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THE RELATIONSHIP BETWEEN ALTITUDES AND THE CONTENTS OF PROTEIN, CARBOHYDRATES, LIPIDS OF PUMPKIN *(Cucurbita moschata)*

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ABSTRACT

Cucurbita moschata or pumpkin can be used as an alternative food mainly due to its carbohydrate content, and it is very easy to grow in many different habitats. The objective of this research was to evaluate the biochemical contents of *C. moschata* based on the altitudes and also to examine whether any relationship between the environmental conditions and protein, carbohydrate and lipid contents. Proximate analysis was used for statistical consideration of the results obtained. Chemical analysis was conducted by using mesocarp of pumpkin after cleaning, peeling and removing seeds from the center of fruits. Kjedahl and soxhlet methods were used to look at the content of protein and lipid respectively. Meanwhile, the method of difference was employed to measure the percentage of carbohydrates. Although there was no significant relationship between the biochemical contents and the environmental conditions, it was recorded that plants grown at higher altitudes with high soil pH and air temperature tended to have higher protein, carbohydrate and lipid contents, compared to that of higher soil moisture. This results showed that the highest biochemical contents of protein, carbohydrate and lipid of two varieties *C. moschata* were evident at the lowest altitude.

Keywords: altitudes, carbohydrates, *Cucurbita moschata*, lipids, protein

INTRODUCTION

Pumpkin or *Cucurbita moschata* Duch. (Cucurbitaceae) was considered as one of the very potential alternatives of food in developing countries. There are two species *C. maxima* and *C. pepo* in Nigeria have been studied intensively, both of morpho-anatomical features and nutrient value (Agbagwa and Ndukwu, 2004). This vegetable plant was very popular due to its usefulness in providing human energy and health. Currently, pumpkin fruits have been consumed widely not only by people in Asian regions but also by many people from America (Kim *et al*., 2012). This plant could grow quite well especially in tropical areas with the height ranging from zero to more than fifteen hundred metres above sea level (1500 m asl) (Radovich, 2010). Due to its very easy growing in the natural habitat, this species has several varieties such as Hai Je Pi from Taiwan or vegetable spaghety squash from Japan (Yenrina *et al*., 2009). Accordingly, Maynard (2001) also noted six cultivars of tropical pumpkin, while in Indonesia, there were many *C. moschata* varieties which grow naturally in several islands, including in Central Java. Based on the basic shape of the fruit, at least three kinds of pumpkin have been recorded, namely oval, rounded and cylindric/snake types (Revans, 2011). The skin of pumpkin fuits varied from rough to very smooth depending on the varieties. The color of fleshy fruits was light orange to yellow (Aruah *et al*., 2010). Two of the three varieties of *C. moschata* planted in Central Java were the oval (locally named kelenting) and rounded (locally named bokor) shapes.

This species of *C. moschata* has been considered to have many functions for human health as antimicrobial and nephroprotective (Caili

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et al., 2006; El-Aziz and El-Kalek, 2011). Because of the high content of carbohydrates and fibre, this vegetable plant has been implemented as a food with valuable source of dietary fibre in human nutrition (Hussain *et al.*, 2010). The fibre content of *C. moschata* may decrease the serum cholesterol level, the risk of coronary heart disease, and hypertension. Besides, the seed, which is known to have high amount of zinc, has been used in treating the early stages of prostate problem (Pandya and Rao, 2010).

Although *C. moschata* has been recorded as one of the most significant species having higher concentration of carbohydrates, fibre and energy compared to other species, its consumption as a food item in the local comunity of Central Java is minimal or very restricted. Because of the plant ability to grow in different habitats, *C. moschata* has wide distribution not only in the lower land but also in mountainous areas. It is one of the species that grows specifically at the the most favorable habitats where more sunlight would determine the quality of the plant growth. It is quite well understood that the higher is the altitude of the plant habitat, the lower is its temperature and viceversa. As expected, these environmental conditions would in some extent influence the activity of the plant's metabolism in producing carbohydrates, lipids, and proteins, also in the *C. moschata* fruit part. The objective of this research was to evaluate the biochemical response of *C. moschata* to the altitude of habitat and also to examine whether there is relationship between the environmental conditions and protein, carbohydrate and lipid contents.

