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Research article

Stability of vitamin c and beta – carotene during the tomato paste lyophilization process

Monika Kosmala, Katarzyna Ulańska

Institute of Food Technology, Lodz University of Technology
Stefanowskiego 4/10, 90-924 Łódź, Poland

*monika.kosmala@p.lodz.pl

Abstract: *The aim of the study was to evaluate the stability of vitamin C and beta-carotene in the freeze-drying process compared to other thermal drying methods. Tomato puree was selected as a model product for testing the stability of vitamin C and vitamin A in drying processes, due to its properties, such as: appropriate consistency, which was favorable for the analyzes performed, and the presence of the above-mentioned vitamins. Model studies were performed on the stability of vitamin C and beta-carotene under the conditions of air-drying at 105°C, drying under reduced pressure at 70°C and freeze-drying on a tomato puree matrix. Results proved that freeze-drying is superior to other drying methods with the lowest losses of both vitamin C (losses of 4%) and beta-carotene (losses of 25%) in tomato purée. In the case of drying at 70°C under reduced pressure, the loss of beta-carotene is 55%, and vitamin C – 78%. In the case of air-drying at 105°C, vitamin C is 100% degraded, and the loss of beta-carotene is 91%. Moreover, it can be concluded that vitamin C in a higher dose has a protective effect on carotenoids.*

Keywords: *freeze-drying; vitamin C; vitamin A; drying.*

Introduction

The main purpose of drying is to ensure the purity and microbiological stability of food products. Drying as a method of food preservation causes evaporation of water from the dried product, which reduces its availability for microorganisms. Lowering water activity limits the growth of undesirable microorganisms and protects against adverse chemical reactions as well as enzymatic and non-enzymatic transformations [1]. In addition, during drying, the product is reduced in volume and weight, which significantly reduces packaging costs and facilitates transport [2].

Drying allows can also cause unfavorable changes, including a significant breakdown of vitamin C. This fact is confirmed by the research carried out by Stanisław Rudy, who showed that L-ascorbic acid is degraded during leek air-drying and that this decrease is the greater the higher the drying temperature [3].

Vacuum drying is often used to dry products rich in heat-sensitive ingredients. This process does not use air as a heating medium, so the product dried with this method retains better properties than the product air-dried. During drying, a

reduced pressure is applied which allows the water vapor pressure to increase. The difference between the ambient temperature and the temperature of the dried product is also reduced, which significantly affects the time of the process. The consequence of this is the preservation of a greater number of heat-sensitive ingredients. Moreover, the color changes of the product dried under reduced pressure are less noticeable compared to air-drying. Despite many advantages, this method is very expensive and requires a large financial contribution [2].

Freeze drying is currently one of the most modern methods of food preservation. It was first developed in the 1950s in the United States to produce light and valuable rations for the military and astronauts. Unlike other, traditional methods of drying, it takes place at a temperature below 0 ° C, using a reduced pressure of 13.3-66.6 Pa [1]. Freeze-drying is currently one of the best drying methods, which is confirmed by numerous studies. Among other things, the analyzes performed on the pumpkin show that the quality of the freeze-dried product is much better than the quality of air-dried one [4]. Thus, freeze-drying enables the preservation of most of the physical, chemical and biological properties of the original product. Moreover, the retention of thermolabile compounds in the raw material is also high [2].

Vitamin C and beta-carotene are examples of labile vitamins from the group of water-soluble and fat-soluble vitamins, respectively, both with antioxidant activity [5-6]. Vitamin C is not only important for human nutrition but is also used as a quality indicator for food processes [7]. Among the environmental variables that affect the vitamin C degradation, temperature and time are the most important parameters [7].

