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Garlic Curing: Post-Harvest Nutrient Remobilization From Leaves to Cloves

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GARLIC CURING: POST-HARVEST NUTRIENT REMOBILIZATION FROM LEAVES TO CLOVES

A Master's Thesis

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In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Plant Science

By

Mary Lee Books

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ABSTRACT

Garlic (*Allium sativum* L.) is grown on small and large scales worldwide. After harvesting, garlic undergoes a drying process for long-term preservation, called curing. Some producers cure with leaves intact, while others will remove and discard the leaves, curing only the bulbs. Due to the ability of plants to remobilize nutrients from leaves to underground storage organs, such as bulbs, the curing method of garlic may affect clove nutrient content. This study explores nutrient remobilization responses in garlic when cured with leaves intact compared to leaves separated from bulbs. Four cultivars of garlic, German White, Chesnok Red, Romanian Red, and Inchelium Red, were grown and harvested for three consecutive years. Plants of each cultivar were randomly assigned to one of three treatments: oven-dried (bulbs separated from leaves and placed directly into a forced air oven), intact (leaves remained intact with bulbs and plants cured in a greenhouse), and separated (leaves were cut from the bulbs to cure separately in a greenhouse). Leaf and clove dry weight were recorded, and nutrient content of boron, calcium, copper, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, sodium, sulfur, and zinc were determined using an ICP-MS. There was no effect of curing treatment on leaf and clove dry weight. Remobilization of phosphorus, potassium, sulfur, and copper was supported by a significant reduction of leaf content in intact plants compared to separated. Similarly, intact cloves had higher concentrations of magnesium, phosphorus, potassium, sulfur, copper, molybdenum, and zinc. Opposite results in leaf and clove calcium and boron concentrations suggest low or no mobility of these nutrients. However, the differences in nutrient concentrations were not consistent in all cultivars and years in this study. Although curing method did not affect overall yield of garlic, keeping leaves intact during curing may result in some remobilization from the leaves and increased nutrients in the cloves. Further research is needed to clarify these findings to determine the extent of nutrient remobilization in garlic.

KEYWORDS: garlic, Allium sativum, garlic curing, curing methods, nutrient remobilization, post-harvest, hardneck, softneck

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INTRODUCTION

Garlic (*Allium sativum* L.) is consumed worldwide due to its flavor and nutritional value. Americans consume on average two pounds a year, a quantity that is steadily increasing (Boriss, 2006; FAOSTAT, 2020). In 2019, over 28 million tons were produced worldwide. China is the lead producer growing more than two-thirds of the world's garlic. Currently, U.S. production is primarily in California, with smaller commercial operations in Oregon, Nevada, Washington, and New York (Ford et al., 2014). There has been an increase in garlic production in the U.S. by approximately 28% since 2010 (FAOSTAT, 2020), one reason being access to cold hardy garlic cultivars, allowing garlic to be produced in more regions across the U.S. (Keene Garlic, 2021; Ford et al., 2014).

The popularity of garlic is attributed to its high nutrient content and its unique composition of organic compounds that contribute to its distinct smell and ability to enhance food taste (Najman et al., 2021). It is also superior in its ability to retain nutrients throughout processing (Lanzotti et al., 2014). Since the beginning of recorded history, people have eaten garlic as a nutritional supplement (Simon, 2020). Its chemical properties reduce blood pressure (Stevinson et al., 2000; Ried et al., 2010), lower cholesterol (Dhawan and Jain, 2005), and shorten the life of the common cold or flu (Josling, 2001; Nantz et al., 2012). In addition, garlic provides fiber, protein, vitamins B and C, essential micronutrients (magnesium, selenium, iron, copper, potassium), free amino acids (Gorinstein et al., 2008), and over 70 fatty acids, which are a major source of energy and precursors of essential structural and metabolic substances (Baggott, 1998).