MATERIALS AND METHODS

Sample Preparation

Samples of *C. moschata* from different altitudes were collected between June and November 2011 from three different districts, two of them were from Central Java Island and the other one was from East Java. Wonogiri and Karanganyar districts of Central Java and Magetan district located at the East Java were taken as sampling locations. Pumpkins were collected from fourteen sub-districts of Wonogiri including Paranggupito, Pracimantoro, Eromoko, Wuryantoro, Wonogiri, Nguntoronadi, Baturetno, Tirtomoyo, Ngadirejo, Girimarto, Jati Purno, Slogohimo, Bulukerto and Puhpelem), six sub-districts of Karanganyar (Jatiyoso, Matesih, Karangpandan, Tawangmangu, Ngargoyoso and Jenawi) and three sub-districts (Poncol, Plaosan and Panekan) of Magetan (Figure 1 A, B, C). The examples of two varieties of pumpkin (*Cucurbita moschata*) are presented in Figure 2. The biochemical tests were conducted in the Plant Biochemistry Laboratory at the Faculty of Agriculture UNS Solo, Indonesia.

The altitudes of plant samples were grouped into 5 sections namely; 1 – 300 meters asl; 301 – 600 meters asl; 601 – 900 meters asl; 901 – 1200 meters asl and 1201 – 1500 meters asl. Samples were cleaned, peeled and the seeds were removed from the center of the fruit. Mesocarp was cut in order to be used for chemical analysis.

Chemical Analyses

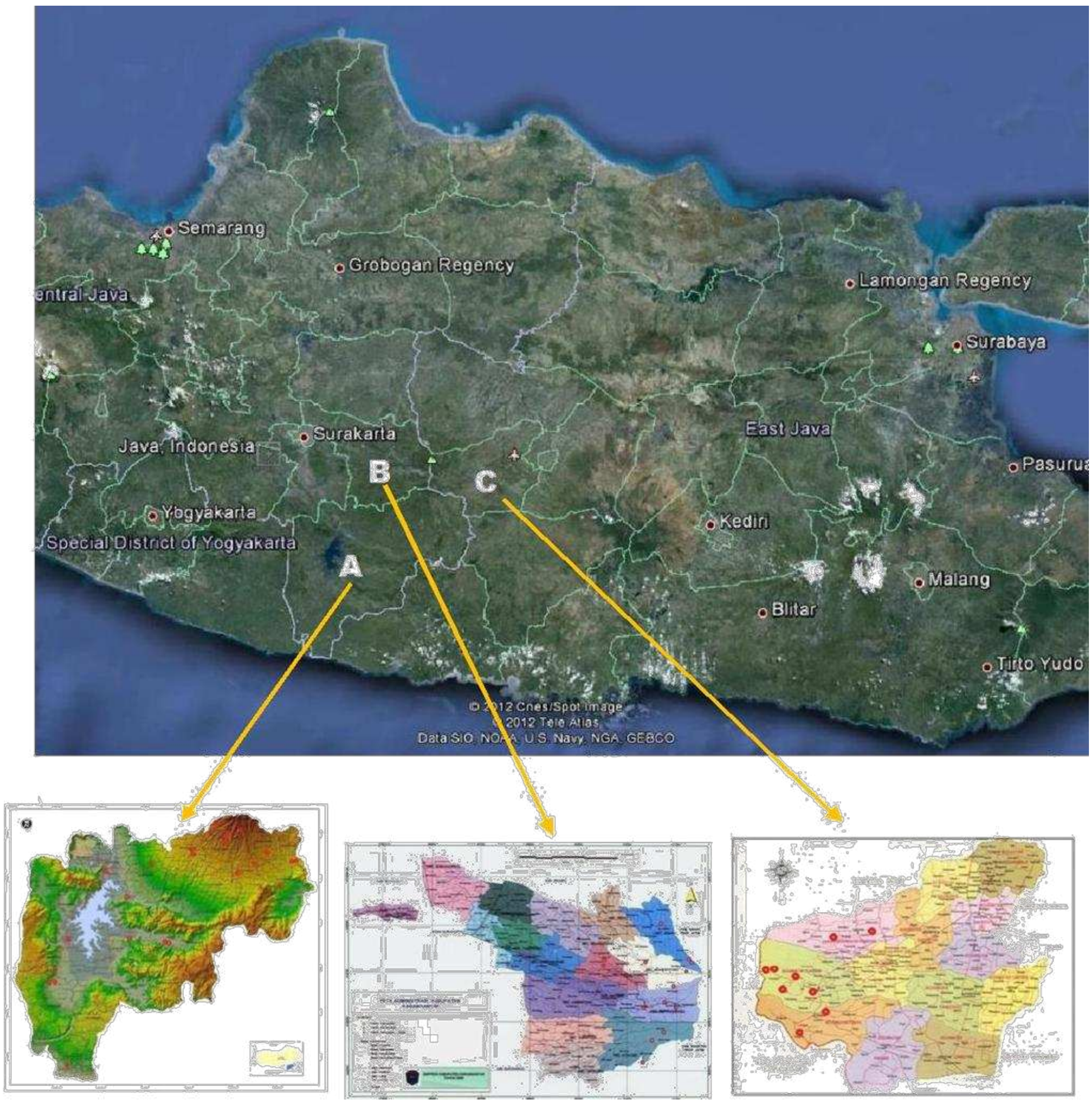
Chemical analysis was performed for the water, ash, lipid and protein contents.

