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STUDY AND COMPARISON OF AMINO ACIDS IN RED AND GREEN APPLES

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ABSTRACT: Apples are rich of vital components to the human health, such as the amino acids, but their presence differ according to the type and colour of the apples. In this study we compared the concentrations of amino acids in the peels and pulps of red and green apples, by using the HLPC for analysis. 16 amino acids were found with different concentrations according to the locality in which they exist in the red and green apples cultivars. The content of amino acids in the pulps was found higher than in the peels in both the red and green apples cultivars. The content of amino acids in the green apples recorded higher value than in the red apples. Higher concentrations of Aspartic acid were found in the peels and pulps of the red and green apples while the content of Methionine acid decreased, which was considered as the lowest amino acid found in the two apples cultivars and Histidine amino acid was not detected in both red and green apples cultivars.

INTRODUCTION: The general perception that apples are good for human health, together with the consumer's increasing demand for functional foods, has encouraged researchers to study in depth the polyphenolic profiles and antioxidant properties of many apple cultivars. It is well known that apples are one of the most important natural sources of polyphenols, exhibiting antioxidant activity, which can potentially prevent chronic diseases. The popularity of apples is not only a result of their taste, but also of their high nutritional value and healthful properties. It is a well-known fact that apples are a rich source of polyphenols [1].

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What is more, the concentration of free phenolics in apples is the highest amongst all fruits and for that reason these Apple juice composition compounds may be assimilated in higher amounts from apples. Phenolics, which are naturally existing antioxidants, demonstrate a variety of biological activities that may protect against the development of some chronic diseases, such as cardiovascular disease, obesity, diabetes, cancer and asthma; they may also have a beneficial influence on preventing premature ageing.

Apples refill the reserves of Vitamins, minerals, phenolic compounds, amino acids, dietary fiber and trace elements in the human body [2] therefore; they have been a highly popular snack for a long time throughout the world. The color, soluble sugars and organic acids are important components of fruit taste, and together with the aroma, they have a strong impact on the overall organoleptic quality of fruits.

Currently, red-fleshed apples are receiving increased attention because of their remarkable anthocyanin contents, as it has been reported that anthocyanin contributes more to hydrogen peroxide scavenging than other phenolic compounds in the apple peel [3], and they are very important natural flavonoid compounds for the apple colour.

Anthocyanins are the main pigments responsible for the colour of apples, where in the previous studies; cy3-gal was reported to be the most abundant anthocyanin inthe red apples cultivars [4]. Moreover, soluble sugars and organic acids also play an important role in fruit coloration. Organic acids could serve to stabilize anthocyanins [5]. Colour is the most important indicator of maturity and quality in many fruit species. Anthocyanins represent a group of natural flavonoid compounds in plants and are responsible for coloration. Development of red colour of fruits is influenced by genetic factors and various environmental factors.

Amino acids in apple juice are main nitrogen source for yeasts. Many amino acids are the intermediates or precursors of some volatile compounds, especially the production of higher alcohols. Nitrogen compounds can be the limiting factor for yeast growth and activity [6]. So far, several researchers have studied cider production from dessert apples [7].

However, none of these studies have attempted to investigate the change profile of chemical compositions, especially volatile compounds and amino acids during the process. Apples are among the main vegetal food sources rich in phenolic compounds, and the schools health programs indicates that the nutrition of school-age children should be supplemented with vegetal products as apples. Malic acid is the main acid in apple fruit and has an important influence on the sour taste of apples. In cultivars with low amounts of malic acid, the sweet taste becomes predominant.

"Starkrimson" is a black red apple cultivar. It was developed a long time ago but is still widely planted in some regions. In the arid area of the northern Wei River of China, "Starkrimson" is a very popular apple cultivar due to its deep red colour, large fruit, appropriate sugar: acid ratio and well-balanced aroma, which all have a strong impact on the overall organoleptic quality of the fruit.

Where the apples fruits are vital nutrients, as they contain several amino acids, the contents of amino acids in the peels and pulps of the red and green apples were examined by HLPC, and then the concentrations of these acids and their presence were compared between peels and pulps. The red and green apples were also compared in terms of their amino acids contents.

