



ORIGINAL SCIENTIFIC PAPER

Antioxidant components and their *in vitro* bioaccessibility in processed and stored chickpea and amaranth greens mix

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Abstracts

The effect of processing and storage on antioxidant components and their *in vitro* bioaccessibility in chickpea and amaranth greens mix prepared with decorticated chickpea (*Cicer arietinum*) and green leaves of amaranth (*Amaranthus caudatus*) was investigated in this study. Food mix was cooked and stored under frozen storage (wet form) or under refrigeration and at room temperature (dehydrated form) and analyzed. Polyphenol (PP), tannin (TN) and flavonoid (FL) contents of food mix pre- and post- dehydration were ranged from 253-341, 357-364 and 35-48mg/100g dry weight respectively. Higher values were observed for PP and FN in dehydrated mix and for TN, total and β -carotene in fresh mix. The bioaccessibility of antioxidant components did not change significantly during frozen storage. The dehydrated mix showed higher reduction in digestibility of PP than fresh sample. Antioxidant components were retained in dehydrated mix. Frozen storage preserved higher antioxidant components in the chickpea+amaranth mix than storage at higher temperature.

Keywords: dehydration; polyphenols; tannins; flavonoids; carotenoids; frozen storage

Introduction

During the last few decades, dietary transition caused due to insufficient consumption of whole grains, legumes and vegetables has resulted in increased incidence of life style diseases among general population. Several reports claim that inclusion of legumes and fruits and vegetables in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer (Devasagayam et al., 2004; Kris-Etherton et al., 2002, Randhir et al., 2008). In addition to protein, starch and dietary fiber, greens and legumes have many other health improving properties, which are due to variety of micronutrients and phytochemicals. Processed ready-to-eat (RTE) products based on such ingredients are also available commercially as consumers demand such products (Tharanathan et al., 2003, Almeida Costa et al., 2006). Different techniques are used to extend the shelf life of processed products, among which dehydration and low temperature storage are very common. The consumer interest for high quality foods that can provide more nutrients and functional components are the reasons for studying effect of storage and its effect on nutrients and bioactive components in foods. The benefit from antioxidant components and their bioaccessibility in RTE foods depends on their content, the processing involved and the storage conditions. The antioxidant components, which are high in fresh foods can be destroyed during food storage due to changes that can happen in their structure. The temperature, light, moisture, exposure to air, etc can affect antioxidant retention and stability during storage (Zerdin et al., 2003).

Legumes and green leafy vegetables are common food ingredients used in South East Asian cuisine. Legumes serve as important source of proteins whereas greens provide many

essential vitamins, minerals and dietary fibre. Thermal processing is essential to convert them to edible forms and many traditional dishes are based on such ingredients. With advances in food processing, ready-to-eat (RTE) shelf stable packaged products based on legumes and greens such as curries or soup mixes have started appearing on market shelves. The present study was planned to investigate the effect of processing (dehydration), storage duration (1-3 months) and temperature on antioxidant components and their bioaccessibility in a mix based on chickpea and amaranth greens which serves as a model representing processed convenience products.

Materials and methods

Materials

The food materials, needed for the study, namely chickpea (*Cicer arietinum*) and fresh amaranth leaves (*Amaranthus caudatus*) were procured from local market in a single lot. These were grown around Mysore city, a district in South India and study was conducted in the year 2011. Chickpea was used in decorticated (without the outer husk) form. All chemicals used for the study were of analytical grade and purchased from Sd Fine Chemicals and Qualigens Chemicals Ltd. Mumbai, India. For the bioaccessibility studies, the enzymes used namely, pepsin (Batch No. 3-0060) and pancreatin (Batch No. 0-0864); and dialysis tubing (molecular mass cut off, 8000 Kda, D-9777, Lot 16H1545) were procured from Sigma Aldrich Co. USA. Glass double distilled water was used and all experiments were carried out in triplicate.

