THE EVOLUTION OF ASCORBIC ACID CONTENT DURING WHITE CABBAGE PICKLING PROCESS

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Abstract. The evolution of ascorbic acid content during white cabbage pickling was the purpose of this paper. White cabbage, purchased from commercial network, was the biological material. Various samples of cabbage (whole and minced) were introduced within glass jars over which was poured brine (made with non-iodized salt), for each type of cabbage. The samples were left to ferment at 18ºC for 5 days, then stored at 10ºC, where each brine was subjected to periodic aeration, by pouring from one container to another in prolonged contact with air. The evaluation of pH was made with a digital pH meter, and ascorbic acid content through a titrimetric method, from fresh raw material (before pickling), as well as from cabbage, minced cabbage and from their sauce, at 2 or 3 days, during the pickling process. In the whole cabbage, the ascorbic acid content significantly decreased up to the 17th day of pickling, while in the minced cabbage up to the 14th day, accumulating gradually, at about the same intervals, within their brine. At the end of the experiment (after 30 days), compared to fresh sample, the total content of ascorbic acid was reduced by 22.3% (within whole cabbage and its brine), and by 27.7% (within minced cabbage and its brine). Cabbage pickling, including mechanical processing, fermentation and periodic aeration has led to reduction of initial content of ascorbic acid especially around pH values of 4.2-4.3.

INTRODUCTION

Cooking, pasteurization and the addition of chemical preservatives guarantee safe vegetables and fruits, but bring about a number of not always desirable changes in their physical characteristics and chemical composition (*Zia-ur-Rehman et al., 2003; Zhang and Hamauzu, 2004*). In order to reduce the drawbacks derived from above mentioned food processing technologies, novel technologies, such as high-hydrostatic pressure processing, ionization radiation and pulsed-electric fields, new packaging systems and the use of natural antimicrobial preservatives are considered (*Devlieghere et al., 2004; Gómez-López et al., 2005; Elmnasser et al., 2007*).

Vegetables and fruits can be preserved, for a long time without modifying their properties, by means of conservation methods based on acidification or pickling method, i.e. lactic acid fermentation, considered as the simple and valuable biotechnology to keep and/or enhance the safety, nutritional, sensory and shelf life properties of vegetables and fruits (*Steinkraus, 1996; Buckenhüskes et al., 1997; Karovičová and Kohajdová, 2003; Demir et al., 2006; Di Cagno et al., 2013*).

Representing the easiest and the most suitable way for increasing the daily consumption of fresh-like vegetables and fruits (*Di Cagno et. al., 2013*), lactic acid fermentation of vegetables has an industrial significance mainly for cabbages, cucumbers and olives (*Montet et al.,* 2006; *Rodrìguez et al., 2009*).

Between Brassica vegetable (cabbage) consumption and cancer risk is an inverse relationship (*Zhang et al., 2006; Higdon et al., 2007*). The beneficial effects of vegetables in general, and cruciferous vegetables, in particular, on human health are due to the presence of high levels vitamins (mainly ascorbic acid), and of glucosinolates, i.e. glucosides containing sulfur (*Moreno et al., 2006; Higdon et al., 2007,* cited by *Martinez-Villaluenga et al., 2009*).

According to *Aleksandrova et al.* (1992), during cabbage shredding, glucobrassicin (a cabbage glucosinolate) is transformed into indole-3-carbinol by the action of myrosinase and during fermentation, as the pH decrease, this indole reacts nonenzymatically with L-ascorbic acid to yield ascorbigen. This last compound is responsible for the anticarcinogenic properties in humans with a high intake in white cabbage (*Lysenkova et al., 2001; Smiechowska et al., 2008*).

In Romania, pickles (cabbage, cauliflower, cucumbers, green tomatoes, carrots, fruits) are widespread and appreciated, being consumed all the year, but especially in winter and spring. For many families, pickled white cabbage is a component of the daily diet (spice and/or food), being almost customary in the daily diet.

The purpose of this work was to study the ascorbic acid evolution during white cabbage pickling, to see to what extent the fermentation process influences the initial content (from the raw material) of this vitamin.

MATERIALS AND METHODS

Research materials. White cabbage *(Brassica oleracea* var. *capitata* L.*)*, purchased from commercial network and selected to meet the quality parameters necessary to conservation, was the biological material. The cabbage with a head weight of between 1-1.5 kg was fresh, well made, no damage and no attack by insects or parasites, clean, odorless and with a good taste. Prior to pickling process, fresh raw material was analyzed, determining the pH, whose value was **7.05±0,08**, and the ascorbic acid content (**40.3±2,74 mg%).** For experiments it was used non-iodized crystallized salt, provided by the National Salt Company, Salina Cacica Branch, and water from the distribution network of the Suceava city.

