Fresh Crushed Garlic Exhibits Superior Allicin and Pyruvic Acid Stability, While Fresh Sliced Garlic Leads in Phenolic and Antioxidant Content

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Running Title: Peak Allicin Content, Antioxidant Activity, and Pyruvic Acid Levels….

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Abstract

This study investigates the stability of allicin, phenols, and antioxidants in different forms of garlic (fresh whole peeled, fresh sliced, fresh crushed, and dried slices) under various storage conditions (0 to 2 days at 4 ºC and 20 ºC). Fresh garlic exhibited allicin levels ranging from 0.6 to 32.14 mg/g, while dried garlic showed significantly lower levels (3.77 to 6.68 mg/g). Maximum allicin stability in fresh garlic was observed after 10 minutes at 20 ºC, with pyruvic acid peaking after 10 minutes at 4 ºC. Freshly sliced and crushed garlic retained the highest phenol content and antioxidant activity immediately after preparation, whereas dried garlic had reduced levels due to thermal processing. Consumer testing revealed that hummus with fresh crushed garlic at 10 minutes and 20 ºC was preferred, while dried garlic at 20 minutes and 4 ºC had the strongest flavor and aroma. In conclusion, fresh crushed garlic is optimal for allicin and pyruvic acid content, while fresh sliced garlic excels in phenol and antioxidant levels; drying notably diminishes these beneficial compounds, affecting flavor and consumer preference.

INTRODUCTION

Garlic, *Allium sativum*, is one of the ancient crops, and it has been named the "aroma" vegetable since it has been used as a flavoring in cuisine and as a medication due to its numerous health and wellness advantages in many civilizations (Botas *et al*., 2019; Netzel, 2020). Garlic has a high concentration of functionally active compounds and a diverse chemical composition (Marchese *et al*., 2016). Multiple research studies have demonstrated that the health benefits attributed to fresh garlic extract are believed to be associated with the presence of a total of 33 sulfur compounds (Amagase, 2006; Bhandari, 2012; Kovarovič *et al*., 2019). Those benefits include antioxidant properties, anti-inflammatory activity, antimicrobial activity, immunological activation, cardiovascular defense, antitumor, anti-carcinogenic, gastric safety, anti-diabetic function, anti-obesity activity, the nervous and endocrine systems (Shang *et al*., 2019; Gam *et al*., 2021).

Garlic has a weak, undetectable odor until it is peeled. When peeled, sliced, or crushed, it forms a compound called allicin, which releases a strong odor containing sulfur glycosides (Yusuf *et al*., 2018). Allicin, or diallyl thiosulfinates, is the most active biological ingredient in garlic (Farías-Campomanes *et al*., 2014), accounting for over 70% of the total thiosulfinates found in cloves (Rahman, 2007), it's highly unstable and can breakdown even at ambient temperature (Ilić *et al*., 2015; Zhu and Zeng, 2020), in a matter of days or hours to far more stable sulfur-containing compounds (Rybak *et al*., 2004). Furthermore, allicin is not present in garlic; it is synthesized from alliin (S-Allyl-L cysteine-sulfoxides), an odorless derivative of cysteine, by the enzyme allinase (Abe *et al*., 2020). Upon processing or consumption, these compounds contribute to the distinct pungent aroma of garlic (Kovarovič *et al*., 2019).

Khar et al., (2011) studied 93 garlic ecotypes for allicin content and observed variability in allicin content in each one. Prati et al., (2014) studied allicin content in 5 cultivars for three forms of processed garlic (unsalted garlic paste, chopped fried garlic, and fried sliced garlic) for a long period of storage reaching 180 days, with the most loss of allicin recorded in fried garlic, followed by paste. Another researcher reported that cooking methods at home (boiling, microwaving, pressure cooking, griddling, frying, and baking) have been demonstrated to affect the important components and antioxidant activity of garlic (Martins et al., 2016). The stability of allicin, which is typically thought of as unstable in nature, has been the subject of several research. The stability of allicin varies depending on environmental parameters including temperature and the solvent used to dissolve it (Chan *et al*., 2012). This unstable chemical has poor solubility in water (Marchese *et al*., 2016). The most prevalent bioactive components of garlic are naturally sensitive to temperature degradation, which reduces their effectiveness (Martins *et al*., 2016). The high volatility and instability of allicin limit the use of garlic as a pharmacologically active drug, which significantly restricts its commercial usage. Also, Allicin's instability is one issue that inhibits its chemical uses (Ilić *et al*., 2011).