Water Content Analysis

To calculate the water content of mesocarp of pumpkin, thermogravimetry was used. One to two grams of fresh mesocarps were blended and the weights were measured. Then, put in the sterile bottle and heated up for period of 8 to 24 hours at 105^{o}C. After the sample was cooled down, it was measured. The sample was further heated for 30 minutes and then cooled down using excicator until the content was able to be calculated by measuring the difference between the fresh and dry sample.

Ash/Dust Content Analysis

To determine the percentage of dust, 3-5 grams of dry mesocarp (Y) was put on mortar and burn at 105^{o}C for 1 hour, and then transferred into 600^{o}C for 12 hours until complete dust/ash was produced. The sample was transfered again to 120^{o}C for 1 hour and then cooled down gently using excicator until the white dust/ash appeared, and it was measured (Z). The percentage (%) of dust/ash was calculated by using the formula :



A. Wonogiri District B. Karanganyar District C. Magetan District

Figure 1: The Map Showing Areas where Samples are Collected

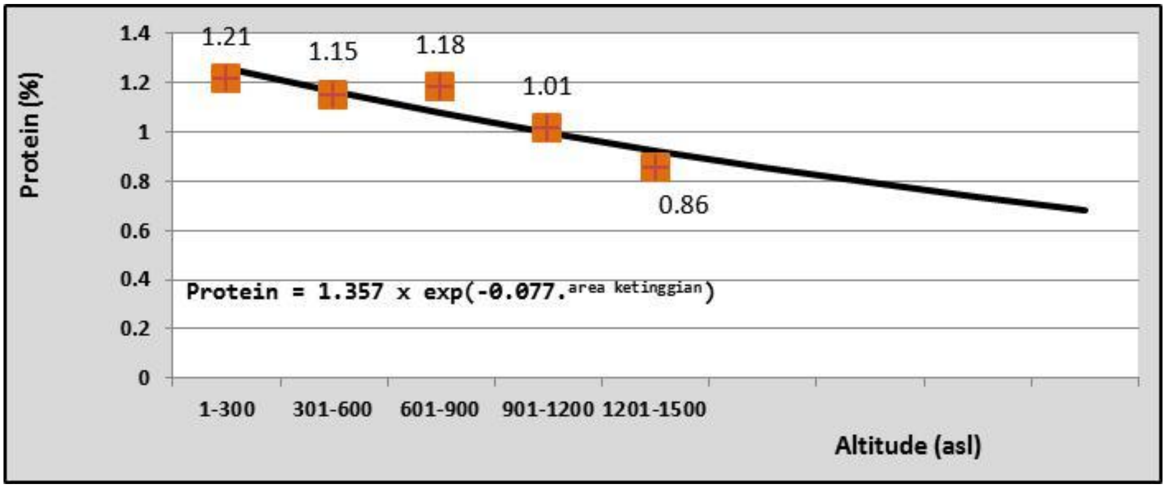


Figure 2. The Protein Trend Toward Various Altitude Areas

Soxhlet Method for Lipid Content

About one or two grams of mesocarp of pumpkin (G) was wrapped with free oil filter paper, then put in 105°C oven for 5 hours, and then the weight was measured after the cooling condition (H). The lipid was extracted by organic solvent (petroleum ether) for 4 – 14 hours until the clear solution appeared. This took about 10 – 15 circulation times, and then soxhlet extraction equipment was switched off. When the ether solution was moving down to reservoir tube, the samples was removed and left dry until all the ether gas completely evaporated, and the weight was measured immedietely (I). The percentage of lipid content was evaluated using a formula:

$$\frac{(\text{H} - \text{I})\text{x } 100\%}{\text{G}} = \%\text{ Of Raw Lipid}$$

Kjedahl Method for Measuring Total Protein Concentration

