











## **ABSTRACT**

**Keywords:** ascorbic acid, dehydroascorbic acid, horticultural products

The ascorbic acid is one of the most important factors of quality horticultural products, being involved in the biological activities of the human body. The vitamin C content of horticultural products may be influenced by various factors, such as genotypic differences, climatic conditions and agricultural practices, as well as storage and processing techniques.

The thesis, "Research on the stability of ascorbic acid content of some fresh and processed horticultural products", approaches a topical issue from the perspective of the studied products. The analyzed assortment is part of canned fruits and vegetables with a high volume of sales due to increased demand both in season and out of season.

This paper aims to investigating the stability of ascorbic acid in fresh fruits and vegetables, during the technological process and on the end of the technological process.

For this purpose were established the following objectives:

- bibliographic study on the current nationally and internationally state of research about the stability of ascorbic acid content of fresh and processed horticultural products;
- establishing of research methods for the determination of ascorbic acid, polyphenolic compounds, flavonoid compounds and enzyme activity (ascorbate oxidase, superoxide dismutase, catalase, polyphenoloxidase and peroxidase);
- identification of the main quality characteristics of fruit and vegetables used to obtain canned food;
- identification, quantification and traceability of ascorbic acid stability during processing vegetal products;
- identification, quantification and traceability of enzymatic activity on ascorbatoxidase, superoxide dismutase, polyphenoloxidase, peroxidase and catalase during processing of fruits and vegetables;
  - identification, quantification and traceability of total polyphenol and flavonoids content













## during processing plant products;

- statistical interpretation of data obtained through chemical determinations achieved on samples of batches of raw material and samples, that underwent heat treatment during the technological flow and the formulation of correlations between the ascorbic acid and the polyphenolic compounds, flavonoid compounds and enzymes studied.

The doctoral thesis is structured in two parts with a total of x pages, which includes y tables, z figures and u references. The first part of the paper refers to the current state of research, following the presentation of the experimental protocol and results are detailed in the second part. The thesis is finalized with some conclusions and recommendations.

In chapter I called "Theoretical studies on ascorbic acid" are presented synthesized bibliographic data on the structure, recycling, degradation and the role of ascorbic acid and also notions about ascorbic acid necessary in the body and its use as a food additive.

Chapter II refers to the current state of research on the dynamics of ascorbic acid in some fresh and processed horticultural products.

The final chapter of the first part reports theoretical studies on enzyme activity, total polyphenol and flavonoid compounds content analyzed on the horticultural products.

In chapter IV called "Purpose, research objectives, research material and methods used in the thesis" are presented in detail the aspects necessary to complete the thesis.

The main objective of the research being the observation of ascorbic acid stability during processing technology, was chosen as the sampling to be conducted in fruit and vegetable processing unit, SC Contec Foods S.R.L. from Tecuci.

The experimental protocol was conducted in the following laboratories: Laboratory of Oenology from the Research Center for Oenology, Romanian Academy - Iaşi Branch; Laboratory of Food Chemistry and Biochemistry, The Department of Food Science and Technology, Aristotle University of Thessaloniki, Greece; Laboratory of Bio-Chemistry of the Faculty of Biology, Alexandru Ioan Cuza University, Iasi; Laboratory of Viticulture and Laboratory of Horticultural Products Technology of the Faculty of Horticulture which belongs to the University of Agricultural Sciences and Veterinary Medicine, Iaşi.

To achieve the thesis purpose were studied the following products: "Grated apple for pie", "Apricot compote", "Cherries compote", "Peach compote", "Sour cherries compote", "Apricot jam", "Peach jam", "Sour cherries jam", "Cherries confiture", "Sour cherries confiture", "Cauliflower in vinegar", "Sweet peppers in vinegar", "Green peas in brine", "Beetroot salad", "Tomato pulp~passata", "Tomato broth", "Green tomatoes in vinegar", "Sauerkraut", and "Cabbage leaves in brine".













As for regards the content ascorbic acid and dehydroascorbic, determinations were carried out after a storage period of 3 months at 10 and 25 °C. Samples were taken for analysis in 2011 and 2012.

