Determination of Changes in Amylose and Amylopectin Percentages of Cowpea and Green Gram during Storage

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Abstract

Freshly harvested Samples of cowpea (Variety Dhawala and Waruni) and green gram (Variety MI-5) were collected from Research, Field Crops Research and Development Institute, Mahailluppallama. Sri Lanka. The objective of the study is to determine the change of amylose and amylopectin percentage in starches of stored Green gram and Cowpea with time and to determine the physio-chemical properties of respective starches and flours accordingly. Study was carried throughout 12 weeks in 2 week intervals starting from the day 1 after the legumes being harvested. A significant change in amylose percentages of the legumes was not resulted within the study period of 12 weeks for all 3 varieties though declining trend with weak correlation was observed. (Declines of 0.14% for Waruni, 0.13% for Dhawala and 0.18% for MI-5) but there was a significant difference among the two cowpea varieties. Highest amylose percentage was shown in cowpea variety Dhawala (35.25-35.12%) and the lowest in Cowpea variety Waruni (28.85-28.71%).

Keywords: Green gram; Cowpea; Amylose percentage; Amylopectin; Waruni; Dhawala; Food legumes

Introduction

Food legumes are grown by farmers all over the world because it provides nutritionally balanced diet. (*Vigana radiate* (L.) R. Wilczek) [1], is often known as green gram or mung bean is native to India and Central Asia. Lots of Asian traditional foods such as porridge, snacks and noodles are made using mung bean seeds or flour [2]. Cowpea, (*Vignaunguiculata* (L.) Walp) [3], is a warm-season, annual legume. It is commonly cultivated as a nutritious andvery palatable food source. Locations where cowpea is grown as a commercial crop include United States, Middle East, Africa, Asia, and majority of tropical and subtropical areas in the world. Starch is widely used in the food industry apart from a staple element in nutrition such as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent [4]. Upon the amylose and amylopectin percentages the physiochemical properties in starches are changed such as swelling factor and amylose leaching, crystalline structure, thermal properties, and pasting properties which are important in food industry [5]. The quality degradation of the grains is quantified via the Dry Matter Loss. What denoted as the Dry Matter Loss is the loss of carbohydrate which is available, due to the respiration [6], so there can be a change in amylose and amylopectin percentages in stored legumes upon time.

Materials and Methods

Sample collection and storage

One kilogram each from freshly harvested Green gram (Variety MI-5) and cowpea (Variety Dhawala and Waruni) were purchased from Field Crops Research and Development Institute, Mahailluppallama, Sri Lanka. All 3 legume samples were kept under normal room condition (28±1 °C, 90% RH).

Sample preparation

All 3 legume samples were ground separately for 5 minutes using the domestic grinder and sieved using 212 micron sieve. Powder sieved through the sieve was used for the determination of amylose percentage. The obtained flour was kept in air tight containers.

Determination of amylose content

Each legume sample was taken and 100.0±0.5mg of each sample was measured in to a clean and dry Erlenmeyer flasks. From one variety 3 samples of 100.0±0.5mg was taken in to 3 flasks each. Then 9ml of 1N NaOH was added to the samples followed by 1ml of 95% ethanol. The samples were kept overnight to completely gelatinize the starch and obtain a clear viscous gelatinous solution. The flasks were washed

several times using distilled water and transferred to 100ml volumetric flasks and they were topped up using distilled water. The solutions were shaken well in order to completely dissolve the starch. Subsequently 5ml of each solution which were prepared latest was transferred to other 100ml volumetric flasks and they were covered via aluminium foils. Another volumetric flask was covered via aluminium foil and 5ml 0.09N NaOH was added to it. Then each of them was fed by 1ml of 1N acetic acid and 2ml of 0.2% Iodine solution. The n all the flasks were topped up by distilled water and shaken well. They were kept in the dark for 20 minutes and absorbance was measured at 620nm using the UVVIS spectrophotometer. The final solution prepared using 0.09N NaOH instead of flour samples was used as the blank solution. A standard curve was drawn against the absorbance and amylose concentration. According to the standard curve the amylose percentage of the whole sample was measured along with the readings of moisture percentage of the samples which were measured parallel, using electric moisture analyzer. Finally according to the total starch percentage of the each sample which was obtained amylose percentage of each legume sample (as per starch) was calculated.

