase and lipase may be effective in the regulation of hyperglycaemia and type 2 diabetes by controlling glucose absorption (*Podsędek et al., 2014; González-Muñoz et al., 2013*). Moreover, as documented by Picot et al. (*2014*) other compounds like fibres or viscous polysacharydes can contribute to the inhibition of digestive enzymes, due to decreases in the postprandial plasma glucose level.

CHAPTER 3.2. - Evaluation of the final products

Three sets of final products were produced using selected plant materials described in **CHAPTER 3.1.**. The first set was based on pure apple juice, the second on apple juice mixed with persimmon purée (75:25) and the third on apple juice mixed with dry strawberry tree fruits (75:25). The composition of the 20 final products is reported in **Annex 1b.**.

The 20 final products were immediately investigated at three different levels: appreciation by consumers (sensory evaluation), evaluation of the most significant chemical compounds (chemical characterization) and assessment of biological activities (antioxidant activity and digestive enzymes inhibitory activity).

To evaluate the stability of these final products, evaluation of main physico-chemical parameters, phenolic compounds and biological activities were performed after 3 and 6 months of storage at room temperature (20 ± 2 °C).

3.2.1. Sensory analysis

The first role of a proper diet is to provide sufficient nutrition to satisfy the metabolic requirements of individuals and to give consumers a feeling of well-being and satisfaction through self-indulgent attributes such as taste, aroma, colour and consistency Nutrition Society (*1999*).

The sensory results obtained by the trained panel were grouped according to complex sensory properties: colour, aroma, taste, consistency and desirability (**Annex 2.**). Sensory evaluation was carried out in the products immediately after processing using 5° hedonic scale (**Table 8.**; **Figure 19.**). Based on the results of the study, significant differences ($p \leq 0.05$) were found between all final products for colour, aroma, taste, consistency and desirability.

Table 8. Consumer evaluation of the final products (5° hedonic scale).

Sample code	Type of product	Qualitative parameters				
		Colour	Aroma	Taste	Consistency	Desirability
B1	juice	3.40±0.70abcd	4.60±0.70a	4.30±0.67a	2.90±1.20a	4.50±0.53a
B1S01	juice	3.25±0.86bcd	3.85±0.58abc	4.00±0.67ab	2.90±1.20a	4.10±0.99ab
B1S05	juice	2.50±0.97cde	2.70±0.67bc	2.95±0.76ab	2.80±1.32a	3.20±0.79abc
B1M5	juice	3.85±1.16abc	2.90±1.10abc	3.40±1.17ab	3.00±1.25a	3.50±1.27abc
B1F5	juice	1.60±0.84e	2.15±1.06c	2.15±1.06b	2.90±1.29a	1.85±1.11c
B1K5	juice	2.95±0.83bcde	3.60±0.52abc	4.15±0.47ab	3.00±1.15a	3.55±1.07abc
B1C5	juice	3.90±0.74abc	4.10±0.74ab	3.75±0.86ab	2.90±0.99a	4.00±1.33abc
B2	smoothie	3.10±0.74bcde	3.40±0.84abc	3.40±0.97ab	2.20±0.63a	3.10±0.57abc
B2S01	smoothie	3.00±0.67bcde	3.30±1.06abc	2.90±1.29ab	2.10±0.88a	3.20±1.03abc
B2S05	smoothie	3.00±0.82bcde	2.90±0.99abc	3.15±1.06ab	2.60±0.84a	3.15±0.88abc
B2M5	smoothie	4.50±0.53ab	3.60±0.97abc	3.15±0.94ab	3.00±0.94a	3.45±0.96abc
B2F5	smoothie	2.10±0.99de	2.75±1.18bc	2.10±0.99b	2.20±1.23a	1.80±1.03c
B2C5	smoothie	4.20±0.42ab	3.50±0.85abc	3.70±0.95ab	2.70±0.95a	3.15±0.94abc
B3	smoothie	4.40±0.70ab	3.60±0.97abc	3.00±1.33ab	2.80±0.63a	4.20±1.23ab
B3S01	smoothie	4.30±0.67ab	3.70±0.95abc	3.40±0.97ab	2.90±0.57a	4.10±1.20ab
B3S05	smoothie	4.50±0.71ab	3.00±0.94abc	2.80±1.14ab	2.70±0.95a	3.80±1.55abc
B3M5	smoothie	4.90±0.32a	3.80±0.92abc	3.30±1.06ab	3.10±0.99a	3.60±1.51abc
B3F5	smoothie	2.10±1.20de	2.90±0.99abc	2.20±1.14b	3.50±1.35a	2.00±0.82bc
B3K5	smoothie	4.30±0.95ab	4.00±1.15ab	3.70±1.25ab	2.70±0.95a	4.30±1.25a
BK	purée	3.50±0.85abcd	2.15±1.00c	3.00±1.49ab	2.00±1.25a	2.55±1.57abc

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-e) are significantly different (homogenous groups) at $p \leq 0.05$. Sample codes have their references in **Annex 1b.**.

The study showed that almost all products were attractive in colour terms (≥ 3.00). However, the highest colour scores (≥ 4.30) were obtained by products with base B3 (strawberry tree fruits 25 % + apple juice 75 %), except product with additional feijoa flowers - B3F5. The good value of colour was also observed in some products with base B2 (persimmon purée 25 % + apple juice 75 %). Among them were products where additional components were purple myrtle berry extract and strawberry tree fruits: B2M5 (score 4.50) and B2C5 (score 4.20), respectively. The lowest scores for the colour

evaluation (≤ 2.10) were obtained by all products containing feijoa flowers as an additional component.

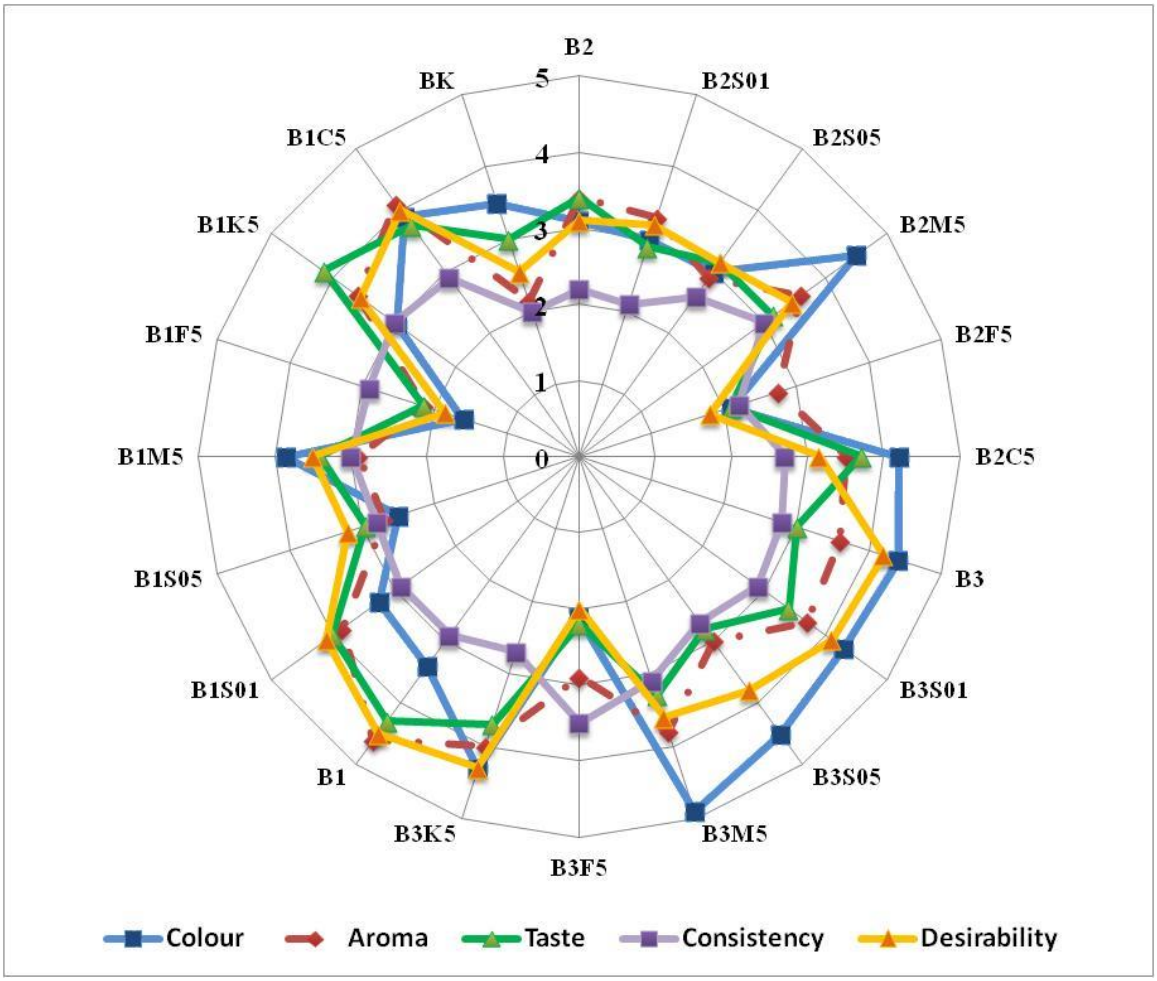


Figure 19. Consumer evaluation of the final products (5° hedonic scale).
Sample codes have their references in **Annex 1b.**.

According to consumers the following products had the best aroma: B3K5 (score 4.00), B1C5 (score 4.10) and B1 (score 4.60). B1F5 and BK had the worst aroma (score 2.15). For taste value the products which came out the best (≥ 4.00) were: B1S01, B1K5 and B1. The ones that tasted the worst (≤ 2.20) were all products with additional feijoa flowers. According to consumers the consistency was the lowest of all measured sensory properties. The highest consistency evaluation (≥ 3.10) was in products with base B3 and addition of myrtle purple berry extract and feijoa flowers, while the lowest scores (≤ 2.20) were for products B2, B2S01, B2F5 and BK. According to consumers the best products

were those with pure base B3 (score 4.20) and B1 (score 4.50) as well as those with additional saffron 0.1 % (score 4.10). Moreover, high values were observed in products: B1C5 (score 4.00) and B3K5 (score 4.30). All products containing feijoa flowers turned out to be definitely unacceptable, probably due to their brown colour and unpleasant solid parts, resembling rotten vegetables. However, due to its significant bioactive potential (*Montoro et al., 2020*), it can be good raw material, enriching the quality of the final product. A more appropriate technological process for preparing the feijoa plant material would probably make it more acceptable to consumers.

Furthermore, the taste types were assessed. Consumers were asked to choose the particular taste types from proposed ones, among which were: lemon, nut, apple, persimmon, strawberry, chocolate, herbs and forest fruits. According to the consumers in all final products it was possible to taste persimmon, even in products which did not contain this fruit. Moreover, the lemon and strawberry taste was noted in all products with base B1 and B3 except final products B1 and B1S01, where it was only possible to identify lemon. Among products with base B2 consumers noted lemon taste in samples B2S01, B2S05, while strawberry taste was not detected in any of them. According to the consumers the taste of chocolate, nut, herbs and forest fruits was noticeable in all products containing feijoa flowers and purple myrtle berry extract as well as in the following samples: B1S05, B1C5 and B1K5. Moreover, the taste of forest fruits was observed in products containing 0.5 % of saffron flower juice B2S05, B3S05, and also in product B3K5. Additionally, in products B3BLC, B3S05 and BK it was possible to taste chocolate, while in products B2C5, B1S01 and BK it was possible to taste nut. Herbal taste was observed in samples B2S01 and B3. The perception of these tastes may make the final product more attractive to consumers.

Finally, consumers were asked about preferred packaging for the final products. Among proposed packages were: plastic bottle, Tetra Pak® with a straw, glass bottle, glass

jar and pouch pack. In general the most desirable were glass and plastic bottle, especially for products based on base B1 and B2. For those products as well as for products with base B3, the glass jar and Tetra Pak® with a straw were also popular. Furthermore, the panel of assessors suggested that products with base B3 should be consumed from a pouch pack.

3.2.2. Physico-chemical investigation

The main physico-chemical parameters (dry matter (DM), ashes, total soluble solids (TSS), total acidity (TA), pH and vitamin C) were evaluated in 20 final products immediately after processing and after storage time (3 and 6 months) at 20 ± 2°C. Statistical differences ($p \leq 0.05$) were found among the analysed mixed plant material products and obtained results were presented in **Table 9.**.

The analyses of obtained final products were focused on the dry matter and ashes content. Differences between analysed fruit products were observed and reflected in their chemical composition. The highest DM content was detected in all products with base B3 in a range between 22.40-25.92 g/100 g fw. Moreover, the products with base B1 and B2 with 5 % addition of strawberry tree fruits and feijoa flowers, and product BK were characterized by dry matter content in a range between 16.97-17.92 g/100 g fw. The lowest values of dry matter (13.54-14.97 g/100 g fw) were observed in other final products with base B1 and B2. In addition, considering storage, slight increments (1-4 %, after 3 months) and decrements (1-8 %, after 6 months) were observed in analysed final products.

The highest values of ashes were observed in all products with base B3 in a range from 0.42 to 0.74 g/100 g fw, as well as in products B1F5, B2F5 and BK (0.53, 0.52 and 0.52 g/100 g fw, respectively). Thus, it was observed that all products with addition of 5 % of feijoa flowers were the richest in minerals. In contrast, the lowest ash content (0.23-0.34 g/100 g fw) was detected in products with base of 100 % of apple juice, while products with base of persimmon purée and apple juice (25:75), were slightly more abundant in ash

content (0.24-0.42 g/100 g fw). Taking storage into account, no significant ($p \geq 0.05$) changes were obesrved in minerals among analysed final products.

The total soluble content (TSS) was also evaluated in this study. It is a characteristic which largely determines the final dry matter content (*Nowicka et al., 2019*). The soluble solids value depends on the content of soluble compounds like dyes, tannins and non-volatile organic acids (citric, tartaric or malic), but as in principally on the total sugar content (*Nowicka et al., 2017*). Thus, TSS content is usually higher in strongly coloured fruits containing more sugars and acids. In this study a relationship was observed between the content of soluble solids in the analysed final products and their solids' content. Therefore, the highest content of soluble solids was found in final products with base B3, where strawberry tree fruits were present. In these products the soluble solids had sugar content in a range between 19.80 and 22.60 °Brix. This is consistent with the findings reported by Vidrih et al. (*2013*). On the other hand, in other final products the content of soluble solids was almost twice as low, except products B2F5, B2C5, B1F5 and B1C5, where values were ≥ 15.40 °Brix, due to 5 % addition of *A. unedo* fruits and *A. sellowiana* flowers. During storage, a slight decrease (1-5 %) was observed in analysed final products. According to Kheiralipour et al. (*2008*) and Zatylny et al. (*2005*), the dry matter content and the total solid content depends not just on the cultivar but may also be influenced by many other factors like the degree of fruit dehydration, harvest time, climatic and agricultural conditions and an increase in the insoluble solids' content of the fruit during maturation.

Regarding both pH and TA values, significant differences were observed among all final products, especially between products with different bases (B1, B2 and B3). Moreover, some significant differences ($p \leq 0.05$) were observed in the final products during storage (3 and 6 months). The highest content of titratable acidity was determined in final products with base B3 (0.66-0.72 g of MA/100 g fw), as well as in products with

base B1 and B2 with addition of 5 % feijoa flowers (0.49 and 0.44 g of MA/100 g fw, respectively) and strawberry tree fruits (0.50 and 0.49 g of MA/100 g fw, respectively). In other samples, TA was at a comparable level, from 0.39 to 0.45, with one exception of final product BK, where the value of TA was the lowest (0.29 g of MA/100 g fw) compared with other products. As seen in **CHAPTER 3.1.**, both feijoa flowers and strawberry tree fruits were characterized in our study by a high content of organic acids (42.31 and 23.21 g/100 g dm, respectively). This has also been confirmed in the literature concerning *A. unedo* berries (*Oliveira et al., 2011*). While *A. sellowiana* flowers have never been investigated before for their organic acid content, their profile and content was similar to that of feijoa fruits (*Castellanos et al., 2016*). Therefore, these two semi-products proved responsible for the acidity of final products. Low total acidity is important in terms of technological preservation, because of possible problems in the pasteurisation process during juices preparation at pH below or above 4.6. (*Wojdyło et al., 2014c*).

Furthermore, TSS and TA parameters as well as the ratio between them are commonly used by the juice industry as quality control indicators. As described by Poll (*1981*) and Jaros et al. (*2009*), TSS/TA ration is the most important parameter, helping to predict the consumers' preferences for cloudy apple juices. Moreover, according to Jaros et al. (*2009*), there was a part of the consumers that in general prefer sweeter juices, with higher ratios of TSS/TA. It was partially confirmed in this study, that the panel of consumers preferred sweeter final products but with lower TSS/TA ratios in general. Therefore, as described by Konić-Ristić et al. (*2011*) industrial processors of commercial juices have different requirements regarding acidity in raw materials because low ratios are a good indicator for prolonging fruit quality during storage. In addition, obtained results show significant differences in the values of these important parameters that influence not only the sensory quality but also colour intensity and microbiological stability of the final products (*Pérez-Magariño and Gonzales-Sanjosé, 2003*).

The pH of all analysed final products immediately after processing varied from 3.31 to 4.61, while during storage time was decreased slightly: 3.25-4.47 (after 3 months) and 3.26-4.36 (after 6 months). Immediately after processing, all smoothies prepared from 25 % of rehydrated strawberry tree fruits and 75 % apple juice as a base had the lowest pH values (generally below 3.58, except for product B3F5 (3.76)). In contrast, the highest pH values were present in product BK (4.61), as well as in products with an added 5 % feijoa flowers: B1F5 (4.00) and B2F5 (4.10).

Analysing the content of vitamin C (ascorbic acid), showed that investigated final products can be divided in three major groups. The lowest content of vitamin C (≤ 1.60 mg/100 g fw) was observed in all products with base B1 and B2 (first group), except those with an added 5 % of strawberry tree fruits, where the amount of vitamin C was 23.68 and 23.59 mg/100 g fw, respectively (second group). Furthermore, in product BK was detected 7.76 mg/100 g fw of vitamin C. In the third group were products with the highest content of vitamin C in a range between 46.32-95.92 mg/100 g fw, including all final products with base B3. High content of vitamin C in these products was due to the presence of *A. unedo* fruits, which are a rich source of this vitamin, according to Vadrih et al. (*2013*) and Morgado et al. (*2018*). Based on the results of the study, significant correlations were found before and after storage of final products between vitamin C and dry matter, total soluble solids, total soluble solid or organic acids (Pearson correlation in a range between 0.7424 and 0.9139). Generally, it was noticed that these parameters correlated more closely, after storage time, than immediately after final product preparation.

Table 9. Physico-chemical parameters of final products before and after storage time (3 and 6 months) at 20±2°C.

Storage time: Immediately after processing (0 months)

Sample code	Physico-chemical parameters						
	DM (g/100 g fw)	Ashes (g/100 g fw)	TSS (°Brix)	TA (g of MA*/100 g fw)	TSS/TA	pH	Vitamin C (mg/100 g fw)
B1	13.54 ± 0.00m	0.28 ± 0.04gh	13.20 ± 0.00o	0.42 ± 0.01fg	32.20	3.31 ± 0.03n	0.90 ± 0.01h
B1S01	13.88 ± 0.07l	0.23 ± 0.00i	13.50 ± 0.02m	0.44 ± 0.01ef	30.68	3.52 ± 0.01jk	0.64 ± 0.01h
B1S05	14.48 ± 0.07j	0.34 ± 0.03ef	14.10 ± 0.03j	0.45 ± 0.00e	31.33	3.58 ± 0.02hi	0.65 ± 0.01h
B1M5	14.85 ± 0.09i	0.33 ± 0.02ef	14.60 ± 0.01i	0.45 ±0.01e	31.74	3.68 ± 0.01f	0.65 ± 0.01h
B1F5	17.59 ± 0.01f	0.53 ± 0.04b	16.00 ± 0.01f	0.49 ± 0.01d	32.65	4.00 ± 0.02c	0.95 ± 0.01h
B1K5	14.49 ± 0.31j	0.28 ± 0.02gh	13.80 ± 0.02kl	0.44 ± 0.03ef	30.00	3.59 ± 0.01h	0.68 ± 0.04h
B1C5	16.97 ± 0.03h	0.31 ± 0.00fg	16.10 ± 0.00f	0.50 ± 0.01d	31.57	3.56 ± 0.04ij	23.68 ± 0.23f
B2	14.39 ± 0.01j	0.24 ± 0.01i	13.30 ± 0.03no	0.42 ± 0.01fg	32.44	3.31 ± 0.03n	1.60 ± 0.37h
B2S01	14.18 ± 0.19k	0.26 ± 0.00hi	13.40 ± 0.01mn	0.39 ± 0.02h	33.50	3.68 ± 0.00f	0.94 ± 0.01h
B2S05	14.50 ± 0.11j	0.33 ± 0.00ef	13.70 ± 0.00l	0.39 ± 0.01gh	36.05	3.74 ± 0.01e	1.43 ± 0.02h
B2M5	14.97 ± 0.01i	0.35 ± 0.00e	13.90 ± 0.05k	0.41 ± 0.01gh	34.75	3.84 ± 0.03d	1.13 ± 0.07h
B2F5	17.92 ± 0.00e	0.52 ± 0.06bc	15.40 ± 0.00h	0.44 ± 0.01ef	35.81	4.10 ± 0.00b	1.25 ± 0.24h
B2C5	17.78 ± 0.13ef	0.42 ± 0.02d	15.60 ± 0.02g	0.49 ± 0.01d	31.84	3.63 ± 0.01g	23.59 ± 0.60f
B3	22.76 ± 0.00c	0.48 ± 0.00c	20.50 ± 0.04d	0.70 ± 0.01ab	28.87	3.46 ± 0.01m	91.92 ± 5.43b
B3S01	22.86 ± 0.00c	0.42 ± 0.00d	20.60 ± 0.12cd	0.69 ± 0.02b	29.43	3.48 ± 0.01ml	95.62 ± 0.60a
B3S05	22.77 ± 0.18c	0.54 ± 0.04b	20.70 ± 0.03c	0.69 ± 0.01b	29.57	3.52 ± 0.01k	86.99 ± 0.78c
B3M5	23.81 ± 0.03b	0.51 ± 0.04bc	21.10 ± 0.00b	0.72 ± 0.00a	29.31	3.58 ± 0.03hi	82.67 ± 4.47d
B3F5	25.92 ± 0.11a	0.74 ± 0.04a	22.60 ± 0.25a	0.70 ± 0.02ab	33.24	3.76 ± 0.01e	46.32 ± 1.45e
B3K5	22.40 ± 0.06d	0.51 ± 0.00bc	19.80 ± 0.15e	0.66 ± 0.03c	30.94	3.51 ± 0.01kl	83.36 ± 3.75d
BK	17.39 ± 0.24g	0.52 ± 0.01b	14.10 ± 0.01j	0.29 ± 0.00i	48.62	4.61 ± 0.02a	7.76 ± 0.15g

MA* : malic acid; Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-s) are significantly different (homogenous groups) at $p \leq 0.05$. Sample codes have their references in **Annex 1b.**.

Storage time: 3 months

Sample code	Physico-chemical parameters						
	DM (g/100 g fw)	**Ashes (g/100 g fw)**	**TSS (°Brix)**	**TA (g of MA*/100 g fw)**	**TSS/TA**	**pH**	**Vitamin C (mg/100 g fw)**
B1	13.47 ± 0.15h	0.28 ± 0.05defg	13.10 ± 0.01r	0.50 ± 0.00fg	26.20	3.34 ± 0.00j	0.84 ± 0.00f
B1S01	13.51 ± 0.55h	0.22 ± 0.03g	13.40 ± 0.01o	0.44 ± 0.00i	30.45	3.34 ± 0.02jk	0.66 ± 0.07f
B1S05	14.49 ± 0.04g	0.34 ± 0.04d	14.00 ± 0.02l	0.48 ± 0.00h	29.17	3.39 ± 0.00i	0.60 ± 0.00f
B1M5	14.85 ± 0.02fg	0.32 ± 0.03de	14.50 ± 0.00k	0.48 ± 0.01h	30.21	3.50 ± 0.00g	0.62 ± 0.01f
B1F5	17.27 ± 0.29de	0.53 ± 0.01b	15.90 ± 0.01h	0.48 ± 0.00h	33.13	3.80 ± 0.00c	0.94 ± 0.00f
B1K5	14.30 ± 0.08g	0.26 ± 0.01efg	13.80 ± 0.00m	0.40 ± 0.00j	34.50	3.42 ± 0.02i	0.60 ± 0.00f
B1C5	15.60 ± 1.92f	0.30 ± 0.00def	16.00 ± 0.01g	0.49 ± 0.01gh	32.99	3.38 ± 0.00i	20.58 ± 0.06e
B2	14.47 ± 0.02g	0.26 ± 0.04efg	13.00 ± 0.05s	0.42 ± 0.01j	31.33	3.67 ± 0.00de	1.54 ± 0.01f
B2S01	14.73 ± 0.02g	0.24 ± 0.01fg	13.10 ± 0.00r	0.41 ± 0.00j	31.95	3.64 ± 0.04e	0.95 ± 0.01f
B2S05	14.77± 0.05g	0.32 ± 0.03de	13.30 ± 0.02p	0.51 ± 0.02f	26.34	3.66 ± 0.00e	1.10 ± 0.24f
B2M5	15.01 ± 0.01fg	0.34 ± 0.03d	13.70 ± 0.04n	0.48 ± 0.02h	28.84	3.70 ± 0.00d	1.04 ± 0.21f
B2F5	17.81 ± 0.01d	0.53 ± 0.01b	15.10 ± 0.12j	0.49 ± 0.02gh	31.13	3.99 ± 0.01b	0.96 ± 0.23f
B2C5	18.05 ± 0.14d	0.41 ± 0.06c	15.40 ± 0.00i	0.49 ± 0.00fgh	31.43	3.47 ± 0.00gh	19.01 ± 0.05e
B3	22.95 ± 0.01b	0.48 ± 0.04bc	20.30 ± 0.02d	0.72 ± 0.00c	28.19	3.25 ± 0.00l	73.13 ± 1.48b
B3S01	22.47 ± 0.07bc	0.42 ± 0.08c	19.80 ± 0.03e	0.71 ± 0.01cd	27.89	3.30 ± 0.00k	70.45 ± 2.41c
B3S05	23.01 ± 0.02b	0.53 ± 0.02b	20.50 ± 0.01c	0.70 ± 0.00de	29.29	3.35 ± 0.00j	73.24 ± 1.03b
B3M5	23.09 ± 0.14b	0.50 ± 0.06b	20.60 ± 0.01b	0.76 ± 0.01b	27.28	3.46 ± 0.07h	75.97 ± 2.38a
B3F5	25.88 ± 0.06a	0.74 ± 0.05a	22.40 ± 0.02a	0.79 ± 0.00a	28.35	3.59 ± 0.00f	28.75 ± 1.17d
B3K5	22.15 ± 0.07c	0.51 ±0.06b	19.70 ± 0.03f	0.69 ± 0.01e	28.55	3.33 ± 0.00jk	70.57 ± 4.84c
BK	16.71 ± 0.20e	0.51 ± 0.01b	14.00 ± 0.02l	0.29 ± 0.00k	48.28	4.47 ± 0.00a	1.93 ± 0.13f

MA* : malic acid; Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-s) are significantly different (homogenous groups) at $p \leq 0.05$. Sample codes have their references in **Annex 1b.**.

Storage time: 6 months

Sample code	Physico-chemical parameters						
	DM (g/100 g fw)	**Ashes (g/100 g fw)**	**TSS (°Brix)**	**TA (g of MA*/100 g fw)**	**TSS/TA**	**pH**	**Vitamin C (mg/100 g fw)**
B1	13.54 ± 0.07o	0.28 ± 0.04fgh	13.00 ± 0.00n	0.50 ± 0.01c	26.26	3.26 ± 0.00i	0.40 ± 0.05k
B1S01	13.92 ± 0.08n	0.24 ± 0.01h	13.30 ± 0.02m	0.44 ± 0.01d	30.57	3.33 ± 0.01fgh	0.58 ± 0.02jk
B1S05	14.49 ± 0.06l	0.34 ± 0.03e	14.00 ± 0.03j	0.48 ± 0.01c	29.47	3.29 ± 0.00ghi	0.50 ± 0.01jk
B1M5	14.85 ± 0.11k	0.33 ± 0.02ef	14.50 ± 0.01i	0.49 ± 0.01c	29.90	3.42 ± 0.01e	0.50 ± 0.04jk
B1F5	17.48 ± 0.04h	0.54 ± 0.01b	15.80 ± 0.03f	0.49 ± 0.01c	32.58	3.64 ± 0.03c	0.79 ± 0.01ijk
B1K5	14.28 ± 0.04m	0.27 ± 0.01gh	13.80 ± 0.01k	0.41 ± 0.01d	34.07b	3.37 ± 0.01f	0.51 ± 0.04jk
B1C5	16.92 ± 0.06i	0.32 ± 0.01efg	16.00 ± 0.01e	0.49 ± 0.01c	32.99	3.27 ± 0.00hi	1.13 ± 0.01hij
B2	14.50 ± 0.01l	0.25 ± 0.01h	12.80 ± 0.01o	0.42 ± 0.01d	30.84	3.57 ± 0.00d	1.32 ± 0.02hi
B2S01	14.77 ± 0.04k	0.25 ± 0.01h	13.00 ± 0.02n	0.41 ± 0.01d	32.10	3.60 ± 0.00cd	0.64 ± 0.01jk
B2S05	14.78 ± 0.04k	0.34 ± 0.01e	13.10 ± 0.03n	0.50 ± 0.01c	26.20	3.45 ± 0.00e	0.80 ± 0.01ijk
B2M5	15.07 ± 0.05j	0.35 ± 0.01e	13.60 ± 0.01l	0.48 ± 0.01c	28.33	3.62 ± 0.00c	0.49 ±0.08jk
B2F5	17.82 ± 0.01g	0.53 ± 0.02bc	15.00 ± 0.25h	0.49 ± 0.01c	30.93	3.87 ± 0.01b	0.37 ± 0.05k
B2C5	18.08 ± 0.13f	0.42 ± 0.04d	15.30 ± 0.15g	0.50 ± 0.01c	30.91	3.37 ± 0.00f	17.26 ± 0.34g
B3	22.99 ± 0.01c	0.48 ± 0.02c	20.20 ± 0.00c	0.72 ± 0.01b	28.06	3.33 ± 0.13fg	71.84 ± 0.77a
B3S01	22.60 ± 0.01d	0.42 ± 0.07d	19.60 ± 0.02d	0.72 ± 0.01b	27.41	3.26 ± 0.01i	70.01 ± 0.94b
B3S05	23.07 ± 0.07bc	0.54 ± 0.01b	20.30 ± 0.03c	0.71 ± 0.01b	28.79	3.28 ± 0.02ghi	68.03 ± 0.04c
B3M5	23.18 ± 0.06b	0.51 ± 0.06bc	20.50 ± 0.02b	0.74 ± 0.01b	27.89	3.36 ± 0.01f	62.95 ± 0.18e
B3F5	25.95 ± 0.06a	0.74 ± 0.03a	22.20 ± 0.04a	0.78 ± 0.02a	28.65	3.46 ± 0.00e	31.28 ± 0.54f
B3K5	22.20 ± 0.07e	0.51 ± 0.04bc	19.60 ± 0.12d	0.72 ± 0.07b	27.22	3.30 ± 0.00ghi	63.64 ± 0.78d
BK	17.03 ± 0.28i	0.53 ± 0.01bc	14.00 ± 0.02j	0.29 ± 0.01e	49.12	4.36 ± 0.00a	1.73 ± 0.01h

MA* : malic acid; Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-s) are significantly different (homogenous groups) at $p \leq 0.05$. Sample codes have their references in **Annex 1b.**.

3.2.2.1. Colour measurement

Colour is one of the most important parameters determining product quality and this parameter is the first stage in sensorial analysis to evaluate the consumer desirability (*Nowicka and Wojdyło, 2015*), as reported in **Paragraph 3.2.1.**. Colorimetric measurements using the colorimeters allowed the detection of differences indiscernible to the human eye and displayed these differences in numerical terms. In the obtained smoothies and juices after processing and storage colour related parameters were measured: colour lightness (L^*), redness (a^*), yellowness (b^*) and total change in colour (ΔE^*) values (**Figure 20.** and **Table 10.**). The study showed that the colour of analysed final products differed significantly ($p \leq 0.05$). In addition, some significant changes in colour were observed during the storage.

The value of L^* parameter in the analysed final products immediately after processing ranged from 29.25 to 63.90, while after 3 and 6 months ranged from 30.31 to 62.78 and 31.18 to 61.52, respectively. The highest value of parameter L^* was detected in persimmon purée (BK), followed by product composed of 25 % persimmon purée and 75 % apple juice (B2); (L^* = 61.11, 60.06, 61.52 for 0, 3 and 6 months, respectively). In turn, the lowest value of parameter L^* (29.25, 30.31 and 31.18 for 0, 3 and 6 months, respectively) was detected in juice with base B1 and addition of 5 % purple myrtle berries extract (B1M5). Taking product B1 as the standard, (present in all final products, except of product BK) only in the case of products B3S05 and B1S01 (products enriched with 0.5 and 0.1 % of saffron flower juice, respectively) was there no significant change in parameter L^*. This was due to the fact that the lightness of the products B1 (L^* = 50.54), B3S05 (L^* = 50.63) and B1S01 (L^* = 49.60) were not significantly different ($p > 0.05$), even after saffron by-product addition immediately after preparation. Similar relationships were observed after 3 and 6 months storage. In general the storage of final products caused

a slight increase in their brightness except in product BK, which appeared to be darker. Moreover, it is worth noting that the L^* parameter also changed in all three bases, when additional components (saffron flower juice, purple myrtle berries, feijoa flowers and strawberry tree or persimmon fruits) were added. It was observed that each final product as pure base (B1, B2 and B3) got darker after addition of plant material components. The exceptions were products B1K5, B1C5, and B3K5 which were brighter than the pure base (L^* = 54.75, 54.92 and 55.51, respectively) immediately after processing. The same relationship was observed during storage.

The value of a^* parameter in the final products immediately after processing ranged from 1.49 to 18.30, while after 3 and 6 months ranged from 2.05 to 14.55 and 0.98 to 13.38, respectively. The most intense red colour (a^*) was detected in product composed of base B3 and 0.5 % of saffron flower juice, while a less intense red colour (a^*) was observed in 100 % apple juice, followed by apple juice with 0.1 % of saffron flower juice (a^* = 2.74, 2.28 and 2.28, for 0, 3 and 6 months, respectively). In general, the storage of final products caused a decrease in their redness. Furthermore, it is worth noting that the value of a^* parameter changed also in all three bases, when semi-products (saffron flower juice, purple myrtle berries, feijoa flowers and strawberry tree or persimmon fruits) were added. It was observed that each final product as pure base (B1, B2 and B3) was redder after addition of plant material components. The exception was the product B3M5, B3F5 and B3K5, which appeared to be less red than the pure base (a^* = 15.59, 10.44 and 16.56, respectively) immediately after processing. Similar relationships were observed during storage.

The value of b^* parameter in the analysed final products immediately after processing ranged from -1.73 to 30.63, while after 3 and 6 months ranged from -1.53 to 33.61 and 0.26 to 36.67, respectively. The most intense yellow colour (b^*) was detected in products composed of pure base B3, followed by product composed of this base and 0.1 % saffron flower juice, followed by product B3K5 (b^* = 30.33; 0 months). In turn the less intense

yellow colour (b^*) was detected in all products containing 5 % of purple myrtle berries (b^*= -1.48, -1.73 and 2.72 for B1M5, B2M5 and B3M5, respectively; 0 months). Furthermore, it is worth noting that the value of b^* parameter also changed in all three bases, when semi-products (saffron flower juice, purple myrtle berries, feijoa flowers and strawberry tree or persimmon fruits) were added. It was observed that each final product as pure base (B1, B2 and B3) was less yellow after the addition of plant material components. The exceptions were the products B1K5, B1C5, B2C5 and B3S01 which appeared to be more yellow than the pure base (b^* = 18.13, 24.99, 25.64 and 30.63, respectively) immediately after processing. Similar relationships were observed during storage.

While analyzing the colour of examined final products, it was possible to distinguish three separate product groups: bright, red and yellow. The first group of products were juices and smoothies of bright colour, comparable to the control product (B1). Among them were all products to which were added 5 % of dry strawberry tree fruits and persimmon purée (B1K5, B1C5, B2C5 and B3K5). The value of L^* parameter in these products ranged from 54.75 to 57.31. The other products brighter than the control were products B2 and B3, as well as four others composed from those two bases (B2 and B3) and with 0.1 and 0.5 % of added saffron flower juice (B2S01, B2S05, B3S01 and B3S05). The value of L^* parameter in these six products ranged from 50.63 to 61.11. It is noteworthy that, the addition of strawberry tree and persimmon fruits made the final products brighter compared to 100 % apple juice. On the other hand, adding purple myrtle berry extract, and feijoa flowers to each base, made the final products darker, producing lower parameter L^* results than in the control. Moreover, a* and b* values of analysed final products had significant differences ($p \leq 0.05$) with those of control juice. The different colour of these products is related to the nature of the additional plant materials. Persimmon and strawberry tree fruits are characterised by red-orange skin and pulp: therefore a^* and b^* value was higher in products containing these semi-products than in the

control juice. However, high a^* and b* value in final products with *D. kaki* and *A. unedo* was probably due to a high content of carotenoids (*Izuchi et al., 2009; Delgado-Pelayo et al., 2016*). Moreover, feijoa flowers, saffron flower juice and myrtle purple berries are characterised by a blue-violet colour parts: thus a^* was higher but b^* value was lower in products containing these semi-products than in the control juice (B1). The only exceptions were products B2 and B3 with 0.1 and 0.5 % of added saffron flower juice, which were redder and more yellow than 100 % apple juice (control). This variation could be due to the nature of the pigments in these plant material cultivars, especially anthocyanins content which produces darker colours.