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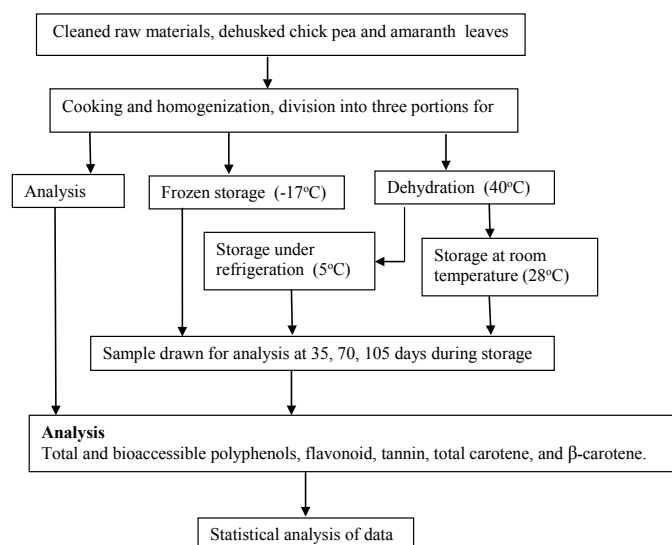


Figure 1. Study Design

Methods

Processing of chickpea and amaranth greens mix

The chickpea and amaranth greens mix chosen for the study was based on common curry or soup preparations used in Asian/ Indian cuisine with addition of spices and other flavour adjuncts. Many RTE processed products or dehydrated convenience products are also processed similarly, stored at varying temperatures and used. Hence the processing variables of mix were chosen to represent such conditions of cooking and storage. The raw materials were cleaned and washed with distilled water. Edible portion of greens was separated and leaves were cut into small pieces. One part of legume (100g), two parts of greens (200g) and 100 ml water were placed in a bowl and cooked under pressure for 20 min on a hot plate. The cooked mass was homogenized well in a mixer and divided into three parts, one part was retained for analysis, second part

was stored in freezer (-17°C) in airtight containers, and third part was dried for 24 h at 40°C. The dried mix was further divided into two parts and stored in airtight jars under refrigeration (5°C) and at room temperature (28°C) for 105 days (Fig 1). Moisture content of fresh and dehydrated samples were determined (AOAC, 2005). All samples were stored in separate polyethylene terephthalate jars and drawn on day 35 (First month), day 70 (Second month) and day 105 (Third month) for analysis. The frozen and refrigerated samples were thawed at room temperature prior to analysis. All mixes were analysed for presence of bioactive components, namely, polyphenols, flavonoids, tannins, total carotenoids, -Carotene and their bioaccessibility in fresh mix (0 day), dehydrated mix stored at room temperature and under refrigeration and frozen wet mix on day 35, 70 and 105.

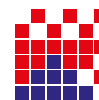
Determination of bioactive components

Bioactive components were measured in aqueous extracts prepared by mixing known amount of sample with glass double distilled water, shaking for 3 hours, centrifugation and filtration. Estimation of polyphenols (PP) was based on their reaction with Folin-Ciocalteu reagent and measurement of colour at 740 nm using spectrophotometer expressed as mg tannic acid equivalent/100g sample (Matthaus, 2002). Total flavonoid (FL) content was estimated by Dowd method as modified by Arvouet-Grand et al (1994) using quercetin as standard. Tannins (TN) were estimated colorimetrically by measurement of blue color formed by the reduction of phosphor-tungstomolybdic acid in alkaline solution (AOAC, 1990). For estimation of total and β-Carotene, samples were extracted in acetone and transferred to petroleum ether phase. Total carotene was read colorimetrically using petroleum ether for baseline correction. β-carotene was separated by column chromatography and read colorimetrically (AOAC, 1990).