Results and Discussion

Determination of amylopectin content

Amylopectin percentage was calculated using the below mentioned equation. The equation was explained by Juan et al. [7]. Average value of amylose % obtained from the equation put up as per total starch was considered for the calculation.

Amylopectin = (100 - Amylose %)

Determining the total starch percentage

Total starch percentage of the legumes was determined according to the method of Sri Lankan Standards 964: 1992.

Data analysis

Results were statistically evaluated from the software Minitab 17 to determine the significant change of amylose percentage from week 0 to week 12 under the following hypotheses under 95% confidence interval.

H_0 = All means of are equal regarding the amylose percentage in the tests carried out from 0 to 12 weeks

H_1 =At least one mean is different regarding the amylose percentage in the tests
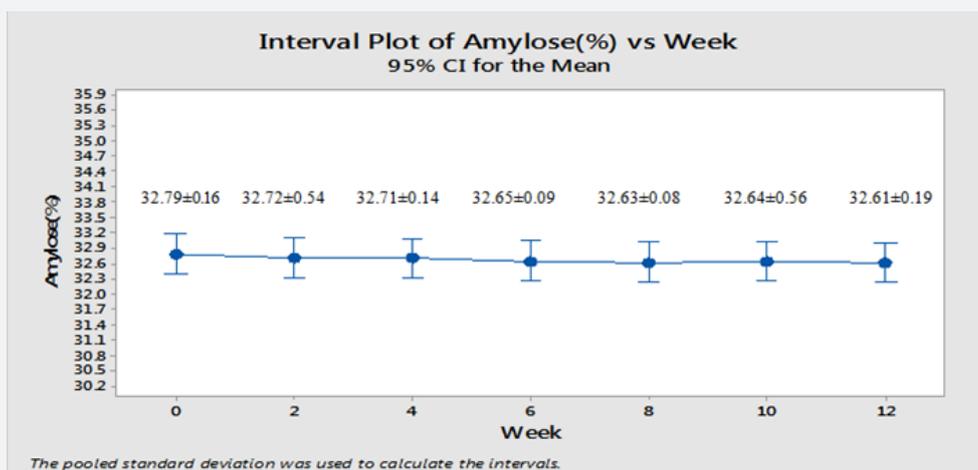

Figure 1: Amylose Percentage in Green gram Variety MI-5.

Amylose percentage (as per Starch) for the green gram variety resulted a mean of 32.67±0.27% (Figure 1). Which is within the range of results observed by Kaur et al. [8] 29.9-33.6% both cowpea varieties showed higher mean amylose percentages (as per starch) (Waruni-28.79 ± 0.13 %, Dhawala -35.16 ± 0.20%) (Table 1) than results obtained in the study done by Huang 25.8% [7]. Varietal difference must have caused the difference as some of the cowpea starches had shown 39.09% to 42.78% of amylose content in their respective starches according to [9]. According to the hypotheses carried out regarding all the 3 legume varieties separately, null hypothesis wasaccepted since all the P values resulted from the tests had a numerical value >0.05 at 95%confidence level (Waruni- 0.862, Dhwala 0.992 and MI-5-0.991). So as per the readings obtained all the varieties had no significant change in their respective amylose percentages (as per starch) upon time period of 12weeks. All the regression equations showed a declining pattern but cannot be taken in to consideration since the R^2 value of all of the varieties (Waruni- 0.373, Dhawala-0.603, MI-50.689) were lower than 0.9 showing weak correlation among the values in the regression model. Within the time period of first four months after harvesting the amylose percentage of legume starches is reduced in insignificant levels. The rate of reduction is increased with