To sum up, the second group of juices and smoothies of red colour, comparable to the control product (B1) were products from apple juice mixed with all analysed plant materials, while the third and the last group of products were the products obtained from three mases (B1, B2 and B3) mixed with 5 % persimmon and/or strawberry tree fruits, as well as products where these two semi-products accounted for 25 % of whole product. Moreover, products composed from base B2 and B3 with 0.1 and 0.5 % of added saffron flower juice belonged to this group. These differences in the results might be attributable to the differences in anthocyanin composition (*Wojdyło et al., 2013b*) in *A. unedo, M. communis, A. sellanowa,* and *C. sativus*. As described by (*Koponen et al., 2007*) in nature, cyanidin has more redness than delphinidin derivatives.

The parameter ΔE^*, describing the human eye's ability to discriminate between the colours of two different products is also important from the perspective of the processing industry. It is well known that a consumer can distinguish the colour of two different samples only when values of ΔE^* are higher or equal 5 (*Pérez-Magariño and Gonzales-Sanjosé, 2003*). The values of ΔE^* parameter in the analysed final products immediately after processing ranged from 1.80 to 28.52, while after 3 and 6 months ranged from 0.80 to 31.19 and 1.89 to 27.75, respectively. The highest value of ΔE^* was detected in B1M5

before and after 3 months storage, while after 6 months storage the highest value of ΔE^* was observed in B3. In turn, the lowest value of ΔE^* was observed in B1S01 before and after 6 months storage, while after 3 months storage the lowest value of ΔE^* was observed in B1K5.

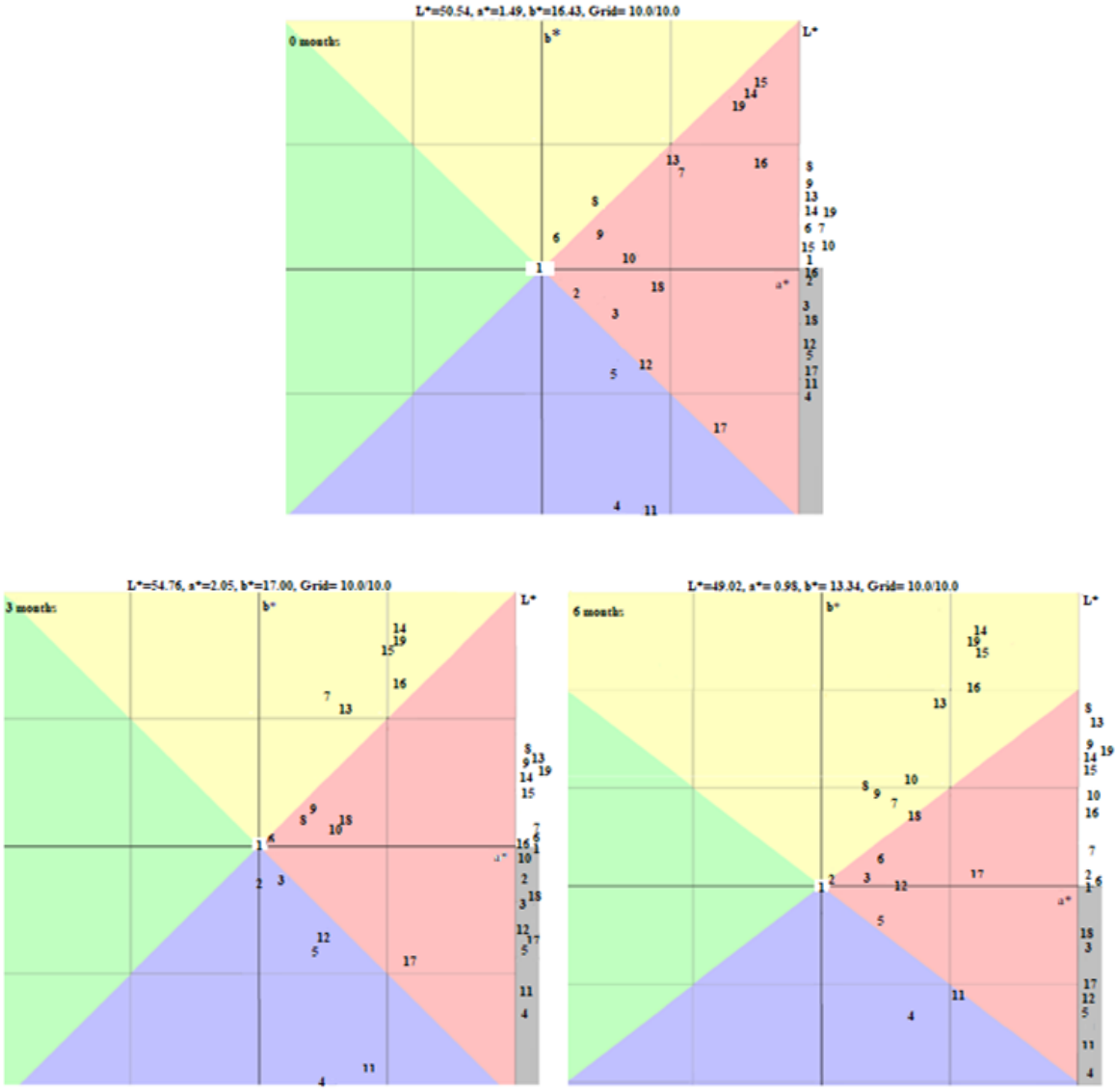


Figure 20. Colour parameters of final products using as a standard 100 % apple juice (B1).

1-B1; 2-B1S01; 3-B1S05; 4-B1M5; 5-B1F5; 6-B1K5; 7-B1C5; 8-B2; 9-B2S01; 10-B2S05; 11-B2M5; 12-B2F5; 13-B2C5; 14-B3; 15-B3S01; 16-B3S05; 17-B3M5; 18-B3F5; 19-B3K5.

Table 10. Colour parameters of final products before and after storage time (3 and 6 months) at 20 ± 2 °C.

Sample code	Colour parameters											
	Immediately after processing (0 months)				3 months				6 months			
	L^*	a^*	b^*	ΔE^*	L^*	a^*	b^*	ΔE^*	L^*	a^*	b^*	ΔE^*
B1	$50.54\pm0.03_{f}$	$1.49\pm0.00_{r}$	$16.43\pm0.05_{i}$	-	$54.76\pm0.06_{ef}$	$2.05\pm0.00_{o}$	$17.00\pm0.02_{j}$	-	$49.02\pm0.08_{g}$	$0.98\pm0.00_{n}$	$13.34\pm0.11_{l}$	-
B1S01	$49.60\pm0.05_{f}$	$2.74\pm0.02_{p}$	$15.53\pm0.02_{j}$	$1.80\pm0.00_{o}$	$50.33\pm0.18_{g}$	$2.28\pm0.00_{n}$	$13.61\pm0.06_{k}$	$5.58\pm0.13_{n}$	$49.60\pm0.14_{g}$	$2.28\pm0.02_{m}$	$14.59\pm0.05_{k}$	$1.89\pm0.00_{p}$
B1S05	$44.13\pm0.06_{g}$	$6.11\pm0.03_{no}$	$13.88\pm0.01_{k}$	$8.30\pm0.02_{m}$	$44.90\pm0.06_{h}$	$4.50\pm0.01_{l}$	$13.67\pm0.02_{k}$	$10.69\pm0.01_{k}$	$43.69\pm0.11_{h}$	$5.12\pm0.06_{l}$	$14.27\pm0.03_{k}$	$6.81\pm0.00_{n}$
B1M5	$29.25\pm0.00_{l}$	$7.78\pm0.00_{l}$	$-1.48\pm0.00_{o}$	$28.52\pm0.03_{a}$	$30.31\pm0.05_{k}$	$7.66\pm0.01_{g}$	$-1.53\pm0.00_{o}$	$31.19\pm0.02_{a}$	$31.18\pm0.03_{m}$	$7.48\pm0.02_{h}$	$0.26\pm0.00_{o}$	$23.06\pm0.12_{f}$
B1F5	$37.29\pm0.13_{i}$	$7.30\pm0.06_{m}$	$8.75\pm0.03_{m}$	$16.38\pm0.11_{f}$	$38.57\pm0.04_{i}$	$6.05\pm0.03_{j}$	$10.07\pm0.12_{l}$	$18.06\pm0.03_{f}$	$37.81\pm0.04_{k}$	$5.79\pm0.02_{k}$	$10.57\pm0.03_{m}$	$12.51\pm0.06_{l}$
B1K5	$54.75\pm0.06_{de}$	$2.83\pm0.02_{p}$	$18.13\pm0.12_{g}$	$4.73\pm0.03_{n}$	$55.03\pm0.03_{e}$	$2.72\pm0.01_{m}$	$17.34\pm0.03_{j}$	$0.80\pm0.00_{o}$	$49.96\pm0.05_{g}$	$5.66\pm0.03_{k}$	$16.30\pm0.06_{j}$	$5.62\pm0.00_{o}$
B1C5	$54.92\pm0.21_{de}$	$12.36\pm0.05_{e}$	$24.99\pm0.02_{d}$	$14.51\pm0.02_{h}$	$57.50\pm0.11_{cd}$	$7.86\pm0.04_{fg}$	$28.67\pm0.06_{d}$	$13.32\pm0.34_{i}$	$51.44\pm0.02_{f}$	$7.48\pm0.12_{h}$	$22.08\pm0.11_{h}$	$11.16\pm0.13_{m}$
B2	$61.11\pm0.05_{b}$	$6.00\pm0.00_{o}$	$21.59\pm0.01_{e}$	$12.60\pm0.06_{j}$	$60.06\pm0.23_{b}$	$5.41\pm0.03_{k}$	$19.38\pm0.012_{h}$	$6.71\pm0.02_{l}$	$61.52\pm0.06_{a}$	$5.79\pm0.02_{k}$	$23.18\pm0.12_{g}$	$16.62\pm0.05_{h}$
B2S01	$58.44\pm0.13_{c}$	$6.35\pm0.01_{n}$	$19.17\pm0.02_{f}$	$9.67\pm0.07_{l}$	$58.82\pm0.11_{bc}$	$6.47\pm0.06_{i}$	$20.01\pm0.02_{g}$	$6.71\pm0.01_{l}$	$59.80\pm0.02_{b}$	$6.62\pm0.03_{j}$	$22.70\pm0.03_{g}$	$15.35\pm0.01_{i}$
B2S05	$53.58\pm0.02_{e}$	$8.81\pm0.01_{j}$	$17.58\pm0.03_{h}$	$8.01\pm0.02_{m}$	$53.65\pm0.12_{f}$	$7.85\pm0.00_{fg}$	$18.24\pm0.03_{i}$	$6.03\pm0.01_{m}$	$56.36\pm0.01_{de}$	$8.36\pm0.01_{g}$	$24.01\pm0.06_{f}$	$14.91\pm0.02_{j}$
B2M5	$31.95\pm0.11_{k}$	$12.05\pm0.03_{f}$	$-1.73\pm0.00_{o}$	$28.05\pm0.01_{b}$	$33.26\pm0.03_{j}$	$10.75\pm0.00_{d}$	$-0.67\pm0.01_{n}$	$29.16\pm0.12_{b}$	$34.49\pm0.03_{l}$	$11.54\pm0.10_{d}$	$2.07\pm0.00_{n}$	$21.20\pm0.05_{g}$
B2F5	$39.79\pm0.02_{h}$	$8.26\pm0.02_{k}$	$10.41\pm0.01_{l}$	$14.06\pm0.05_{i}$	$39.80\pm0.04_{i}$	$6.99\pm0.02_{h}$	$10.55\pm0.01_{l}$	$17.02\pm0.10_{g}$	$39.86\pm0.05_{j}$	$7.10\pm0.02_{i}$	$12.94\pm0.03_{l}$	$11.02\pm0.00_{m}$
B2C5	$57.31\pm0.05_{c}$	$11.50\pm0.05_{g}$	$25.64\pm0.03_{c}$	$15.19\pm0.03_{g}$	$59.75\pm0.06_{b}$	$9.15\pm0.03_{e}$	$27.64\pm0.00_{e}$	$13.73\pm0.06_{h}$	$61.24\pm0.10_{a}$	$9.70\pm0.01_{e}$	$31.86\pm0.05_{d}$	$23.84\pm0.06_{e}$
B3	$55.18\pm0.08_{d}$	$16.76\pm0.02_{c}$	$30.61\pm0.04_{a}$	$21.35\pm0.01_{d}$	$57.54\pm0.05_{cd}$	$12.59\pm0.04_{b}$	$33.61\pm0.02_{a}$	$19.87\pm0.00_{d}$	$58.41\pm0.11_{c}$	$12.72\pm0.02_{bc}$	$36.67\pm0.02_{a}$	$27.75\pm0.02_{a}$
B3S01	$54.59\pm0.14_{de}$	$17.05\pm0.01_{b}$	$30.63\pm0.01_{a}$	$21.45\pm0.02_{d}$	$56.67\pm0.02_{d}$	$12.29\pm0.01_{c}$	$32.81\pm0.05_{b}$	$18.93\pm0.02_{e}$	$57.40\pm0.06_{cd}$	$12.78\pm0.03_{b}$	$35.61\pm0.03_{b}$	$26.56\pm0.01_{c}$
B3S05	$50.63\pm0.13_{f}$	$18.30\pm0.02_{a}$	$25.27\pm0.10_{cd}$	$18.99\pm0.10_{e}$	$54.50\pm0.03_{ef}$	$12.69\pm0.01_{b}$	$30.72\pm0.03_{c}$	$17.36\pm0.03_{g}$	$55.24\pm0.02_{e}$	$12.75\pm0.05_{bc}$	$33.81\pm0.02_{c}$	$24.42\pm0.00_{d}$
B3M5	$33.38\pm0.02_{j}$	$15.59\pm0.12_{d}$	$2.72\pm0.00_{n}$	$26.10\pm0.12_{c}$	$38.69\pm0.02_{i}$	$14.55\pm0.00_{a}$	$8.71\pm0.00_{m}$	$21.98\pm0.10_{c}$	$41.75\pm0.03_{i}$	$13.38\pm0.11_{a}$	$14.75\pm0.01_{k}$	$14.44\pm0.01_{k}$
B3F5	$42.95\pm0.03_{g}$	$10.44\pm0.03_{h}$	$15.80\pm0.11_{j}$	$11.75\pm0.05_{k}$	$44.66\pm0.10_{h}$	$8.04\pm0.05_{f}$	$18.40\pm0.02_{i}$	$11.83\pm0.06_{j}$	$44.78\pm0.05_{h}$	$8.46\pm0.03_{fg}$	$20.45\pm0.04_{i}$	$11.16\pm0.03_{m}$
B3K5	$55.51\pm0.02_{d}$	$16.56\pm0.03_{c}$	$30.33\pm0.06_{a}$	$21.10\pm0.06_{d}$	$57.73\pm0.12_{cd}$	$12.56\pm0.06_{b}$	$33.46\pm0.15_{a}$	$19.75\pm0.01_{d}$	$58.42\pm0.13_{c}$	$12.53\pm0.06_{c}$	$35.95\pm0.10_{b}$	$27.07\pm0.14_{b}$
BK	$63.90\pm0.12_{a}$	$9.54\pm0.03_{i}$	$28.16\pm0.24_{b}$	-	$62.78\pm0.15_{a}$	$9.01\pm0.00_{e}$	$25.50\pm0.11_{f}$	-	$60.85\pm0.01_{ab}$	$8.64\pm0.03_{f}$	$25.83\pm0.02_{e}$	-

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-p) are significantly different (homogenous groups) at $p \leq 0.05$. Sample codes have their references in **Annex 1b.**. STD parameter is B1. L*: lightness; a*: indicates red for positive value and green for negative value; b* - indicates yellow for positive value and blue for negative value; ΔE* - total colour difference.

3.2.3. Chemical investigation

3.2.3.1. Sugar content

The analysed 20 final products were tested for sugar content. For this purpose, the standard mixture of selected sugars was prepared and presented in **Figure 15.** (**Paragraph 3.1.2.1.**). **Table 11.** reports the exact profile of sugars in all analysed final products according to their retention time (Rt) order and the total content of these compounds.

Table 11. Sugar content (g/100 g fw) of final products immediately after processing.

Sample code	Sugar content (g/100 g fw)					
	Rhamnose	**Fructose**	**Sorbitol**	**Glucose**	**Sucrose**	**Total**
B1	nd	6.73 ± 0.23l	0.07 ± 0.00efg	0.97 ± 0.05i	0.32 ± 0.02e	8.10 ± 0.03o
B1S01	nd	6.77 ± 0.15l	0.07 ± 0.01efgh	1.02 ± 0.10i	0.32 ± 0.01e	8.17 ± 0.04o
B1S05	nd	9.34 ± 0.10h	0.10 ± 0.01cd	1.33 ± 0.00hi	0.36 ± 0.02c	11.13 ± 0.03k
B1M5	nd	11.47 ± 0.05d	0.12 ± 0.02bc	2.57 ± 0.01cde	0.23 ± 0.00hi	14.40 ± 0.03f
B1F5	nd	10.93 ± 0.42e	0.11 ± 0.00cd	2.03 ± 0.02fg	0.50 ± 0.01b	13.56 ± 0.02h
B1K5	nd	11.02 ± 0.01e	0.14 ± 0.01ab	0.98 ± 0.00i	0.34 ± 0.01d	12.48 ± 0.00j
B1C5	nd	11.72 ± 0.22d	0.16 ± 0.02a	2.40 ± 0.00cdef	0.57 ± 0.02a	14.85 ± 0.03e
B2	nd	5.94 ± 0.21m	0.03 ± 0.00j	1.59± 0.05gh	0.01 ± 0.00j	7.57 ± 0.02p
B2S01	nd	8.06 ± 0.34j	0.04 ± 0.00ij	2.62 ± 0.80cde	nd	10.72 ± 0.01m
B2S05	nd	9.42 ± 0.02h	0.08 ± 0.00def	2.92 ± 0.68bc	nd	12.43 ± 0.05j
B2M5	nd	13.12 ± 0.05b	0.10 ± 0.05cd	4.20 ± 0.04a	nd	17.42 ± 0.06a
B2F5	nd	7.72 ± 0.10k	0.04 ± 0.00hij	2.85 ± 0.03bcd	nd	10.61 ± 0.15n
B2C5	nd	8.69 ± 0.01i	0.06 ± 0.00fghi	2.19 ± 0.02ef	nd	10.94 ± 0.10l
B3	nd	10.11 ± 0.12g	0.04 ± 0.00ij	2.86 ± 0.00bcd	0.26 ± 0.01f	13.27 ± 0.00i
B3S01	nd	11.08 ± 0.26e	0.10 ± 0.01cd	2.67 ± 0.00cde	0.25 ± 0.00fg	14.10 ± 0.03g
B3S05	nd	12.39 ± 0.06c	0.09 ± 0.00de	2.64 ± 0.01cde	0.24 ± 0.01gh	15.36 ± 0.03d
B3M5	nd	13.49 ± 0.05a	0.03 ± 0.00j	2.67 ± 0.52cde	0.31 ± 0.02e	16.50 ± 0.02b
B3F5	nd	12.37 ± 0.00c	0.08 ± 0.00def	3.19 ± 0.43b	0.22 ± 0.00hi	15.87 ± 0.08c
B3K5	nd	10.60 ±0.02f	0.05 ± 0.00ghij	2.47 ± 0.04cdef	0.22 ± 0.00i	13.34 ± 0.01i
BK	nd	3.00 ± 0.00n	nd	2.35 ± 0.01def	nd	5.35 ± 0.12r

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-r) are significantly different (homogenous groups) at $p \leq 0.05$. Sample codes have their references in **Annex 1b.**.

The study showed that sugar content of analysed final products differed significantly ($p \leq 0.05$). In fact, by mixing various plant materials, specific sugars can be present in the

final products. For example, using apple juice as a basic semi-product for final products preparation enriched them in sorbitol and sucrose.

Generally, fructose, glucose, sucrose and sorbitol were the main sugars detected in final products, while no traces of rhamnose were detected. The total sugar content in all analysed final products immediately after processing ranged from 5.35 to 17.42. The highest total sugar content was found in product composed of base B2 with 5 % of purple myrtle berry extract added, followed by product composed of base B3 with the same semi-product added (B3M5; 16.50 g/100 g fw). In contrast, the lowest total content of sugar characterized product BK (100 % persimmon purée), followed by product B1 (100 % apple juice; 8.10 g/100 g fw), which were the simplest versions of prepared final products, and were not enriched with other additional components. Moreover, in final product B2 low sugar content (7.57 g/100 g fw) was observed, which could be connected to diluting the persimmon purée with apple juice. Analysis of sugar content after storage time (3 and 6 months) was not performed because, as observed in **Paragraph 3.2.2.**, no significant changes were observed in total soluble content (TSS), strongly connected to sugar content. Moreover, the aim of this thesis project was more connected with bioactive organic compounds like polyphenols.

Fructose and glucose were the abundant sugars, while sorbitol and sucrose content was much lower and was not detected in BK, nor was sucrose detected in products from set B2, barring very low amounts in the pure base (0.01 g/100 g fw). However, analyzing every single sugar, some interesting results were observed. The fructose content in all final products immediately after processing ranged from 3.00 to 13.49 g/100 g fw. The highest fructose content was detected in product B3M5, followed by product B2M5 (13.12 g/100 g fw), while the lowest fructose content was found in persimmon purée (BK). It is noteworthy that, the addition of purple myrtle berries to all bases (B1, B2 and B3), made them the richest version of the final product among each set, except set B1, where product

with an added of 5 % strawberry tree fruits was also rich in fructose (11.72 g/100 g fw). On the other hand, the glucose content of all final products immediately after processing ranged from 0.97 to 3.19 g/100 g fw. The highest glucose content was detected in product B3F5, composed of the base containing 25 % of strawberry tree fruits and 75 % apple juice with an added 5 % of feijoa flowers. In turn, the lowest content of glucose was observed in 100 % apple juice, followed by product - B1K5, this juice containing an additional component of persimmon purée (0.98 g/100 g fw). It is worth noting, that the presence of *A. unedo* fruits made the final product rich in glucose. Moreover, feijoa flower and saffron flower juice addition increased the glucose content in all sets, while enrichment in purple myrtle berry extrac increased the glucose content in products from set B1 and B2, but decreased its content in set B3. Next, sorbitol and sucrose content in all final products ranged from 0.03 to 0.16 g/100 g fw and 0.01 to 0.57 g/100 g fw, respectively. The highest content of sorbitol was detected in product B1C5, while the lowest in product B2 and B3M5. Regarding sucrose, the highest content was evaluated in product B1C5, followed by product B1F5 (0.50 g/100 g fw), while the lowest was in product B2. The absence of sucrose in the products from set B2 could be the result of abundant dilution of apple juice by other semi-products.

It should be noted that the final products are rich in fructose that has better metabolic properties than glucose and sucrose due to its lower glycemic index (**CHAPTER 3.1.**). Moreover, regarding sugar composition in the investigated plant materials, it is known that fructose and glucose are sweeter than sucrose (*Wojdyło et al., 2017*), and in addition fructose has higher relative sweetness than glucose (*Veberic et al., 2008*). Therefore, consumers' perception of sweetness in analysed final products, in all likelihood, is mostly related to their content of fructose and glucose. Mixing different plant material semi-products may be a good way to correct overly sour or sweet tastes, which would lead to obtaining an attractive product, as in the present study. Moreover, the impact of sugar-

containing foods varies according to food class. Given that excessive dietary sugar intake may be associated with an increased risk of cardiovascular and metabolic diseases. Therefore, it is necessary to limit the dialy consumption of food products containing particular quantities of total sugar content to the healthy recommended sugar limits (*Medicinal News Today, 2019*). Finally, the quality of foods should be assessed not only based on their sugar content, but also on their micronutrient and dietary fibre contents (*Tappy, 2018*).

3.2.3.2. Organic acid content

The analysed 20 final products were tested for organic acid content according to the standard mixture of selected organic acids that was prepared and presented in **Figure 16.** (**Paragraph 3.1.2.2.**). **Table 12.**. reports the quantitative analysis of organic acids in all analysed final products, and the results were presented according to their retention time (Rt) order. Moreover, the total content of these compounds was reported**.** Nine organic acids were identified and quantified in analysed final products: oxalic, citric, isocitric, tartaric, malic, quinic, ascorbic, shikimic and fumaric acid. Lactic and succinic acid were not detected. The study showed that the organic acid content of analysed beverages differed significantly ($p \leq 0.05$). Generally, the total organic acid content in all analysed final products immediately after processing ranged from 0.94 to 3.09 g/100 g fw. The highest total organic acid content was found in product composed of base B3 with an added 5 % of feijoa flowers. In contrast, pure apple juice (B1) being the simplest version among all analysed functional products, had the lowest total content of organic acids. Moreover, regarding each set of products, the highest organic acid content was found in all beverages based on mixed of strawberry tree fruits and apple juice in ratio 25:75 (B3). The results were in the range from 2.12 to 3.09 g/100 g fw. In contrast, the lowest content of

organic acid (0.94-1.15 g/100 g fw) was detected in the set of products based on 100 % apple juice (B1) and on mixes of persimmon purée and apple juice in ratios of 25:75 (B2), excluding final products with 5 % of feijoa flowers (B1F5 and B2F5) and 5 % strawberry tree fruits (B1C5 and B2C5), in which organic acid content was detected in a range from 1.26 to 1.58 g/100 g fw.

Analysis of organic acid content after storage time (3 and 6 months) was not performed, because, as observed in **Paragraph 3.2.2.**, only slight changes were observed in total acidity (TA) and pH. Organic acids have lower receptivity to change during processing and storage than other fruit components and thus they are a convenient index of authenticity in fruit products (*Nour et al., 2010b*). However, according to Igual et al. (*2010*), who used different method of grapefruit juice preservation (conventional treatment, microwave pasteurisation and frozen storage), some significant changes in organic acid content can be observed.

Quinic and malic acid were the most abundant organic acids in all analysed functional beverages, while other acids were detected in much lower quantities or not detected at all (lactic and succinic acid). Although, analyzing every single organic acid, some interesting results were observed. Quinic and malic acid were present in all final products in the highest quantities, in a range from 0.39 to 1.76 g/100 g fw and from 0.33 to 0.75 g/100 g fw, respectively. The highest content of quinic acid was detected in product composed of base B3 with 5 % addition of feijoa fruits, while the lowest content of quinic acid was observed in product composed of base B2 and the same additional component as above. In the case of malic acid, the highest content was found in the same product as quinic acid (B3F5), while the lowest content of malic acid was detected in pure persimmon purée. Moreover, all final products with base B3 were the richest in these two acids.

On the other hand, oxalic and citric acids were detected in all final products in much lower quantities (0.01-0.22 and 0.01-0.42 g/100 g fw, respectively), than the other two

acids. Generally, oxalic acid was detected in quantities ≤ 0.04 g/100 g fw in all final products, except in those with an additional 5 % of feijoa flowers (c.a. 0.20 g/100 g fw), while citric acid was detected in quantity ≤ 0.13 g/100 g fw in all final products, except of products B1F5, B2F5 and B3K5 (0.29, 0.42 and 0.20 g/100 g fw, respectively).

Tartaric acid was detected in pure apple juice and in all final products containing persimmon purée, except for B3K5, which could be connected to diluting the persimmon purée with apple juice and strawberry tree fruits. The values of this acid ranged from 0.01 to 0.20 g/100 g fw. Furthermore, the quantity of this acid was ≤ 0.03 g/100 g fw in all products with base B2, while in a case of product B4 the amount of tartaric acid was slightly higher (0.20 g/100 g of fw).

Regarding isocitirc acid, this compound was specific to some final products containing strawberry tree fruits (B3, B3M5 and B3F5). The amount of isocitiric acid present in these three products was 0.39, 0.25 and 0.31 g/100 g of fw, respectively. Finally, the presence of ascorbic, shikimic and fumaric acids was confirmed in all final products. The quantity of these three acids was very low (≤ 0.02 or traces).

The presence of particular organic acids guarantees specific tastes, flavours and aromas, as well as playing a role in stabilising and preserving foodstuffs (*Theron and Rykers Lues, 2011*). The appearance of different organic acids has a significant influence on the fruits' taste and colour (depending on pH), also taking into account the sugar/acid ratio (*Formica-Oliveira et al., 2017; Castillejo et al., 2016*). Recent studies (*Nowicka et al., 2017*) have shown that, by mixing different fruits, final products with specific qualities can be designed. Therefore, organic acid profile can be influenced by the ratios in which different fruits are mixed and used for final product preparation. Moreover, the use of feijoa flowers led to the highest content of citric and oxalic acid, while the use of strawberry tree fruits led to the highest content of isocitric and quinic acids. Furthermore,

use of purple myrtle berries, saffron flower juice, feijoa flowers and strawberry tree fruits led to the highest concentrations of malic acid.

Each organic acid occurring in fruits, posseses particular health benefits as described in **CHAPTER 3.1.**; **Paragraph 3.1.2.2.**. Moreover, these compounds stimulate the secretion of digestive enzymes, as well as regulate the proper chemical reactions of the body (*Seymour et al., 2008*).

Table 12. Organic acid content (g/100 g fw) of final products immediately after processing (0 months).

Sample code	Organic acid content (g/100 g fw)											
	Oxalic	**Citric**	**Isocitric**	**Tartaric**	**Malic**	**Quinic**	**Ascorbic**	**Lactic**	**Shikimic**	**Succinic**	**Fumaric**	**Total**
B1	0.01±0.00$_{g}$	0.01±0.00$_{i}$	nd	0.01±0.00$_{cd}$	0.48±0.01$_{fghi}$	0.44±0.01$_{hijk}$	tr	nd	tr	nd	tr	0.94±0.01$_{l}$
B1S01	0.01±0.00$_{g}$	0.01±0.00$_{hi}$	nd	nd	0.51±0.02$_{efg}$	0.44±0.01$_{hijk}$	tr	nd	tr	nd	tr	0.98±0.02$_{kl}$
B1S05	0.01±0.00$_{g}$	0.02±0.00$_{ghi}$	nd	nd	0.52±0.03$_{ef}$	0.52±0.01$_{gh}$	tr	nd	tr	nd	tr	1.07±0.06$_{j}$
B1M5	0.02±0.01$_{fg}$	0.03±0.00$_{ghi}$	nd	nd	0.56±0.02$_{de}$	0.53±0.03$_{g}$	tr	nd	0.01±0.00$_{a}$	nd	tr	1.15±0.03$_{i}$
B1F5	0.20±0.03$_{b}$	0.29±0.03$_{b}$	nd	nd	0.57±0.02$_{d}$	0.48±0.06$_{ghij}$	tr	nd	0.02±0.00$_{a}$	nd	tr	1.57±0.00$_{f}$
B1K5	0.02±0.01$_{fg}$	0.02±0.00$_{ghi}$	nd	0.06±0.01$_{b}$	0.50±0.02$_{fgh}$	0.47±0.04$_{ghijk}$	tr	nd	tr	nd	tr	1.07±0.01$_{j}$
B1C5	0.02±0.00$_{efg}$	0.03±0.00$_{ghi}$	nd	nd	0.58±0.03$_{cd}$	0.84±0.01$_{e}$	tr	nd	tr	nd	tr	1.48±0.02$_{g}$
B2	0.03±0.00$_{def}$	0.03±0.00$_{ghi}$	nd	0.03±0.01$_{c}$	0.47±0.02$_{ghi}$	0.43±0.01$_{ijk}$	tr	nd	tr	nd	tr	0.99±0.00$_{kl}$
B2S01	0.04±0.01$_{de}$	0.03±0.01$_{ghi}$	nd	0.03±0.00$_{c}$	0.46±0.02$_{hi}$	0.42±0.03$_{jk}$	tr	nd	tr	nd	tr	0.98±0.00$_{kl}$
B2S05	0.04±0.00$_{d}$	0.03±0.00$_{ghi}$	nd	0.03±0.00$_{c}$	0.43±0.03$_{i}$	0.42±0.05$_{ijk}$	tr	nd	tr	nd	tr	0.96±0.02$_{kl}$
B2M5	0.02±0.00$_{defg}$	0.04±0.00$_{g}$	nd	0.02±0.00$_{c}$	0.45±0.01$_{hi}$	0.46±0.03$_{ghijk}$	tr	nd	0.01±0.00$_{a}$	nd	tr	1.00±0.00$_{k}$
B2F5	0.22±0.02$_{a}$	0.42±0.03$_{a}$	nd	0.02±0.00$_{c}$	0.50±0.02$_{fgh}$	0.39±0.03$_{k}$	tr	nd	0.02±0.00$_{a}$	nd	0.01±0.00$_{a}$	1.58±0.05$_{f}$
B2C5	0.02±0.00$_{defg}$	0.04±0.00$_{gh}$	nd	0.02±0.01$_{c}$	0.47±0.04$_{ghi}$	0.70±0.01$_{f}$	tr	nd	tr	nd	tr	1.26±0.02$_{h}$
B3	0.04±0.00$_{c}$	tr	0.39±0.02$_{a}$	nd	0.71±0.03$_{ab}$	1.57±0.00$_{bc}$	tr	nd	tr	nd	tr	2.72±0.06$_{b}$
B3S01	0.01±0.00$_{g}$	0.01±0.00$_{hi}$	nd	nd	0.69±0.06$_{b}$	1.41±0.02$_{d}$	tr	nd	tr	nd	tr	2.12±0.01$_{e}$
B3S05	0.03±0.00$_{def}$	0.13±0.03$_{d}$	nd	nd	0.63±0.06$_{c}$	1.47±0.03$_{d}$	tr	nd	tr	nd	tr	2.27±0.00$_{d}$
B3M5	0.04±0.01$_{d}$	0.07±0.01$_{f}$	0.25±0.02$_{c}$	nd	0.70±0.02$_{ab}$	1.64±0.12$_{b}$	tr	nd	tr	nd	tr	2.70±0.01b
B3F5	0.19±0.01$_{b}$	0.08±0.01$_{ef}$	0.31±0.02$_{b}$	nd	0.75±0.03$_{a}$	1.76±0.06$_{a}$	tr	nd	tr	nd	tr	3.09±0.02$_{a}$
B3K5	0.04±0.00$_{cd}$	0.20±0.03$_{c}$	nd	nd	0.70±0.01$_{ab}$	1.54±0.05$_{c}$	tr	nd	0.01±0.00$_{a}$	nd	tr	2.50±0.05$_{c}$
BK	0.02±0.00$_{fg}$	0.09±0.01$_{e}$	nd	0.20±0.04$_{a}$	0.33±0.01$_{j}$	0.50±0.00$_{ghi}$	tr	nd	tr	nd	tr	1.15±0.03$_{i}$

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-l) are significantly different (homogenous groups) at $p \leq 0.05$. tr= ≤ 0.0001; nd ≤ LOD. Sample codes have their references in **Annex 1b.**.

3.2.3.3. Qualitative identification of phenolic compounds

The identification of polyphenolic compounds by LC-PDA-QTof/MS analysis of extracts from 20 final products was conducted in negative and positive ion mode, as described in **CHAPTER 3.1.** for qualitative identification of polyphenols in analysed plant materials. Obtained results were presented in **Table 13.** and **Figure 21.**.

The LC/MS analysis of all final products revealed the presence of 91 compounds in total, deriving from all studied plant materials in **CHAPTER 3.1.**. Moreover, these compounds varied between final products, starting from the three bases (B1, B2 and B3). In fact, base B1 (100 % apple juice) was characterised by the presence of all 17 compounds (5 hydroxycinnamic acids, 2 dihydrochalcones, 5 flavan-3-ols and 5 quercetin derivatives), which were detected also in the extract of dry apple. On the other hand, base B2 was rich in compounds deriving from both mixed plant materials (*M. domestica* and *D. kaki* fruits). All 17 compounds present in apple fruit were detected as well in product B2. In turn, persimmon guaranteed the presence of salicylic and syringic acid, as well as 2 hydroxybenzoic acid derivatives (galloyl glucoside II and III). Finally, as with base B2, in base B3 all 17 compounds deriving from apple were detected. Furthermore, strawberry tree fruits enriched the final product in other 20 polyphenols. Among these detected compounds were 2 anthocyanins (cyanidin-3-*O*-galactoside and -arabinoside), 10 hydroxybenzoic acid derivatives (gallic acid glucoside I and II, galloyl glucoside I, 3-*O*-galloylquinic acid, gallic acid 4-*O*-*β*-D-glucopyranoside, galloyl shikimic acid, digalloyl shikimic acid I and II, digalloyl quinic acid I, and ellagic acid arabinoside), 1 flavan-3-ol (procyanidin B3). Other 7 were flavonols, among which were present 2 quercetin derivatives, quercetin galloylhexose, myricetin-3-*O*-xyloside, myricetin-3-*O*-rhamnoside and 2 myricetin-3-*O*-hexosides (galactoside and glucoside). In addition, the polyphenol profile was investigated in BK (persimmon purée). In this final product 2 hydroxybenzoic

acid derivatives (galloyl glucoside II and III), syringic and salicylic acid, 3 hydroxycinnamic acids (chlorogenic, caffeic and *p*-coumaric acid), two flavan-3-ols (procyanidin B1 and (+)-catechin), and 5 flavanols (kaempferol-3-*O*-glucoside, quercetin-derivative, quercetin-3-*O*-rutinoside, -galactoside and -glucoside) could be found.

