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Research Article

Influence of the Preservation Method on the Nutritional Profile of Elephant Garlic (*Allium ampeloprasum* L.) Grown in Valdichiana, a Traditional Cultivation Area of Tuscany, Italy

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Abstract

This study investigated the effect of two different methods, i.e. air-drying and freeze-drying, of preservation of elephant garlic (*Allium ampeloprasum* L.) cultivated in Valdichiana, a traditional cultivation area of Tuscany, Italy, on its nutritional characteristics (i.e. soluble proteins. polyphenols, flavonoids, free radical scavenging activity, starch, soluble sugars, allicin). The results showed that compared to the fresh product, the traditional method of air-drying causes significant reductions only in the total antioxidant power (-46%) and in the content of starch (-22%), while the modern method of freeze-drying negatively affects almost all the parameters investigated, overall by about 40%. The content of allicin is not changed by the preservation method. Although freeze-drying is considered one of the best methods for preserving plant-based food, attention should be paid to the effect of this process on the nutritional characteristics of the product.

Keywords: Food; Freeze-drying; Giant garlic; Great-headed garlic; Air-drying

Introduction

The application of preservation methods is of fundamental importance to protect food from spoiling (i.e., its natural degradation). However, modification in physical, chemical, and biological features of food, as well as alterations in taste, color, smell, and nutritional profile, may inevitably occur (Amit et al., 2017).

Air-drying is the most ancient and traditional method for the conservation of agricultural products (Esper et al., 1998), such as vegetables (onion, garlic; (Abdou Bouba et al., 2017; Papu 2014)) and fruits (dates, figs, tomatoes; (Al-Farsi et al., 2015; Sohail et al., 2011). This method allows the reduction or the almost complete removal of water, which is the driving factor for the development of microorganisms and ferments inside food (Priecina and Karklina, 2014), in a relatively short time. However, after its application, changes in the quality of the product, both from a visual (i.e., colour) and chemical (i.e. nutrient content) point of view can occur (Amit et al., 2017; Oliveira et al., 2016).

Over the years, studies on food preservation have brought enormous advancements in this area, generating modern, very efficient, but expensive, methods of maintaining food integrity. Among them, freeze-drying, or lyophilization, is considered the most promising one (Novak et al., 2020), being able to almost completely dry the product with just small alterations to its nutritional value (Bhatta et al., 2020). Drying under freezing (i.e., sublimation) and in anoxic conditions may, in fact, ensure the complete dehydration of the product with very small influence on its nutritional characteristics (Bhatta et al., 2020). However, negative changes in the quality of some vegetable products, after freeze-drying, have also been highlighted (Poiana et a., 2020; Shofian et al., 2011).

The consumption of vegetables is essential for the achievement of human health and well-being (Grumezescu et al., 2018; Javed et al., 2019) because they contain numerous health-promoting molecules (such as vitamins, organosulphur, carotenoids, carbohydrates, and antioxidants;(Singh et al., 2015; Arya et al.,2019) that the human body is unable to produce itself. The regular consumption of vegetables, in fact, may ensure physiological well-being and reduce the risk of incidence of pathologies, such as cardiovascular diseases, diabetes, and cancer (Ramya et al., 2019). However, following the application of preservation methods, the nutritional profile of vegetables can be altered, either from a positive or negative point of view (Vyankatrao et al., 2014; Kamiloglu et al., 2016; Neri et al., 2020). Therefore, the choice of the best method to preserve the quality of plant food should be carefully investigated on a case-by-case basis.

The elephant garlic (*Allium ampeloprasum* L.) cultivated in Valdichiana (locally known as "Aglione della Valdichiana"), an agricultural area located in SE Tuscany (central Italy), is a traditional crop plant, which has recently undergone an important re-evaluation both from the nutritional and commercial point of view (Loppi et al.,2021; Vannini et al., 2021). From the nutritional point of view, elephant garlic is similar to common garlic, but with a much milder effect on human breath due to a much lower allicin content and a better digestibility owed to the lower fiber content (Ceccanti et al., 2021). The overall production of Aglione della Valdichiana is about 150 t y-1 and the product is commonly placed on the market about one month from harvest, after a period of air-drying, the traditional drying process. In this way, post-harvest preservation may last 6-8 months. Air-drying elephant garlic heads reduces the water content by approximately 20% (Loppi et al., 2021), but the effect of this process on the nutritional profile, to the best of our knowledge, has never been investigated. Similarly, also the effect of lyophilization has never been investigated. This study thus aimed to investigate if air-drying and freeze-drying change the nutritional properties of elephant garlic from Valdichiana.

