Babes-Bolyai University
Faculty of Chemistry and Chemical Engineering
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PhD Thesis Abstract

EVALUATION OF BIOACCESSIBILITY AND BIOAVAILABILITY OF PHYTOCHEMICALS FROM DIFFERENT FRUITS AND VEGETABLES

JURY

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Keywords

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α-glucosidase
cytotoxicity
functional foods

ABBREVIATIONS LIST

ABTS	2, 2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
AC	antioxidant capacity
Caco-2 cells	cancer colon cells
DPPH	1,1-diphenyl-2-picrylhydrazyl
FAES	flame atomic emission spectroscopy
FRAP	ferric reducing antioxidant power
G	gastric
GAE	gallic acid equivalent
GI	gastrointestinal
HPLC	high performance liquid chromatography
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma - optical emission spectrometry
PCA	principal component analysis
RDA	recommended daily allowance
SGF	simulated gastric fluid
SIF	simulated intestinal fluid
SSF	simulated salivary fluid
TA	titratable acidity
THQ	target hazard quotient
TLC	thin-layer chromatography
TPC	total phenolic content
TSC	total sugars content
TSS	total soluble solids
UAE	ultrasound assisted extraction

1. Introduction

Fruits and vegetables represent a major source of chemical compounds with numerous health benefits. Recently, many analytical methods have been developed for the identification and quantification of these compounds. Developing efficient extraction methods that improve the extraction yield without reducing the health benefits of phytochemicals food matrices is also of a great demand. In this sense, one of the main objective of this research was the evaluation of the extraction kinetics of phytochemicals contained in broccoli florets and evaluation of food matrix effect on the stability of phytochemicals contained in different types of lingonberry jams in order to optimize the extraction yield.

Furthermore, despite the beneficial properties provided by phytochemicals, their effectiveness depends on their digestibility and possibility to be absorbed by humans. Since knowledge on the phytochemicals availability to exert their biological functions in the human body is still limited, the present thesis provides information regarding phytochemicals' digestion and is focused on investigating minerals and different phytochemicals' stability, such as carotenoids, chlorophylls, polyphenols, anthocyanins, sugars or vitamin C from different fruits or vegetables during gastrointestinal (GI) digestion using *in vitro* or *in vivo* simulated models. In addition, evaluation of the changes in the antioxidant capacity during the simulated digestion represents an important aspect of the thesis.

Finally, since the consumers are looking for products with added nutritional value and food companies are nowadays interested in designing rich antioxidant beverages and functional foods, another objective of this study was to assess the digestive stability of phytochemicals contained in lingonberry jams in the presence of different sweeteners, as well as to evaluate *in vitro* antidiabetic activity and cytotoxicity of lingonberry jams formulated with different sweeteners, as a future perspective for the development of new functional foods.

Original Contributions

2. Extraction kinetics of chlorophylls and carotenoids from Brassica Oleracea florets[1]

In this chapter, a special attention is paid to the extraction of chlorophylls and carotenoids by ultrasounds and to evaluate the kinetic model, in order to find the optimum extraction conditions. The extraction of chlorophylls and carotenoids broccoli using an economical and sustainable alternative extraction method, such as ultrasounds could be of great interest from the industrial point of view. The results of this research involve potential applications for natural additives in food industry.

2.3.1 Ultrasound assisted extraction optimization

Chlorophylls and carotenoids determination was carried out according to the spectrophotometric methods described by Lichtenthaler et al., (2001). In order to optimize the extraction process of chlorophylls and carotenoids from *Brassica oleracea*, parameters such as extraction time, temperature and solvent type have been evaluated. The acetone and ethanol were chosen as extraction solvents because these solvents are often used for extraction of food pigments (Fernandez-Leon et al., 2015). A comparative study of the extraction of chlorophylls and carotenoids from broccoli with both solvents was performed. The experimental results obtained after extraction with ethanol and acetone are represented in Tables 2.1-2.3. Ethanol revealed a better efficiency for chlorophylls and carotenoids extraction. Ethanol is a preferred solvent taking into account its recommendation as an eco-friendly solution. Moreover, ethanol is cheaper than other solvents, non-toxic and safe. It is important when choosing an extraction solvent to consider the possible toxicity associated with the residues of some of these solvents in the extracts, even more if extracts are used as additives or food dyes, such as chlorophylls and carotenoids.

Another parameter that influences the extraction process of natural pigments from vegetables such as broccoli is extraction time. The tested extraction time was chosen between 5 and 40min because some studies report that the effect of ultrasound assisted extraction is more effective in the first 30min (Zhang et al., 2008).

[1] Parts of this chapter were published in Scrob et al., 2019b.

Table 2.1 Chl-A concentration (mg/g) at different extraction times and temperatures

Extraction time (min)	**Acetone (30^0C)**	**Ethanol (30^0C)**	**Ethanol (50^0C)**	**Ethanol (80^0C)**
0	0	0	0	0
5	4.29	5.02	4.60	3.98
10	6.48	7.35	6.10	5.49
15	7.39	8.40	6.87	6.33
20	7.15	8.53	7.32	6.56
30	7.00	8.27	7.62	6.69
40	7.07	8.13	7.74	6.79

Table 2.2 Chl-B concentration (mg/g) at different extraction times and temperatures

Extraction time (min)	**Acetone (30^0C)**	**Ethanol (30^0C)**	**Ethanol (50^0C)**	**Ethanol (80^0C)**
0	0	0	0	0
5	2.34	3.19	3.23	2.72
10	3.27	4.99	4.55	4.10
15	3.58	5.92	5.26	4.75
20	3.34	6.13	5.65	4.90
30	3.23	6.11	5.77	4.98
40	3.27	6.06	5.82	5.02

Table 2.3 Carotenoids concentration (mg/g) at different extraction times and temperatures

Extraction time (min)	**Acetone (30^0C)**	**Ethanol (30^0C)**	**Ethanol (50^0C)**	**Ethanol (80^0C)**
0	0	0	0	0
5	1.46	1.45	1.15	1.02
10	1.64	1.93	1.53	1.42
15	1.63	2.15	1.63	1.49
20	1.63	2.16	1.63	1.48
30	1.62	2.12	1.62	1.47
40	1.62	2.08	1.60	1.45

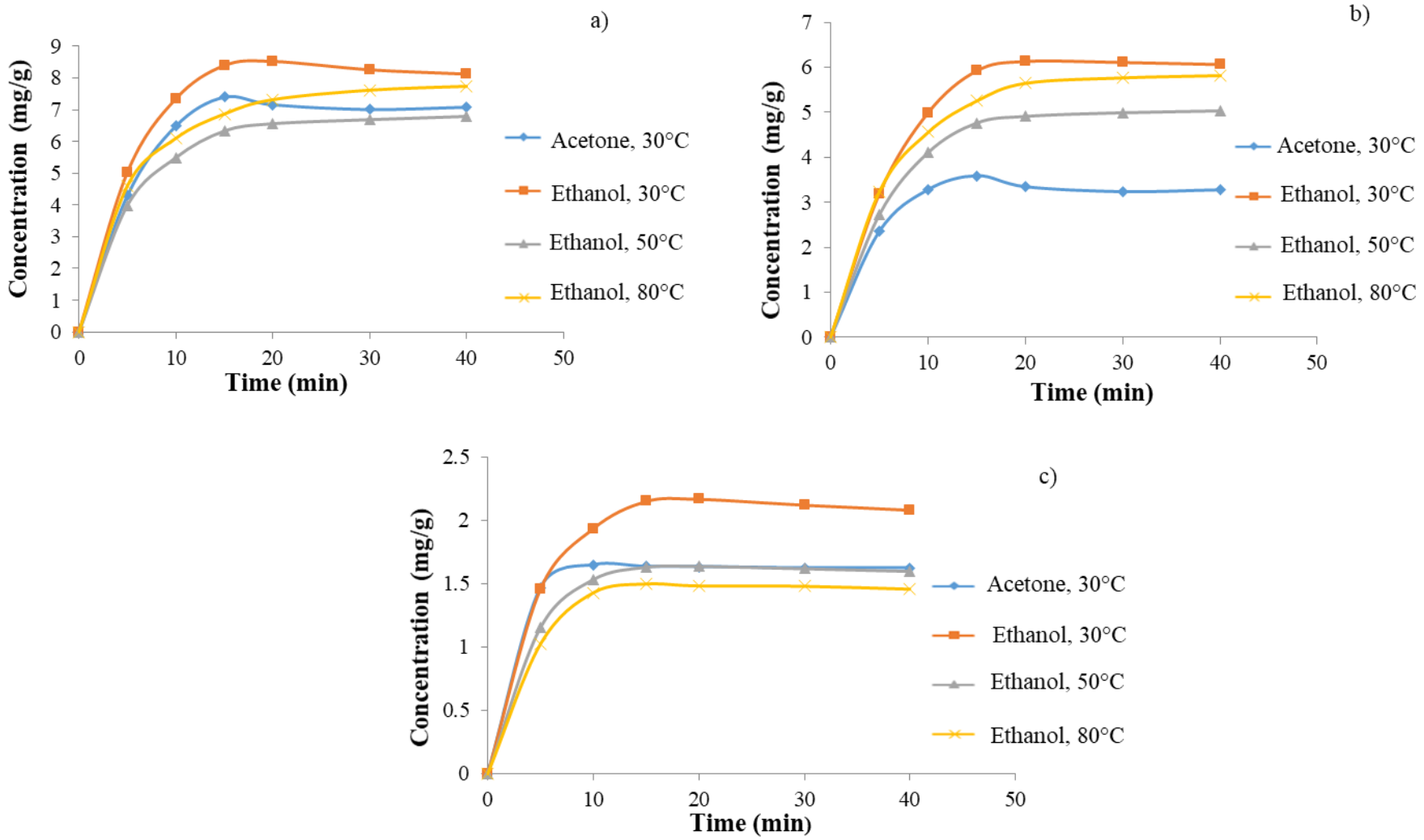


Figure 2.1 Influence of extraction time, solvent and temperature on Chl-A (a), Chl-B (b) and carotenoids (c) extraction

2.3.2 Kinetic modeling

The determination of kinetic parameters is extremely important for efficient ultrasonic assisted extraction. The use of kinetic models, the extraction rate and stationary times required to complete the extraction process involving ultrasounds could be predicted, these data being extremely useful when technological transfer is desired (Lazar et al., 2016).

The process of extracting chlorophylls and carotenoids from broccoli takes place in two steps: a rapid one, in the first 20min, followed by a slower step, which approaches the equilibrium concentration. In these circumstances, extraction rate follows a second-order law. The experimental data obtained from the UAE were processed and represented graphically in the coordinate specific to the second order kinetic model (Lazar et al., 2016). The validation of the kinetic model for all experimental data is confirmed in each case by the mathematical value of the determination coefficient (R^2>0.98) (Figures 2.2-2.4).

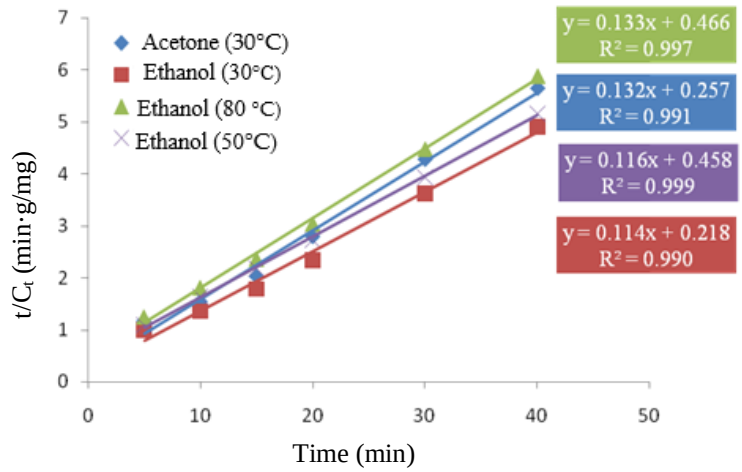


Figure 2.2 Validation of the second order kinetic model for Chl-A

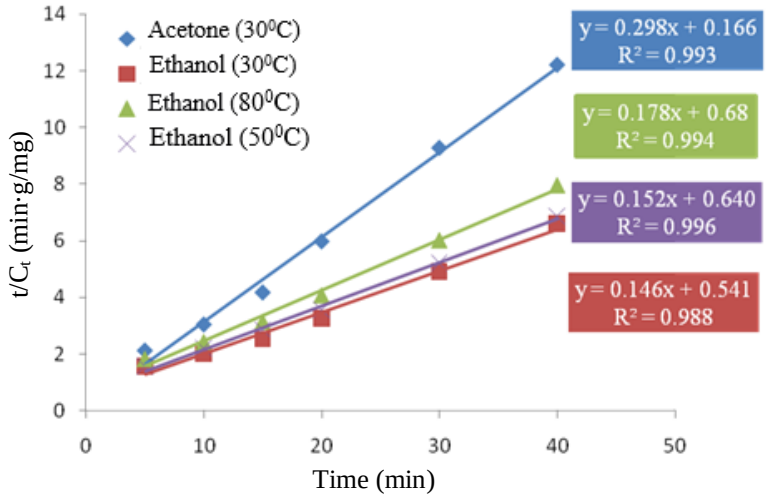


Figure 2.3 Validation of the second order kinetic model for Chl-B

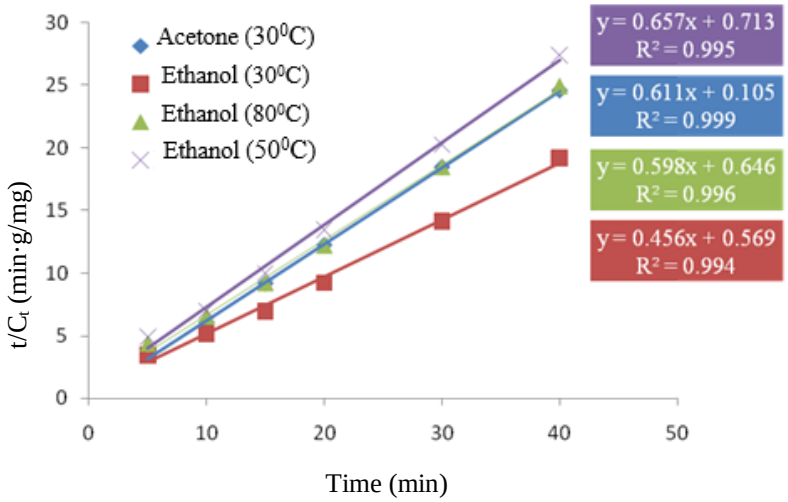


Figure 2.4 Validation of the second order kinetic model for carotenoids

In conclusion, kinetic ultrasound assisted extraction study of chlorophylls and carotenoids from broccoli (*Brassica oleracea*) was performed in order to determine the main factors and mechanisms involved for further possible food industry applications. Chlorophylls and carotenoids extraction follows a second-order kinetic model (R^2>0.98). Ethanol extracted a higher amount of natural pigments than acetone. Extraction with acetone determined higher values of the rate constants k than ethanol. Although acetone extracted faster chlorophylls and carotenoids from broccoli, ethanolic extracts are preferred due to their use as natural additives in the food industry. Extraction with ethanol at 30°C was the most efficient and higher temperatures determined a decrease of the amount of these pigments. The results of this research involve potential applications for natural additives in food industry.

3. *In vitro* gastrointestinal digestion of *Brassica oleracea* florets[2]

Broccoli (*Brassica oleracea)* is a vegetable that became very popular nowadays mainly due to its protection against various diseases and its wide variety of antioxidants content. In this sense, knowledge of the changes that occur during the digestion process is of great interest. This chapter comprises the results obtained from the investigation of *in vitro* gastrointestinal digestion of broccoli on the antioxidant activity, chlorophylls, carotenoids and total phenolic content. The antioxidant capacity was monitored by ABTS assay and the content of target compounds were investigated by UV-Vis spectrophotometry and thin-layer chromatography. The experimental results highlight that gastrointestinal digestion may substantially affect the absorption of phenolics, chlorophylls and carotenoids present in *Brassica oleracea*, representing a basis for further studies on the stabilization of these phytochemicals.

3.3.1 Bioaccessibility of chlorophylls and carotenoids

In order to evaluate the content of phytochemicals from broccoli following the digestion process, the samples were analyzed before and after *in vitro* digestion. The static *in vitro* digestion method used in this study included the salivary, gastric and intestinal phases (Minekus et al., 2014). The detailed composition of simulated fluids, namely salivary fluid - SSF, gastric fluid- SSG and intestinal fluid - SIF is given in Table 3.1.

Table 3.1 Preparation of simulated digestion fluids

	Volume of constituents (mL)					
Simulated digestion fluid	KCl (37.3 g/L)	KH_2PO_4 (68 g/L)	$NaHCO_3$ (84 g/L)	NaCl (117 g/L)	$MgCl_2(H_2O)_6$ (30.5 g/L)	$(NH_4)_2CO_3$ (48 g/L)
SSF (pH=7)	15.1	3.7	6.8	—	0.5	0.06
SGF (pH=3)	6.9	0.9	12.5	11.8	0.4	0.5
SIF (pH=7)	6.8	0.8	42.5	9.6	1.1	—

Variations in the content of carotenoids, chlorophylls and phenolics, as well as the changes in AC during *in vitro* digestion are presented in Table 3.2.

[2] Published in Scrob et al., 2019b.

Table 3.2 Total content of carotenoids, chlorophylls and phenolics, and antioxidant capacity in non-digested and *in vitro* digested samples.

Digestion Phase	Concentration			Antioxidant capacity (µmol Trolox/mL)
	Carotenoids (mg/mL)	Chlorophylls (mg/mL)	Polyphenols (µg GAE/mL)	
Initial	6.11±0.98	18.21±1.21	136.44±9.85	1.05±0.03
Gastric	1.34±0.12	7.49±0.84	126.36±5.16	0.43±0.01
Intestinal	1.34±0.14	5.09±0.62	69.24±4.25	0.20±0.00

Compared to the amount of carotenoids present in non-digested samples, the content of these compounds decreased after the digestion process. It was reported a loss of 78.07% of carotenoids after both gastric and intestinal digestion compared to content before digestion (6.11 mg/mL). The decrease of carotenoids during digestion has been also confirmed by other authors (Courraud et al., 2013; Fernández-García et al., 2012). The chlorophylls content followed a similar pattern with carotenoids and decreased after *in vitro* digestion compared to the initial amount (72.05%). It has been reported that chlorophylls are compounds sensitive to extreme pH and high temperatures.

The changes occurring in the content of chlorophylls and carotenoids before and after each step of *in vitro* digestion was also evaluated by TLC method that has increased the interest of researches in the last decades, being fast and relatively inexpensive in separating complex mixtures. With the high development of stationary phases and the possibility to be combined with accurate detection equipment, the technique is still increasing its uses in many research fields (Scrob et al., 2019a). Samples obtained after simulated digestion - salivary phase digesta (P1), gastric phase digesta (P2) and intestinal phase digesta (P4) were analyzed by TLC and a decreasing trend in chlorophylls and carotenoids content after *in vitro* digestion process was observed compared to non-digested ethanolic extract (P6). Samples (P3) and (P5) represent the ethanolic residues obtained after gastric and intestinal digestion, respectively; these samples were analyzed in order to verify if the target compounds remained in the solid matrix during digestion. The separations were visualized in UV light at a wavelength of 366nm (Figure 3.1). The color intensity of spots shows that the highest amount of compounds is found in the non-digested sample (P6), followed by ethanolic extracts of the residual solid material resulting from oral (P1), gastric digestion (P3) and intestinal digestion (P5).

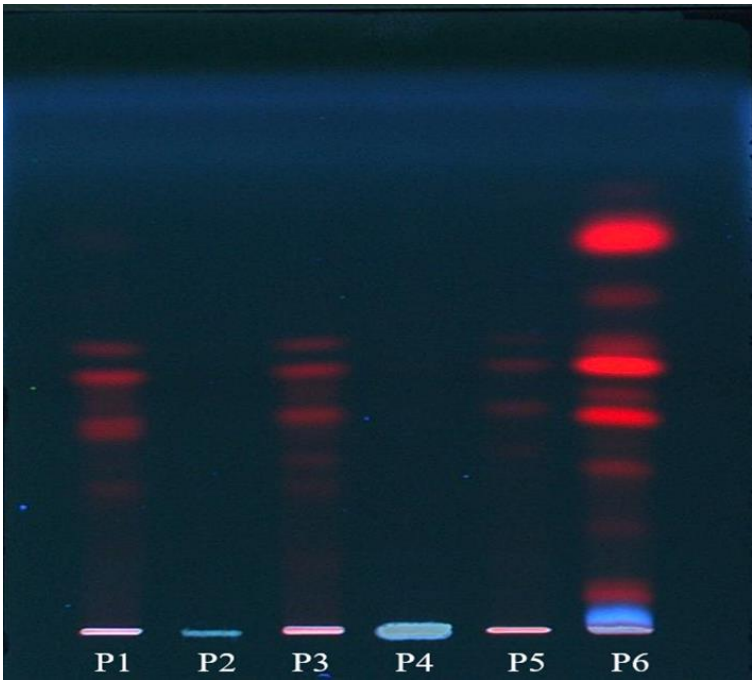


Figure 3.1 TLC separation in UV light at 366 nm of salivary phase digesta (P1), gastric phase digesta (P2), gastric ethanolic extract (P3), intestinal phase digesta (P4), intestinal ethanolic extract (P5) and non-digested ethanolic extract (P6).