It was noticed that, during the process of base preparation some detected in plant materials (**CHAPTER 3.1.**) compounds were not detected. In the case of base B2, enriched with persimmon fruits these compounds were: protocatechuic acid, myricetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, quercetin-derivative III, quercetin-pentoside, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rhamnoside, while in a case of base rich strawberry tree fruits was not possible to find 3 anthocyanis (delphinidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside and delphinidin), 5 hydroxybenzoic acid derivatives (digalloylquinic acid II, strictinin ellagitannin, gallotannin derivative, ellagic acid xyloside and gallotannin), and kaempferol. Furthermore, in product BK protocatechuic acid, myricetin-3-*O*-glucoside, quercetin-pentoside and kaempferol-3-*O*-rhamnoside could not be found. The lack of these polyphenols was probably caused by dilution of the fruits with apple juice, or high temperatures used in pasteurization.

Based on LC/MS analysis of obtained final products, further identification of polyphenols in 3 sets of final products was performed. Apart from polyphenolic profile in 3 pure bases, additionally it was possible to identify other compounds deriving from each additional component (strawberry tree fruits, persimmon fruits, saffron flower juice, purple myrtle berry extract and feijoa flowers). The use of the LC-PDA-QTof/MS enabled confirmation of the presence of 81 (B1), 75 (B2) and 75 (B3) different phenolic compounds, belonging to seven subclasses (anthocyanins, hydroxybenzoic and hydroxycinnamic acids, dihydrochalcones, flavan-3-ols, flavonols and flavones).

Regarding each particular enriched functional juice with base B1, LC-MS metabolic profiles highlighted the presence of a large group of polyphenols, precisely: 25 in juice

with 0.1 % of saffron flower juice, 29 in juice with 0.5 % saffron flower juice, 34 in juice with 5 % of myrtle purple berry extract, 35 in juice with 5 % of feijoa flowers, 19 in juice with 5 % of persimmon purée and 38 in juice with 5 % of strawberry tree fruits.

The positive LC-MS metabolic profiles highlighted the presence of a total of 9 anthocyanins. According to the results obtained in **CHAPTER 3.1.**; **Paragraph 3.1.2.3.**, apple and persimmon extracts do not have anthocyans, therefore, the presence of these compounds in analysed final products was due to their enrichment with other plant materials (*A. unedo* fruits, *M. communis* berries, *A. sellowiana* flowers and *C. sativus* flower juice). The addition of 5 % strawberry tree fruits enriched apple juice in cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside, while the addition of 5 % purple myrtle berry extract, enriched apple juice in delphinidin-pentoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside. Moreover, delphinidin-3,5-*O*-diglucoside was detected only in juices containing 0.1 and 0.5 % of saffron flower juice, while delphinidin-3-*O*-glucoside was detected in juice BM5 and BS05. Cyanidin-3-*O*-glucoside was detected in product B1M5 and B1F5. The 5 % addition of *A. unedo* fruits was not enough to enrich the final juice in delphinidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside and delphinidin. On the other hand, enrichement in 5 % of purple myrtle berry extract did not result in finding in final juice three 3-*O*-arabinosides of delphinidin, petunidin and malvidin in the final juice. Moreover, the addition of saffron flower juice in amounts of 0.1 and 0.5 % was too little to enrich juice in petunidin-3,5-*O*-diglucoside and two 3-*O*-glucosides of petunidin and malvidin. Delphinidin-3-*O*-glucoside was not detected in B1S01, and the 5 % addition of feijoa flowers was not enough to enhance final products in this compound.

The negative LC-MS metabolic profiles highlighted the presence of a total of 28 hydroxybenzoic acids and their derivatives, 5 hydroxycinnamic acids, 2 dihydrochalcones, 6 flavan-3-ols, 30 flavonols in investigated final products with base B1. All hydroxycinnamic acids and dihydrochalcones were detected in all final products from this

set. Among detected hydroxycinnamic acids were neochlorogenic acid and *p*-coumaroyloquinic acid, most likely derived solely from apple fruits, while chlorogenic acid, caffeic acid and *p*-coumaric probably only derived from apple and persimmon fruits. Two dihydrochalcones (phloretin-2'-*O*-xyloglucoside and phloretin-2'-*O*-glucoside), were present in our juices probably solely from apples. In a case of flavan-3-ols only procyanidin B3 was characteristic of product B1C5 and other 5 (procyanidin B1, (+)-catechin, (-)-epicatechin, procyanidin B2, and procyanidin C1) were present in all products from the set B1.

In juice with addition of saffron flower juice no hydroxybenzoic acid derivatives were detected. Therefore, the presence of these compounds in analysed products was due to their enrichment with other plant materials (*A. unedo* fruits, *M. communis* berries, *A. sellowiana* flowers and *D. kaki* purée). Among detected hydroxybenzoic acids and their derivatives were 11 compounds detected only in juice enriched with strawberry tree fruits: gallic acid glucoside I and II, galloyl glucoside I, 3-*O*-galloylquinic acid (theogallin), gallic acid 4-*O*-*β*-D-glucopyranoside, galloyl shikimic acid, digalloylquinic acid I and II, digalloyl shikimic acid I and II, and strictinin ellagitannin. Moreover, another 10 hydroxbenzoic derivatives were found only in juices enriched with 5 % feijoa flowers. Among these were castalagin, casuarin, ellagitannin II and IV, nilocitin, casuarinin, ellagic acid and its two pentosides (arabinoside and xyloside) and methyl ellagic acid II. In addition, five hydroxybenzoic acid derivatives were detected only in juice with an additional 5 % purple myrtle berry extract. Among these were galloyl-HHDP-glucose I and II, digalloyl-HHDP-glucose I, ellagitannin I and III. Finally, in juice with an additional 5 % persimmon purée two hydroxybenzoic acid derivatives (galloyl glucoside II and III) were found. The addition of plant materials in selected quantities did not enrich product B1C5 in gallotannin and its derivative, and two ellagic acid pentosides (arabinoside and xyloside),

B1M5 in digalloyl-HHDP-glucose II and quinic acid 3,5-di-*O*-gallate, B1F5 in methyl ellagic acid I, and B1K5 in protocatechuic, syringic and salicylic acid.

Flavonols were the last group of polyphenols identified in all tested juices from set B1. In all these final products were found two quercetin-3-*O*-hexosides (galactoside and glucoside) and quercetin-3-*O*-rhamnoside. Furthermore, analysis in the LC-MS confirmed the presence of two quercetin-*O*-pentosides (arabinoside and xyloside) in all final products from set B1. In addition, another quercetin pentoside was detected only in juice with 5 % addition of fejioa flowers.

Unique to juices enriched with saffron flower juice were ten other flavonols. The mass spectrometric characterization of compounds, provided evidence for the presence of three kaempferol derivatives: kaempferol-3-*O*-sophoroside-7-*O*-glucoside, kaempferol-3,7-*O*-diglucoside, and kaempferol-3-*O*-sophoroside in both B1S01 and B1S05. Another five were detected as isorhamnetin derivatives: isorhamnetin-3,7-*O*-digalactoside, isorhamnetin-3,7-*O*-diglucoside and isorhamnetin-3-*O*-rutinoside in both B1S01 and B1S05, while isorhamnetin-3-*O*-sophoroside and isorhamnetin-3-*O*-glucoside were only detected in B1S05. Moreover, quercetin-3,7-*O*-digalactoside was found in juice enriched with 0.5 % saffron flower juice, while quercetin-3,7-*O*-diglucosidase was found in both juices (B1S01 and B1S05), as well as in juice with an added 5 % feijoa flowers. Moreover, other four flavonols: quercetin-pentoside, kaempferol-hexoside I, quercetin and kaempferol were detected only in juice enriched with 5 % fejoa flowers. In contrast, in juice enriched with 5 % of purple myrtle berry extract were present myricetin gallactoside-gallate, myricetin-3-*O*-arabinoside and myricetin, while in juices enriched with strawberry tree fruits quercetin galloylhexose, quercetin derivative II, and myricetin-3-*O*-xyloside were found. Furthermore, myricetin-3-*O*-galactoside, myricetin-3-*O*-glucoside and myricetin-3-*O*-rhamnoside were found in juices enriched in 5 % purple myrtle berry extract and strawberry tree fruits, while quercetin derivative I was present in juices BF5 and BC5. It is

noteworthy that, isorhamnetin derivatives were found only in juices containing *C. sativus* flower juice, while myricetin derivatives were unique to juices enriched in *M. communis* berries and *A. unedo* fruits. The addition of plant materials in selected quantities did not enrich products B1S01, B1S05, B1C5 and B1M5 in kaempferol, nor products B1S01 and B1S05 in kaempferol-3-*O*-rutinoside and kaempferol-3-*O*-glucoside, nor B1S01 in quercetin-3,7-*O*-digalactoside, isorhamnetin-3-*O*-sophoroside and isorhanetin-3-*O*-glucoside, nor product B1F5 in kaempferol-hexoside II and apigenin, nor product B1K5 in myricetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, quercetin derivative III, quercetin-pentoside, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rhamnoside.

Comparing the LC-MS phenolic profiles of products from set B2 to the above results (set B1) no differences in anthocyanin, hydroxycinnamic acid, dihydrochalcone, and flavan-3-ol profile were noticed. In turn, some differences were noticed regarding hydroxybenzoic acid derivatives. The presence of persimmon purée in each final product from this set enriched some of them in particular polyphenolic compounds for *D. kaki* fruit. Therefore, regarding the presence of hydroxybenzoic derivatives, in products B2S01, B2S05 and B2F5 galloyl glucoside II and III were also detected, while in product B2F5 syringic acid was detected. In contrast, products with base B2 lack some of the compounds detected in products with base B1. For example, in product B2F3 methyl ellagic acid II was not detected, while in product B1M5 digalloyl-HHDP-glucose I and ellagitanin I were not detected. Moreover, digalloylquinic acid II, digalloyl shikimic acid II and strictinin ellagitannin were not detected in product B2C5. Other hydroxybenzoic acids were similar to the products from set B1.

The LC/MS analysis also showed some differences in the flavonols profile between set B1 and B2. In product B2S01, it was not possible to detect kaempferol-3,7-*O*-diglucoside, isorhamnetin-3,7-*O*-digalactoside and quercetin-3-*O*-glucoside, but it was possible to identify isorhamnetin-3-*O*-sophoroside and isorhamnetin-3-*O*-glucoside.

Moreover, some differences were noticed in product containing 0.5 % saffron juice. In product B2S05, quercetin-3,7-*O*-digalactoside was missing. Another difference was a lack of quercetin derivative II and myricetin-3-*O*-xyloside in B2C5 and enrichement in apigenin and kaempferol-hexoside II in product B2F5. All other flavonols were the same as in products from set B1.

In the third set of products (B3) the LC/MS analysis helped to reveal some differences compared with the two previous sets. This set was much varied than set B1 and B2. Regarding the anthocyanins profile, it was observed that all products were rich in cyanidin-3-*O*-galactoside. Moreover, products containing saffron flower juice (B3S01 and B3S05) showed the presence of petunidin-3-*O*-glucoside, while in product with an added 5 % of feijoa cyanidin-3-*O*-arabinoside was identified. Only in product B3S01 it was not possible to detect delphinidin-3,5-*O*-diglucoside, and in product B3M5 was not possible to detect delphinidin-pentoside. In contrast, in product B3M5 was possible to find delphinidin-3-*O*-galactoside.

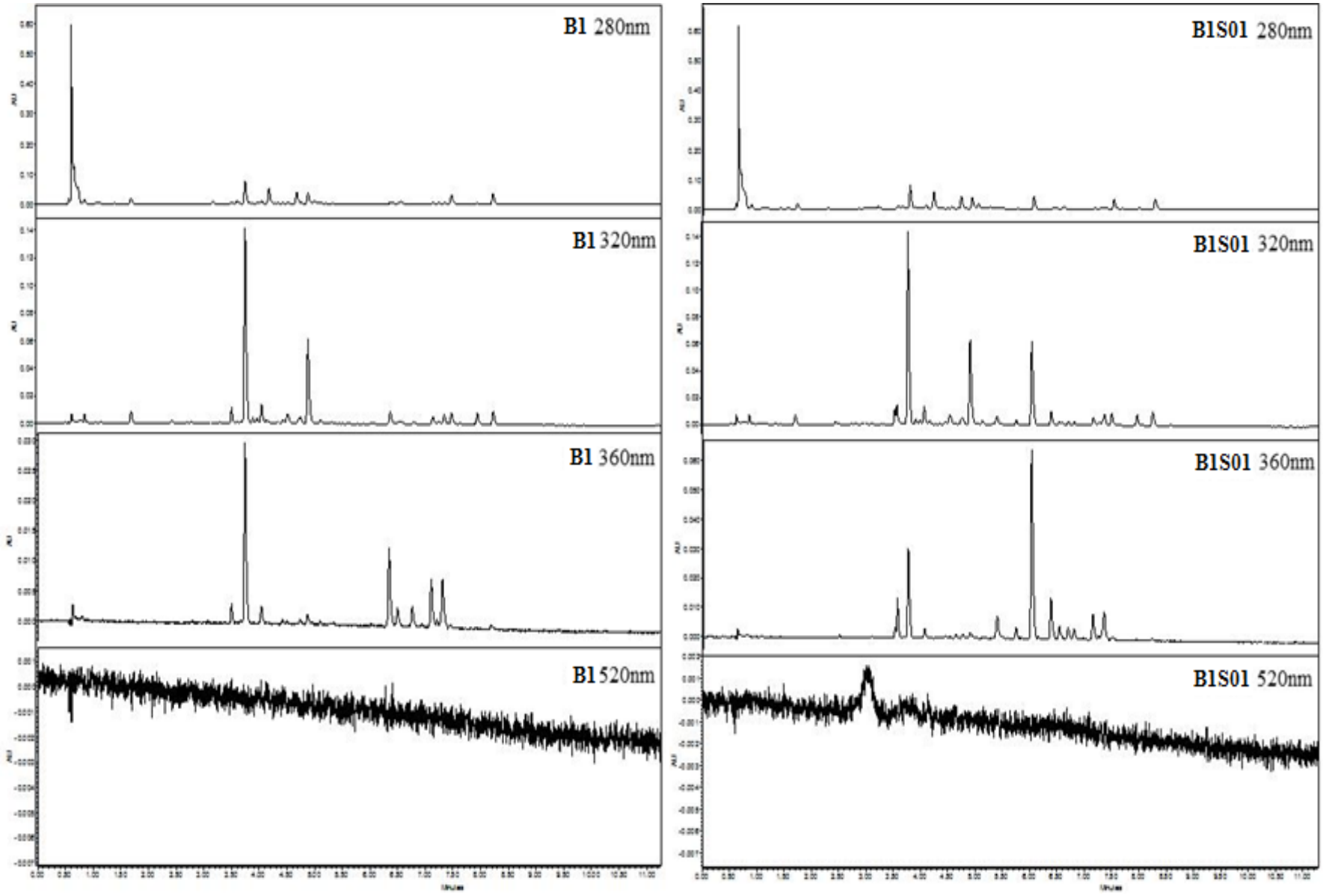
Regarding the composition of hydroxybenzoic acids and their derivatives in products from set B3, interesting differences were observed. All final products from this set contained galic acid glucoside I and II, galloyl glucoside I, theogallin, galloyl shikimic acid, digalloylquinic acid I and digalloyl shikimic acid I. Furthermore, galloyl glucoside III and salicylic aid were detected only in B3K5. Gallic acid 4-O-*β*-D-glucopyranoside was detected in all final products from this set, except product B3F5 in which digalloylquinic acid II was found. Digalloyl shikimic acid II was found in all final products from this set, except products B3M5 and B3F5. It was also possible to detect quinic acid 3,5-di-*O*-gallate in product with an additional 5 % of purple myrtle berry extract (B3M5), in contrast to products containing this additional component in base B1 and B2. Interestingly gallotannin derivative (in product B3S05) and ellagic acid arabinoside (in product B3M5) were found to be present. Finally, in product B3K5 salicylic acid and ellagic acid were detected, as

well as its two pentosides (arabinoside and xyloside), while galloyl glucoside II, galloyl-HHDP-glucoside I and II were not detected in products from set B3.

As in the case of products from the two previous sets, no differences in hydroxycinnamic acid and dihydrochalcone profiles were noticed. In contrast, flavan-3-ols and procyanidn B3 were detected in all listed products of set B3.

Finally, some unususal differences were observed in the profile of flavonols. Myricetin-3-*O*-galactoside, myricetin-3-*O*-xyloside, 3-*O*-rhamnoside of myricetin and quercetin, two quercetin-3-*O*-hexosides (galactoside and glucoside), two quercetin-3-*O*-pentosides (arabinoside and xyloside) were found in all products from set B3. Moreover, in products enriched in saffron flower juice myricetin-3-*O*-glucoside and quercetin galloylhexose were detected. The second compound, and quercetin derivative I, were also identified in product B3M5. In contrast, isorhamnetin-3-*O*-sophoroside and kaempferol-3-*O*-rutinoside were not detected in the products with an additional saffron flower juice (B3S01 and B3S05). Finally, the addition of 5 % persimmon purée enriched base B3 in quercetin-3-*O*-rutinoside, while addition of 5 % feijoa flowers did not enriched final product in kaempferol-hexoside II and apigenin.

To sum up, the set B3 showed the greatest variety in polyphenols among all three sets of products. In this way it appeared to be the most interesting in terms of phenolic profile. Hence, mixing plant materials with different polyphenolic profiles leads to diversity in chemical composition, which gurantees specific biological advantages (**CHAPTER 3.1.**; **Paragraph 3.1.2.4.**). The principal benefit of mixing different types of plant materials is the high quality of the final product. It improves healthy properties and nutritional values of the final product. This was also confirmed by Nowicka et al. (*2017*), who studied different kinds of fruit juices mixed with sour cherry pure.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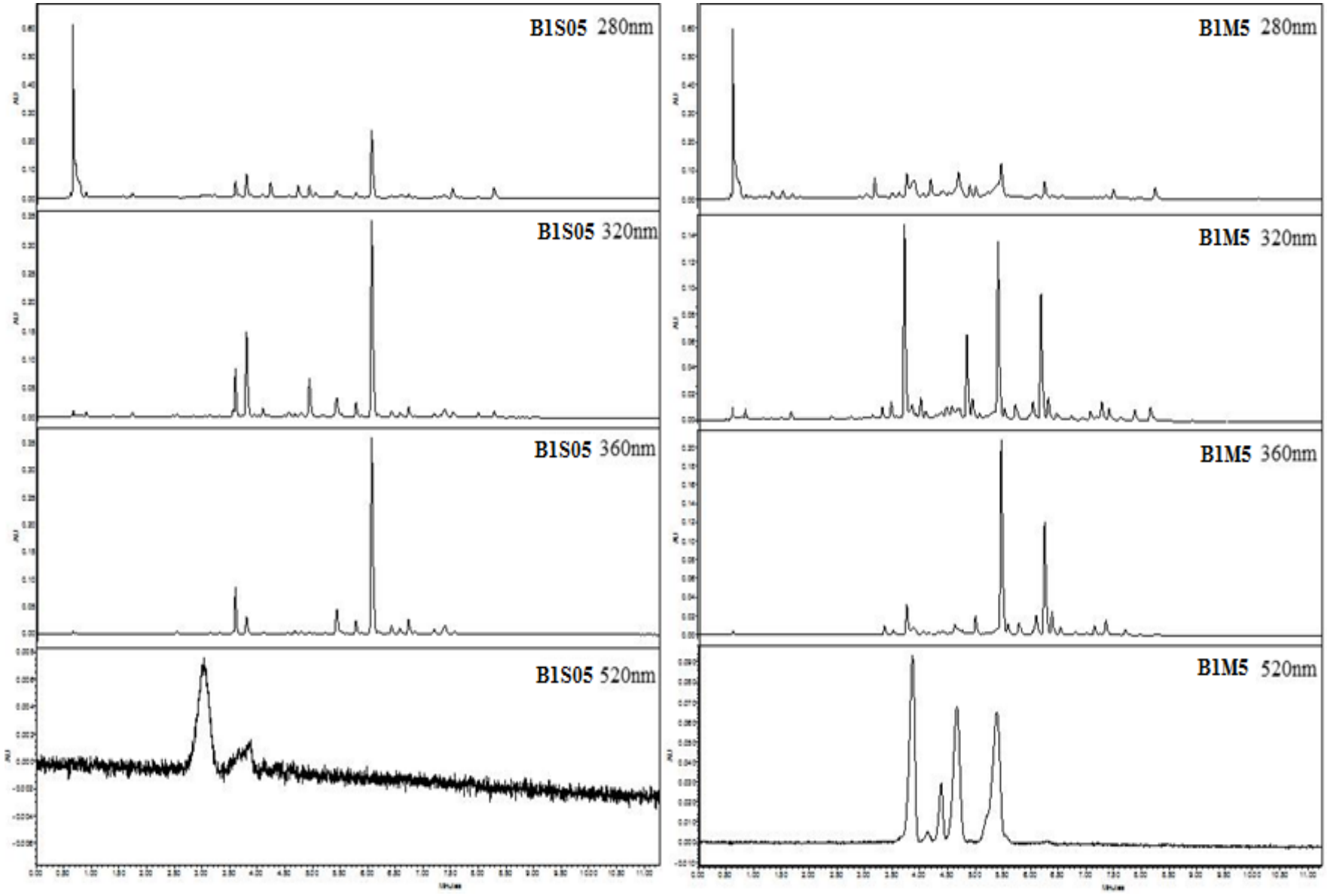
B1 320nm
B1 360nm
B1 520nm
B1S01 280nm
B1S01 320nm
B1S01 360nm
B1S01 520nm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B1M5 320nm
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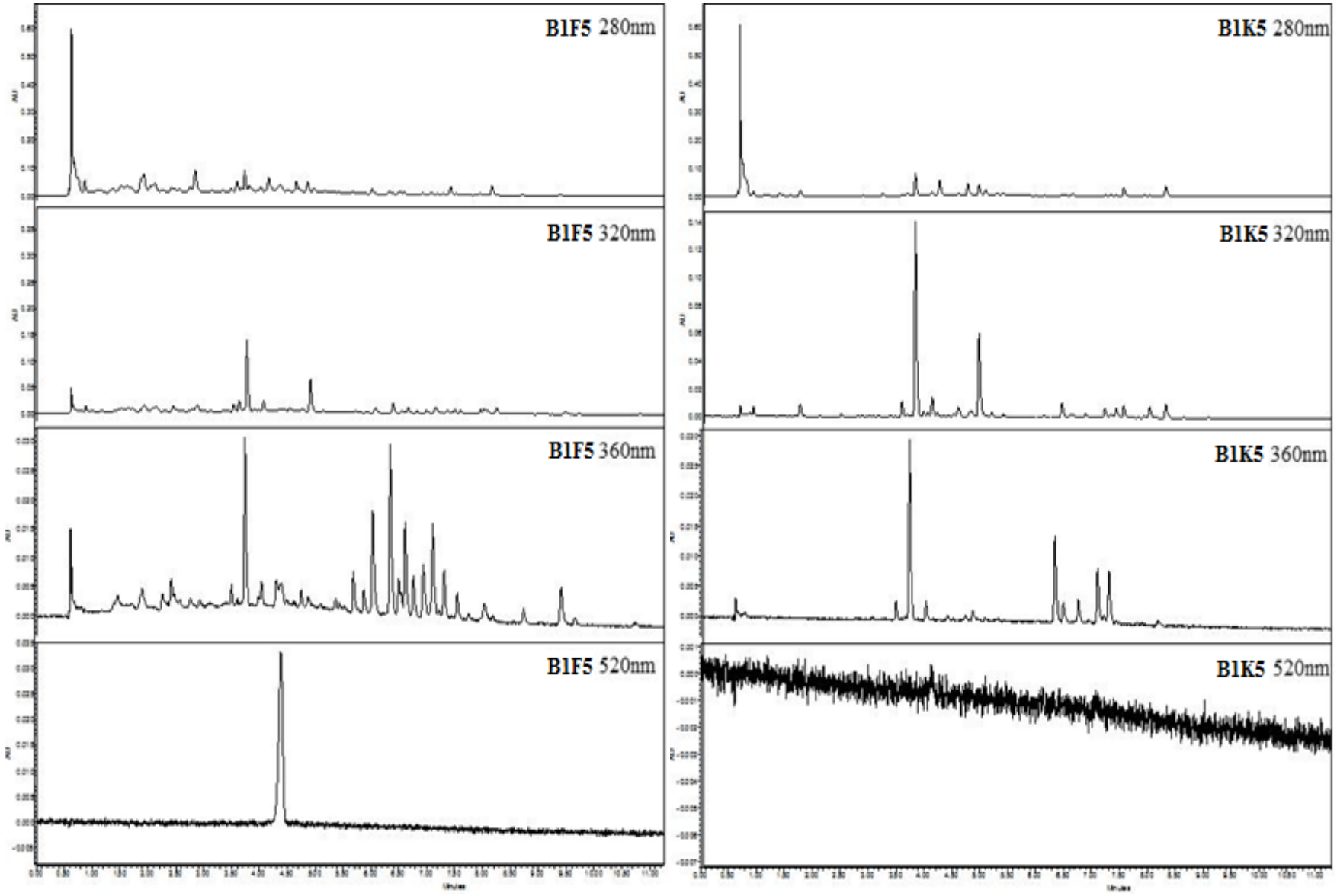
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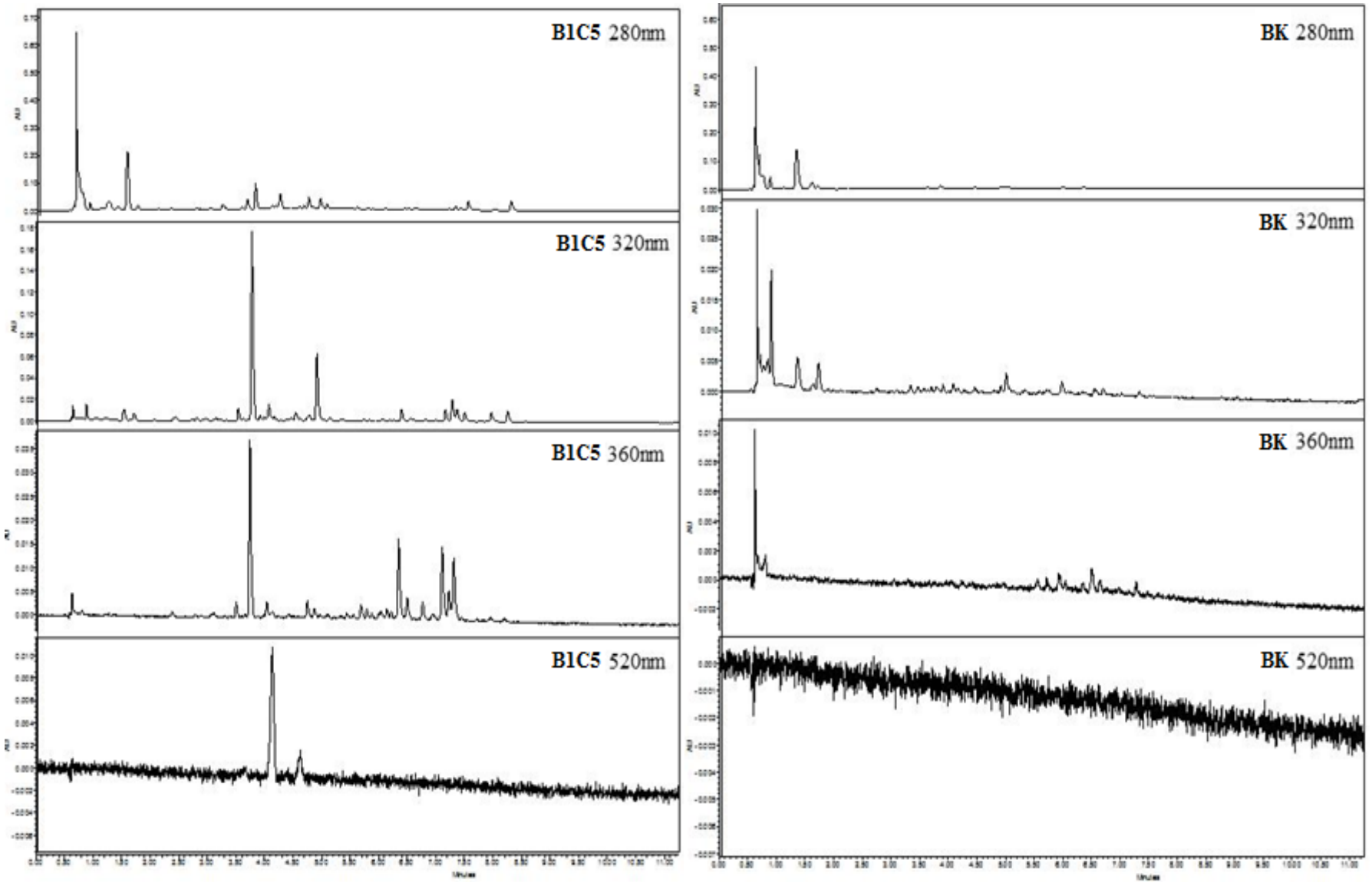
B1F5 320nm
B1F5 360nm
B1F5 520nm
B1K5 280nm
B1K5 320nm
B1K5 360nm
B1K5 520nm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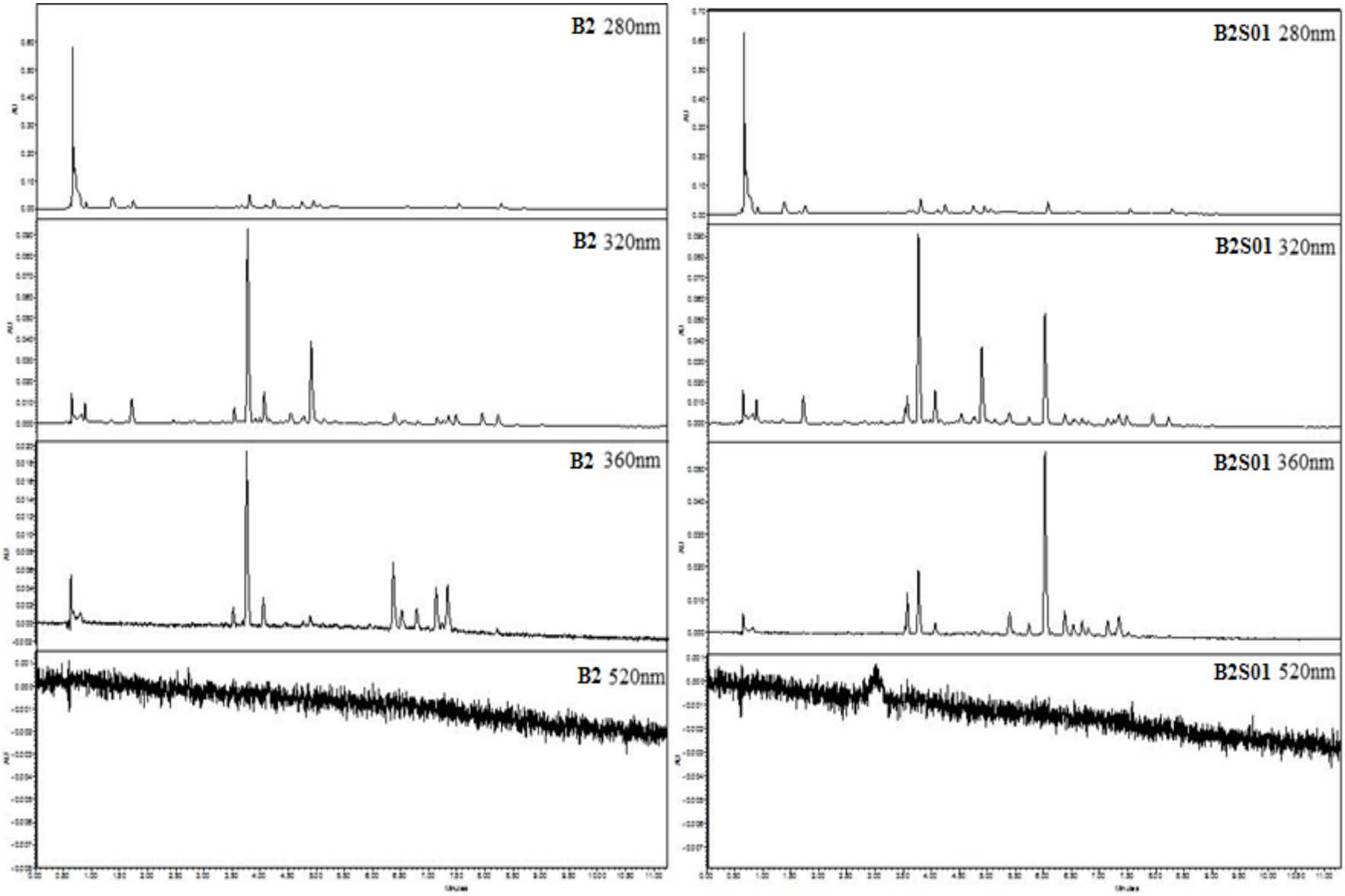
B1C5 320nm
B1C5 360nm
B1C5 520nm
BK 280nm
BK 320nm
BK 360nm
BK 520nm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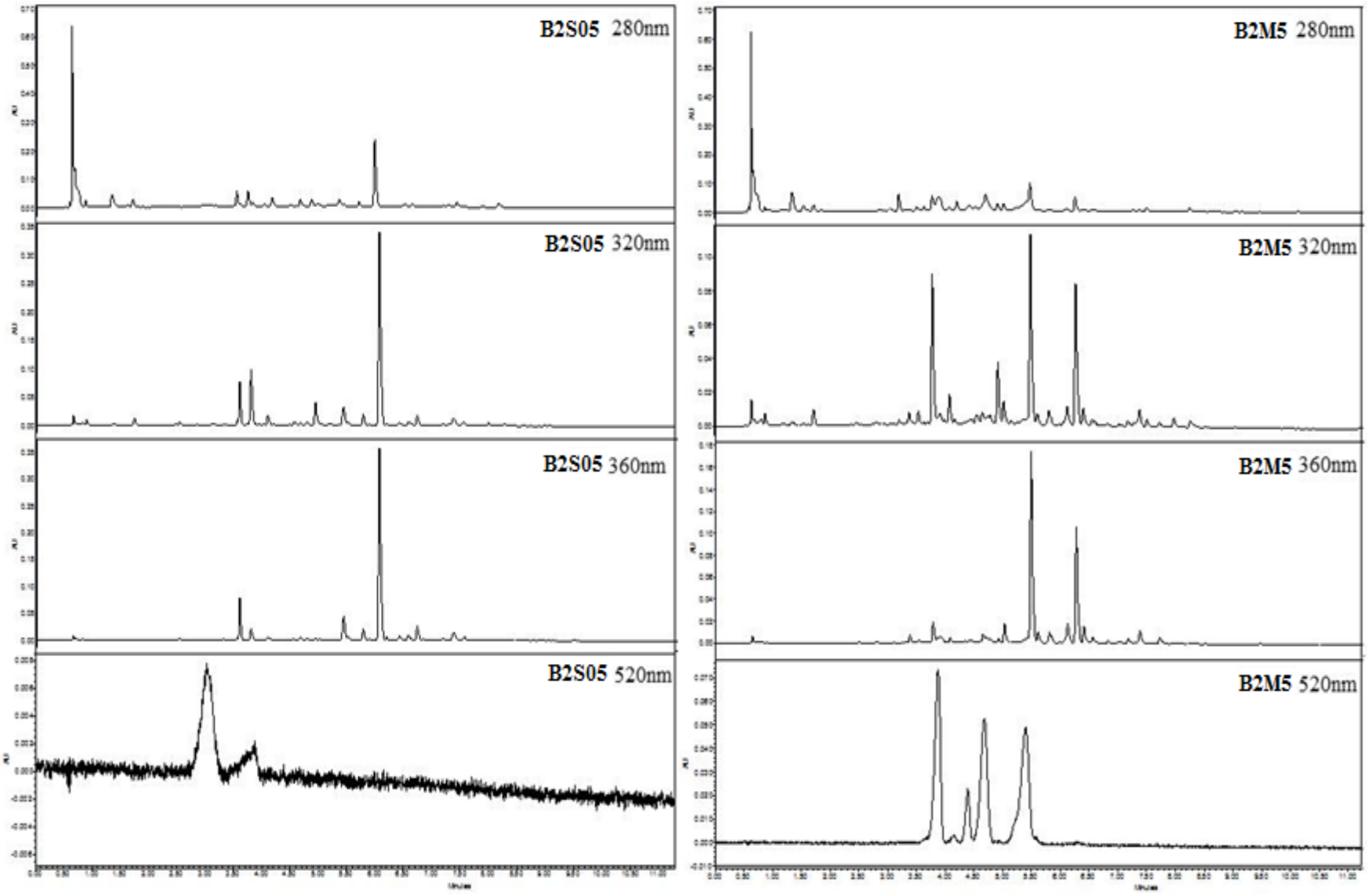
B2 320nm
B2 360nm
B2 520nm
B2S01 280nm
B2S01 320nm
B2S01 360nm
B2S01 520nm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B2S05 320nm
B2S05 360nm
B2S05 520nm
B2M5 280nm
B2M5 320nm
B2M5 360nm
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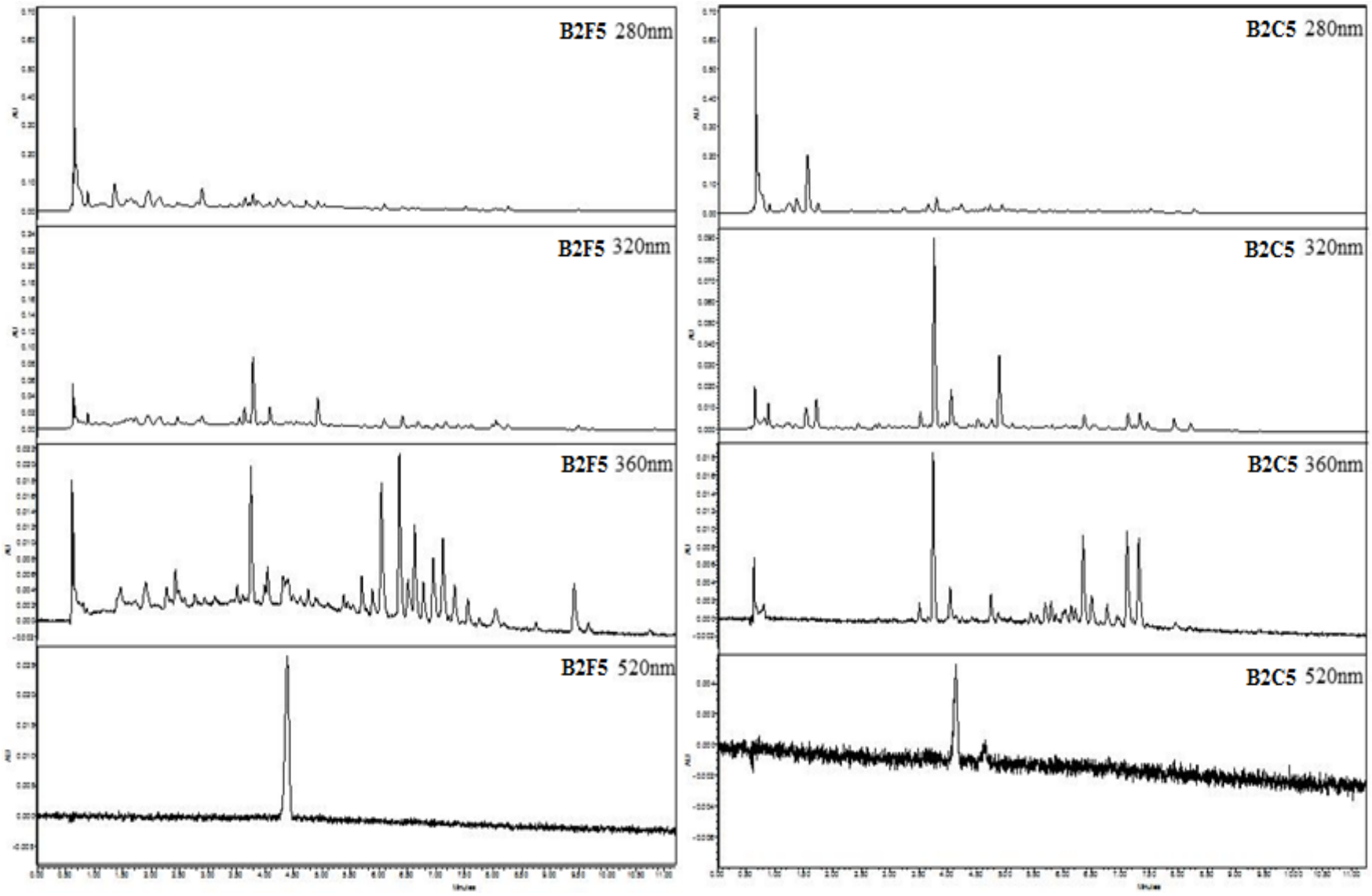
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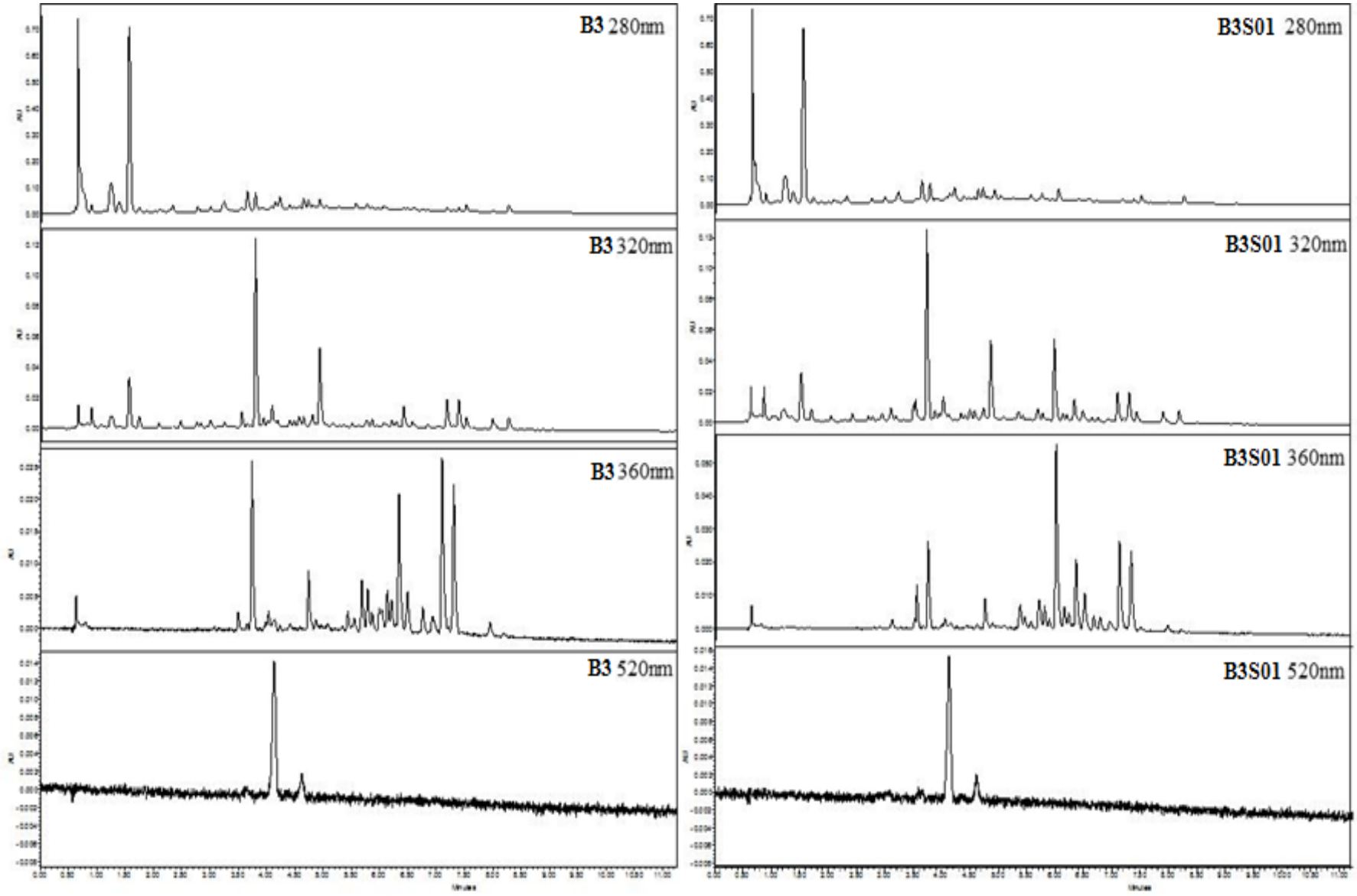
B2C5 280nm
B2F5 320nm
B2C5 320nm
B2F5 360nm
B2C5 360nm
B2F5 520nm
B2C5 520nm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B3 320nm
B3 360nm
B3 520nm
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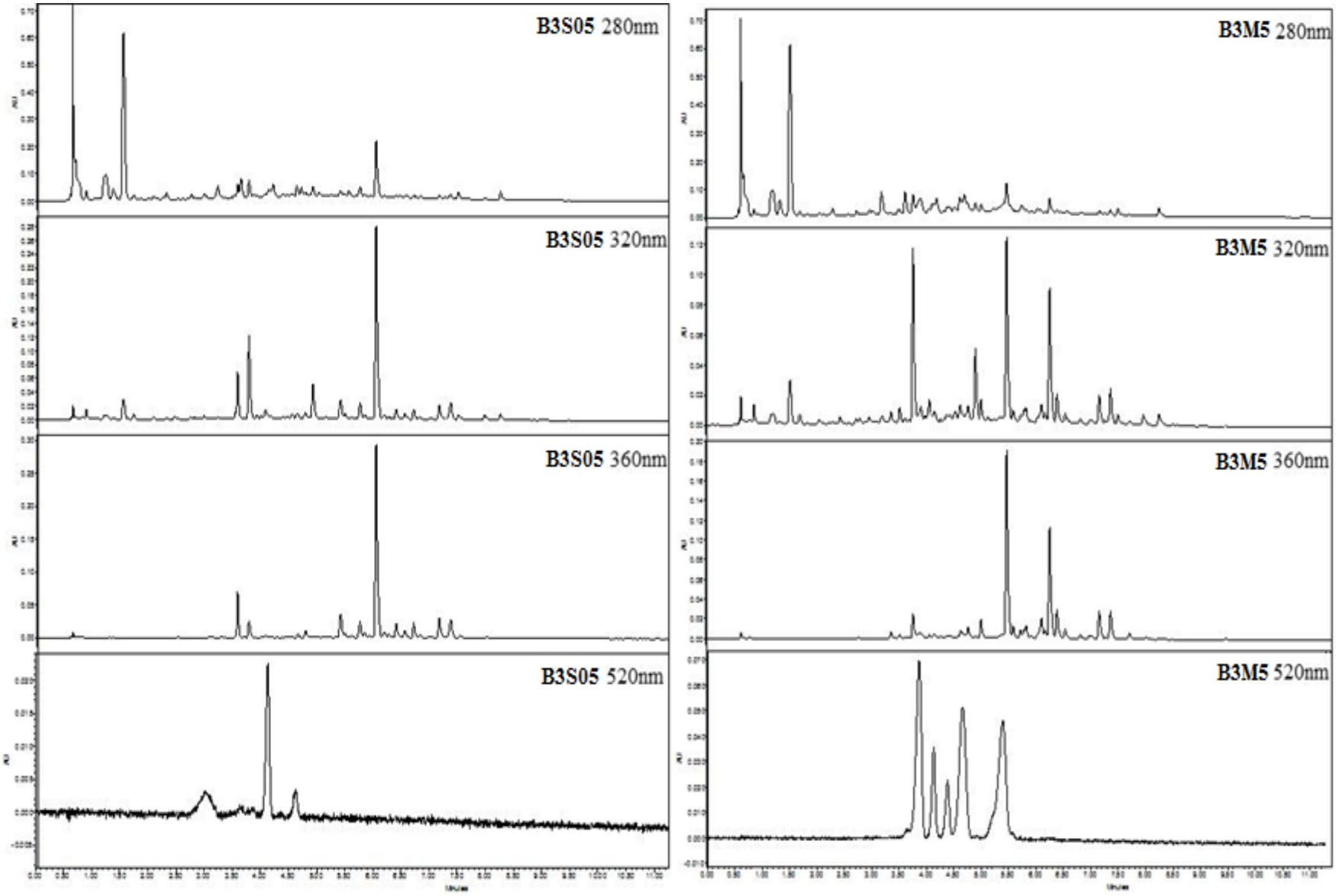
B3S01 520nm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B3S05 360nm
B3S05 520nm
B3M5 280nm
B3M5 320nm
B3M5 360nm
B3M5 520nm