Materials and Methods

Sample collection

Plants of elephant garlic *Allium ampeloprasum* (L.) were collected from three traditional cultivation fields located in the Valdichiana (Chiana plain) during June 2021. Each farmer provided five randomly selected fresh heads of elephant garlic, each composed of five cloves. In the laboratory, one clove was randomly taken from each head (statistical replicate) and then immediately analyzed for its nutritional properties (fresh samples). At the same time, another clove was randomly taken from each head and then freeze-dried (freeze-dried samples). The remaining samples were left in a sun-sheltered and ventilated place at a temperature of 25 ± 5°C and relative humidity in the range 55-60% for four weeks. After this time, another clove was randomly taken from each head and analyzed for the content of nutritional molecules; these cloves (air-dried samples) simulate the product in its "market conditions". The experiment was replicated three times.

Sample preparation

Both fresh and air-dried samples were peeled, finely chopped using a stainless-steel knife, and immediately analyzed for their nutritional content. Instead, freeze-dried samples were peeled, finely chopped, frozen at -80°C for 24 hours, and then freeze-dried for 48 hours using a freeze-dryer Lio SP (5Pascal, Milan, Italy) equipped with a Edwards RV3 oil vacuum pump (operating conditions: T = - 50°C, P = 0.2 mbar).

Nutraceutical analysis

The analyses carried out in this work followed the methods reported by Loppi et al. (2021) for the investigation of the nutritional features of elephant garlic cultivated in Valdichiana. The water content of samples was determined after oven-drying at 65°C for 24h. All values are expressed on an oven-dry wet basis. All analyses were run in triplicate.

Soluble proteins

The method described by Bradford (1976) was followed. Samples (approx. 0.1 g) were homogenized in 4 mL H_2O and centrifuged at 4000 rpm for 5 min.; subsequently 0.2 mL of the supernatant were added to 0.8 mL of Bradford solution (Sigma-Aldrich, Darmstadt, Germany). The absorbance of the samples was read at 595 nm with a UV-VIS spectrophotometer (Agilent 8453, Santa Clara, CA, USA). The quantification was obtained with a 10-100 µg/mL calibration curve with bovine albumin (Sigma-Aldrich, Darmstadt, Germany). The results are expressed as mg g-1 of bovine albumin equivalent (BAE).

Polyphenols

The method described by Henriquez et al. (2010) was followed, with minor modifications. Samples (approx. 0.1 g) were homogenized in 4 mL of 70% acetone and centrifuged at 4000 rpm for 5 min. 0.5 mL of the supernatant were added to 0.125 mL of the Folin-Denis' reagent (Sigma-Aldrich, Darmstadt, Germany), 0.750 mL of a saturated NaCO3 solution, and 0.950 mL of deionized water. The solution was then left at 36° for 30 min and then centrifuged again. The absorbance of the samples was read at 750 nm with a spectrophotometer (Agilent 8453, Santa Clara, CA, USA). The quantification was obtained with 30-300 µg/mL a calibration curve with gallic acid (Sigma-Aldrich, Darmstadt, Germany). The results are expressed as mg g-1 of gallic acid equivalent (GAE).

Flavonoids

The method described by Heilmer et al. (2005) was followed. Samples (approx. 0.5 g) were homogenized in 2 mL of 80% ethanol and then centrifuged at 15,000 rcf for 5 min. The supernatant (300 µL) was added to 45 µL of a 10% $AlCl_3$ solution, at which 300 µL of a 1M NaOH solution and 360 µL of deionized H_2O were then added. The absorbance of the samples was read at 517 nm with a spectrophotometer (Agilent 8453, Santa Clara, CA, USA). The quantification was done by using a calibration curve (5-200 µg/mL) of quercetin (Sigma-Aldrich, Darmstadt, Germany). The results were expressed as mg g^{-1} of quercetin equivalent (QE).