The behavior of chlorophylls and carotenoids during gastric and intestinal digestion was better highlighted by analyzing the image of separation at 366nm in which the color intensity of spots shows that the highest amount of compounds is found in the non-digested sample (P6), followed by ethanolic extracts of the residual solid material resulting from oral (P1), gastric digestion (P3) and intestinal digestion (P5). Regarding the fluids obtained from gastric (P2) and intestinal (P4) digestion phases, respectively, the content of chlorophylls and carotenoids decreased so that the compounds could no longer be detected by TLC.

3.3.2 Bioaccessibility of total phenolics

Regarding TPC it is well known that these phytochemicals can interact with different dietary constituents such as proteins, fibers, lipids, changing their chemical structure and affecting in this way their bioavailability (Bouayed et al., 2011). In the present study, the release of phenolics from *Brassica oleracea* following simulated digestion was mainly achieved during the gastric phase (Table 3.2).

From experimental data it can be seen that the polyphenols from broccoli are more stable to changes in pH and enzymatic activities (pepsin and pancreatin) during GI digestion than chlorophylls and carotenoids. The results of this study highlight that the bioaccessibility of polyphenols from *Brassica oleracea* is closely related to transformations taking place during the digestion process.

3.3.3 Changes in antioxidant capacity during simulated digestion

The AC of all the samples was decreased after *in vitro* digestion as compared to the non-digested samples (Table 3.2). The AC followed a similar pattern with that of tested compounds. This fact may indicate that antioxidant capacity in vegetables such as broccoli could be attributed to the presence of these phytochemicals. The low AC at the end of the simulated digestion process could be associated with the low stability of these compounds (Gayoso et al., 2016).

In conclusion, the study of phytochemicals bioaccessibility from broccoli plays an important role because only the molecules released from the plant matrix as a result of the digestion process will subsequently be bioavailable for absorption. The results indicate that the simulated digestion decreased total carotenoids and chlorophylls content from *Brassica oleracea*, as well as total phenolics content and antioxidant capacity. All changes were statistic significantly ($p<0.05$), with the exception of carotenoids modifying during intestinal digestion. The experimental results showed a slight decrease in the content of polyphenols after the gastric and intestinal stages, indicating a higher level of bioavailability of these compounds in comparison to carotenoids and chlorophylls; thus, *Brassica oleracea* remains an important source of compounds with antioxidant properties.

4. Effect of *in vitro* simulated gastrointestinal digestion on nutritional characteristics of several dried fruits[3]

Dried fruits are highly consumed nowadays due to the healthy properties they possess. To provide new information on potential health benefits of dried fruits, this chapter covers the determination of *in vitro* digestion effects on minerals, total phenolics, total sugars and the antioxidant capacities. Determination of Mg, Ca, Mn, Fe, Cu, and Zn was performed by ICP-OES, whereas the determination of Na and K was performed by flame atomic emission spectrometry (FAES). Toxic elements were determined by inductively coupled plasma mass spectrometry (ICP-MS). Bioaccessibility of these compounds was assessed also by multivariate statistical analysis. The similarities/dis-similarities in pattern of analyzed parameters during digestion was revealed by heat map. Most relevant parameters in each digestion phase were highlighted by two-way joining cluster and principal component analysis. This research is also aimed to generate interest in studying dried fruits as functional foods.

4.3.1 Validation of ICP-OES, ICP-MS and FAES methods

The LODs in solid sample were calculated considering the sample preparation protocol for the determination of total and bioaccessible fraction (Table 4.4).

Table 4.4 Limits of detection in ICP-OES, ICP-MS and FAES for multielemental determination in dried fruits.

Determination	**Methods**													
	ICP-OES						**ICP-MS**						**FAES**	
	Mg	**Ca**	**Mn**	**Fe**	**Cu**	**Zn**	**As**	**Cd**	**Co**	**Hg**	**Pb**	**Ni**	**Na**	**K**
Liquid (ng/mL)	0.50	0.50	0.60	0.20	3.3	3.5	0.0006	0.02	0.02	0.04	0.10	0.06	100	20
Solid (total) (µg/kg)	50	50	60	20	330	350	0.06	2	2	4	10	6	100000	2000
Gastric (µg/kg)	1	1	1	0.4	6.6	7.0	-	-	-	-	-	-	200	40
Intestinal (µg/kg)	2	2	2	0.8	13.2	14.0	-	-	-	-	-	-	400	80

4.3.2 Characterization of dried fruits samples

[3] Published in Scrob et al., 2022a.

Table 4.5 Elemental composition (mg/kg d.b) of the 24 studied dried fruits samples. Bioaccessibility on gastric phase (Bioaccessibility$_{(G)}$) and gastrointestinal phase (Bioaccessibility$_{(GI)}$) are expressed in percentages (%). Co and Cd are < LOD in all of the samples.

Sample	**Na**	**K**	**Mg**	**Ca**	**Mn**	**Fe**	**Cu**	**Zn**	**As**	**Pb**	**Ni**	**Hg**
Dates (D)												
Min.	2074	33357	426	333	4.82	8.06	9.76	16.5	0.00018	0.149	0.478	0.0085
Max.	3092	36503	610	820	7.48	27.2	18.0	38.0	0.0173	0.481	0.664	0.0747
Average	2427	35575	524	550	6.02	18.3	12.6	26.5	0.0112	0.268	0.580	0.0260
CI[b]	983	3202	213	463	2.95	16.9	8.17	24.7	0.0176	0.314	0.171	0.0680
Median	2271	36220	529	522	5.88	19.0	11.4	25.7	0.0140	0.221	0.590	0.0125
Bioaccessibility$_{(G)}$	48.5	59.5	42.5	51.6	65.3	12.7	4.10	<	c	c	c	c
Bioaccessibility$_{(GI)}$	20.3	34.2	36.6	40.1	<	2.65	<	<	c	c	c	c
Raisins (R)												
Min.	2302	25866	303	555	5.22	29.6	9.08	10.1	0.0151	0.144	0.314	0.0086
Max.	6555	32345	397	632	6.34	73.3	10.5	14.7	0.0354	0.862	0.371	0.0168
Average	3931	29671	345	608	5.63	43.6	9.85	12.1	0.0280[a]	0.397	0.350	0.0100
CI[b]	4156	5880	84.9	75.8	1.12	20.0	1.38	4.96	0.0240	0.336	0.0540	0.0083
Median	3432	30235	339	621	5.47	35.7	9.92	11.75	0.0350	0.291	0.358	0.008
Bioaccessibility$_{(G)}$	54.3	66.1	53.8	28.8	50.0	27.7	11.3	2.39	c	c	c	c
Bioaccessibility$_{(GI)}$	24.8	74.2	51.9	29.2	37.6	10.2	<	<	c	c	c	c

Coconut (K)												
Min.	2058	17247	554	92.1	17.1	24.2	11.7	16.4	0.0021	0.0503	0.357	-
Max.	2869	19100	861	162	23.7	31.6	16.2	19.7	0.0397	0.252	3.17	-
Average	2480	18056	713	123	20.3	28.1	12.8	18.8	0.0209[a]	0.131[a]	1.63	0.0096[a]
CI[b]	714	1648	273	76.3	6.08	6.51	5.29	3.50	0.0572	0.228	3.08	0.0000
Median	2496	17938	718	119.3	20.1	28.4	12.3	19.6	0.0209	0.0931	1.51	0.0096
Bioaccessibility$_{(G)}$	74.1	58.8	47.7	75.2	92.5	26.2	16.7	12.7	c	c	c	c
Bioaccessibility$_{(GI)}$	51.1	37.3	33.4	50.3	3.27	43.8	20.1	<	c	c	c	c
Cranberries (C)												
Min.	3003	10055	35.7	72.6	2.80	2.47	4.34	2.73	0.0011	-	0.130	0.0086
Max.	4013	11784	53.7	94.5	3.51	4.52	5.21	3.72	0.0396	-	0.269	0.0260
Average	3455	10619	43.3	78.1	3.20	3.56	4.88	3.17	0.0124	-	0.183	0.0160
CI[b]	898	1849	18.3	24.2	0.676	1.81	0.866	0.887	0.0380	-	0.129	0.0212
Median	3402	10398	41.8	74.2	3.25	3.63	5.00	3.12	0.0053	0.0520	0.168	0.0170
Bioaccessibility$_{(G)}$	46.8	68.5	53.9	74.3	17.5	9.36	<	<	c	c	c	c
Bioaccessibility$_{(GI)}$	24.1	40.8	42.3	61.9	7.55	6.53	<	<	c	c	c	c
Prunes (P)												
Min.	10528	17923	262	523	2.86	5.73	5.11	9.43	0.0154	0.145	0.218	-

Max.	20791	33586	337	634	5.26	13.7	7.63	24.7	0.0542	0.391	0.653	-
Average	15618	24065	300	520	3.91	9.66	6.39	16.3	0.0373[a]	0.261	0.367	-
CI[b]	9050	15347	81.2	217	2.38	7.17	2.85	16.3	0.0432	0.280	0.419	-
Median	15576	22376	301	529	3.77	9.62	6.41	15.6	0.0430	0.255	0.300	0.0070
Bioaccessibility$_{(G)}$	53.5	72.3	56.3	31.4	57.9	12.4	10.4	17.9	c	c	c	c
Bioaccessibility$_{(GI)}$	39.0	53.0	38.4	25.0	36.2	9.35	39.7	<	c	c	c	c
Dried banana (B)												
Min.	4758	22335	547	157	6.67	4.89	4.81	9.54	0.00021	0.0426	0.275	-
Max.	7857	26483	610	183	17.4	10.5	5.72	15.6	0.0020	0.195	0.937	-
Average	5802	23768	577	165	10.5	6.39	5.27	11.5	0.00065	0.0850	0.494	0.0090[a]
CI[b]	3002	4528	61.8	26.4	10.2	4.56	0.809	6.00	0.0019	0.159	0.660	0.0000
Median	5295	23165	575	159	9.01	7.38	5.29	10.4	0.00021	0.0525	0.383	0.0090
Bioaccessibility$_{(G)}$	77.0	72.0	39.9	21.6	33.4	34.4	5.26	13.7	c	c	c	c
Bioaccessibility$_{(GI)}$	55.1	46.3	25.7	17.0	11.0	16.7	43.7	<	c	c	c	c

Values are presented as average for 4 samples and 3 parallel measurements for each sample.

[a] Calculated without values below limit of detection (LOD).

[b] Confidence interval (CI) (95%, n=4).

[c] Toxic metals were not taken into consideration for bioaccessibility measurements.

d.b: dry basis

a) Essential minerals and toxic elements

Total concentrations of Na, K, Mg, Ca, Mn, Fe, Cu, Zn, Co, As, Pb, Cd, Ni and Hg, expressed as mean from three independent experiments are given in Table 4.5. In all cases, acceptable values for errors between measurements (RSD) were smaller than 10.0%.

According to the results obtained for total content, one can conclude that K is the most abundant macronutrient, while among the microelements Zn and Fe are present at a higher concentration. High concentrations of K have been also reported in other types of fruits by Perreira et al., (2015). The elemental concentrations decreased in the following order: K > Na > Ca > Mg > Zn > Fe > Cu > Mn, for dates and prunes; K > Na > Mg > Ca > Fe > Zn > Cu > Mn in the case of raisins; K > Na > Mg > Ca > Fe > Zn > Mn > Cu, for coconut; K > Na > Ca > Mg > Cu > Fe > Mn > Zn, for cranberries; K > Na > Mg > Ca > Zn > Mn > Fe > Cu, for dried bananas.

Present in high concentrations, toxic elements such as Co, As, Pb, Cd, Ni and Hg may result in various adverse health effects. For example, the accumul ation of cadmium in the human body may affect the pulmonary, renal or hepatic system (Tokalioglu et al., 2014), while lead is involved in many adverse health effects including neurotoxicity and nephrotoxicity (Gercia-Leston et al., 2010). Therefore, evaluation of the potential health risks of heavy metals from foods is important. The non-carcinogenic risk of As, Cd, Ni, Co, Pb and Hg from dried fruits was assessed using the target hazard quotient (THQ). THQs of individual toxic elements are presented in Figure 4.2.

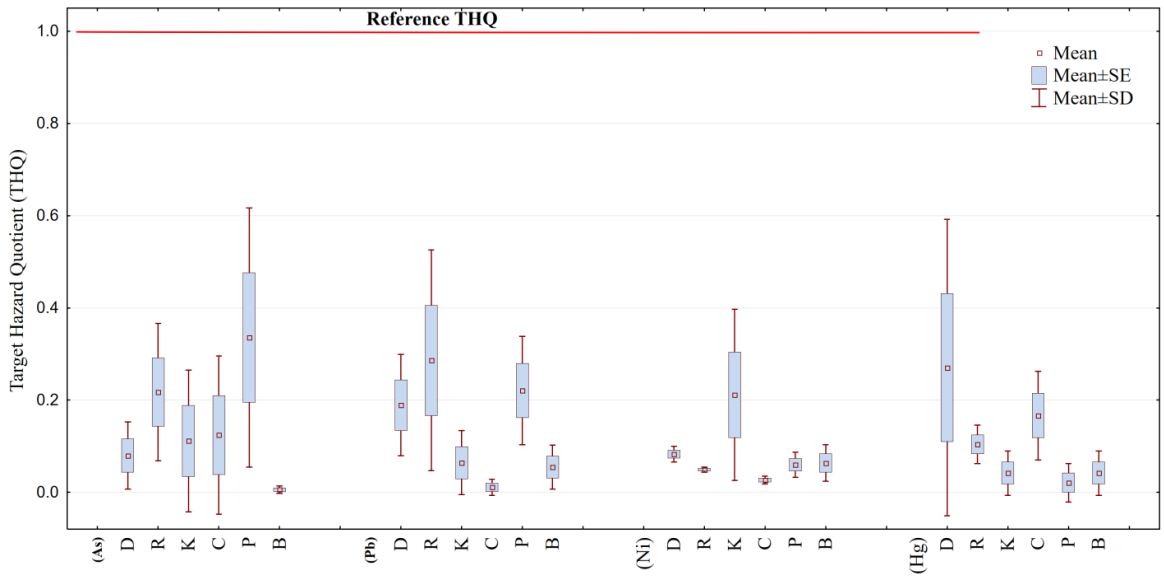


Figure 4.2 The health risk exposure to As, Pb, Ni and Hg by occasional consumption of dried fruits: D - dates; R - raisins; K - coconut; C – cranberries; P - prunes; B – dried banana. Cd and Co are below LOD in all of the samples.

THQ value of each metal was much lower than 1 suggesting that exposed population is unlikely to experience any adverse health hazard. The cumulative health risk was also evaluated by summing THQ value of individual metal and expressed as total THQ (TTHQ). The observation is of concern especially for vegetarians. Due to the low concentration values in the non-digested sample and because they do not represent a potential health risk, As, Cd, Ni, Co, Pb and Hg were not taken further into consideration in the bioaccessibility measurements.

b) TPC, TSC and AC of dried fruits

The experimental results for TPC, TSC and AC of selected dried fruits obtained before *in vitro* digestion are presented in Table 4.6. Among the studied dried fruits, raisins contain the highest amount of TPC, followed by cranberries, dates, prunes and dried banana. The lowest TPC among the tested dried fruits is found in coconut. The obtained results are in line with those reported by others (Ishiwata et al., 2004; Wu et al., 2004).

The TSC in varied in all the analyzed dried fruits. Dates are the fruit with the smallest content of sugars. The highest concentration of sugars is found in dried banana (Table 4.6). This fact could be attributed to the sugar and honey added by the producers to these fruits, as is specified on the label. Moreover, fruits drying significantly increases solids concentration (Chang et al., 2016) and this can be correlated also to the high sugar content. Prunes and cranberries also represent a high source of sugars, after dried banana. All cranberries types used in this study contained added sugar and this fact could be correlated with the high sugars content obtained. Interestingly, prunes did not contain added sugar; thus, these dried fruits represent an important source of natural sugars, such as fructose and glucose. The AC of dried fruit samples was determined by ABTS, DPPH and FRAP assays. The highest AC_{ABTS} values being found for cranberries and dates, while the coconut samples have the lowest AC_{ABTS}.

4.3.3 In vitro GI digestion of dried fruits

a) Bioacessibility of essential elements

In general, the total content of a compound in a food sample is not enough for evaluating the real nutritional value and studies of bioaccessibility are required. Determinations of bioaccessibility of essential elements play an important role as these elements are involved in a variety of biological functions. The bioaccessibilities (%) of the essential elements after the gastric and gastrointestinal digestion are given in Table 4.5. The variations of essential elements content during *in vitro* digestion approach are given in Figure 4.3.

Table 4.6 TPC (mg Gallic acid/g d.b), TSC (mg D-glucose/g d.b), AC_{ABTS} (µmol Trolox/g d.b), AC_{DPPH} (µmol Trolox/g d.b) and AC_{DPPH} (mg ascorbic acid/g d.b) of the 24 studied dried fruits samples. Bioaccessibility in gastric phase ($Bioaccessibility_{(G)}$) and gastrointestinal phase ($Bioaccessibility_{(GI)}$) are expressed in percentages (%).

Sample	**TPC**	**TSC**	**AC_{ABTS}**	**AC_{DPPH}**	**AC_{FRAP}**	**Sample**	**TPC**	**TSC**	**AC_{ABTS}**	**AC_{DPPH}**	**AC_{FRAP}**
Dates (D)						**Cranberries (C)**					
Min.	0.625	2042	2.05	1.38	0.310	Min.	1.43	5770	5.02	2.36	0.215
Max.	3.95	2875	5.57	2.45	0.483	Max.	3.05	14220	7.48	3.06	0.267
Average	1.92	2348	3.63	1.99	0.381	Average	2.04	9116	6.34	2.70	0.242
CI[a]	4.00	886	3.13	0.540	0.0160	CI[a]	2.00	10242	4.19	0.596	0.0030
Median	1.55	2237	3.44	2.07	0.366	Median	1.85	8238	6.43	2.70	0.243
$Bioaccessibility_{(G)}$	42.3	109	265	103	203	$Bioaccessibility_{(G)}$	27.5	23.7	46.1	74.9	66.8
$Bioaccessibility_{(GI)}$	62.2	193	401	87.7	172	$Bioaccessibility_{(GI)}$	63.2	60.0	202	182	100
Raisins (R)						**Prunes (P)**					
Min.	1.30	2429	0.889	2.19	0.117	Min.	0.804	9265	0.791	1.78	0.128
Max.	3.04	5339	3.88	2.39	0.204	Max.	2.26	13625	1.75	2.89	0.223

Average	2.25	3454	2.36	2.27	0.158	Average	1.60	11595	1.40	2.39	0.160
CI[a]	2.00	3536	2.03	0.115	0.0030	CI[a]	1.00	2763	0.344	0.642	0.0000
Median	2.33	3024	2.33	2.25	0.155	Median	1.67	11746	1.54	2.44	0.145
Bioaccessibility$_{(G)}$	110	104	255	66.9	299	Bioaccessibility$_{(G)}$	152	10.3	589	189	801
Bioaccessibility$_{(GI)}$	112	121	2074	88.3	234	Bioaccessibility$_{(GI)}$	209	26.0	1412	381	818
Coconut (K)						**Dried banana (B)**					
Min.	0.241	7494	0.0466	0.868	0.0596	Min.	0.445	12653	1.30	0.952	0.110
Max.	0.485	9424	0.744	1.06	0.0970	Max.	0.665	19280	2.78	1.65	0.172
Average	0.360	8096	0.239	0.945	0.0740	Average	0.549	16770	1.74	1.32	0.139
CI[a]	0.0000	1989	0.0460	0.0481	0.0000	CI[a]	0.0000	6791	2.00	0.223	0.0000
Median	0.358	7732	0.0840	0.926	0.0690	Median	0.543	17574	1.44	1.33	0.137
Bioaccessibility$_{(G)}$	91.0	55.0	3948	46.0	91.7	Bioaccessibility$_{(G)}$	66.8	21.4	189	36.7	35.1
Bioaccessibility$_{(GI)}$	195	139	26792	406	371	Bioaccessibility$_{(GI)}$	123	50.0	775	62.3	57.5

Values are presented as average for 4 samples and 3 parallel measurements for each sample.

[a] Confidence interval (CI) (95%, n=4).