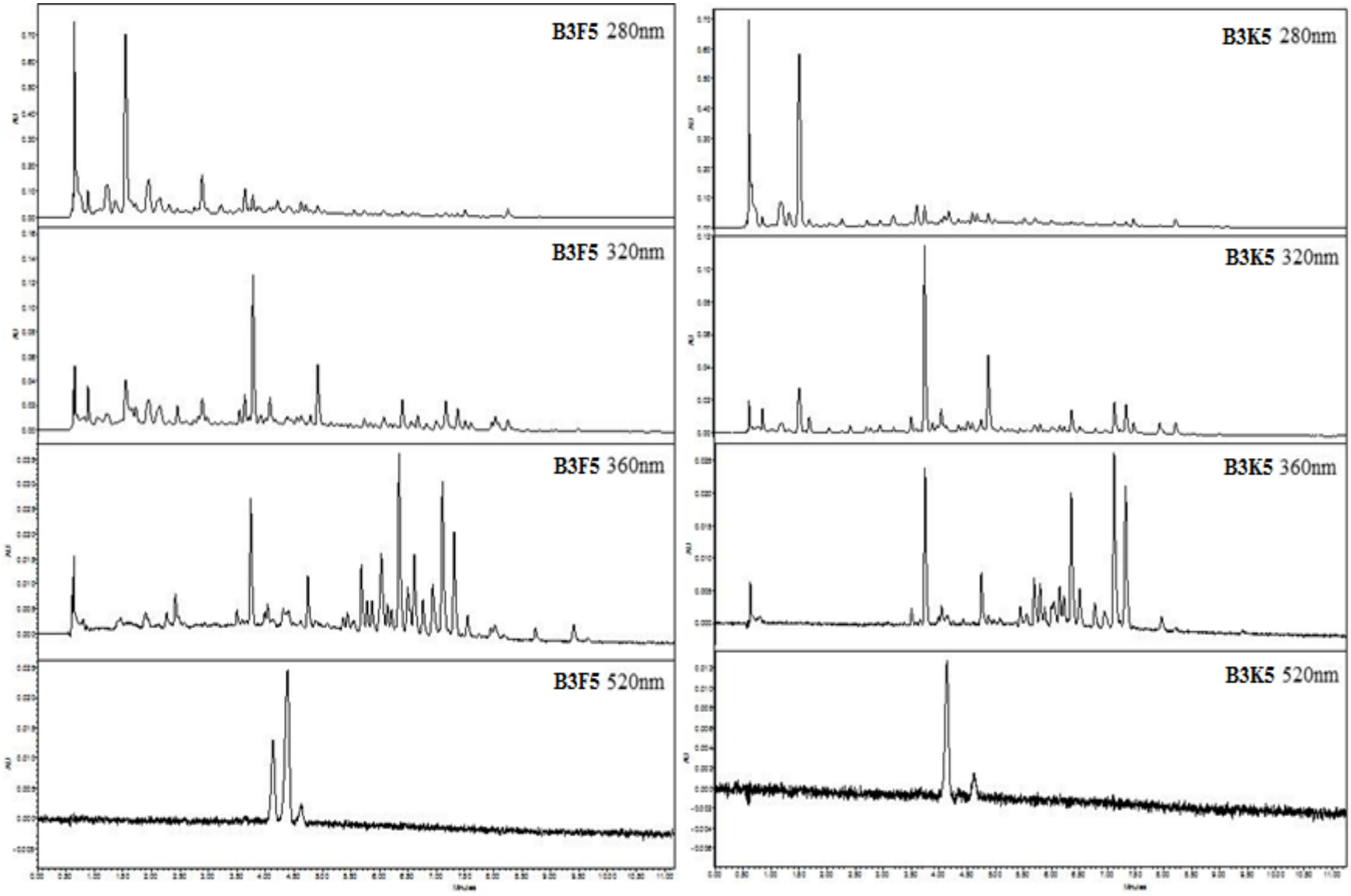

Figure 21. UPLC-PDA fingerprinting of final products detected at 280, 320, 360 and 520 nm.

Table 13. Identification of phenolic compounds by UPLC-PDA QTof/MS method in final products.

Code	Final product																			
	B1	B1S01	B1S05	B1M5	B1F5	B1K5	B1C5	B2	B2S01	B2S05	B2M5	B2F5	B2C5	B3	B3S01	B305	B3M5	B3F5	B3K5	BK
Anthocyanins																				
A1	-	X	X	-	-	-	-	-	X	X	-	-	-	-	-	X	-	-	-	-
A2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
A3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A4	-	-	X	X	-	-	-	-	-	X	X	-	-	-	-	-	X	-	-	-
A5	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
A6	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	X	-
A7	-	-	-	X	X	-	-	-	-	-	X	X	-	-	-	-	X	X	-	-
A8	-	-	-	-	-	-	X	-	-	-	-	-	X	X	-	-	-	X	X	-
A9	-	-	-	X	-	-	-	-	-	-	X	-	-	-	X	X	X	-	-	-
A10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A11	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-
A12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A13	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-
A14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxybenzoic acids																				
B1	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	X	-
B2	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	X	-
B3	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
B4	-	-	-	-	-	X	-	X	X	X	-	X	-	-	-	-	-	-	-	X
B5	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	X	-
B6	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	X	-
B7	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
B8	-	-	-	-	-	X	-	X	X	X	-	X	-	-	-	-	-	-	X	X
B9	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B10	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	-	X	-

B11	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
B12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B13	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	X	-
B14	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
B15	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B16	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	X	-
B17	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
B18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
B19	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-
B20	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	X	-	-
B21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B22	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
B23	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
B24	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	X	X	-
B25	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-	-	-	-	X
B26	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
B27	-	-	-	-	-	-	X	-	-	-	-	-	-	X	X	X	-	-	X	-
B28	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
B29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-
B30	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	X	X
B31	-	-	-	-	X	-	-	-	-	-	-	X	-	X	-	-	X	X	X	-
B32	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	X	-
B33	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	X	-
B34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B36	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxycinnamic acids																				
C1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	_
C2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C5	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-

Dihydrochalcones																			
D1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
D2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
Flavan-3-ols																			
E1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
E2	-	-	-	-	-	-	X	-	-	-	-	-	-	X	X	X	X	X	X	-
E3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
E4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
E5	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
E6	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
Flavonols and Flavones																			
F1	-	X	X	-	-	-	-	-	X	X	-	-	-	-	X	X	-	-	-	-
F2	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F3	-	X	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-
F4	-	X	X	-	-	-	-	-	-	X	-	-	-	-	X	X	-	-	-	-
F5	-	-	-	-	X	-	X	-	-	-	-	X	X	X	-	-	X	X	X	-
F6	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-
F7	-	X	X	-	X	-	-	-	X	X	-	X	-	-	X	X	-	X	-	-
F8	-	-	-	X	-	-	X	-	-	-	X	-	X	X	X	X	X	X	X	-
F9	-	-	-	X	-	-	X	-	-	-	X	-	X	X	X	X	X	-	X	-
F10	-	X	X	-	-	-	-	-	X	X	-	-	-	-	X	X	-	-	-	-
F11	-	-	-	-	-	-	X	-	-	-	-	-	X	X	X	X	X	-	X	-
F12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	X
F13	-	X	X	-	-	-	-	-	X	X	-	-	-	-	X	X	-	-	-	-
F14	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-
F15	-	-	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	-
F16	-	-	X	-	-	-	-	-	X	X	-	-	-	-	-	-	-	-	-	-
F17	-	-	-	-	-	-	X	-	-	-	-	-	-	X	X	X	X	X	X	-
F18	-	-	-	X	-	-	X	-	-	-	X	-	X	X	X	X	X	X	X	-
F19	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F20	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

F21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F22	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
F23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
F24	-	X	X	-	-	-	-	-	X	X	-	-	-	-	X	X	-	-	-	-
F25	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
F26	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	X	-
F27	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
F28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
F29	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-
F30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F31	-	-	X	-	-	-	-	-	X	X	-	-	-	-	X	X	-	-	-	-
F32	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
F33	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-
F34	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-
F35	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
F36	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-
F37	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-

$[M+H]^+$ (*m/z*) for anthocyanins were obtained in the positive ion mode; X = detected;- = not detected (< LOD); * = tentatively attribution; Sample codes have their references in **Annex 1b.**. Phenolic compounds have their references in the **Table 4.**.

3.2.3.4. Quantification of phenolic compounds

The results of the quantification of polyphenols found in all final products made from investigated in **CHAPTER 3.1.** plant materials were shown in **Table 14.**. For a more sensitive identification of proanthocyanidins (PAC), the quantitative analysis was performed using the UPLC-FL method described in **Paragraph 2.10.**, based on the comparison standards (**Figure 18.**) and published data, if available. The quantitative analysis revealed the presence of a wide range of polyphenolic compounds in all investigated matrixes immediately after processing and during storage time. Statistically significant differences ($p \leq 0.05$) were observed between obtained results of all analysed final products.

The sum of polyphenols evaluated by UPLC-PDA analysis varied in all investigated beverages, due to a wide concentration range depending on particular plant material. The greatest differences were observed between three sets of final products (B1, B2 and B3). Depending on the type of plant material additive, the total quantity of detected polyphenols in final products (0 month) varied and was the lowest in set B1 (131.93-274.32 mg/100 g fw), higher in set B2 (227.63-372.34 mg/100 g fw) and the greatest in set B3 (530.72-635.96 mg/100 g fw); (immediately after processing). Moreover, in product BK the sum of detected polyphenols was 382.16 mg/100 g fw. Generally, based on the analysis of total polyphenols it was possible to see some trends. They indicate that supplementing of the final products with additional components increases the total content of these compounds, especially in the case of products from set B3. Furthermore, supplementation with 5 % addition of purple myrtle berry extract or feijoa flowers significantly enriches the final products in natural antioxidants.

Regarding products from set B1, characteristic of B1C5 was the presence of two cyanidin derivatives, of which the most abundant was cyanidin-3-*O*-galactoside (1.04

mg/100 g fw), while cyanidin-3-*O*-arabinoside was found in much lower amounts (0.04 mg/100 g fw, respectively). On the other hand, considering the results of juice B1M5, cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside and malvidin-3-*O*-glucoside were three main compounds responsible for the red-purple colour of the juices (3.32, 10.55 and 17.05 mg/100 g fw, respectively). The values of the other three anthocyanins detected in B1M5 ranged from 0.04 (peonidin-3-*O*-glucoside) to 0.83 mg/100 g fw (petunidin-3-*O*-glucoside). Furthermore, in juices containing floral by-products (B1S01, B1S05 and B1F5) few different anthocyanins were found. In juices with 0.1 and 0.5 % saffron flower juice delphinidin-3,5-*O*-diglucoside (0.17 and 1.17 mg/100 g fw, respectively) was detected, while 0.37 mg/100 g fw of delphinidin-3-*O*-glucoside was detected in B1S05. In turn, in juice B1F5 3.69 mg/100 g fw of cyanidin-3-*O*-glucoside was found.

Hydroxybenzoic acids and their derivatives were found in B1M5, B1F5, B1K5 and B1C5. The highest concentration of this subclass was detected in juices enriched in *A. unedo* fruits and *A. sellowiana* flowers (29.28 and 28.37 mg/100 g fw, respectively). Juice B1C5 was the most abundant in theogallin (23.10 mg/100 g fw), while other compounds were detected in much lower amounts in a range between 0.03 to 2.06 mg/100 g fw. On the other hand, regarding juice B1F5, the most representative hydroxybenzoic acid derivative was castalagin (7.78 mg/100 g fw) and ellagitannin II (9.34 mg/100 g fw), while other compounds were present in lower amounts (0.26-3.56 mg/100 g fw). Moreover, in juice B1M5 and B1K5 some amounts of these compounds were detected. The first juice was rich in two galloyl HHDP-glucoses (c.a. 2.50 mg/100 g fw), and other derivatives were detected in amount between 0.13 to 1.50 mg/100 g fw. The final juice in which hydroxybenzoic acid derivatives were found was juice B1K5. Two galloyl glucosides (II and III) were found in this functional juice in amount 1.10 and 1.70 mg/100 g fw, respectively.

Hydroxycinnamic acids were presented in all analysed functional juices. The total content of these compounds in set B1 ranged between 6.58 to 8.42 mg/100 g fw. The most abundant in this subclass of polyphenols juice was B1C5, while the pure apple juice was the least. Chlorogenic acid was present in all juices in the highest quantity (5.00 to 6.62 mg/100 g fw), while values of other acids ranged between 0.10 to 1.01 mg/100 g fw. Obtained results showed that each additional component enriched apple juice in hydroxycinnamic acids.

Dihydrochalcones were the subclass of detected polyphenols which was found in the present study in all products from set B1. The total amount of these compounds was present in analysed juices in quantities ranging from 4.32 to 5.19 and mg/100 g dm, respectively (immediately after processing).

Flavan-3-ols (monomers, dimers, trimer and polymeric proanthicyanidins) were the major subclass in analysed juices. The highest total amount of these compounds was detected in juice B1F5 (223.21 mg/100 g fw) and in B1K5 (255.59 mg/100 g fw). In turn, the poorest in these compounds was pure apple juice (119.11 mg/100 g fw). In addition, monomers (1.44-5.72 and 5.98-7.94 mg/100 g fw for (+)-catechin and (-)-epicatechin, respectively) and dimers (procyanidin B1 and B2; 1.09-11.25 mg/100 g fw) were detected among all juices from set B1 in larger amounts than trimer (procyanidin C1; 0.40-0.80 mg/100 g fw). The procyanidin B1 and B2 were found in product B1M5 in much higher quantities than in other products from this set. Moreover, 0.06 mg/100 g fw was detected only in procyanidin B3. In addition, the total amount of polymeric procyanidins present in all analysed juices fromset B1 ranged between 108.11 to 242.68 mg/100 g fw. The richest in these compounds was juice B1K5, followed by B1F5, while the poorest was 100 % apple juice. This confirms that each additional component enriched juices in polymeric procyanidins, or that mixing two components leads to the creation of new polymeric compounds.

Our analysis revealed statistically significant differences in flavonol concentrations in analysed juices from set B1. The flavonol content ranged from 1.63 to 22.72 mg/100 g fw (immediately after processing). The highest value of these compounds were detected in juices enriched with 0.5 % saffron flower juice, followed by 5 % of purple myrtle berry extract, while the lowest was found in pure apple juice. In all functional juices quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside and quercetin-3-*O*-rhamnoside were found in amounts ranging from 0.57 to 1.28, 0.10 to 0.40 and 0.50 to 1.52 mg/100 g fw, respectively. Furthermore, in all juices two flavonol-*O*-pentosides, such a quercetin-3-*O*-arabinoside and -xyloside were present (0.14-0.27 and 0.32 to 0.74 mg/100 g fw, respectively). All the above compounds were present in B1, but it was noticed that each additional component increased the amount of these compounds in final juices from set B1.

In B1S01 and B1S05 kaempferol derivatives in amounts between 0.04 to 2.13 and 0.15 to 11.84 mg/100 g fw, respectively were present. Kaempferol-3-*O*-sophoroside proved to be the major representative among all detected kaempferol derivatives. Isorhamnetin derivatives were the other particular group of compounds detected in juices B1S01 and B1S05 in a range between 0.17 to 0.24 and 0.20 to 1.32 mg/100 g fw, respectively. Moreover, small amounts (0.06 mg/100 g fw) of quercetin-3,7-*O*-digalactoside were found in B1S05, while quercetin-3,7-*O*-diglucosidase was detected in juice B1S01 and B1S05 (0.35 and 2.26 mg/100 g fw, respectively), as well as in much lower quantities in B1M5 (0.09 mg/100 g fw, respectively).

Juice B1F5 was rich in kaempferol-3-*O*-galactoside, kaempferol-hexoside I, quercetin-pentoside, quercetin and kaempferol (0.53, 0.21, 0.39, 0.05 and 3.78 mg/100 g fw, respectivley), while B1M5 was characterized by the presence of myricetin gallactoside-gallate, myricetin-3-*O*-arabinoside and myricetin (1.75, 1.18 and 0.33 mg/100 g fw).

In turn, only in juices enriched with 5 % of strawberry tree fruits were found 0.03-0.27 mg/100 g fw of quercetin galloylhexose, quercetin derivative II and myricetin-3-*O*-xyloside. Furthermore, myricetin-3-*O*-galactoside myricetin-3-*O*-glucoside and myricetin-3-*O*-rhamnoside were found in B1M5 (9.85, 0.50 and 5.88 mg/100 g fw, respectivley) and B1C5 (0.33, 0.25 and 0.02 mg/100 g fw, respectively). In B1F5 and B1C5 quercetin derivative I was detected in quantities 0.09 and 0.21 mg/100 g fw, respectively.

The next set of products was B2 in which some variations were noticed compared with products from set B1. In the second set the total quantity of anthocyanins was detected in a range between 0.23 to 22.54 mg/100 g fw (0 months). As in the first set the highest amount of these compounds was detected in product enriched with 5 % of purple myrtle berry extract (B2M5), while the lowest was in product enriched in 0.1 % of saffron flower juice (B2S01). The compounds tendency was similar to that reported for set B1. Regarding anthocyanins deriving from purple myrtle berry extract, generally a decrease was noticed. The values of these compunds in product B2M5 ranged between 0.03 to 10.96 mg/100 g fw. On the other hand, the amount of delphinidin derivatives from saffron flower juice slightly increased in products B2S01 and B2S05 and ranged between 0.23 to 1.73 mg/100 g fw. Moreover, some significant decrement in amount of cyanidin-3-*O*-glucoside was detected in products B2M5 and B2F5 (2.21 and 2.84 mg/100 g fw, respectively), as well as in the quantity of cyanidin-3-*O*-galactoside in product enriched with 5 % of *A. unedo* fruits (B2C5; 0.41 mg/100 g fw).

Regarding the amount of hydroxybenzoic acids and their derivatives in set B2, a different trend to set B1 was observed. Generally, in all products from this set these compounds were detected. The total amount of this subclass of polyphenols, varied in a range between 5.51 to 33.08 mg/100 g fw. The highest quantity was found in products B2F5, followed by B2C5, while the lowest was in both products with saffron flower juice. Moreover, ellagitannin I, digalloylquinic acid II, digalloyl shikimic acid II, strictinin

ellagitanin and methyl ellagic acid were not detected in products from set B2, while syringic acid was detected in product B2F5 (0.39 mg/100 g fw). In product B2C5 a slight increase of gallic acid glucoside I, galloyl glucoside I, gallic acid 4-*O*-*β*-D-glucopyranoside, digalloylquinic acid I (1.09, 2.19, 0.06, and 0.27 mg/100 g fw, respectively) was noticed compared with product B1C5, while in product B2F5 a slight increase of ellagic acid (3.13 mg/100 g fw) was observed compared to product B1F5. Greater increases were observed in the quantities of galloyl HHDP-glucose I, gallic acid glucoside II, ellagitannin IV, nilocitin, (7.33, 2.18, 0.95, 1.02 mg/100 g fw, respectively). In turn, decreases were observed evaluating the quantity of 3-*O*-galloylquinic acid, galloyl shikimic acid and digalloyl shikimic acid I in product B2C5 (21.30, 0.58 and 0.79 mg/100 g fw, respectively), castalagin, casuarin, ellagitannin II, casuarinin, ellagic acid arabinoside and xyloside in product B2F5 (2.29, 0.95, 7.75, 2.98, 0.62 and 0.19 mg/100 g fw, respectively), and galloyl HHDP-glucose II and ellagitannin III in product B2M5 (2.36 and 0.84 mg/100 g fw). Finally, in all products from set B2, (except product B2C5 and B2M5) galloyl glucoside II and III were detected in a range between 5.06 to 10.06 and 0.45 to 2.77 mg/100 g fw, respectively. The largest amount of these two compounds was detected in product B1F5.

The total hydroxycinnamic acid content in products from set B2 was lower than its equivalents from set B1. The total quantity ranged between 4.27 and 4.76 mg/100 g fw. Moreover, it was noticed that each additional component decreased the amount of these compounds. Similarly, chlorogenic acid was the major representative of this group of polyphenols (3.09-3.53 mg/100 g fw), while four other acids were detected in much lower quantities (0.05-0.63 mg/100 g fw).

In the case of dihydrochalcones a very significant reduction of the level of these compounds in set B2 (0.90-1.32 and 0.83-1.37 mg/100 g fw for phloretin-2'-*O*-

xyloglucoside and -glucoside, respectively) was observed. The total content of these compounds varied from 1.73-2.66 mg/100 g fw (0 months).

Evaluating the results for flavan-3-ols quantity, significant decrease was noted compared with set B1. The total content of these compounds (Total E) ranged between 210.33 to 334.87 mg/100 g fw (0 months). On the other hand, some similarities were noticed. As in the set B1 the greatest quantiy of flavan-3-ols was detected in product enriched in 5 % purple myrtle berry extract (B2M5). Also regarding each particular compound, it was observed that monomers and dimmers were the major flavan-3-ols. In contrast to set B1, the absence of procyanidin B3 was noticed in product with 5 % of strawberry tree fruits added (B2C5). Furthermore, regarding polymeric procyanidins, they were observed in c.a. twice the concentrations in which they were observed in set B1. Among the products from set B2 the highest amount of PP was detected in product B2C5 (325.63 mg/100 g fw).

The last group of polyphenols from set B2 which was compared with set B1 were flavonols. Similar trends were noticed in the profile of this subclass of polyphenols. The total content of these compounds (Total F) was lower than in set B1 and ranged from 1.11 to 21.61 mg/100 g fw. As in juices from set B1, the most abundant in flavonols were products B2M5 followed by B2S05, while the poorest, pure base B2. Two quercetin-3-*O*-hexosides (galactoside and glucoside), -pentosides (arabinoside and xyloside) and -rhamnoside were detected in lower quantities than in the first set (0.02-1.34 mg/100 g fw). Almost all flavanols present in products B2S01 and B2S05 were found in lower quantities than in their equivalents from set B1. In contrast, isorhamnetin-3-*O*-glucoside was detected in higher quantities (compared with set B1). In general, other flavonols were detected in lower quantities than in the previous set. Just few exceptions were noticed. In product B2F5 the higher quantity of quercetin pentoside and kaempferol (0.43 and 4.04 mg/100 g fw) was observed, while in product B2M5 0.02 mg/100 g fw of myricetin was detected.

Generally, considering each particular subclass of polyphenols, except anthocyanins, dihydrochalcones and hydroxycinnamic acids, set B3 was the richest in these compounds. A pecularity of this set was the presence of cyanidin-3-*O*-galactoside in all products (1.04-3.16 mg/100 g fw), and delphinidin-3-*O*-galactoside in B2M5 (0.44 mg/100 g fw). Other differences from the other two sets were observed in the quantity of some derivatives of hydroxybenzoic acids. Gallic acid glucoside I and II, galloyl glucoside I, theogallin, galloyl shikimic acid, digalloyl shikimic acid I and digalloylquinic acid I were not only evaluated in all products from set B3 but also detected in larger amounts. In particular, 3-*O*-galloylquinic acid was present in last set in quantities c.a. 3 times higher than in product B1C5 and B2C5. Furthermore, digalloyl shikimic acid II was detected in all products from set B3 (2.96-3.39 mg/100 g fw), except product B3F5 and B3M5, while ellagic acid arabinoside was detected in all products from set B3 (0.92-4.81 mg/100 g fw), except final smoothies with addition of saffron flower juice. Also, gallic acid 4-*O*-*β*-D-glucopyranoside was present in all iterations of set B3, except B3F5 (0.08-0.41 mg/100 g fw). Another important dissimilarity was absence of galloyl-HHDP-glucose I and II, galloyl glucoside II in the set B3. In turn, only in product B3M5, B3S05 and B3K5 was 0.31 mg/100 g fw of quinic acid 3,5-di-*O*-gallate, 0.43 mg/100 g fw of gallotannin derivative and 0.08 mg/100 g fw of salicylic acid, respectively, detected.

Hydroxycinnamic acids presented in the products with base B3 were detetected higher amounts and showed a similar tendency to the two previous sets, while regarding dyhydrochalcones it was noticed that phloridzin was detected in higher quantities (2.03-2.22 mg/100 g fw), than phloretin-2'-*O*-xyloglucoside (1.42-1.87 mg/100 g fw).

In the terms of flavan-3-ols, interesting results were observed. The quantitative analysis showed that procyanidin B1 and (+)-catechin were detected in third set in c.a. 2-10 and 3-12 times larger quantities than in the other two sets. Furthermore, regarding

polymeric procyanidins, it was observed that comparing the results with the set B1 and B2, the quantity of PP was c.a. 2-4 times greater in products from the last set.

Considering the differences in flavonls between set B3 and other two, some enrichement could be seen. The total content of this subclass of polyphenols (Total F) varied from 5.90 to 25.03 mg/100 g fw, with the highest in product B3M5. The presence of myricetin-3-*O*-derivatives (galactoside, xyloside and rhamnoside) in all products from set B3 and quercetin galloylhexose and myricetin-3-*O*-glucoside (except in B3F5) in quantities ranging from 0.04 to 9.32 mg/100 g fw was unusual. Moreover, 0.86 mg/100 g fw of quercetin-3-*O*-rutinoside was detected in product B3K5.