Free radical scavenging activity

The method described by Brand-Williams et al. (1995) was followed, with modifications. Samples (approx. 0.1 g) were homogenized in 2 mL of 80% ethanol and then centrifuged at 15,000 rcf for 5 min. The supernatant (100 µL) was added to 1 mL of a DPPH (Sigma-Aldrich, Darmstadt, Germany) solution made by dissolving 3.9 mg of this reagent in 0.1 L of methanol/water (80:20v/v). All preparations were left in dark conditions (1 h) and their absorbance read at 517 nm with a spectrophotometer (Agilent 8453, Santa Clara, CA, USA). Results were expressed as % Antiradical Activity (ARA%) according to the formula:

$$ARA\% = 100_x(1–sample/control)$$

Where control indicates the absorbance of the reagents only.

Starch

The method described by Lebon et al. (2004) was followed. Samples of (approx. 0.1 g) were homogenized in 4 mL of DMSO and then 0.5 mL of 8 M HCl were added. The extract was left at 60°C for 30 min, and, 0.5 mL of 8 M NaOH were then added. The resulting mixture was brought to 10 mL with deionized water. Subsequently, the samples were centrifuged at 4000 rpm for 5 min and 0.5 mL of the supernatant were added to 2.5 mL of a Lugol solution (HCl 0.05 M-0.03% I2, 0.06% KI); the mixture was left to react for 15 min. Samples were read at 605 nm with a spectrophotometer (Agilent 8453, Santa Clara, CA, USA). The content of starch was quantified using a 10.0-440 µg/mL calibration curve prepared with pure starch (Merck KGaA, Darmstadt, Germany).

Soluble sugars and Total Sweetness Index (TSI)

The method described by Fedeli et al. (2022) was followed. Samples (approx. 0.1 g) were homogenized in 2 mL of deionized water and then centrifuged at 15,000 rcf for 5 min. The supernatant was filtered using a syringe filter (0.45 µm) and then analyzed by HPLC

(Waters 600 system, MA, USA) combined with a Waters 2410 refractive index detector (MA, USA). Deionized water was used as mobile, eluted at 0.5mL/min, and a Waters Sugar-Pak I ion-exchange column (6.5 × 300 mm) maintained at 90°C using an external temperature controller (Waters Column Heater Module, MA, USA), as separator. Quantification of sucrose, glucose, and fructose was obtained using 0.1-20 mg/mL calibration curves prepared by diluting the 3 pure sugars (Merck KGaA, Darmstadt, Germany) in deionized water. The total sweetness index (TSI) was calculated according to the formula proposed by Clarke et al. (1995), often used in past studies on different food products (Fedeli et al.,2022; Magwaza et al., 2015; Chen et al., 2019).

Content of allicin

The method described by de Diego et al. (2007) was followed. Samples (approx. 0.5 g) were homogenized in 2 mL of an ethanol/water solution (50%/50%). Then, samples were centrifuged at 4000 rpm for 10 minutes, filtered at 0.45 µm and directly analyzed by means of a Waters 600 HPLC system (MA, USA). Allicin was separated using methanol/water (50%/50%) eluted at 1.0 mL/min and an Agilent C18 column (150x4.6 mm with 5 µm of particle size). Runs were monitored at 242 nm using a Waters 996 Photodiode Array Detector (PAD). Allicin was quantified by means of a calibration curve (40–160 µg/mL) prepared by dissolving the pure compound (DBA Italia s.r.l.; Milan, Italy) in an ethanol/water solution (50%/50%).

Statistical analysis

The significance ($p<0.05$) of differences between the drying methods was checked by the pair wise t-test, correcting for multiple comparisons according to Benjamini and Hochberg (1995). Data normality was checked using the Shapiro-Wilk test. All calculations were run using the R software (2022).