To achieve the objectives, the research methods included:

- chemical determinations carried out on the samples: determination of moisture (%), total dry matter, soluble dry matter, titratable acidity, NaCl determination by Mohr's method and determination of the pH;
- determination of ascorbic acid: analysis by high performance liquid chromatography (HPLC), reflectometry method, 2.6 diclorfenolindofenol titrimetric method;
- determining the enzymes activity: ascorbatoxidase determination (MF Oberbacher and HM Vines method), assessment of polyphenoloxidase (Ermakov, AI), the determination of peroxidase activity (LV Gudkova and RG method Degtiari), superoxide dismutase activity determination (SOD) (method Winterbourne, Hawkins Brian and Carrel adapted by Vlad Artenie), determination of catalase activity trough Lobeck gasometric method;
  - determination of total polyphenol content (Folin-Ciocalteu Photometric method);
  - determination of total flavonoids (Tibiri method).

Chapters V and VI represent the largest part of the thesis, where are interpreted the results obtained from the analysis of the studied products.

Regarding the product "Grated apple for pie" has shown a significant decrease in ascorbic acid content during processing, so at the raw material were obtained values of approximately 6,5 mg/100 g sample, according to the analyzed method; at the end of the technological process was found 0,25 mg/100 g sample, and during of the finished product storage and were recorded about 0,1 mg/100 g sample.

Dehydroascorbic acid content showed an upward trend until the stage of division and begining with blanching operation is recorded a significant degradation; during storage at 25 °C was determined an increase of this compound, thus the determined quantitative value is about 0,1 mg/100 g sample.

Regarding the total content of polyphenols and flavonoids, which had a slightly downward trend during processing, being evidenced greater stability of ascorbic acid in apple fruit during processing. The correlation between the content of ascorbic acid and phenolic compounds analyzed highlight the same dynamic compounds during processing fruit. As a result of interpretation, flavonoid compounds and ascorbic acid content shows a very good correlation.

The enzymatic activity was evidenced in samples taken from batches of raw material, while the other samples analyzed on the technological flow highlight enzyme inactivation.













After analyzing samples from the technological process of obtaining compote of apricots, cherries, peaches and cherries, the results have revealed a decreasing trend of ascorbic acid content. In comparison with the products studied (compotes) of the finished product with the highest content of ascorbic acid was the cherry compote, about 3 mg/100 g processed fruit, as the raw material showed about 17 mg/100 g. Reduced values were obtained from samples taken from the obtaining flow of apricot compote (2 mg/100 g in samples analyzed in 2011 and 1,5 mg/100 g compotes studied in 2012) and peaches compote (1,5 mg/100 g in samples analyzed in 2011 and 1.2 mg/100 g for the compotes studied in 2012) and the average determined in the two years of study in samples from the technological process of producing cherry compote have revealed a ascorbic acid content of 2 mg/100 g of processed fruit.

Concerning dehydroascorbic acid determination, is observed at all compotes tipes an increase during processing, followed by a significant decrease due to pasteurization, then returns to the upward trend due to ascorbic acid degradation during storage.

The data obtained from research indicates a total content of polyphenols and flavonoids slightly reduced at the end of the technological process compared with analyzed samples of raw material batches. According to research conducted, the highest concentration of polyphenol compounds and flavonoids were identified in both cherry fruit samples of process flow steps and the finished product.

The enzymatic activity was revealed in samples of raw material, after blanching operation, where it were partially inactivated, observing that the following samples analyzed on the flow, from the finished product category, point out the inactivation of these compounds.

Regarding the data obtained after analysis of samples taken from the technological process of obtaining jam from: apricots, peaches and cherries were noted significant reductions ascorbic acid content. The decrease in ascorbic acid content was highlighted in particular the stages of blanching, chopping and pasteurization. During storage is recorded a downward trend depending on storage temperature. Analyzing the values obtained from the determination of ascorbic acid and dehydroascorbic there is an inverse proportionality thus in stages ascorbic acid content in significant losses, dehydroascorbic acid has an upward trend. The common point is observed the same trend is pasteurization product stage where both compounds show a considerable reduction.

The results of research conducted on the total content of polyphenols and flavonoids revealed slightly downward trend in all fruit samples taken during the technological process. A major influence has a concentration step where it observed increased concentrations of phenolic compounds and flavonoids. The highest concentration of these compounds was identified in both













cherry fruit samples of process flow steps and the finished product. The correlation between the values obtained from the determination of ascorbic acid and polyphenolic flavonoid compounds shows an acceptable combination of these compounds. Better results were obtained for the correlation of flavonoid compounds ascorbic acid compared with the combination of ascorbic acid and phenolic compounds from all kinds of jam analyzed.

As is the case of compotes, the enzymatic activity was observed in samples taken from batches of raw material, followed by blanching operation that inactivate thes enzymes.