Product BK was not attractive in terms of anthocyanins, hydroxycinnamic acids, dihydrochalcones, flavan-3-ols and flavonols. On the other hand, it was interesting in terms of hydroxybenzoic acids and their derivatives, as well as polymeric proantocyanidins.

Following 3 and 6 months of storage at 20 ± 2°C, a significant ($p \leq 0.05$) change was observed in the content of phenolic compounds. Generally, after storage, the content of total polyphenols decreased in all final products. The total content of polyphenols in juices and smoothies after 3 months storage ranged from 103.33 to 199.52, 192.44 to 300.09 and 333.26 to 465.11 mg/100 g fw for set B1, B2 and B3, respectively. Moreover, after 6 months the total polyphenol content decreased more and ranged from 76.34 to 149.31, 189.42 to 236.41 and 301.75 to 388.72 mg/100 g fw for set B1, B2 and B3, respectively.

The highest stability during storage, both after 3 and 6 months, was observed in the case of polymeric proanthocyanidins. The content of PP after 3 months of storage ranged from 71.98 to 152.93, 169.20 to 263.48 and 224.64 to 329.00 mg/100 g fw for set B1, B2 and B3, respectively. After 6 months storage the content of PP ranged from 54.84 to 129.69, 160.98 to 202.22 and 199.02 mg/100 g fw for set B1, B2 and B3, respectively. The stability of the polymeric flavan-3-ols meant that the final products from set B2 and B3, after storage were, characterised by the highest content of polyphenols. According to the

findings of Nowicka and Wojdyło (*2016*), the stability of PP depends on food structure (liquid or semi-liquid form). These compounds are more stable in mixed products, than in fruit juices. Probably in mixed products (smoothies or purée) the PP are surrounded by the cell wall structure, which inhibits their degradation. Therefore, the degradation of PP in products from set B2 and B3 was slower.

During storage an outstanding decrease of anthocyanins in all final products containing 5 % of purple myrtle berry extract was noticed. After 3 months the total amount of these compounds was c.a. 3 times lower in the products B1M5 and B2M5, while c.a. 7 times lower in product B3M5. After 6 months, their total amount decreased to 6.74, 5.49 and 0.72 mg/100 g fw in set B1, B2 and B3. Moreover, in terms of total content of hydroxybenzoic acid derivatives, a large decrease was noticed in product B1C5.

The study showed that the most attractive final products in terms of phenolic compounds, both immediately after processing and after storage were smoothies from the set B3. It is noteworthy, that each additional component has a positive influence on the phenolic composition of functional juices. Thanks to each particular plant matrix, apple juice became a more desirable nutraceutical product.

Table 14. Quantification of phenolic compounds (mg/100 g fw) in final products before and after storage time (3 and 6 months) at 20±2°C.

Storage time: immediately after processing (0 months)

Code	Final product (mg/100 g fw)																			
	B1	**B1S01**	**B1S05**	**B1M5**	**B1F5**	**B1K5**	**B1C5**	**B2**	**B2S01**	**B2S05**	**B2M5**	**B2F5**	**B2C5**	**B3**	**B3S01**	**B3S05**	**B3M5**	**B3F5**	**B3K5**	**BK**
Anthocyanins																				
A1	nd	0.17 ±0.01c	1.77 ±0.02a	nd	nd	nd	nd	nd	0.23 ±0.02c	1.73± 0.16a	nd	nd	nd	nd	nd	0.35 ±0.02b	nd	nd	nd	nd
A2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.44 ±0.03a	nd	nd	nd
A3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A4	nd	nd	0.37 ±0.04e	10.55 ±0.31a	nd	nd	nd	nd	nd	0.57 ±0.11d	8.42 ±0.22b	nd	nd	nd	nd	nd	8.03 ±0.22c	nd	nd	nd
A5	nd	nd	nd	0.23 ±0.02b	nd	nd	nd	nd	nd	nd	0.31 ±0.03a	nd	nd	nd	nd	nd	nd	nd	nd	nd
A6	nd	nd	nd	nd	nd	nd	1.04 ±0.11d	nd	nd	nd	nd	nd	0.41 ±0.01e	1.25 ±0.05c	1.31 ±0.11c	1.79 ±0.11b	3.16 ±0.11a	1.04 ±0.11d	1.23 ±0.10c	nd
A7	nd	nd	nd	3.32 ±0.26b	3.69 ±0.36a	nd	nd	nd	nd	nd	2.21 ±0.12d	2.84 ±0.10c	nd	nd	nd	nd	2.34 ±0.15d	2.79 ±0.14c	nd	nd
A8	nd	nd	nd	nd	nd	nd	0.04 ±0.00d	nd	nd	nd	nd	nd	0.07 ±0.00b	0.22 ±0.02a	nd	nd	nd	0.04 ±0.00d	0.06 ±0.01c	nd
A9	nd	nd	nd	0.83 ±0.11a	nd	nd	nd	nd	nd	nd	0.61 ±0.01b	nd	nd	nd	0.02 ±0.00c	0.03 ±0.00c	0.65 ±0.06b	nd	nd	nd
A10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A11	nd	nd	nd	0.04 ±0.00a	nd	nd	nd	nd	nd	nd	0.03 ±0.00b	nd	nd	nd	nd	nd	0.03 ±0.01b	nd	nd	nd
A12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A13	nd	nd	nd	17.05 ±0.24a	nd	nd	nd	nd	nd	nd	10.96 ±0.32b	nd	nd	nd	nd	nd	10.47 ±0.44c	nd	nd	nd
A14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total A	**nd**	**0.17 ±0.01k**	**2.14 ±0.03g**	**32.02 ±0.15a**	**3.69 ±0.36e**	**nd**	**1.08 ±0.05i**	**nd**	**0.23 ±0.02k**	**2.30 ±0.12g**	**22.54 ±0.11c**	**2.84 ±0.10f**	**0.48 ±0.01j**	**1.47 ±0.03h**	**1.33 ±0.06h**	**2.17 ±0.07g**	**25.12 ±0.18b**	**3.87 ±0.05d**	**1.29 ±0.06h**	**nd**
Hydroxybenzoic acids																				
B1	nd	nd	nd	nd	nd	nd	1.03 ±0.02f	nd	nd	nd	nd	nd	1.09 ±0.02f	3.91 ±0.04b	3.39 ±0.22d	3.34 ±0.12de	3.75 ±0.06c	4.71 ±0.11a	3.25 ±0.10e	nd
B2	nd	nd	nd	nd	nd	nd	2.06 ±0.02f	nd	nd	nd	nd	nd	2.19 ±0.03f	7.84 ±0.11b	6.79 ±0.32d	6.69 ±0.21de	7.51 ±0.22c	9.44 ±0.42a	6.52 ±0.15e	nd

B3	nd	nd	nd	2.52 ±0.03b	nd	nd	nd	nd	nd	nd	7.33 ±0.12a	nd	nd	nd	nd	nd	nd	nd	nd	nd
B4	nd	nd	nd	nd	nd	1.10 ±0.01e	nd	5.15 ±0.03cd	5.31 ±0.15c	5.06 ±0.02d	nd	10.06 ±0.36b	nd	nd	nd	nd	nd	nd	nd	17.89 ±0.22a
B5	nd	nd	nd	nd	nd	nd	0.54 ±0.01e	nd	nd	nd	nd	nd	2.18 ±0.03a	1.68 ±0.12cd	1.66 ±0.03cd	1.62 ±0.09d	1.71 ±0.02c	1.64 ±0.03cd	1.80 ±0.10b	nd
B6	nd	nd	nd	nd	nd	nd	23.10 ±0.74d	nd	nd	nd	nd	nd	21.30 ±0.56e	73.86 ±1.22a	73.03 ±2.14a	66.75 ±1.03c	69.94 ±0.42b	73.53 ±0.34a	66.42 ±1.23c	nd
B7	nd	nd	nd	2.43 ±0.06a	nd	nd	nd	nd	nd	nd	2.36 ±0.11b	nd	nd	nd	nd	nd	nd	nd	nd	nd
B8	nd	nd	nd	nd	nd	1.70 ±0.12d	nd	2.37 ±0.19b	0.54 ±0.04f	0.45 ±0.03f	nd	2.77 ±0.12a	nd	nd	nd	nd	nd	nd	2.01 ±0.22c	1.09 ±0.06e
B9	nd	nd	nd	0.39 ±0.02a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B10	nd	nd	nd	nd	nd	nd	0.03 ±0.00cd	nd	nd	nd	nd	nd	0.06 ±0.00bc	0.10 ±0.00b	0.10 ±0.00b	0.41 ±0.06a	0.10 ±0.01b	nd	0.08 ±0.01bc	nd
B11	nd	nd	nd	nd	7.87 ±0.52a	nd	nd	nd	nd	nd	nd	2.29 ±0.22c	nd	nd	nd	nd	nd	4.26 ±0.42b	nd	nd
B12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B13	nd	nd	nd	nd	nd	nd	0.70 ±0.09f	nd	nd	nd	nd	nd	0.58 ±0.08g	2.74 ±0.11b	2.42 ±0.21d	2.33 ±0.08e	2.73 0.12b	3.37 ±0.11a	2.55 ±0.17c	nd
B14	nd	nd	nd	nd	1.15 ±0.03b	nd	nd	nd	nd	nd	nd	0.95 ±0.05c	nd	nd	nd	nd	nd	1.69 ±0.08a	nd	nd
B15	nd	nd	nd	0.13 ±0.01a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B16	nd	nd	nd	nd	nd	nd	0.21 ±0.03d	nd	nd	nd	nd	nd	0.27 ±0.03c	1.15 ±0.11a	1.11 ±0.08a	0.99 ±0.05b	0.23 ±0.01cd	0.12 ±0.01e	0.26 ±0.04cd	nd
B17	nd	nd	nd	nd	9.34 ±0.12b	nd	nd	nd	nd	nd	nd	7.75 ±0.42c	nd	nd	nd	nd	nd	15.28 ±0.22a	nd	nd
B18	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.31 ±0.05a	nd	nd	nd
B19	nd	nd	nd	1.50 ±0.21a	nd	nd	nd	nd	nd	nd	0.84 ±0.09b	nd	nd	nd	nd	nd	0.11 ±0.02c	nd	nd	nd
B20	nd	nd	nd	nd	nd	nd	0.26 ±0.02b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.26 ±0.11a	nd	nd
B21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B22	nd	nd	nd	nd	0.64 ±0.03b	nd	nd	nd	nd	nd	nd	0.95 ±0.15a	nd	nd	nd	nd	nd	0.71 ±0.12b	nd	nd
B23	nd	nd	nd	nd	0.26 ±0.02c	nd	nd	nd	nd	nd	nd	1.02 ±0.10a	nd	nd	nd	nd	nd	0.41 ±0.05b	nd	nd
B24	nd	nd	nd	nd	nd	nd	0.98 ±0.08e	nd	nd	nd	nd	nd	0.79 ±0.12f	2.61 ±0.34bc	2.74 ±0.22b	2.92 ±0.11a	0.80 ±0.09f	1.72 ±0.10d	2.46 ±0.06c	nd
B25	nd	nd	nd	nd	nd	nd	nd	0.04 ±0.00b	nd	nd	nd	0.39 ±0.03a	nd	nd	nd	nd	nd	nd	nd	0.03 ±0.00c
B26	nd	nd	nd	nd	3.56 ±0.23a	nd	nd	nd	nd	nd	nd	2.98 ±0.33b	nd	nd	nd	nd	nd	0.28 ±0.04c	nd	nd
B27	nd	nd	nd	nd	nd	nd	0.18 ±0.02c	nd	nd	nd	nd	nd	nd	3.02 ±0.22b	2.96 ±0.12b	3.39 ±0.43a	nd	nd	3.12 ±0.21b	nd

B28	nd	nd	nd	nd	nd	nd	0.21 ±0.01a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.43 ±0.03a	nd	nd	nd	nd
B30	nd	nd	nd	nd	nd	nd	nd	0.06 ±0.00c	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.08 ±0.00b	0.12 ±0.01a
B31	nd	nd	nd	nd	0.87 ±0.06de	nd	nd	nd	nd	nd	nd	0.62 ±0.11e	nd	2.91 ±0.42c	nd	nd	4.81 ±0.44a	0.92 ±0.13d	3.65 ±0.33b	nd
B32	nd	nd	nd	nd	0.52 ±0.03c	nd	nd	nd	nd	nd	nd	0.19 ±0.02d	nd	nd	nd	nd	nd	0.88 ±0.11a	0.74 ±0.11b	nd
B33	nd	nd	nd	nd	3.04 ±0.13b	nd	nd	nd	nd	nd	nd	3.13 ±0.12b	nd	nd	nd	nd	nd	4.30 ±0.31a	0.81 ±0.12c	nd
B34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B36	nd	nd	nd	nd	1.11 ±0.24a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total B	**nd**	**nd**	**nd**	**6.97 ±0.13m**	**28.36 ±0.15i**	**2.80 ±0.05p**	**29.28 ±0.11h**	**7.62 ±0.04l**	**5.85 ±0.10n**	**5.51 ±0.06o**	**10.53 ±0.10k**	**33.08 ±0.11g**	**28.46 ±0.13i**	**99.84 ±0.31b**	**94.19 ±0.14c**	**88.87 ±0.24f**	**91.99 ±0.21e**	**125.51 ±0.34a**	**93.74 ±0.13d**	**19.14 ±0.08j**
Hydroxycinnamic acids																				
C1	0.33 ±0.03def	0.55 ±0.09a	0.27 ±0.02efghi	0.46 ±0.05bc	0.45 ±0.02bc	0.37 ±0.01cd	0.42 ±0.02bc	0.25 ±0.03fghi	0.20 ±0.03hi	0.19 ±0.01i	0.30 ±0.02defg	0.31 ±0.01defg	0.23 ±0.01	0.35 ±0.05de	0.29 ±0.07defgh	0.22 ±0.00ghi	0.48 ±0.04ab	0.46 ±0.02bc	0.32 ±0.01def	nd
C2	5.00 ±0.11c	5.43 ±0.06b	5.42 ±0.22b	5.46 ±0.32b	4.81 ±0.16cd	5.10 ±0.21bc	6.62 ±0.21a	3.53 ±0.22f	3.48 ±0.34fg	3.48 ±0.12fg	3.44 ±0.03fg	3.09 ±0.22g	3.13 ±0.11fg	4.57 ±0.32de	4.58 ±0.44de	4.28 ±0.30e	4.47 ±0.11de	4.43 ±0.22de	4.27 ±0.31e	0.40 ±0.02h
C3	0.20 ±0.02g	0.21 ±0.02fg	0.21 ±0.01fg	0.27 ±0.03cdef	0.33 ±0.01bc	0.23 ±0.01efg	0.24 ±0.03defg	0.24 ±0.03efg	0.26 ±0.03defg	0.28 ±0.01bcdef	0.31 ±0.05bcd	0.34 ±0.11b	0.26 ±0.01defg	0.24 ±0.04def	0.29 ±0.02bcde	0.25 ±0.02defg	0.29 ±0.00bcde	0.41 ±0.05a	0.24 ±0.02defg	0.02 ±0.00h
C4	0.13 ±0.00de	0.14 ±0.01cd	0.15 ±0.01c	0.28 ±0.02a	0.10 ±0.02fg	0.13 ±0.01de	0.15 ±0.00cd	0.08 ±0.00g	0.09 ±0.00g	0.08 ±0.01g	0.15 ±0.01c	0.05 ±0.00h	0.15 ±0.00c	0.14 ±0.02cd	0.12 ±0.00ef	0.18 ±0.01b	0.08 ±0.00g	0.10 ±0.01fg	0.18 ±0.03b	0.05 ±0.00h
C5	0.92 ±0.11ab	0.99 ±0.03a	1.01 ±0.00a	0.97 ±0.11ab	0.93 ±0.08ab	0.93 ±0.11ab	0.99 ±0.03a	0.63 ±0.12defg	0.59 ±0.03efg	0.57 ±0.06efg	0.56 ±0.12fg	0.53 ±0.02g	0.50 ±0.08g	0.77 ±0.13cd	0.82 ±0.13bc	0.72 ±0.11cde	0.70 ±0.10cdef	0.76 ±0.09cd	0.76 ±0.04cd	nd
Total C	**6.58 ±0.05d**	**7.32 ±0.03b**	**7.06 ±0.02c**	**7.44 ±0.04b**	**6.62 ±0.10d**	**6.76 ±0.13d**	**8.42 ±0.11a**	**4.73 ±0.08g**	**4.62 ±0.21g**	**4.59 ±0.03g**	**4.76 ±0.06g**	**4.32 ±0.05h**	**4.27 ±0.08h**	**6.07 ±0.13e**	**6.10 ±0.18e**	**5.65 ±0.11f**	**6.02 ±0.06e**	**6.16 ±0.11e**	**5.77 ±0.22f**	**0.47 ±0.01i**
Dihydrochalcones																				
D1	2.08 ±0.42bc	2.22 ±0.04ab	2.43 ±0.10a	2.27 ±0.01ab	1.78 ±0.11d	2.10 ±0.04b	2.23 ±0.06ab	1.29 ±0.06ghi	1.22 ±0.11ghi	1.32 ±0.08fgh	1.15 ±0.04hi	1.09 ±0.12ij	0.90 ±0.22j	1.79 ±0.03d	1.53 ±0.03ef	1.87 ±0.11cd	1.84 ±0.11d	1.42 ±0.03fg	1.71 ±0.01de	nd
D2	2.53 ±0.13c	2.74 ±0.17ab	2.76 ±0.05ab	2.89 ±0.12a	2.54 ±0.08c	2.61 ±0.11bc	2.72 ±0.11abc	1.37 ±0.02e	1.22 ±0.05ef	1.07 ±0.03f	1.13 ±0.10f	1.11 ±0.02f	0.83 ±0.09g	2.20 ±0.11c	2.13 ±0.04c	2.17 ±0.22c	2.22 ±0.15c	2.05 ±0.11c	2.03 ±0.11c	nd
Total D	**4.61 ±0.10c**	**4.96 ±0.08b**	**5.19 ±0.11a**	**5.16 ±0.09a**	**4.32 ±0.10d**	**4.71 ±0.12c**	**4.95 ±0.12b**	**2.66 ±0.08h**	**2.44 ±0.06i**	**2.39 ±0.06ij**	**2.27 ±0.10jk**	**2.20 ±0.04k**	**1.73 ±0.15l**	**3.99 ±0.10e**	**3.66 ±0.05f**	**4.04 ±0.12e**	**4.06 ±0.11e**	**3.47 ±0.08g**	**3.74 ±0.10f**	**nd**
Flavan-3-ols																				
E1	1.49 ±0.06hi	1.61 ±0.10h	1.13 ±0.11jk	11.25 ±0.23b	1.09 ±0.11jk	1.60 ±0.06h	2.00 ±0.02g	0.90 ±0.10jk	0.88 ±0.03jk	0.82 ±0.04k	10.31 ±0.54c	1.20 ±0.03ij	1.12 ±0.04jk	6.70 ±0.42e	2.90 ±0.11f	8.60 ±0.32d	19.36 ±0.15a	2.20 ±0.04g	3.08 ±0.02f	0.34 ±0.00l
E2	nd	nd	nd	nd	nd	nd	0.06 ±0.00d	nd	nd	nd	nd	nd	nd	0.06 ±0.00d	0.05 ±0.00d	0.13 ±0.00c	0.38 ±0.01b	0.90 ±0.11a	0.06 ±0.00d	nd
E3	1.48 ±0.11kl	1.79 ±0.05jk	1.44 ±0.14kl	2.18 ±0.22i	4.17 ±0.32gh	1.92 ±0.06ij	5.72 ±0.14f	1.19 ±0.12lm	1.44 ±0.08kl	0.93 ±0.03m	1.68 ±0.21jk	4.39 ±0.22g	4.02 ±0.13h	10.96 ±0.24d	11.97 ±0.32b	10.79 ±0.11d	11.54 ±0.08c	13.03 ±0.54a	10.14 ±0.21e	0.56 ±0.15n

E4	5.98 ±0.04e	7.20 ±0.22b	6.60 ±0.03c	7.20 ±0.0b	6.07 ±0.12de	6.83 ±0.11c	7.94 ±0.21a	3.76 ±0.22f	3.52 ±0.04fg	3.53 ±0.11fg	3.60 ±0.42f	3.74 ±0.10f	3.28 ±0.12g	6.64 ±0.22c	6.77 ±0.18c	6.83 ±0.11c	1.05 ±0.06h	6.28 ±0.16d	6.10 ±0.11de	nd
E5	1.65 ±0.11de	1.79 ±0.06d	1.80 ±0.02d	6.43 ±0.11a	1.45 ±0.12f	1.76 ±0.04de	1.62 ±0.12e	1.08 ±0.11hi	1.06 ±0.15hij	0.96 ±0.06ijk	4.62 ±0.12b	0.83 ±0.02k	0.56 ±0.03l	0.91 ±0.04jk	1.31 ±0.12fg	1.18 ±0.01gh	3.47 ±0.14c	1.12 ±0.03h	1.14 ±0.04h	nd
E6	0.40 ±0.01gh	0.79 ±0.10a	0.61 ±0.03de	0.74 ±0.04ab	0.44 ±0.02fgh	0.80 ±0.03a	0.62 ±0.04de	0.58 ±0.02e	0.38 ±0.00h	0.56 ±0.03e	0.66 ±0.05cd	0.50 ±0.04f	0.26 ±0.00i	0.44 ±0.01fgh	0.46 ±0.03fg	0.49 ±0.00f	0.71 ±0.04bc	0.44 ±0.00fgh	0.56 ±0.03e	nd
PP	108.11 ±1.25k	109.15 ±2.56k	88.69 ±3.64l	118.40 ±2.54k	209.99 ±7.45i	242.68 ±3.56g	160.66 ±4.56j	223.40 ±9.53h	203.05 ±2.38i	204.72 ±5.63i	205.39 ±4.91i	281.04 ±11.22f	325.63 ±8.64e	408.62 ±9.31b	402.90 ±12.11b	384.42 ±5.42c	387.13 ±6.81c	463.40 ±3.12a	398.55 ±4.12b	361.31 ±2.08d
Total E	**119.11 ±1.14o**	**122.33 ±2.15o**	**100.27 ±2.54p**	**146.20 ±3.15n**	**223.21 ±4.64k**	**255.59 ±3.18i**	**178.62 ±3.21m**	**230.91 ±4.51j**	**210.33 ±3.14l**	**211.52 ±2.55l**	**226.26 ±3.27jk**	**291.70 ±4.16h**	**334.87 ±5.24g**	**434.33 ±3.12b**	**426.36 ±4.22c**	**412.44 ±2.55e**	**423.64 ±5.44cd**	**487.37 ±4.13a**	**419.63 ±4.45d**	**362.21 ±3.11f**
DP	3.53	3.64	3.28	3.66	4.78	4.10	4.11	7.54	6.90	6.95	7.75	7.82	7.00	4.61	4.85	4.58	4.67	4.95	4.81	55.67
Flavonols and Flavones																				
F1	nd	0.39 ±0.02c	2.06 ±0.11a	nd	nd	nd	nd	nd	0.33 ±0.03c	1.81 ±0.22b	nd	nd	nd	nd	0.35 ±0.04c	0.34 ±0.02c	nd	nd	nd	nd
F2	nd	nd	0.06 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F3	nd	0.04 ±0.00c	0.15 ±0.01a	nd	nd	nd	nd	nd	nd	0.12 ±0.01b	nd	nd	nd	nd	nd	0.12 ±0.00b	nd	nd	nd	nd
F4	nd	0.24 ±0.03c	0.41 ±0.05b	nd	nd	nd	nd	nd	nd	0.26 ±0.03c	nd	nd	nd	nd	0.42 ±0.05b	0.75 ±0.12a	nd	nd	nd	nd
F5	nd	nd	nd	nd	0.09 ±0.00g	nd	0.21 ±0.02e	nd	nd	nd	nd	0.06 ±0.00g	0.12 ±0.01f	0.36 ±0.03c	nd	nd	0.41 ±0.02b	0.46 ±0.06a	0.30 ±0.02d	nd
F6	nd	nd	nd	1.75 ±0.10a	nd	nd	nd	nd	nd	nd	1.19 ±0.02c	nd	nd	nd	nd	nd	1.35 ±0.12b	nd	nd	nd
F7	nd	0.35 ±0.02d	2.26 ±0.22a	nd	0.09 ±0.02e	nd	nd	nd	0.31 ±0.03d	2.09 ±0.12b	nd	0.05 ±0.00e	nd	nd	0.36 ±0.07d	1.80 ±0.11c	nd	0.10 ±0.00e	nd	nd
F8	nd	nd	nd	9.85 ±0.54a	nd	nd	0.33 ±0.02d	nd	nd	nd	8.69 ±0.34c	nd	0.26 ±0.04de	0.11 ±0.01de	0.16 ±0.03de	0.25 ±0.02de	9.32 ±0.23	0.16 ±0.02de	0.13 ±0.00	nd
F9	nd	nd	nd	0.50 ±0.08b	nd	nd	0.25 ±0.03d	nd	nd	nd	0.40 ±0.01c	nd	0.03 ±0.00f	0.10 ±0.00e	0.85 ±0.11a	0.86 ±0.03a	0.48 ±0.05b	nd	0.04 ±0.00f	nd
F10	nd	0.17 ±0.00e	1.18 ±0.02b	nd	nd	nd	nd	nd	0.10 ±0.00f	0.95 ±0.11c	nd	nd	nd	nd	0.60 ±0.06d	1.66 ±0.12a	nd	nd	nd	nd
F11	nd	nd	nd	nd	nd	nd	0.08 ±0.00d	nd	nd	nd	nd	nd	0.10 ±0.00d	0.26 ±0.01c	0.32 ±0.03b	0.30 ±0.03bc	0.51 ±0.15a	nd	0.31 ±0.02bc	nd
F12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.86 ±0.04a	0.23 ±0.00b
F13	nd	2.13 ±0.02d	11.84 ±0.35a	nd	nd	nd	nd	nd	1.89 ±0.12e	11.34 ±0.24b	nd	nd	nd	nd	1.91 ±0.12e	9.57 ±0.16c	nd	nd	nd	nd
F14	nd	nd	nd	1.18 ±0.01a	nd	nd	nd	nd	nd	nd	1.09 ±0.03b	nd	nd	nd	nd	nd	1.06 ±0.02c	nd	nd	nd
F15	nd	nd	nd	nd	nd	nd	0.27 ±0.01a	nd	nd	nd	nd	nd	nd	0.21 ±0.00b	nd	nd	nd	nd	nd	nd
F16	nd	nd	0.20 ±0.01a	nd	nd	nd	nd	nd	0.03 ±0.00c	0.18 ±0.00b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F17	nd	nd	nd	nd	nd	nd	0.03 ±0.00e	nd	nd	nd	nd	nd	nd	0.30 ±0.02b	0.28 ±0.01c	0.32 ±0.02a	0.31 ±0.01ab	0.24 ±0.02d	0.24 ±0.01d	nd
F18	nd	nd	nd	5.88 ±0.22a	nd	nd	0.02 ±0.00e	nd	nd	nd	5.47 ±0.24b	nd	0.07 ±0.00de	0.23 ±0.01c	0.18 ±0.02cd	0.12 ±0.01cde	5.78 ±0.15a	0.18 ±0.00cd	0.12 ±0.01cde	nd

F19	0.57 ±0.07f	0.67 ±0.12ef	0.57 ±0.03f	1.05 ±0.02c	1.28 ±0.04b	0.66 ±0.02ef	0.75 ±0.11e	0.31 ±0.02h	0.28 ±0.02h	0.29 ±0.03h	0.62 ±0.14f	0.99 ±0.12cd	0.45 ±0.02g	0.92 ±0.06d	0.94 ±0.04cd	0.88 ±0.04d	1.34 ±0.01b	1.65 ±0.11a	0.93 ±0.03cd	0.02 ±0.00i
F20	0.10 ±0.00i	0.21 ±0.02f	0.40 ±0.01d	0.21 ±0.02f	0.23 ±0.01f	0.14 ±0.00h	0.17 ±0.01g	0.07 ±0.00j	0.49 ±0.03b	0.39 ±0.03d	0.17 ±0.01gh	0.28 ±0.02e	0.16 ±0.01gh	0.26 ±0.02e	0.53 ±0.03a	0.49 ±0.02b	0.40 ±0.01d	0.45 ±0.04c	0.23 ±0.00f	0.02 ±0.00k
F21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F22	nd	nd	nd	nd	0.53 ±0.03b	nd	nd	nd	nd	nd	nd	0.42 ±0.02c	nd	nd	nd	nd	nd	0.55 ±0.04a	nd	nd
F23	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.05 ±0.00a
F24	nd	0.22 ±0.02d	1.32 ±0.06a	nd	nd	nd	nd	nd	0.07 ±0.00f	1.21 ±0.03b	nd	nd	nd	nd	0.18 ±0.01e	1.01 ±0.06c	nd	nd	nd	nd
F25	0.14 ±0.00de	0.18 ±0.03cde	0.16 ±0.00cde	0.17 ±0.04cde	0.27 ±0.03b	0.15 ±0.01de	0.15 ±0.01de	0.14 ±0.01de	0.02 ±0.00gh	1.26 ±0.12a	0.05 ±0.00gh	0.19 ±0.01cd	0.07 ±0.00fg	0.11 ±0.00ef	0.17 ±0.01cde	0.14 ±0.01de	0.17 ±0.02cde	0.27 ±0.04b	0.22 ±0.02bc	nd
F26	nd	nd	nd	nd	0.39 ±0.02c	nd	nd	nd	nd	nd	nd	0.43 ±0.03b	nd	nd	nd	nd	nd	0.50 ±0.05a	0.11 ±0.00d	nd
F27	0.32 ±0.04f	0.41 ±0.03ef	0.36 ±0.04f	0.42 ±0.01ef	0.68 ±0.10c	0.41 ±0.01ef	0.74 ±0.02c	0.21 ±0.02g	0.18 ±0.00g	0.11 ±0.01g	0.17 ±0.02g	0.53 ±0.06d	0.47 ±0.04de	1.31 ±0.12b	1.33 ±0.03ab	1.37 ±0.06ab	1.35 ±0.07ab	1.43 ±0.05a	1.29 ±0.15b	nd
F28	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.02 ±0.00a
F29	0.50 ±0.03hi	0.84 ±0.05g	1.52 ±0.11d	1.27 ±0.12e	0.53 ±0.03hi	0.68 ±0.04gh	1.07 ±0.06f	0.38 ±0.03i	0.42 ±0.05i	1.34 ±0.20e	0.84 ±0.05g	0.34 ±0.03i	0.79 ±0.04g	1.73 ±0.11c	1.90 ±0.03c	2.54 ±0.22a	2.19 ±0.13b	1.52 ±0.21d	1.77 ±0.13c	nd
F30	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F31	nd	nd	0.23 ±0.02b	nd	nd	nd	nd	nd	0.04 ±0.00e	0.26 ±0.01a	nd	nd	nd	nd	0.10 ±0.01c	0.07 ±0.00d	nd	nd	nd	nd
F32	nd	nd	nd	nd	0.21 ±0.01a	nd	nd	nd	nd	nd	nd	0.10 ±0.00c	nd	nd	nd	nd	nd	0.15 ±0.01b	nd	nd
F33	nd	nd	nd	0.33 ±0.03b	nd	nd	nd	nd	nd	nd	0.37 ±0.02a	nd	nd	nd	nd	nd	0.36 ±0.03a	nd	nd	nd
F34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.02 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd
F35	nd	nd	nd	nd	0.05 ±0.00b	nd	nd	nd	nd	nd	nd	0.04 ±0.00c	nd	nd	nd	nd	nd	0.12 ±0.01a	nd	nd
F36	nd	nd	nd	nd	3.78 ±0.12b	nd	nd	nd	nd	nd	nd	4.04 ±0.24a	nd	nd	nd	nd	nd	1.80 ±0.12c	nd	nd
F37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.02 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd
Total F	**1.63 ±0.06m**	**5.85 ±0.10j**	**22.72 ±0.13b**	**22.61 ±0.11b**	**8.12 ±0.05g**	**2.04 ±0.03l**	**4.07 ±0.06**	**1.11 ±0.03n**	**4.16 ±0.11**	**21.61 ±0.08c**	**19.06 ±0.10d**	**7.51 ±0.08h**	**2.52 ±0.03k**	**5.90 ±0.12j**	**10.58 ±0.08e**	**22.59 ±0.14b**	**25.03 ±0.22a**	**9.58 ±0.13f**	**6.55 ±0.09i**	**0,34 ±0.02o**
Total All	**131.93 ±3.45n**	**140.64 ±5.12m**	**137.37 ±3.44mn**	**220.40 ±8.21l**	**274.32 ±4.22j**	**271.90 ±3.03j**	**226.41 ±4.21l**	**247.03 ±5.32k**	**227.63 ±3.26l**	**247.92 ±4.12k**	**285.42 ±5.26i**	**341.65 ±2.12h**	**372.34 ±3.22g**	**551.60 ±4.51c**	**542.23 ±2.14d**	**535.75 ±3.11de**	**575.86 ±1.66b**	**635.96 ±5.21a**	**530.72 ±6.11e**	**382.16 ±5.01f**

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-p) are significantly different (homogenous groups) at $p \leq 0.05$; nd ≤ LOD. DP = degree of polymeryzation. Total All = sum of all detected polyphenols. Sample codes have their references in **Annex 1b.**. Phenolic compounds have their references in the **Table 4.**.