Results

The water content of fresh, air-dried, and freeze-dried samples was remarkably different (Tab. 1), with reductions by ca. 20% and 80% for air- and freeze-dried samples, respectively.

Compared to fresh samples, air-dried samples showed reductions in the content of starch (-22%) and the expression of the total antioxidant power (-46%), while freeze-dried samples showed reductions for all the investigated parameters (ca. -40%), except for the content of sucrose (Tab. 1).

Differences between air-dried and freeze-dried samples emerged for the content of polyphenols (32%), soluble proteins (36%), glucose (37%), fructose (37%), as well as in the expression of the total sweetness index (27%), with lower values always observed in freeze-dried samples (Tab. 1). Statistically significant differences in the content of allicin did not emerge (Tab. 1).

	Fresh	***Air-dried***	***Freeze-dried***
Water content %	84.1 ± 0.1 a	64.1 ± 0.1 b	4.0 ± 0.1 c
Antioxidants (ARA%)	24.0 ± 2.1 a	12.8 ± 1.4 b	16.2 ± 2.7 b
Polyphenols (mg g^{-1})	2.0 ± 0.1 a	1.9 ± 0.2 a	1.3 ± 0.1 b
Soluble proteins (mg g^{-1})	25.8 ± 0.9 a	26.0 ± 0.8 a	16.7 ± 0.3 b
Starch (mg g^{-1})	3.6 ± 0.1 a	2.8 ± 0.5 b	2.1 ± 0.1 b
Sucrose (mg g^{-1})	12.6 ± 1.4 a	11.3 ±0.5 a	11.1 ± 0.9 a
Glucose (mg g^{-1})	5.5 ± 0.3 a	4.8 ± 0.2 a	3.0 ± 0.2 b
Fructose (mg g^{-1})	5.6 ± 0.3 a	4.8 ± 0.2 a	3.0 ± 0.2 b
Total sweetness index (TSI)	25.2 ± 1.7 a	22.1 ± 0.6 a	16.2 ± 1.0 b
Allicin (mg g^{-1})	0.36 ± 0.12 a	0.29 ± 0.12 a	0.38 ± 0.02 a

Table 1: Nutraceutical parameters (mean ± standard error) in fresh, air-dried, and freeze-dried samples of Allium ampeloprasum. Different letters indicate statistically significant ($p<0.05$) differences.

Discussion

The total antioxidant power of plant-based products or whole plants can correspond to the sum of the individual amounts of compounds with an antioxidant activity present in their tissues, generally resumed as carotenoids, polyphenols, alkaloids, nitrogen-containing, and organosulphur compounds (Nayak et al., 2015). Those compounds are, perhaps, very labile since the application of common food preservation methods, such as drying and freezing, may alter their content. In fact, both drying and freezing can reduce the total antioxidant power of both fruits and vegetables as a consequence of reactive oxygen species (ROS) overproduction (Bhatta et al., 2020; Lohachoompol et al. 2004; Hung et al., 2012; Tan et al., 2021). Dehydration, increases in respiration, and cellular damage are the main features responsible for this process (Baek et al., 2012; Meitha et al., 2020), which, in turn, causes the utilization of the whole antioxidant pool to counteract the oxidizing action. However, also food manipulation, such as cutting, can have a relevant role in reducing the total antioxidant power of plant-based food (Opara et al., 2010; Cocetta et al., 2014) since, after cutting, the oxidation of the sample increases and oxidizing enzymes from damaged cells can be released (Opara et al., 2010; Bravo and Mateos, 2008). Hence, reductions in the anti-radical scavenging activity of both air-dried and freeze-dried samples of *A. ampeloprasum* may have occurred following two main processes: either the application of the drying and freezing method or the manual chopping of the samples that happened before (in the case of freeze-dried samples) or after (in the case of air-dried samples) the application of the preservation methods.