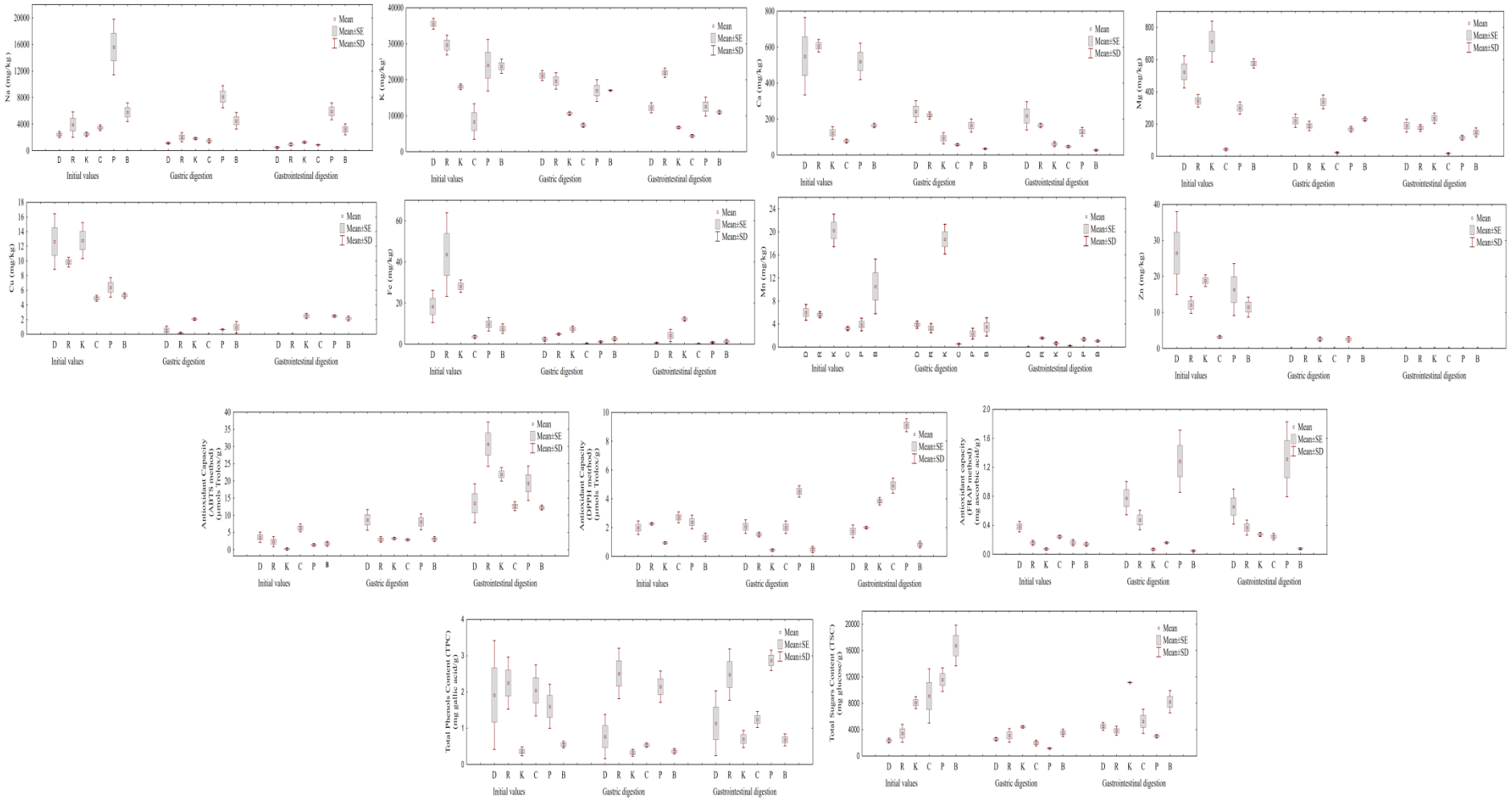
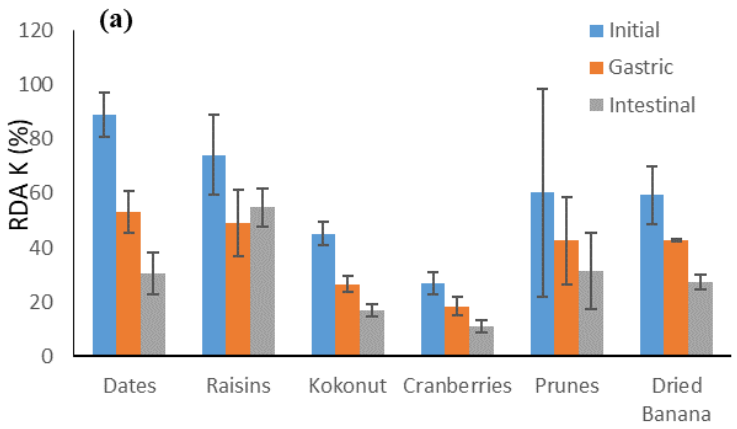


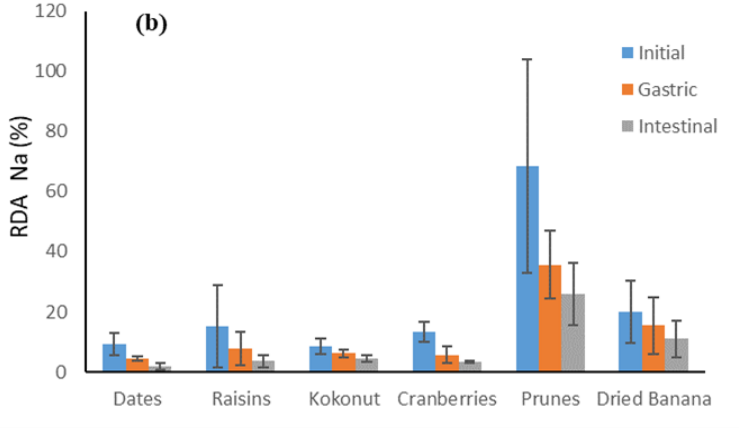
Figure 4.3 The concentration of essential elements: (a)-K, (b)-Na, (c)-Ca, (d)- Mg, (e)- Cu, (f)-Fe, (g)-Mn, (h)-Zn in dried fruit samples before digestion and after gastric (G) and gastrointestinal (GI) digestion phases

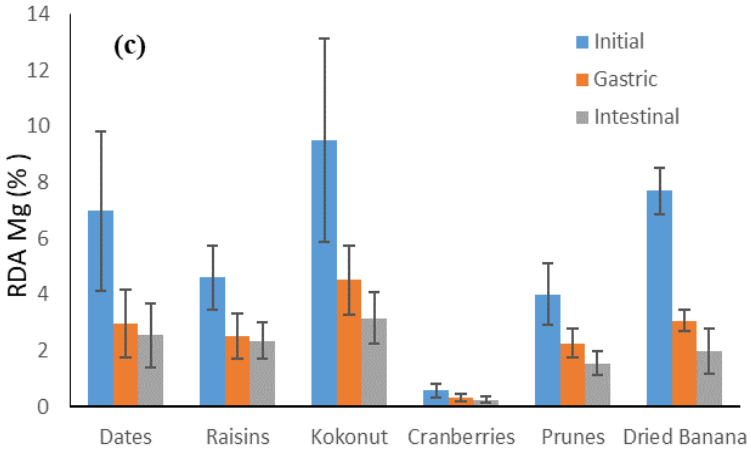
The highest K bioaccessibility after GI phase was observed for raisins, ranging from 68.17 to 78.84%, while dates represented a poor source of bioaccessible K at the end of digestion phase, with bioaccessibility values between 30.64 and 37.12%. Mg bioaccessibility following gastrointestinal digestion varied from 25.7% in dried banana to 51.9% in raisins, whereas that of Ca ranges from 17.0% in dried banana to 62.0% in cranberries. Fe, Mn and Zn has much higher bioaccessibility values in the gastric phase compared with the gastrointestinal phase, except for Fe from coconut samples, which is more bioaccessible after GI phase (37.87-52.72%). Zinc has the lowest bioaccesibility of all the fruits and after the GI stage, its concentration being smaller than LOD for all samples. From statistical point of view, elements Mg, Fe, Cu and Zn are found in a statistically significant lower concentration (test t, $p < 0.05$) after both G and GI digestion.

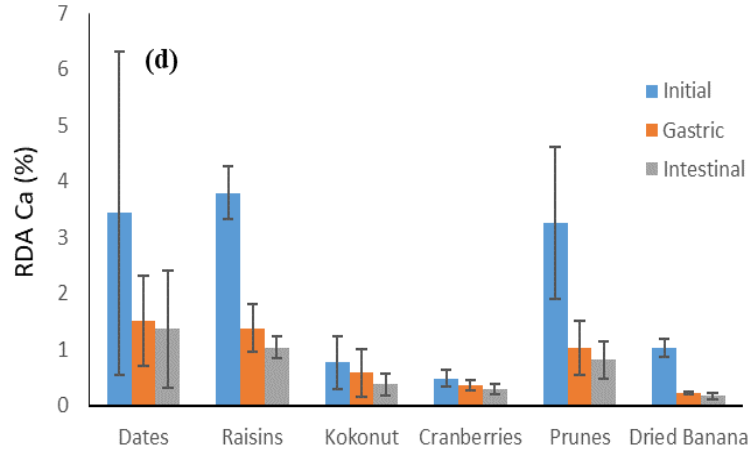
b) Contribution of dried fruits consumption to the Recommended Dietary Allowance (RDA) of essential elements

As discussed above, essential elements present in dried fruit samples are not completely released from the matrix during *in vitro* digestion, some of them presenting low bioaccessibility levels. Therefore, it is important when estimating dietary intakes to take into account not the total content of elements, but the content obtained after digestion. Regulation (EU) No. 1169/2011 established that a significant intake for minerals and micronutrients needs a value of at least 15% RDA supplied per single portion, while below 5% it is rather modest. The %RDA contributions from a daily serving of 50g of dried fruits at the end of G and GI digestion phase are presented in Figure 4.4. It can be seen from this figure that dried fruits could be considered important sources of essential elements such as K, Na, Mg, Ca, Mn, Fe, Cu and Zn, taking into consideration the total metals concentration. However, considering the bioaccessible fraction, the concentration values to the contribution are reduced. It was shown that, excepting cranberries, all analyzed dried fruits remain a great source of bioaccessible potassium following the GI digestion (RDA >15%). Consumption of 50g of raisins would provide approximately 63.6% of the amount of K required for good health, followed by prunes (31.33% RDA), dates (30.5% RDA), dried bananas (27.4 %RDA) and coconut (16.8 % RDA). Regarding sodium, the results reflected a higher contribution to the diet of prunes (RDA >15%), followed by dried bananas, coconut, dried cranberries, raisins and dates. Such values are quite promising and can prove special importance of dried fruits in human nutrition, since potassium is an essential element for human health.


(a)
RDA K (%)
120
100
80
60
40
20
0
Initial
Gastric
Intestinal
Dates
Raisins
Kokonut
Cranberries
Prunes
Dried Banana


(b)
RDA Na (%)
120
100
80
60
40
20
0
Initial
Gastric
Intestinal
Dates
Raisins
Kokonut
Cranberries
Prunes
Dried Banana


(c)
RDA Mg (%)
14
12
10
8
6
4
2
0
Initial
Gastric
Intestinal
Dates
Raisins
Kokonut
Cranberries
Prunes
Dried Banana


(d)
RDA Ca (%)
7
6
5
4
3
2
1
0
Initial
Gastric
Intestinal
Dates
Raisins
Kokonut
Cranberries
Prunes
Dried Banana

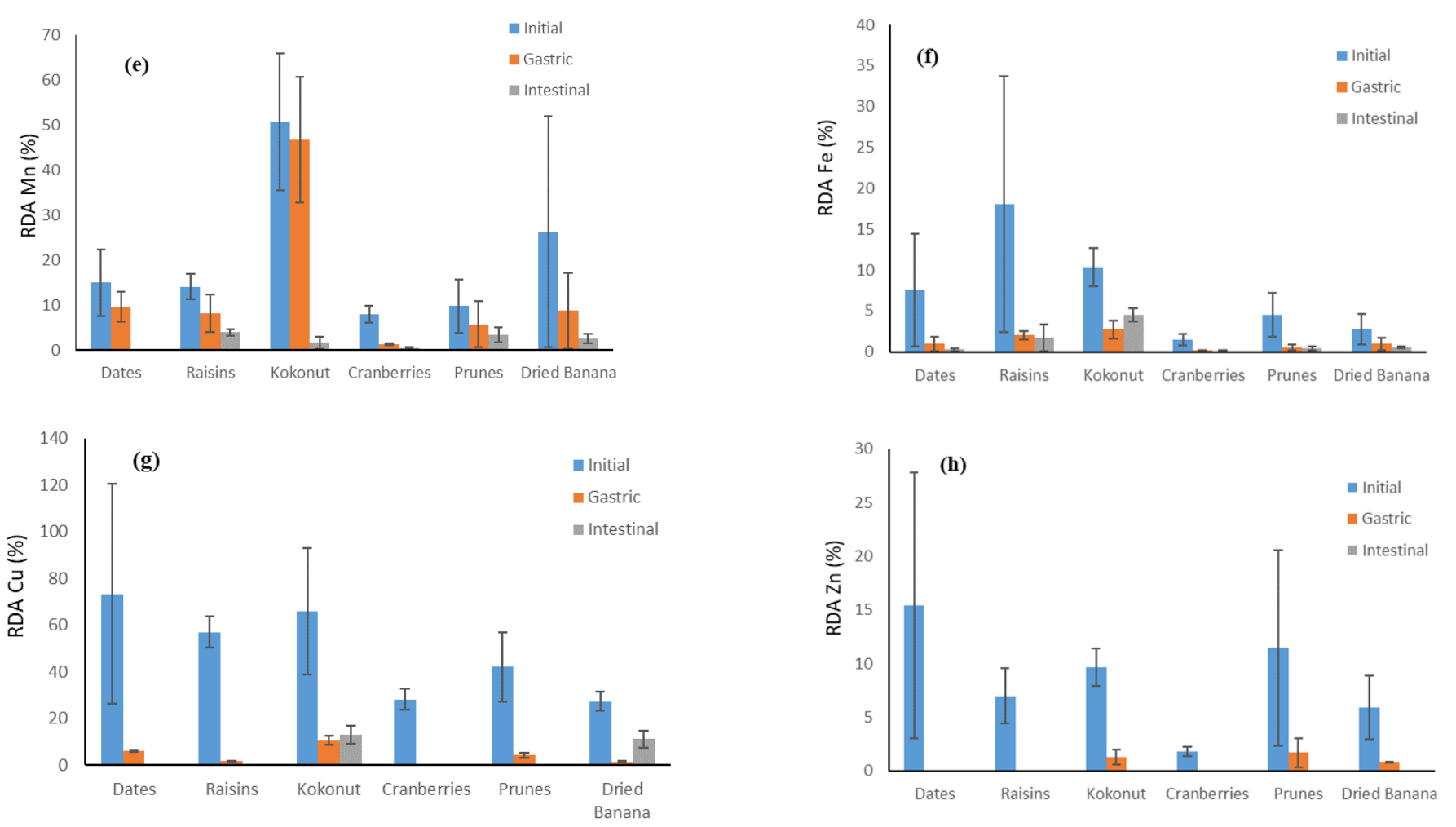


Figure 4.4 The % RDA contributions of K (a), Na (b), Mg (c), Ca (d), Mn (e), Fe (f), Cu (g), Zn (h) from a daily serving of 50g of dried fruits at different phases of *in vitro* digestion.

c) Bioaccessibility of total phenolics and total sugars

Many literature data offer information regarding the total content and composition of readily extractable food polyphenols, but few studies are reported regarding their bioaccessibility (Saura-Calixto et al., 2007). Figure 4.5 shows the total phenolic content found in non-digested dried fruit samples and after each step of digestion.

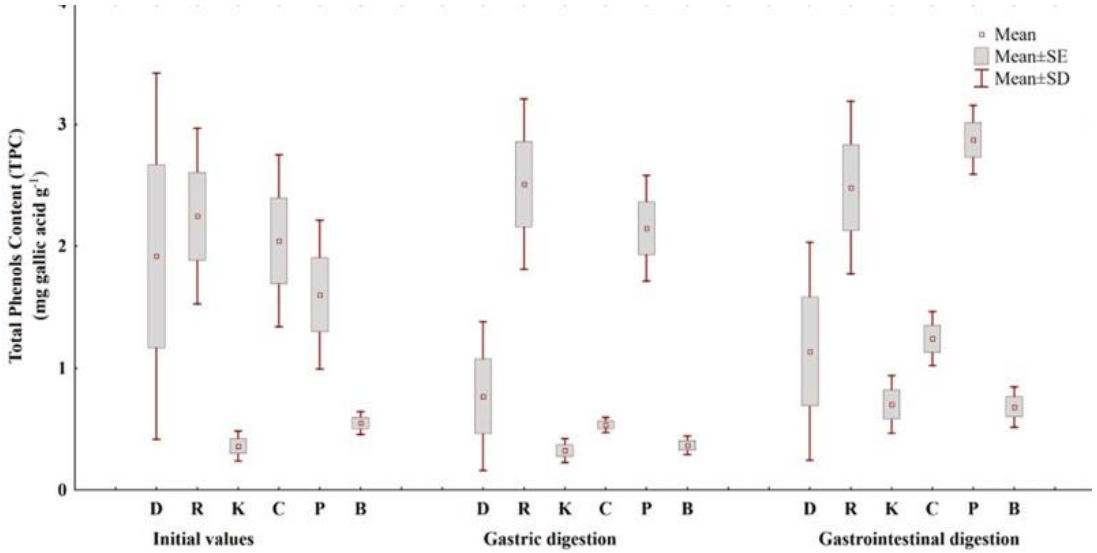


Figure 4.5 The concentration of total phenolic content in dried fruit samples before digestion and after gastric and gastrointestinal digestion phases.

After gastric digestion no statistically differences were revealed ($p > 0.05$, Table 4.7) with the exception of gastric digested cranberries and dried banana. In the case of cranberries and dates, only 27.5% and 42.3% of the total phenolics were released, these dried fruits presenting the lowest gastric bioaccessibility. Remarkable, after both gastric and intestinal digestion phases, TPC of all dried fruits increased compared with their gastric TPC content (Figure 4.6).

The highest GI bioaccessibility was observed in the case of prunes, coconut and dried banana, whereas dates and cranberries present a lower bioaccessibility level (Table 4.5). Although dates and cranberries were not extracted as well as the other fruits, their bioaccessibility was still maintained higher than 50%. Other authors reported high GI bioaccessibility levels (Kamiloglu et al., 2014). This fact could be attributed to a longer time of extraction comparative to gastric step. In addition, intestinal digestion is accomplished by the effect of intestinal digestive enzyme on the food matrix (Kamiloglu et al., 2014).

The changes in TSC during each step of GI digestion are shown in Figure 4.7. It was found that sugars are highly extracted after GI digestion and in smaller amounts in gastric phase. Half of the analyzed dried fruits show a gastric bioaccessibility lower than 50% (Table 4.5): prunes, dried bananas and cranberries. Coconut exhibits a slightly higher bioaccessibility (55.0%). This could be possible because in this phase of digestion sugars are bond with phenolic molecules (Coe et al., 2013). Variations of TSC are statistically significant (test t, $p < 0.05$, Table 4.7) for cranberries, prunes and bananas samples.

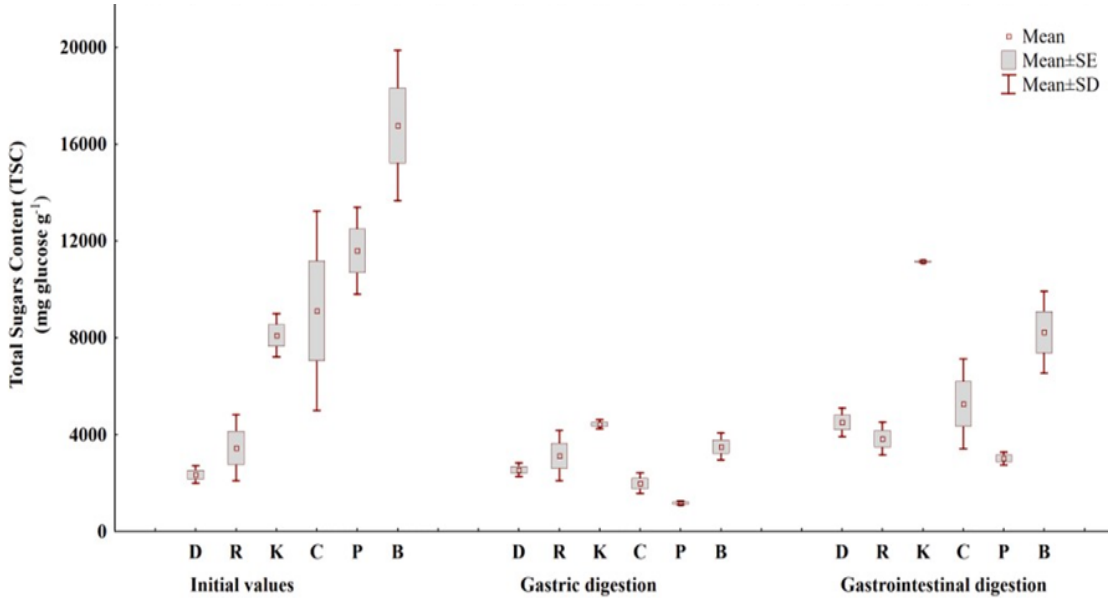


Figure 4.7 The concentration of total sugars content (TSC) in dried fruit samples before digestion and after gastric (G) and gastrointestinal (GI) digestion phases.

d) Changes in AC during in vitro digestion

The AC of samples was determined using three different assays, namely ABTS, DPPH and FRAP. The bioaccessibility of antioxidant compounds assessed by these methods is shown in Table 4.5.

AC_{ABTS} of all dried fruits increased significantly (t test, $p < 0.05$, Table 4.7) after *in vitro* GI digestion, indicating that antioxidants present in dried fruits have the ability to scavenge $ABTS^{\bullet+}$ free radical during digestion. Following GI digestion, AC_{ABTS} significantly increased in the case of all samples, especially in the case of coconut and raisins. Noteworthy, antioxidant compounds found in dried fruits exhibit lower bioaccessibility values as assessed by DPPH and FRAP methods compared to ABTS method (Figure 4.10).