MATERIALSAND METHODS:

Sampling Method: Tow cultivars of imported apples were obtained including the red and green apples, as the study aimed to compare amino acids found in the peels and pulps of both red and green apples.

Sample Preparation: The exterior peel of both samples was separated and seeds were removed, so we have two samples of each cultivar for analysis which are the peel and pulps. The samples were crushed using electric blender, and each type of samples was homogenized separately.

Experimental:

- Proportion of water was determined in each sample so as calculate the results of analysis in a form of a dry matter.
- Analysis of amino acids in the four samples using the standard methods AOAC-9772.
- **Materials:** All the chemicals used in the analysis are of high purity and solid materials were obtained from Sigma Corporation.
- HPLC apparatus was used in determination of amino acids with the following specifications and operating conditions:

- Waters e2695 Separation Module (alliance).
- Waters 2475 FLR detector (Excitation wavelength 250nm and Emission wave-length 395nm).
- **Column:** AccQ Tag 3.9mm for hydrolyzed amino acids analysis.
- **Mobile Phase:** Eluent A: Aqueous buffer, Waters AccQ. Tag, Eluent B: HLPC grade acetonitrile, Eluent C: Milli-Q water.
- **Preparation of the Samples:** According to AccQ Tag Method.

Identification of the chromatographic peaks of different amino acids in the 4 samples was achieved by comparing the retention times and spectral characteristics UV-Vis of them with the peaks of the reference compounds of amino acids. With regard to the quantitative analysis, a calibration curve of amino acids was obtained by the injection of known concentrations of different standard materials of amino acids. The concentrations of amino acids identified in the four samples of apples were within the limits of the calibration curves. 17 amino acids represented in the four samples of the peels and pulps of red and green apples were examined and including: Glutamic acid (Glu), Serine (SER) Aspartic acid (Asp), Proline (Pro), Alanine (Ala), Threonine (THR), Arginine (ARG), Histidine (HIS), Glycine (GLY), Leucine **(**LEU), Lysine (LYS), Methionine (MET), Valine (VAL), Tyrosine (TYR), Cysteine (CYS), Isoleucine (IEU), Phenylalanine (PHE), as shown in **Table 1, Fig. 1.**

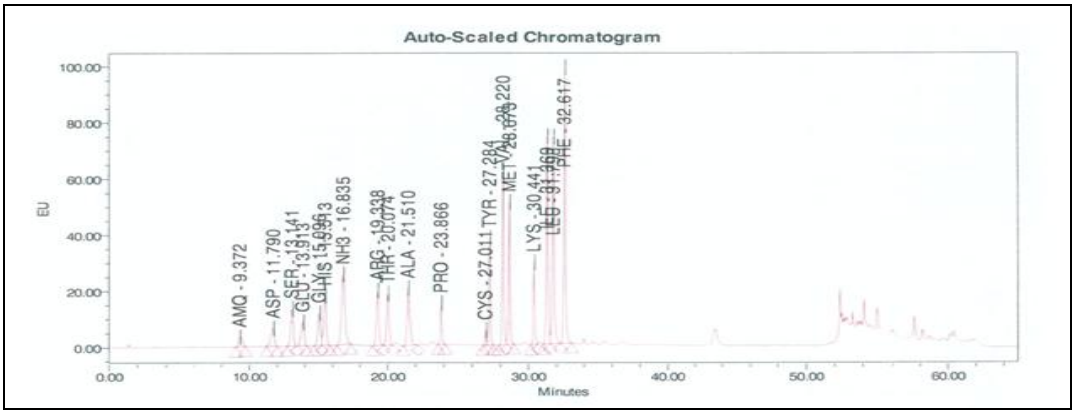

FIG. 1: HLPC CHROMATOGRAMS OF STANDARD AMINO ACIDS USING THE COLUMN; 3.9×105mm FOR HYDROLYZED AMINO ACIDS ANALYSIS AND USING DETECTOR (EXCITATION WAVELENGTH 250nm and EMISSION WAVELENGTH 395nm)