time till the time period of 12 months [10]. When comparing the mean value percentage loss of amylose percentage happened within the time period of 12 weeks considering the mean values of respective weeks regarding all legume varieties (The percentage difference is considered regarding the mean amylose percentage value at harvesting and the lowest mean amylose percentage recorded in a week within the time frame of the study. Because of the change of amylose percentage is lower than 0.5% in every legume variety the percentage of error regarding readings must be high, which causes apparent slight increases in some respective weeks.) the highest reduction of amylase percentage was recorded is variety MI-5 (0.18%) and two cowpea varieties had almost similar reductions of amylose content (Waruni - 0.14% and Dhawala-0.13%) during the study period of 12 weeks. According to the study carried out by Pushpamma & Vimala [10] Green Gram had shown a 0.49% reduction of amylose in its starch within the first 4 months after harvesting and rest of the legumes subjected to the study had delivered similar results -reduction lower than 0.5% (Pigeon-pea (Cajanuscajan) -0.41%, Chick-pea (Ciceraritinum) -0.17%, and Black gram (Phaseolusmungo) -0.10%) [10]. Since there is no significant change in the amylose and amylopectin percentage in each legume upon time within the study of 12 periods according to the study there are no specific recommendations which can be formed for specific food making upon their storage time. Specifications and recommendations which are made according to their respective general amylose and Amylopectin percentages are valid for all 3 legume varieties where the readings are taken at any time during the period of the first 12weeks after their harvest under ambient storage.

Table 1: Amylose percentage in Cowpea varieties.

Cowpea Varieties (Amylose Content %)		
Week	Waruni	Dhawala
0	28.85±0.15	35.25±0.24
2	28.84±0.21	35.17±0.21
4	28.82±0.15	35.14±0.22
6	28.71±0.16	35.19±0.13
8	28.78±0.07	35.12±0.14
10	28.82±0.14	35.12±0.38
12	28.74±0.10	35.13±0.21

Since there is no significant effect by the time spent after the harvest within the 12weeks regarding the results of this study to the amylose percentage of the starch in all three legume varieties, cross comparison was done to two cowpea varieties considering the effect of time is negligible regarding their respective amylose percentage (as per starch). The comparison was done to determine whether there is a significant difference of the amylose percentages (in starch) among two cowpea varieties,

H_0 = All means of are equal regarding the amylose percentage in the tests carried out for all 3 legume varieties.

H_1 =At least one mean is different regarding the amylose percentage in the tests.

The statistical test results a P value of 0.000 stating that according to the results the Null hypothesis is rejected and alternative hypothesis is accepted under 95% confidence. Since all the data from each variety is distributed normally a two sample T test was carried out between two cowpea varieties Dhawala and Warunito determine what the varieties are showing significant difference in amylose content between each other. Two sample T test granted P value of 0.000 under 95% confidence interval, and therefore according to the data obtained via the test two cowpea varieties showing significant differences in Amylose content between each other. According to the mean amylose percentage values regarding each variety as per starch, according to the data Cowpea variety Dhawala (35.16 ± 0.20%) has the higher amylose percentage among 2 cowpea varieties while variety Waruni has the lowest (28.71 ± 0.16%) Since the amylopectin content in starch can be calculated in each variety by deducting from 100% the higher amylopectin percentage (as per starch) is present in variety Waruni (71.29 ± 0.16%) and Lowest in Variety Dhawala (64.84 ± 0.20%) As there is a significant difference between two cowpea varieties regarding their respective amylose and amylopectin contents there is a difference between their several physiochemical properties [5] and might be in their GI even though not significant [11]. Swelling factor of starches is decreased upon the increase of amylose percentage in starches at a certain temperature above 55 °C. Swelling is facilitated by amylopectin and disturbed by amylose. Amylose leaching is increased upon the amylose percentage of starches [5]. According to the results obtained from the study the higher swelling factor can be expected from the variety Waruni while a lower swelling factor can be expected from the cowpea variety Dhawala at a similar temperature. Leaching of amylose should be the higher in variety Dhawala between the two cowpea varieties while variety Waruni being lower. Regarding the formation of crystalline structure of a food product dominated by starches the crystallinity of the respective starch should be taken in to account. The relative crystallinity in the starches varies with the amylose content and branch chain length distribution of amylopectin. Amylopectin is generally providing the crystallinity of starch while amylopectin is considered as a disrupter of the crystalline packing of amylopectin. High amylose starches are showing less crystalline structure than Low amylose starches [5]. According to the results if to get a high crystalline structure in the final product of a process food the Variety Waruni is the recommended variety between two cowpea varieties in order to obtain starch as it has the highest amylopectin percentage. Variety Dhawala is the more suitable variety if to get the least crystalline structure regarding a processed food material. Regarding thermal properties of

