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CHEMICAL COMPOSITION, ASCORBIC ACID AND ANTIOXIDANT ENZYMES OF SOME POTATO VARIETIES

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ABSTRACT

Chemical composition and ascorbic acid were estimated in tubers of four potatoes varieties (Oceania, Pamina, Lady Rosetta, and Lady Balfour) collected from storage refrigerator. The study showed differences in chemical composition between the four varieties. The protein, fat, carbohydrates, ash, fiber, moisture and ascorbic acid content ranged respectively from 6.78-8.2, 0.2-0.3, 81.01-84.1, 5.36- 6.97, 3.47-471, 78.1-86.21, and 18.92-25.08 % in the varieties investigates. The statistical analysis show highly differences between the four kinds of potatoes. High percent of protein, fat and ascorbic acid were detected in Lady Rosetta potato, of ash and moisture in Pamina potato, of carbohydrates in Oceania potato, while of fiber in Lady Balfour potato. The antioxidant enzymes peroxidase, Catalase and Ascorbate peroxidase were determined, showing ranges of 0.05-0.13, 0.15-0.2 and 0.12- 0.31 Unit min^{-1} gm^{-1} in Oceania, Pamina, Lady Rosetta and Lady Balfour**,** respectively.

Keywords: Potato, Oceania, Pamina, Lady Rosetta, Lady Balfour, Chemical composition, ascorbic acid.

INTRODUCTION

One practical goal of food and nutrition policy planners in developing countries is to reduce disparities between requirement and intakes of nutrients. Potato (*Solanum tuberosum L*.) is one of the most important staple crops grown worldwide. Because of its low cost, low fat content and a good source of carbohydrates, high quality protein, fiber, vitamins and minerals, it plays an important role in human nutrition (FAO, 2005 - 2006). Potato ranks fifth in terms of human consumption and fourth in worldwide production (Horton, 1987). However, its value within the human diet —particularly as a source of vitamin C— is often underestimated or ignored (Woolfe, 1987; Dale *et al*., 2003). There are two major forms of vitamin C: L-ascorbic acid (AA) and L-dehydroascorbic acid (DHAA); however, the terms vitamin C and ascorbic acid are frequently used as synonyms (Bates, 1997). The protein efficiency ratio, an important nutritional parameter, of potato protein was higher than that attributed to many other plant proteins (Kelly, 1972).

The continuous increase of the human population over the last decades has considerably influenced the demand for food and food products. Annual world production of Potato is more than 300 million tonnes. Potato tuber is considered the most important vegetable in many developing and developed countries. Potatoes are used for several purposes, including human consumption as fresh or processed produce (French fries, mashed potato), industrial processing (potato starch, alcohol, etc.) and recultivation (potato seed) (Feustel, 1987; Talburt, 1987).

One of the richest sources of antioxidants in the human diet is potato tubers (Lachman *et al*., 2000). Their antioxidant content decreases a great deal from atherosclerotic processes, and is inhibited from cholesterol accumulation in blood serum and enhances the resistance of the vascular

walls. Many antioxidants decrease risk of coronary heart disease and have free radical scavenging effect. The main potato antioxidants are polyphenols, ascorbic acid, carotenoids, tocopherols, α-lipoic acid, and selenium. Polyphenolic compounds, esp. flavonoids are effective antioxidants (Bors and Saran 1987) due their capability to scavenge free radicals of fatty acids and oxygen (Good 1994). AA is an essential component of most living tissues. As an antioxidant, it plays an important role in protection against oxidative stress. AA is an important scavenger of free radical species, such as reactive oxygen species (Bates, 1997) which achieve cellular saturation and reduce risk of heart disease, stroke and cancer in healthy individuals (Naidu, 2003).

Peroxidase (POD) is a ubiquitous enzyme that reduces H_20_2 in the presence of electron donor. POD is widely used as an important reagent for clinical diagnosis and microanalytical immunoassay because of its high sensitivity in reaction. New applications for POD have been suggested in the field of medicinal, chemical and food industry including elimination of phenolic and aromatic compounds in waste treatment (Krell, 1991).

The present study aimed to determine the chemical composition and antioxidants in four potato varieties (Oceania, Pamina, Lady Rosetta, Lady Balfour) grown in Egypt.

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MATERIALS AND METHODS

Plant material

Potatoes tubers of 4 different termed as varieties Oceania, Pamina, Lady Rosetta, and Lady Balfour were collected from a local supplier in El-Minia city, Egypt.

Chemical analysis

The moisture, Nitrogen, fat, fiber and ash content of potato tubers were determined according to standard ACAC method (AOAC, 2000).