Polyphenols are the main representative family of antioxidants in vegetables, composed of phenolic acids, flavonoids, stiblins, phenolic alcohols, and lignans, well-known molecules which provide beneficial effects for human health (Abbas et al., 2017). Although their amount seems related to the expression of the total antioxidant power, their concentration can remain unchanged even if reductions in the total antioxidant power can be observed (Sang et al., 2014; Santarelli et al., 2020). Consistently, a reduction in the content of polyphenols was not observed in air-dried elephant garlic samples. Nevertheless, polyphenol decrease in freeze-dried samples may have been caused by the additional release of enzymes from the cells following their destruction as a result of freezing (Shofian et al., 2011). In support of this view, reductions in the content of polyphenols following freeze-drying were observed in fruits of mango (*Mangifera indica* L.), tomato (*Lycopersicon esculentum* L.), strawberry (*Fragaria ananassa* L.), sweet cherry (*Prunus avium* L.), and sour cherry (*Prunus cerasus* L.) (Poiana et al., 2010; Tan et al., 2021; Rahman et al., 2015).

Starch is an insoluble polysaccharide consisting of polymers of α-glucose, synthesized by plants as a storage carbohydrate, and therefore, as an energy source for cellular metabolism (Raigond et al., 2015). A decrease in starch content after harvest can occur in fruits as a consequence of its use for sugar production (MacRae et al., 1992; Wegrzvn and Elspeth, 1995), but in samples of air-dried elephant garlic, this starch reduction was not observed. Nevertheless, a reduction in starch without an increase in soluble sugars was observed in samples of common garlic (*A. sativum* L.) even several months after harvest (Mashavekhi et al., 2016), suggesting that a decrease in starch content but not followed by an increase in sugar may be the rule for Allium species. The reduction of starch in freeze-dried samples, instead, may have occurred as a consequence of starch degradation generated by freezing (Feng et al., 2020), a process well-known to alter its physical properties, as well as its chemical reactivity (Oyinloye et al., 2020).

A similar effect of freezing on molecules with nutritional value was also observed for the content of soluble proteins, which decreased by ca. 35%. Proteins are thermolabile molecules, easily subjected to unfolding after cooling and freezing, a consequence of the combination of three main factors, technically called low temperature, freeze-concentration, and ice formation (Bhatnagar et al., 2007). Following these processes, in fact, proteins face structural damage which may subsequently affect their chemical reactivity (Roy and Munishwar, 2004). Sugars can protect protein unfolding during drying (Tang et al., 2005; Tonnis et al., 2015), but this protection is not enough to totally protect from the damage that occurs as a consequence of the stronger action of freeze-drying (Allison et al., 1999). Following freeze-drying, reductions in the content of some soluble sugars (such as glucose and fructose) were also observed. We speculate that this effect is most likely due to the binding of sugars with proteins, which occurred as a consequence of the sample freezing (Tang et al., 2005). We assume that this binding, although partially reversible, would have been able to reduce the content of sugars that could be separated, and therefore, analyzed by HPLC. However, further evidence of this process is necessary.

The concentration of soluble sugars and the taste of plants are strictly intercorrelated (McCaughey et al., 2008). Freeze-drying reduced the total sweetness of elephant garlic, thus reducing also its palatability, a fundamental feature for the enjoyment of the product.

Allicin is an organosulphur compound produced by garlic following its conversion from alliin, which occurs after clove breakage. This molecule, naturally produced as a defense against predation, has important features for human health, such as anti-cancer, antibacterial, and anti-diabetic properties (Borlinghaus et al., 2014). The amount of allicin measured in this study is consistent with the 0.4 mg/g reported in the literature (Kim et al., 2018). Differences in the content of allicin between fresh, air-dried, and freeze-dried samples were not observed, suggesting that the preservation method does not influence the concentration of this compound.

Conclusion

Two different methods, i.e. air-drying and freeze-drying, of preservation of elephant garlic cultivated in Valdichiana have different effects on the nutritional traits of this traditional crop plant. Compared to fresh samples, the traditional air-drying method causes significant reductions only in the total antioxidant power (-46%) and in the starch content (-22%), while the modern freeze-drying method negatively affects almost all the investigated parameters overall by about 40%. The content of allicin is not changed by the preservation method.

