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*Edited by*

**C. Arteaga, C. Franco, J. Silva, D. Terán**

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# INTRODUCTION

The first international congress of Food Science and Biotechnology (CICABI from the Spanish) – 2018 was organized at the Ambato city in Ecuador, between the 25th and 29nd of June of 2018. The aim of the congress was to promote the careers of Food sciences and biotechnology of the Universidad Técnica de Ambato (UTA). Also this event promoted the presentation of the milestones of the different researches that are carried out at the UTA, but also the presentation of senior and young researchers of Food sciences and Biotechnology from different countries.

This international congress had the honour of hold oral presentation from well-known international researchers. Among these international researchers we can mention, Dra. Rosa Lamuela and Yolanda Cajal from the Universidad de Barcelona - España, Dr. Diego Hidalgo from the Universidad de California - Berkeley, Dra. Silvina Drago from the Universidad del Litoral de Argentina and the Msc. Gabriele Bigoni from the Universidad Nacional Autónoma de México. Also this congress had the honour to have the oral presentation from Jhonn Pressing representative de FAO – ECUADOR, Dr. César Paz y Miño and other well-known researchers that shared their knowledge with the participants of this congress.

It is important to mention that CICABI – 2018 had the support of different administrative and technical direction from the UTA. Especially, the Direction of Research and Development (DIDE from the Spanish) and the public company from the UTA (UTA-EP from the Spanish), that provided the economical support for this especial edition.

This special edition is the result of the participation of academics, researchers and students in this international congress. Also this special edition highlights Food sciences and Biotechnology as principal topics for this volume. Also these topics had high relevance and attention for the Central Andean Region in South America. Therefore, this edition highlights researches that show technics developed for the region such as, technics that allows the improvement of food processing and experiments in the search of primary material with higher nutritional components for the development of new food products. In addition, this special edition presents studies that had as base line the analysis of the native microorganisms and application of them in an industrial level.

CICABI – 2018 made a precedent of scientific production not only for Ecuador but also for other countries in the region. Thus, CICABI constitute a space for knowledge interchange between scientists in the field of Food sciences and Biotechnology.

*C. Arteaga, C. Franco. J. Silva, D. Terán*

# THE EFFECT OF SUNLIGHT ON THE CONTENT OF THIOCYANATES, SUGARS AND STARCHES IN ACCESSIONS OF *TROPAEOLUM TUBEROSUM* RUÍZ & PAVÓN

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## ABSTRACT

The objective of the study was to identify accessions of *T. tuberosum* with highest quantities of thiocyanates, starches and sugars. The study was performed in Cevallos, Ecuador. Six accessions were cultivated in six individual plots and processed. The Yellow, White and Purple accessions were exposed to sunlight for periods of 3, 6 and 9 days. The resulting biomass was analysed: The Purple variety presented the greatest quantity of thiocyanates (663 mg/100g) after three days' exposure. Meanwhile, the Yellow variety showed the highest amount of starches (47%) and total sugars in freshly-harvesting. The results reveal the potential of these accessions as a source of starch and thiocyanate.

*Keywords*: sunlight, sweeting, thiocyanates, starches and sugars, secondary metabolites

## 1. INTRODUCTION

*Tropaeolum tuberosum* (mashua) has been grown in the Andean region for thousands of years (CONDORI *et al*., 2008). It is traditionally cultivated - as a single crop, or together with other Andean tubers, such as the potato, oca and ullucus - between 2,400 and 4,300 metres of altitude (MANRIQUE *et al*., 2014). Despite the importance of *T. tuberosum* as a staple of ancestral communities, in recent decades it has been largely replaced in the diet and agriculture of most rural families (GRAU *et al*., 2003). However, it has established its importance in natural medicine for its therapeutic properties in the treatment of genitourinary disease (MANRIQUE *et al*., 2014) as well. Among the Andean countries there are a great number of varieties, but in Ecuador the cultivable genotypes are limited in number. The yellow variety is most common and may be found in the marketplace (ESPIN *et al*., 2003). Mashua is known for its primary and secondary constituents (isothiocyanates) of interest to both industry and medicine. However, the variability of these in different crops is unknown, as is the effect of sunlight on their levels of concentration.