Enzyme assays

All spectrophotometric assays were performed by using a Unicom™ UV/VIS 2100 spectrophotometer.

Extraction of antioxidant enzyme activities

The potato samples were frozen in liquid nitrogen, then stored frozen for later analysis. Samples were prepared for, Peroxidase (POD), Catalase (CAT) and Ascorbate peroxiase (APX) activity analyses by homogenizing 1 g of frozen powder in 10 mL of phosphate buffer (100 mmol L^{-1}, pH 7.8) containing 0.1 mM Na_2EDTA and 0.1gm of polyvinyl pyrrolidone. The homogenate was filtered thought cheese cloth then centrifuged and blended for 5 min in blender at minimal speed. The homogenate was centrifuged for 10 min at 18 000 × g and the supernatant removed for POD, Catalase, Ascorbate POD activity assay.

Assay of antioxidant enzyme activities

Peroxidase (POD)

Peroxidase activity was assayed according to the method of Günes & Bayindirli (1993).One mL of extract was mixed with 1 mL of guaiacol (45 mmol L^{-1}), 1 mL of H_2O_2 (200 mmol L^{-1}) and 18 mL of KH_2PO_4 buffer (65 mmol L^{-1}, pH 6.5). After incubation at room temperature for 10 min, absorbance was measured at 430 nm. One unit of the enzyme activity was

defined as change in absorbance of 0.001 min^{-1}.

Catalase (CAT)

Sample extract was homogenized in 966 mM KH_2PO_4 buffer (pH 7.0). The supernatants were used for the assay. The reaction mixture was composed of 66mM phosphate buffer (pH 7.0), sample extract and 3 % hydrogen peroxide. Boiled samples with no CAT activity served as controls. Change in absorbance was measured at 240 nm using spectrophotometer over a 3 min period and the decrease in absorbance recorded. CAT activity was expressed as micromoles of hydrogen peroxide reduced per minute per milligram of protein, using an extinction coefficient of 39.4mM^{-1} cm^{-1} (Aebi, 1984).

Ascorbate peroxidase (APX)

Samples extract was homogenized in 50mM KH_2PO_4 buffer (pH 7.0) containing 0.5mM ascorbic acid. The reaction mixture consisted of 50mM phosphate buffer containing 0.5mM ascorbic acid, 0.3% H_2O_2 and supernatant of sample. Boiled samples served as controls. The rate of change in absorbance (decrease) at 290 nm was measured for 10 min in a spectrophotometer. Enzyme activity was expressed as micromolar ascorbate oxidized per minute per mg protein using a molar extinction coefficient of 2.8mM^{-1} cm^{-1} (Asada, 1984).

Ascorbic acid

Ascorbic acid concentrations in potato tubers were evaluated by the spectrophotometric method of Egoville *et al. (*1988). The method is based on the ability of ascorbic acid to reduce the dye 2,6 dichloroindophenol. Since L-dehydroascorbic acid (DHAA) is present in very low amount in potatoes we do not evaluate its concentration. Briefly, the 15 g laboratory sample was extracted with an oxalic acid and acetone solution (0.4 and 20%, respectively) by homogenizing in a Sorvall Omni Mixer during 5 minutes at 4000 rpm. The extract was filtered under vacuum through filter paper Whatman 2 and brought to 100 ml with the same extracting solution. One ml of the extract was reacted with 9 ml of 2,6-dichloroindophenol (1.6%) during 1 minute and read at 520 nm on a Unicom™ UV/VIS 2100 spectrophotometer. The ascorbic acid concentration was quantified through comparison with a standard curve of L-AA (MERCK).

Statistical analysis

Randomized complete block design with 4 replications was used and the analysis of variance was performed according to Gomez and Gomez (1984). Data for Fat and enzymes percent were subjected to statistical analysis.

RESULTS AND DISCUSSION

Chemical analysis

Data in Table (1) show that the percentage of protein in dry matter content of potato tubers was 6.79, 6.78, 8.20 and 7.57 for Oceania, Pamina, Lady Rosetta and Lady Balfour, respectively. This result is in agreement with those reported by Lisi and Leszcy, 1989; Abd Elfattah, (1988) but exceed those obtained by MAFF (1996) .Statistical analysis of the present results

showed that protein content of Oceania, Pamina, Lady Rosetta and Lady Balfour had highly significant (P<0.01) differences for Lady Rosetta and Lady Balfour while potato varieties had no significant differences between Oceania and Pamina.