In conclusion, although freeze-drying is considered one of the best methods for preserving plant-based food, attention should be paid to the effect of this process on the nutritional characteristics of the product. Lastly, since freeze-drying is a widely-used method of sample preparation prior to nutritional analysis, its use for this objective should be strongly reconsidered.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Abdou Bouba Armand., et al. "Effect of solar and electric drying on the content of the phenolic compounds and antioxidant activity of three varieties of onion (Allium cepa L)". International journal of biology, pharmacy and allied sciences (2012).
2. Abbas Munawar., et al. "Natural polyphenols: An overview". International Journal of Food Properties 20.8 (2017): 1689-1699.
3. Al-Farsi Mohamed., et al. "Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactylifera L.) varieties grown in Oman". Journal of agricultural and food chemistry 53.19 (2005): 7592-7599.
4. Allison S Dean., et al. "Hydrogen bonding between sugar and protein is responsible for inhibition of dehydration-induced protein unfolding". Archives of Biochemistry and Biophysics 365.2 (1999): 289-298.
5. Amit Sadat Kamal., et al. "A review on mechanisms and commercial aspects of food preservation and processing". Agriculture & Food Security 6.1 (2017): 1-22.
6. Arya MS., et al. "Nutraceuticals in vegetables: New breeding approaches for nutrition, food and health: A review". Journal of Pharmacognosy and Phytochemistry 8.1 (2019): 677-682.
7. Baek Kwang-Hyun and Daniel Z Skinner. "Production of reactive oxygen species by freezing stress and the protective roles of antioxidant enzymes in plants". Journal of Agricultural Chemistry and Environment (2012).
8. Benjamini Yoav and Yosef Hochberg. "Controlling the false discovery rate: a practical and powerful approach to multiple testing". Journal of the Royal statistical society: series B (Methodological) 57.1 (1995): 289-300.

9. Bhatnagar Bakul S, Robin H Bogner and Michael J Pikal. "Protein stability during freezing: separation of stresses and mechanisms of protein stabilization". Pharmaceutical development and technology 12.5 (2007): 505-523.
10. Bhatta Sagar, Tatjana Stevanovic Janezic and Cristina Ratti. "Freeze-drying of plant-based foods". Foods 9.1 (2020): 87.
11. Bradford Marion M. "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". Analytical biochemistry 72.1-2 (1976): 248-254.
12. Brand-Williams Wendy, Marie-Elisabeth Cuvelier and CLWT Berset. "Use of a free radical method to evaluate antioxidant activity". LWT-Food science and Technology 28.1 (1995): 25-30.
13. Bravo Laura and Raquel Mateos. "Analysis of flavonoids in functional foods and nutraceuticals". Methods Anal Funct Foods Nutraceuticals 17 (2008): 147-206.
14. Borlinghaus Jan., et al. "Allicin: chemistry and biological properties". Molecules 19.8 (2014): 12591-12618.
15. Ceccanti Costanza., et al. "Comparative phytochemical profile of the elephant garlic (Allium ampeloprasum var. holmense) and the common garlic (Allium sativum) from the Val di Chiana area (Tuscany, Italy) before and after in vitro gastrointestinal digestion". Food Chemistry 338 (2021): 128011.