About 0.2 grams (J) of smooth grounded mesocarp was put in the Kjedahl tubes and then 0.7 grams of mixed catalyst and 5 ml of solid H_2SO_4 were poured. Destruction of the sample was done by gently heating it up. After heating, clear colorless solution appeared, the flowing heater was stopped. The cooled solution was diluted with 20 – 60 ml of distilled water, and several granular zincs were added, followed by the addition of 15 ml of boric acid given gently. The distilled water solution of Erlenmeyer tube was removed and then 3 drops of mixed red methyl and blue methyl were added and titrated gently with 0.02 N (K ml) HCl. This result was compared to the blank titrate (L). The percentage (%) of protein was calculated using the following formula:

$$\frac{(\text{K} - \text{L})\text{x N HCl x } 0.14 \text{ x } 6.25 \text{ x } 100\%}{\text{J}} = \%\text{ Of Protein}$$

Measuring The Percentage (%) of Carbohydrate Using the Method of Difference

The percentage of carbohydrate was calculated by subtracting 100% - % H_2O - % Dust - % Lipid - % Protein.

Statistical Analyses

Proximate analysis was used to test the chemical contents of proteins, lipids and carbohydrates, respectively (Sudarmadji *et al.*, 1996). In order to investigate the relationship between chemical contents of *C. moschata* and the environmental conditions, Pearsons' correlation test was used, while the statistical test of ANOVA was employed to obtain the influence of altitudes on the protein, lipid, and carbohydrate contents.

Table 1. The result of chemical analysis of protein, carbohydrate and lipids of *Cucurbita moschata* mesocarp at different level of altitudes

Varieties	Altitude (m asl)[1]	Biochemical contains (%)[2]				
		Protein	Carbohydrate	Lipids	Total of mineral	Water
Cucurbita moschata var. Bokor/bowl	1 – 300 asl	1.45	9.16	0.24	0.94	88.21
	301 – 600 asl	1.22	10.79	0.19	0.70	87.11
	601 – 900 asl	1.11	7.25	0.20	0.59	90.85
	901 – 1200 asl	1.03	8.52	0.21	0.86	89.37
	1201 – 1500 asl	0.92	7.38	0.10	0.58	91.02
Cucurbita moschata var. Kelenting/oval	1 – 300 asl	0.98	10.88	0.20	0.85	87.12
	301 – 600 asl	1.08	8.31	0.16	0.75	89.70
	601 – 900 asl	1.24	10.84	0.19	0.76	86.96
	901 – 1200 asl	1.00	7.88	0.15	0.79	90.19
	1201 – 1500 asl	0.79	8.32	0.16	0.54	90.18

Remarks : [1] asl = above sea level, [2] % expressed on dried tissue

RESULTS AND DISCUSSION

Biochemical Contents of Protein, Carbohydrates, and Lipids

Table 1 shows the highest carbohydrate contents of two varieties harvested at 301 - 900 meters asl. Besides, the lipid and protein were also found at that altitudes. This occurrence may be due to the fact that in such an environmental condition it would result in high efficiency of photosynthesis for the plant.