TABLE 1: CONCENTRATION AND RETENTION TIME OF STANDARD AMINO ACIDS

S. No.	Name	RT	Area	Height	Amount	Units
1	AMQ	9.372	688355	33552		
2	ASP	11.790	1149899	64061	1.660	ppm
3	SER	13.141	1754208	133019	1.310	ppm
4	GLU	13.913	1369261	84300	1.840	ppm
5	GLY	15.096	1605592	115215	0.940	ppm
6	HIS	15.513	2440928	175830	1.940	ppm
7	NH_3	16.835	4552448	175830	1.940	ppm
8	ARG	19.338	2447348	196066	2.180	ppm
9	THR	20.074	2280114	184825	1.490	ppm
10	ALA	21.510	296314	203711	1.110	ppm
11	PRO	23.866	1214510	147374	1.440	ppm
12	CYS	27.011	376914	53643	1.500	ppm
13	TYR	27.284	2785871	381574	2.270	ppm
14	VAL	28.220	4919667	611070	1.470	ppm
15	MET	28.673	3954250	507688	1.870	ppm
16	LYS	30.441	2276070	295086	1.830	ppm
17	ILE	31.369	6369855	742151	1.640	ppm
18	LEU	31.798	6616421	737970	1.640	ppm
19	PHE	32617	8396392	982254	2.070	ppm

RESULTS AND DISCUSSION: Chemical analyses of amino acids in peels and pulps of the red apples were done by HLPC system. Chromatograms of amino acids in peels and pulps of red apples were obtained, where 16 peaks of amino acids were detected as shown in **Fig. 2** in red apples peels and **Fig. 3** in red apples pulps, these amino acids include: Glutamic acid (GLU), Serine (SER) Aspartic acid (ASP), Proline (PRO), Alanine (ALA), Threonine (THR), Arginine (ARG), Glycine (GLY), Leucine (LEU), Lysine (LYS), Methionine (MET), Valine (VAL), Tyrosine

(TYR), Cysteine (CYS), Isoleucine (IEU), Phenylalanine (PHE). **Table 2** shows amino acids content in the red apples peels and **Table 3** shows the concentration of amino acids in red apples pulps.

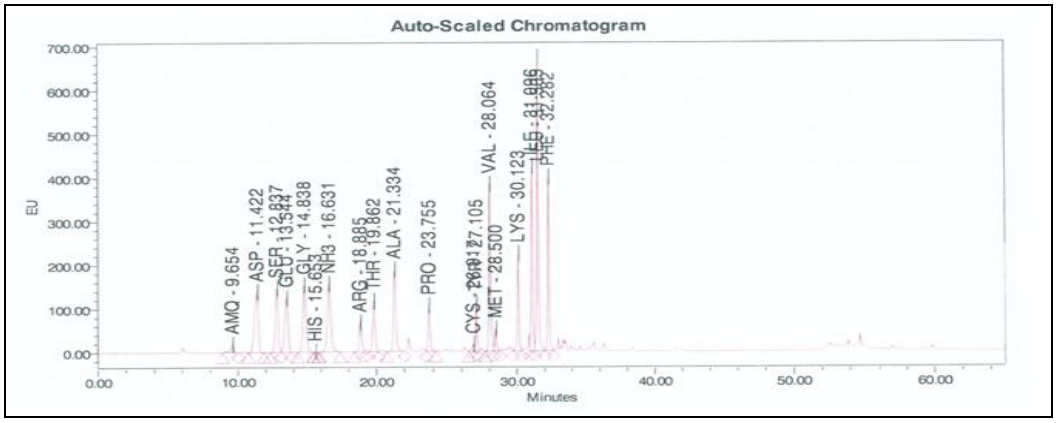

FIG. 2: HLPC CHROMATOGRAMS OF AMINO ACIDS IN THE PEELS OF RED APPLESUSING THE COLUMN; 3.9×105mm FOR HYDROLYZED AMINO ACIDS ANALYSIS AND USING DETECTOR (EXCITATION WAVE-LENGTH 250nm AND EMISSION WAVELENGTH 395nm)