16. Chen Xiao-li., et al. "Sugar accumulation and growth of lettuce exposed to different lighting modes of red and blue LED light". Scientific Reports 9.1 (2019): 1-10.
17. Clarke, M. Carbohydrates, Industrial; Wiley-VCH: New York, NY, USA, (1995).
18. de Diego Marta., et al. "Validated liquid chromatographic method for quantitative determination of allicin in garlic powder and tablets". Journal of separation science 30.16 (2007): 2703-2707.
19. Cocetta Giacomo., et al. "Effect of cutting on ascorbic acid oxidation and recycling in fresh-cut baby spinach (Spinacia oleracea L.) leaves". Postharvest Biology and Technology 88 (2014): 8-16.
20. Esper A and W Mühlbauer. "Solar drying-an effective means of food preservation". Renewable energy 15.1-4 (1998): 95-100.
21. Fedeli Riccardo., et al. "Bio-Based Solutions for Agriculture: Foliar Application of Wood Distillate Alone and in Combination with Other Plant-Derived Corroborants Results in Different Effects on Lettuce (Lactuca Sativa L.)". Biology 11.3 (2022): 404.
22. Feng Wenjuan, Sen Ma and Xiaoxi Wang. "Recent advances in quality deterioration and improvement of starch in frozen dough". Grain & Oil Science and Technology 3.4 (2020): 154-163.
23. Grumezescu Alexandru Mihai and Alina Maria Holban, eds. Food quality: balancing health and disease, Academic Press 13 (2018).
24. Henríquez Carolina., et al. "Determination of antioxidant capacity, total phenolic content and mineral composition of different fruit tissue of five apple cultivars grown in Chile". Chilean journal of agricultural research 70.4 (2010): 523-536.
25. Heimler Daniela., et al. "Rapid tests to assess the antioxidant activity of Phaseolus vulgaris L. dry beans". Journal of Agricultural and Food Chemistry 53.8 (2005): 3053-3056.
26. Hung PV and TL Duy. "Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants". International Food Research Journal 19.1 (2012).
27. Javed I, Waseem A and Ammad R. "Vegetables as a source of important nutrients and bioactive compounds: their human health benefits". MOJ Food Process Technol 7.4 (2019): 136-146.
28. Kamiloglu Senem., et al. "A review on the effect of drying on antioxidant potential of fruits and vegetables". Critical reviews in food science and nutrition 56.sup1 (2016): S110-S129.
29. Kim Sunyoung., et al. "Comparative studies of bioactive organosulphur compounds and antioxidant activities in garlic (Allium sativum L.), elephant garlic (Allium ampeloprasum L.) and onion (Allium cepa L.)". Natural product research 32.10 (2018): 1193-1197.
30. Lebon Gaël., et al. "Flower abscission and inflorescence carbohydrates in sensitive and non-sensitive cultivars of grapevine". Sexual Plant Reproduction 17.2 (2004): 71-79.
31. Lohachoompol Virachnee, George Srzednicki and John Craske. "The change of total anthocyanins in blueberries and their antioxidant effect after drying and freezing". Journal of Biomedicine and Biotechnology 2004.5 (2004): 248-252.
32. Loppi Stefano., et al. "Comparison of the Mineral and Nutraceutical Profiles of Elephant Garlic (Allium ampeloprasum L.) Grown