Based on the average carbohidrate contents, it was recorded that in all altitudes, the variety of klenting *C. moschata* was lower compared to the bokor. The difference in each level of altitude was quite high, ranging from a couple of hundreds asl until fifteen hundreds above sea level (as shown at Figure 3). That occurance was also found in the lipid content, except at that level of 601 - 900 asl, it was found that there was no difference between two varieties. Although statistical data showed that altitude did not gave any real significant value for the carbohydrate and lipid contents, but those were also recorded for proteins (see Figures 2 and 4). This result confirms that for some reasons, *C. moschata* fruit would be useful for people who are on diet of cholesterol, especially for those with heart attack. This pumpkin fruit was one of the fruits with very low percentage of lipid, and it only had about 2,18% of lipid content, while in peanut, the lipid content was reported to be around 45%. This lipid content was even lower than that of *Cucumis sativus* (2,50%) (Hussain *et al*., 2010).

The highest percentage of biochemical content in this plant was carbohydrate reaching nearly 70% after harvesting and this high percentage would decrease to a percentage of 58% after 3 months storage (Loy, 2004). During harvesting the *C. moschata* contains starch and sugar with around 52% and 15%, respectively, but after 3 months of storage, the percentage changed drastically in which the starch remained about 19% and the sugar became 43%. It was noted that variety of *C. moschata* klenting almost had a higher biochemical content than the bokor one. And this result has been recorded at every single altitude. It was predicted that the ability of klenting variety to adjust to a lower level of carbohydrate content would be able to produce more protein by using up the preserved and accumulated protein. Therefore, more C and N components would be used to synthesize the carbohydrate when unfavorable condition occurred (Mapegau, 2006).