TABLE 2: CONCENTRATION OF AMINO ACIDS IN THE PEELS OF RED APPLES

S. No.	Name	RT	Area	Height	Amount	Units
1	AMQ	9.654	3072757	182468		
2	ASP	11.422	26250956	1380635	37.896	ppm
3	SER	12.837	19859636	1463675	14.831	ppm
4	GLU	13.544	20973998	1248874	28.185	ppm
5	GLY	14.838	26045351	1539891	15.248	ppm
6	HIS	15.653	9835	1141	0.008	ppm
7	NH_3	16.631	27659393	1557757	15.248	ppm
8	ARG	18.885	8845601	670194	7.879	ppm
9	THR	19.862	14644399	1168118	9.570	ppm
10	ALA	21.334	23439503	1882394	8.789	ppm
11	PRO	23.755	9624123	1050255	11.411	ppm
12	CYS	26.917	1837759	160176	7.314	ppm
13	TYR	27.105	8394140	1134944	6.840	ppm
14	VAL	28.064	32697175	3835838	9.770	ppm
15	MET	28.500	4236252	511611	2.003	ppm
16	LYS	30.123	18115260	2243863	14.565	ppm
17	ILE	31.096	34452493	4241366	8.870	ppm
18	LEU	31.505	55719108	6724067	13.811	ppm
19	PHE	32.282	31895754	4005562	7.863	ppm

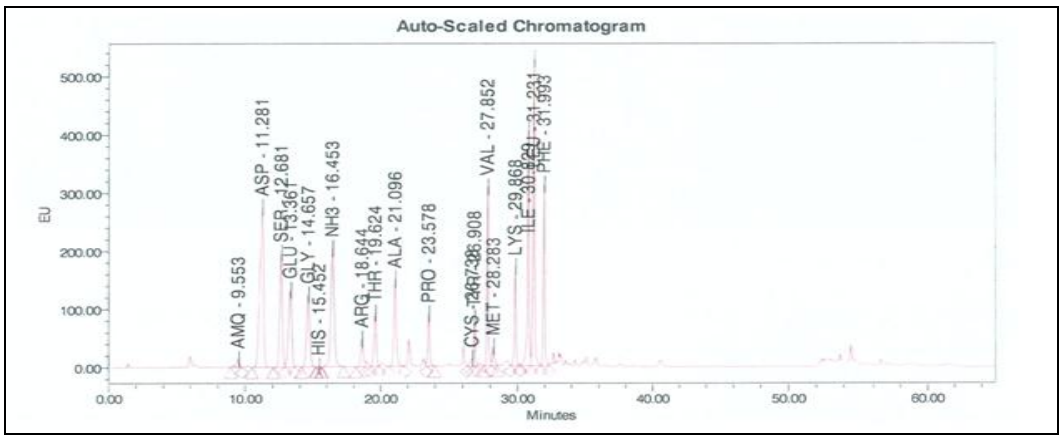

FIG. 3: HLPC CHROMATOGRAMS OF AMINO ACIDS IN THE PULPS OF RED APPLESUSING THE COLUMN; 3.9×105mm FOR HYDROLYZED AMINO ACIDS ANALYSIS AND USING DETECTOR (EXCITATION WAVELENGTH 250nm AND EMISSION WAVELENGTH 395nm)

TABLE 3: CONCENTRATION OF AMINO ACIDS IN THE PULPS OF RED APPLES

S. No.	Name	RT	Area	Height	Amount	Units
1	AMQ	9.553	2323405	136695		
2	ASP	11.281	52869813	2757939	76.323	ppm
3	SER	12.681	26716042	1961840	19.951	ppm
4	GLU	13.361	22362117	1330209	30.050	ppm
5	GLY	14.657	20576494	1240381	12.047	ppm
6	HIS	15.452	47461	4487	0.038	ppm
7	NH_3	16.453	36049035	2037798	5.305	ppm
8	ARG	18.644	7013231	483485	6.247	ppm
9	THR	19.624	11571644	922535	7.562	ppm
10	ALA	21.096	19398889	1499521	7.274	ppm
11	PRO	23.578	8499018	897900	10.077	ppm
12	CYS	26.738	1588411	134491	6.321	ppm
13	TYR	26.908	6091567	827424	4.964	ppm
14	VAL	27.852	24731133	3091628	7.390	ppm
15	MET	28.283	3120765	332802	1.476	ppm
16	LYS	29.868	13622084	1707332	10.952	ppm
17	ILE	30.829	35064807	4054117	9.028	ppm
18	LEU	31.231	42961232	5272281	10.649	ppm
19	PHE	31.993	24881007	3125171	6.134	ppm