The preparation of pickles. Whole cabbages were cleaned and placed in colored glass jars of 15 liters each, well stuffed (with pressure) without goals. Using non iodized salt it was prepared brine in some pots, by introducing of 25 g salt per liter of water. Although *Banu et al.* (2000) recommended as among rows of sprouts to be placed pieces of horseradish roots and dried dill weed flowers, in order to improve the flavor of the product and to give a preservative role to the broth, in this experiment there was not used horseradish, because it has an appreciable content of ascorbic acid which would influence test results. Before closing the jars, there were placed two small wooden boards (cross) to prevent cabbage to rise above, then the jars were left to ferment at 18ºC for 5 days. Because the anaerobic environment may favor butyric fermentation, the cabbage brine was subjected to periodic aeration (*Guţulescu, 1973*), by pouring from one container to another in prolonged contact with air. After completion of pickling tank, pickled cabbage was stored at 10°C.

Minced cabbage was obtained from fresh cabbage, healthy, clean, without outer leaves, which was cut with a steel knife in long thin strips. The cabbage fragments were transferred within two metal flasks and were salted, separately, with non-iodized salt (25-30 g of salt per 1 kg of cabbage), and then were rubbed to leave enough juice. After approx. 30-60 minutes, the cabbage shredded and salted from containers was introduced in colored glass jars (5 liters each), squeezed by hand, and covered (2-3 cm liquid) with the soup made. Over minced cabbage were put two small wooden boards in cross. The initial fermentation temperature was 18°C for 5 days, after which the jars were transferred to a room at a temperature of 10°C. During fermentation it was added brine, made up of 15 g of salt per liter of water. The determinations of chemical and biochemical parameters were carried out to each 2-3 days, using the whole or chopped pieces of cabbage or brine.

Research methods. The evaluation of pH and ascorbic acid content was made from fresh raw material (before pickling) as well as from cabbage, minced cabbage and from their sauces, at 2 or 3 days, during the pickling process. **PH** was determined with a digital pH meter supplied by Hanna, and **ascorbic acid** content was determined through a method based on reduction (by ascorbic acid) of 2.6-dichlorphenol-indophenol (2.6-DCPIP) to the corresponding leucoderivate (*Artenie and Tănase, 1980; Indyk and Konings, 2000*). The result was expressed as mg ascorbic acid per 100 g or per 100 ml (mg%) product.

Statistical analysis. The data of experiments, consisting in four replicates for each determination, were statistically processed using SAS Version 8.02**.** In order to analyze the significance of differences among samples, generalized linear model analysis was carried out, and for multiple comparisons was used Duncan's multiple range test ($P<0.05$).

RESULTS AND DISCUSSIONS

The table 1 indicates the evolution of ascorbic acid content in cabbage and cabbage brine, during pickling.

Table 1. The ascorbic acid and pH values in cabbage and its brine, during pickling

Time (days)	2d	5d	8d	11d	14d	17d	20d	23d	26d	30d
Sample/Par.	Ascorbic acid (mg %)									
Cabbage (C)	34.51 ±3.72 AB*	32.95 ±4.15 B*	31,56 3.93 B	31.41 ±2.58 B	27.65 ±1.98 B	24.73 ±3.61 BC	24.51 ±1.76 BC	24.82 ±2.53 BC	24.13 ±3.04 BC	24.20 ±3.16 BC
Cabbage brine (CB)	5.02 ±0.86 c*	6.28 ±0.53 bc*	6.34 ±0.97 bc	8.07 ±0.65 ab	7.92 ±1.27 ab	7.30 ±0.95 b	7.50 ±0.74 b	7.08 ±1.03 b	7.25 ±0.79 b	7.10 ±0.54 b
Total AA** (C+CB)	39.35	39.23	37.90	36.78	35.57	32.03	32.01	31.90	31.38	31.30
	pH									

Time (days)	2d	5d	8d	11d	14d	17d	20d	23d	26d	30d
Cabbage brine	6.78 ±0.16 b*	6.52 ±0.29 b	5.80 ±0.06 bc*	4.89 ±0.08 c	4.50 ±0.27 c	4.36 ±0.15 d	3.97 ±0.07 d	3.85 ±0.09 d	4.07 ±0.10 d	4.05 ±0.14 d

*Means with different letters within a row are statistically diferent (P<0.05); Par.= parameter; AA**= Ascorbic acid; d=days

As compared to fresh cabbage (40.3±2.74 mg%), the ascorbic acid content in pickling cabbage has recorded significant decrease up to the 17th day of the analyzed interval (24.73±3.61 mg%), from which the values of this compound have not anymore presented significant variations (P<0.05). The largest percentage reduction of ascorbic acid content in cabbage was after the first 2 days of pickling (14.37%) and between 14-17 days (10.4%).