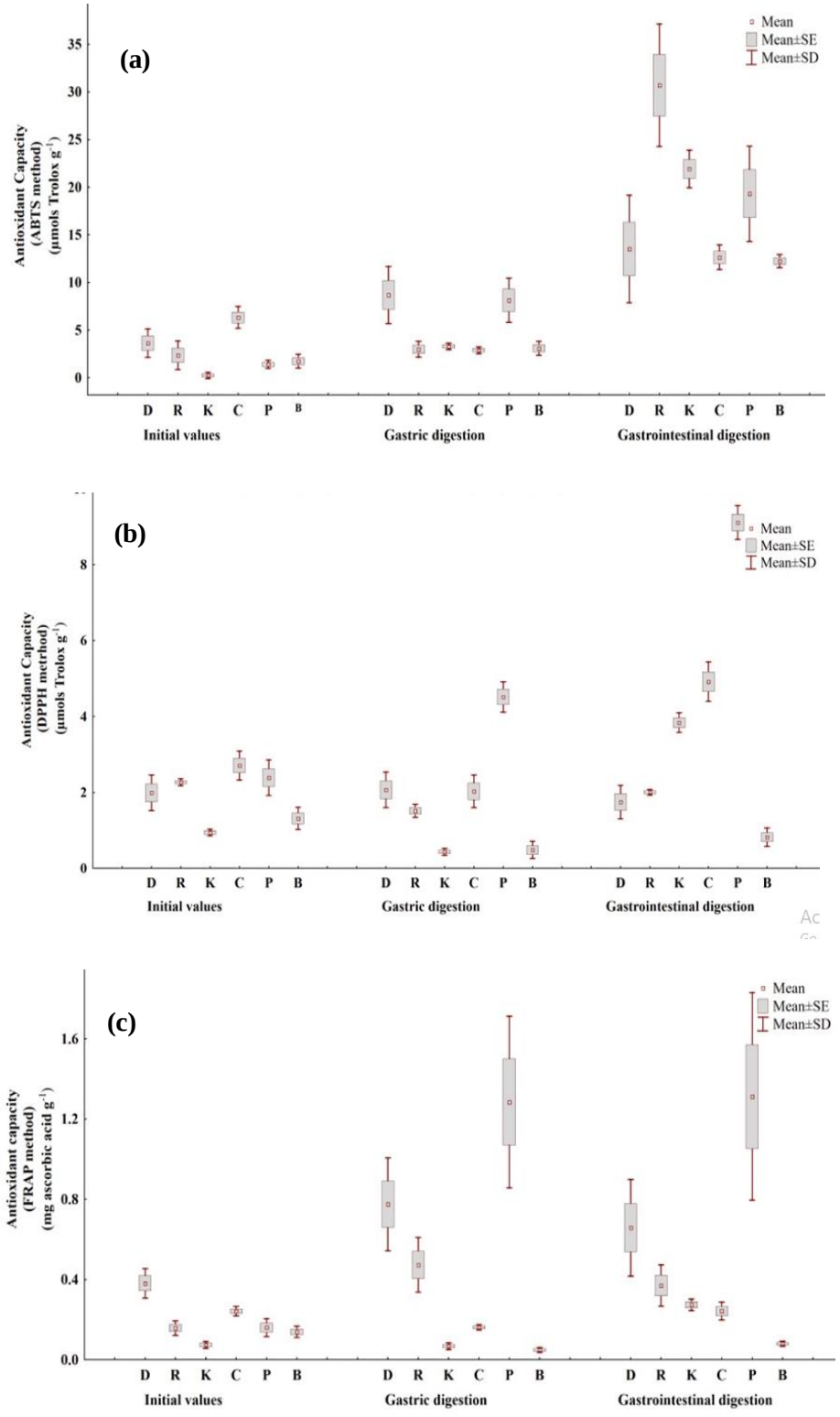


Figure 4.10 The AC tested by ABTS (a), DPPH (b) and FRAP (c) assays in dried fruit samples before digestion and after gastric (G) and gastrointestinal (GI) digestion phases.

4.3.4 Statistical evaluation of nutritional and functional properties of dried fruits

In order to accomplish dried fruits behavior during the digestion process, two-way joining cluster analysis and principal component analysis (PCA) methods were applied considering the minerals (as %RDA) and functional properties (AC_{DPPH}, AC_{ABTS}, AC_{FRAP}, TPC and TSC) parameters. Two-way joining cluster analysis may yield very useful results. The heat map graphs ordering cases and variables based on similarity patterns are presented in Figure 4.11. The PCA score plots graphs were used to reveal the classification of the samples associated with the retained PCs. In the PC1-PC2 model the determined parameters are responsible for the patterns seen among the samples. According to the PCs loadings, the variables that are closer to the center are not relevant for explaining the variation associated with this pair of PCs (Figure 4.12).

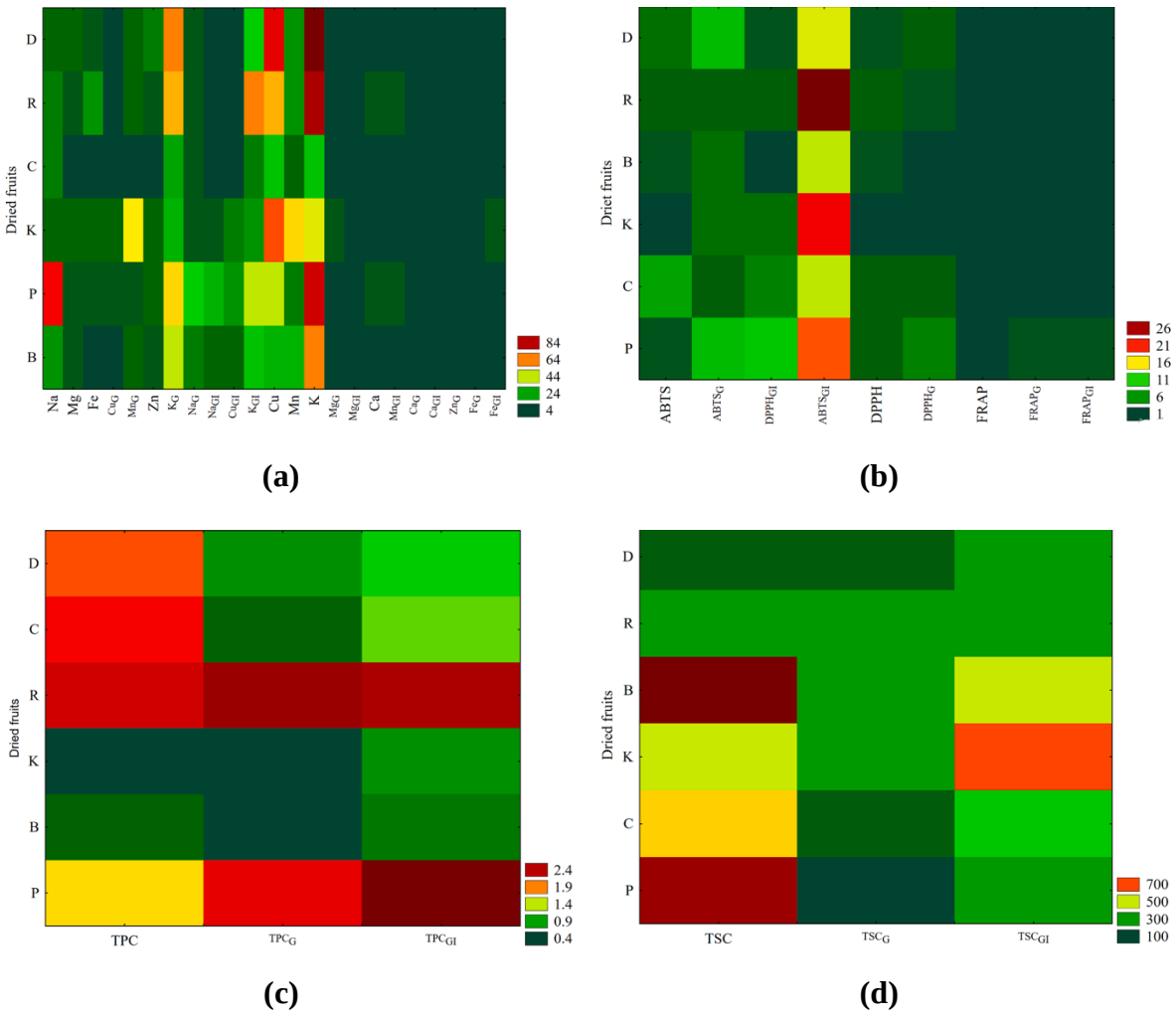


Figure 4.11 Two-way joining heat map revealing the variation of: (a) essential elements; (b) AC; (c) TPC and (d) TSC.

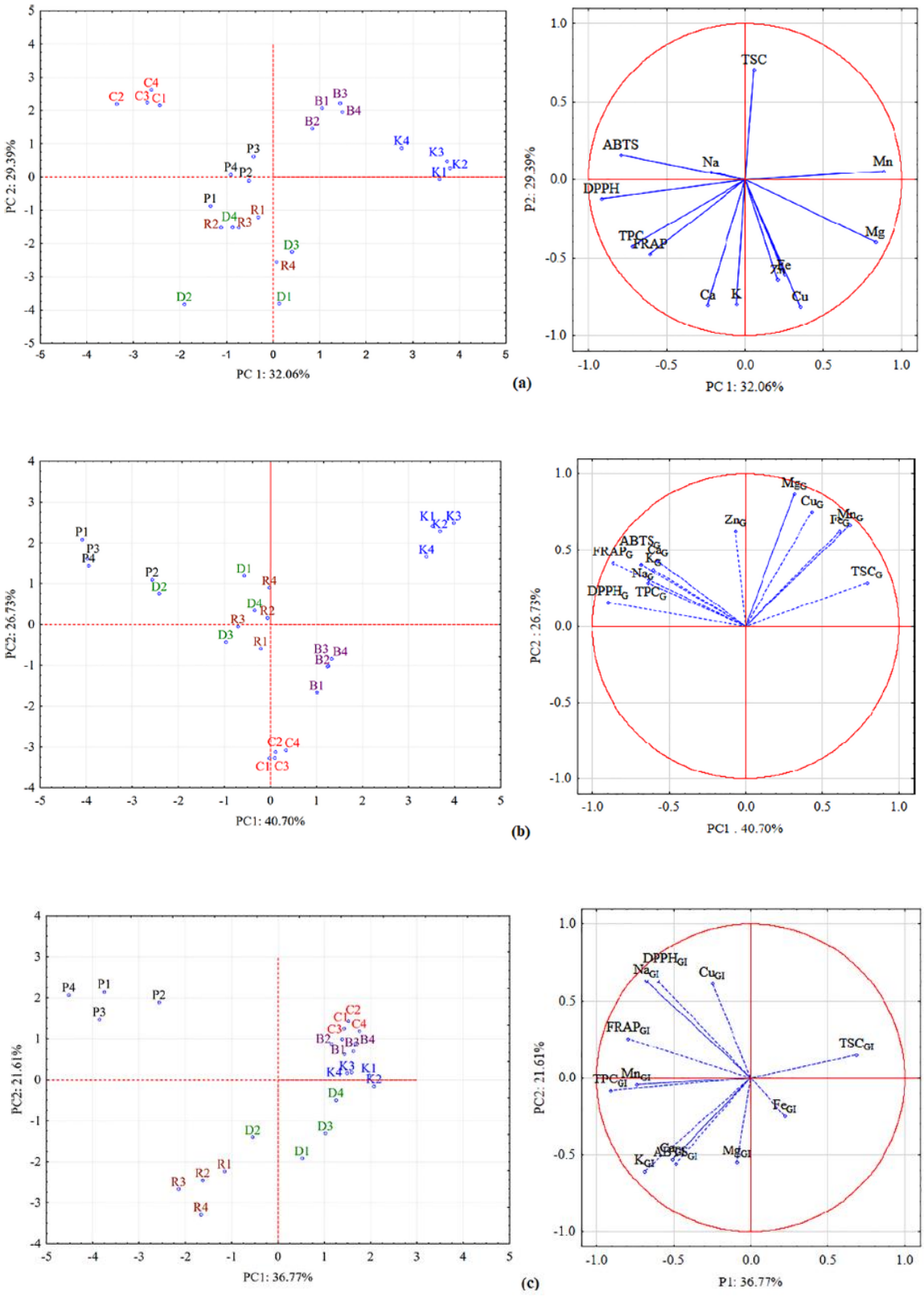


Figure 4.12 The PCA score plot of data from the analyzed dried fruit samples (four samples in each case) and the corresponding PCA loading plot of the first two PCs comparing the analyzed parameters: a) before digestion; b) after G digestion; c) after GI digestion.

In conclusion, following the GI digestion, changes are observed for almost all of the essential elements studied, resulting in a smaller bioaccessible fraction after GI digestion in comparison with the gastric and initial values. The studied dried fruits have been reported to be a good source of minerals such as Na, K, Mg, Fe, Mn and Cu, presenting moderate bioaccessibility levels for these elements, excepting Zn that has shown the lowest bioaccessibility. Non-carcinogenic risk of dried fruits was also revealed (THQ<1). Prunes, coconut, dried banana and raisins can be considered as important sources of phenolics, which show high bioaccessibility after digestion. Following *in vitro* simulation of digestion the AC increased in the case of the majority of dried fruits, making them as valuable in prevention of some diseases correlated with the oxidative stress. Results of this study have been accomplished by two-way joining cluster and PCA that showed the similarities/dis-similarities in pattern of analyzed parameters during simulated digestion. Although the results obtained following *in vitro* GI digestion cannot directly predict the human *in vivo* conditions, these results might be helpful in designing epidemiological studies or investigating the effect of food matrix on phytochemicals bioaccessibility.

5. Degradation kinetics of vitamin C, anthocyanins, phenolics and reducing sugars from different lingonberry jams during storage[4]

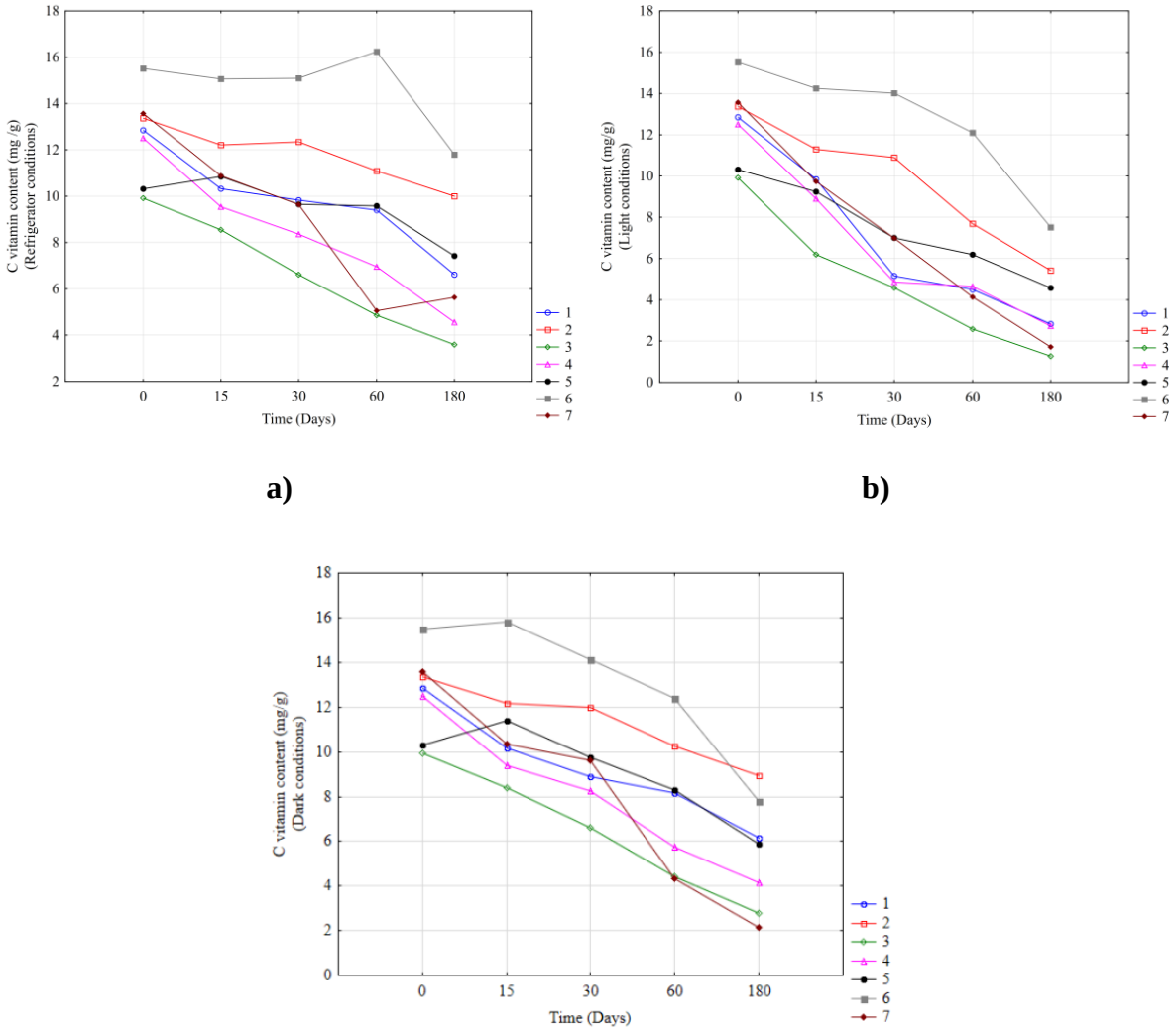
Lingonberry fruits *(Vaccinium vitis-idaea)* are considered as a valuable dietary source of phytochemicals but due to the fact that they are perishable, they are mostly used in the form of jams. This chapter comprises the changes in anthocyanins, vitamin C, total phenolics, total reducing sugars and antioxidant capacity of several lingonberry jams formulated with different sweeteners (sucrose, fructose, erythritol, brown sugar, coconut sugar, stevia, saccharine). Lingonberry jams were prepared in the laboratory, according to a traditional procedure. The recipe of jams did not use stabilizers or other preservatives. The jams were stored for 180 days at 4°C and 25°C (both under light and dark conditions). Vitamin C, TAC, TPC, AC and TSC were determined spectrophotometrically. The rate constants (k) and the half time values ($t_{1/2}$) of the degradation processes were determined. Degradation kinetics of vitamin C, TAC, TPC, AC and TSC was described by fitting the experimental data with different kinetic models. Also, the protective effects of some additives on the total phenolics content and antioxidant capacity are investigated here. The results provide useful information for understanding some phytochemicals degradation in real foods, contributing to the development of new food products.

5.3.1 Variations in phytochemicals content during different storage conditions of lingonberry jams

a) Vitamin C

Vitamin C is a phytochemical that accomplishes the nutritional properties of lingonberry fruits due to its health benefits (Zanini et al., 2018). Unfortunately, this vitamin is the most difficult to be preserved during storage because it is rapidly oxidized to dehydroascorbic acid (Sutwal et al., 2019). Taking into consideration the above mentioned, storing the jams under appropriate conditions become relevant to get the maximum benefit of the vitamin C. The behavior of vitamin C during 180 days of storage under different conditions is shown in Figure 5.1. It can be noted gradual decreases of vitamin C content that is also supported by statistical data.

[4] Parts of this chapter were published in Scrob et al., 2022c



a) **b)**

c)

Figure 5.1. Variation of vitamin C content in jams samples during 180 days in different conditions of storage: (a) refrigerator, (b) light and (c) dark conditions (1-sugar; 2-fructose, 3-erythritol; 4-brown sugar; 5-coconut sugar; 6-stevia; 7-saccharine).

b) Total anthocyanin content and individual anthocyanins

Among phytochemicals present in lingonberries, anthocyanins play an important role. Although are considered important quality indicators with potential antioxidant effects, they have the disadvantage of being sensitive to exposure to light, high temperatures, acids etc. (Hou et al., 2013). The degradation of these phytochemicals in lingonberries may be partly attributed to indirect oxidation reactions or polymerization that occurs during storage (Benedek et al., 2020). It was found that all jam samples present a significant decrease in TAC after only 15 days under light conditions (Table 5.2) This is

not surprisingly since the susceptibility of anthocyanins to light is well known (Hou et al., 2013). Even dark conditions of storage determined a significant decrease of TAC following 30 days of storage. In the case of jams stored under refrigeration, the decrease of TAC became significant only after 60 days of storage. This fact indicates again the importance of low temperatures of storage for smaller loss of phytochemicals in jams.

The change of TAC concentration in lingonberry jams during 180 days of storage under different conditions is shown in Figure 5.2. The highest loss of TAC, by approximately 90% it was observed in the case of coconut sugar based jam stored under dark.