Tomatoes and the tomato puree obtained from them contain 0.107 mg/100 g and 0.575 mg/100 g of vitamin A, as well as 0.640 mg/100 g and 3.448 mg/100 g beta-carotene, respectively [8-10]. Tomatoes are also a natural source of vitamin C (5-33 mg/100 g FW) [11]. The natural presence of these compounds in tomatoes makes tomato puree an ideal matrix to study the influence of selected drying processes on these vitamins stability. Such tests are especially important because the freeze-drying process is more and more often used before analyzes in scientific research as a process that has the least impact on the composition of the product while depriving it of water and extending its shelf life.

That is why, the aim of the study was to evaluate the stability of vitamin C and beta-carotene in the freeze-drying process in comparison to traditional methods of thermal drying (air-drying at 105°C, drying under reduced pressure at 70°C) on a tomato puree matrix.

Experimental

Materials

Commercial tomato puree 30% (Kotlin, Poland). Ascorbic acid, metaphosphoric acid, potassium dihydrogen phosphate (KH_2PO_4) were purchased from Sigma-Aldrich (Poland), hexane, and acetone (Poch, Poland).

Methods

Sample preparation

Appropriate doses of L-ascorbic acid were added to 30 g of purée, in two technological repetitions. The vitamin was added in such a way as to obtain five different levels of saturation of the samples with vitamin C (0, 30, 77, 170, 330 mg/100 g FW).

Drying

Three types of drying were used in the research, such as: air-drying at 105 ° C for 16 h, drying under reduced pressure 60 mbar at 70° C and freeze drying for 72 h at -35° C and pressure 0.22 mbar, freeze dryer Christ Alpha 1-2 LD plus (Osterode am Harz, Germany). Drying was carried out in duplicate analytical replications, the samples were weighed in the amount necessary to perform the determination of vitamin C (1 g) or beta-carotene (0.5 g) with a double weight of sand in order to evenly grind the product. This method of carrying out the experiment allowed for the conversion of the content of vitamin C and beta-carotene to the fresh mass of the product.

Determination of beta-carotene content

The principle of beta-carotene determination is based on extracting the analyzed component with hexane, and then determining its content in the obtained extract using the colorimetric method at a wavelength of 450 nm.

5 mL of acetone was added to the samples after drying, i.e. samples with 0.5 g of puree and sand, and mixed thoroughly, thoroughly rubbing the puree with sand. The resulting acetone extract was then transferred to a glass separating funnel and the remaining precipitate was washed five more times with 5 mL of acetone. Then 5 mL of hexane and about 4 mL of distilled water were added to the obtained extract and shaken. This caused the beta-carotene to move to the upper hexane layer. Then, 5 mL of hexane was added to the bottom acetone/water layer that was drained into a beaker. The upper hexane layer was poured into a 50 mL volumetric flask. A second extraction was performed. The two hexane layers were connected to each other and the volumetric flask containing them was filled up to the mark with acetone. The absorbance of the resulting solution was measured at a wavelength of 450 nm.

Determination of vitamin C by HPLC

1 g of samples was extracted with 3% metaphosphoric acid into a 10 mL flask. Mixed and centrifuged in a MPW-260R laboratory centrifuge (Med Instruments, Warsaw, Poland) at 16,000 g, transferred to chromatographic vials, and subjected to HPLC analysis.

The HPLC phase was potassium dihydrogen phosphate (KH_2PO) buffered to pH 2.5. A Knauer Smartline chromatography set (Berlin, Germany) equipped with a degasser, two pumps, a mixer, an autosampler, a thermostat and a PDA detector was used. The separation was carried out in a Phenomenex Gemini 5u C18 110A 250x4.60 mm, 5 µm column (Phenomenex, Torrance, CA, USA).

Column temperature 25 °C, flow rate 1 mL/min, injection volume: 20 µl, detection: 254 nm, analysis time: 10 min. A vitamin C standard curve ranging from (0 to 400 mg / L) was used. The formula was y = 48,094x + 1226.6 R2 = 0.9972

Statistical analysis

The results are expressed as means and pooled SEM. Two-way and one-way analysis of variance (ANOVA) and the post-hoc Duncan test at a statistical significance of $p \leq 0.05$ were used.