Table 1. Effect of dehydration on Bioactive components in chick pea amaranth mix

Components (mg/100g)	Total		Bioaccessible	
	Fresh	Dehydrated	Fresh	Dehydrated
Polyphenols	88.7± 0.05 (323.01)	331.0 ± 1.41 (341.48)	44.7 ± 1.73 (162.79)	130.1 ± 1.11 (134.28)
P Value	-	0.020ns	-	0.091
Tannins	102.6± 0.28 (373.54)	353.5 ± 1.20 (364.75)	64.7 ± 0.30 (235.75)	238.2 ± 3.81 (245.75)
P Value	-	0.034	-	0.109
Flavonoids	10.3± 0.71 (37.57)	47.2 ± 0.10 (48.74)	4.1 ± 0.52 (14.99)	18.3 ± 0.86 (18.95)
P Value	-	0.058	-	0.148
Carotenoids	8.0± 0.26 (29.18)	13.8 ± 0.84 (14.32)	0.24± 0.02 (0.90)	0.70 ± 0.03 (0.72)
P Value	-	0.028	-	0.153
β-carotene	2.1± 0.34 (7.84)	5.2 ± 0.59 (5.38)	-	-
P Value	-	0.093	-	-

Initial moisture content of fresh mix was 72.5±0.21 % and that of dehydrated mix was 3.0±0.02 %. Figures in parenthesis indicate values on dry weight basis. **P value** refers to comparison between fresh and dehydrated samples. $P \leq 0.05$: marginally significant, $P \leq 0.01$: significant, $P \leq 0.001$: highly significant, $P > 0.05$: not significant.

**Table 2.** Effect of frozen storage (-17 °C) on Bioactive components in fresh chick pea amaranth mix

Components (mg/100g)	Total				Bioaccessible			
	0 day	I month	II month	III month	0 day	I month	II month	III month
Polyphenols	88.7 ^a ± 0.05	80.6 ^b ± 0.00	78.5 ^b ± 0.98	72.08 ^c ± 1.67	44.7 ^a ± 1.73	44.1 ^a ± 2.90	41.3 ^a ± 1.04	38.5 ^a ± 1.85
Tannins	102.6 ^a ± 0.28	91.7 ^b ± 0.42	85.8 ^c ± 0.63	81.3 ^d ± 1.13	64.7 ^a ± 0.30	68.0 ^a ± 0.00	32.9 ^c ± 1.78	36.9 ^b ± 0.64
Flavonoids	10.3 ^a ± 0.71	8.9 ^a ± 0.63	6.4 ^b ± 0.00	6.2 ^b ± 0.17	4.1 ^a ± 0.52	2.2 ^b ± 0.48	1.6 ^b ± 0.29	1.3 ^b ± 0.10
Carotenoids	8.0 ^a ± 0.26	7.2 ^{ab} ± 0.28	6.7 ^b ± 0.23	6.3 ^b ± 0.15	0.24 ^a ± 0.02	0.17 ^a ± 0.02	0.12 ^a ± 0.02	0.14 ^a ± 0.04
β-carotene	2.1 ^a ± 0.34	1.8 ^a ± 0.02	1.9 ^a ± 0.11	1.9 ^a ± 0.19	-	-	-	-

Different superscripts in rows indicate significant differences month wise on application of Tukey's test.

In Vitro Bioaccessibility of Bioactive components

An *In vitro* digestion process using semi-permeable membrane was used to determine bioaccessibility of polyphenols, tannins and flavonoids (Luten et al., 1996). The samples were digested with enzymes and proportion of antioxidant components diffused through a semi-permeable membrane was estimated by methods mentioned earlier. For studying *in vitro* bioaccessibility of total carotenoids and β-carotene, the method of Miller as modified by Gayathri et al., (2004) was used. Known amount of sample was treated with enzymes and bile salts to simulate human digestion system and the digesta was used for colorimetric measurement of total and β carotene (AOAC, 1990).

Statistical analysis

The data was subjected to statistical analysis to test the difference between samples using Students 'T' test, Analysis of variance, and posthoc Tukey's test for multiple comparisons using SPSS 18 (SPSS Inc.Chicago IL, USA). Since moisture was a variable in samples, moisture free values were used for all statistical comparisons for validity.

