*The Annals of the University Dunarea de Jos of Galati*
*Fascicle VI – Food Technology (2013), 37(2), 93-99*

**ORIGINAL RESEARCH PAPER**

# EVOLUTION OF ANTIOXIDANT CAPACITY OF BLEND JUICE MADE FROM BEETROOT, CARROT AND CELERY DURING REFRIGERATED STORAGE

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Received on 31st August 2013
Revised on 16th December 2013

Vegetable juice is a valuable source of antioxidants because it contains a significant amount of bioactive compounds and it can be a convenient way of consumption. The antioxidant capacity of vegetables depends on a wide range of compounds like flavonoids, phenolic acids, aminoacids, ascorbic acid, tocopherols and pigments. The levels of individual antioxidants in food do not reflect their total antioxidant capacity, which could also depend on their synergism. The aim of this study is to compare the changes occurred on antioxidant capacity of vegetables juice during refrigerated storage after two different preservation processes: pasteurization and fermentation. A blend of juice obtained from beetroot, carrot and celery has been stored at temperature of 4ºC for two weeks. During this period of time antioxidant activity, total flavonoids and polyphenolic compounds have been quantified. Our results have shown that fermented juice is an important source of antioxidant compounds with a good stability during storage.

**Keywords**: vegetable juice, antioxidant capacity, lactic fermentation

## Introduction

Antioxidant compounds from vegetables play an important role in maintaining human health. Antioxidant capacity is the ability of a compound to reduce pro-oxidant free radicals. These compounds are able to block or delay the reaction of a substrate with molecular oxygen or reactive oxygen species. Compounds such as polyphenols and flavonoids act against free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that cause many diseases. They reduce the chronic diseases like cancer and heart disease.

Polyphenols compounds of plant origin with a wide variety of molecules have a structure with several hydroxyl groups on aromatic rings, but also molecules with

one phenol ring, such as phenolic acids and phenolic alcohols. Polyphenols are divided according to the number of phenol rings that they contain and to the structural elements that bind these rings. The fundamental groups of polyphenols are: flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans.

Flavonoids are plant phytochemicals that can be classified into six classes: flavanones, flavones, flavonols, isoflavonoids, anthocyanins and flavans, their structural characteristics vary around the heterocyclic oxygen ring. Different processing methods can affect the phenolic acid content and antioxidant activity in distinct ways (Đordevic´ et al., 2010; Ravichandran et al., 2012; Maa et al., 2013). According to Peterson and Dwyer (1998), the food preparation and processing of vegetables may decrease flavonoid content by 50% owing to leaching into water or removal of portions of the plant that are rich in flavonoids. *In vitro* and animal studies have demonstrated that flavonoids have antioxidant and antimutagenic activities. Case control studies suggested that flavonoids may reduce the risk of cardiovascular disease and stroke. Flavonoids may vary in their absorption and their metabolism is still obscure. They are conjugated in the liver or kidney and excreted into bile or urine. Colonic bacteria split the heterocyclic ring and degrade the flavonoids to phenyl acids which may be absorbed, conjugated, and excreted or metabolized further by colonic bacteria.

Vegetables are a source of antioxidants that belongs to various classes of compounds with a wide variety of physical and chemical properties (D'Achivio et al., 2007; Denga et al., 2013; Lopez and Denicola, 2013). Their consumption in a proper manner can have a positive impact on maintaining health condition. Lactic acid fermentation represents the easiest and the most suitable way for increasing the daily consumption of fresh-like vegetables and fruits (Cagno et al., 2013).

Antioxidants are part of diet but their bioavailability depends on several factors. This can be influenced by poor solubility, inefficient permeability, food processing, low stability during storage and degradation in gastrointestinal tract. Therefore, information on stability of antioxidant during storage is important for evaluating the potential health benefits of foods. Our research is a comparative study between the influences of a thermal and a non-thermal preservation process on the antioxidant capacity of a blend vegetable juice.

## Materials and methods

### *Material*

The vegetables used for this study: carrot, beetroot and celery, were purchased from a local market. After the preliminary operations like washing, peeling and slicing, the vegetables were juiced with a centrifugal juicer provided by Philips.

In order to compare the influence of preservation processes on antioxidant capacity, the blend juice was pasteurized or subjected to lactic fermentation. Pasteurization process was carried out using a water bath at temperature of 70ºC for 3 minutes. For fermentation process the juice was inoculated with a lyophilized termophilic culture provided by Enzymes & Derivates: DIPROX YBA 986, which

contains *Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus termophilus.*

After this preservation processes, the juice was stored for two weeks at temperature of 4ºC.

***Methods***

Measurement of total flavonoid content was determined by spectrophotometry using Aluminum Chloride Colorimetric Method described by Vardapetyan, H. et al. (2013) with some modification. This method is based on the formation of complex flavonoid-aluminium with the maximum absorbtivity at 430 nm. 1 mL of juice was mixed with 1 mL of $AlCl_3$ (2.5%), 2mL of $CH_3COONa$ (10%) and 6 mL of ethanol (70%). After incubation at room temperature for 30 min, the absorbance of the reaction mixtures was measured at 430 nm. The flavonoids content was expressed as quercetin equivalents in mg/100 mL juice. The concentration of flavonoids was derived from a standard curve of quercetin.