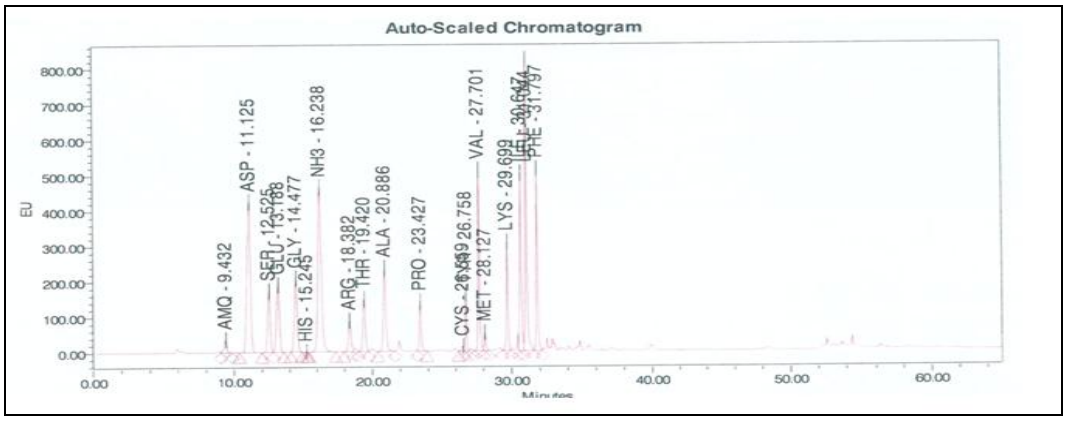

FIG. 4: HLPC CHROMATOGRAMS OF AMINO ACIDS IN THE PEELS OF GREEN APPLES USING THE COLUMN; 3.9×105mm FOR HYDROLYZED AMINO ACIDS ANALYSIS AND USING DETECTOR (EXCITATION WAVELENGTH 250nm AND EMISSION WAVELENGTH 395nm)

TABLE 4: CONCENTRATION OF AMINO ACIDS IN THE PEELS OF GREEN APPLES

S. No.	Name	RT	Area	Height	Amount	Units
1	AMQ	9.432	4956444	365983		
2	ASP	11.125	75459665	4255769	108.934	ppm
3	SER	12.525	22561756	1728763	16.849	ppm
4	GLU	13.188	29317754	1900082	39.397	ppm
5	GLY	14.477	30879725	2068241	18.179	ppm
6	HIS	15.245	1609	272	0.001	ppm
7	NH_3	16.238	81631737	4645773	12.014	ppm
8	ARG	18.382	1198711	886799	10.678	ppm
9	THR	19.420	18472660	1491167	12.071	ppm
10	ALA	20.886	29964641	2364021	11.236	ppm
11	PRO	23.427	12849826	1412146	15.236	ppm
12	CYS	26.559	1573871	131101	6.264	ppm
13	TYR	26.758	11861995	1636610	9.665	ppm
14	VAL	27.701	40042083	5131013	11.965	ppm
15	MET	28.127	4416540	538814	2.089	ppm
16	LYS	29.699	23897981	3099135	19.214	ppm
17	ILE	30.647	40152123	5037384	10.338	ppm
18	LEU	31.044	67584077	8228758	16.752	ppm
19	PHE	31.797	40297361	5161557	9.935	ppm