Results and discussion

The results of the study are summarized in Table 1-4 and Figure 2. The sample stored at -17°C represents freshly cooked mix on 0 day and samples stored at 5°C and 28°C represent dehydrated samples. Storage data is for days 35, 70 and 105. All comparisons and statistical computations are done using dry weight values due to varying moisture content of samples. Footnotes below the table indicate the level of significance for statistical treatment of data.

Effect of processing and storage of chickpea amaranth mix on polyphenols

The effect of dehydration on bioactive components of chickpea+amaranth mix is presented in Table 1. All results are discussed on dry weight basis. The PP content of fresh sample was 323.01mg /100 g and increased by 5.7% on dehydration. The increase was marginally significant (P value: 0.020), though for tannins, a slight decrease (marginally significant with P value of 0.034) was observed. Flavonoids did not differ markedly but carotenoids decreased by 51% on dehydration which was a considerable decrease in practical terms. β-carotene also decreased by 31% in dried sample. When bio-

active components were analysed for bioaccessibility using simulated digestion system, small differences were observed in fresh and dehydrated samples. For bioaccessible PP the value for fresh sample was 162.79 mg which decreased to 134.28 mg/100g on dehydration. Tannins showed a small difference (235.75 and 245.25 mg/100g) whereas carotenoids differed by 20.9% with dry sample showing higher bioaccessibility. Statistically, there were no significant differences in fresh and dry samples indicating that dehydration process had no effect on the bioaccessibility of bioactive components. According to Kalt et al (2005) some antioxidant components such as vitamin C are more susceptible to loss during postharvest handling and storage of fruits and vegetables. In contrast, certain carotenoids and phenolics are more stable and can actually increase under appropriate storage conditions.

Effect of frozen storage (-17°C) on bioactive components in fresh chickpea+amaranth mix

The data on bioactive components in frozen samples is compiled in Table 2 and results indicate that as the storage duration increased, there was a gradual decrease in bioactive components. For PP, the initial value in the fresh mix 88.7 mg/100g and decreased to 72.08 mg /100g at the end of storage duration. For tannins, the corresponding values were 102.6 and 81.4 mg /100g. ANOVA revealed significant differences in PP and tannins. Marginal decrease was also seen in carotenoids on frozen storage for 3 months. However, β-carotene was very stable and did not show any changes on storage with values ranging between 1.8-2.1mg/100g. The bioaccessible constituents did not show much differences in frozen samples. PP and carotenoids ranged from 38.5-44.7 mg/100g and 0.14- 0.24 mg / 100g. Hence, the differences on storage were marginal. Results indicate that frozen storage did not affect the bioaccessibility of bioactive components to a great extent. Ready-to-eat shredded orange and purple carrots, packed in air (control), or in modified atmosphere packaging, and stored chilled for up to 13 days, were examined for their antioxidant activity and components by Alasalvar et al (2005). The content of anthocyanin, found only in purple carrots, decreased slightly during the storage period. In both orange and purple carrots, loss of total carotenoids occurred in the modified atmosphere treatment. Total phenolic content of purple carrots increased at a much higher rate during storage than orange carrots.

Table 3. Effect of room and cold temperature storage on Bioactive components in dehydrated chick pea amaranth mix