Preliminary investigations indicate that mashua plants contain, among other substances, isothiocyanates, proteins, starches, and sugars (KING and GERSHOFF, 1987). The principle glucosinolates identified were: 4-Hydroxybenzyl GSL (OHB, Glucosinalbin), Benzyl GSL (B, Glucotropaeolin), and m-Methozybenzyl GSL (MOB, Glucolimnathin) (ORTEGA *et al*., 2006). Isothiocyanates have properties which enhance the prostate treatment in men (AIRE-ARTEZANO *et al*., 2013). Mashua plants contain high levels of glucosinalates (methoxybenzyl glucosilanate and benzyl glucosilanate). These compounds produce isothiocyanates in the presence of the mirosinase enzyme. Benzyl isothiocyanate appears to be most abundant in mashua plants and has been reported to be an inhibitor in the development of breast and stomach cancer (QUIROS and ORTEGA, 2004). Furthermore, mashua plants release glucose, sulphates, and toxic compounds such as nitriles, thiocyanates and oxazolidines, through hydrolysed glucosinalate (BELL *et al*., 2015).

In another study, in Colombian mashua accessions, isothiocyanates were quantified through the method of High-Performance Liquid Chromatography (HPLC). From the four accessions studied, Yellow yielded between 12.96 and 27.69 μmoles/g; Purple 19.32 and 22.54 μmoles/g; Purple-White 11.47 and 20.05 μmoles/g; and Red-White yielded between 4.86 and 45 μmoles/g (ARIAS, 2011). Additionally, there is evidence that polyphenols derived from mashua plants are effective antioxidants, combatting the damage caused by oxidizing processes in biological structures. These properties may be utilized in the food and cosmetics industry (CHIRINOS *et al*., 2008). In Ecuador, the yellow variety known as *zapallo* has been shown to consist of 9.17% protein; 46.92% starch; and 42.81% (ESPÍN, VILLACRÉS and BRITO, 2003).

There are evidence that mashua plants reduce sperm production (CÁRDENAS-VALENCIA *et al*., 2008). Extracts of *T. tuberosum* have been evaluated in the effect on the male reproductive system of mice, narrowing sperm-production parameters without any toxic effects (VÁSQUEZ *et al*., 2012). The use of the plant as an aphrodisiac is thought to derive from these qualities (JONHS *et al*., 1982).

Mashua plants take various forms and colours (MELCHIORRE, 1985). According ancestral knowledge, the darker varieties (purple and black) are considered to have greater medicinal value. However, these are poorly cultivated, so as consequence rarely seen in local markets, and in danger of disappearing (KING and GERSHOFF, 1987). The yield of the mashua crop is generally some 36 t/ha when grown with suitable fertilizers (VALDIVIA *et al*., 1999).

In some indigenous communities, mashua tubers are still consumed once they have been exposed to sunlight (called sweetening). This constituting a part of the alimentary diet with other Andean tubers (GONZALES *et al*., 2003). Furthermore, artisanal agroindustry has developed some mashua by-products for medicinal purposes. Herbalists frequently elaborate mashua extracts or dehydrated mass of tubers for sale.
The object of this study was to identify which accessions of mashua contained the highest levels of thiocyanates, sugars and starches in freshly harvested under exposed to sunlight, in order to better understand the traditional practices of rural areas, and to define the optimum exposure to sunlight that produces the greatest yield of medicinal compounds.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Six mashua were recollected from indigenous communities within the provinces of Tungurahua and Chimborazo. All the accessions were sowed in sandy loam and grown in separate plotin in the experimental farm of the Agricultural Sciences Faculty-Technical University of Ambato, Ecuador, located at 2740 metres of altitude. These accessions were named Yellow (A1), White (A2), Purple (A3), Milicia Red (A4) Poza Rondador (A5) and Green Yellow (A6). From the freshly-harvested tubers, samples were taken for an analysis of their thiocyanate, starch and sugar content. In addition, the samples were subjected to phytochemical analysis to evaluated the presence of secondary metabolites based in the method proposed by CASTILLO *et al*. (2014).

### 2.2. Methods

2.2.1 Natural sweetening

Three of the accessions (A1, A2, and A3) were subjected to the natural sweetening process in nine days. After harvesting the three varieties were washed and exposed to sunlight in an open area with no danger of interference. The levels of solar radiation are expressed in hours of sunlight per days, as follows: 11.6h/ after 3days; 21.1h/ after 6 days and 29.3 h/ after 9 days.
The factors studied were the accessions and the length of time exposure to sunlight. As a control sample (time=0) an unexposed root of the plant was used. The three accessions were subjected to three lengths of exposure to sunlight: T1=3 days; T2=6 days; T3=9 days. The experimental model consisted of 3 accessions in three exposures (3x3) + 1 sample with 3 repetitions, making a total of 30 experimental units. The biomass collected from the experiment was sliced into thin sections and dried at a temperature of 40°C for 72 hours, and then ground into powder for analysis.