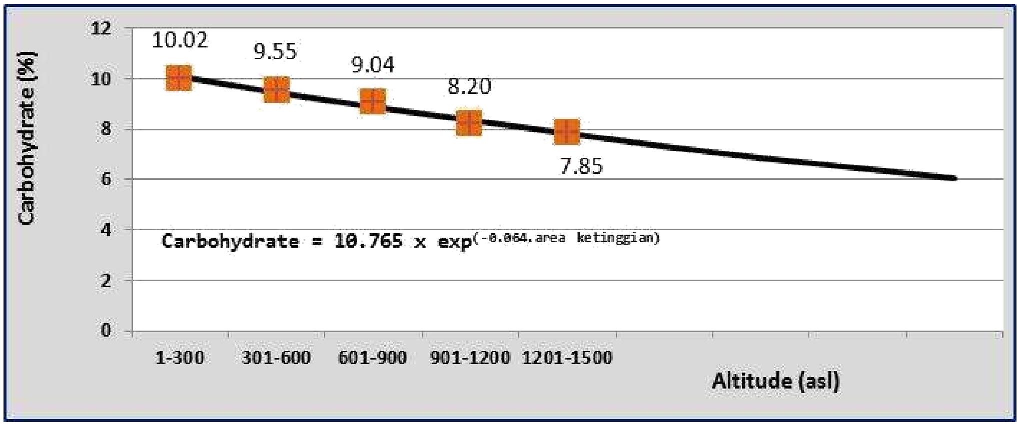


Figure 3. The Carbohydrate Trend Towards Various Altitude Areas

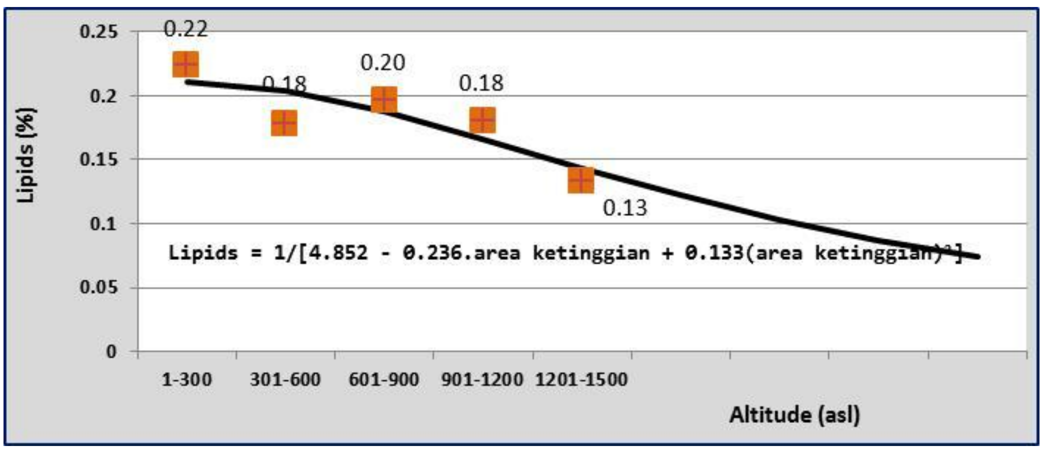


Figure 4. The Lipid Trend Towards Various Altitude Areas

Table 2. Correlation of Protein, Carbohydrates, Lipids Contents and Environment Conditions

Variables		**Altitudes**	**Protein**	**Carbohydrates**	**Lipids**
Altitudes	Pearson Correlation	1	-.277	-.248	-.334
	Sig. (2-tailed)		.052	.082	.018
	N	50	50	50	50
PH of Soil	Pearson Correlation	-.281*	.336	.124	.079
	Sig. (2-tailed)	.048	.017	.389	.584
	N	50	50	50	50
Temperature	Pearson Correlation	-.769**	.256	.381	.219
	Sig. (2-tailed)	.000	.073	.006	.127
	N	50	50	50	50
Soil Moisture	Pearson Correlation	.030	-.117	-.146	-.051
	Sig. (2-tailed)	.837	.420	.313	.725
	N	50	50	50	50

Correlation Between Environmental Condition (Altitudes) and Biochemical Contents

Based on the above data there was no significant relationship between the contents of protein, carbohydrate, lipid and the environmental conditions, such as; the altitudes, soil pH, temperature, relative humidity or even soil moisture. The result of Pearson Correlation Test showed that in all cases the relationship between altitudes and the content of carbohydrate, protein, and lipid were only -0,24%, -0,27%, and -0,33%, respectively (see Table 2). This, therefore, means that the altitudes where the plants were grown did not give any different results of their biochemical contents. Beside, that phenomenom was also shown in the results of environmental condition in producing the protein, lipid, as well as the carbohydrate.

These results showed that the pH of soil did not statistically give any strong relationship to biochemical contents of *C. moschata*. However it put forward the trend that the highest pH would usually result in the highest carbohydrate, protein, and lipid as well.

Altitude did not significantly affect the biochemical content of protein, carbohydrates, lipid in pumpkin fruit or *Cucurbita moschata*. However, it was interesting to note that the plants which grew at a higher altitude were found to have less percentage of biochemical content compared to the lower land. The best area where pumpkin produced a higher content of proteins, carbohydrates and lipids were at the level of 1-600 m asl. The highest and the lowest protein content were 2.45% and 0.31%, respectively, and the carbohydrate content was 16.65% and 4.05%; the lipid content was 0.45% and 0.03%. These results confirmed that *Cucurbita moschata* was one of the most significant species, which had high percentage (%) of carbohydrate compared to other species as reported by Hussain *et al.* (2010). Further studies of the nutrition values of this species, Kim *et al*. (2012) predicted that the high content of β-carotene in the fruit, would be useful in reducing the skin damage from the sun, while the α-carotene has been considered to have function in preventing or slowing the aging process. This fruit could also reduce the risk of developing cataracts and tumor growth.