Results and Discussion

Table 1 shows the content of beta-carotene and vitamin C (mg/100 g FW) obtained for samples after air-drying at 105 °C, drying under reduced pressure at 70°C and after freeze-drying depending on the dose of added vitamin C.

Table 1. The content of vitamin C and beta-carotene after drying depending on the dose of added vitamin C, mg/100 g FW

Drying temperature, °C	Added vitamin C dose [mg/100 g]	Vitamin C determined [mg/100 g]	Beta-carotene [mg/100 g]
Without drying	0.0	0.0±0.0g	32.7±0.2a
105° C	0.0	0.0±0.0g	2.4±0.3f
105° C	30.0	0.0±0.0g	2.5±0.2f
105° C	77.0	0.0±0.0g	2.9±0.1f
105° C	169.0	0.0±0.0g	3.7±0.8f
105° C	334.5	0.0±0.0g	3.1±0.2f
70° C	0.0	0.0±0.0g	11.4±0.8e
70° C	30.0	0.0±0.0g	12.8±0.3e
70° C	77.0	15.0±21.2fg	13.5±0.8de
70° C	169.0	44.5±8.9e	15.9±0.7d
70° C	334.5	128.5±25.6c	20.2±3.1c
Freeze-drying	0.0	0.0±0.0	30.2±2.6a
Freeze-drying	30.0	29.6±9.9ef	21.1±2.1c
Freeze-drying	77.0	87.0±20.2d	21.9±0.7c
Freeze-drying	169.0	168.9±17.3b	25.5±1.1b
Freeze-drying	334.5	332.1±10.8a	26.0±1.4b
Interaction (temperature*added vitamin C dose)		*0.000000*	*0.000170*

Two-way anova (temperature*added vitamin C dose) with Duncan test. The results marked with different letters in the columns differ statistically significantly at $p \leq 0.05$.

The planned test method, taking into account the weighing of the analytical weight (1 or 0.5 g) for the determination of vitamin C or beta-carotene before the drying process, made it possible to convert the analyte (vitamin C, beta-carotene) into the fresh mass of the puree. Two-factor analysis, in which the effect of dose and temperature on the content of beta-carotene and vitamin C was examined, confirms that there are interactions between dose and temperature.

The obtained results indicate that each type of drying used in the research results in a loss of beta-carotene content. The greatest losses occur in the case of air-drying at 105°C (the carotene content has decreased ten times), and the lowest in the case of freeze-drying. On the other hand, drying under reduced pressure at 70°C causes a loss of 50-60% of the initial beta-carotene content. Thus, as the drying temperature increases, the loss of beta-carotene content increases. This statement is confirmed by the results obtained in [12] where the influence of air-drying and freeze drying conditions on the quality of carrot root dried was investigated.

Moreover, in the case of drying at 70°C, correlation was found between the dose of added vitamin C and the content of beta-carotene ($y = 0.0254x + 11.657$, $R^2 = 0.9959$). However, in the other two cases, this linearity was not found. The conducted research confirms the thesis presented in the article [11] that vitamin C may have a protective effect on other ingredients contained in food.

The obtained results indicate that air-drying at 105°C causes complete decomposition of vitamin C in all samples regardless of its initial content. The high lability of this vitamin and its sensitivity to high temperature were also found earlier [11]. Also in the case of drying at 70°C, the loss of vitamin C content is also very high, although it does not completely degrade. In studies on apple puree [13] it was found that temperature had the highest impact on degradation in the range 40–60°C implying a mechanism change above 60°C. Oxygen availability in the medium decreases with temperature and oxidation reactions and might have been the limiting factor in apple purée serum between 60 and 80°C, as further supply of energy did not increase anymore the degradation pace. In the case of freeze-drying, vitamin C loss practically does not occur. Similar data were obtained earlier [2].

In order to investigate whether the drying temperature or the dose of vitamin C has a greater influence, a one-way analysis was carried out, in Table 2 and Table 3, respectively.