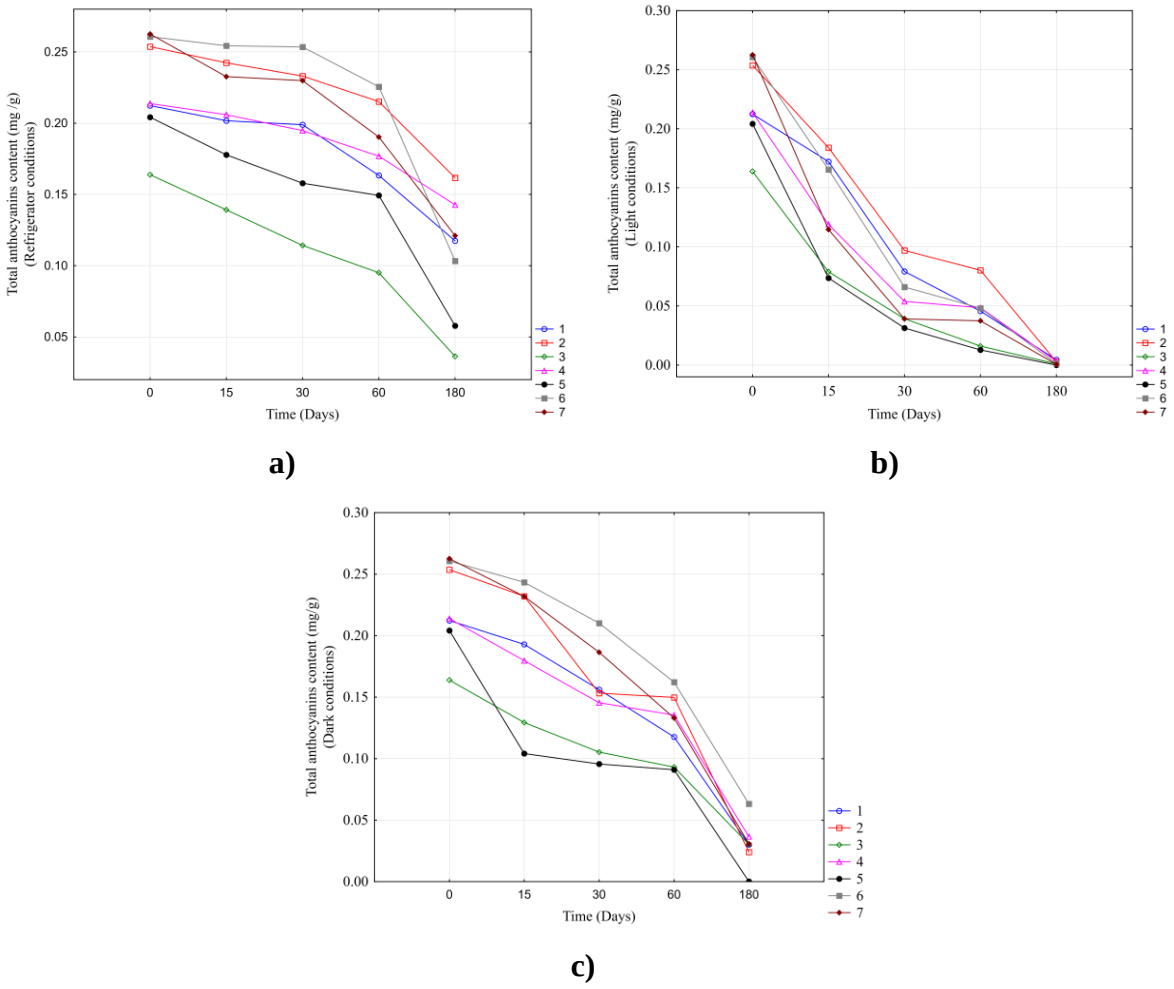


Figure 5.2. Variation of TAC in jams samples during 180 days in different conditions of storage: (a) refrigerator, (b) light and (c) dark conditions

As regarding the individual anthocyanins, three main anthocyanins were identified, namely: cyanidin 3-galactoside (Peak 1), cyanidin 3-glucoside (Peak 2) and cyanidin 3-arabinoside (Peak 3) (Figure 5.3).

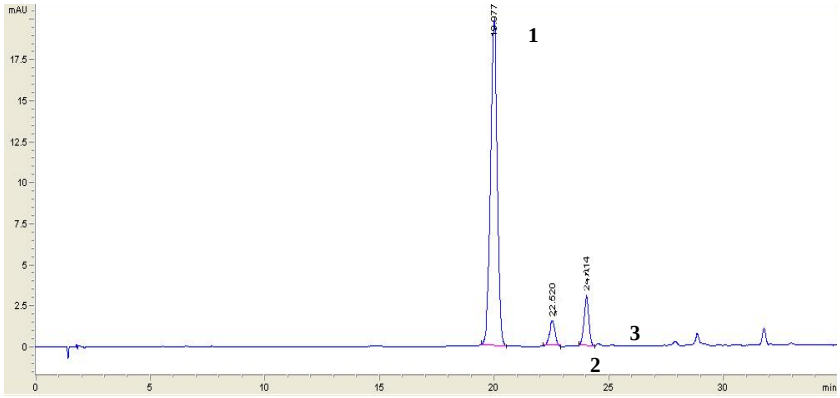
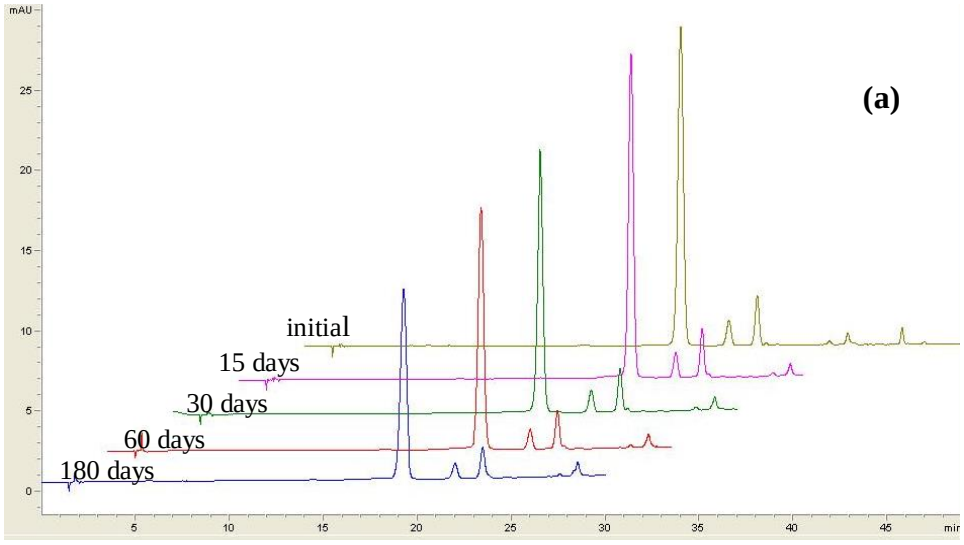
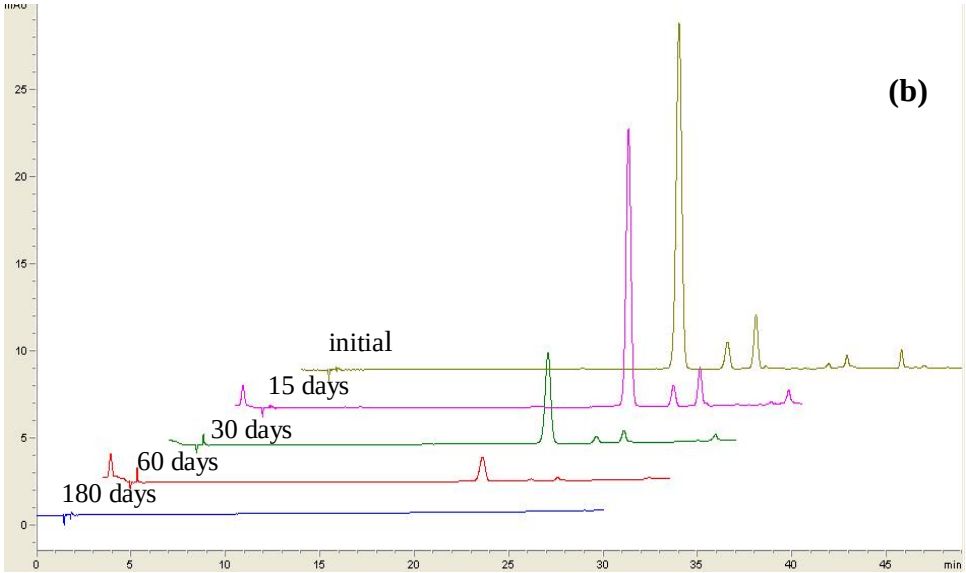


Figure 5.3 HPLC profile (532 nm) of anthocyanins in lingoberry jams: (1) cyanidin 3-galactoside; (2) cyanidin 3-glucoside; (3) cyaniding 3-arabinoside.

Individual anthocyanins followed a similar behavior with that of TAC. As it can be seen from Figures 5.4, each anthocyanin was clearly affected by storage conditions.





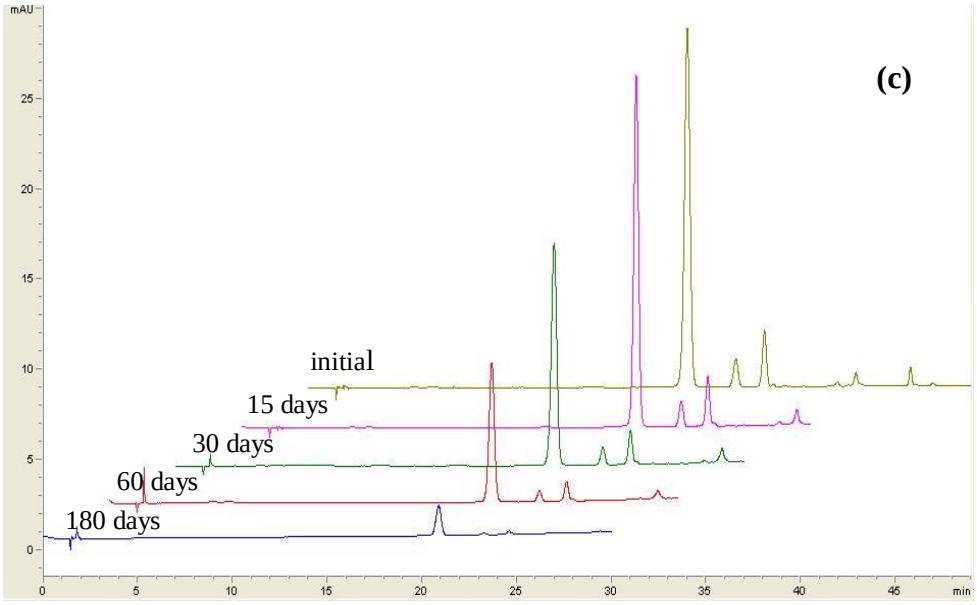


Figure 5.4. Changes in HPLC profile of individual anthocyanins from fructose based jam under different storage conditions: (a)-refrigeration; (b)-light; (c)-dark.

c) Total phenolic content (TPC)

It is well known that phenolics have an important role in human life quality. However, these phytochemicals may suffer different degradation reactions during processing or storage and, therefore, the quality of products may be decreased. In this sense, knowledge on the impact of processing and storage on these bioactive compounds should be very well documented (Brown et al., 2016).

d) Antioxidant Capacity (AC)

AC is directly correlated with degradation of anthocyanins, phenolics and other phytochemicals that possess antioxidant properties. A decreasing trend of AC was observed in the case of all jams, regardless of storage conditions (Figure 5.6). Light stored jams show the most pronounced decline in AC during the storage. No significant decreases in AC of lingonberry jams (Table 5.2), are noted in the first 60 days of storage under refrigeration but significant losses in AC are observed when storage at 4°C is extended to 180 days. This fact shows that storage time is a factor that negatively affects the stability of antioxidant compounds. As in the case of TAC, fructose showed a protective effect on AC during storage, presenting a loss of only 30.5% after 180 days. These results may suggest that AC of lingonberry jams could be attributed in a higher part to anthocyanins content. Brown sugar negatively affects the antioxidants profile, but, according to our knowledge, no literature studies have been previously reported regarding the influence of brown sugar upon antioxidants behavior.

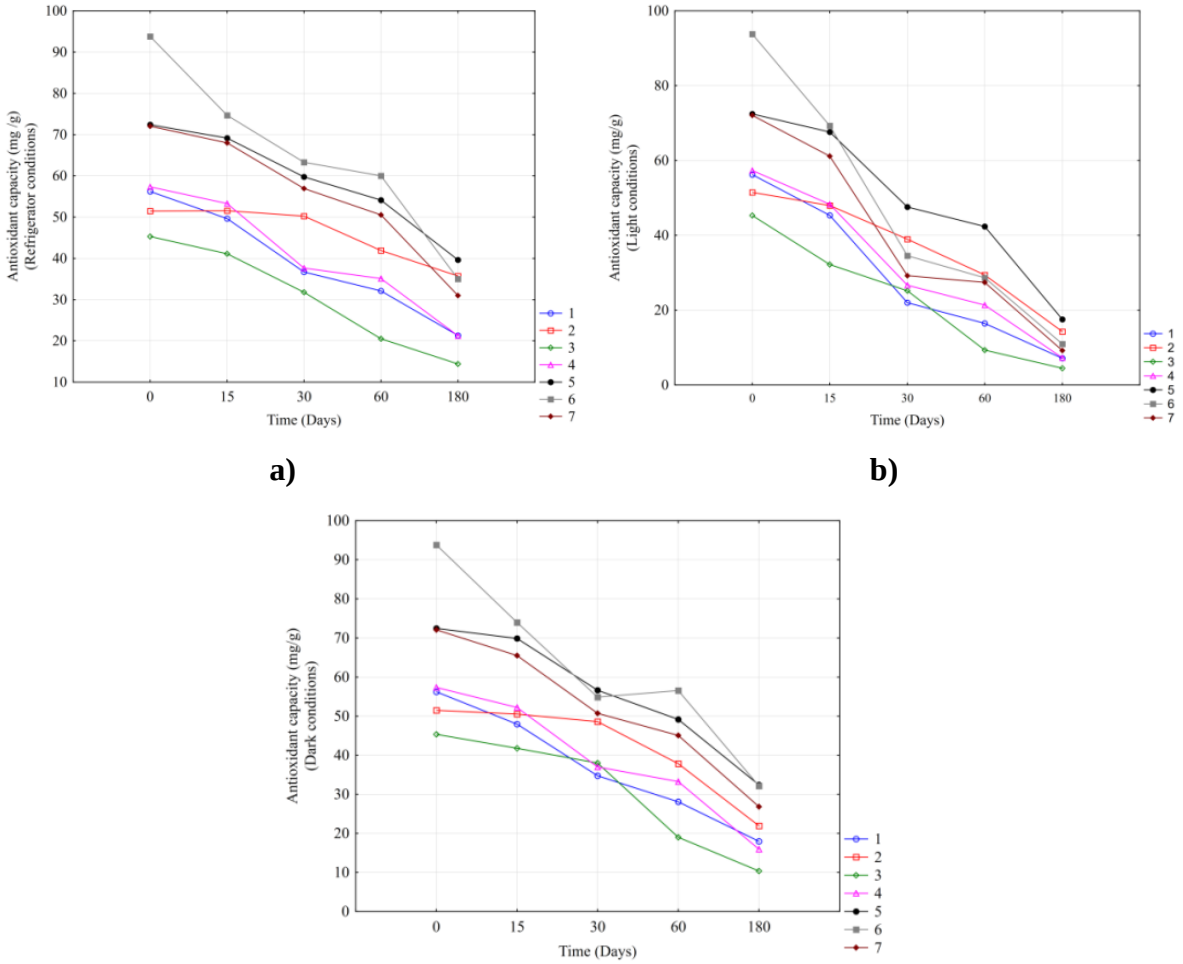


Figure 5.6. Changes in AC of jams samples during 180 days in different conditions of storage: (a) refrigerator, (b) light and (c) dark conditions (1-sugar; 2-fructose, 3-erythritol; 4-brown sugar; 5-coconut sugar; 6-stevia; 7-saccharine).

e) Reducing sugars

The behavior of sugars during storage may be important predictor in processed foods quality, since these phytochemicals act as natural food preservatives and influence the flavor of the food products (Sutwal et al., 2019). Thus, monitoring reducing sugars is an important step for food industry. The concentration of reducing sugars before storage ranged between 95.1mg/g in the case of erythritol based jam to 415mg/g in the case of fructose based jam. During storage, the reducing sugars content increased in the all cases (Figure 5.7).

5.3.2 Kinetic analysis

For vitamin C, a second-order kinetic model was found to best describe the degradation mechanism of this phytochemical from lingonberry jams (R^2 =0.91-0.99). The

kinetic parameters determined for vitamin C degradation during storage are showed in Table 5.3.

Table 5.3. Kinetic parameters for the degradation process of Vitamin C and TAC from lingonberry jams during different periods of storage under refrigerator (a), light (b) and dark (c) conditions respectively. Rate constants are expressed as $k \cdot 10^{-3}$ (day^{-1}) and half-life values ($t_{1/2}$) are expressed in days.

Sample	Vitamin C						TAC					
	A		b		c		a		b		C	
	K	$t_{1/2}$	k	$t_{1/2}$	k	$t_{1/2}$	k	$t_{1/2}$	k	$t_{1/2}$	k	$t_{1/2}$
Jam 1	0.43	180	1.68	46.3	0.52	150	3.37	206	22.1	31.4	10.7	64.8
Jam 2	0.16	467	0.64	117	0.23	325	2.54	273	24.7	28.0	12.7	54.5
Jam 3	1.08	93.4	3.93	25.7	1.49	67.7	8.55	81.1	30.0	23.1	9.55	72.6
Jam 4	0.82	97.5	1.72	46.5	0.98	81.6	2.35	295	24.1	28.8	9.67	71.7
Jam 5	0.20	485	0.74	131	0.39	249	6.89	101	46.7	14.8	35.8	19.4
Jam 6	0.11	586	0.37	174	0.34	190	4.75	146	29.9	23.2	7.83	88.5
Jam 7	0.59	124	2.81	26.2	2.21	33.3	4.43	156	34.5	20.1	11.8	58.6

Anthocyanins from lingonberry jams follow a first-order kinetic model under the three different storage conditions (R^2=0.97-0.99). Other authors have been reported a similar kinetic model for these phytochemicals (Benedek et al., 2020; Moldovan et al., 2020; Hou et al., 2013). TAC highest stability was found in jams sweetened with fructose, brown sugar and sucrose, respectively, during storage under refrigeration.

As regarding TPC, a second-order model best suits the experimental data (R^2=0.89-0.99). It was reported that phytochemicals such as phenolics do not follow a specific reaction order (Benedek et al., 2020). Total phenolics are most affected by temperature and exposure to light, according to kinetic parameters.

Changes in AC are best fitted to a second-order kinetic model (R^2=0.93-0.99). Fructose, coconut sugar and stevia determine the slowest degradation of antioxidant compounds during 180 days of storage under refrigeration. On the opposite side, erythritol, sucrose and brown sugar accelerate the degradation process. The stability of antioxidants in

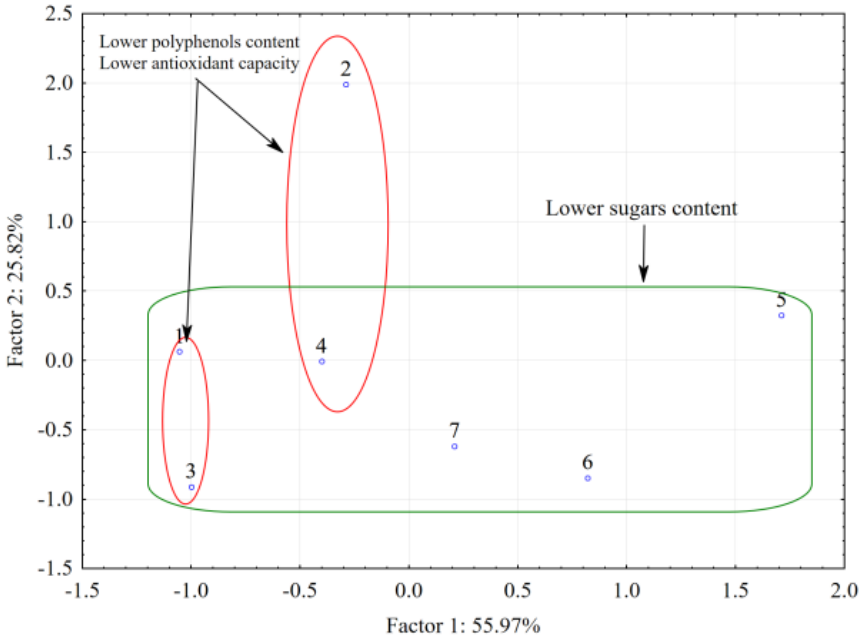
the presence of fructose and coconut sugar is 5.60-fold and 4.00-fold, respectively, higher than that observed by storage in the presence of erythritol.

As regarding reducing sugars, these phytochemicals were not supposed to degrade, but to accumulate. A zero-order kinetic model best described the accumulation of reducing sugars during storage (R^2=0.85-0.99). These results are in agreement with the results reported by other authors (Mane et al., 2011).

5.3.3 Statistical analysis

Factor analysis (FA) method was applied to identify specific patterns in degradation rate of the determined parameters (Vitamin C, TAC, TPC, AC and reducing sugars) during 180 days of storage. The FA performed on the principal components (PCs) shows that the studied jam samples are described about 92.15% - 93.19% by the first three factors (Table 5.6) with a strong contribution (loadings > 0.700), namely TPC, AC and reducing sugars. Factor 1 and Factor 2 describe between 70.92% - 81.79% of samples characteristics and were associated with the same parameters (TPC, AC and reducing sugars). On the other hand, Factor 3 accounts between 11.41% - 25.99% from characteristics and revealed a high variation of vitamin C content under light condition. A strong contribution of TAC variation was associated with the last factor.