Garlic is in the onion family (*Amaryllidaceae*), a relative to leeks, chives, and shallots. Even though garlic is a perennial, it is commonly grown as an annual. The plant is composed of a leafed stock and a bulb. The bulb can have one to as many as 25 cloves, individually wrapped in a papery skin called clove wrappers. There are two major subspecies of garlic, hardneck (*Allium sativum* var. *ophioscorodon* L.) and softneck (*Allium sativum* var. *sativum* L.). Hardneck garlic is more closely related to wild garlic and is found to be more cold-hardy than softneck cultivars (Frederick et al., 2014). Hardneck cultivars are also known as bolting garlic because it produces an inflorescence, a long flowering stock called a scape. The scape is topped with an umbel containing small aerial cloves called bulbils. The scape is edible, and removal is common to reduce nutrient and resource competition to the developing bulb (Ford et al., 2014). If a scape is left on the plant, a flower will bloom, bearing tiny bulbils, which can be used for propagation. Hardneck cultivars tend to produce larger cloves but fewer in number, as a result, cloves are easier to peel, but have shorter storage life (Frederick et al., 2014; Smith and Garden-Robinson, 2021). Thus, hardneck garlic is commonly used as fresh garlic for cooking or processing (Frederick et al., 2014). Softneck cultivars, or nonbolting, garlic does not traditionally produce a scape, although in cases of high environmental stresses it has been noted to bolt (Takagi, 1990; Ford et al., 2014). Softneck cultivars tend to produce a high number of small cloves with a longer shelf life than hardneck cultivars, making it the garlic commonly found in grocery stores (Frederick et al., 2014).

Garlic is traditionally planted in the late fall or early spring and grown up to nine months to maturity (Frederick et al., 2014). The plants require vernalization, a period of cold temperatures to induce flowering. Vernalization is necessary for scape and flower production, but it also increases leaf emergence and clove development (Fenwick and Hanley, 1990b).

Cloves can be stored in cold before planting or planted in late fall or early spring to experience winter conditions in the field (Brewster and Rabinowitch, 1990).

Garlic's biological elasticity gives it the ability to acclimate to many environments, regions, altitudes, and soil conditions (Ford et al., 2014; Fredrick et al., 2014). The ideal soil for garlic production has a high water holding capacity but is well-drained for proper aeration. Soil should also be high in fertility with pH near 5.5-7 (pHs near 5.0-6.5). Both pre-plant and during peak vegetative growth, fertilizer applications are often applied based on soil tests and regional fertilizer recommendations. Cloves are planted upright, 3-6 cm deep, at a rate of 360-910 kg/ha, depending on cultivar. Irrigation is commonly used to achieve well-watered conditions up until a month before harvest. Weed barriers are also utilized to reduce moisture loss and competition for underground space and nutrients (Boyhan et al., 2017; Ford et al., 2014). Harvest can start as early as when the foliage droops and starts to senesce (Takagi, 1990). Many university extension sources recommend harvesting when 40-60% of leaves have yellowed (Boyhan et al., 2017; Ford et al., 2014; Frederick et al., 2014).

Post-harvest treatment of garlic depends on its intended use. Garlic can be consumed fresh or processed to be powdered, granulated, minced, chopped, or diced, or it can also be used to create oil or juice. Most processed products are made by dehydrating garlic in forced air ovens at 55-75 °C (Fenwick and Hanley, 1990b). To preserve garlic bulbs for long-term storage or transportation from the producer to the consumer, it undergoes a process called curing. Even though storage conditions can affect bulb factors such as moisture content, protein, crude fat, carbohydrates, ash, fatty acids, lipids, amino acids, anthocyanins, flavanols, and phenolics (Fenwick and Hanley, 1990a), there are conflicting ideas about the best curing methods (Martins et al., 2016).

Curing is the process of drying the plants over time until the bulb wrappers are fully dried to preserve and protect the cloves. The clove wrappers provide mechanical protection and limit gas exchange and the entry of rot-causing organisms into the fleshy pseudo stems or injured tissues, helping maintain cosmetic quality (Randle and Lancaster, 2002). When curing garlic, factors to be considered are post-harvest treatments, relative humidity, temperature, ventilation, and light exposure (Diriba-Shiferaw, 2016). These factors have an impact on storage life (Ford et al., 2014), disease prevalence (Madhu et al., 2019), nutritional content (Alam et al., 2016; Downes et al., 2009), color, moisture, sprouting, and rooting (Ichikawa et al., 2006). Curing conditions can ultimately affect yield, quality (Desta et al., 2021a; Lynch et al., 2012), and propagation potential of cloves for future crops (Desta et al., 2021a; Lynch et al., 2012; Walters, 2008).