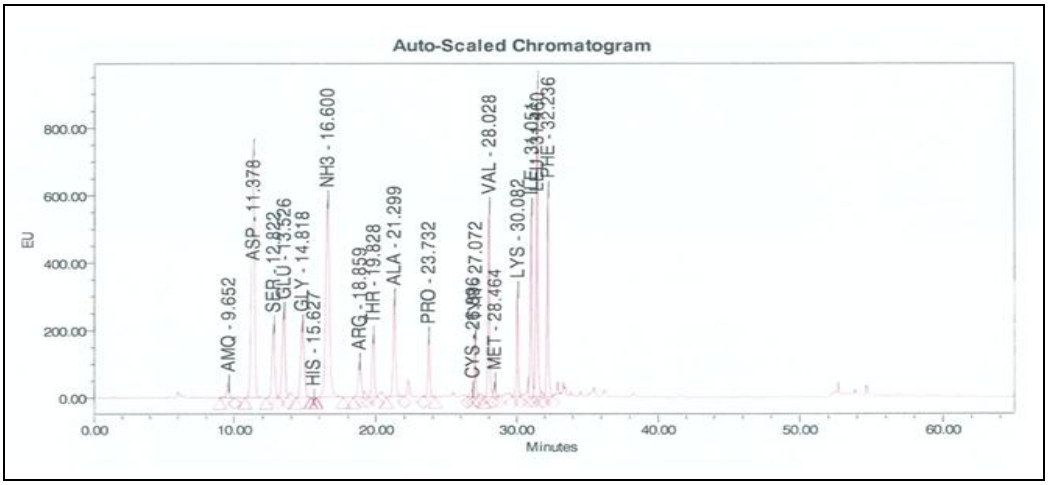

FIG. 5: HLPC CHROMATOGRAMS OF AMINO ACIDS IN THE PULPS OF GREEN APPLES USING THE COLUMN; 3.9×105mm FOR HYDROLYZED AMINO ACIDS ANALYSIS AND USING DETECTOR (EXCITATION WAVELENGTH 250nm AND EMISSION WAVELENGTH 395nm)

TABLE 5: CONCENTRATION OF AMINO ACIDS IN THE PULPS OF GREEN APPLES

No.	Name	RT	Area	Height	Amount	Units
1	AMQ	9.652	5569973	430814		
2	ASP	11.378	11951077	7436795	172.296	ppm
3	SER	12.822	28815146	2179250	21.518	ppm
4	GLU	13.526	37049643	2577513	49.787	ppm
5	GLY	14.818	37671449	2201735	22.055	ppm
6	HIS	15.627	9062	1098	0.007	ppm
7	NH_3	16.600	106832249	5882309	15.723	ppm
8	ARG	18.859	14880472	1066717	13.255	ppm
9	THR	19.828	22864193	1846143	14.941	ppm
10	ALA	21.299	36844797	2968373	13.815	ppm
11	PRO	23.732	18106440	1823906	21.468	ppm
12	CYS	26.896	2889320	216558	11.499	ppm
13	TYR	27.072	14234582	1934424	11.599	ppm
14	VAL	28.028	45626342	5683693	13.633	ppm
15	MET	28.464	3993151	460132	1.888	ppm
16	LYS	30.082	25773966	3181714	20.723	ppm
17	ILE	31.051	45787999	5653998	11.789	ppm
18	LEU	31.460	77292156	9403041	19.158	ppm
19	PHE	32.236	49347340	6165726	12.166	ppm

From **Table 2** it became obvious that Aspartic acid was the predominant amino acid in red apples peels with the concentration of 37.896 ppm, followed by Glutamic acid with the concentration of 28.185 ppm, then Glycine, Serine and Lysine. Methionine was the minority amino acid with the concentration of 2.003 ppm, which is considered as the lowest content in the red apples peels. Histidine was not detected in red apples peels as it was shown in **Table 2**. The total concentration of amino acids in red apples peels was approximately 204.844 ppm. When examining the amino acids in red apples When the concentrations of amino acids were compared between the red apples peels, **Table 2** and red apples pulps, **Table 3** the prominent result was that, the red apples pulps contained Aspartic acid more than twice its content in the peels of the same apples cultivar. Small differences were

pulps, high contents were found, and aspartic acid was the predominant amino acid in red apples pulps with the concentration of 76.323 ppm, followed by Glutamic acid with the concentration of 30.050 ppm, then Serine and Glycine. Methionine was the minority amino acid with the concentration of 1.476 ppm, which is considered as the lowest content in the red apples pulps. Histidine amino acid was not detected in red apples pulps as it was shown in **Table 3**. The total concentration of amino acids in red apples pulps was 226.445 ppm.

observed when other amino acids contents in red apples peels and pulps were compared. No statistically significant differences were observed in concentration difference of the remaining amino acids in the red apples peels and pulps. The higher total content of amino acids was found in the red apples pulps more than in the peels.