It was found that the addition of sucrose and erythritol have a similar negative effect on TPC and AC under refrigeration (Figure 5.9).



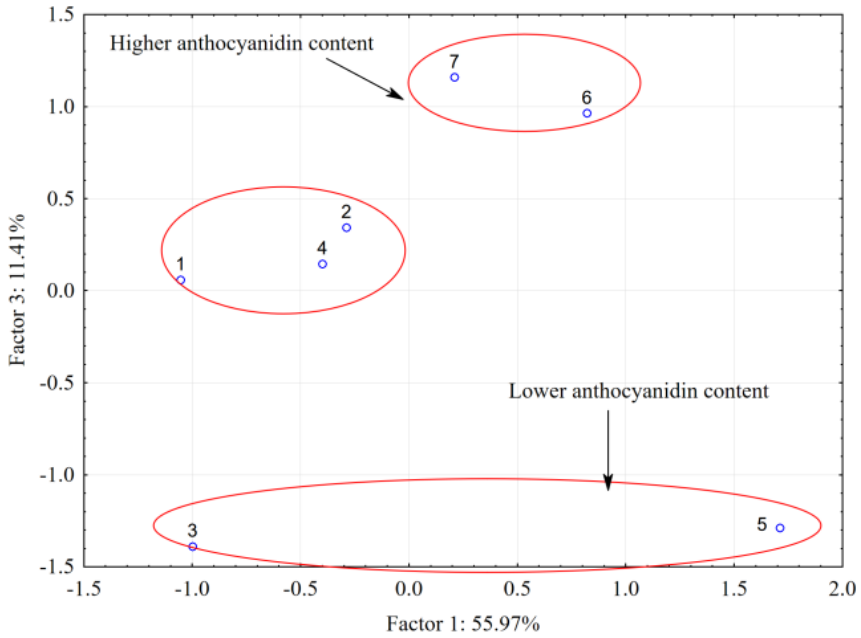


Figure 5.9. Classification of the jam samples based on the variation of the analyzed parameters during storage under refrigeration.

Fructose and brown sugar addition also show a similar effect in decreasing the TPC and AC of jams during storage. The addition of coconut sugar and stevia presents a contrary effect under refrigeration. It can be also seen from Figure 5.9 that stevia addition has a positive effect on TAC degradation and vitamin C.

The pattern observed in the case of jams stored under refrigeration is also observed under light conditions, where degradation of these phytochemicals was accelerated (Figure 5.10). Erithrytol and coconut sugar based jams are associated with lower vitamin C content during storage under light. Stevia based jam presents a different behavior regarding the other samples, indicating that this sweetener may affect the degradation process following a distinct mechanism.

The factors describing the degradation rate parameters were also plotted (Figure 5.12). From these results, it can be observed that the addition of sweeteners such as stevia and fructose decrease the degradation of antioxidant compounds, vitamin C and TAC. Since stevia and fructose are non-synthetic sweeteners that possess beneficial health properties (Heacock et al., 2002), results that their incorporation in food products such as jams is beneficial in providing beverages with a high nutritional value. On the opposite side, erythritol accelerates the degradation of phytochemicals in lingonberry jams. In addition, degradation rate of this jam is not associated with the other jams rate. Saccharine

and coconut sugar are associated regarding their effect upon degradation rate of phytochemicals. Since these two sweeteners have similar effects, it is important to note that saccharine is a synthetic sweetener with additional taste, whereas coconut sugar is a natural sweetener that might be preferred in jam's formulation instead of saccharine.

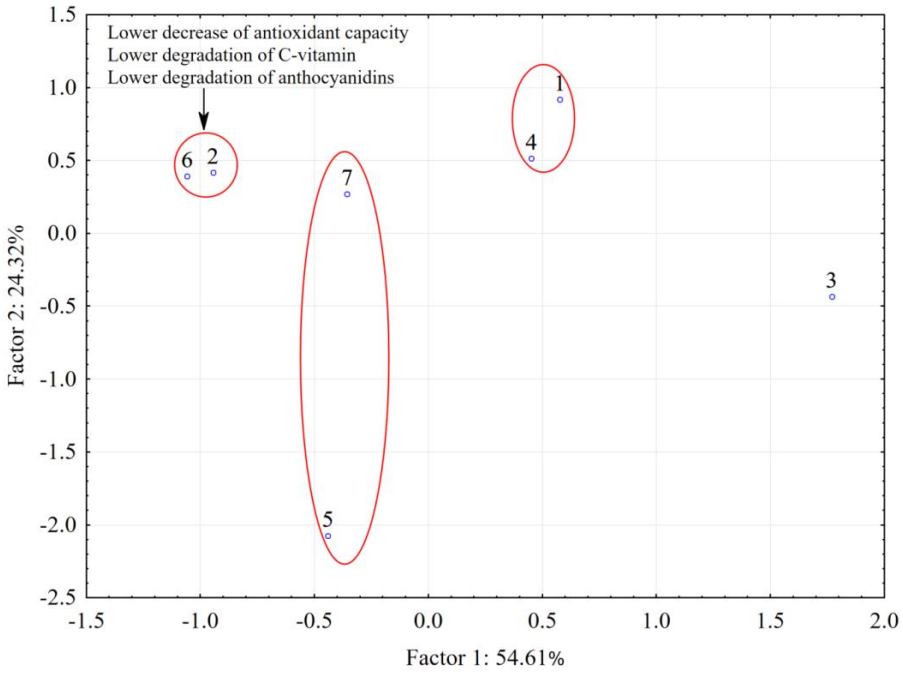


Figure 5.12. Classification of the jam samples based on the degradation rate parameters during storage: 1-sugar; 2-fructose, 3-erythritol; 4-brown sugar; 5-coconut sugar; 6-stevia; 7-saccharine.

In conclusion, for vitamin C, a second-order kinetic model was found to describe the degradation mechanism of this phytochemical from lingonberry jams, whereas TAC and individual anthocyanins degradation follow a first-order kinetic model. TPC and antioxidants both are described by a second-order kinetic model. The accumulation of reducing sugars was lower in the presence of erythritol, stevia and saccharin, indicating that these sweeteners best suit for formulation of jams for patients with diabetes. Erythritol determined the most pronounced degradation of vitamin C and anthocyanins at both investigated temperatures. Fructose proved to produce the slowest degradation rate of antioxidants. Preservation of phytochemicals during storage is of commercial importance and information about the effect of sweeteners on the preservation of jams are necessary before manufacturing these products at industrial scale.

6. Effect of sweeteners on physicochemical properties and bioaccessibility of some phytochemicals from lingonberry jams[5]

Sucrose is the main sugar used in jams preparation, but its excessive consumption is associated to several diseases and replacing white sugar with other sweeteners play an important role, especially for food industry development. This chapter presents the influence of seven different sweeteners (sucrose, fructose, erythritol, brown sugar, coconut sugar, stevia and saccharine) on titratable acidity (TA) and total soluble solids (TSS), as well as on the bioaccessibility of vitamin C, anthocyanins and the antioxidant capacity (AC) of lingonberry jams under *in vitro* gastrointestinal digestion. In addition, synthetic sweeteners may influence the release of target phytochemicals from solid matrix and, in this way, their bioaccessibility.

6.3.1 Titratable acidity (TA) of jams samples

The TA values of jams samples obtained during 60 days of storage are presented in Table 6.1. Jams' acidity increases in the case of all samples regardless of temperature conditions. Evaluating this parameter is an important predictor in the quality of fruit based products because acidity protects against the development of microorganisms.

6.3.2 Total soluble solids (TSS) of jams samples

TSS represents an important predictor for the quality of a jam. In addition, here TSS represents the index of sweetness being express as °Brix. The values of the jams samples determined during 60 days are presented in Table 6.1. Prior storage, TSS values of lingonberry jams were: 56.2°Brix (Jam 1), 56.7°Brix (Jam 5), 56.8°Brix (Jam 2 and 3), 57°Brix (Jam 4), while in the case of Jams 6 and 7, Brix values were much lower (22 and 21.7°Brix, respectively). It can be noticed from Table 6.1 that TSS did not significantly change following 60 days of storage under refrigeration. Stevia based jam presents the highest increase of TSS (3.63%), whereas fructose based jam presented an opposite effect (0.18%). In another study (Muhammad et al., 2008), an increase in TSS of apple jams during storage was noticed. The jams constituents might be solubilized during storage and this fact may increase the TSS (Muhammad et al., 2008).

[5] Published in Scrob et al. 2021 and in Scrob et al. 2022b

Table 6.1 The TA and TSS values of jams samples obtained during storage.

Time (days)	Titratable acidity (%)			Total soluble solids (°Bx)		
	refrigerated	dark	light	refrigerated	dark	Light
Jam 1						
0	0.161	0.161	0.161	56.2	56.2	56.2
15	0.168	0.168	0.175	56.3	56.4	56.6
30	0.189	0.189	0.192	56.4	56.8	56.8
60	0.192	0.195	0.203	56.5	56.9	56.9
Jam 2						
0	0.182	0.182	0.182	56.8	56.8	56.8
15	0.196	0.210	0.224	56.8	56.8	56.9
30	0.245	0.248	0.315	56.8	56.8	57.0
60	0.273	0.280	0.315	56.9	56.9	57.4
Jam 3						
0	0.168	0.168	0.168	56.9	56.9	56.9
15	0.175	0.175	0.182	43.4	44.3	45.2
30	0.182	0.182	0.196	43.7	44.6	44.8
60	0.203	0.189	0.217	44.0	44.1	44.2
Jam 4						
0	0.161	0.161	0.161	57.0	57.0	57.0
15	0.175	0.182	0.280	57.1	57.1	57.3
30	0.189	0.199	0.343	57.2	57.2	57.6
60	0.196	0.210	0.343	57.3	57.3	57.7
Jam 5						
0	0.175	0.175	0.175	56.7	56.7	56.7
15	0.210	0.217	0.315	56.8	56.8	56.8
30	0.224	0.238	0.332	56.9	56.9	56.9
60	0.252	0.273	0.364	56.9	57.0	57.2
Jam 6						
0	0.210	0.210	0.210	22.0	22.0	22.0
15	0.280	0.311	0.343	23.2	23.4	23.4
30	0.287	0.318	0.350	22.8	23.0	22.9
60	0.301	0.343	0.385	22.9	22.9	23.0
Jam 7						
0	0.210	0.210	0.210	21.7	21.7	21.7
15	0.273	0.273	0.315	21.7	21.7	22.0
30	0.294	0.308	0.322	21.9	21.9	22.0
60	0.301	0.308	0.336	22.1	22.0	22.0

6.3.3 Sensorial properties of jams samples

Sensorial properties such as color, taste, texture, spreadability and overall acceptability have been evaluated in this study and compared to the control jam prepared with sucrose. The sensory evaluation indicates that the greater part of jams are acceptable to the consumers after 180 days under refrigeration (Table 6.2). Coconut sugar based jam recordes the best sensory evaluations, except for the color. Color is an important sensory attribute that improve the quality of a jam and this jam had a dark brown color that was not appreciated by the panelists. Stevia and saccharine based jams recorded the highest values for color acceptability.

Table 6.2. Sensorial parameters of jams.

Jam / **Parameter**	1	2	3	4	5	6	7
Color	8.60	8.00	5.00	8.60	6.20	8.80	8.80
Taste	7.80	7.00	4.20	7.20	8.80	8.00	6.80
Texture	8.80	7.40	4.20	8.40	8.80	8.00	8.00
Spreadability	8.80	6.40	4.00	8.60	8.80	8.40	8.20
Overall Acceptability	8.20	7.60	3.80	8.20	8.80	8.40	8.00

6.3.4 Bioaccessibility studies

a) Vitamin C

Before digestion, vitamin C concentration in lingonberry jams decreased in the following order: Jam 6 > Jam 7 > Jam 2 >Jam 1 > Jam 4 > Jam 5 > Jam3 (Table 6.3). Stevia based jam presents the highest vitamin C concentration, whereas erythritol based jam is the jam with lowest vitamin C content.

The content of vitamin C varies during digestion in the case of all prepared jams and its bioaccessibility at the end of gastrointestinal digestion ranges from 42.8% in the case of coconut sugar based jam to 62.9% in the case of stevia based jam (Figure 6.2). The protective effect of stevia components on the degradation of ascorbic acid might be correlated with the high bioaccessibility of vitamin C (Kroyer, 2010). No literature studies have been reported regarding the low bioaccessibility of vitamin C in the presence of coconut sugar.

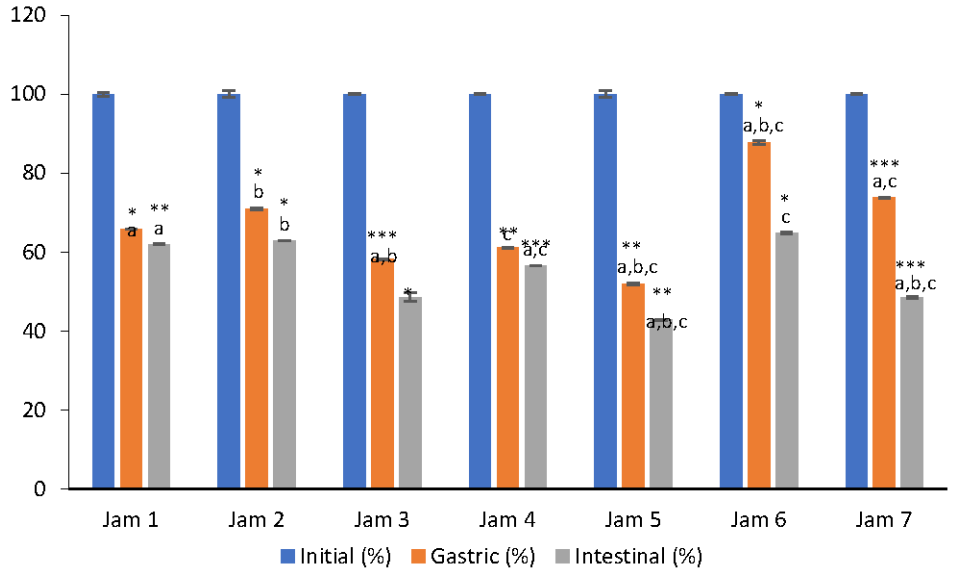


Figure 6.2 Changes in bioaccessibility (%) of vitamin C during *in vitro* digestion of lingonberry jams. Data represent average values ± standard deviation of three independent measurements and initial bioaccessibility determined in non-digested jams is set as 100%. Letters a, b, c represent statistically significant differences between the values for each phase of digestion ($p < 0.05$) according to Student's *t* tests. Asterisk symbols signify the following levels of statistical significance of differences according to one-way ANOVA and Student's *t* tests: *** $p < 0.0001$, ** $p < 0.001$, * $p < 0.05$.

b) TAC and individual anthocyanins

Before digestion, the jams with highest TAC are those prepared with saccharine and stevia, whereas erythritol based jam is the jam with lowest TAC. As in the case of vitamin C, TAC is also affected by *in vitro* digestion (Figure 6.3). Since anthocyanins are generally instable compounds and their stability can be influenced by various factors (David et al., 2019), their degradation during digestion is expected.

Anthocyanins are stable under gastric conditions since most of the jam samples had the anthocyanins bioaccessibility higher than 50%. Other authors (Han et al., 2019) reported a similar behavior of anthocyanins under acidic gastric environment, finding bioaccessibilities equal to 75–88%. The high stability of anthocyanins could be due to the low pH value in the stomach (David et al., 2019). High gastric bioaccessibility is desired due to the fact that it was found that anthocyanins may be also absorbed through gastric cells (Han et al., 2019) and exert their health benefits without being intestinally absorbed. Among sweeteners used in this study, fructose shows the highest protection upon TAC degradation.

Cyanidin-3-galactoside was found to be the most abundant anthocyanin (84.61% from total anthocyanin content). Studying the bioaccessibility of cyanidin-3-galactoside might be important taking into consideration that this phytochemical is one of the most widespread anthocyanins with positive impact on human health.

Regarding cyanidin-3-arabinoside, the highest bioaccessibility is found in fructose based jam (56.4%), followed by sucrose based jam (41.8%) and erythritol based jam (25.9%). Saccharine determines the lowest bioaccessibility of cyanidin-3-arabinoside at the end of digestion (8.3%).

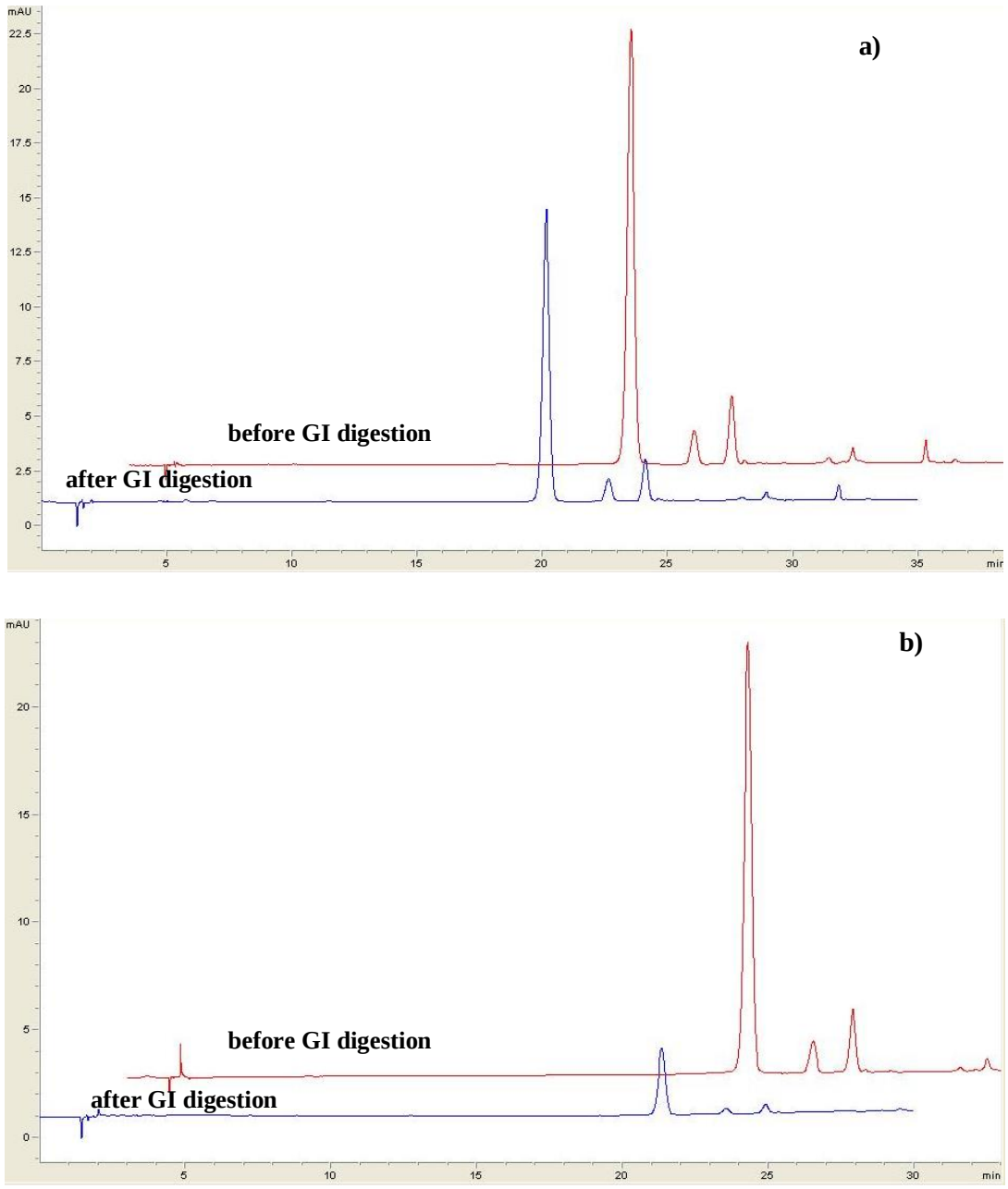


Figure 6.5. HPLC profiles (532 nm) of anthocyanins from fructose (a) and coconut sugar (b) lingonberry jam before and after *in vitro* GI digestion

c) Antioxidant capacity (AC)

It is well known that lingonberries possess antioxidant properties (Dróżdż et al., 2018), so it is expected that the jams also mantain this capacity ABTS and FRAP assays were used to assess the AC of jams during digestion. Before digestion, the AC_{ABTS} of lingonberry jam extracts ranges from 66.6 µmol Trolox/g of jam to 109 µmol Trolox/g of jam, while AC_{FRAP} varies from 4.64 mg vit C/g of jam to 9.37 mg vit C/g of jam (Table 6.5). Stevia based jam presents the highest AC regardless the used methods, whereas erythritol based jam presents a contrary behavior.

Table 6.5. AC of lingonberry jams before digestion

Lingonberry Jam	**AC_{ABTS} (µmols Trolox/g Jam)**	**AC_{FRAP} (mg Vitamin C/g Jam)**
Jam 1 (white sugar)	56.2 ± 2.0 ^{a}	5.39 ± 0.10 ^{a}
Jam 2 (fructose)	51.4 ± 0.4 ^{b}	5.64 ± 0.08 ^{b}
Jam 3 (erythritol)	45.3 ± 2.7 ^{a}	4.64 ± 0.02 ^{a,b,c}
Jam 4 (brown sugar)	57.3 ± 6.6 ^{c}	5.36 ± 0.06 ^{c}
Jam 5 (coconut sugar)	72.4 ± 1.3 ^{a,b}	7.82 ± 0.10 ^{a,b,c}
Jam 6 (stevia)	93.8 ± 1.1 ^{a,b,c}	9.37 ± 0.23 ^{a,b,c}
Jam 7 (saccharine)	72.1 ± 0.2 ^{a,b}	8.85 ± 0.68 ^{a,b,c}

Values represent mean ± SD (n = 3). Superscripts (a, b, c, d) indicate statistically significant differences ($p < 0.05$) according to Student's t tests.