Post-harvest management practices of garlic vary in the industry (Lynch et al., 2012), especially with curing methods (Martins et al., 2016). Post-harvest treatments such as leaf removal, sometimes called top removal, can play an important role in the curing process. However, depending on climate and drying resources, top removal is variable. In warm dry climates, plants can be removed from the ground and left in the field to be cured in windrows for three weeks, and in some cases, tops are removed 13 cm above the bulb before being collected. In wet climates or conditions, it is recommended to remove tops in the field and cure the bulbs indoors (Brewster and Rabinowitch, 1990). Removal of leaf material will reduce moisture and lower the likelihood of disease. Higher humidity and lack of ventilation can result in fugus such as *Embellisia allii* (Keene Garlic, 2021), leading up to 40% bulb loss (Madhu et al., 2019). Madhu et al. (2019) also found garlic storage longevity to increase up to eight months when removing leaves and storing bulbs in nylon netted bags compared to curing with leaves intact for

hanging. However, when grown for propagation cloves, most plants are stored with leaves attached in unheated warehouses (Diriba-Shiferaw et al., 2016). There are exceptions for propagation clove production, such as at Keene Garlic, who removes tops, leaving 15-23 cm of stalk above the bulb, and the bulbs are stored in crates in the dark (Keene Garlic, 2021).

For local sales and home-grown garlic, leaves can be left attached to the bulbs for weaving or bunching. Keene Garlic (2021) recommends hanging garlic bundles by the leaves, which condenses storage and increases ventilation for home gardeners. The New Jersey Agricultural Experiment Station recommends hanging from the stalks for 4-6 weeks then cutting the stalk back for long-term storage (Frederick et al., 2014).

Ease of plant harvest, reduced moisture, and reduced curing space are the main reported justifications for top removal before curing. Many garlic management reviews, such as Diriba-Shiferaw et al. (2016) and Madhu et al. (2019) present methods to cure with leaves. However, little information is known about the impact of the curing method on the nutritional aspects of garlic products.

While there are several studies on the chemical and nutritional composition of garlic, many do not identify a curing method or relate results to a specific curing method. Martins et al. (2016) review of pre-and post-harvest condition effects on chemical composition made no mention of leaf removal when presenting curing methods. Additionally, garlic nutrient studies such as Mochizuki et al. (1998) and Varhan-Oral et al. (2019) made no mention of top removal.

Top removal was studied in onions by Nega et al. (2015), finding a reduction in rotting rates from keeping leaves intact. In onions, leaves naturally senesce when intact, and substances in the leaves inhibit sprouting and promote resistance to disease-causing fungi into the bulbs (Randle and Lancaster, 2002). The sealing of the neck left attached also limits disease entry

(Diriba-Shiferaw et al., 2016). However, onions respond differently to extended curing compared to garlic, onion being one fleshy bulb instead of individual cloves and having round hollow leaves instead of flat leaves like garlic (Boyhan et al., 2017; Smith and Graden-Robinson, 2021; Wright et al., 2001). In addition, garlic does not exhibit a breakdown of the neck tissue to signal maturity like an onion (Boyhan et al., 2017). Since the vascular connections between the leaves and bulbs could be maintained during the curing process in garlic, research is needed to determine if leaf removal could impact bulb development and nutritional content.