Storage time: 3 months

Code	Final product (mg/100 g fw)																			
	B1	**B1S01**	**B1S05**	**B1M5**	**B1F5**	**B1K5**	**B1C5**	**B2**	**B2S01**	**B2S05**	**B2M5**	**B2F5**	**B2C5**	**B3**	**B3S01**	**B3S05**	**B3M5**	**B3F5**	**B3K5**	**BK**
Anthocyanins																				
A1	nd	0.09 ±0.00c	0.38 ±0.02b	nd	nd	nd	nd	nd	0.07 ±0.00c	0.57 ±0.06a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A4	nd	nd	nd	4.21 ±0.12a	nd	nd	nd	nd	nd	0.38 ±0.02d	2.98 ±0.11b	nd	nd	nd	nd	nd	1.10 ±0.08c	nd	nd	nd
A5	nd	nd	nd	0.10 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A6	nd	nd	nd	nd	nd	nd	0.12 ±0.01d	nd	nd	nd	nd	nd	0.05 ±0.00e	0.04 ±0.00e	0.15 ±0.01c	0.16 ±0.02c	0.49 ±0.03a	0.18 ±0.02b	0.15 ±0.02c	nd
A7	nd	nd	nd	1.06 ±0.08a	0.69 ±0.02b	nd	nd	nd	nd	nd	0.67 ±0.04b	0.44 ±0.03c	nd	nd	nd	nd	0.28 ±0.03d	0.30 ±0.03d	nd	nd
A8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.06 ±0.01a	nd	nd	nd	nd	nd	nd
A9	nd	nd	nd	0.33 ±0.02a	nd	nd	nd	nd	nd	nd	0.23 ±0.02b	nd	nd	nd	nd	nd	0.09 ±0.00c	nd	nd	nd
A10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A11	nd	nd	nd	0.01 ±0.00a	nd	nd	nd	nd	nd	nd	0.01 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd	nd
A12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A13	nd	nd	nd	5.64 ±0.22a	nd	nd	nd	nd	nd	nd	3.60 ±0.12b	nd	nd	nd	nd	nd	1.58 ±0.10c	nd	nd	nd
A14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total A	**nd**	**0.09 ±0.00hij**	**0.38 ±0.02g**	**11.35 ±0.10a**	**0.69 ±0.02e**	**nd**	**0.12 ±0.01hij**	**nd**	**0.07 ±0.00ijk**	**0.95 ±0.05d**	**7.49 ±0.10b**	**0.44 ±0.03fg**	**0.05 ±0.00jk**	**0.10 ±0.01hij**	**0.15 ±0.01hi**	**0.16 ±0.02h**	**3.54 ±0.11c**	**0.48 ±0.03f**	**0.15 ±0.02h**	**nd**
Hydroxybenzoic acids																				
B1	nd	nd	nd	nd	nd	nd	0.87 ±0.06f	nd	nd	nd	nd	nd	1.05 ±0.11e	2.90 ±0.12b	2.57 ±0.13d	2.84 ±0.11bc	3.21 ±0.12a	2.77 ±0.11c	2.85 ±0.13bc	nd
B2	nd	nd	nd	nd	nd	nd	1.74 ±0.11f	nd	nd	nd	nd	nd	2.10 ±0.22e	5.81 ±0.22b	5.16 ±0.32d	5.70 ±0.34bc	6.44 ±0.22a	5.55 ±0.32c	5.72 ±0.22bc	nd
B3	nd	nd	nd	2.00 ±0.12b	nd	nd	nd	nd	nd	nd	6.41 ±0.22a	nd	nd	nd	nd	nd	nd	nd	nd	nd

B4	nd	nd	nd	nd	nd	0.65 ±0.03e	nd	4.14 ±0.22d	4.33 ±0.31c	4.26 ±0.11cd	nd	7.77 ±0.32b	nd	nd	nd	nd	nd	nd	13.23 ±1.02a
B5	nd	nd	nd	nd	nd	nd	0.36 ±0.01f	nd	nd	nd	nd	nd	2.26 ±0.32a	0.80 ±0.03d	0.70 ±0.03e	0.75 ±0.03de	0.74 ±0.03de	0.98 ±0.06c	1.08 ±0.11b	nd
B6	nd	nd	nd	nd	nd	nd	19.67 ±0.56c	nd	nd	nd	nd	nd	18.50 ±0.41c	60.31 ±2.33b	60.91 ±3.11b	61.00 ±0.41b	60.82 ±4.22b	66.48 ±1.22a	60.71 ±2.22b	nd
B7	nd	nd	nd	2.07 ±0.11a	nd	nd	nd	nd	nd	nd	0.49 ±0.02b	nd	nd	nd	nd	nd	nd	nd	nd	nd
B8	nd	nd	nd	nd	nd	1.74 ±0.12b	nd	0.47 ±0.02de	0.54 ±0.03d	0.39 ±00.02e	nd	1.31 ±0.06c	nd	nd	nd	nd	nd	nd	2.17 ±0.06a	0.47 ±0.02de
B9	nd	nd	nd	0.33 ±0.02a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B10	nd	nd	nd	nd	nd	nd	0.01 ±0.00de	nd	nd	nd	nd	nd	0.03 ±0.00cd	0.07 ±0.00b	0.05 ±0.00bc	0.22 ±0.02a	0.06 ±0.00b	nd	0.05 ±0.00bc	nd
B11	nd	nd	nd	nd	8.44 ±0.22a	nd	nd	nd	nd	nd	nd	1.26 ±0.03c	nd	nd	nd	nd	nd	3.52 ±0.11b	nd	nd
B12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B13	nd	nd	nd	nd	nd	nd	0.27 ±0.01d	nd	nd	nd	nd	nd	0.37 ±0.02d	1.26 ±0.13c	2.23 ±0.22a	1.22 ±0.11c	2.30 ±0.11a	1.97 ±0.12b	2.22 ±0.02a	nd
B14	nd	nd	nd	nd	0.79 ±0.03b	nd	nd	nd	nd	nd	nd	0.50 ±0.03c	nd	nd	nd	nd	nd	0.98 ±0.06a	nd	nd
B15	nd	nd	nd	0.13 ±0.01a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B16	nd	nd	nd	nd	nd	nd	0.12 ±0.01de	nd	nd	nd	nd	nd	0.17 ±0.02d	0.71 ±0.06b	1.07 ±0.05a	0.44 ±0.02c	0.10 ±0.01e	0.07 ±0.00ef	0.18 ±0.01d	nd
B17	nd	nd	nd	nd	6.99 ±0.22b	nd	nd	nd	nd	nd	nd	0.24 ±0.02c	nd	nd	nd	nd	nd	15.00 ±0.41a	nd	nd
B18	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.23 ±0.02a	nd	nd	nd
B19	nd	nd	nd	1.09 ±0.12a	nd	nd	nd	nd	nd	nd	1.02 ±0.21a	nd	nd	nd	nd	nd	0.06 ±0.00b	nd	nd	nd
B20	nd	nd	nd	nd	nd	nd	0.07 ±0.00b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.26 ±0.08a	nd	nd
B21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B22	nd	nd	nd	nd	0.37 ±0.01b	nd	nd	nd	nd	nd	nd	0.40 ±0.02b	nd	nd	nd	nd	nd	0.56 ±0.04a	nd	nd
B23	nd	nd	nd	nd	0.32 ±0.02b	nd	nd	nd	nd	nd	nd	0.27 ±0.01c	nd	nd	nd	nd	nd	3.96 ±0.23a	nd	nd
B24	nd	nd	nd	nd	nd	nd	0.39 ±0.01c	nd	nd	nd	nd	nd	0.37 ±0.03c	0.99 ±0.03b	1.00 ±0.06b	1.26 ±0.13a	0.37 ±0.03c	0.26 ±0.01c	1.14 ±0.06ab	nd
B25	nd	nd	nd	nd	nd	nd	nd	0.03 ±0.00b	nd	nd	nd	0.28 ±0.03a	nd	nd	nd	nd	nd	nd	nd	nd
B26	nd	nd	nd	nd	0.37 ±0.03b	nd	nd	nd	nd	nd	nd	1.68 ±0.12a	nd	nd	nd	nd	nd	0.13 ±0.01c	nd	nd
B27	nd	nd	nd	nd	nd	nd	0.07 ±0.00c	nd	nd	nd	nd	nd	nd	2.31 ±0.11b	2.66 ±0.21a	2.78 ±0.22a	nd	nd	2.22 ±0.33b	nd
B28	nd	nd	nd	nd	nd	nd	0.18 ±0.01a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

B29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.17 ±0.00a	nd	nd	nd	nd
B30	nd	nd	nd	nd	nd	nd	nd	0.04 ±0.00b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.04 ±0.00b	0.08 ±0.01a
B31	nd	nd	nd	nd	0.50 ±0.06d	nd	nd	nd	nd	nd	nd	0.34 ±0.05d	nd	1.49 ±0.15c	nd	nd	2.97 ±0.22a	0.59 ±0.02d	1.79 ±0.06b	nd
B32	nd	nd	nd	nd	0.33 ±0.03b	nd	nd	nd	nd	nd	nd	0.15 ±0.01c	nd	nd	nd	nd	nd	0.33 ±0.02b	0.57 ±0.03a	nd
B33	nd	nd	nd	nd	2.93 ±0.22b	nd	nd	nd	nd	nd	nd	2.42 ±0.22c	nd	nd	nd	nd	nd	3.10 ±0.11a	0.85 ±0.06d	nd
B34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B36	nd	nd	nd	nd	0.79 ±0.03a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total B	**nd**	**nd**	**nd**	**5.62 ±0.08l**	**21.83 ±0.10h**	**2.39 ±0.05o**	**23.75 ±0.12g**	**4.68 ±0.03mn**	**4.87 ±0.10m**	**4.65 ±0.08n**	**7.92 ±0.06k**	**16.62 ±0.14i**	**24.85 ±0.16f**	**76.65 ±0.13d**	**76.35 ±0.11e**	**76.38 ±0.21e**	**77.30 ±0.15c**	**107.51 ±0.23a**	**81.59 ±0.15b**	**13.78 ±0.10j**
Hydroxycinnamic acids																				
C1	0.34 ±0.02b	0.34 ±0.04b	0.32 ±0.02bc	0.35 ±0.03b	0.49 ±0.01a	0.34 ±0.01b	0.25 ±0.02d	0.23 ±0.01de	0.25 ±0.01d	0.22 ±0.01de	0.18 ±0.11e	0.35 ±0.02b	0.21 ±0.02de	0.27 ±0.02cd	0.26 ±0.01cd	0.26 ±0.01cd	0.34 ±0.01b	0.36 ±0.02b	0.27 ±0.02cd	nd
C2	4.39 ±0.12a	4.58 ±0.11a	4.66 ±0.03a	1.86 ±0.06d	4.49 ±0.03a	4.25 ±0.11a	4.40 ±0.11a	2.87 ±0.05c	3.37 ±0.11b	2.89 ±0.03c	2.89 ±0.12c	2.64 ±0.22c	2.60 ±0.11c	3.50 ±0.11b	3.53 ±0.22b	3.62 ±0.11b	3.55 ±0.05b	3.58 ±0.01b	3.54 ±0.21b	0.29 ±0.02e
C3	0.16 ±0.01d	0.19 ±0.03cd	0.19 ±0.02cd	0.21 ±0.02cd	0.31 ±0.02a	0.21 ±0.01cd	0.17 ±0.02d	0.25 ±0.03bc	0.23 ±0.01cd	0.19 ±0.03cd	0.21 ±0.01cd	0.33 ±0.01a	0.21 ±0.02cd	0.19 ±0.01cd	0.21 ±0.11cd	0.19 ±0.03cd	0.22 ±0.01cd	0.30 ±0.01ab	0.19 ±0.01cd	0.02 ±0.00e
C4	0.10 ±0.01ef	0.12 ±0.01cd	0.13 ±0.00bc	0.13 ±0.01bc	0.08 ±0.00f	0.08 ±0.01f	0.09 ±0.01ef	0.05 ±0.00g	0.08 ±0.00f	0.07 ±0.00f	0.07 ±0.01f	nd	0.15 ±0.01ab	0.09 ±0.01ef	0.09 ±0.01ef	0.11 ±0.02de	0.05 ±0.00g	0.16 ±0.00a	0.11 ±0.02de	0.04 ±0.00g
C5	0.79 ±0.11ab	0.84 ±0.02a	0.87 ±0.03a	0.80 ±0.03ab	0.79 ±0.01ab	0.76 ±0.06ab	0.76 ±0.11ab	0.09 ±0.03g	0.52 ±0.03cdef	0.47 ±0.06def	0.48 ±0.01def	0.44 ±0.02ef	0.40 ±0.02f	0.57 ±0.03cdef	0.63 ±0.02bcd	0.65 ±0.04bc	0.56 ±0.00cdef	0.60 ±0.03cde	0.58 ±0.03cde	nd
Total C	**5.78 ±0.10b**	**6.07 ±0.08a**	**6.17 ±0.06a**	**3.35 ±0.05i**	**6.16 ±0.08a**	**5.64 ±0.10b**	**5.67 ±0.11b**	**3.49 ±0.06hi**	**4.45 ±0.09f**	**3.84 ±0.10g**	**3.83 ±0.08g**	**3.76 ±0.05g**	**3.57 ±0.10h**	**4.62 ±0.08e**	**4.72 ±0.10de**	**4.83 ±0.17d**	**4.72 ±0.04de**	**5.00 ±0.09c**	**4.69 ±0.10de**	**0.35 ±0.02j**
Dihydrochalcones																				
D1	1.80 ±0.14c	1.74 ±0.07cd	2.08 ±0.07b	1.64 ±0.18cd	1.48 ±0.13a	1.52 ±0.02de	1.80 ±0.11c	0.88 ±0.03i	1.14 ±0.09gh	0.85 ±0.01i	0.70 ±0.01i	0.17 ±0.00j	0.74 ±0.04i	1.30 ±0.04efgh	1.28 ±0.11fgh	1.39 ±0.11ef	1.37 ±0.04efg	1.13 ±0.04h	1.26 ±0.09fgh	nd
D2	2.31 ±0.06a	2.31 ±0.08a	2.27 ±0.11ab	2.11 ±0.31bc	1.69 ±0.21d	1.95 ±0.06c	2.19 ±0.31ab	0.85 ±0.05i	1.08 ±0.02h	0.69 ±0.01ij	0.68 ±0.02ij	0.68 ±0.06ij	0.60 ±0.08j	1.38 ±0.06fg	1.47 ±0.21efg	1.51 ±0.03def	1.59 ±0.03de	1.40 ±0.04efg	1.31 ±0.04g	nd
Total D	**4.11 ±0.10b**	**4.05 ±0.09b**	**4.35 ±0.12a**	**3.75 ±0.20c**	**3.17 ±0.05e**	**3.47 ±0.05d**	**3.99 ±0.10b**	**1.73 ±0.04j**	**2.22 ±0.03i**	**1.54 ±0.03k**	**1.38 ±0.02l**	**0.85 ±0.03m**	**1.34 ±0.10l**	**2.68 ±0.04gh**	**2.75 ±0.15g**	**2.90 ±0.06f**	**2.96 ±0.05f**	**2.53 ±0.06h**	**2.57 ±0.10**	**nd**
Flavan-3-ols																				
E1	0.98 ±0.06hij	0.96 ±0.02hij	0.93 ±0.04ij	9.12 ±0.18b	0.97 ±0.03hij	1.21 ±0.05ghi	1.32 ±0.11fgh	0.50 ±0.05k	0.53 ±0.06k	0.55 ±0.02k	8.10 ±0.44c	0.56 ±0.08k	0.75 ±0.03jk	4.20 ±0.12e	1.57 ±0.23f	5.26 ±0.24d	9.90 ±0.11a	1.31 ±0.03fgh	1.50 ±0.07fg	0.16 ±0.01l
E2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.04 ±0.00cd	0.03 ±0.00cd	0.02 ±0.00cd	0.14 ±0.01b	0.75 ±0.05a	0.06 ±0.00c	nd
E3	0.74 ±0.03hi	1.39 ±0.04g	0.40 ±0.03ij	0.95 ±0.01h	1.58 ±0.03g	3.51 ±0.04e	3.76 ±0.06e	0.36 ±0.04ij	0.80 ±0.02h	0.62 ±0.03hi	0.91 ±0.24h	2.26 ±0.06f	2.44 ±0.04f	4.38 ±0.12d	6.80 ±0.41a	5.72 ±1.24b	5.35 ±0.12c	5.74 ±0.42b	6.73 ±0.14a	0.17 ±0.01j
E4	4.02 ±0.01a	4.11 ±0.12a	3.57 ±0.41b	4.28 ±0.11a	3.07 ±0.12c	3.10 ±0.11c	3.74 ±21b	1.85 ±0.12ef	1.88 ±0.11ef	1.46 ±0.03g	1.49 ±0.03g	1.81 ±0.24f	1.62 ±0.11fg	2.74 ±0.24d	2.64 ±0.12d	2.73 ±0.22d	0.48 ±0.10h	2.12 ±0.11e	2.74 ±0.03d	nd

E5	1.23 ±0.02b	1.20 ±0.06b	1.17 ±0.08b	2.41 ±0.07a	0.56 ±0.01fg	0.81 ±0.02d	0.97 ±0.04c	0.06 ±0.00k	0.59 ±0.02fg	0.14 ±0.00jk	1.18 ±0.02b	0.24 ±0.07ij	0.36 ±0.01hi	0.44 ±0.02gh	0.63 ±0.10ef	0.63 ±0.04ef	0.77 ±0.02de	0.61 ±0.02ef	0.45 ±0.02gh	nd
E6	0.46 ±0.04a	0.37 ±0.01ab	0.31 ±0.01bc	0.23 ±0.02bcde	0.30 ±0.02bc	0.29 ±0.01bc	0.35 ±0.01ab	0.02 ±0.00f	0.15 ±0.00cdef	0.07 ±0.00ef	0.12 ±0.00def	0.27 ±0.02bcd	0.16 ±0.01cdef	0.15 ±0.01cdef	0.14 ±0.00cdef	0.22 ±0.01bcde	0.47 ±0.01a	0.15 ±0.02cdef	0.14 ±0.02cdef	nd
PP	113.28 ±1.26j	80.45 ±2.12lm	86.06 ±2.54l	71.98 ±1.15m	97.98 ±1.26k	119.07 ±2.15j	152.93 ±1.33i	179.22 ±3.37gh	183.33 ±3.44g	184.32 ±2.11g	169.20 ±5.41h	221.42 ±8.12f	263.48 ±2.54d	278.77 ±3.21c	230.44 ±2.55f	232.03 ±4.22f	224.64 ±3.02f	333.77 ±4.12b	245.32 ±5.12e	362.83 ±5.26a
Total E	**120.71 ±1.15m**	**88.48 ±1.54o**	**92.44 ±2.11o**	**88.97 ±1.08o**	**104.46 ±2.14n**	**127.99 ±1.59l**	**163.07 ±2.33k**	**182.01 ±1.64j**	**187.28 ±2.99i**	**187.16 ±3.04i**	**181.00 ±1.87j**	**226.56 ±4.15h**	**268.81 ±2.34d**	**290.72 ±3.01c**	**242.25 ±2.12fg**	**246.61 ±2.44f**	**241.75 ±2.75g**	**344.45 ±3.12b**	**256.94 ±4.41e**	**363.16 ±4.15a**
DP	29.71	42.18	42.33	32.17	36.08	78.18	39.52	61.93	70.12	70.33	73.23	65.41	165.56	81.73	74.30	61.42	73.50	96.38	85.20	62.01
Flavonols and Flavones																				
F1	nd	0.32 ±0.01c	1.85 ±0.12a	nd	nd	nd	nd	nd	0.33 ±0.02c	1.60 ±0.03b	nd	nd	nd	nd	0.29 ±0.02c	0.17 ±0.01d	nd	nd	nd	nd
F2	nd	nd	0.06 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F3	nd	0.03 ±0.00d	0.16 ±0.01a	nd	nd	nd	nd	nd	nd	0.13 ±0.00b	nd	nd	nd	nd	nd	0.11 ±0.01c	nd	nd	nd	nd
F4	nd	0.18 ±0.01d	0.41 ±0.02a	nd	nd	nd	nd	nd	nd	0.17 ±0.01d	nd	nd	nd	nd	0.24 ±0.01c	0.34 ±0.02b	nd	nd	nd	nd
F5	nd	nd	nd	nd	0.06 ±0.00e	nd	0.14 ±0.01d	nd	nd	nd	nd	0.04 ±0.00e	0.06 ±0.00e	0.20 ±0.02c	nd	nd	0.26 ±0.03b	0.31 ±0.02a	0.19 ±0.01c	nd
F6	nd	nd	nd	1.14 ±0.15a	nd	nd	nd	nd	nd	nd	0.86 ±0.05b	nd	nd	nd	nd	nd	0.70 ±0.04c	nd	nd	nd
F7	nd	0.33 ±0.02d	1.90 ±0.11a	nd	0.06 ±0.00f	nd	nd	nd	0.18 ±0.01e	1.75 ±0.06b	nd	0.05 ±0.00f	nd	nd	0.24 ±0.01de	1.50 ±0.21c	nd	0.06 ±0.00f	nd	nd
F8	nd	nd	nd	7.97 ±0.12a	nd	nd	0.20 ±0.01c	nd	nd	nd	6.54 ±0.12b	nd	0.22 ±0.01c	0.06 ±0.00c	0.09 ±0.00c	0.18 ±0.01c	6.69 ±0.20b	0.09 ±0.00c	0.06 ±0.00c	nd
F9	nd	nd	nd	0.44 ±0.02c	nd	nd	0.23 ±0.01e	nd	nd	nd	0.31 ±0.01d	nd	0.02 ±0.00f	0.07 ±0.01f	0.61 ±0.03a	0.53 ±0.03b	0.41 ±0.02c	nd	0.03 ±0.00f	nd
F10	nd	0.15 ±0.01e	0.99 ±0.09b	nd	nd	nd	nd	nd	0.13 ±0.01e	0.72 ±0.02c	nd	nd	nd	nd	0.36 ±0.02d	1.37 ±0.11a	nd	nd	nd	nd
F11	nd	nd	nd	nd	nd	nd	0.05 ±0.00c	nd	nd	nd	nd	nd	0.04 ±0.00c	0.18 ±0.01b	0.19 ±0.01b	0.19 ±0.00b	0.39 ±0.01a	nd	0.14 ±0.01b	nd
F12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.71 ±0.03a	0.19 ±0.01b
F13	nd	1.84 ±0.20d	10.37 ±0.41a	nd	nd	nd	nd	nd	1.76 ±0.02d	9.38 ±0.22b	nd	nd	nd	nd	1.49 ±0.11e	8.10 ±0.13c	nd	nd	nd	nd
F14	nd	nd	nd	0.73 ±0.06a	nd	nd	nd	nd	nd	nd	0.66 ±0.02b	nd	nd	nd	nd	nd	0.66 ±0.02b	nd	nd	nd
F15	nd	nd	nd	nd	nd	nd	0.17 ±0.01a	nd	nd	nd	nd	nd	nd	0.17 ±0.01b	nd	nd	nd	nd	nd	nd
F16	nd	nd	0.25 ±0.02a	nd	nd	nd	nd	nd	0.04 ±0.00c	0.10 ±0.00b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F17	nd	nd	nd	nd	nd	nd	0.05 ±0.00f	nd	nd	nd	nd	nd	nd	0.17 ±0.01c	0.21 ±0.02b	0.29 ±0.01a	0.22 ±0.01b	0.15 ±0.01d	0.13 ±0.01e	nd
F18	nd	nd	nd	4.82 ±0.12a	nd	nd	0.04 ±0.00cd	nd	nd	nd	4.45 ±0.20b	nd	0.04 ±0.00cd	0.16 ±0.01c	0.15 ±0.01cd	0.13 ±0.03cd	4.34 ±0.22b	0.12 ±0.01cd	0.07 ±0.00cd	nd
F19	0.45 ±0.02fg	0.48 ±0.02f	0.45 ±0.02fg	0.77 ±0.06c	1.04 ±0.11ab	0.35 ±0.01gh	0.55 ±0.02def	0.20 ±0.01i	0.23 ±0.01i	0.17 ±0.01i	0.49 ±0.02ef	0.65 ±0.03cd	0.26 ±0.01hi	0.66 ±0.13cd	0.62 ±0.02d	0.61 ±0.03de	0.96 ±0.13b	1.09 ±0.12a	0.61 ±0.03de	nd

F20	0.09 ±0.00gh	0.14 ±0.01ef	0.34 ±0.02a	0.19 ±0.01d	0.22 ±0.01b	0.12 ±0.00fg	0.13 ±0.01ef	0.02 ±0.00i	0.12 ±0.01fg	0.23 ±0.01b	0.13 ±0.01ef	0.14 ±0.01ef	0.08 ±0.01h	0.19 ±0.01d	0.21 ±0.01cd	0.27 ±0.01b	0.27 ±0.01b	0.25 ±0.02b	0.16 ±0.01e	nd
F21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F22	nd	nd	nd	nd	0.34 ±0.02a	nd	nd	nd	nd	nd	nd	0.24 ±0.02c	nd	nd	nd	nd	nd	0.31 ±0.0b	nd	nd
F23	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F24	nd	0.15 ±0.01d	1.12 ±0.03a	nd	nd	nd	nd	nd	0.04 ±0.00f	0.79 ±0.03c	nd	nd	nd	nd	0.10 ±0.00e	0.84 ±0.02b	nd	nd	nd	nd
F25	0.09 ±0.00efg	0.13 ±0.02d	0.09 ±0.00efg	0.09 ±0.00fg	0.23 ±0.01b	0.11 ±0.00de	0.11 ±0.01def	0.03 ±0.00lm	0.01 ±0.00mn	0.84 ±0.04a	0.04 ±0.00kl	0.10 ±0.00efg	0.04 ±0.00jkl	0.06 ±0.00hi	0.08 ±0.00gh	0.06 ±0.00ij	0.09 ±0.00efg	0.15 ±0.02c	0.05 ±0.00ijkl	nd
F26	nd	nd	nd	nd	0.33 ±0.01a	nd	nd	nd	nd	nd	nd	0.20 ±0.02c	nd	nd	nd	nd	nd	0.30 ±0.01b	0.07 ±0.00d	nd
F27	0.30 ±0.02e	0.29 ±0.01e	0.29 ±0.01e	0.24 ±0.02f	0.51 ±0.02d	0.48 ±0.01d	0.48 ±0.01d	0.11 ±0.01g	0.10 ±0.01g	0.05 ±0.00h	0.08 ±0.00gh	0.29 ±0.01e	0.26 ±0.02ef	0.79 ±0.02b	0.80 ±0.03ab	0.80 ±0.05ab	0.84 ±0.04a	0.81 ±0.03ab	0.71 ±0.05c	nd
F28	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F29	0.47 ±0.03jk	0.60 ±0.05ij	0.93 ±0.07ef	0.81 ±0.08fg	0.52 ±0.03ijk	0.76 ±0.05gh	0.77 ±0.06gh	0.17 ±0.02m	0.31 ±0.02lm	0.64 ±0.0hi	0.49 ±0.02ijk	0.22 ±0.01m	0.45 ±0.03kl	1.18 ±0.11c	1.32 ±0.10b	1.70 ±0.10a	1.45 ±0.21b	1.02 ±0.15de	1.09 ±0.12cd	nd
F30	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F31	nd	nd	0.11 ±0.01b	nd	nd	nd	nd	nd	nd	0.16 ±0.02a	nd	nd	nd	nd	0.04 ±0.00d	0.06 ±0.00c	nd	nd	nd	nd
F32	nd	nd	nd	nd	0.09 ±0.01a	nd	nd	nd	nd	nd	nd	0.05 ±0.00c	nd	nd	nd	nd	nd	0.08 ±0.00b	nd	nd
F33	nd	nd	nd	0.22 ±0.01a	nd	nd	nd	nd	nd	nd	0.14 ±0.01b	nd	nd	nd	nd	nd	0.12 ±0.01c	nd	nd	nd
F34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.01 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd
F35	nd	nd	nd	nd	0.07 ±0.01a	nd	nd	nd	nd	nd	nd	0.02 ±0.00c	nd	nd	nd	nd	nd	0.06 ±0.00b	nd	nd
F36	nd	nd	nd	nd	0.99 ±0.06b	nd	nd	nd	nd	nd	nd	1.20 ±0.12a	nd	nd	nd	nd	nd	0.36 ±0.03c	nd	nd
F37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total F	**1.40 ±0.05o**	**4.64 ±0.06h**	**19.32 ±0.11a**	**17.42 ±0.10b**	**4.46 ±0.08i**	**1.82 ±0.02n**	**2.92 ±0.13m**	**0.53 ±0.02p**	**3.25 ±0.04l**	**16.73 ±0.08d**	**14.19 ±0.06e**	**3.21 ±0.10l**	**1.47 ±0.04o**	**3.89 ±0.11k**	**7.04 ±0.06f**	**17.25 ±0.10c**	**17.40 ±0.11b**	**5.14 ±0.04g**	**4.02 ±0.02j**	**0.19 ±0.01r**
Total All	**132.00 ±1.22k**	**103.33 ±1.22m**	**122.67 ±1.41l**	**130.46 ±1.25k**	**140.77 ±2.47j**	**141.31 ±3.14j**	**199.52 ±5.22h**	**192.44 ±2.33i**	**202.14 ±3.15h**	**214.87 ±2.11g**	**215.82 ±3.15g**	**251.44 ±2.51f**	**300.09 ±4.12e**	**378.65 ±2.01b**	**333.26 ±3.55d**	**348.13 ±4.25c**	**347.67 ±7.24c**	**465.11 ±2.15a**	**349.96 ±2.11c**	**377.48 ±3.12b**

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-r) are significantly different (homogenous groups) at $p \leq 0.05$; nd ≤ LOD. DP = degree of polymeryzation. Total All = sum of all detected polyphenols. Sample codes have their references in **Annex 1b.**. Phenolic compounds have their references in the **Table 4.**.

Storage time: 6 months

Code	Final product (mg/100 g fw)																			
	B1	**B1S01**	**B1S05**	**B1M5**	**B1F5**	**B1K5**	**B1C5**	**B2**	**B2S01**	**B2S05**	**B2M5**	**B2F5**	**B2C5**	**B3**	**B3S01**	**B3S05**	**B3M5**	**B3F5**	**B3K5**	**BK**
Anthocyanins																				
A1	nd	nd	nd	nd	nd	nd	nd	nd	0.02 ±0.00b	0.18 ±0.01a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A4	nd	nd	nd	2.47 ±0.11a	nd	nd	nd	nd	nd	0.47 ±0.02c	2.01 ±0.10b	nd	nd	nd	nd	nd	0.20 ±0.01d	nd	nd	nd
A5	nd	nd	nd	0.07 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.03 ±0.00b	nd	nd	0.12 ±0.01a	nd	nd	nd
A7	nd	nd	nd	0.68 ±0.03a	0.17 ±0.01c	nd	nd	nd	nd	nd	0.67 ±0.05a	0.24 ±0.02b	nd	nd	nd	nd	0.09 ±0.00d	nd	nd	nd
A8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.05 ±0.00a	nd	nd	nd	nd	nd	nd
A9	nd	nd	nd	0.20 ±0.01a	nd	nd	nd	nd	nd	nd	0.16 ±0.01b	nd	nd	nd	nd	nd	0.03 ±0.00c	nd	nd	nd
A10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A11	nd	nd	nd	0.01 ±0.00a	nd	nd	nd	nd	nd	nd	0.01 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd	nd
A12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A13	nd	nd	nd	3.31 ±0.22a	nd	nd	nd	nd	nd	nd	2.64 ±0.11b	nd	nd	nd	nd	nd	0.28 ±0.02c	nd	nd	nd
A14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total A	**nd**	**nd**	**nd**	**6.74 ±0.10a**	**0.17 ±0.01f**	**nd**	**nd**	**nd**	**0.02 ±0.00h**	**0.65 ±0.02d**	**5.49 ±0.11b**	**0.24 ±0.02e**	**nd**	**0.08 ±0.00g**	**nd**	**nd**	**0.72 ±0.02c**	**nd**	**nd**	**nd**
Hydroxybenzoic acids																				
B1	nd	nd	nd	nd	nd	nd	0.13 ±0.00f	nd	nd	nd	nd	nd	0.90 ±0.06e	3.00 ±0.12b	2.38 ±0.14d	2.79 ±0.13c	3.20 ±0.15a	3.09 ±0.17ab	2.82 ±0.34c	nd
B2	nd	nd	nd	nd	nd	nd	0.25 ±0.02f	nd	nd	nd	nd	nd	1.80 ±0.20e	6.02 ±0.23b	4.77 ±0.03d	5.59 ±0.24c	6.42 ±0.32a	6.20 ±0.28ab	5.66 ±0.24c	nd
B3	nd	nd	nd	2.11 ±0.03b	nd	nd	nd	nd	nd	nd	6.05 ±0.20a	nd	nd	nd	nd	nd	nd	nd	nd	nd

B4	nd	nd	nd	nd	nd	1.31 ±0.11f	nd	4.48 ±0.20c	3.46 ±0.13e	3.90 ±0.24d	nd	8.90 ±0.34b	nd	nd	nd	nd	nd	nd	nd	13.54 ±0.56a
B5	nd	nd	nd	nd	nd	nd	0.62 ±0.03f	nd	nd	nd	nd	nd	2.03 ±0.06a	1.85 ±0.24b	1.10 ±0.02e	1.72 ±0.12c	1.52 ±0.07d	1.02 ±0.04e	1.13 ±0.03e	nd
B6	nd	nd	nd	nd	nd	nd	0.23 ±0.01e	nd	nd	nd	nd	nd	18.05 ±0.21d	62.92 ±1.54b	62.31 ±1.42b	62.11 ±2.14b	60.14 ±2.45c	65.46 ±2.13a	58.64 ±1.64c	nd
B7	nd	nd	nd	2.07 ±0.14b	nd	nd	nd	nd	nd	nd	0.48 ±0.02a	nd	nd	nd	nd	nd	nd	nd	nd	nd
B8	nd	nd	nd	nd	nd	1.21 ±0.13b	nd	0.46 ±0.03e	0.64 ±0.06d	nd	nd	1.27 ±0.13b	nd	nd	nd	nd	nd	nd	2.58 ±0.21a	0.86 ±0.06c
B9	nd	nd	nd	0.30 ±0.04a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.03 ±0.00e	0.07 ±0.00b	0.06 ±0.00d	0.22 ±0.01a	0.06 ±0.00cd	nd	0.06 ±0.00c	nd
B11	nd	nd	nd	nd	5.62 ±0.20a	nd	nd	nd	nd	nd	nd	1.31 ±0.15c	nd	nd	nd	nd	nd	4.40 ±0.06b	nd	nd
B12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B13	nd	nd	nd	nd	nd	nd	0.59 ±0.02f	nd	nd	nd	nd	nd	0.96 ±0.02e	3.25 ±0.21a	3.24 ±0.11a	3.05 ±0.14b	2.97 ±0.12bc	2.15 ±0.02d	2.90 ±0.21c	nd
B14	nd	nd	nd	nd	0.64 ±0.03b	nd	nd	nd	nd	nd	nd	0.41 ±0.02c	nd	nd	nd	nd	nd	0.78 ±0.05a	nd	nd
B15	nd	nd	nd	0.12 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B16	nd	nd	nd	nd	nd	nd	0.15 ±0.00f	nd	nd	nd	nd	nd	0.39 ±0.02c	1.13 ±0.06a	1.12 ±0.03a	0.49 ±0.02b	0.23 ±0.01e	0.07 ±0.00g	0.32 ±0.03d	nd
B17	nd	nd	nd	nd	4.04 ±0.12b	nd	nd	nd	nd	nd	nd	0.18 ±0.01c	nd	nd	nd	nd	nd	13.42 ±0.21a	nd	nd
B18	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.17 ±0.01a	nd	nd	nd
B19	nd	nd	nd	0.73 ±0.03a	nd	nd	nd	nd	nd	nd	0.76 ±0.11a	nd	nd	nd	nd	nd	0.06 ±0.00b	nd	nd	nd
B20	nd	nd	nd	nd	nd	nd	0.04 ±0.00b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.39 ±0.11a	nd	nd
B21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B22	nd	nd	nd	nd	0.21 ±0.01c	nd	nd	nd	nd	nd	nd	0.40 ±0.02b	nd	nd	nd	nd	nd	0.76 ±0.03a	nd	nd
B23	nd	nd	nd	nd	0.19 ±0.01a	nd	nd	nd	nd	nd	nd	0.13 ±0.00c	nd	nd	nd	nd	nd	0.16 ±0.01b	nd	nd
B24	nd	nd	nd	nd	nd	nd	0.67 ±0.02c	nd	nd	nd	nd	nd	0.20 ±0.02f	0.71 ±0.03b	0.63 ±0.04d	0.86 ±0.02a	0.03 ±0.00g	0.49 ±0.03e	0.86 ±0.01a	nd
B25	nd	nd	nd	nd	nd	nd	nd	0.04 ±0.00b	nd	nd	nd	0.22 ±0.01a	nd	nd	nd	nd	nd	nd	nd	nd
B26	nd	nd	nd	nd	0.36 ±0.02a	nd	nd	nd	nd	nd	nd	0.09 ±0.00c	nd	nd	nd	nd	nd	0.16 ±0.01b	nd	nd
B27	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.42 ±0.24c	2.65 ±0.13b	2.97 ±0.15a	nd	nd	2.27 ±0.13d	nd
B28	nd	nd	nd	nd	nd	nd	0.23 ±0.01a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