Given the significant volatility of organosulfur compounds in garlic, such as allicin, proper storage conditions are critical for retaining the excellent quality of garlic bulbs and by-products. The period of storage is critical for garlic's bioactive qualities (Martins et al., 2016). Also, storage temperature is particularly significant since it can influence the chemical structure and, as a result, the ultimate bioactivity intensity of garlic and allicin content over time (Veríssimo et al., 2010). Most of the previous work available in the literature relates to the evaluation of allicin at high temperatures, long storage interval periods (up to 45 days or 180 days), and the comparison of different ecotypes or cultivars of garlic. From another point of view, for processed foods, the approach has a substantial impact on the retention of bioactive chemicals from garlic, especially those connected to antioxidant properties like the one achieved by allicin from garlic (Prati *et al*., 2014). The previous work available in the literature relates to the evaluation of allicin stability in different solvent extractions, showing allicin stability in polar and nonpolar solvents after extraction (Zalepugin et al., (2015). In our study, we evaluated allicin stability before the extraction process in different forms (freshly sliced, freshly crushed, and dried slices) during short storage intervals, which were considered practical for daily use. Based on the above, the aims of this study are to evaluate the stability of allicin in fresh sliced garlic, freshly crushed garlic, and dried garlic slices under different storage conditions of time and temperature (from zero time to two days at 4 ºC and 20 ºC).

MATERIALS AND METHODS

Materials

Fresh white garlic (*Allium sativum L.*) bulbs and hummus (a dip made from pureed chickpeas sesame seed paste and lemon) were purchased from the local market in Irbid, Jordan. Allicin standard (purity 98%) was purchased from (TargetMol, EU). All chemical reagents utilized in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA).

Sample Preparation

This study analyzed three different forms of garlic: freshly sliced, freshly crushed, and dried garlic slices. Fresh whole garlic was used as a control. Thirteen samples were prepared, each weighing 10 g, and stored under different conditions. Fresh garlic was cut into uniform slices (3.5 mm) using a kitchen knife (Fresh sliced), crushed into a paste using a garlic press (Fresh crushed), and dried slices were dried in the oven at 60 ºC for 24 hours (Dried garlic). The garlic samples were prepared, labeled, and stored at different temperatures for different times as described in Table 1.

Garlic Extraction

Garlic was extracted according to the method described by Bat-Chen *et al*., (2010) with some appropriate modifications. The residue was reconstituted with 5 ml of the mobile phase (50 % Water: 50 % Methanol) and homogenized by placing it in a closed beaker, then sonicated before filtration.

Identification of Allicin in Garlic Extract by HPLC

Allicin stock solutions were prepared with 50 mg of allicin (Commercially available) dissolved in 5 ml of 60:40 (mixtures of water and methanol) as described by Rybak *et al*., (2004). The identification of the allicin peak was at 220 nm wavelength. Two vails were injected in the HPLC to determine the allicin content in the samples. The first vial was garlic extract without the standard, and the second was garlic extract with the standard. Then, the intensity of the peak was compared to the standard.

Determination of Allicin Content in Garlic Extract by HPLC

The identification and quantification of allicin were performed in a high-performance liquid chromatography (HPLC) system (SHIMADZU, Japan). The determination of allicin in the garlic sample was performed according to the procedure described by de Diego et al., (2007) with some modifications in column diminution (100 mm x 3 mm).

Determination of Pyruvic Acid Content in Garlic

The pyruvic acid content in garlic extract was measured according to a procedure described by Anthon and Barrett, (2003).

Determination of Total Phenol Content

The total phenol content of the garlic extract was determined using the Folin–Ciocalteu method, as previously described by Alu'datt *et al*., (2010).

Determination of Antioxidant Activity

Anti-oxidant activity of the garlic extract was determined using a method described by (Brand-Williams *et al*., 1995).

Sensory Evaluation

The study was reviewed and approved by the scientific research committee at the Department of Nutrition and Food Technology, Jordan University of Science and Technology to perform the sensory evaluation. The samples are hummus with different treatments of garlic prepared according to the method conducted by Olaimat *et al*., (2017). A sensory evaluation of hummus samples was conducted using a hedonic scale of acceptance (Yilmaz and Aydeniz, 2012), and Just About Right (JAR) scales to assess whether the intensity of an attribute is appropriate (Lawless and Heymann, 2010). A total of 40 participants from Jordan University of Science and Technology, aged between 18 and 30 years participated in this study. Different samples of hummus were prepared and garlic from different treatments was added to the hummus samples. Participants were directed to taste samples and evaluate them at individual tables, each participant was provided with thirteen plates. To eliminate carry-over factors, participants were also provided with green apples and water for mouth cleaning between samples. The participants were asked to record their acceptability scores (Hedonic scale) of samples for overall appearance, overall color, overall aroma, overall pungency, and overall flavor (9-point scale with 9 = extremely like and 1 = extremely dislike). Acceptability intensity scores (Just About Right (JAR) scales) of samples for flavor, aroma, and pungency (5-point scale with 5 = normal and 1 = too much).