Table1: Chemical composition of four varieties of potato tubers (Oceania, Pamina, Lady Rosetta and Lady Balfour) (values are means's. of four samples)

Chemical composition / Potato variety	Protein (Nx6.25) (% dm) Mean±SE	Fat (% dm) Mean±SE	carbohydrates (% dm) Mean±SE	Ash (% dm) Mean±SE	Fiber (% dm) Mean±SE	Moisture (%) Mean±SE	Ascorbic acid (%) Mean±SE
Oceania	6.79±0.01	0.28±0.02	84.10±0.01	5.36±0.0	3.47±0.01	78.51±0.01	22.8±0.02
Pamina	6.78±0.03	0.20±0.02	82.57±0.01	6.97±0.02	3.48±0.01	86.21±0.02	20.32±0.01
Lady Rosetta	8.20±0.02	0.30±0.01	82.59±0.02	5.37±0.01	3.54±0.02	78.18±0.02	25.08±0.01
Lady Balfour	7.57±0.01	0.23±0.02	81.01±0.01	6.48±0.01	4.71±0.01	84.66±0.01	18.92±0.01
L.S.D. 5%	0.14	0.16	0.25	0.15	0.08	0.10	1.34
L.S.D. 1%	0.19	0.23	0.36	0.21	0.11	0.15	1.93

The fat content ranged from 0.20% in Pamina to 0.30% in Lady Rosetta these results are in harmony with those of Burton (1966). The statistical analysis showed that the fat content had no significant differences among Lady Rosetta and Lady Balfour potatoes while had significant (P<0.05) differences between Oceania, Pamina.

Carbohydrates provide the bulk of the calories (4 kcal/gram) in most diets and starches provide the bulk of that. Age, sex, size, health, and the intensity of physical activity strongly affect the daily need for calories. Moderately active females (19–30 years old) need 1500–2500 kcal/day, while males of the same age need 2500–3300 kcal/day. In this study the potatoes carbohydrate was 84.10% in Oceania potatoes but the lowest percentage was 81.01 % in Lady Balfour. These results are in agreement with those of Lisi and Leszcy (1989). The statistical analysis showed that carbohydrate content had highly significant (P<0.01) differences among different variant kind of potato varieties.

On the other hand the percentage of ash content was 5.36, 6.97, 5.37 and 6.48% for Oceania, Pamina, Lady Rosetta and Lady Balfour, respectively, this results are in harmony with those reported by Burton (1966) Lisi and Leszcy (1989). Statistically the Ash content between of Oceania, Pamina, Lady Rosetta and Lady Balfour had highly significant (P<0.01) in differences Pamina and Lady Balfour potatoes while had no significant differences between Oceania, and Lady Rosetta.

The fiber content ranged from 3.47% in Oceania to 4.71±0.00 in Lady Rosetta, These results are in harmony with those of Lisi and Leszcy (1989) Burton (1966). The statistical analysis showed that fiber had highly significant (P<0.01) differences among differences variant kind of potato except between Oceania and other varieties investigate.

The high percentage of carbohydrate was determined respectively in for Oceania, Pamina, Lady Rosetta and Lady Balfour 6.79, 6.78, 8.20and 7.57. The statistical analysis showed that carbohydrate had highly significant

(P<0.01) differences among different potato varieties. On the other hand The Moisture content of potatoes samples were 78.51, 86.21, 78.18 and 84.66 % for Oceania, Pamina, Lrady Rosetta and Lady Balfour, respectively. Statistically the moisture content between of Oceania, Pamina, Lady Rosetta and Lady Balfour had highly significant (P<0.01) differences while no significant differences was found between Pamina and Lrady Rosetta.

Ascorbic acid content of potatoes samples was 22.8, 20.32, 25.08 and 18.92% for Oceania, Pamina, Lady Rosetta and Lady Balfour, respectively., these results are in agreement with those of Burgos *et al*.(2008) which showed that the ascorbic acid (AA) concentration in freshly harvested raw, peeled tubers ranged from 22.2 to 121.4 mg/100 g on dry weight basis (DW) and from 6.5 to 36.9 mg/100 g on fresh weight basis (FW). However, The statistical analysis showed that Ascorbic acid had highly significant (P<0.01) differences among different potato varieties except between Oceania and Lady Balfour had significant (P<0.05) differences.