Following G digestion, the ACABTS significantly decreases ($p < 0.05$) in the case of all jams (Figure 6.6a). Stevia shows a negative effect upon antioxidants that interact with ABTS, presenting a bioaccessibility level of 32.8%. Fructose instead presents a protective effect upon this type of antioxidants (59.9%). The ACABTS significantly increases ($p < 0.05$) from the gastric to the intestinal step, especially in the case of stevia based jam.

Regarding AC_{FRAP}, the bioaccessibilities of antioxidant compounds assayed by this method are significantly reduced following gastric digestion and the highest decrease can be observed in the case of stevia based jam (47.3%). In comparison with gastric AC_{ABTS}, fructose based jam has a higher bioaccessibility at gastric level when tested by FRAP method (90.8%) (Figure 6.6b). If in the case of AC_{ABTS} after GI digestion no statistically significant differences were observed between jams ($p > 0.05$), the bioaccessibilities of antioxidants assessed by FRAP method reveal a distinct behavior.

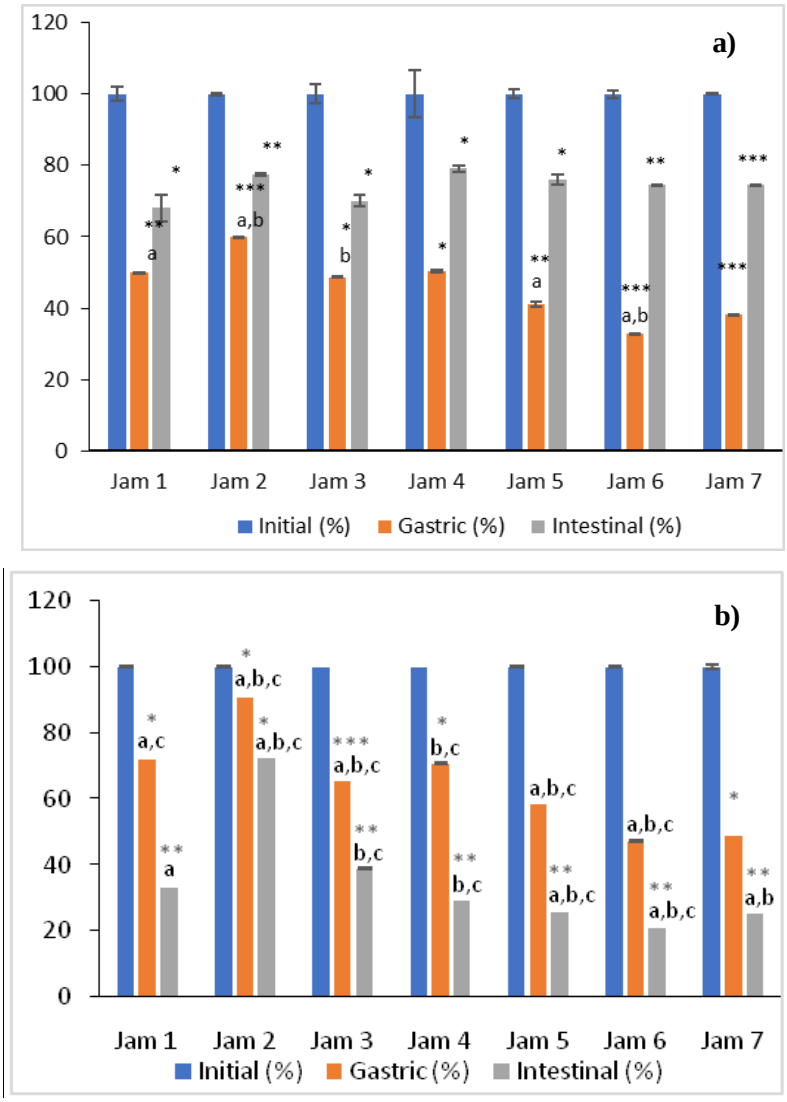


Figure 6.6. Changes in bioaccessibility (%) of antioxidants assessed by AC_{ABTS} (a) and AC_{FRAP} (b) during *in vitro* digestion of lingonberry jams.

In conclusion, the use of several sweeteners in of lingonberry jams formulation determined the obtaining of products similar to conventional jam with sucrose, excepting jam prepared with erythritol. Coconut sugar led to the best scores for lingonberry jams, mainly due to its distinctive flavor. The vitamin C was not highly affected in the gastric phase (<50%), but its concentration decreased following intestinal step. GI digestion resulted in a high loss of TAC. Antioxidant capacity was also affected by GI conditions. Stevia showed its protective effect upon vitamin C, determining the highest GI bioaccessibility. Fructose protected anthocyanins during digestion. This study could make a valuable contribution in designing new functional foods.

7. Impact of different sweeteners on *in vitro* α-glucosidase inhibitory activity, biocompatibility of lingonberry jams and *in vivo* bioavailability of their anthocyanins[6]

Although anthocyanins behavior during digestion has been extensively studied by *in vitro* methods, little is known about their outcome after *in vivo* digestion. The last chapter of this thesis comprises the results obtained regarding the bioavailability of anthocyanins from lingonberries jams and the impact of used sweeteners (sucrose, fructose, erythritol, brown sugar, coconut sugar, stevia and saccharine) on their absorption. The α-glucosidase inhibitory activity, as well as the cytotoxicity on human endothelial and colon cancer cells of lingonberry jams was investigated. The jam extracts were administered to rats by gavage and then the blood samples were collected after 1h, 2h, 6h and 24h. The three main anthocyanins in jams (cyanidin 3-galactoside, cyanidin 3-glucoside and cyanidin 3-arabinoside) were determined by high performance liquid chromatography.

7.3.1 Inhibitory activity of lingonberry jams against α-glucosidase

In this research, the enzyme α-glucosidase was used to study the ability of lingonberry jams extracts, taking into consideration its commonly use for investigating α-glucosidase inhibitors from different sources (Mohamed Sham Shihabudeen et al., 2011). The inhibition degrees of lingonberry jams on α-glucosidase were determined and the results are comparatively presented in Figure 7.1. The equivalent concentration of acarbose (µg/mL) was also determined based on calibration curve. The highest value of the equivalent acarbose concentration is observed in the case of unsweetened jam (132.1µg/mL). Thus, consuming 113.5g unsweetened lingonberry jam would be equivalent to the recommended daily dose of acarbose. In the case of sweetened jams, it is necessary a higher dose: 186.8g fructose-based jam or 210.5g stevia-based jam. Similar behavior was also observed by Jan et al., (2021), that has shown the increasing inhibition capacity of α-glucosidase of stevia compared to other sweeteners, being an excellent alternative in preventing or controlling diabetes.

[6] Manuscript submitted to publication

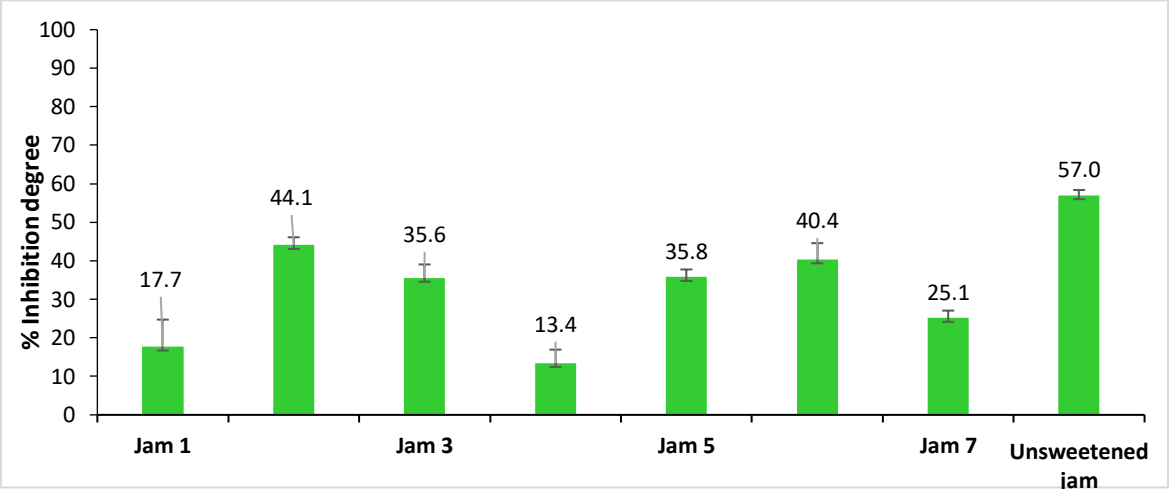
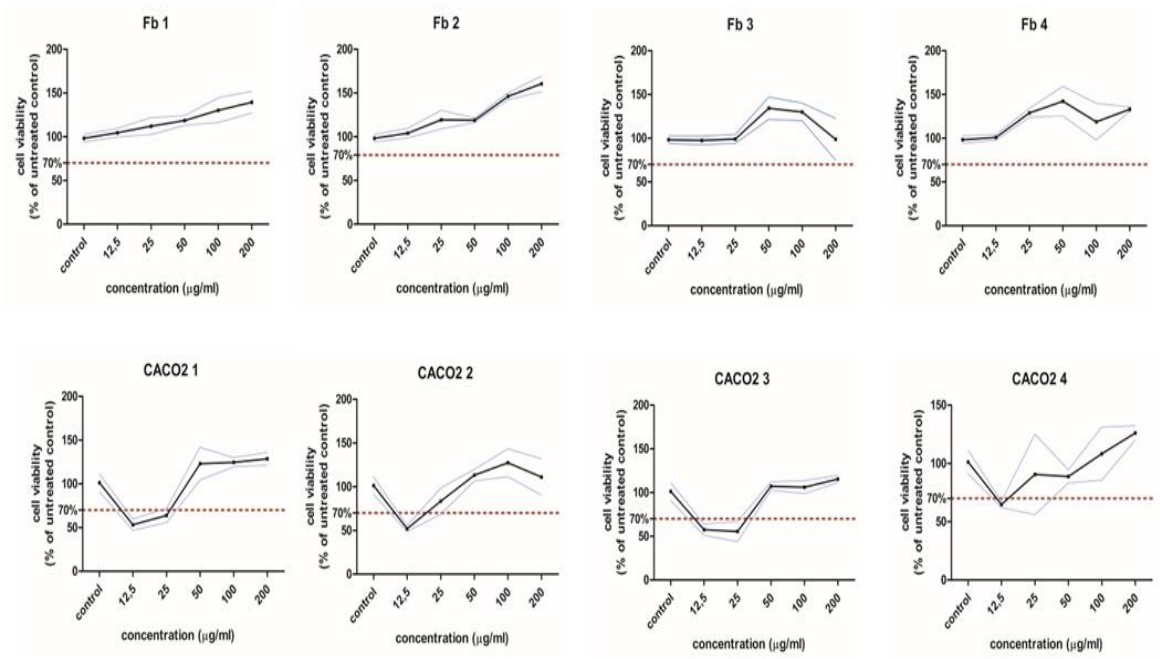


Figure 7.1 The percentage enzyme inhibition of lingonberry jams.

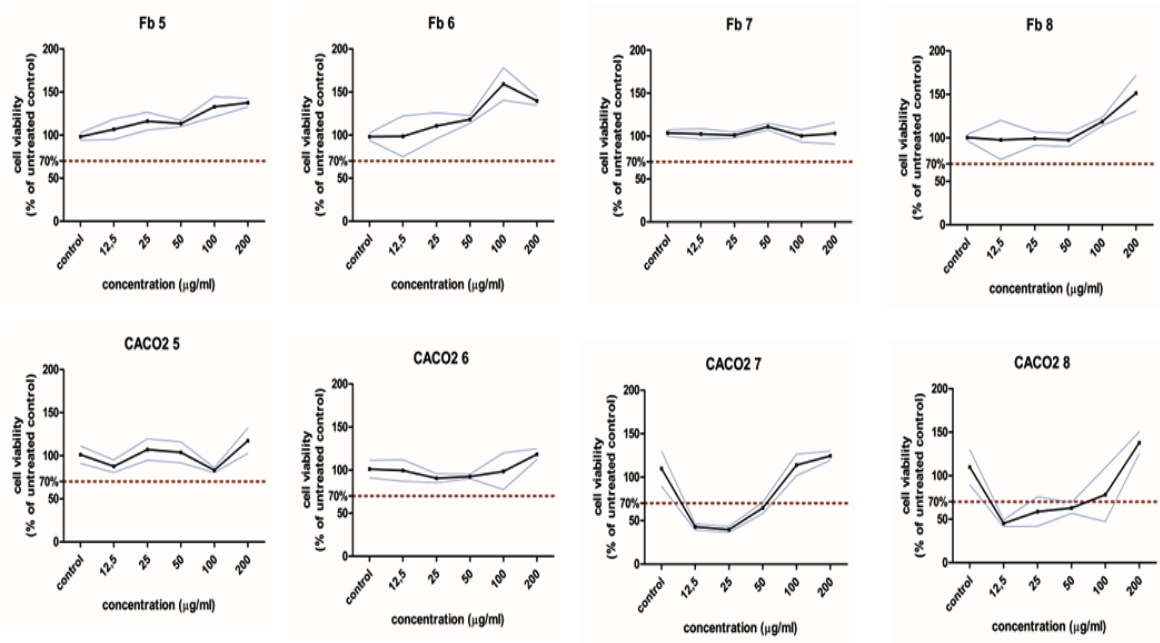
7.3.2 Cytotoxicity and cell viability in normal and CaCo-2 cancer cells

Cytotoxicity studies were done on normal dermal human fibroblast cells (BJ) and colon cancer (Caco-2) cells (Figure 7.2). Both normal and cancer cells were exposed to different lingonberry jams extracts at concentrations ranging between 12.5 – 200µg/mL. Viability data show differences between normal and cancerous cells ($p < 0.05$). Lingonberry jams extracts show no cytotoxic effects on normal fibroblastic cells, regardless of the sweetener used in the jam formulation (cell viability > 70% for all treated cells) and regardless the concentration used. However, they exhibited a dose dependent stimulatory effect and most of lingonberry jams extracts exerted a stimulatory effect upon normal cells up to the concentration of 200µg/mL. Significant differences ($p < 0.05$) have been observed between jam 7 and all other jams (excepting jam 3), whereas no significant differences ($p > 0.05$) were noticed compared to jam without sweetener.

When colon cancer cells were exposed to jams extracts, they presented a higher vulnerability than normal cells, the cell viability being lower than 70% in the case of all jams, excepting coconut sugar and stevia-based jams. Colon cancer cell viability is lower than 70% only at lower concentrations (12.5-25µg/mL), excepting saccharine-based jam and unsweetened jam, where cell viability was lower than 70% in a higher range of concentrations (12.5 - 50µg/mL). The strongest inhibition of cancer cell viability can be observed for saccharine-based jam (Figure 7.2), but not at high concentrations (100 - 200µg/mL).



a)



b)

Figure 7.2 Cell viability assay: jams 1-4 (a) and jams 5-8 (b). Human dermal fibroblasts (Fb) and colon cancer cells (Caco-2) were exposed for 24h to different concentrations of the jam extracts (12.5-200 µg/ml), untreated cultures were used as controls. Data are presented as % of untreated controls, (mean ± sd, n = 3).

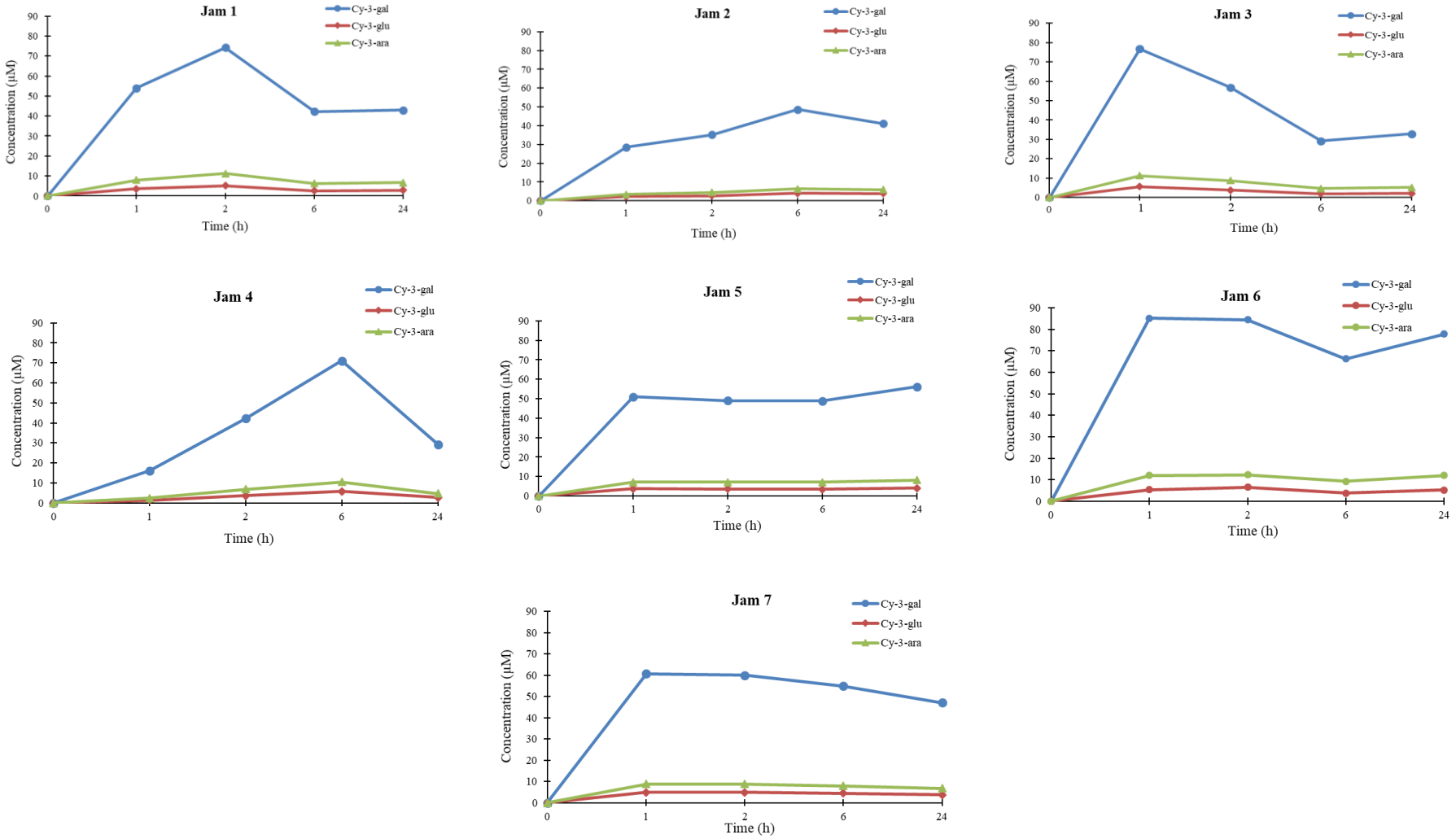


Figure 7.3 Plasma anthocyanin concentration profiles in rats after administration of lingonberry jams. Cy-3-gal: cyanidin-3-galactoside; Cy-3-glu: cyanidin-3-glucoside; Cy-3-ara: cyanidin-3-arabinoside

7.3.3 In vivo bioavailabiliy of anthocyanins from lingonberry jams

For a better understanding of the action and potential protective effects of dietary anthocyanins in human body, it is necessary to investigate their fate following ingestion. Although studies on the anthocyanins content and their *in vitro* bioaccessibility from lingonberry jams have been reported (Scrob et al., 2022), the bioavailability and potential absorption of anthocyanins from these products remain unknown.

The plasma concentration profiles of the three major anthocyanins at 1, 2, 6 and 24h after administration are shown in Figure 7.3.

At only 1 h after administration, all three peaks were detected in all plasma samples in a similar peak pattern to that of the administered lingonberry jams extracts, indicating their intact absorption. Talavera et al., (2003) did not also reveal the presence of anthocyanins metabolites, suggesting that anthocyanins are unmetabolised absorbed. In this research, plasma appearance rate of each anthocyanin is different depending on type of administrated lingonberry jam. This fact indicates the influence of sweetener upon anthocyanins absorption. All three anthocyanins are absorbed following administration and are still detectable in the bloodstream after 24h, indicating a prolonged bioavailability in rats.

In the case of white sugar, stevia and saccharine-based jams, anthocyanins are rapidly absorbed, reaching their maximum level at 1-2h after administration (Figure 7.3). Ichiyanagi et al., (2006) found a similar pattern and indicate that this fast appearance of anthocyanins could be due to their ability to permeate the gastric barrier. In the case of fructose and brown sugar-based jams, anthocyanins reached their maximum level at 6h after ingestion, indicating that these sweeteners could slow the absorption of anthocyanins.