Components (mg/100g)	Temp (°C)	Total				Bioaccessible			
		0 day	I month	II month	III month	0 day	I month	II month	III month
Polyphenols	5	331.0 ^a	330.1 ^a ±0.10	312.4 ^b ±3.16	315.2 ^b ±0.00	130.1 ^d ± 1.11	231.6 ^a ±0.79	210.9 ^b ±1.76	184.2 ^c ±0.03
	28	± 1.41	302.9 ^b ±3.52	271.8 ^c ±3.53	271.8 ^d ±3.54		227.5 ^a ±3.91	181.28 ^b ±6.20	173.7 ^b ±5.96
	P value	-	0.029	0.021	0.018	-	0.183	0.037	0.121
Tannins	5	353.5 ^b	365.0 ^a ±2.28	364.5 ^a ±1.83	360.0 ^a ±1.06	238.2 ^b ± 3.81	299.7 ^a ±3.45	167.5 ^c ±12.7	160.0 ^c ±8.69
	28	± 1.20	357.5 ^a ±1.84	348.5 ^b ±0.98	344.9 ^b ±1.62		298.3 ^a ±8.34	146.2 ^c ±17.1	157.5 ^c ±3.97
	P value	-	0.037	0.010	0.007	-	0.460	0.153	0.378
Flavonoids	5	47.2 ^a	43.1 ^b ±0.14	38.2 ^c ±0.14	29.6 ^d ±1.34	18.3 ^a ± 0.86	8.3 ^b ±0.94	5.7 ^{bc} ±0.78	4.2 ^c ±0.00
	28	± 0.10	33.2 ^b ±0.21	26.2 ^c ±1.41	19.1 ^d ±1.31		5.9 ^b ±0.19	3.5 ^{bc} ±0.74	2.2 ^c ±0.28
	P value	-	0.000	0.025	0.008	-	0.080	0.053	0.032
Carotenoids	5	13.8 ^a	13.0 ^a ±0.23	12.9 ^a ±0.48	12.3 ^a ±0.00	0.70 ^a ± 0.03	0.32 ^b ±0.03	0.40 ^b ±0.13	0.31 ^b ±0.07
	28	± 0.84	10.2 ^b ±0.40	10.0 ^b ±0.26	9.6 ^b ±0.18		0.33 ^b ±0.04	0.27 ^b ±0.04	0.30 ^b ±0.048
	P value	-	0.023	0.004	0.015	-	0.485	0.050	0.212
β-carotene	5	5.2 ^a	5.8 ^a ±0.16	5.3 ^a ±0.00	4.6 ^a ±0.11	-	-	-	-
	28	± 0.59	4.12 ^{ab} ±0.25	3.7 ^b ±0.05	3.7 ^b ±0.21	-	-	-	-
	P value	-	0.025	0.008	0.045	-	-	-	-

Different superscripts in rows indicate significant differences month wise on application of Tukey's test. **P value** refers to comparison between samples stored at 28 and 5°C. $P \leq 0.05$: marginally significant, $P \leq 0.01$: significant, $P \leq 0.001$: highly significant, $P > 0.05$: not significant.

Effect of room (28°C) and cold temperature (5°C) storage on bioactive components in fresh chickpea+amaranth mix

The dehydrated chickpea and amaranth mix was stored at room temperature and low temperature and the effect of storage on total and bioaccessible components is given in Table 3. The bioactive components exhibited varying results. PP values decreased to 315.2 mg after 3 months from the 0 day value of 331.0 mg at 5°C. The decrease was higher in II and III month in comparison to I month with marginal significance. At room temperature the value decreased to 271.8 mg after three months which was a significant lowering of PP on storage. Between cold and room temperature storage, significantly higher losses were seen at room temperature. Low temperature storage had no effect on tannin content, though a decrease was seen in room temperature stored products. Flavonoids exhibited a continued incremental decrease on storage in all samples. Carotenoids and β-carotenes were retained in low temperature stored samples, and showed a marginally significant decline in room temperature storage. The bioaccessible components followed a slightly different trend wherein for all constituents a decreased bioaccessibility was observed in stored samples. The differences were more obvious in second and third month of storage. The overall results indicate that sample stored at low temperature retained the components better in comparison to room temperature storage, however, bioaccessibility was affected in both types of storage showing a decrease. Effects of processing on the carotenoids of fruits and vegetable has been reported by many workers. Mayer-Miebach (2003) noted that eight weeks of cold storage of carrot reduced its total carot-

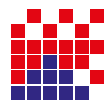
enoids by 30% and β-carotene by 20%. In our study also, loss of total carotenoids was more than β-carotene.