B29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.22 ±0.01a	nd	nd	nd	nd
B30	nd	nd	nd	nd	nd	nd	nd	0.05 ±0.00c	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.06 ±0.00b	0.10 ±0.01a
B31	nd	nd	nd	nd	0.54 ±0.05e	nd	nd	nd	nd	nd	nd	0.35 ±0.01f	nd	1.52 ±0.06b	nd	nd	2.21 ±0.06a	0.75 ±0.05d	1.30 ±0.11c	nd
B32	nd	nd	nd	nd	2.19 ±0.12a	nd	nd	nd	nd	nd	nd	0.23 ±0.00d	nd	nd	nd	nd	nd	0.76 ±0.04b	0.53 ±0.02c	nd
B33	nd	nd	nd	nd	3.13 ±0.11b	nd	nd	nd	nd	nd	nd	4.05 ±0.02a	nd	nd	nd	nd	nd	0.63 ±0.03c	0.43 ±0.01d	nd
B34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B36	nd	nd	nd	nd	0.59 ±0.02a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total B	**nd**	**nd**	**nd**	**5.33 ±0.10j**	**17.51 ±0.12g**	**2.52 ±0.03l**	**2.91 ±0.04l**	**5.03 ±0.06j**	**4.10 ±0.04k**	**3.90 ±0.26k**	**7.29 ±0.15i**	**17.54 ±0.22g**	**24.36 ±0.31f**	**82.89 ±0.26b**	**78.26 ±0.31d**	**80.02 ±0.21c**	**77.01 ±0.14e**	**101.69 ±1.05a**	**79.56 ±0.26c**	**14.50 ±0.17h**
Hydroxycinnamic acids																				
C1	0.40 ±0.05b	0.37 ±0.01bc	0.35 ±0.02c	0.41 ±0.02b	0.57 ±0.05a	0.36 ±0.02bc	0.37 ±0.03bc	0.28 ±0.02d	0.24 ±0.02de	0.23 ±0.02de	0.25 ±0.02de	0.40 ±0.02b	0.23 ±0.01e	0.33 ±0.02c	0.28 ±0.01d	0.27 ±0.03de	0.33 ±0.02c	0.39 ±0.04b	0.34 ±0.01c	nd
C2	4.31 ±0.23abc	4.50 ±0.12ab	4.40 ±0.03ab	4.56 ±0.13a	4.19 ±0.11bc	4.00 ±0.15c	4.24 ±0.15abc	2.88 ±0.10e	2.88 ±0.15e	2.80 ±0.22e	2.84 ±0.24e	2.43 ±0.11f	2.55 ±0.23ef	3.53 ±0.31d	3.54 ±0.19d	3.50 ±0.22d	3.43 ±0.13d	3.58 ±0.24d	3.49 ±0.33d	0.30 ±0.02g
C3	0.20 ±0.01bcd	0.21 ±0.02bcd	0.19 ±0.05cd	0.22 ±0.02bce	0.30 ±0.01a	0.21 ±0.03bcd	0.18 ±0.03cd	0.19 ±0.01cd	0.20 ±0.01bcd	0.20 ±0.02bcd	0.21 ±0.03bcd	0.30 ±0.01a	0.21 ±0.02bcd	0.22 ±0.02bc	0.23 ±0.02bc	0.17 ±0.01d	0.24 ±0.06b	0.31 ±0.02a	0.21 ±0.02bcf	0.02 ±0.00e
C4	0.12 ±0.01cde	0.12 ±0.00cd	0.12 ±0.01c	0.08 ±0.00i	0.10 ±0.00fgh	0.09 ±0.00hi	0.11 ±0.00cde	0.06 ±0.00k	0.07 ±0.01j	0.07 ±0.00j	0.10 ±0.01gh	nd	0.15 ±0.01b	0.10 ±0.01efg	0.08 ±0.00i	0.11 ±0.01def	0.05 ±0.00k	0.16 ±0.01a	0.09 ±0.01hi	0.05 ±0.00k
C5	0.77 ±0.13a	0.83 ±0.03a	0.81 ±0.03a	0.78 ±0.06a	0.76 ±0.06a	0.74 ±0.08a	0.77 ±0.06a	0.48 ±0.03def	0.47 ±0.03ef	0.45 ±0.02f	0.46 ±0.03f	0.41 ±0.02f	0.40 ±0.03f	0.63 ±0.03b	0.56 ±0.12bcd	0.57 ±0.02bcd	0.59 ±0.06b	0.58 ±0.02bc	0.56 ±0.03bcd	nd
Total C	**5.80 ±0.11a**	**6.03 ±0.08a**	**5.87 ±0.16a**	**6.05 ±0.04a**	**5.92 ±0.22a**	**5.40 ±0.09a**	**5.67 ±0.11a**	**3.89 ±0.12a**	**3.86 ±0.08a**	**3.75 ±0.10a**	**3.86 ±0.15a**	**3.54 ±0.13a**	**3.54 ±0.20a**	**4.81 ±0.18a**	**4.69 ±0.20a**	**4.62 ±0.10a**	**4.64 ±0.14a**	**5.02 ±0.21a**	**4.69 ±0.12a**	**0.37 ±0.03b**
Dihydrochalcones																				
D1	1.81 ±0.05b	1.93 ±0.11a	1.77 ±0.04b	1.58 ±0.03c	1.54 ±0.08c	1.33 ±0.02e	1.62 ±0.02c	0.96 ±0.05gh	1.04 ±0.04g	0.91 ±0.05h	0.78 ±0.03i	0.76 ±0.01i	0.65 ±0.06j	1.17 ±0.02f	1.19 ±0.05f	1.42 ±0.03d	1.19 ±0.10f	1.21 ±0.05f	1.23 ±0.06f	nd
D2	2.18 ±0.10bc	2.32 ±0.08a	2.20 ±0.07b	2.11 ±0.02c	1.94 ±0.06d	1.60 ±0.06e	2.01 ±0.04d	1.03 ±0.06i	0.97 ±0.02i	0.82 ±0.01j	0.80 ±0.05j	0.77 ±0.03j	0.53 ±0.02k	1.34 ±0.01h	1.43 ±0.05fgh	1.45 ±0.02f	1.44 ±0.06fg	1.43 ±0.10fgh	1.35 ±0.03gh	nd
Total D	**3.99 ±0.18b**	**4.25 ±0.09a**	**3.97 ±0.10b**	**3.69 ±0.07c**	**3.48 ±0.10d**	**2.93 ±0.08e**	**3.63 ±0.16cd**	**1.99 ±0.06g**	**2.01 ±0.10g**	**1.73 ±0.05h**	**1.58 ±0.11hi**	**1.53 ±0.04i**	**1.18 ±0.08j**	**2.51 ±0.03f**	**2.62 ±0.10f**	**2.87 ±0.11e**	**2.63 ±0.08f**	**2.64 ±0.11f**	**2.58 ±0.07f**	**nd**
Flavan-3-ols																				
E1	0.76 ±0.02i	1.05 ±0.04h	0.63 ±0.03j	8.60 ±0.20b	0.31 ±0.02m	0.98 ±0.03h	0.54 ±0.01jk	0.36 ±0.03lm	0.51 ±0.02k	0.45 ±0.02kl	7.91 ±0.12c	0.85 ±0.01i	0.77 ±0.02i	2.97 ±0.03e	1.63 ±0.02f	5.27 ±0.10d	11.32 ±0.06a	1.45 ±0.08g	1.37 ±0.02g	0.15 ±0.01n
E2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.06 ±0.00c	0.03 ±0.00d	0.02 ±0.00d	0.10 ±0.01b	0.75 ±0.03a	0.07 ±0.00c	nd
E3	0.68 ±0.04j	1.13 ±0.03h	0.36 ±0.01kl	1.42 ±0.06g	1.45 ±0.04g	2.72 ±0.04d	0.73 ±0.04j	0.32 ±0.02kl	0.24 ±0.01lm	0.19 ±0.01m	0.64 ±0.03j	2.03 ±0.04f	2.32 ±0.08e	6.00 ±0.13c	6.51 ±0.21b	6.09 ±0.03c	6.56 ±0.04b	8.25 ±0.14a	0.95 ±0.03i	0.39 0.01k
E4	3.32 ±0.08c	3.27 ±0.02c	1.72 ±0.04h	4.37 ±0.07b	1.91 ±0.06fg	5.49 ±0.12a	3.01 ±0.08d	1.41 ±0.02i	1.42 ±0.03i	1.24 ±0.04j	1.39 ±0.03i	1.43 ±0.11i	0.34 ±0.02k	2.11 ±0.14e	1.80 ±0.06gh	1.96 ±0.04f	0.04 ±0.00l	1.90 ±0.02fg	1.87 ±0.02fg	nd

E5	1.08 ±0.02b	1.01 ±0.04c	0.62 ±0.02e	1.52 ±0.07a	0.22 ±0.08j	0.39 ±0.03h	0.69 ±0.04d	0.42 ±0.02gh	0.39 ±0.01h	0.27 ±0.04ij	1.06 ±0.04bc	0.23 ±0.01j	0.27 ±0.02ij	0.37 ±0.03h	0.46 ±0.02g	0.45 ±0.02g	0.55 ±0.03f	0.47 ±0.01g	0.30 ±0.01i	nd
E6	0.26 ±0.02bc	0.27 ±0.02b	0.08 ±0.00gh	0.19 ±0.01e	0.22 ±0.01d	0.03 ±0.00j	0.25 ±0.02c	0.07 ±0.00h	0.20 ±0.01e	0.05 ±0.00i	0.09 ±0.01g	0.25 ±0.02bc	0.06 ±0.00hi	0.08 ±0.00gh	0.08 ±0.00gh	0.07 ±0.00h	0.44 ±0.01a	0.14 ±0.01f	0.07 ±0.00gh	nd
PP	64.05 ±1.15k	54.84 ±1.24l	65.88 ±2.14k	49.86 ±2.36m	82.04 ±1.02j	127.22 ±2.05i	129.69 ±6.12i	175.16 ±2.54g	173.92 ±1.15g	160.98 ±2.11h	164.38 ±5.41h	164.62 ±8.12h	202.22 ±6.81ef	211.75 ±3.12d	199.02 ±2.22f	208.87 ±3.15d	207.05 ±2.45de	258.91 ±3.33b	237.66 ±2.20c	329.00 ±2.13a
Total E	**70.15 ±1.08k**	**61.57 ±1.11l**	**69.29 ±0.98k**	**65.96 ±0.54kl**	**86.15 ±1.15j**	**136.83 ±2.26i**	**134.91 ±3.24i**	**177.74 ±2.11f**	**176.68 ±3.74f**	**163.18 ±1.75h**	**175.47 ±4.54f**	**169.41 ±3.68g**	**205.98 ±4.22e**	**223.34 ±3.47d**	**209.53 ±5.11e**	**222.73 ±3.89d**	**226.06 ±4.64d**	**271.87 ±4.24b**	**242.29 ±3.55c**	**329.54 ±4.63a**
DP	26.86	57.88	36.55	37.22	42.20	117.29	88.54	68.61	84.43	95.32	55.76	69.89	176.40	80.14	61.74	76.94	74.95	63.94	76.48	105.61
Flavonols and Flavones																				
F1	nd	0.31 ±0.02c	1.85 ±0.10a	nd	nd	nd	nd	nd	0.34 ±0.03c	1.60 ±0.05b	nd	nd	nd	nd	0.35 ±0.03c	0.19 ±0.01d	nd	nd	nd	nd
F2	nd	nd	0.05 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F3	nd	0.03 ±0.00d	0.19 ±0.01a	nd	nd	nd	nd	nd	nd	0.17 ±0.00b	nd	nd	nd	nd	nd	0.15 ±0.01c	nd	nd	nd	nd
F4	nd	0.20 ±0.01d	0.35 ±0.02b	nd	nd	nd	nd	nd	nd	0.19 ±0.01e	nd	nd	nd	nd	0.27 ±0.01c	0.41 ±0.03a	nd	nd	nd	nd
F5	nd	nd	nd	nd	0.07 ±0.00f	nd	0.15 ±0.01e	nd	nd	nd	nd	0.07 ±0.00f	0.05 ±0.00g	0.23 ±0.01c	nd	nd	0.26 ±0.01b	0.36 ±0.02a	0.18 ±0.01d	nd
F6	nd	nd	nd	0.90 ±0.05a	nd	nd	nd	nd	nd	nd	0.69 ±0.04b	nd	nd	nd	nd	nd	0.64 ±0.03c	nd	nd	nd
F7	nd	0.29 ±0.02d	1.81 ±0.08a	nd	0.06 ±0.00ef	nd	nd	nd	0.24 ±0.02d	1.66 ±0.11b	nd	0.05 ±0.00ef	nd	nd	0.22 ±0.01d	1.47 ±0.11c	nd	0.07 ±0.00e	nd	nd
F8	nd	nd	nd	7.01 ±0.22a	nd	nd	nd	nd	nd	nd	6.68 ±0.15b	nd	0.20 ±0.01e	0.08 ±0.00ef	0.10 ±0.00ef	1.59 ±0.12d	6.25 ±0.18c	0.10 ±0.00ef	0.08 ±0.00ef	nd
F9	nd	nd	nd	0.38 ±0.03c	nd	nd	nd	nd	nd	nd	0.35 ±0.01d	nd	0.05 ±0.00ef	0.07 ±0.00e	0.60 ±0.04b	0.69 ±0.02a	0.39 ±0.01c	nd	0.04 ±0.00f	nd
F10	nd	0.13 ±0.01e	0.88 ±0.02b	nd	nd	nd	nd	nd	0.13 ±0.01e	0.72 ±0.03c	nd	nd	nd	nd	0.37 ±0.01d	1.22 ±0.06a	nd	nd	nd	nd
F11	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.06 ±0.00e	0.18 ±0.01c	0.19 ±0.01c	0.22 ±0.01b	0.31 ±0.01a	nd	0.12 ±0.01d	nd
F12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.41 ±0.02a	0.24 ±0.01b
F13	nd	1.75 ±0.15d	9.74 ±0.32a	nd	nd	nd	nd	nd	1.54 ±0.02e	9.21 ±0.22b	nd	nd	nd	nd	1.44 ±0.09e	8.00 ±0.22c	nd	nd	nd	nd
F14	nd	nd	nd	0.69 ±0.04b	nd	nd	nd	nd	nd	nd	0.72 ±0.03a	nd	nd	nd	nd	nd	0.63 ±0.03c	nd	nd	nd
F15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.13 ±0.01a	nd	nd	nd	nd	nd	nd
F16	nd	nd	0.22 ±0.01a	nd	nd	nd	nd	nd	nd	0.21 ±0.02b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F17	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.19 ±0.01c	0.17 ±0.01d	0.28 ±0.01a	0.18 ±0.01d	0.21 ±0.02b	0.13 ±0.01e	nd
F18	nd	nd	nd	4.44 ±0.15a	nd	nd	nd	nd	nd	nd	4.33 ±0.15b	nd	0.03 ±0.00ef	0.15 ±0.01d	0.12 ±0.01de	0.12 ±0.01de	3.96 ±0.15c	0.16 ±0.01d	0.09 ±0.00def	nd
F19	0.48 ±0.02hi	0.45 ±0.01i	0.43 ±0.03i	0.79 ±0.05c	0.87 ±0.04b	0.41 ±0.02i	0.49 ±0.02ghi	0.23 ±0.02j	0.22 ±0.01j	0.21 ±0.01j	0.49 ±0.03ghi	0.67 ±0.05d	0.25 ±0.02j	0.64 ±0.08de	0.56 ±0.03fg	0.59 ±0.02ef	0.93 ±0.11b	1.17 ±0.08a	0.55 ±0.01fgh	nd

F20	0.11 ±0.00i	0.13 ±0.01h	0.27 ±0.02c	0.19 ±0.01d	0.17 ±0.01e	0.11 ±0.00i	0.11 ±0.00i	0.06 ±0.00k	0.02 ±0.00l	0.28 ±0.01c	0.14 ±0.01g	0.16 ±0.01e	0.08 ±0.00j	0.16 ±0.01e	0.15 ±0.01f	0.29 ±0.01b	0.30 ±0.01a	0.30 ±0.01a	0.16 ±0.01e	nd
F21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F22	nd	nd	nd	nd	0.29 ±0.01b	nd	nd	nd	nd	nd	nd	0.26 ±0.02c	nd	nd	nd	nd	nd	0.37 ±0.01a	nd	nd
F23	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F24	nd	0.16 ±0.01d	1.08 ±0.04a	nd	nd	nd	nd	nd	0.08 ±0.00e	0.95 ±0.04b	nd	nd	nd	nd	0.15 ±0.01d	0.88 ±0.03c	nd	nd	nd	nd
F25	0.09 ±0.00fg	0.11 ±0.01d	0.08 ±0.00gh	0.07 ±0.00h	0.17 ±0.01a	0.09 ±0.00ef	0.13 ±0.00c	0.05 ±0.00j	nd	0.15 ±0.01b	0.05 ±0.00j	0.12 ±0.00c	0.04 ±0.00k	0.10 ±0.00de	0.09 ±0.00fg	0.08 ±0.00g	0.10 ±0.00de	0.17 ±0.01a	0.06 ±0.00i	nd
F26	nd	nd	nd	nd	0.28 ±0.02b	nd	nd	nd	nd	nd	nd	0.21 ±0.02c	nd	nd	nd	nd	nd	0.40 ±0.01a	0.09 ±0.00d	nd
F27	0.23 ±0.01hi	0.25 ±0.02gh	0.24 ±0.02ghi	0.25 ±0.02gh	0.37 ±0.02e	0.37 ±0.02e	0.31 ±0.02f	0.14 ±0.02j	0.14 ±0.02j	0.11 ±0.00j	0.06 ±0.00k	0.28 ±0.02fg	0.21 ±0.01i	0.70 ±0.04c	0.71 ±0.02c	0.78 ±0.04b	0.74 ±0.02c	0.95 ±0.03a	0.63 ±0.01d	nd
F28	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F29	0.47 ±0.01hi	0.68 ±0.07g	0.92 ±0.10ef	0.82 ±0.08f	0.41 ±0.03ij	0.65 ±0.02g	0.55 ±0.06gh	0.30 ±0.03jk	0.40 ±0.03ij	0.91 ±0.07ef	0.43 ±0.02hij	0.24 ±0.01k	0.38 ±0.02ij	1.07 ±0.10cd	1.12 ±0.09c	1.75 ±0.14a	1.35 ±0.15b	1.14 ±0.06c	0.99 ±0.11de	nd
F30	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
F31	nd	nd	0.11 ±0.01b	nd	nd	nd	nd	nd	nd	0.14 ±0.01a	nd	nd	nd	nd	0.04 ±0.00d	0.06 ±0.00c	nd	nd	nd	nd
F32	nd	nd	nd	nd	0.09 ±0.00	nd	nd	nd	nd	nd	nd	0.07 ±0.00b	nd	nd	nd	nd	nd	0.10 ±0.01a	nd	nd
F33	nd	nd	nd	0.05 ±0.00	nd	nd	nd	nd	nd	nd	0.19 ±0.01b	nd	nd	nd	nd	nd	0.21 ±0.01a	nd	nd	nd
F34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.02 ±0.00a	nd	nd	nd	nd	nd	nd	nd	nd
F35	nd	nd	nd	nd	0.04 ±0.00	nd	nd	nd	nd	nd	nd	0.02 ±0.00b	nd	nd	nd	nd	nd	0.08 ±0.01a	nd	nd
F36	nd	nd	nd	nd	0.81 ±0.04c	nd	nd	nd	nd	nd	nd	2.04 ±0.14a	nd	nd	nd	nd	nd	1.92 ±0.07b	nd	nd
F37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total F	**1.38 ±0.06n**	**4.49 ±0.10i**	**18.22 ±0.14b**	**15.59 ±0.21e**	**3.63 ±0.05k**	**1.63 ±0.04m**	**1.74 ±0.08m**	**0.78 ±0.02o**	**3.11 ±0.05l**	**16.51 ±0.12c**	**14.13 ±0.09f**	**4.21 ±0.03j**	**1.35 ±0.06n**	**3.70 ±0.10k**	**6.65 ±0.11h**	**18.77 ±0.12a**	**16.25 ±0.21d**	**7.50 ±0.08g**	**3.53 ±0.11k**	**0.24 ±0.02p**
Total	**81.33 ±0.33o**	**76.34 ±0.45p**	**97.35 ±1.10n**	**103.36 ±1.16m**	**116.86 ±0.36l**	**149.31 ±1.14k**	**148.86 ±1.24k**	**189.42 ±1.02j**	**189.77 ±0.54j**	**189.72 ±2.13j**	**207.81 ±0.87h**	**196.46 ±0.45i**	**236.41 ±1.22g**	**317.34 ±2.46e**	**301.75 ±3.12f**	**329.01 ±1.47d**	**327.31 ±0.56d**	**388.72 ±2.33a**	**332.65 ±6.15c**	**344.65 ±3.11b**

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-p) are significantly different (homogenous groups) at $p \leq 0.05$; nd ≤ LOD. DP = degree of polymeryzation. Total All = sum of all detected polyphenols. Sample codes have their references in **Annex 1b.**. Phenolic compounds have their references in the **Table 4.**.

3.2.4. Antioxidant activity

Antioxidant activity of all final products was measured by cupric reducing antioxidant activity (CUPRAC), ferric reducing/antioxidant power (FRAP), and free radical-scavenging activity ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) and oxygen radical absorbance capacity (ORAC) assays. Moreover, the total polyphenol content was evaluated in all juices and smoothies Results of all performed assays (**Table 15.** and **Figure 22.**) showed approximately the same trends among final products, both before and after storage at room temperature. Pearson correlation between total phenol content measured by Folin-Ciocalteu method and five antioxidant assays immediately after final product preparation was 0.9339, 0.9081, 0.9041, 0.8974 and 0.8971 for FRAP, ORAC, CUPRAC, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, respectively. Furthermore, a positive increase in correlations between antioxidant activity and total content of polyphenols in final products after storage of 3 months up to 0.9894 for CUPRAC and 6 moths up to 0.9865 for $ABTS^{\bullet+}$ was noticed.

Among analysed final products immediately after processing the highest antioxidant activity (CUPRAC method) was determined in all products with base B3 (25 % of strawberry tree fruits and 75 % of apple juice) in a range from 6.98 mmol Fe^{2+}/100 g fw (B3) to 8.75 mmol Fe^{2+}/100 g fw (B3M5). Furthermore, antioxidant analysis (FRAP, ORAC, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$) showed that the greatest antioxidant activity appears in 6 products made from base B3, especially in those with added purple myrtle berry extract (2.28 mmol Fe^{2+}/100 g fw; $ABTS^{\bullet+}$) and feijoa flowers (1.49 and 2.51 mmol Fe^{2+}/100 g fw; $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively). These 6 final products were also characterized by the highest stability of antioxidant activity (all methods) and total polyphenols content, approximately from 61 to 98 % in the case of storage 3 months and in a range from 62 % to 98 % in the case of storage 6 months. Furthermore, it was observed that the addition of 5 % dry feijoa flowers or extract of purple myrtle berries positively influenced the storage of

the final products. Greater antioxidant activity in final products was positively associated with a significant increase of total polyphenols content. As a consequence, smoothies with base B3 had the highest content of polyphenols, among which the most abundant were B3M5, B3F5 and B3K5 (288.15, 285.68, 276.17 mg GAE/100 g fw).

In contrast, the lowest antioxidant activity was found in final products based on 100 % of apple juice. In these juices the antioxidant activity measured by the CUPRAC method ranged from 1.81 mmol Fe^{2+}/100 g fw (B1) to 7.04 mmol Fe^{2+}/100 g fw. However, the addition of 5 % feijoa flowers or 5 % persimmon fruits showed high antioxidant activity compared with other products from this set (6.01 mmol Fe^{2+}/100 g fw and 7.04 mmol Fe^{2+}/100 g fw, respectively). Furthermore, the other antioxidant tests showed similar results. According to FRAP, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, product B1 had the lowest values of the antioxidant activity: 0.58, 047 and 0.48 mmol Fe^{2+}/100 g fw, respectively. Final products with base B2 were more attractive than products with base B1, while less attractive than products with base B3, regarding antioxidant activity.

It is worth noting that the addition of 0.1 and 0.5 % of saffron flower juice significantly decreased the antioxidant activity and total polyphenols content of final products based on 25 % of persimmon fruit purée and 75 % of apple juice. In addition, final product B1K5 was characterized by the lowest stability of antioxidant activity (all methods, apart the ORAC test) and total polyphenols content, approximately from 27 % ($ABTS^{\bullet+}$) to 38 % (FRAP) in the case of storage for 3 months and in a range from 22 % ($ABTS^{\bullet+}$) to 33 % (FRAP) in the case of storage for 6 months.

According to Ou et al. (*2002*), antioxidant activity is determined not only by the total content of polyphenols, but also by their types. Moreover, as suggested by Wojdyło et al. (*2014a*) and Nowicka et al. (*2016a*) antioxidant potential depends on the presence of anthocyanins, flavonols and polymeric procyanidins. The results presented in this study showed that the antioxidant activity of the obtained final products during storage correllate

well with the total hydroxybenzoic acid content and its derivatives (Pearson correlation from 0.5786 to 0.9318), polymeric proanthocyanidins (Pearson correlation from 0.5168 to 0.9318 for all anthioxidant assays, except ORAC assay, where the values of correlation were in the range from 0.3321 to 0.84337), and total polyphenol content measured by UPLC-PDA method (Pearson correlation: 0.6157-0.9419). Furthermore, a significant positive correlation was found in final products between the antioxidant activity and organic acids content (Pearson correlation: 0.5575-0.9338). Moreover, in the case of the antioxidant activity measured by five different methods (CUPRAC, FRAP, ORAC, $DPPH^{\bullet}$, $ABTS^{\bullet+}$) correlations with vitamin C were observed: 0.5262, 0.5722, 0.6503, 0.4262 and 0.5303 (0 months); 0.7502, 0.7786, 0.7028, 0.6015 and 0.7371 (after 3 months storage); 0.7812, 0.7552, 0.7340, 0.6268 and 0.7300 (after 6 months), respectively.

To sum up, the antioxidant activity depended on different chemical features. Mixing the different plant materials increased the quantity of powerful antioxidants (e.g. polyphenols), which plays significant role in antioxidant power.

Table 15. Antioxidant activity of the final products before and after storage time (3 and 6 months) at 20 ± 2 °C.

Storage time: immediately after processing (0 months)

Sample code	Antioxidant activity					
	TP	**CUPRAC**	**FRAP**	**ORAC**	**DPPH$^{\bullet}$**	**ABTS$^{\bullet+}$**
	mg GAE/100 g fw	mmol Fe^{2+}/100 g fw	mmol Trolox/100 g fw			
B1	82.25 ± 2.36i	1.81 ± 0.09k	0.58 ± 0.00j	2.17 ± 0.17i	0.47 ± 0.01m	0.48 ± 0.00k
B1S01	91.19 ± 6.78i	4.77 ± 0.06h	1.00 ± 0.01i	2.23 ± 0.10i	0.89 ± 0.03j	1.11 ± 0.01h
B1S05	98.08 ± 7.79i	3.08 ± 0.04j	0.65 ± 0.01j	2.81 ± 0.19gh	0.50 ± 0.03m	0.57 ± 0.00j
B1M5	132.20 ± 9.43h	3.61 ± 0.21i	1.15 ± 0.04h	3.07 ± 0.16fg	0.73 ± 0.04k	1.14 ±0.01h
B1F5	179.84 ± 9.95fg	6.01 ± 0.04f	1.77 ± 0.02e	3.51 ± 0.23e	1.26 ± 0.01de	2.21 ± 0.01bc
B1K5	194.06 ± 1.39ef	7.04 ± 0.09cd	1.44 ± 0.01f	2.96 ± 0.13gh	1.05 ± 0.04h	1.82 ± 0.13f
B1C5	115.89 ± 4.57h	3.05 ± 0.02j	0.95 ± 0.11i	2.10 ± 0.15i	0.60 ± 0.02l	0.93 ± 0.03i
B2	238.04 ± 10.16d	7.01 ± 0.07cd	1.78 ± 0.16e	4.47 ± 0.28c	1.26 ± 0.02de	1.83 ± 0.04f
B2S01	171.48 ± 3.78g	6.76 ± 0.07de	1.37 ± 0.05fg	2.87 ± 0.06gh	0.96 ± 0.03i	1.53 ± 0.01g
B2S05	173.23 ± 8.73g	6.36 ± 0.05ef	1.25 ± 0.02gh	2.70 ± 0.01h	0.92 ± 0.02ij	1.09 ± 0.02h
B2M5	210.20 ± 10.41e	5.50 ± 0.11g	1.91 ± 0.04bcde	3.34 ± 0.21ef	1.25 ± 0.07def	2.06 ± 0.02d
B2F5	198.20 ± 5.37e	6.15 ± 0.04f	1.83 ± 0.01cde	3.63 ± 0.22de	1.24 ± 0.03ef	2.01 ± 0.02de
B2C5	207.01 ± 5.62e	5.09 ± 0.09gh	1.83 ± 0.01cde	3.64 ± 0.27de	1.20 ± 0.04fg	1.97 ± 0.03e
B3	247.97 ± 1.49cd	6.98 ± 0.26d	1.95 ± 0.05bcd	4.21 ± 0.21c	1.25 ± 0.02def	2.07 ± 0.02d
B3S01	262.10 ± 8.20bc	7.07 ± 0.38cd	1.89 ± 0.27bcde	4.94 ± 0.12b	1.18 ± 0.04g	2.20 ± 0.08c
B3S05	258.63 ± 5.75bc	7.18 ± 0.73cd	1.97 ± 0.05abc	3.88 ± 0.06d	1.16 ± 0.05g	2.06 ± 0.03d
B3M5	288.15 ± 3.13a	8.75 ± 0.07a	2.02 ± 0.02ab	4.22 ± 0.25c	1.30 ± 0.02cd	2.28 ± 0.05b
B3F5	285.68 ± 19.22a	8.20 ± 0.64b	2.12 ± 0.10a	4.49 ± 0.13c	1.49 ± 0.01a	2.51 ± 0.01a
B3K5	276.17 ± 19.73ab	8.17 ± 0.65b	2.04 ± 0.01ab	5.25 ± 0.27a	1.37 ± 0.04b	2.19 ± 0.05c
BK	271.88 ± 20.77ab	7.53 ± 0.17c	1.80 ± 0.12de	4.50 ± 0.10c	1.34 ± 0.01bc	1.94 ± 0.03e

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-m) are significantly different (homogenous groups) at $p \leq 0.05$; nd ≤ LOD. Sample codes have their references in **Annex 1b.**.

Storage time: 3 months

Sample code	Antioxidant activity					
	TP	**CUPRAC**	**FRAP**	**ORAC**	**DPPH$^{\bullet}$**	**ABTS$^{\bullet+}$**
	mg GAE/100 g fw	mM Fe^{2+}/100 g fw	mM Trolox/100 g fw			
B1	60.07 ± 2.01lm	1.73 ± 0.15j	0.48 ± 0.00mn	2.29 ± 0.21ef	0.32 ± 0.02i	0.41 ± 0.00r
B1S01	54.46 ± 0.56m	2.00 ± 0.08j	0.46 ± 0.16mn	1.84 ± 0.18ghi	0.30 ± 0.01i	0.38 ± 0.04s
B1S05	76.15 ± 6.49k	2.10 ±0.06j	0.49 ± 0.01mn	2.57 ± 0.10de	0.30 ± 0.01i	0.48 ± 0.01p
B1M5	115.22 ± 2.18h	2.92 ± 0.15i	1.04 ± 0.05i	2.71 ± 0.15d	0.56 ± 0.01h	1.02 ± 0.00k
B1F5	147.37 ± 2.41fg	4.61 ± 0.21f	1.33 ±0.00g	3.30 ± 0.23bc	0.86 ± 0.02e	1.32 ± 0.02h
B1K5	67.26 ± 0.65kl	1.99 ±0.10j	0.55 ± 0.01m	1.79 ± 0.04hi	0.32 ± 0.01i	0.50 ± 0.00p
B1C5	113.95 ± 2.37h	2.99 ± 0.06i	0.92 ± 0.01j	2.06 ± 0.12fgh	0.56 ± 0.00h	0.89 ± 0.00l
B2	94.98 ± 2.92j	2.82 ± 0.02i	0.77 ± 0.02j	1.63 ± 0.11i	0.59 ± 0.02h	0.84 ± 0.03m
B2S01	98.35 ± 3.45ij	2.64 ± 0.10i	0.65 ± 0.01l	1.55 ± 0.13i	0.58 ± 0.01h	0.70 ± 0.01o
B2S05	106.08 ± 6.70hi	2.91 ± 0.13i	0.73 ± 0.00j	1.99 ± 0.05fgh	0.56 ± 0.05h	0.77 ± 0.00n
B2M5	153.13 ± 9.11ef	4.56 ± 0.14f	1.19 ± 0.02h	1.95 ± 0.18gh	0.81 ± 0.01f	1.28 ± 0.01i
B2F5	158.58 ± 7.88e	4.66 ± 0.26f	1.45 ± 0.01f	2.54 ± 0.16de	1.04 ± 0.02c	1.61 ± 0.03f
B2C5	139.96 ± 5.13g	3.92 ± 0.18g	1.05 ± 0.01i	2.82 ± 0.16d	0.73 ± 0.02g	1.16 ± 0.01j
B3	216.72 ± 4.17c	6.31 ± 0.10c	1.78 ± 0.01c	3.14 ± 0.11c	0.98 ± 0.04d	1.87 ± 0.03d
B3S01	201.90 ± 3.60d	6.06 ± 0.18cd	1.53 ± 0.03e	3.30 ± 0.17c	0.95 ± 0.02d	1.63 ± 0.01f
B3S05	216.16 ± 7.39c	5.68 ± 0.20de	1.87 ± 0.07b	3.58 ± 0.26b	0.97 ± 0.02d	2.02 ± 0.00c
B3M5	256.00 ± 6.40b	7.25 ± 0.19b	1.99 ± 0.03a	4.15 ± 0.19a	1.13 ± 0.03b	2.08 ± 0.00b
B3F5	275.00 ± 12.78a	8.01 ± 0.63a	2.02 ± 0.06a	4.38 ± 0.21a	1.46 ± 0.02a	2.47 ± 0.00a
B3K5	206.41 ± 5.46d	5.39 ± 0.57e	1.63 ± 0.02d	3.22 ± 0.26c	0.96 ± 0.02d	1.74 ± 0.03e
BK	112.43 ± 2.33h	3.50 ± 0.11h	0.89 ± 0.01j	2.14 ± 0.11fg	0.77 ± 0.02g	0.88 ± 0.02l

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-s) are significantly different (homogenous groups) at $p \leq 0.05$; nd ≤ LOD. Sample codes have their references in **Annex 1b.**.

Storage time: 6 months

Sample code	Antioxidant activity					
	TP	**CUPRAC**	**FRAP**	**ORAC**	**DPPH$^{\bullet}$**	**ABTS$^{\bullet+}$**
	mg GAE/100 g fw	mM Fe^{2+}/100 g fw	mM Trolox/100 g fw			
B1	37.71 ± 0.47k	1.65 ± 0.07m	0.42 ± 0.00j	1.06 ± 0.08g	0.28 ± 0.00j	0.35 ± 0.01l
B1S01	53.26 ± 4.50j	1.98 ± 0.14l	0.45 ± 0.01j	1.59 ± 0.07f	0.20 ± 0.07k	0.36 ± 0.00l
B1S05	68.48 ± 2.82i	2.08 ±0.04kl	0.48 ± 0.00j	2.10 ± 0.15d	0.29 ± 0.01j	0.37 ± 0.00l
B1M5	100.83 ± 4.45g	2.52 ± 0.18j	0.99 ± 0.02g	2.44 ± 0.24c	0.55 ± 0.03h	0.91 ± 0.01h
B1F5	146.81 ± 4.61e	4.51 ±0.16f	1.32 ± 0.02d	3.10 ± 0.24b	0.82 ± 0.03f	1.27 ± 0.09f
B1K5	49.09 ± 0.00j	1.91 ± 0.08lm	0.48 ± 0.00j	1.52 ± 0.06f	0.31 ± 0.00j	0.39 ± 0.00l
B1C5	100.20 ± 3.92g	2.95 ± 0.05i	0.78 ± 0.03h	2.01 ± 0.13de	0.49 ± 0.02i	0.67 ± 0.00jk
B2	81.34 ± 5.52h	2.35 ± 0.02jk	0.62 ± 0.01i	1.61 ± 0.20f	0.55 ± 0.01h	0.61 ± 0.00k
B2S01	74.95 ± 1.69hi	2.17 ± 0.04kl	0.62 ± 0.01i	1.49 ± 0.17f	0.53 ± 0.01h	0.65 ± 0.01k
B2S05	93.96 ± 4.23g	2.85 ± 0.20i	0.72 ± 0.02h	1.98 ± 0.10de	0.55 ± 0.01h	0.74 ± 0.00i
B2M5	140.95 ± 3.90e	4.31 ± 0.19fg	1.17 ± 0.03f	1.62 ± 0.15f	0.80 ± 0.02f	1.24 ± 0.02f
B2F5	147.37 ± 6.38e	4.05 ± 0.14g	1.43 ± 0.03d	2.42 ± 0.05c	1.03 ± 0.03c	1.45 ± 0.03e
B2C5	112.58 ± 5.79f	3.24 ± 0.12h	0.95 ± 0.01g	1.77 ± 0.04ef	0.69 ± 0.01g	1.01 ± 0.01g
B3	194.82 ± 3.46c	5.87 ± 0.27c	1.69 ± 0.01b	3.04 ± 0.24b	0.97 ± 0.01d	1.70 ± 0.02c
B3S01	197.61 ± 2.69c	5.79 ± 0.20cd	1.50 ± 0.08c	3.06 ± 0.25b	0.91 ± 0.02e	1.59 ± 0.01d
B3S05	202.18 ± 2.44c	5.55 ± 0.20d	1.57 ± 0.02c	3.03 ± 0.11b	0.95 ± 0.03d	1.65 ± 0.05cd
B3M5	231.35 ± 12.19b	7.12 ± 0.25b	1.98 ± 0.02a	3.75 ± 0.27a	1.12 ± 0.01b	2.02 ± 0.13b
B3F5	254.90 ± 8.79a	7.58 ± 0.18a	2.01 ± 0.13a	3.87 ± 0.28a	1.46 ± 0.03a	2.45 ± 0.00a
B3K5	180.13 ± 6.48d	5.21 ± 0.23e	1.54 ± 0.03c	3.26 ± 0.19b	0.95 ± 0.01d	1.61 ± 0.03d
BK	81.90 ± 1.49h	3.29 ± 0.14h	0.78 ± 0.03h	1.53 ± 0.04f	0.67 ± 0.01g	0.73 ± 0.01ij

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-m) are significantly different (homogenous groups) at $p \leq 0.05$; nd ≤ LOD. Sample codes have their references in **Annex 1b.**.

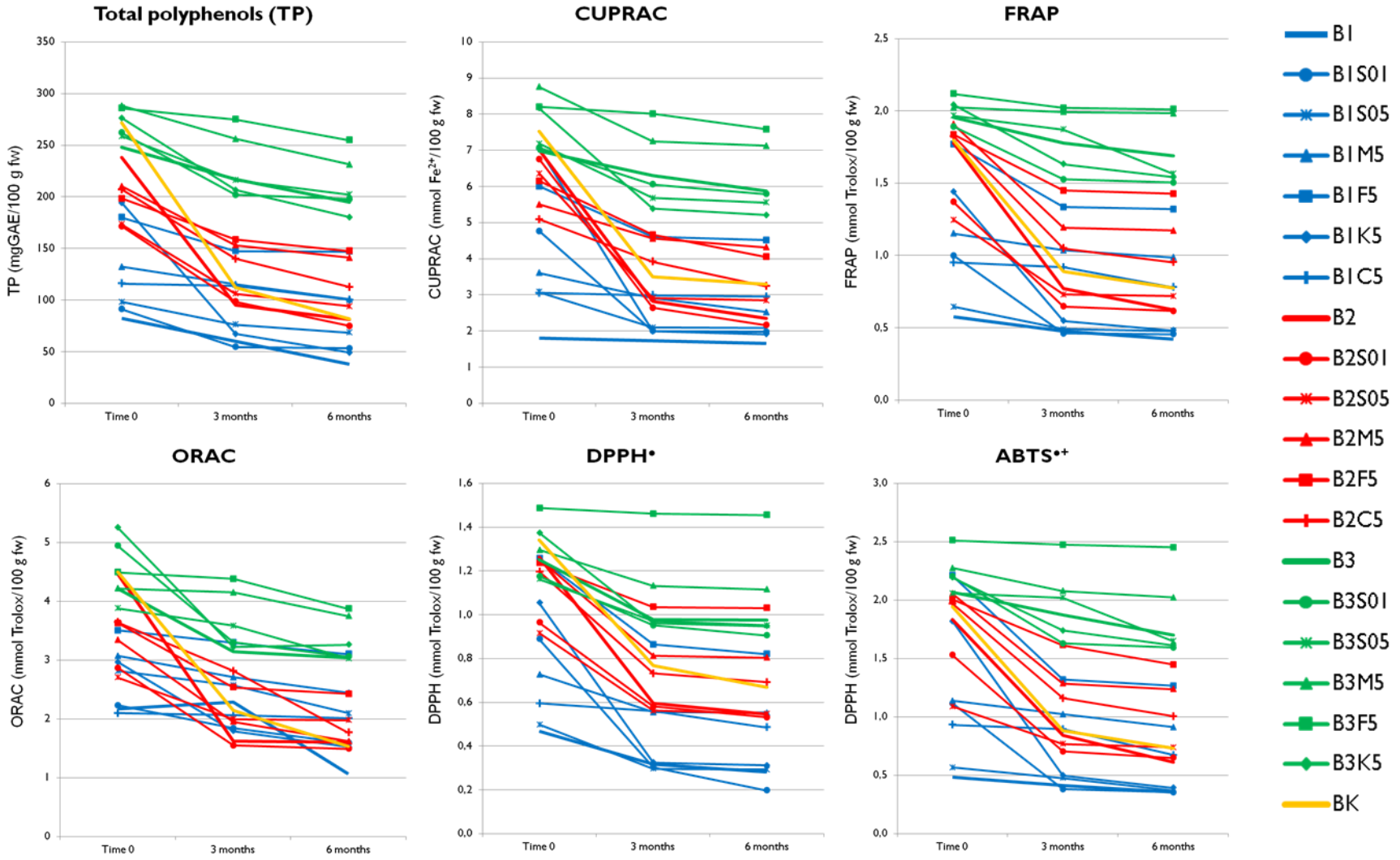

Figure 22. Evolution of the antioxidant activity of the final products before and after storage time (3 and 6 months) at 20 ± 2 °C.