Potatoes are substantial sources of several vitamins, one of Ascorbic acid (vitamin C) is found in the freshly harvested raw tuber in both the reduced and oxidized forms. In the freshly harvested raw tuber the reduced form L-ascorbic acid is quantitatively the most important or is the only form present (Wills *et al*. 1984). Newly harvested potato tubers have been reported to contain up to 46 mg AA /100 g FW (Mishra, 1985; Mullin *et al*., 1991; Nordbotten *et al*., 2000; Han *et al*., 2004). Functions of Ascorbic acid is coenzyme in the synthesis of collagen .

Enzyme activities

From data presented in Table (2), the peroxidase content ranged from 0.05 in Lady Balfour to 0.13 Unit min^{-1} gm^{-1} fresh weight in Oceania. The statistical analysis showed that peroxidase had highly significant (P<0.01) differences among differences variant kind of potato and significant (P<0.05) differences between Oceania and Lady Rosetta but had not significant differences between Oceania and (Pamina, Lady Rosetta).

Table 2: Enzyme activities of four varieties of potato tubers (Oceania, Pamina, Lady Rosetta and Lady Balfour).

Enzymes / potato tubers	Peroxidase (Unit min^{-1} gm^{-1} fresh weight)	Catalase (Unit min^{-1} gm^{-1} fresh weight)	Ascorbate Peroxidase (Unit min^{-1} gm^{-1} fresh weight)
	Mean±SE	Mean±SE	Mean±SE
Oceania	0.13±0.01	0.19±0.02	0.15±0.02
Pamina	0.11±0.01	0.20±0.03	0.17±0.03
Lady Rosetta	0.09±0.02	0.15±0.02	0.3±1E-3
Lady Balfour	0.05±0.01	0.170.01	0.12±0.01
L.S.D. 5%	0.20	0.24	0.27
L.S.D. 1%	0.29	0.34	0.39

Peroxidase (POD) is a ubiquitous enzyme that reduces H_20_2 in the presence of electron donor. POD is widely used as an important reagent for clinical diagnosis and microanalytical immunoassay because of its high

sensitivity in reaction. New applications for POD have been suggested in the field of medicinal, chemical and food industry including elimination of phenolic and aromatic compounds in waste treatment (Krell, 1991).

On the other hand, the highly content of Catalase was 0.20 Unit min^{-1} gm^{-1} in Pamina and the lower content was in 0.15 Unit min^{-1} gm^{-1}. The statistical analysis showed that catalase had highly significant ($P<0.01$) differences among differences variant kind of potato except between Oceania and Pamina, Lady Rosetta had not significant differences.

The percentage of Ascorbate Peroxidase contant was 0.15,0.17, 0.31and 0.12 for Oceania, Pamina, Lady Rosetta and Lady Balfour, respectively, The statistical analysis showed that of Ascorbate Peroxidase had highly significant ($P<0.01$) differences between Oceania and Lrady Rosetta potato only and hand no significant differences between other samples .

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التركيب الكيميائى وحامض الاسكوربيك وبعض إنزيمات الأكسده فى بعض أصناف البطاطس

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تم فى هذه الدراسة تقدير التركيب الكيميائي وحامض الأسكوربك والانزيمات المؤكسدة في أربع أصناف من البطاطس وهى (Oceania, Pamina, Lady Rosetta, Lady Balfour والتي تم تجميعها من ثلاجات التخزين .وقد أظهرت الدراسة اختلافات في التركيب الكيميائي بين الأنواع الأربعة في البروتين والدهن و الكربوهيدرات والرماد والألياف والرطوبة وحامض الاسكوبك فكان متوسط هذه النسب يقع على التوالى مـــا بـــين ٦,٧٨- ٨,٢ و ٠,٢ – ٠,٣ و ٨١,١ – ٨٤,١ و ٥,٣٦- ٦,٩٧ و ٣,٤٧ – ٤,٧١ و ٧٨,١- ٨٦,٢١ و ١٨,٩٢- ٢٥,٠٨ % في كل من (Oceania, Pamina, Lady Rosetta, Lady Balfour). كما اظهر التحليل الأحصائى فروق معنوية جدا بين جميع الأصناف فاعلي نسبة للبروتين والدهن وحامض الاسكوربك كانت في بطاطس Lady Rosetta واعلي نسبه للرماد والرطوبة كانت في بطاطس Pamina واعلي نسبة للكربوهيدرات في بطاطس Lady Rosetta واعلي نسبة في الألياف في بطاطس Lady Balfour. كما تم تقدير ثلاثة من اللأنزيمات المؤكسدة فى درنات هذة الأصناف وكانت مقاديرها كالآتى 0.05-0.13 للبروكسيديز ،0.15-0.2 للكتـــاليز ، 0.12- 0.31Unit min-1 gm-1Ascorbate peroxidase.