The total polyphenol content of juices was determined according to the method described by the International Organization for Standardization (ISO) 14502-1. Briefly, 1.0 mL of juice was transferred to separate tubes containing 5.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 mL of a $Na_2CO_3$ solution (7.5% w/v) was added. The tubes were allowed to stand at room temperature for 60 min before absorbance at 765 nm (in a UV–Vis spectrophotometer) was measured against water. The total polyphenol content of samples was expressed as gallic acid equivalents in mg/100 mL juice. The concentration of polyphenols in samples was derived from a standard curve of gallic acid.

The antioxidant capacity was determined using DPPH method (Ramadan-Hassanien, 2008). The samples were obtained by adding 1 mL of juice in a hydroalcoholic water:ethanol=1:1 (w:w) solution that was homogenized through ultrasonication for 1 minute and filtered with Whatman filter paper. 0.1 mL of sample was added on 1 mL DPPH methanolic solution (3.5 mg/100mL methanol) and was strongly stirred. The tubes were filled with methanol to a 4 mL volume. The blends were kept in darkness for 60 minutes, after which the absorbance was measured at 515 nm. The antiradical activity (RAC%) was estimated with the following relation:

$$RAC\% = 100 \times (A_{control} - A_{sample})/A_{control} \quad (1)$$

where: $A_{sample}$ is the absorbance of the sample with DPPH;

$A_{control}$ is the control absorbance of a blend formed from 1mL DPPH solution + 3 mL methanol.

The chemicals and reagents were of analytical grade. All the determinations were made in duplicate and the standard deviation was less than 0.7.

## Results and discussion

The compounds with antioxidant properties can be influenced by the preservation processes of raw materials of food systems. A better stability of flavonoids in pasteurized juice has been observed compared to the juice after fermentation. According to Svensson et al. (2010) the stability of antioxidants compounds in fermented juice can be influenced by pH condition and the microorganism use to conduct the fermentation. Our results are different from those obtained by Kwon and Ha (2012) who observe an increase of flavonoid content due to the increase of rutin, quercitrin, and quercetin levels after fermentation of *Houttuynia cordata*.

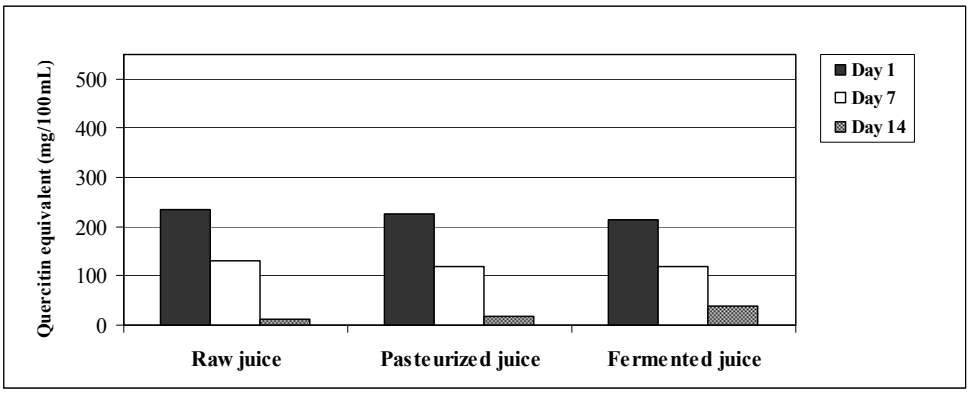


**Figure 1.** Evolution of flavonoids during storage

After 7 days of storage no significant difference between the juices has been noted, as you can see in Fig.1. A loss of 45% flavonoids compared to the initial amount has been observed. At the end of the storage period, the quantity of flavonoids was insignificant. The amount of flavonoids from fermented juice was higher than untreated and pasteurized juice. These results are correlated with the conclusion of Hostetler et al. (2013) who observed that a low pH increases the stability of flavonoids.

The initial amount of total polyphenols (Fig. 2) was similar to the amount of flavonoids contained by juices (Fig.1). After the application of the preservation processes the losses in polyphenols were of 9% for pasteurization and 13% for fermentation. In comparison, Yao and Ren (2011) demonstrate that the contents of phenolic acids, total phenolic compounds, and antioxidant activities are affected significantly by temperature and time variables of the thermal treatments.

Antioxidant capacity of fermented juice was higher than the one of pasteurized juice with a loss of 4.9%, respectively 15.8% compared to the initial antioxidant capacity of raw juice (Fig. 3). Antioxidant capacity can be influenced by other compounds that are found in vegetable juice, like vitamin C. Our previous research demonstrates that vitamin C has a better stability in fermented juice compared to unfermented juice (Profir and Vizireanu, 2013).