Based on the above data, this species would further remain very atractive to be studied, especially to identify genetic and environmental variation. For example, correlation between biochemical contents and altitudes. Early results showed that using polyacrylamide gel electrophoresis (PAGE), for both izosyme and protein patterns indicated that there was relatioship between the above two factors.

CONCLUSIONS

The level of the relationship between environmental factors and the biochemical content of the pumpkin fruit or *Cucurbita moschata* grown in many areas was quite interesting to note. The plants grown at a higher altitude, pH value of soil as well as air temperatures, showed a tendency that the content of protein, carbohydrates, lipids were also quite high. While the influence of higher soil moisture resulting in the contents of protein, carbohydrate, and lipid tended to be low too. The level of biochemical content of pumpkin in higher altitude tended to be lower compare to the lower one. Finally, this evidence confirmed that *C. moschata* was typical tropical plants.

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REFERENCES

Agbagwa, I.O. and B.C. Ndukwu. 2004. The value of morpho-anatomical feature in the systematics of *Cucurbita landraces* (Cucurbitaceae) species in Nigeria. Afr. J. Biotechnol. 3(10): 541-546.

Aruah, C.B., M.I. Uguru and B.C. Oyiga. 2010. Variations among some Nigerian *Cucurbita landraces*. Afr. J. Plant Sci. 4(10): 374-386.

El-Aziz, A.B. Abd. and H.H. Abd. El-Kalek. 2011. Antimicrobial proteins and oil seeds from pumpkin (*Cucurbita moschata*). Nature and Sci. 9(3): 105-119.

Caili, F., Shi H. and L. Quanhong. 2006. A Review on pharmacological activaties and utilization technologies of pumpkin. Plant Food Hum. Nutr. 61(2): 70-77.

Hussain, J., N.U. Rehman, A.L. Khan, M. Hamayun and Z.K. Shinwari. 2010. Proximate and essensial nutrients evaluation of selected vegetables species from Kohat region, Pakistan. Pak. J. Bot., 42(4): 2847-2855.

Kim, M.Y., E.J. Kim, Y.N. Kim, C. Choi and B.H. Lee. 2012. Comparison of the chemical compositions and nutritive values of various pumpkin (*Cucurbitaceae*) species and parts. Nutr. Res. Pract. 6(1): 21-27.

Loy, J. B. 2004. Morpho-physiological aspects of productivity and quality in squash and pumpkins *(Cucurbita spp).* Critical Reviews in Plant Sci. 23(4): 337-363.

Mapegau. 2006. The influence of water stress on the growth and harvesting seeds of soybean (*Glycine max* L. Merr.). KULTURA Sci. J. Agr. 41(1): 43-51.

Maynard, D.N.. 2001. Variation among tropical pumpkin (*Cucurbita moschata*) cultivars in susceptibility to silverleaf. Cucurbits Genetic Cooperative Report. 24: 71-72.

Pandya, J.B. and T.V.R. Rao. 2010. Analysis of certain biochemical changes with growth and ripening of pumpkin fruit in relation to its seed development. PRAJNA J. Pure Appl. Sci. 18: 34-39.

Revanz, R.. 2011. The yellow plant of pumpkin. http://rachmadrevanz.com/2011/tanaman-labu-kuning.html. Accessed on December 7, 2011.

Radovich, T.. 2010. Farm and forestry production and marketing profile for pumpkin and squash (*Cucurbita spp*). Permanent Agriculture Resource (PAR). USA.

Sudarmadji, S., Haryono and B. Suhardi. 1996. Analysis of food materials and agriculture. (in Indonesian). Liberty, Yogyakarta

Yenrina, R., N. Hamzah and R. Zilkia. 2009. The quality of jam made up from the mixture of *Ananas comosus* and *Cucurbita moschata.* (in Indonesian). J. Educ. Fam. 1(2): 33-42