starches increase of amylose percentage is resulted in high onset temperatures of gelatinization, peak and conclusion temperatures of gelatinization. The cause of the result is because crystalline regions of higher amylose starches restricted the hydration of amorphous regions, resulting retardation of swelling and gelatinization, whereas lower amylose rice starch consisted mostly of crystalline regions and thus could begin gelatinization at a lower temperature. The amylopectin molecular structure is related to crystalline structure of starch granules [5]. According to the results obtained by the tests processed foods which are in need of gelatinization at lower temperatures variety Waruni is the more recommended while variety Dhawala is the less recommended cowpea variety in corresponding their amylose and amylopectin percentages. Regarding the pasting properties of a processed food or a food paste there are few factors which should be considered such as amylose content, lipid content and amylopectin branch chain distribution. Since all the legume varieties have almost similar, low lipid percentages effect caused by lipid is minimum. Along with the increase of amylose percentage of the starches pasting temperature, set back and the final viscosity are increased while peak viscosity is decreased. If a certain process food is in need of physiochemical properties like high pasting temperature and high final viscosity cowpea variety Dhawala is the recommended variety between two cowpea varieties while cowpea variety Waruni is the non-recommended variety. If a processed food is in need of high peak viscosity variety Waruni is more recommended legume while variety Dhawala is the less recommended one. There is no significant relationship between the amylose or amylopectin content and glycemic index. But there is a negative insignificant trend in association with increasing amylose content and Glycemic index [11] and studies have indicated that chronic consumption of high amylose diets have a strong possibility to occur lower postprandial blood glucose levels [12]. Both of these trends are beneficial for diabetic patients. According to the results obtained Variety Dhawala can be considered to be recommended to be included in diets of diabetic patients (considering the composition of starch) and its starches can be recommended to be used in foods for diabetic patients over other two legume varieties. Other cowpea variety Waruni is the less recommended variety between the two.

Conclusion

According to the results obtained for the amylose percentages in legumes all 3 legume samples (Dhawala, Waruni and MI-5) did not show a significant change within 12 weeks but granted negative regression models with weak correlations. According to the results Variety Waruni showed the maximum change of 0.14% within the study period (28.85 ± 0.15% to 28.71 ± 0.16%) while having the mean amylose percentage of 28.79 ± 0.13% throughout the study. Variety Dhawala showed the maximum change of 0.13% within the study period (35.25 ± 0.24% to 35.12 ± 0.14%, 35.12 ± 0.38%) while having the mean amylose percentage of 35.16 ± 0.20% throughout the study. Variety MI-5 showed the maximum change of 0.18% within the study (32.79 ± 0.16% to 32.61 ± 0.19%) while having the mean amylose percentage of 32.67 ± 0.27% throughout the study. Even though neither of the 3 legume samples showed a significant change in their amylose content within time, two cowpea varieties showed significant differences between the varieties as higher being the variety Dhawala while the lower being variety Waruni.

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