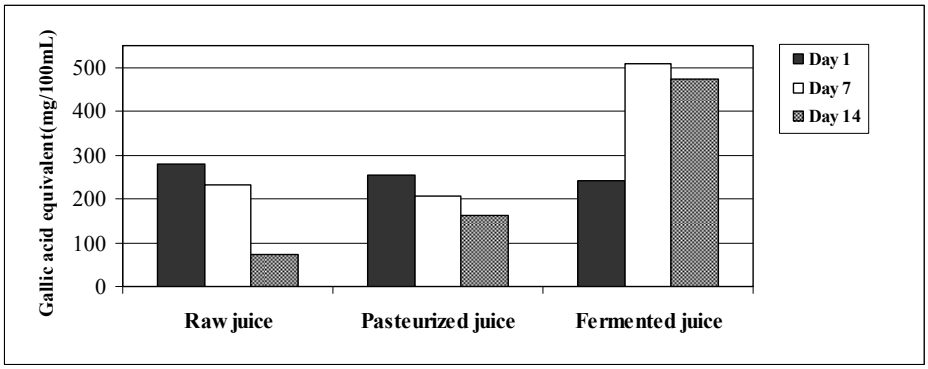


**Figure 2.** Evolution of polyphenols during storage

An increase of 210 % in the amount of total polyphenols contained in the fermented juice appeared after 7 days of storage, as you can see in Fig. 2. The losses of total polyphenols content from the pasteurized juice are similar with the losses of untreated juice during the storage period.

At the end of 14 days of storage the highest concentration in polyphenols was found in fermented juice, where the low pH of the juice influence in a positive way the stability of the product. The greatest losses were observed in untreated juice, where 73% of total polyphenols were lost compared with the initial period.

After a week of storage, the pasteurized juice had a high antioxidant capacity comparative to the untreated juice (Fig. 3). The increase of the antioxidant capacity in the fermented juice might be explained by the increase of the total polyphenols amount. Heo et al. (2007) concluded that the total antioxidant capacity in complex extracts of phenolics from fruits and vegetables could be estimated if the concentrations of individual phenolics and their own antioxidant capacities analyzed by using ABTS radicals are known.

The antioxidant capacity found at the end of the storage period in our juice can also be correlated with the quantities of the polyphenols contained. These results are different from the results obtained by Hua-Bin et al. (2007) who found that the correlation coefficient between the antioxidant capacities and the phenolic contents was very small, and phenolic compounds were not a major contributor to the antioxidant capacities of these microalgae.

In literature, there are several explanations of the ambiguous relationship between the antioxidant activity and total phenolics: (1) Total phenolic content did not include all antioxidants, such as ascorbic acid, carotenoids and tocopherols; (2) The synergism between antioxidants in a food system made the antioxidant activity dependent on antioxidant concentration and the structure and interactions among antioxidants as well. That is why samples with similar concentrations of total phenolics may vary remarkably in their antioxidant activity; (3) The use of different methods for measuring antioxidant activity based on different

mechanisms may conduct to different observations (Sun and Ho, 2005). Wouters et al. (2013) obtained different results according to the test applied to analyze the antioxidant capacity of the fermented leek samples (ORAC analyses or DPPH analyses).

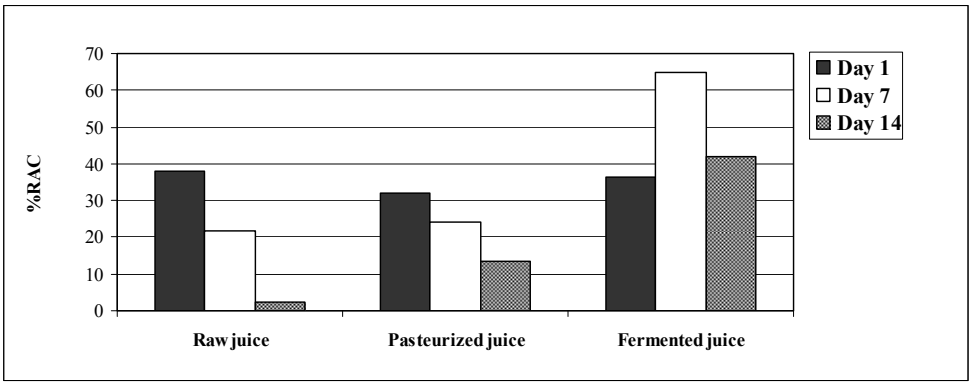


**Figure 3.** Evolution of antioxidant capacity during storage

## Conclusion

Lactic fermentation may offer a way to preserve fresh-like vegetable juices with a small loss in bioactive compounds.

Antioxidant capacity was influenced by the total amount of polyphenols and the content in other bioactive compounds. This observation is correlated with the one of Yao (2011) who concludes that there is a significantly positive correlation between antioxidant capacity (DPPH and ABTS) values and the contents of flavonols or total phenolics acids.

Due to the complex structure of vegetable juice, our following studies will focus on the analysis of different aspects of the antioxidant capacity. In order to accurately determine different aspects of antioxidant properties of the vegetable juices, many *in vitro* chemical based assays should be considered. For testing natural products as antioxidants, a sequential multifaceted approach is highly recommended.

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