Table 2. One-way Anova analysis considering only the drying method without taking into account the dose of added vitamin C, mg/100 g FW

Drying temperature, °C	Vitamin C determined [mg/100 g]	Beta-carotene [mg/100 g]
105° C	0.0±0.0b	2.9±0.6c
70° C	37.6±52.2b	14.8±3.4b
Freeze-drying	129.6±131.4a	24.9±3.7a
P	*0.004405*	*0.000000*

One-way anova with Duncan test. The results marked with different letters in the columns differ statistically significantly at $p \leq 0.05$.

The influence of temperature is statistically significant for both the content of beta-carotene and vitamin C. The high values of the standard deviation are due to the fact that the analysis did not take into account the additionally added dose of vitamin C, which resulted in divergent results in the group.

Table 3. One-way Anova analysis considering only the dose of added vitamin C without drying method, g/100 g FW

Added vitamin C dose	Vitamin C determined [mg/100 g]	Beta-carotene [mg/100 g]
0	0.0±0.0b	14.7±12.8
30	10.0±15.9b	12.1±8.4
77	34.0±43.6b	12.8±8.5
169	77.8±88.8b	15.0±9.8
335	156.8±155.1a	16.4±10.8
P	*0.018795*	*0.947558*

One-way anova with Duncan test. The results marked with different letters in the columns differ statistically significantly at $p \leq 0.05$.

The effect of the dose on the vitamin C content is statistically significant. However, in the case of carotenes, $p>0.05$, which means that there are no significant differences between the groups. Large values of the standard deviation are due to the fact that the analysis did not take into account the drying temperature, so the results in the group were inconsistent.

In the case of vitamin C content, this effect is very large, while in the case of carotenes it is slightly less profound, but also occurs. This analysis also shows that freeze-drying is a process superior from other drying methods and preserves the highest content of both vitamin C and beta-carotene. These results are confirmed by the studies of other authors, who also found that the vacuum freeze-drying process has the least impact on the loss of vitamin C and other labile compounds compared to other drying methods such as atmospheric freeze-drying, accelerated by using ultrasound, microwave, infrared heating, or other techniques [14]. The results also show that vitamin C added in a higher dose causes less loss in the content of beta-carotene, ie it has a protective effect.

To sum up, drying at 105°C degrades more compounds than drying at 70°C. The research also shows that each type of drying applied causes a decrease in the beta-carotene content. Moreover, as the drying temperature increases, the losses in the beta-carotene content are increasing, while in the case of lyophilization the decrease is insignificant, while in the case of air-drying and drying at 70°C, significantly greater losses are observed. The greatest losses in the content of beta-carotene occur in the samples dried at 105°C, and the lowest in the samples subjected to freeze-drying. Moreover, the influence of the vitamin C dose applied on the final carotene content is statistically significant. Vitamin C in a higher dose has a protective effect on carotenoids. The drying temperature also affects the final vitamin C content of the tomato puree. Air-drying at 105°C causes its complete decomposition. The loss of vitamin C in tomato purée after the freeze-drying process is the lowest, in comparison with drying at 70°C under reduced pressure and air-drying at 105°C. The influence of the applied drying temperature and the introduced dose of vitamin C on the content of beta-carotene and vitamin C

is statistically significant. Summing up, freeze-drying is superior to other drying methods, because vitamin C losses are practically non-existent, and beta-carotene losses are also reduced.

Conclusions

Freeze-drying causes the lowest loss of vitamin C in tomato puree, in comparison to other drying methods (the loss of the initial vitamin C content is on average 4%). In the case of drying at 70°C under reduced pressure, losses reach 78%, and in the case of convection drying at 105°C, vitamin C is in 100% degraded.

- The greatest losses in the content of beta-carotene occur in samples dried at 105°C (average loss of 91% of the initial carotene content), and the lowest in the samples subjected to lyophilization (loss of about 25% of the initial carotene content).
- Vitamin C in a higher dose has a protective effect on carotenoids.

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