Moreover, amino acids in green apples peels and pulps were analyzed using HLPC, as it was shown in **Fig. 4, 5**. The chromatographic profile was complex to the green apples peels and pulps in terms of the numbers of compounds and peak areas. 16 amino acids were extracted with different concentrations as it was shown in **Fig. 4** which represents the HPLC chromatogram of amino acids present in the green apples peels, and **Fig. 5** which represents the HPLC chromatogram of amino acids present in the green apples pulps.

When amino acids contents in green apples peels were investigated it became obvious from **Table 4**, which represent the changes in theamino acids concentrations in green apples peels, a significant increase in the concentration of Aspartic acid has occurred which is considered as the main amino acid in green apples peels with the concentration of (108.934 ppm) followed by a Glutamic acid which was present with the concentration of (39.397ppm) then Lysine and Glycine followed by Leucine and Serine acids with nearly similar concentrations. Methionine was the minority amino acid in green apples peels with the concentration of2.089 ppm. Histidine amino acid was not detected in green apples peels. The total concentration of amino acids in green apples peels recorded 318.702 ppm.

When examining the concentration of amino acids in green apples pulps as indicated in **Table 4**, Aspartic acid was the predominant amino acid in green apples pulps with the concentration of 172.296 ppm, followed by glutamic acid with the concentration of 49.787 ppm, then Glycine followed by Proline and Serine acids with nearly similar concentrations, then Lysine. Methionine was the minority amino acid in green apples pulps with the concentration of1.888 ppm. Histidine amino acid was not detected in green apples pulps. The total concentration of amino acids in green apples pulps recorded 431.59 ppm. When comparing the changes of amino acids concentrations between the green apples peels, **Table 4** and green apples pulps, **Table 5** the higher content of amino acids was found in the green apples as it was significantly concentrated in the pulps than in peels, where the concentrations of all amino acids in pulps were much higher than in peels and Aspartic acid was the predominant amino acid which represent a higher content in green apples peels and pulps. When comparing the total contents of all amino acids concentrations in the green apples pulps (431.59 ppm), they were found significantly exceeding amino acids concentrations in the green apples peels which recorded (318.702 ppm).

When comparing the red and green apples cultivars, it was found that the green apples outmatched the red apples as they recorded higher content of amino acids in both the peels and pulps, The total concentration of amino acids in green apples reached nearly twice its content in red apples as it recorded 750.292 ppm while the total concentration of amino acids in red apples recorded 431.289 ppm, which revealed to us the importance of this apples cultivar as a valuable source of amino acids. The total amino acids concentration was found higher in pulps than in peels of both red and green apples, which proved the importance of the apples pulps in obtainment of amino acids in both cultivars.

Significance of aspartic acid became evident, as it was considered the predominant amino acid in the peels and pulps of red and green apples, and which was found with higher concentrations especially in the green apples pulps as it recorded 172.296 ppm compared to the concentrations of other acids. Moreover, Glutamic acid showed a relatively high content in both apples cultivars, but a significant decline was observed in Methionine acid compared to the contents of other acids in both apples cultivars. Interestingly, no Histidine acid was found in the peels and pulps of red and green apples. A comparison was carried out between the ratios of amino acids in the peels and pulps of both red and green apples before and after drying as shown in **Table 6, Fig. 6**. The ratio of amino acids contents in the samples recorded significant increase after drying, where the total ratio of amino acids in the green apples peels recorded 2.41% after drying compared to 0.47% before drying. The total ratio of

amino acids in the green apples pulps amounted to 3.06% compared to 0.40% before drying. Furthermore, we observed increase in the total ratio of amino acids in the peels and pulps of red apples after drying compared to their ratio before drying as indicated in **Table 6, Fig. 6**.