2.2.2 Starch content

Starch content was measured using the polarimetry method described by HAROLD *et al.* (1993). In accordance with this method, the starch was treated with a dilute solution of hydrochloric acid in water bath and determine the rotation angle with the polarimeter. The percentage of starch was calculated using the mathematical formula: % starch = (a-b)f, where a = the rotation angle of the sample in degrees, b = the rotation angle of the blank in degrees, and f = the starch factor.

2.2.3 Total sugars

The measurement of total sugars was developed by the method of DUBOIS *et al*. (1956) with modifications. This method consists in performing a hydrolysis of the polysaccharides in a warm acidic medium. The anthrone reacts with the hexoses and the aldopentoses to produce a blue-green complex with a maximum absorbency at 625 mm. The Indian Standards Institute method was used for the measurement of the thiocyanates, as proposed by the FAO. The isothiocyanate contained in the matrix was distilled with water vapour and collected in a solution of silver nitrate. The excess of the solution of $AgNO_3$ was determined by volumetric method with a standard solution of ammonium thiocyanate. The content of isothiocyanate was calculated with the following mathematical formula: isothiocyanate (g/kg) = 9.915(Vo-V1) x N x 10/M, where Vo = the volume of the standard solution of ammonium thiocyanate needed to determine the blank (ml); V1 = the volume of the solution of ammonium thiocyanate needed to establish the sample (ml); N = the normality of the standard solution of ammonium thiocyanate; and M = the weight of the sample.

## 3. RESULTS AND DISCUSSION

### 3.1. Analysis of thiocyanates

The analysis of thiocyanates, total sugars and total starch carried out in six samples of recently-harvested mashua accessions show differences in the accessions of the study. In regard to the content of thiocyanates, the sample of Poza Rondador showed the greatest content (574.2 mg/100g). Meanwhile the Yellow accession showed the lowest content (240.9 mg/100 g). On the other hand, the Green accession showed the greatest total sugar content (26.9%) and the White accession the least amount (17.9%). As to total starch content, the Yellow accession showed the greatest content (46.8%) and Red Milicia showed the least (2.5%).

The presence of a low content of thiocyanate and a high content of starch in the Yellow accession could be an excellent option to improve the cultivation and commercialization. The accessions with a high thiocyanate content present unpleasant taste and are little accepted to consumption (ESPINOSA *et al*., 1996). Based in previous studies that mashua can be effective in prostate infections treatment, the Yellow variety has the lowest content of thiocyanates, so it would be the least suitable for pharmaceutical uses. The Poza Rondador and Red Milicia varieties could be the most suitable option due to their high thiocyanate content after harvesting. Table 1 shows the content of thiocyanates per 100 g of dry plat material, sugars and starches are expressed in percentage.

### 3.2. Screening

The phytochemical screening demonstrated that the Poza Rondador accession had the greatest content of alkaloids, sterols and 2 – deoxy sugars (Table 2). Meanwhile, the Green-Yellow accession shows the lowest ocontent of all secondary metabolites evaluated in this study. The White and Yellow varieties showed a high content of anthocyanins (CHIRINOS *et al*., 2006).

**Table 1.** Content of thiocyanates, sugars and starches in fresh samples, after harvesting.

| Accessions | Thiocyanate* (mg/100 g) | Total sugars (%) | Total starches (%) |
|---|---|---|---|
| Yellow | 240.9[c] | 18.838[de] | 46.842[a] |
| White | 511.5[b] | 17.936[e] | 13.642[b] |
| Purple | 491.7[b] | 20.188[d] | 14.493[b] |
| Red Milicia | 504.9[b] | 22.733[c] | 2.525[d] |
| Poza Rondador | 574.2[a] | 24.528[b] | 3.063[d] |
| Green-Yellow | 534.6[ab] | 26.908[a] | 9.977[c] |

*Expressed as allyl thiocyanate.

**Table 2.** Phytochemical screening of various mashua accessions.

| | Alkaloids | Sterols | 2 – deoxy sugars | Leucoanthocyanins | Flavonoids | Tannins | Anthraquinones | Saponins |
|---|---|---|---|---|---|---|---|---|
| White | ++ | +++ | + | +++ | + | - | - | + |
| Poza Rondador | +++ | +++ | +++ | ++ | + | - | - | + |
| Purple | ++ | + | ++ | + | ++ | - | - | ++ |
| Green-Yellow | + | + | + | + | + | - | - | + |
| Yellow | ++ | ++ | ++ | +++ | + | - | - | + |
| Red Milicia | +++ | ++ | +++ | ++ | ++ | - | - | + |

## 3.3. Sunlight on the content of thiocyanates

Regarding the effect of sunlight on the content of thiocyanates, starches and total sugars were analysed. The results show the content of thiocyanate under sunlight increase the first three days, reaching 548.9 mg/100 g and decline after this. In contrast, fresh harvested mashua tubers showed the highest level of starch (24.9%), and by the ninth day of exposure, starch content decreased significantly. Meanwhile sugar content showed a increase over the time of exposure to sunlight (Table 3). The results in general evidence the importance of sunlight exposure in the carbohydrates transformation and explains why rural communities in producing countries prefer to consume these tubers after sweetening process.