The research conducted on samples of the obtaining processes for the confiture of cherries and sour cherry showed results similar trend analyzed compounds in fruit jam. During the processing it observes the decrease of the ascorbic acid content and the increases of the dehydroascorbic acid content. After the sterilization both compounds show a significant decrease, following an increase of dehydroascorbic acid during storage while ascorbic acid dynamics is declining.

Significant differences were found in polyphenolic compounds and flavonoids, where it due to concentration, their content was augmented compared to the values obtained from the raw material. The obtained value (R<sup>2</sup>) as a result of the correlation of polyphenolic compounds with ascorbic acid shows a good correlation between the two compounds, flavonoid compounds while the combination of ascorbic acid with a weak correlation shape.

The enzymatic activity was observed in the raw material samples after blanching operation showed partial inactivation of enzymes, and the final product samples after enzymatic activity is not recorded. The associations made between ascorbic acid and enzymes studied showed a very good correlation, showing similar behavior during processing of fruit.

Regarding the results obtained after analyzing products artificially acidified ("Cauliflower in vinegar", "Sweet pepper in vinegar", "Beetroot salad", "Green tomatoes in vinegar"), it was found that they show a downward trend in most parameters studied .

In the case of ascorbic acid content due to acidic medium, was observed a higher concentration of the finished product and lower losses during storage the chemical compound as compared with the other studied products. Horticultural product with the highest ascorbic acid content in the raw material was the sweet peppers during processing while preserving a high proportion.

Compared with other products artificially acidified, sweet peppers preserved in vinegar solution showed an increase in total polyphenol content and flavonoiode. For this reason, the combination of these compounds with ascorbic acid results in a weak correlation (different behavior of ascorbic acid, and flavonoids and polyphenolic compounds during processing).













The studies carried out to determine the enzymatic activity have revealed its inactivation after blanching operation, and at the final product has not been indicated enzymatic activity. The correlations made between ascorbic acid and studied enzymes, highlights the different behavior of the analysed compounds, because of the different response when processing of horticultural products.

Through research carried out at the canned green peas beans, stood out significant reductions of ascorbic acid content both during processing and during storage. The decrease in ascorbic acid content decreases progressively during processing, leading to loss of 80% in the finished product. Dehydroascorbic acid content is inversely proportional to the development trend of ascorbic acid, registering upward trend during processing, sterilization step downward trend, following that during storage to observe an upward trend more pronounced at higher temperatures.

The results showed a trend of decreasing abundance polyphenols and flavonoids during processing peas beans and a very good correlation of these compounds with ascorbic acid.

Regarding enzymatic activity, it was noted in samples taken at the beginning of the technological flow, the inactivation is observed after blanching operation. The finished product does not record enzymatic activity. As a result of statistical interpretation positive correlations were obtained between the studied enzymes and ascorbic acid.

The experimental data obtained after analysis of samples taken from the technological process of obtaining pulp and tomato broth revealed decrease in ascorbic acid content. Significant decreases were determined in steps of chopping, blanching and pasteurization. These values show a decreasing trend according to the storage temperature during storage. Dehydroascorbic acid content shows oscillatory trend during processing tomatoes, while finished products recorded a dehydroascorbic acid content to rise. This growth is influenced by the time and temperature of storage.

The samples after blanching operation revealed no enzymatic activity. The correlations made between the studied enzymes and ascorbic acid content showed a very good combination of compounds, they have the same trend during processing.

In case of lactic acid fermentation products ("Sauerkraut") has been found that less degradation of the ascorbic acid content, reduction of this compound is due to the dilution of the coating liquid in a proportion of about 50% compared with the value obtained from the cabbage raw material. Dehydroascorbic acid content showed a rising trend both during fermentation and during storage.

Polyphenolic compounds and flavonoids have decreased slightly during lactic acid













fermentation process. The correlation obtained between the values of these compounds and ascorbic acid content outlined very good results.

Compared with all the products studied, the pickled cabbage was the only finished product which has been found enzymatic activity, having a very good correlation between the enzyme and the ascorbic acid content.

Regarding product "Cabbage leaves in brine", it was found that after processing, the values obtained from measurements ascorbic acid content of significant reductions due to product pasteurization.

The values obtained in the determination of polyphenolic compounds and favonoidici showed downward trend during processing. Through correlations made between compounds analyzed stood out very good association parameters correlated with similar behavior in the course of processing.

During the process flow of production the "Cabbage leaves in brine" there was inactivating enzymes. The associations made between ascorbic acid and enzymes studied showed a very good correlation, demonstrating the same response of the compounds analyzed in the process.