During a plant's life cycle, nutrients are moved throughout the plant. As nutrients are initially taken in by the plant and used to develop and maintain tissues, the plant may move them, primarily through the phloem, to a different location within the plant, in a process known as remobilization (Marschner, 2012). Remobilization is based on physiological and biochemical processes involving the utilization of nutrients stored in vacuoles, breakdown of storage proteins, and breakdown of cell structures and enzymes. For instance, the breakdown of chloroplasts involves the transformation of structurally bound nutrients like magnesium in chlorophyll, making the nutrient mobile within the cell. Short-distance transport in the symplasm will move the nutrient to the phloem where long-distance transport is employed for nutrient remobilization to different plant parts (Marschner, 2012).

Nutrients within plants differ on their ability to be remobilized. Marschner (2012) groups elements into three categories based on their phloem mobility within the plant. Magnesium, nitrogen, phosphorus, potassium, sodium, and sulfur to have high mobility. Boron, copper, iron, molybdenum, and zinc have intermediate mobility, while calcium and manganese have low mobility.

Plants utilize remobilization during seed germination, periods of nutrient deficiency, reproductive growth, and in perennials the period before leaf drop. During reproductive stages nutrient content of vegetative parts decline, and nutrients are remobilized to be allocated to reproductive tissues to support the development of the next generation. The demand of reproductive organs like the seed, fruit, or tuber and nutrient status of the vegetative parts affect the amount of remobilization. This is related to the ratio between vegetive mass and the number reproductive organs (Marschner, 2012).

In perennial woody species in temperate climates, as leaves senesce and discolor in the fall, nutrients (boron, calcium, iron, magnesium, and manganese) will remobilize from leaves to woody parts until leaf drop. Remobilization commonly takes place during leaf senescence. This is a degenerative process of breaking down cellular organelles and biomolecules so the resultant catabolites can be remobilized to other tissues. Remobilization of micronutrients copper, iron, and zinc have been linked to leaf senescence, with a positive correlation between nitrogen and copper during times of deficiency (Marschner, 2012). Maillard et al. (2015) also found net mobilization of 13 nutrients during leaf senescence in crop and tree species, measured by regular leaf sampling throughout plant life span. The remobilization of nitrogen occurred in the largest amounts, although magnesium, phosphorus, potassium, and sulfur also remobilized in most species.

In perennial grasses, winter remobilization from leaves to rhizomes occurs for overwintering, and nutrients are again remobilized to support spring shoot regrowth. Nassi o Di Nasso et al. (2013) found nitrogen, phosphorus, and potassium remobilized from leaves to rhizomes in Giant Reed (*Arundo donax* L.) and Remley (2010) reported nitrogen remobilization in tall fescue (*Lolium arundinaceum* L.). Nutrient remobilization from leaves to seed in grain

crops, such as maze and soybean, has also been studied. In soybeans (*Glycine max* L.), nutrients move from the leaves to seeds as the growing season progresses (Bender et al., 2015) and corn (*Zea mays* L.) was found to have peak grain nutrients at seed maturity, just before harvest (Bender et al. 2013).

Garlic research related to nutrient movement includes fertilizer application studies and biochemical analysis of specific compounds. Diriba-Shiferaw et al. (2013b) found fertilizer applications to increase garlic bulb pyruvate content which remained post-harvest through the third month of storage, indicating bulbs will take up and store available nutrients. Additionally, the effects of fertilizer and harvest time on the compound alliin have been studied.

Alliin is a sulfur containing compound with biological properties responsible for the distinct flavor and pungency, along with antimicrobial properties of garlic (Borlinghaus et al., 2014; Diriba-Shiferaw et al., 2013a). Sulfur is a nutrient studied in garlic because of its role in alliin. Bloem et al. (2011) found fertilization of sulfur along with post-harvest curing increased alliin concentrations by about 50% in garlic bulbs. They did not indicate if leaves were intact during the curing process or not. However, Bloem et al. (2010) found an increasing trend in bulb content of nitrogen and sulfur, along with translocation of alliin from leaves to bulbs between start of foliage death to almost full foliage death before harvest. This research supports remobilization of highly mobile nutrients, like nitrogen and sulfur, from leaves to bulbs during leaf senescence before harvest.