**Table 3**: Variation in thiocyanates, starch and total sugars according to length of exposure to sunlight*.

| Parámetros | Unit | Days | | | |
|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 |
| Thiocyanate + | mg/100g | 414.7 | 548.9 | 523.49 | 481.58 |
| Total starch | % | 24.992 | 18.173 | 16.472 | 14.529 |
| Total sugar | % | 19 | 20.57 | 21 | 21.7 |

*Average of three accessions.
+ Expressed as allyl thiocyanate.

### 3.4. Quantity of thiocyanates per accession

Regarding the behaviour of the quantity of thiocyanates per accession, it was found that the Purple accession, after three days of exposure, showed the highest quantity (663 mg/100 g) of all the accessions studied. After this time, the content of thiocyanate decline. The White accession present a different pattern, with a steady decline during the first three days, and gradually became to its initial thiocyanate content (Fig. 1). Previous studies in Peru, report differences in accessions exposed to sunlight freshly harvesting, where the antioxidant capacity and the content of bioactive compounds changed. Meanwhile the anthocyanins showed a marked decline (CHIRINOS *et al*., 2007). A similar pattern was found in the thiocyanates content of the Purple variety, with a significant decrease over time of exposure to sunlight. In regard to glucosinolates, the Purple varieties have been found to contain between 19.32 and 22.54 mg/100 g (ARIAS, 2011).

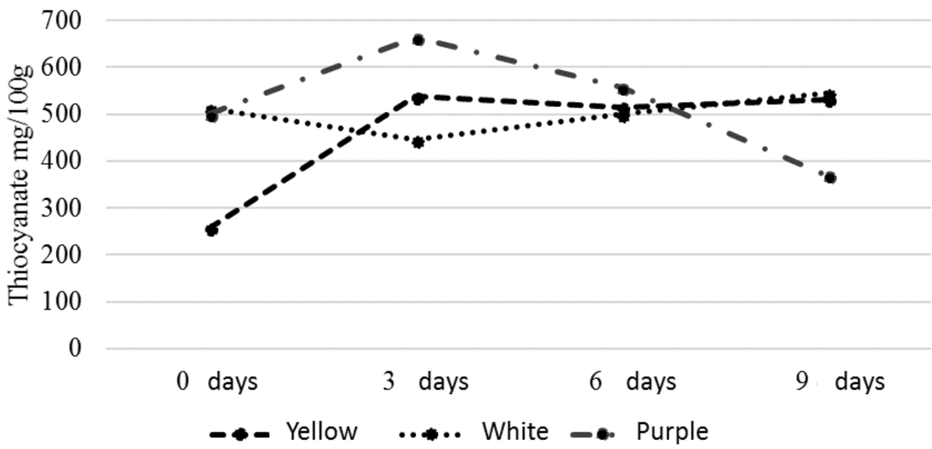


**Figure 1.** Variation in thiocyanates with time of exposure to sunlight.

### 3.5. Total sugars

In reference to total sugars, White accession showed the highest content of sugar (23%) after three days of exposure under sunlight. Purple accessions present a slight increase between the first and ninth day (Fig. 2). This suggests, a transformation from starches to sugars through the effect under sunlight. For example, in mashua accessions a 27.44% content of amylose has been observed (VALCÁRCE-YAMANI *et al*., 2013). The sugars in oca (*Oxalis tuberosa*) showed 53% sugar content after 9 days, and 70% after 15 days of exposure, especially in white variety (BRITO *et al*., 2003) as our results in the mashua tubers. So as consequence, we can infer that mashua tubers might reach a greater sugar content with longer exposure to sunlight.