Thermal processing can give rise to degradation by products, which may behave like phenolic components. Thermal treatments could degrade conjugated polyphenolic such as tannins to simple phenolics (Cheng et al., 2006). It is reported that thermal processing increased flavonoid content of different vegetables (Miglio et al., 2007, Wachtel-Galor et al., 2008). In present study, after pressure cooking due to breakage of cell wall and complexes, there was an increase in free antioxidant components, which could be affected during further processing.

Effect of storage on percent retention of antioxidant components

Table 4 presents data on the percent retention of total and bioaccessible antioxidant components after 3 months of storage to show inter-sample comparisons. Loss of total PP, FL, TN and carotenoids (4.8, 0.0, 37.2 and 11.0%) was minimum in sample stored at 5°C. β-carotene was retained more at -17°C. Bioaccessible PP and TN followed the same trend as total but bioaccessible FL and bioaccessible carotenoids were retained better when they were stored at -17°C. It was observed that storage at ambient temperatures lead to highest loss of total and bioaccessible components when compared with frozen and refrigerated sample.

Gupta and Prakash (2013) analysed the total carotenoids and β-carotene content in two variants of amaranth greens on dehydration and reported a range of values of 68-69% retention for *Amaranthus gangeticus* and 63% for *Amaranthus tri-*

**Table 4.** Percent retention of total/ bioaccessible antioxidant components in chick pea and amaranth mix after storage duration

Components	Total components			Bioaccessible components		
	-17	5	28	-17	5	28
Polyphenol	81.2 ^b ±1.4	95.2 ^a ±0.3	82.1 ^b ±1.0	86.2 ^b ±5.3	141.6 ^a ±0.9	133.4 ^a ±2.4
Tannin	79.2 ^b ±0.6	101.8 ^a ±0.9	97.6 ^a ±0.3	57.0 ^b ±0.9	67.2 ^a ±1.8	66.1 ^a ±0.4
Flavonoid	60.4 ^{ab} ±1.7	62.8 ^a ±1.9	40.6 ^b ±2.0	33.8 ^a ±4.9	23.1 ^{ab} ±0.8	12.2 ^b ±1.5
Carotenoids	79.5 ^a ±3.3	89.0 ^a ±3.8	69.6 ^a ±3.9	57.8 ^a ±3.1	45.6 ^b ±4.4	43.2 ^b ±6.2
B-carotene	91.1 ^a ±2.7	90.0 ^a ±8.8	72.5 ^b ±8.7	-	-	-

Rows with different superscript are statistically different.

colour. These observations were similar to the present study. Darshan et al., (2014) formulated a product using dehydrated *Centella Asiatica* leaves and studied the bioaccessibility of β -carotene. They reported a range of bioaccessible β -carotene of 60-64% in dehydrated leaf powder and 18-22% in a formulated product containing legumes and spices along with leaves. Food processing can affect the polyphenols in different ways depending upon their concentration, chemical structure, oxidation state, localization in the cell, possible interactions with other food components and type of thermal processing applied. Food processing may account for a decrease, increase or minor changes in content and in functionality of polyphenols (Boekel et al., 2010).

Effect of dehydration and storage on percent bioaccessibility of antioxidant components

Effect of dehydration and storage on percent bioaccessibility of antioxidant components of the food mix is depicted