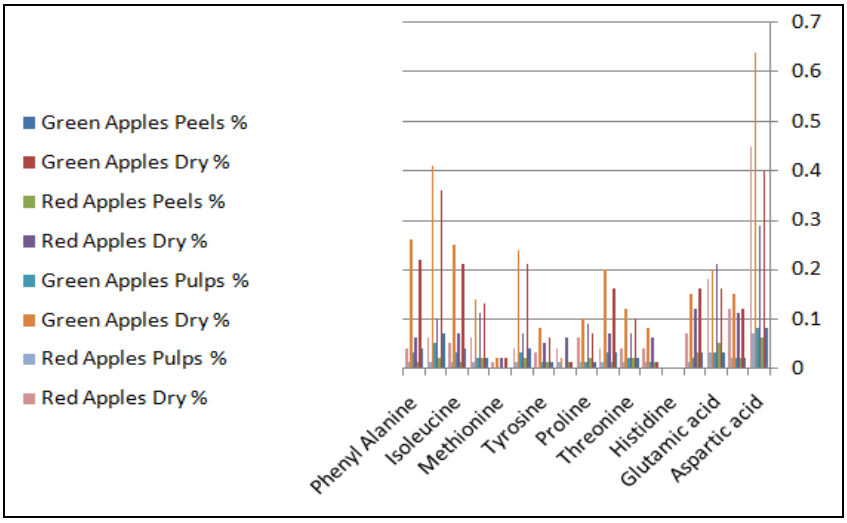

FIG. 6: RATIO OFAMINO ACIDS IN PEELS AND PULPS OF THE RED AND GREEN APPLES BEFORE AND AFTER DRYING

TABLE 6: RATIO OF AMINO ACIDS IN PEELS AND PULPS OF THE RED AND GREEN APPLES BEFORE AND AFTER DRYING

Amino acid title	Green Apples		Red Apples		Green Apples		Red Apples	
	Peels %	Dry %	Peels %	Dry %	Pulps %	Dry %	Pulps %	Dry %
Aspartic acid	0.08	0.40	0.06	0.29	0.08	0.64	0.07	0.45
Serine	0.02	0.12	0.02	0.11	0.02	0.15	0.02	0.12
Glutamic acid	0.03	0.16	0.05	0.21	0.03	0.20	0.03	0.18
Glycine	0.03	0.16	0.03	0.12	0.02	0.15	0.01	0.07
Histidine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arginine	0.00	0.01	0.01	0.06	0.01	0.08	0.01	0.04
Threonine	0.02	0.10	0.02	0.07	0.02	0.12	0.01	0.04
Alanine	0.03	0.16	0.01	0.07	0.03	0.20	0.01	0.04
Proline	0.01	0.07	0.02	0.09	0.01	0.10	0.01	0.06
Cysteine	0.00	0.01	0.01	0.06	0.00	0.02	0.01	0.04
Tyrosine	0.01	0.06	0.01	0.05	0.01	0.08	0.00	0.03
Valine	0.04	0.21	0.02	0.07	0.03	0.24	0.01	0.04
Methionine	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.01
Lysine	0.02	0.13	0.02	0.11	0.02	0.14	0.01	0.06
Isoleucine	0.04	0.21	0.01	0.07	0.03	0.25	0.01	0.05
Leucine	0.07	0.36	0.02	0.10	0.05	0.41	0.01	0.06
Phenyl Alanine	0.04	0.22	0.01	0.06	0.03	0.26	0.01	0.04
Total	0.47	2.41	0.34	1.56	0.40	3.06	0.22	1.34

CONCLUSION: In this study the variation in amino acids content in green and red apples pulps and peels were determined by HPLC system. The results showed the distribution patterns of amino acids in both apples cultivars, as 16 amino acids were present. For both cultivars, the qualitative and quantitative distribution of amino acids varied between the peels and pulps. The high total amino acids content was found in apples pulps. Moreover, amino acids content was by far greater in green apples than in red apples, where this type of apples is considered as incomparable variety and a valuable source of amino acids. Aspartic acids had the higher concentration in both cultivars while Methionine was the minority amino acid in both apples cultivars.

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CONFLICTS OF INTEREST: Nil.

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