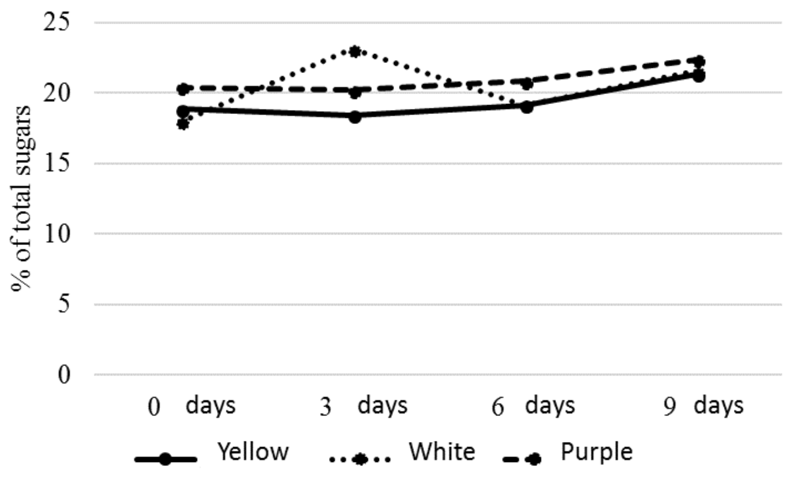


**Figure 2.** Variation in total sugars with length of exposure to sunlight.

### 3.6. Total starch

In regard to the total starch, the freshly-harvested Yellow accession present the highest quantity (47%). Following exposure under sunlight, the initial starch content decreased significantly. A similar pattern was observed in the Purple and White accessions (Fig. 3). This suggests that freshly-harvested tubers can be used as the best sugar, specially the Yellow accession is the most suitable in regard of this parameter. The percentage of starch observed in mashua crops in Peru was 3.65% (HERMOSA, 2013).

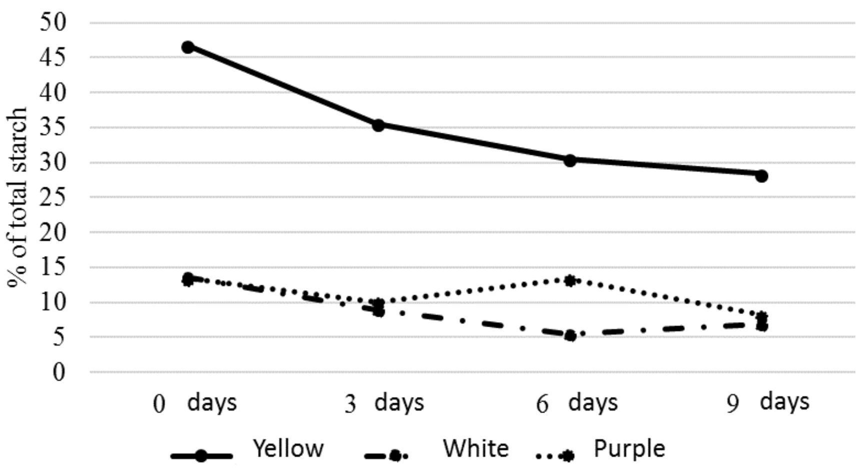


**Figure 3.** presence of starches according to mashua accessions.

### 3.7. Variation analysis between the accessions

The variation analysis shows highly significant differences between the accessions, the number of days and the contrast of accession versus days of exposure for thiocyanate, starch and total sugar content (Table 4). On the other hand, the coefficient of variation is low, which reflects a low variability between data.

**Table 4.** Analysis of variance in the factors studied.

| Source of variation | gl | Thiocyanates | Starch | Sugar |
|---|---|---|---|---|
| Repetitions | 5 | 1633.60 | 41.39 | 1.81 |
| Accession | 2 | 25208.9** | 5128.73** | 25.17** |
| Days | 3 | 61877.9** | 372.77** | 24.49** |
| Accession*Days | 6 | 82150.3** | 79.57** | 13.65** |
| Mean | | 492.17 | 18.542 | 20.58 |
| CV % | | 7.43 | 5.3 | 7.04 |

** Highly significant at a level of 0.05.

Finally, the Pearson correlation tests demonstrate an inverse relationship between the total starch and thiocyanates (-0.40), and likewise between total sugars and total starches (-0.49). Starches are also known as polysaccharides in nutritional terms and are formed by the linkages of glucose in position $\alpha$1-4 and branches in position $\alpha$1-6 (PLAZA-DÍA *et al.*, 2013).

## 4. CONCLUSIONS

The Purple accession, after three days of exposure to sunlight, was most suitable for obtaining thiocyanates. Meanwhile, the Yellow accession is most apt as a food source, due to its high content of starches and sugars, and low concentrations of thiocyanates. The results suggest two industrial applications for mashua plants: on the one hand as a source of starch and sugar nutrients, and on the other hand as a source of thiocyanates for medicinal purposes.

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