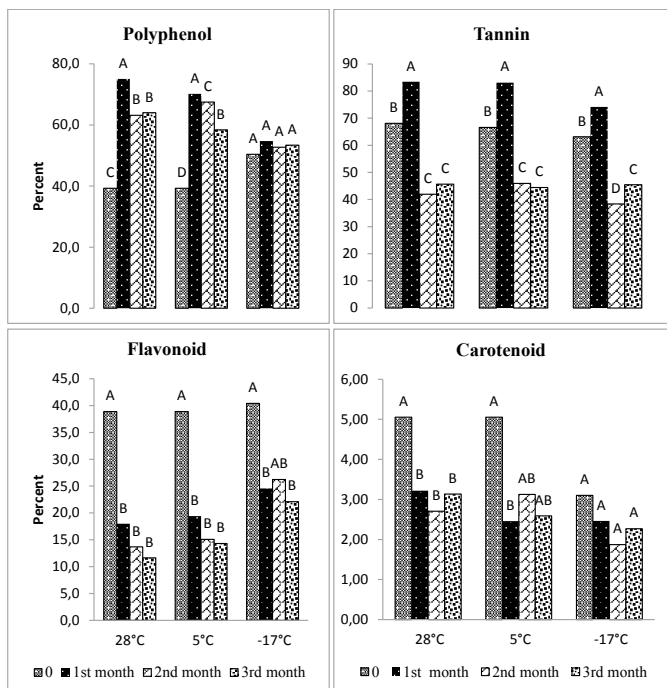


Figure 2. Effect of dehydration and storage on percent bioaccessibility of antioxidant components in sample stored at different temperature (as % total component present) [Bars with different superscript are statistically significantly different.]

in Fig. 2. Percent bioaccessible PP and bioaccessible FL were significantly higher in fresh mix than dehydrated mixes but TN and carotenoids behaved in reverse order. Percent bioaccessibility of PP and TN was found to be in range of 39.3-75.1 and 38.4-83.4% respectively and it was maximum in dry mix after a month (as observed for their total content). PP showed least bioaccessibility on 0 day but the least bioaccessibility for TN was either on 2nd and 3rd month of storage with slight differences, which indicates that major changes in bioaccessibility happened during 1st month of storage. FL bioaccessibility was highest on 0 day, fresh samples (40.4%) and least in the batch stored at 28°C after 3rd month (11.6%). It reduced steadily from 0 day to 3rd month and changes during 1st month were highly significant.

Percent bioaccessibility of carotene in mixes was very low (<6%). Carotenoids at 0 day (5.0 and 3.1% in fresh and dehydrated) was significantly higher than other months and further changes over continued storage were insignificant. McInerney et al. (2007) reported 3-35% of carotenoid bioaccessibility in vegetables treated with high pressure processing. It is also said that the carotenoids are released from plant foods only when the cells in the food matrix are disrupted, as is usually the case during food preparation, processing, and/or mastication, but not during digestion in the ileum of humans (Faulks et al., 2005, Van Buggenhout et al., 2010).

Wide range of bioaccessibility of antioxidant components shows that these compounds are sensitive to storage duration and temperature, so their bioaccessibility varies according to time and temperature of storage. Temperature did not affect percent bioaccessibility of antioxidant components extensively. Inter sample comparisons of samples stored at three temperatures showed that percent bioaccessibility of antioxidant components of mixes was not affected by temperature. Retention of PP was much better in dry form (28 & 5°C) than frozen form.

Endogenous enzyme levels, water activity, oxygen pressure, thermal and mechanical energy are the variables which dictate the content, bioavailability and activity of bioactive compounds. Processing activates enzymes which break the cell walls, and brings together the substrates and the enzymes. As the structure is broken down, and there is greater extractability and enzymatic breakdown, thermal processing increases carotenoid concentration. Freezing retains carotenes but long thawing is believed to decrease the content (Puupponen Pimiä et al., 2003).



Conclusion

Following conclusions can be drawn from the results of the study; dehydration of food mix resulted in increased total PP and FN and decreased TN and carotenoids. Bioaccessible components were not influenced by dehydration except for carotenoids. Storage duration decreased the total and bioaccessible antioxidant components to varying extent depending upon the temperature. All components retained better under refrigeration and exhibited better bioaccessibility. It can be said that considerable amount of antioxidant components were retained in processed food mix even after storage which were bioaccessible in human *in vitro* digestion system.

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