For coconut sugar-based jam, all three anthocyanins appeared in a high concentration in the bloodstream after only 1 h, maintained a plateau and then reach a maximum concentration at 24 h. Thus, the occurrence of anthocyanisn in plasma is governed by food matrix. In study reported by Ichiyanagi et al. (2006), the plasma concentration of anthocyanins maximum occurred at only 15 min after oral administration of bilberry extract and then decreased with time. On the other hand, a study on lingonberry fruits (Lehtonen et al., 2009) testifys that anthocyanins are slowly absorbed. From these results, one can conclude that anthocyanins absorption depends on the provenience source and interaction with other compounds, such as sweeteners.

The bioavailability of anthocyanins was also calculated and the results are given in Table 7.1 The bioavailability calculated ranges from 4.85 to 32.7%.

Table 7.1 Bioavailability (%) of anthocyanins in the plasma of rats 1–24 h after the administration of lingonberry jams

Anthocyanins	Bioavailability (%)						
	Jam 1	Jam 2	Jam 3	Jam 4	Jam 5	Jam 6	Jam 7
1h							
Cy-3-gal	15.2	8.10	28.3	4.91	28.1	28.0	20.3
Cy-3-glu	12.4	8.21	26.1	5.30	29.1	23.8	22.6
Cy-3-ara	15.5	7.11	27.9	5.11	28.5	29.1	20.6
2h							
Cy-3-gal	20.9	10.0	20.9	12.7	27.0	27.8	20.0
Cy-3-glu	18.0	10.2	17.8	14.9	28.0	28.7	22.8
Cy-3-ara	21.6	9.01	21.7	14.4	28.0	29.7	20.3
6h							
Cy-3-gal	11.9	13.8	10.7	21.4	26.9	21.8	18.3
Cy-3-glu	9.11	15.3	9.28	23.0	27.3	16.9	20.3
Cy-3-ara	12.1	13.1	11.5	22.3	28.0	22.4	18.5
24h							
Cy-3-gal	12.1	11.6	12.1	8.81	30.8	25.6	15.7
Cy-3-glu	9.80	14.6	9.71	10.9	32.0	22.8	17.1
Cy-3-ara	12.9	12.0	12.8	9.90	32.7	28.8	15.7

In conclusion, this is the first research describing the influence of different sweeteners upon stability and *in vivo* bioavailability of anthocyanins from lingonberry jams. Moreover, this research brings important knowledge regarding the interaction of two human cell lines with lingonberry jams extracts, as well as the impact of these extracts upon α-glucosidase enzyme. Anthocyanins were significantly decreased as a result of jam storage and this brings challenges to the food industry. Bioavailability and absorption of anthocyanins was mainly governed by the sweeteners used in jam formulation. The highest bioavailability of anthocyanins was observed in the presence of coconut sugar. Colon cancer cells were more vulnerable when exposed to jams extracts than normal cells. Cancer cells inhibition was highly influenced by the administrated dose of extract, but also by the type of sweetener used in jam formulation. Understanding these processes will enable the development more stable food products with healthier benefits.

General conclusions

This thesis mainly aimed to investigate the bioaccessibility and bioavailability of different phytochemicals found in fruits and vegetables, which is essential in evaluating their health benefits after ingestion. The study of phytochemicals outcome after digestion was accomplished by both *in vitro* and *in vivo* methods and focused on chlorophylls, carotenoids, phenolics (especially anthocyanins), vitamin C, sugars, but also minerals. Furthermore, the current data raises important points regarding the development of several lingonberry jams, as a future perspective for designing new functional foods.

Broccoli florets (*Brassica oleracea*) have been subjected to an *in vitro* simulated digestion procedure and the experimental results showed that total carotenoids and chlorophylls and total phenolic content is significantly decreased after digestion. A higher stability during digestion was observed in the case of total phenolics, indicating that these compounds may be better absorbed than carotenoids and chlorophylls and *Brassica oleracea* remains an important source of compounds with antioxidant properties. In addition, it was shown that chlorophylls and carotenoids extraction follows a second-order kinetic model and extraction with ethanol at 30°C was the most efficient.

Total essential elements (Na, K, Mg, Ca, Fe, Mn, Cu and Zn) content and their bioaccessible fraction in six classes of the most common dried fruits have been analyzed. The studied dried fruits have been reported to be a good source of minerals such as Na, K, Mg, Fe, Mn and Cu, presenting moderate bioaccessibility levels for these elements, excepting Zn that has shown the lowest bioaccessibility. Non-carcinogenic risk of dried fruits was also revealed (THQ < 1). Prunes, coconut, dried banana and raisins can be considered as important sources of phenolics, which show high bioaccessibility after digestion. Following *in vitro* simulation of digestion, the AC increased in the case of the majority of dried fruits, making them as valuable in prevention of some diseases correlated with the oxidative stress.

In order to evaluate the behavior of phytochemicals in different food matrices, seven lingonberry jams have been developed, using different sweeteners: white sugar, fructose, erythritol, brown sugar, coconut sugar, stevia and saccharine. The use of other sweeteners in lingonberry jams formulation determined the obtaining of products similar to conventional jam with sucrose, excepting jam prepared with erythritol. Coconut sugar led

to the best scores for lingonberry jams, mainly due to its distinctive flavor. Stevia based jam also recorded a higher overall acceptability than jam prepared with sucrose.

The degradation process of phytochemicals in lingonberry jams was also studied and it was found that vitamin C follow a second-order kinetic model whereas TAC and individual anthocyanins degradation follow a first-order kinetic model. TPC and antioxidants both were described by a second-order kinetic model. The accumulation of reducing sugars during storage was lower in the presence of erythritol, stevia and saccharin, indicating that these sweeteners best suit for formulation of jams for patients with diabetes. The stability of bioactive compounds was highly influenced by the type of sweetener used in jam formulation. Erythritol determined the most pronounced degradation of vitamin C and anthocyanins, while phenolics were better preserved in the presence of this sweetener at 4°C. Under refrigeration, anthocyanins were best preserved in the presence of fructose. Regarding AC, fructose proved to produce the slowest degradation rate of antioxidants, while the degradation was fastest in erythritol containing jams. In turn, the addition of coconut sugar resulted in lower degradation rate constants of total phenolics, antioxidant compounds and vitamin C. Preservation of phytochemicals, such as anthocyanins, vitamin C, phenolics or antioxidant compounds in lingonberry jams during storage is of commercial importance and information about the effect of sweeteners on the preservation of jams are necessary before manufacturing these products at industrial scale.

Following *in vitro* GI simulated digestion of lingonberry jams, vitamin C was not highly affected by gastric phase, but its concentration decreased following intestinal step. Although TAC and all three anthocyanins found in lingonberry jams were quite stable under gastric conditions, GI digestion resulted in a high loss of these important phytochemicals. Antioxidant capacity was also affected by gastrointestinal conditions. AC_{ABTS} was higher after the GI phase than after the gastric phase, indicating that antioxidant compounds are progressively released from the food matrix and can exert their radical scavenging activity and biological effects. On the contrary, AC_{FRAP} decreased in the case of all samples after the GI digestion step, indicating that antioxidants from lingonberry jams have a higher radical scavenging ability than a reducing power. The importance of food matrix upon the stability of phytochemicals from lingonberry jams during digestion was also revealed. Stevia showed its protective effect upon vitamin C, determining the highest GI bioaccessibility. Fructose showed a positive effect upon anthocyanins during digestion. Stevia, fructose and coconut sugar exhibited high protection of the AC of lingonberry jams during digestion.

The last part of this thesis investigated the influence of different sweeteners upon *in vivo* bioavailability of anthocyanins from lingonberry jams. Furthermore, the interactions of two human cell lines with lingonberry jams extracts, as well as the impact of these extracts upon α-glucosidase enzyme were also studied. Bioavailability and absorption of anthocyanins was mainly governed by the sweeteners used in jam formulation. Glucosides were absorbed more effectively than galactosides in the majority of cases. The highest bioavailability of anthocyanins was observed in the presence of coconut sugar. Fructose and stevia-based jams were found to possess the highest inhibition activity upon α-glucosidase enzyme, indicating their benefits in preventing or controlling diabetes. Colon cancer cells were more vulnerable when exposed to jams extracts than normal cells. Cancer cells inhibition was highly influenced by the administrated dose of extract, but also by the type of sweetener used in jam formulation. Understanding these processes will enable the development more stable food products with healthier benefits and could make a valuable contribution in designing new functional foods.

Selected References

Benedek, C., Bodor, Z., Merrill, V. T., Kókai, Z., Gere, A., Kovacs, Z., Dalmadi, I., Abrankó, L. Effect of sweeteners and storage on compositional and sensory properties of blackberry jams. *European Food Research and Technology*, **2020**, *246*, 2187–2204.

Bouayed, J., Hoffmann, L., Bohn, T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. *Food Chemistry*, **2011**, *128*, 14–21.

Brown, E., Gill, C., Stewarta, D., McDougall, G. Lingonberries (*Vaccinium vitis-idaea* L) and blueberries (*Vaccinium corymbosum*) contain discrete epicatechin anthocyanin derivatives linked by ethyl bridges. *Journal of Berry Research*, **2016**, *6*, 13–23.

Chang, S. K., Alasalvar, C., Shahidi, F. Review of dried fruits: Phytochemicals, antioxidant efficacies, and health benefits. *Journal of Functional Foods*, **2016**, *21*, 113–132.

Coe, S., Fraser, A., Ryan, L. Polyphenol Bioaccessibility and Sugar Reducing Capacity of Black, Green, and White Teas. *International Journal of Food Science*, **2013**, 2013, 238216.

Courraud, J., Berger, J., Cristol, J. P., Avallon, S. Stability and bioaccessibility of different forms of carotenoids and vitamin A during *in vitro* digestion. *Food Chemistry*, **2013**, *136*, 871–877.

David, L., Danciu, V., Moldovan, B., Filip, A. Effects of *in vitro* gastrointestinal digestion on the antioxidant capacity and anthocyanin content of cornelian cherry fruit extract. *Antioxidants*, **2019**, *8*, 114–123.

Dróżdż, P., Šėžiene, V., Wójcik, J., Pyrzyńska, K. Evaluation of bioactive compounds, minerals and antioxidant activity of lingonberry (*Vaccinium vitis-idaea* L.) fruits. *Molecules*, **2018**, *23*, 53-62.

Fernández-García, E., Carvajal-Lérida, I., Jarén-Galán, M., Garrido-Fernández, J., Pérez-Gálvez, A. Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. *Food Research International*, **2012**, *46*, 438–450.

Fernandez-Leon, M. F., Lozano, M., Ayuso, M. C, Fernandez-Leon, A. M., Gonzalez Gomez, D. Fast and accurate alternative UV-chemometric method for the determination of

chlorophyll A and B in broccoli (*Brassica oleracea Italica*) and cabbage (*Brassica oleracea Sabauda*) plants. *Journal of Food Composition and Analysis,* **2010**, *5,* 809–813.

Gayoso, L., Claerbout, A. S., Calvo, M. I., Cavero, R. Y., Astiasarán, I., Ansorena, D. Bioaccessibility of rutin, caffeic acid and rosmarinic acid: Influence of the *in vitro* gastrointestinal digestion models. *Journal of Functional Foods*, **2016**, *26*, 428–438.

Gercia-Leston, J., Mendez, J., Pasaro, E., Laffon, B. Genotoxic effects of lead: an updated review. *Environment International,* **2010**, *36,* 623–636.

Han, P., Yang, P., Wang, H., Fernandes, I., Mateus, N., Liu, Y. Digestion and absorption of red grape and wine anthocyanins through the gastrointestinal tract. *Trends in Food Science & Technology,* **2019**, *83*, 211–224.

Heacock, P. M., Hertzler, S. R., Wolf, B. W. Fructose prefeeding reduces the glycemic response to a high-glycemic Index, starchy food in humans. *Journal of Nutrition*, **2002**, *132*, 2601–2604.

Hou, Z., Qin, P., Zhang, Y., Cui, S., Ren, G. Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. *Food Research International,* **2013**, *50,* 691–697.

Ichiyanagi, T., Shida, Y., Rahman, M. M., Hatano, Y., Konishi, T. Bioavailability and tissue distribution of anthocyanins in bilberry (*Vaccinium myrtillus L*.) extract in rats. *Journal of Agricultural and Food Chemistry*, **2006**, *54*, 6578-6587.

Ishiwata, K., Yamaguchi, T., Takamura, H., Matoba, T. DPPH radical-scavenging activity and polyphenol content in dried fruits. *Food Science and Technology Research,* **2004**, *10,* 152–156.

Kamiloglu, S., Pasli, A. A., Ozcelik, B., Capanoglu, E. Evaluating the in vitro bioaccessibility of phenolics and antioxidant activity during consumption of dried fruits with nuts. *LWT - Food Science and Technology*, **2014**, *56*, 284-289.cpe

Kroyer, G. Stevioside and stevia-sweetener in food: application, stability and interaction with food ingredients. *Journal fur Verbraucherschutz und Lebensmittelsicherheit*, **2010**, *5,* 225–229.

Lazar, L., Talmaciu, A. I., Volf, I., Popa, V. I. Kinetic modeling of the ultrasound-assisted extraction of polyphenols from Picea abies bark. *Ultrasonics Sonochemistry,* **2016**, *32,* 191–197.

Lehtonen, H. M., Rantala, M., Suomela, J. P., Viitanen, M., Kallio, H. Urinary excretion of the main anthocyanin in lingonberry (Vaccinium vitis-idaea), cyanidin 3-O-galactoside, and its metabolites. *Journal of Agricultural and Food Chemistry*, **2009**, *57*, 4447–4451.

Lichtenthaler, H. K., Buschmann, C. Chlorophylls and carotenoids: measurement and characterization by UV–Vis spectroscopy. *Current Protocols in Food Analytical Chemistry*, **2001**, *1*, 431-438.

Mane, C., Loonis, M., Juhel, C., Dufour, C., Malien-Aubert, C. Food grade lingonberry extract: polyphenolic composition and *in vivo* protective effect against oxidative stress. *Journal of Agricultural and Food Chemistry*, **2011**, *59*, 3330–3339.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R.P., Vegarud, G.E., Wickham, M.S., Weitschies, W., Brodkorb, A. A standardised static in vitro digestion method suitable for food - An international consensus. *Food & Function*, **2014**, *5*, 1113–1124.

Mohamed Sham Shihabudeen, H., Hansi Priscilla, D., Thirumurugan, K. Cinnamon extract inhibits a-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. *Nutrition & Metabolism*, **2011**, *8*, 46-57.

Muhammad, A., Durrani, Y., Zeb, A., Ayub, M., Ullah, J. Development of diet jam from apple grown in swat (NWFP). *Sarhad Journal of Agriculture*, **2008**, *24*, 461-467.

Pereira, C. C., do Nascimento da Silva, E., de Souza, A. O., Vieira, M. A., Ribeiro, A. S., Cadore, S. Evaluation of the bioaccessibility of minerals from blackberries, raspberries, blueberries and strawberries. *Journal of Food Composition and Analysis*, **2016**, *68*, 73–78.

Saura-Calixto, F., Serrano, J., Goni, I. Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chemistry*, **2007**, *101*, 492–501.

Scrob, T., Hosu, A., Cimpoiu, C. Trends in analysis of vegetables by high performance TLC. *Journal of Liquid Chromatography and Related Technologies*, **2019a**, *42*, 249-257. (IF=1.312)

Scrob, T., Hosu, A., Cimpoiu, C. The Influence of in vitro gastrointestinal digestion of Brassica oleracea florets on the antioxidant activity and chlorophyll, carotenoid and phenolic Content. *Antioxidants*, **2019b**, 212-223.

Scrob, T., Varodi, S. M., Vintila, G. A. Effects of sweeteners and storage on the acidity, soluble solids and sensorial profile of lingonberry jams. *Studia UBB Chemia*, **2021**, 66, 97-106.

Scrob, T., Covaci, E., Hosu, A., Tanaselia, T., Casoni, D., Torok, A. I., Frentiu, T., Cimpoiu, C. Effect of in vitro simulated gastrointestinal digestion on some nutritional characteristics of several dried fruits. *Food Chemistry*, **2022a**, 385, 132713.

Scrob, T., Hosu, A., Cimpoiu. C. Sweeteners from different lingonberry jams influence on bioaccessibility of vitamin C, anthocyanins and antioxidant capacity under in vitro gastrointestinal digestion. *Antioxidants*, **2022b**, 442-456.

Scrob, T., Varodi, S. M., Vintila, G. A., Casoni, D., Cimpoiu, C. Estimation of degradation kinetics of bioactive compounds in several lingonberry jams as affected by different sweeteners and storage conditions. *Food Chemistry X*, **2022c**, *16*, 100471.

Sutwal, R., Dhankhar, J., Kind, P., Mehla, R. Development of low calorie jam by replacement of sugar with natural sweetener stevia. *International Journal of Current Research and Review*, **2019**, *11*, 9-16.

Talavera, S., Felgines, C., Texier, O., Besson, C., Lamaison, J. L., Remesy, C. Anthocyanins are efficiently absorbed from the stomach in anesthetized rats. *Journal of Nutrition*, **2003**, *133*, 4178-4182.

Tokalioglu, S., Clough, R., Foulkes, M., Worsfold, P. Bioaccessibility of Cr, Cu, Fe, Mg, Mn, Mo, Se and Zn from nutritional supplements by the unified BARGE method. *Food Chemistry*, **2014**, *150*, 321–327.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., Prior, R. L. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry*, **2004**, *52*, 4026–4037.

Zanini, D. J., Silva, M. H., Aguiar-Oliveira, E., Mazalli, M. R., Setsusko Kamimura, E., Resende Maldonado, R. Spectrophotometric analysis of vitamin C in different matrices utilizing potassium permanganate. *European International Journal of Science and Technology*, **2018**, *7*, 70-84.

Zhang, Z. S., Wang, L. J., Li, D., Jiao, S. S., Chen, X. D., Mao, Z. H. Ultrasound assisted extraction of oil from flaxseed. *Separation and Purification Technology*, **2008**, *62*, 192–198.

List of published papers

1. **Teodora Scrob**, Anamaria Hosu, Claudia Cimpoiu. Trends in analysis of vegetables by high performance TLC. *Journal of Liquid Chromatography and Related Technologies*, **2019**, *42*, 249-257. (**IF=1.312**)
2. **Teodora Scrob**, Anamaria Hosu, Claudia Cimpoiu. The Influence of *in vitro* gastrointestinal digestion of *Brassica oleracea* florets on the antioxidant activity and chlorophyll, carotenoid and phenolic Content. *Antioxidants,* **2019**, *8(7)*, 212. (**IF=7.675)**
3. **Teodora Scrob**, Sanziana Maria Varodi, Georgiana Alexandra Vintila. Effects of sweeteners and storage on the acidity, soluble solids and sensorial profile of lingonberry jams. *Studia UBB Chemia*, **2021**, *66(4)*, 97-106. (**IF=0.447**)
4. **Teodora Scrob**, Eniko Covaci, Anamaria Hosu, Claudiu Tanaselia, Dorina Casoni, Anamaria Iulia Torok, Tiberiu Frentiu, Claudia Cimpoiu. Effect of in vitro simulated gastrointestinal digestion on some nutritional characteristics of several dried fruits. *Food Chemistry*, **2022**, *385*, 132713. (**IF=9.231**)
5. **Teodora Scrob**, Anamaria Hosu, Claudia Cimpoiu. Sweeteners from different lingonberry jams influence on bioaccessibility of vitamin C, anthocyanins and antioxidant capacity under *in vitro* gastrointestinal digestion. *Antioxidants,* **2022**, *11(3)*, 442. (**IF=7.675**)
6. **Teodora Scrob,** Sânziana Maria Varodi, Georgiana Alexandra Vintila, Dorina Casoni, Claudia Cimpoiu. Estimation of degradation kinetics of bioactive compounds in several lingonberry jams as affected by different sweeteners and storage conditions. *Food Chemistry X*, **2022**, *16*, 100471. (**IF=6.443**)

List of attended conferences

1. **Teodora Scrob**, Cimpoiu Claudia, Hosu Anamaria. Bioaccessibility and potential gastro-intestinal uptake of chlorophylls, carotenoids and polyphenols from *Brassica oleracea*. 9th International Conference of the Chemical Societes of the South-Eastern European Contries "Chemistry a Nature Challenger", 8-11 may, 2019, Târgoviște, Romania.

2. **Teodora Scrob**, Cimpoiu Claudia, Hosu Anamaria. Effect of *in vitro* simulated gastrointestinal digestion on total phenolics, total sugars content and antioxidant capacity of several dried fruits. 3rd International Conference on Natural and Applied Science and Engineering (ICNASEN-2021), 16-18 April, 2021, Turkey.

3. **Teodora Scrob**, Claudia Cimpoiu, Anamaria Hosu. Degradation of bioactive compounds of several lingonberry jams during storage. Young Researchers' International Conference on Chemistry and Chemical Engineering (YRICCCE III), 4-5 June, 2021, Cluj-Napoca.

4. Georgiana-Alexandra Vintila, Sanziana-Maria Varodi, **Teodora Scrob**. Preliminary phytochemical investigation of Vaccinum spp. leaves. International Conference "Students for Students", XVIIth Edition, 21-24 April, 2021, Cluj-Napoca.

5. Sanziana-Maria Varodi, Georgiana-Alexandra Vintila, **Teodora Scrob**. The influence of sweeteners on the characteristics of lingonberries jams. International Conference "Students for Students", XVIIth Edition, 21-24 April, 2021, Cluj-Napoca.