3.2.5. *In-vitro* analysis on Caco-2 cell lines

The best six final product extracts, according to the highest values of antioxidant activity (set B3), were investigated with in cell model evaluating the absence of cytotoxic activity and the ability to scavenge reactive oxygen species.

3.2.5.1. Cytotoxic activity

According to the findings of De Francisco et al. (*2018*), Caco-2 cell lines are often used as intestinal models to investigate the effect of novel food ingredients on cell viability. Moreover, as described by Meunier et al. (*1995*), the functional characteristics and morphology of Caco-2, when used as differentiated cells, are very alike enterocytes (tight junctions, apical and basolateral layers as well as in the microvilli presenton the apical surface).

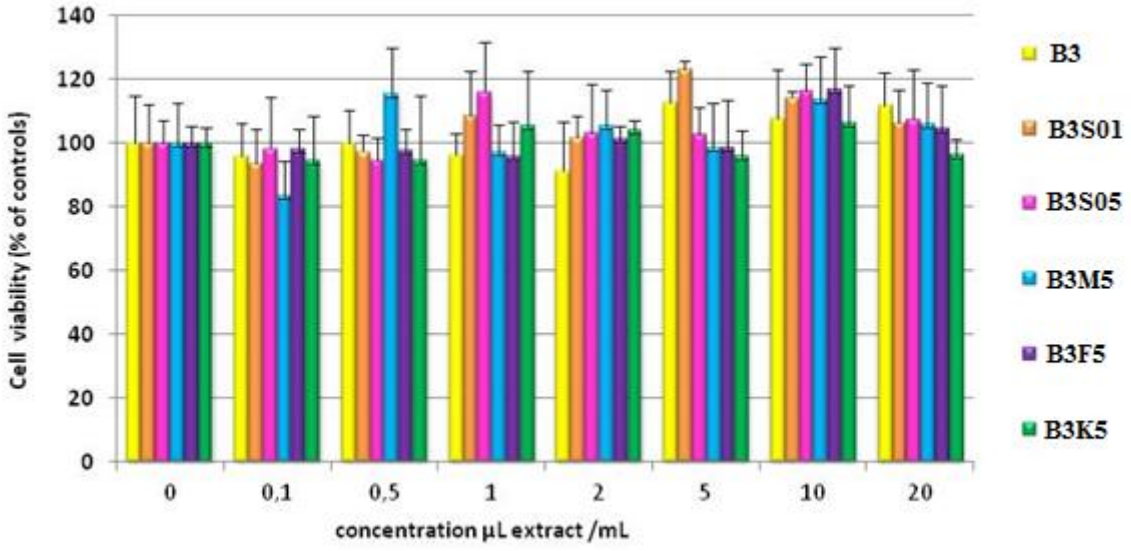

Figure 23. Cell viability of the best six extracts of final products.

Results expressed as % of the control samples, measured in Caco-2 cells after incubation with different concentrations of the extracts of the six selected fresh final products or equivalent volume MeOH:H_2O 80:20 for the controls, and incubated for 24 h. Values were shown as mean ± SD (n = 6 per group). Sample codes have their references in **Annex 1b.**.

The effect of the six selected final product extracts was assessed on differentiated Caco-2 cells at concentrations between 0.1 and 20 μL/mL through MTT assay. **Figure 23.** summarizes the cell viability results. As it is possible to observe, the extracts of different final products did not cause to a decrease in the cellular viability of Caco-2 cells, showing results of around 100 % of the control. The statistical analysis showed that there are no significant differences ($p > 0.05$) in Caco-2 cell viability when exposed to six final product extracts with different concentrations. In spite of that, analysed extracts at 0.5, 1 and 5 μL/mL, showed slight differences when compared to the other concentrations under study for these extracts. When the viability of the different samples at the same concentration is analysed, it is possible to notice differences ($p < 0.05$) between investigated final product extracts. Nevertheless, it should be highlighted that none of the samples caused a decrease of Caco-2 viability.

3.2.5.2. Determination of intracellular ROS production

The ability of six selected final product extracts to scavenge reactive species was also tested in Caco-2 cell cultures (**Figure 24.**). This human colonic epithelial cell line goes through full differentiation to enterocytes *in vitro* and it is extensively used to investigate the effect of nutrient components, for drugs, toxicants and contaminants, as well as normal dietary constituents and additives (*Deiana et al., 2019*).

Determination of intracellular ROS production and subsequent oxidative damage to cell membranes was induced by the organic hydroperoxide (TBH) (*Incani et al., 2016*). In cells pretreated with all phenolic extracts, inhibition in the ROS formation was observed, as indicated by the lower emission of fluorescence. Furthermore, the total phenolic concentration of tested extracts (1, 5, 10 and 20 μg/mL) showed growing, effective inhibition of ROS formation. Three tested extracts (B3M5, B3F5 and B3K5; concentration

1 μg/mL) had slightly lower ROS levels (c.a. < 10-20 %), than the other three. Moreover, the % of ROS production in extracts with concentration 10 μg/mL was equal to or slightly lower than in the control, while in some 20 μg/mL extracts, the concentration of ROS slightly increased. The best protective against oxidative damage appeared to be all extracts with concentration 10 μg/mL, and, significantly, pretreatment with the phenolic extracts significantly attenuated the TBH-induced oxidative process in the cell monolayers.

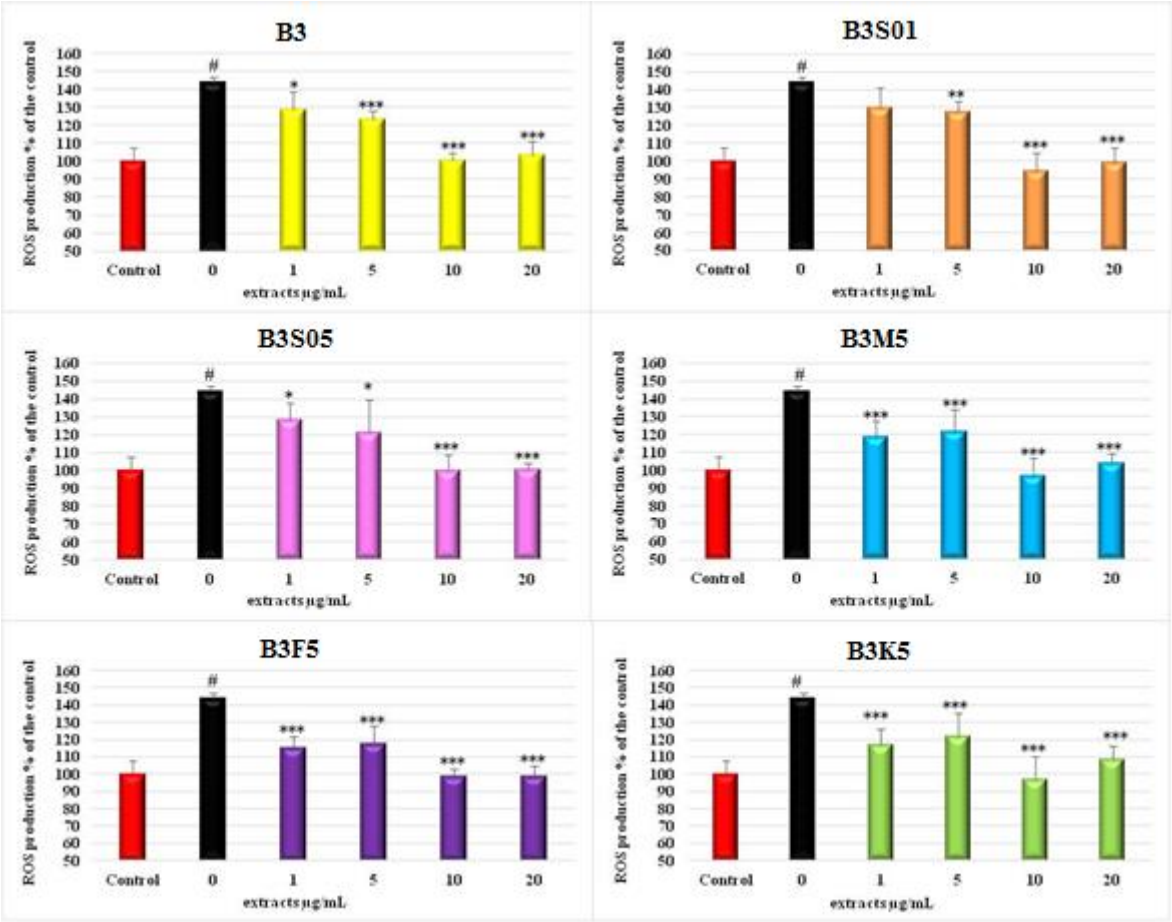

Figure 24. Determination of intracellular ROS production.

ROS level, visualized as H_2-DCF-DA fluorescence and expressed as % of the control samples (non oxidized nor pretreated samples), in Caco-2 after 60 min incubation with TBH 2.5 mmol/L and pretreated with the extracts of six selected fresh final products (B3, B3S01, B3S05, B3M5, B3F5 and B3K5) extracts (μg/mL, 30 min). # = p< 0.001 vs control; * = p< 0.05 vs TBH 2.5 mmol/L; ** = p< 0.01 vs TBH 2.5 mmol/L; *** = p< 0.001 vs TBH 2.5 mmol/L. Sample codes have their references in **Annex 1b.**.

After evaluating both the scavenging properties and the protective properties against oxidative damage (ROS) through a fluorescence probe, all the extracts showed a comparable efficacy, in spite of the differences in the content of polyphenols. A similar

protective activity in intestinal lumen was observed in natural table olives phenolic extracts (*Serreli et al., 2017*), which showed prevention effect against oxidative stress and membrane damage in Caco-2 cells treated with TBH.

To sum up, all the six phenolic extracts of final products (set B3) showed, that they are able to protect enterocytes from oxidative stress (free radicals). The obtained in this study results were biologically relevant. Moreover, Halliwell et al. (*2005*) showed that polyphenols and their metabolites, present in the interior of gastrointestinal trak, may protective human body form oxidative damage-related diseases.

3.2.6. Inhibitory activity toward digestive enzymes

In this study, the inhibitory activity against α-amylase, α-glucosidase and pancreatic lipase were measured in 20 analysed final products, before and after storage (3 and 6 months at 20 ± 2 °C). Obtained results were presented as IC_{50} values in **Table 16.** and in **Figure 25.**. In general, significant differences ($p \leq 0.05$) were found among the analysed final products in inhibitory activities toward these 3 digestive enzymes immediately after processing and after storage.

At 0 months the inhibitory activity against α-amylase, presented as IC_{50} values, ranged from 61.37 to 312.14 mg of final product/mL, while the values for α-glucosidase were from 15.32 to 76.75 mg of final product/mL. Moreover, the inhibitory activity against pancreatic lipase ranged from 2.93 to 19.21 mg of final product/mL. The strongest inhibition of pancreatic α-amylase was found in final products B1K5 and B3K5 in quantities 61.37 and 65.62 mg of final product/mL, respectively. Furthermore, product BK (100 % of persimmon purée) was characterised by strong inhibition of α-amylase (66.72 mg of final product/mL). Moreover, to the group of strong α-amylase inhibitors belong also products composed of base B2 and B3 with an added of 5 % purple myrtle berry

extract (69.11 and 64.70 mg of final product/mL, respectively), as well as product B2C5 and B3F5 (63.98 and 64.83 mg of final product/mL, respectively). The fact that the products with added persimmon purée were the most powerful inhibitors against α-amylase was also confirmed by the results obtained in **CHAPTER 3.1.**, where persimmon had one of the strongest inhibition activities.

In contrast, final products which were characterised by the weakest inhibition of pancreatic α-amylase, were products based on 100 % apple juice (B1, 214 mg of final product/mL) with added of 0.5 % saffron flower juice, 5 % of purple myrtle berry extract, strawberry tree fruits and feijoa flowers (312.14, 306.42, 177.95 and 134.28 of final product/mL).

Regarding inhibition of α-glucosidase, the strongest IC_{50} values (in a range from 15.32 to 16.00 mg of final product/mL) were shown by the same group of final products as in case of α-amylase inhibition, as well as products with pure base B2, where 25 % of persimmon was present, and base B2 with 0.1 an 0.5 % addition of saffron flower juice (15.37, 16.73 and 20.08 mg of final product/mL, respectively). In addition, product B2M5, B3M5 and B3F5 displayed good inhibition activity against α-glucosidase (15.32, 15.84 and 15.86 mg final product/mL). As in the case of α-amylase, these results regarding α-glucosidase inhibitory activity were confirmed by the results obtained in **CHAPTER 3.1.**, where purple myrtle berry extract, feijoa flowers, persimmon and strawberry tree fruits showed the strongest inhibitory activity against this enzyme.

In contrast, the weakest inhibitors of α-glucosidase, having IC_{50} values in a range from 72.10 to 76.75 mg of final product/mL, were all products based on 100 % apple juice, except product B1K5 (15.90 mg of final product/mL), where 5 % of persimmon purée had been added. In addition, product B1F5 appeared to be one of the weakest inhibitors of α-glucosidase (72.10 mg of final product/mL).

Inhibition of pancreatic lipase of final products was confirmed by results obtained for plant materials, as well. IC_{50} values ranged from 2.93 to 3.11 mg of final product/mL, and the most powerful appeared to be all products based on 25 % of strawberry tree fruits and 75 % of apple juice, as well as the product B2C5 (3.17 mg of final product/mL) in which 5 % of *A. unedo* fruits had been added. Also in **Paragraph 3.1.4.** the strongest inhibitior was *A. unedo* fruits.

In contrast, the weakest product was B1M5 BK (10.94 and 12.15 mg of final product/mL), as well as all products with base B2 (7.23-10.97 mg of final product/mL). These results were confirmed by the above data for pancreatic lipase inhibition activity of plant materials.

Regarding the storage parameter, some peculiarities were observed. In general, with time, most of analysed final products lost their inhibitive effect on digestive enzymes. Moreover, some of them maintained stable inhibition levels toward these three enzymes, after a slight initial loss of inhibiting activity. Among these final products (concerning anti-α-amylase) were all products based on base B3, as well as B1C5, B2S01 and B1F5. Furthermore, regarding anti-α-glucosidase, these final products were all products based on 100 % of apple juice. In contrast, some slight increase in inhibiting power towards α-amylase was observed in products containing saffron flower juice and purple myrtle berry extract (B2S01, B1S05 and B1M5), as well as toward pancreatic lipase in product B1M5.

The inhibition of the triglycerides-hydrolysing enzymes (e.g. pancreatic lipase) and carbohydrates-hydrolysing enzymes (e.g. α-amylase and α-glucosidase) can slow the digestion of such nutrients and, consequently their absorption into the body. This lowers fat intake and postprandial plasma glucose rise, respectively. Long-lasting and slow release of glucose into bloodstream is particularly crucial in the treatment of hyperglycaemia and type 2 diabetes. Furthermore, inhibition of these digestive enzymes may help to obtain

satiety and weight loss in overweight and obese people (*Tkacz et al., 2019; Nowicka et al., 2016b*).

Synchronous and strong inhibition of *α*-amylase and *α*-glucosidase can lead to disturbances of the gastrointestinal system, because of the presence of undigested carbohydrates in the colon leading to negative bacterial fermentation. Therefore, a strong inhibition of one of the enzymes and moderate inhibition of the other is desirable (*Unuofin et al., 2018*). It is worth noting that the presence of *D. kaki* and *A. unedo* fruits in products has a positive influence on the digestive enzyme inhibition. According to Wang et al. (*2015*) inhibition of *α*-glucosidase is associated with the content of hydroxycinnamic derivatives, like *p*-coumaric or ferulic acid acids, but in this study, no correlation was found between phenolic acids and inhibition against *α*-glucosidase. This suggests that other polyphenols (anthocyanins, flavonols or polymeric procyanidins) might be involved in the inhibitory effect on digestive enzymes. Moreover, obtained results were in agreement with the data for investigated plant materials in **Paragraph 3.1.4.**.

To sum up, we can see that some correlation was observed for analysed final products. After mixing the components of bases (apple juice, strawberry tree fruits and persimmon purée) and other plant additives, polyphenols of apple juice interacted with polyphenols contained in other plant materials, and as a consequence, showed high anti-*α*-amylase and anti-*α*-glucosidase activity, thereby creating a highly valuable final product with the potential to lower the risk of obesty and *diabetes*.

Table 16. Digestives enzymes inhibitory activities in final products before and after storage time (3 and 6 months) at 20±2 °C.

Sample code	Enzyme inhibition IC_{50} (mg of final product/mL)								
	α-amylase			*α*-glucosidase			pancreatic lipase		
	0 months	3 months	6 months	0 months	3 months	6 months	0 months	3 months	6 months
B1	214.10 ± 0.22c	243.65 ± 0.24e	321.82 ± 0.04a	75.91 ± 0.01b	103.42 ± 0.00c	98.53 ± 0.02g	3.31 ± 0.06i	10.70 ± 0.06f	18.50 ± 0.11h
B1S01	71.16 ± 0.06m	268.88 ± 0.15c	278.02 ± 0.09b	73.32 ± 0.12d	101.18 ± 0.11e	104.11 ± 0.26a	4.53 ± 0.04e	6.80 ± 0.11k	7.51 ± 0.12j
B1S05	312.14 ± 0.31a	314.37 ± 0.08a	277.54 ± 0.22c	72.22 ± 0.11e	100.71 ± 0.08f	94.83 ± 0.11j	9.36 ± 0.12b	21.13 ± 0.14a	20.94 ± 0.08c
B1M5	306.42 ± 0.02b	259.42 ± 0.14d	256.60 ± 0.11e	76.75 ± 0.08a	105.83 ± 0.14b	97.10 ± 0.21h	19.21 ± 0.05a	21.06 ± 0.10a	20.10 ± 0.06l
B1F5	134.28 ± 0.03f	218.10 ± 0.44f	206.81 ± 0.18g	72.21 ± 0.11e	100.32 ± 0.22f	102.42 ± 0.08d	3.75 ± 0.02g	7.50 ± 0.09j	7.33 ± 0.02k
B1K5	61.37 ± 0.11t	286.23 ± 0.12b	257.05 ± 0.31d	15.90± 0.03k	106.87 ± 0.05a	98.65 ± 0.05g	3.61 ± 0.01h	7.71 ± 0.01i	6.13 ± 0.04n
B1C5	177.95 ± 0.01d	204.65 ± 0.19g	208.92 ± 0.21f	75.24 ± 0.02c	102.51 ± 0.08d	103.39 ± 0.11c	3.80 ± 0.01g	6.60 ± 0.03l	6.33 ± 0.01m
B2	111.68 ± 0.03h	93.21 ± 0.11p	104.51 ± 0.15p	15.37 ± 0.03l	68.82 ± 0.12h	99.29 ± 0.01f	6.94 ± 0.11d	4.85 ± 0.01n	9.11 ± 0.11f
B2S01	133.20 ± 0.12g	110.37 ± 0.23k	100.30 ± 0.22r	16.73 ± 0.14j	34.75 ± 0.04l	102.02 ± 0.12e	8.31 ± 0.05c	5.71 ± 0.05m	9.53 ± 0.08d
B2S05	72.49 ± 0.01l	99.34 ± 0.09n	105.89 ± 0.09o	20.08 ± 0.01i	55.75 ± 0.06i	98.81 ± 0.22g	3.78 ± 0.04g	4.52 ± 0.11p	8.87 ± 0.02g
B2M5	69.11 ± 0.04n	86.02 ± 0.08t	91.05 ± 0.14s	15.32 ± 0.00l	24.88 ± 0.01r	36.07 ± 0.11m	3.93 ± 0.03f	4.67 ± 0.08o	9.24 ± 0.05e
B2F5	155.46 ± 0.22e	175.19 ± 0.15h	198.85 ± 0.21h	72.10 ± 0.23e	99.51 ± 0.00g	98.80 ± 0.12g	3.38 ± 0.11i	4.81 ± 0.11n	10.97 ± 0.03c
B2C5	63.98 ± 0.01s	87.92 ± 0.11s	121.97 ± 0.54k	27.32 ± 0.05g	27.10 ± 0.42n	103.71 ± 0.31b	3.17 ± 0.08j	8.74 ± 0.09h	8.23 ± 0.05k
B3	94.90 ± 0.06i	98.13 ± 0.08o	126.51 ± 0.21j	26.53 ± 0.17h	25.41 ± 0.03p	97.17 ± 0.12h	3.01 ± 0.06kl	12.66 ± 0.03e	15.61 ± 0.03o
B3S01	92.31 ± 0.01j	107.40 ± 0.12l	107.71 ± 0.09n	31.60 ± 0.60f	45.09 ± 0.31j	96.75 ± 0.05i	2.93 ± 0.10l	13.89 ± 0.01d	16.66 ± 0.11a
B3S05	79.92 ± 0.10k	113.86 ± 0.03j	115.87 ± 0.15l	27.12 ± 0.06g	26.18 ± 0.16o	97.13 ± 0.04h	3.03 ± 0.08kl	18.80 ± 0.11b	18.92 ± 0.08g
B3M5	64.70 ± 0.01r	92.08 ± 0.09r	87.98 ± 0.18t	15.84 ± 0.04k	23.60 ± 0.02s	36.09 ± 0.09m	3.14 ± 0.03jk	6.75 ± 0.08k	6.05 ± 0.06n
B3F5	64.83 ± 0.01r	148.66 ± 0.15i	141.14 ± 0.23i	15.86 ± 0.03k	38.24 ± 0.06k	99.11 ± 0.03f	3.09 ± 0.11jk	15.33 ± 0.04c	17.64 ± 0.04i
B3K5	65.62 ± 0.08p	106.24 ± 0.16m	114.20 ± 0.12m	16.00 ± 0.00k	29.51 ± 0.91m	90.46 ± 0.37k	3.11 ± 0.08jk	10.17 ± 0.06g	9.62 ± 0.03d
BK	66.72 ± 0.07o	86.00 ± 0.31t	100.13 ± 0.15r	15.39 ± 0.02l	20.87 ± 0.10t	80.08 ± 0.12l	3.31 ± 0.11i	6.49 ± 0.08l	12.15 ± 0.03b

Data are given as mean ± standard deviation (n=3). Mean values within a column with different letters (a-t) are significantly different (homogenous groups) at $p \leq 0.05$; nd ≤ LOD. Sample codes have their references in **Annex 1b.**.

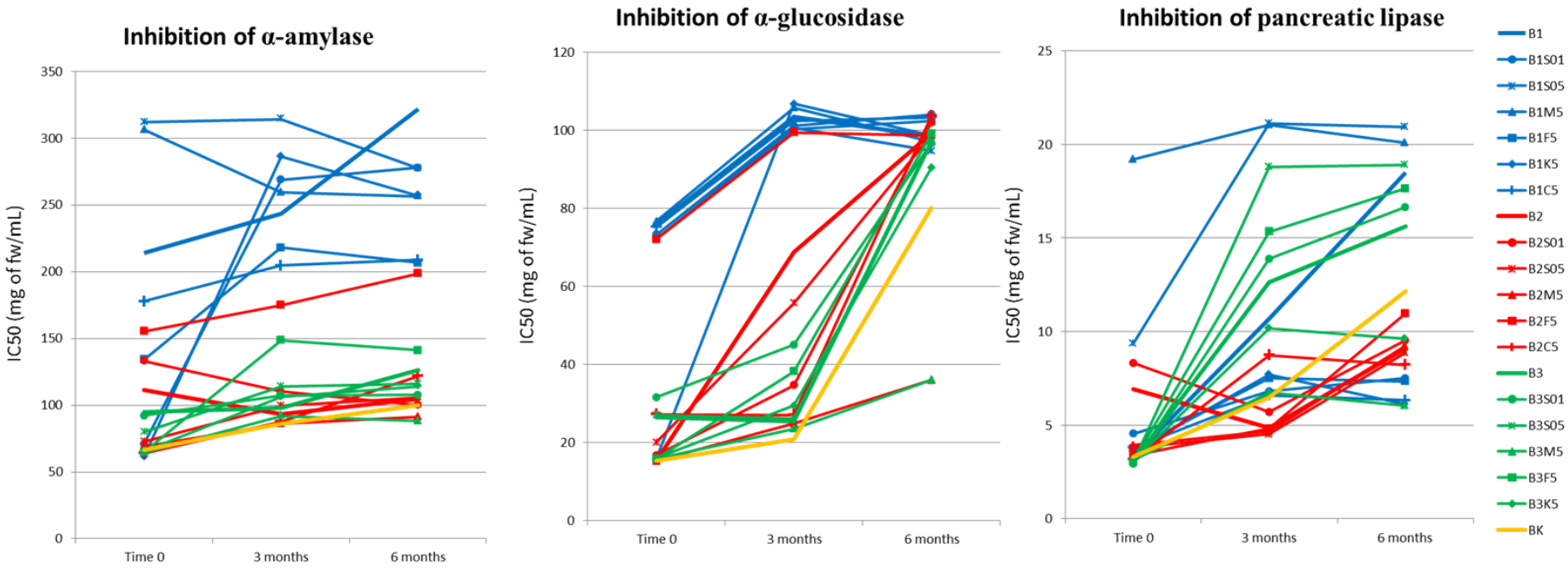

Figure 25. Evolution of the digestive enzymes inhibition of final products before and after storage time (3 and 6 months) at 20 ± 2 °C.

4. CONCLUSIONS

The results of this project showed that it is possible to improve nutritional properties of plant juices/smoothies through addition of plant materials. The six best final products were: **B3, B3S01**, **B3S05, B3M5, B3F5**, and **B3K5**. From the polyphenolic point of view, the chosen products were interesting because each brought with it valuable polyphenolic compounds that are important for human health. Base B3 was already rich in phenols derived from apple juice (chlorogenic acid and quercetin derivatives) and strawberry tree fruits (quercetin-hexose galloyl derivative and anthocyanins like cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside). Other valuable compounds found in base B3 were proanthocyanidins (proanthocyanidin B1, B2 C1 and (-)-epicatechin) and dihydrochalcones (phloretin-2'-*O*-xyloglucose and phloretin-2'-*O*-glucose). After the addition of saffron flower juice, the final products were enriched with kaempferol-3-*O*-sophoroside, quercetin-3,7-*O*-diglucoside, isorhamnetin-3,7-*O*-diglucoside and delphinidin-3,7-*O*-diglucoside, while the addition of myrtle extract enriched the products with group of anthocyanins (delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and malvidin-3-*O*-glucoside) and flavonols (myricetin-3-*O*-galactoside, myricetin-3-*O*-rhamnoside and myricetin). The feijoa flowers enhanced the products content of galloyltannins (casuarina, castalagin and casuarinin), ellagic acid and its pentosides and kaempferol, while persimmon fruits enriched the products in galloyl glucoside, salicylic and syringic acid. Furthermore, the polymeric proanthocyanidins analysis showed that the richest in these compounds are products with base B3. Finally, plant material addition has made six final products (set B3) more interesting than other two sets, in terms of their polyphenol content and worth a deeper investigation.

The sensory evaluation showed that the most desirable final products from set B3 were: base **B3** (score 4.20), as well as those with an additional component of saffron

flower juice (**B3S01**; score 4.10 and **B3S05**; score 3.80), purple myrtle berry extract (**B3M5**; score 3.60) and persimmon pureé (**B3K5**; score 4.30).

The values of total sugars in set B3 were detected in range between (13.27-16.50 g/100 g fw). Moreover, among the investigated sugars, fructose and glucose were present in the largest amounts. The presence of these simple sugars can be positive for functional food preparation, because the addition of extra sweeteners will not be necessary. Among the products from set B3 the richest in sugars were: **B3S05**, **B3M5** and **B3F5**. While, in the case of organic acids, the highest values were found in all products with base B3 in a range between (2.12-3.09 g/100 g fw). Furthermore, among the most common organic acids found in final products were: quinic and malic acid. The presence of these compounds is significant in the preparation of our functional foods, because they promote desirable pH (environment), which is important for product conservation, and are responsible for range of biologic activities.

Moreover, products: **B3M5**, **B3F5**, **B3K5** and **B3S01**, showed the strongest antioxidant activity in a range between (2.20-2.51 mmol Trolox/100 g fw) for $ABTS^{\bullet+}$; (1.89-2.12 mmol Trolox/100 g dm) for FRAP and (4.22-5.25 mmol Trolox/100 g fw) for ORAC assay (before storage). Regarding *in vitro* analysis on Caco-2 cells, all set B3 showed not to be toxic and that they are able to protect enterocytes from oxidative stress. The results of inhibitory activity toward 3 digestive enzymes showed that the most valuable products were: **B3M5**, **B3F5** and **B3K5** in ranges between 64.70-65.62 and 15.84-16.00 mg of final product/mL for α-amylase and α-glucosidase inhibition assay, respectively. While in a case of pancreatic lipase inhibition assay, the best results were obtained by all products with base B3 in a range (2.93-3.14 mg of final product/mL).

To sum up, the addition of saffron flower juice, purple myrtle berry extract, feijoa flowers and persimmon fruits, made products with base B3 the most interesting from a nutritional point of view, and worthy of marketing exploitation.

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Annex 1. Samples

Annex 1a. PLANT MATERIAL

Code	**Note**
Apple	
Af	Apple fresh fruit
Aj	Apple fresh juice
Ad	Apple dry fruit
Feijoa	
Ff	Feijoa fresh flowers
Fd	Feijoa dry flowers
Fc	Feijoa dry flowers after purification on the column
Fe	Feijoa fresh flowers extract
Fpj	Feijoa fresh petal juice
Fpe	Feijoa fresh petal extract
Myrtle	
MPf	Myrtle purple fresh berries
MPd	Myrtle purple dry berries
MPc	Myrtle purple dry berries after purification on the column
MPe	Myrtle purple fresh berries extract 96 % EtOH
MWf	Myrtle white fresh berries
MWd	Myrtle white dry berries
MWc	Myrtle white dry berries after purification on the column
MWe	Myrtle white fresh berries extract
Persimmon	
Kf	Persimmon fresh fruit
Kd	Persimmon dry fruit
Kp	Persimmon fresh fruit purée
Saffron	
Sf	Saffron flower fresh juice
Sd	Saffron flower dry juice
Strawberry tree	
Cf	Corbezzolo/Strawberry tree fresh fruits
Cd	Corbezzolo/Strawberry tree dry fruits
Cc	Corbezzolo/Strawberry tree dry fruits after purification on the column

Annex 1b.

BASE COMPOSITION

B1 Apple juice **100 %**
B2 Persimmon purée **25 %** + Apple juice **75 %**
B3 Strawberry tree fruits (rehydrated with apple juice) **25 %** + Apple juice **75 %**

BEVERAGE COMPOSITION / VARIETY OF SAMPLES

N°	Code	Type of product	Composition
1	**B1**	juice	BLANC (Apple juice 100 %)
2	**B1S01**	juice	Saffron flower juice 0.1 %
3	**B1S05**	juice	Saffron flower juice 0.5 %
4	**B1M5**	juice	Myrtle 5 %
5	**B1F5**	juice	Feijoa flowers 5 %
6	**B1K5**	juice	Persimmon purée 5 %
7	**B1C5**	juice	Strawberry tree dry fruits 5 %
8	**B2**	smoothie	BLANC (Persimmon purée 25 % + Apple juice 75 %)
9	**B2S01**	smoothie	Saffron flower juice 0.1 %
10	**B2S05**	smoothie	Saffron flower juice 0.5 %
11	**B2M5**	smoothie	Myrtle 5 %
12	**B2F5**	smoothie	Feijoa flowers 5 %
13	**B2C5**	smoothie	Strawberry tree dry fruits 5 %
14	**B3**	smoothie	BLANC (Strawberry tree fruits rehydrated with apple juice 25 % + Apple juice 75 %)
15	**B3S01**	smoothie	Saffron flower juice 0.1 %
16	**B3S05**	smoothie	Saffron flower juice 0.5 %
17	**B3M5**	smoothie	Myrtle 5 %
18	**B3F5**	smoothie	Feijoa flowers 5 %
19	**B3K5**	smoothie	Persimmon purée 5 %
20	**BK**	purée	Persimmon purée 100 %

Annex 2. Model of the sensory evaluation form used for the description of the final products

SENSORY ANALYSIS FOR BEVERAGES (JUICE/ SMOOTHIE) B1/B2/B3

Metrics:

Gender: F M

Age: 20-30 31-40 41-50 51-60 >60

In terms of sensory, you prefer the taste: sweet / salty / sour / bitter / tart

COLOUR, AROMA, TASTE, CONSISTENCY, DESIRABILITY EVALUATION

In front of you, there are 6/7 samples of beverages (juice / smoothie) encoded from N1 to N6/N7. Please, evaluate **colour, aroma, taste, consistency, desirability** using a 5° hedonic scale with boundary indications described in the table below. In the comments you can give justification for your choice.

The scale of the assessment of colour, aroma, taste, consistency, desirability	
1	I do not like very much
2	I do not like
3	neither I do not like nor I like
4	I like
5	I like very much

Sample code	**Five-point COLOUR evaluation scale**				
N1	1	2	3	4	5
N2	1	2	3	4	5
N3	1	2	3	4	5
N4	1	2	3	4	5
N5	1	2	3	4	5
N6	1	2	3	4	5
N7	1	2	3	4	5

COMMENTS:……...……

Sample code	Five-point **AROMA** evaluation scale				
N1	1	2	3	4	5
N2	1	2	3	4	5
N3	1	2	3	4	5
N4	1	2	3	4	5
N5	1	2	3	4	5
N6	1	2	3	4	5
N7	1	2	3	4	5

COMMENTS:……...……

Sample code	Five-point **CONSISTENCY** evaluation scale				
N1	1	2	3	4	5
N2	1	2	3	4	5
N3	1	2	3	4	5
N4	1	2	3	4	5
N5	1	2	3	4	5
N6	1	2	3	4	5
N7	1	2	3	4	5

COMMENTS:……...……

Sample code	Five-point **TASTE** evaluation scale				
N1	1	2	3	4	5
N2	1	2	3	4	5
N3	1	2	3	4	5
N4	1	2	3	4	5
N5	1	2	3	4	5
N6	1	2	3	4	5
N7	1	2	3	4	5

COMMENTS:……...……

Sample code	Five-point **DESIRABILITY** evaluation scale				
N1	1	2	3	4	5
N2	1	2	3	4	5
N3	1	2	3	4	5
N4	1	2	3	4	5
N5	1	2	3	4	5
N6	1	2	3	4	5
N7	1	2	3	4	5

COMMENTS:……...……

FLAVOUR TYPE EVALUATION

In front of you, there are 6/7 samples of beverages (juice / smoothie) encoded from N1 to N7. Please, evaluate the **aroma type** of all analysed samples by ticking "X" if you sense the flavours from those listed in the table. In your comments, you can justify your assessment.

Sample code	Perceptibility of individual aroma types							
	lemon	nuts	persimmon	apple	chocolate	strawberry	herb	forest fruits
N1								
N2								
N3								
N4								
N5								
N6								
N7								

COMMENTS:……

PREFERENCE OF THE PACKAGE

In front of you, there are 6/7 samples of drinks (juice / smoothie) encoded from N1 to N6/N7. Please specify what would be the most advantageous form of the packaging of the above mentioned product. Please indicate the type of packaging by marking "X" among the proposed examples or suggest other ones. In the comments you can give justification for your choice.

Sample code	Preferred packaging					
	plastic bottle	tetra pak with straw	glass bottle	glass jar	pouch pack	different package
N1						
N2						
N3						
N4						
N5						
N6						
N7						

COMMENTS:……

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