in Organic and Conventional Fields of Valdichiana, a Traditional Cultivation Area of Tuscany, Italy". Biology 10.10 (2021): 1058.

33. MacRae Elspeth., et al. "Carbohydrate metabolism during postharvest ripening in kiwifruit". Planta 188.3 (1992): 314-323.
34. Mashayekhi Kambiz., et al. "Change in carbohydrate and enzymes from harvest to sprouting in garlic". Food Science & Nutrition 4.3 (2016): 370-376.
35. McCaughey Stuart A. "The taste of sugars". Neuroscience & Biobehavioral Reviews 32.5 (2008): 1024-1043.
36. Magwaza, Lembe Samukelo and Umezuruike Linus Opara. "Analytical methods for determination of sugars and sweetness of horticultural products-A review". Scientia Horticulturae 184 (2015): 179-192.
37. Meitha Karlia, Yonadita Pramesti and Sony Suhandono. "Reactive oxygen species and antioxidants in postharvest vegetables and fruits". International Journal of Food Science (2020).
38. Nayak Balunkeswar, Rui Hai Liu and Juming Tang. "Effect of processing on phenolic antioxidants of fruits, vegetables, and grains-a review". Critical reviews in food science and nutrition 55.7 (2015): 887-918.
39. Neri Lilia., et al. "Antioxidant activity in frozen plant foods: Effect of cryoprotectants, freezing process and frozen storage". Foods 9.12 (2020): 1886.
40. Nowak Dorota and Ewa Jakubczyk. "The freeze-drying of foods-The characteristic of the process course and the effect of its parameters on the physical properties of food materials". Foods 9.10 (2020): 1488.
41. Oliveira Sara M, Teresa RS Brandao, and Cristina LM Silva. "Influence of drying processes and pretreatments on nutritional and bioactive characteristics of dried vegetables: A review". Food Engineering Reviews 8.2 (2016): 134-163.
42. Opara Umezuruike Linus and Majeed R Al-Ani. "Antioxidant contents of pre-packed fresh-cut versus whole fruit and vegetables". British Food Journal (2010).
43. Oyinloye Timilehin Martins and Won Byong Yoon. "Effect of freeze-drying on quality and grinding process of food produce: A review". Processes 8.3 (2020): 354.
44. Papu Singh., et al. "Effect of drying characteristics of garlic-a review". Journal of Food Processing & Technology 5.4 (2014): 1.
45. Sohail Muhammad., et al. "Physicochemical and microbiological evaluation of sun dried tomatoes in comparison with fresh tomatoes". Pakistan Journal of Biochemistry and Molecular Biology 44.3 (2011): 106-109.
46. Priecina Liga and Daina Karklina. "Natural antioxidant changes in fresh and dried spices and vegetables". International Journal of Nutrition and Food Engineering 8.5 (2014): 492-496.
47. Poiana MA., D Moigradean and E Alexa. "Influence of home-scale freezing and storage on antioxidant properties and color quality of different garden fruits". Bulgarian Journal of Agricultural Science 16.2 (2010): 163-171.
48. R Core Team. "R: A language and environment for statistical computing". R Foundation for Statistical Computing, Vienna, Austria (2022).
49. Rahman MM., et al. "Effect of freeze drying on antioxidant activity and phenolic contents of Mango (Mangifera indica)". International Food Research Journal 22.2 (2015): 613.
50. Raigond Pinky, Rajarathnam Ezekiel and Baswaraj Raigond. "Resistant starch in food: a review". Journal of the Science of Food and Agriculture 95.10 (2015): 1968-1978.
51. Ramya V and Priya Patel. "Health benefits of vegetables". IJCS 7.2 (2019): 82-87.
52. Roy Ipsita and Munishwar Nath Gupta. "Freeze-drying of proteins: Some emerging concerns". Biotechnology and applied biochemistry 39.2 (2004): 165-177.
53. Sang Saw Yei., et al. "Influence of drying treatments on antioxidant capacity of forage legume leaves". Journal of food science and technology 51.5 (2014): 988-993.
54. Santarelli Veronica., et al. "Response of organic and conventional apples to freezing and freezing pre-treatments: Focus on polyphenols content and antioxidant activity". Food chemistry 308 (2020): 125570.
55. Shofian Norshahida Mohamad., et al. "Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits". International Journal of molecular sciences 12.7 (2011): 4678-4692.
56. Singh SAURABH and Mayanglambam Bilashini Devi. "Vegetables as a potential source of nutraceuticals and phytochemicals: A review". Int. J. Med. Pharm. Sci 5 (2015): 1-14.

57. Tan Si., et al. "Lycopene, polyphenols and antioxidant activities of three characteristic tomato cultivars subjected to two drying methods". Food Chemistry 338 (2021): 128062.
58. Tang Xiaolin Charlie and Michael J Pikal. "The effect of stabilizers and denaturants on the cold denaturation temperatures of proteins and implications for freeze-drying". Pharmaceutical research 22.7 (2005): 1167-1175.
59. Tonnis Wouter F., et al. "Size and molecular flexibility of sugars determine the storage stability of freeze-dried proteins". Molecular pharmaceutics 12.3 (2015): 684-694.
60. Vannini Andrea., et al. "Potentially Toxic Elements (PTEs) in Soils and Bulbs of Elephant Garlic (Allium ampeloprasum L.) Grown in Valdichiana, a Traditional Cultivation Area of Tuscany, Italy". Applied Sciences 11.15 (2021): 7023.
61. Vyankatrao Naikwade Pratap, Nya Tyatyasaheb Aathalye Arts and VSRS Commerce. "Effect of drying methods on nutritional value of some vegetables". Proceeding of the National Conference on Conservation of Natural Resources & Biodiversity for Sustainable Development. Biosci. Discov 6 (2014).
62. Wegrzyn Teresa and Elspeth MacRae. "Alpha-amylase and starch degradation in kiwifruit". Journal of Plant Physiology 147.1 (1995): 19-28.

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