Analyzing the evolution of ascorbic acid in cabbage brine, one can note that with time the concentration of this compound increased significantly until the 14th day of, then decreased after the 17th day of pickling, preserving then unchanged (without significant variations) to the end of the period (30 days).

After *Banu et al.* (2003), the maintaining of some fruit and vegetables (apples, pears, apricots, potatoes etc.) cut under water, and the pickling process of vegetables (cabbage, cucumbers etc.) causes loss of vitamins by leaching, or partial loss of nutrients of raw material (carbohydrates, vitamins, amino acids and minerals), passing the fermentation broth.

Since ascorbic acid was identified both in solid material (cabbage) and in brine, it was totaled ascorbic acid content of the product and brine at each period analyzed (Table. 1). The results show that greater losses of ascorbic acid, versus fresh sample, have emerged since the 8th day of pickling, and after 17 days the losses were biggest. Then, within 17 to 30 days, ascorbic acid values have remained stable, suffering minor changes (32.03 to 31.3 mg%).

Compared with the fresh sample, after 30 days of pickling, the total content of ascorbic acid (cabbage + brine) was reduced by 22.3%.

As seen from Tab. 1, pH values of cabbage brine decreased significantly until the 17th day (P<0.05), after which no longer had significant variations until the end of the analyzed period (30 days).

The decreases of ascorbic acid content in cabbage from 34.51±3.72 (after 2 days) to 24.73±3.61 (after 17 days), corresponded to a decrease of pH from 6.78±0.16 (after 2 days) to 4.36±0.15 (after 17 days).

According to *Banu et al.* (2003), the oxidation of ascorbic acid depends on pH, being very rapid at pH 4.3.

In the table 2 is reproduced the evolution of ascorbic acid content in minced cabbage and minced cabbage brine, during pickling.

Table 2.The ascorbic acid and pH values in minced cabbage and its brine, during pickling

Time (days)	2d	5d	8d	11d	14d	17d	20d	23d	26d	30d
Sample/Par.	Ascorbic acid (mg %)									
Minced cabbage (MC)	30.71 ±3.44 B*	27.64 ±2.67 B	26.07 ±1.95 BC*	25.53 ±3.08 BC	21.42 ±1.66 C	21.41 ±2.57 C	21.38 ±1.33 C	21.34 ±2.09 C	20.96 ±0.94 C	19.85 ±1.38 C
Minced cabbage brine (MCB)	6.12 ±0.68 b*	7.78 ±0.95 ab*	8.80 ±1.09 a	8.23 ±0.97 ab	8.77 ±0.44 a	8.27 ±0.88 ab	8.16 ±1.05 ab	8.22 ±0.97 ab	8.64 ±0.63 a	9.30 ±1.14 a

Time (days)	2d	5d	8d	11d	14d	17d	20d	23d	26d	30d
Total AA** (MC+MCB)	36.83	35.42	34.87	33.76	30.19	29.68	29.54	29.56	29.60	29.15
	pH									
Minced cabbage brine	6.17 ±0.35 bc*	5.70 ±0.16 bc	5.18 ±0.05 c*	4.45 ±0.14 d	4.23 ±0.37 d	4.17 ±0.21 d	3.65 ±0.28 d	3.87 ±0.19 d	3.94 ±0.09 d	4.12 ±0.38 d

*Means with different letters within a row are statistically diferent (P<0.05); Par.=parameter; AA**= Ascorbic acid; d=days

As compared to fresh cabbage, the ascorbic acid content in minced cabbage has recorded significant decrease up to the 14th day of the analyzed interval (21.42±1.66 mg%), from which the values of this compound have not anymore presented significant variations (P<0.05) up to the end of the experiment (30 days). The largest percentage reduction of ascorbic acid content in chopped cabbage was after the first 2 days of pickling (23.8%) and between 11-14 days (16.1%).

Analyzing the ascorbic acid of brine from shredded cabbage, one can observe that, during the pickling, the concentration of this vitamin recorded the highest values on days 8, 14, 26 and 30, with similar values, undifferentiated significantly between them (P<0.05).