Statistical Analysis

Results were analyzed as the mean and standard deviation using the general linear model (GLM) method with JMP statistical package (JMP Institute Inc., Cary, NC, USA). Means were isolated using the least significant difference (LSD) test procedure. Statistical difference was considered significant if p-value ≤ 0.05.

RESULTS AND DISCUSSION

Identification of Allicin in Garlic Extract by HPLC

The main peaks in the chromatograms of garlic extract (GE) without standard and GE with standard are shown in **fig 1** and **fig 2**. The peak of allicin was identified by comparing peaks for GE without a standard in chromatograms with peaks for GE with a standard in chromatograms. So, the peak that increased with the increase in the amount of allicin (standard) is the peak that expresses allicin which was observed at retention time between 6 and 7 min (approximately 6.5 min).

Determination of Allicin Content in Garlic Extract by HPLC

Tables 2 and 3 demonstrate the mean of allicin content in fresh (sliced and crushed) and dried garlic slices with the effect of different storage conditions (time and temperature). The allicin contents were significantly varied ($p<0.05$) among treatments and forms. The highest allicin content between all treatments is (32.14 mg/g) and was found in fresh crushed garlic in Trt 3 (20 ºC, 10 min), whereas, the lowest allicin content (0.6 mg/g) was also found in fresh crushed garlic in Trt 13 (20 ºC, 2 days). According to several studies, there is significant variability in the garlic content of organic sulfur profiles between previous studies and our study due to two factors. The first cause was the use of different extraction solvents, which affects the degradation of allicin into other compounds such as dichloromethane. The second is an analysis of the many garlic genotypes utilized in the tests (Martins *et al*., 2016; Liu *et al*., 2020).

Table 2 shows that the amount of allicin in fresh (sliced and crushed) garlic in Trt 1 (20 ºC, time 0) is low because the enzymatic reaction that converts alliin to allicin takes about 10 minutes from the moment it is cut. The concentration of allicin in sliced and crushed fresh garlic increased after that in the following treatments, Trt 2 (4 ºC, 10 min) and Trt 3 (20 ºC, 10 min), and then the concentrations of allicin in the treatments began to decrease. These results match findings by Nguyen *et al*., (2021) who found that the allicin content was around 8.3 mg/g at the first measurement (0.5 min), but after 5 minutes, the allicin concentration rose significantly. When we compared the treatments of fresh sliced garlic and fresh crushed garlic, we found that the amount of allicin in the first form is less, but it is more stable. This corresponds to Thuwapanichayanan *et al*., (2014), who confirmed that fresh garlic slices did not create as much allicin as crushed or diced garlic because allinase did not come into contact with alliin as frequently. The result, the content of allicin decreased with increasing time and temperature of storage, and this shows a relation between the decrease in stability of allicin with increased temperature and time of storage, and the opposite is true. these results agree with Fujisawa *et al*., (2008), who found that the different conditions of temperature and time affect the stability of allicin, which is considered unstable. Table 3 shows that when we compare treatments of fresh sliced garlic with dried garlic slices, the allicin content of the first form is much higher. So, the shift in enzyme activity during the drying process may be responsible for the high reduction of allicin in dried garlic slices. Also, high temperatures may accelerate the degradation of allicin, which has been reported to be a heat-sensitive compound whose subsequent evaporation during drying was responsible for the loss (Zhou *et al*., 2016). Our results in dried treatments were within the range reported by Rahman *et al*., (2009), who showed allicin content in dried garlic is 4.932 mg/g. While drying garlic powder causes significant changes to the allinase, it still can convert alliin to allicin. The decrease of allicin in all treatments may be due to the natural variability of the degradation phenomenon and may also be attributed to the unstable allicin hydrolyzed and the decomposition to mercaptans, disulfides, trisulfides, and thiophenes (Henrıquez *et al*., 2014).

Pyruvic Acid Content

The mean of pyruvic acid content in all treatments is given in tables 4 and 5. The remaining flavor aptitude is represented by enzymatic pyruvic acid, as reported by Ammarellou *et al*., (2022). The results in tables 4 and 5 show that the content of pyruvic acid in the treatments differs according to the form of garlic (fresh sliced, fresh crushed, or dried slices). The pyruvic acid content varied substantially across all the treatments, with the highest value being 30.77 µmol/g (wet basis) in fresh crushed garlic in Trt 2 (4 ºC, 10 min storage). Based on the results of our study, the highest values of pyruvic acid were found in fresh crushed garlic, followed by fresh sliced, and dried slices. According to the results shown in table 4, the amount of pyruvic acid in fresh (sliced and crushed) in Trt 1(20 ºC, 0 min storage) is low, and reached its highest value in in Trt 2 (4 ºC, 10 min storage), and then begins to gradually decrease with the passage of time and the increase in temperature, as confirmed by Metrani *et al*., (2018). Concerning the pyruvic acid content in dried garlic, based on the results of our study, all treatments had a low percentage of pyruvic acid content and this decrease might caused by the thermal process. Loss of pungency in garlic may be caused by the destruction of precursors and partial inactivation of the enzyme allinase under drying conditions (Ratti *et al*., 2007).