Gathering (mathematically) the values of ascorbic acid content from chopped cabbage and its brine, at each period analyzed (tab. 2), one can see that greater losses of ascorbic acid, compared to fresh sample, appeared after the first 2 days of pickling and within interval 11-14 days.

Further, between 14-30 days, the ascorbic acid content remained stable, suffering minor changes (30.19 - 29.15 mg%). As compared with the fresh sample, after 30 days of pickling, the total content of ascorbic acid (minced cabbage and its brine) was reduced by 27.7%.

Regarding the chopped cabbage brine, its pH values had significant decreases only up to the 11th day, with insignificant variations up to the end of the review period (P<0.05).

The decrease of ascorbic acid content in minced cabbage from 30.71±3.44 (after 2 days) to 21.42±1.66 (after 14 days), corresponded to a drop in pH from 6.17±0.35 (after 2 days) to 4.23±0.37 (after 14 days).

Once begun the pickling process, the lactic acid accumulation has led to progressively decreasing of pH.

In the table 3 are reproduced ***r*** coefficient values for the correlation between pH and ascorbic acid, during the pickling process.

Table 3. *r* coefficient values for correlations between pH and ascorbic acid content in cabbage and brine

Correlations	pH CB – AAC	pH CB – AACB	pH CB – AAT (C+CB)	pH MCB – AAMC	pH MCB – AAMCB	pH MCB – AAT (MC+MCB)
r	0.942	–0.774	0.937	0.939	–0.676	0.939

CB=cabbage brine; AAC=ascorbic acid in cabbage; AACB=ascorbic acid in cabbage brine; AAT (C+CB) =Ascorbic acid total (cabbage+cabage brine); MCB=minced cabbage brine; AAMC=Ascorbic acid in minced cabbage; AAMCB= Ascorbic acid in minced cabbage brine; AAT (MC+MCB)= Ascorbic acid total (minced cabbage+minced cabage brine)

As seen from table 3, between the pH of cabbage brine and the ascorbic acid content of the cabbage, and the ascorbic acid total (cabbage and its brine), on the one hand, and between the pH of minced cabbage brine and the ascorbic acid content of minced cabbage and the ascorbic acid

total (minced cabbage and its brine), on the other hand, there were positive correlations with close values of ***r*** (0.937-0.942), because progressive lowering of pH during pickling has emphasized the oxidation process of this vitamin, especially at pH 4.23-4.36

Between pH and the ascorbic acid content in cabbage brine, on the one hand, and between pH and ascorbic acid content in minced cabbage brine were negative correlations with a higher value of ***r*** for cabbage brine, because a part (unoxidized) of ascorbic acid passed gradually in broth, accumulating there.

The conditions during pickling process (temperatures of 15-18ºC and periodic aeration to avoid butyric fermentation) made that total content of ascorbic acid to decrease, both in the (whole) cabbage and in the minced one.

In the case of chopped cabbage, the reducing of the total content of this vitamin has been even more pronounced, because of the product fragmentation, which favored easier access of oxygen to the tissues, and the oxidation of a higher percentage of ascorbic acid.

Losses in vitamin C occur when vegetables (e.g. cabbage) are severely cut or shredded (*Mozafar, 1994*). Simply cut of cabbage, carrots leads to losses of up to 75% ascorbic acid (*Banu et al.,* 2003), which is very susceptible to chemical and enzymatic oxidation by ascorbic oxidase during processing (*Lee and Kader, 2000,* cited by *Martinez-Villaluenga et al., 2009*).

After *Martinez-Villaluenga et al.* (2009), loss of vitamin C due to cabbage fermentation may be explained partially to be a result of ascorbic acid involvement in ascorbigen formation. But the decrease of ascorbic content as a consequence of its binding into ascorbigen will not achieve more than 10% of its total amount (*Hrncirik et al., 2001*).

CONCLUSIONS

The pickling process of white cabbage (*Brassica oleracea* var. *capitata* L) under certain conditions (5 days fermentation at 18°C, storage at 10°C, 1.5-2.5% salt in brine, and periodic aeration), has influenced the ascorbic acid content in this vegetable.

During pickling process, the ascorbic acid content significantly decreased up to the 17th day of pickling (whole cabbage) and up to the 14th day (minced cabbage), accumulating gradually, at about the same intervals, within their brine.

After 30 days of pickling, as compared with the fresh sample, the total content of ascorbic acid (cabbage and brine) was reduced by 22.3% (whole cabbage), and by 27.7% (minced cabbage).

Mechanical processing (cutting, shredding), progressively increasing of environmental acidity during fermentation, and periodic aeration emphasized the oxidation of this vitamin, especially at pH 4.2-4.3.

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