Total Phenol Content

The mean of total phenol content (TPC) in fresh (sliced and crushed) and dried garlic slices with the effect of different storage conditions (time and temperature) is given in tables 6 and 7. The total phenolic concentration of all dried treatments was considerably less than that of fresh treatments (sliced and crushed). Heat treatment might create permanent chemical changes and a reduction in total phenolic compounds since they are heat-labile (Djendoubi *et al*., 2012). The TPC is affected by a number of variables, including temperature, light, water, and nutrient availability (Bystrická *et al*., 2013). As shown in table 6 and 7, the result matches the study done by Pedisić *et al*., (2018), who found that TPC in crushed garlic at room temperature for different exposure times (0, 2, 5, and 8 min) decreased. Given that TPC acts as an antioxidant, its possible oxidation to quinones can be used to explain why TPC levels decreased during storage (Jastrzebski *et al*., 2007). During processing and storage, many polyphenols are unstable and highly susceptible to oxidation and degradation (Chandra *et al*., 2021). When plant cells are destroyed, the polyphenol oxidation and degradation reactions of polyphenols will be released and catalyzed, releasing the polyphenol oxidase, polyphenol peroxidase, polyphenol glycosidase, and polyphenol esterase in fruits and vegetables (Montoya *et al*., 2021). This elucidates the rationale behind the elevated levels of total phenol content observed in whole garlic, followed by sliced garlic, and followed by crushed garlic.

Antioxidant Activity

The mean of antioxidant activity in fresh (sliced and crushed) and dried garlic slices with the effect of different storage conditions (time and temperature) is presented in tables 8 and 9. There were significant variances in antioxidant activity between garlic forms. Various parameters, such as heat treatment and extracted solvent, can alter the antioxidant activity of garlic (Songsungkan and Chanthai, 2014). The antioxidant activity comes from total phenol and organosulfur compounds (allyl cysteine, alliin, allicin, and allyl disulfide) in garlic. Total phenolic and antioxidant activity have a positive relationship (Kyung, 2012), and the thiosulfinates, in particular allicin, are capable of scavenging the peroxyl radical and serving as antioxidants (Okada *et al*., 2005). Temperature caused chemical oxidation and released oxidative and hydrolytic enzymes that might degrade the antioxidant chemicals in 46 vegetables, and air exposure also accelerated oxidation processes and enzyme activity (Altemimi *et al*., 2017).

Sensory Evaluation

As the hedonic scale results showed in table 10, there were some significant differences ($P<0.05$) between all samples for overall appearance, overall aroma, overall pungency, and overall flavor. while there were no differences between samples for overall color compared to the control. The results of consumer testing as hedonic scale showed that the highest value of overall appearance was found in sample C3 'hummus with crushed garlic at 10 min 20 ºC' (7.63), the highest value of overall aroma found in sample C3 'hummus with crushed garlic at 10 min 20 ºC' (7.63), the highest value of overall flavor found in sample C3 'hummus with crushed garlic at 10 min 20 ºC' (7.53), the highest value of overall pungency found in sample C3 'hummus with crushed garlic at 10 min 20 ºC' (7.63). Table 11 shows the just-about-right scale according to flavor, aroma, and pungency. The highest value of flavor, aroma, and pungency are found in sample D4 'hummus with dried garlic at 20 min 4 ºC', while the lowest value was found in 'hummus with crushed garlic at 10 min 4 ºC' (1.13). These data also agreed with Al-Nabulsi *et al*., (2022), who found that hummus without garlic was rated 6.9 in color, 6.9 in aroma, 6.9 in overall flavor, and 6.7 in Overall impression. In the same study, when adding 1% garlic to hummus, the sample was rated 6.9 in color, 5.9 in aroma, 5.9 in overall flavor, and 4.5 in the overall impression.

CONCLUSIONS

This study investigated different forms of garlic (freshly sliced, freshly crushed, and dried slices). The rate of allicin formation was significantly affected by temperature, and fresh mashed garlic had more content of allicin and pyruvic acid than fresh sliced garlic. On the other hand, fresh sliced garlic has a higher content of allicin and pyruvic acid than dried garlic slices. Optimum time and temperature in which a large amount of allicin is obtained after 10 minutes of mashing fresh garlic at room temperature. Total phenol content, antioxidant activity, pyruvic acid, and allicin decrease with increased time and temperature. For sensory attributes fresh mashed garlic after 10-minute storage at 20 ºC have the most acceptable pungency attributes.

Data availability:

Data is available at the following link: https://data.mendeley.com/datasets/scgr52tw9h/1.

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