Overall, there is limited information on nutrient remobilization in garlic from leaves to cloves, especially post-harvest. Although a few studies have focused on nutrient movement in garlic plants pre-harvest, it has yet to be determined if the curing method post-harvest affects this movement. Therefore, the objectives of this study were to determine if the method of curing with

leaves intact or curing with leaves separated from the bulbs impact nutrient content of tissues and yield of cloves. This study examined three years of garlic production and compared nutrient content of leaves and cloves from plants cured with leaves intact to plants cured with the leaves removed. It is hypothesized that if garlic leaf nutrients remobilize during the curing process, post-harvest, then curing with leaves intact will result in an increase in nutrients and yield in the cloves.

MATERIALS AND METHODS

Four cultivars of garlic were grown for three consecutive years in southwest Missouri for this study. A variety of types and colors of cultivars were chosen based on recommendations for this growing region and popularity (Alsup-Egbers et al., 2020; Volk and Stern, 2009). Hardneck cultivars of Chesnok Red, German White, and Romanian Red, and softneck cultivar Inchelium Red were included each year. All garlic cloves were obtained from Keene Organics (Sun Prairie, WI). The garlic was planted in October, determined by Alsup-Egbers et al. (2020) to be the most productive planting date of these cultivars for this region.

The first year of this study was planted on October 2, 2018, near Rogersville, MO in raised garden beds containing about a 45 cm depth of mixed media of soil, potting mix, vermiculite, and pearlite. The slow-release fertilizer Osmocote (14-14-14) at 80 kg/ha nitrogen, phosphate, and potash equivalent was mixed into the media before planting. Garlic was planted 3 cm deep, with 15 cm in row spacing and 10 cm between rows. Cultivars were grouped in plots containing 45-60 plants.

The following two years of garlic were planted on October 9, 2019, and October 30, 2020, at the Missouri State University William H. Darr Agricultural Center (Darr Center) in Springfield, MO (lat. 37.172°N, long. 93.317°W, altitude 1243 ft). The planting sites contained a combination of Wanda silt loam (fine-silty, mixed, superactive, thermic typic Paleudolls) and Newtonia silt loam (fine-loamy, mixed, active, mesic typic Paleudolls) (U.S. Dept. Agr., 2017). Before planting, approximately 20 soil cores to a 16 cm depth were randomly collected from the planting bed and combined into one composite sample. Soil pHs (Starter 300 pH meter, Ohaus), % organic matter (loss on ignition), Bray I phosphorus (GENESYS 30 Visible

Spectrophotometer, ThermoFisher Scientific), and calcium, magnesium, and potassium (Atomic Absorption/ Flame Emission Spectrophotometry, Agilent Technology 200 Series AA) were determined as outlined in the University of Missouri Soil Testing Handbook (Nathan et al., 2012). For the October 2019 planting, the soil tests indicated pHs of 5.8, 3.6 % organic matter, 25 kg/ha Bray I phosphorus, 4,228 kg/ha calcium, 243 kg/ha magnesium, 183 kg/ha potassium with a CEC of 14.4 meq/100g. The October 2020 planting site soil had pHs of 6.0, 2.9% organic matter, 29 kg/ha Bray I phosphorus, 4213 kg/ha calcium, 196 kg/ha magnesium, 252 kg/ha potassium, and a CEC of 11.4 meq/100g.

A pre-plant application of fertilizer was applied at a rate of 84 kg/ha nitrogen, 37 kg/ha phosphate, 69 kg/ha potash (Twin Pine; Knot Fertilizer Co., Knox, IN) fertilizer was applied at planting in 2019 and 2020. An additional 84 kg/ha nitrogen, 14 kg/ha phosphate, 21 kg/ha potash (Nature's Source; Ball DPF, Sherman, TX) was applied in early March of both 2019 and 2020. Beds were mounded 6 cm high and 72 cm wide and covered with 1.23 mm black plastic mulch. Cultivars were grouped in plots containing 45-60 plants. Garlic cloves were planted 3 cm deep, with 15 cm in-row and 10 cm between-row spacing, plants offset between rows.

During the three growing seasons, plots were hand-weeded as needed and scapes were removed once they emerged from the leaves. The harvest date was determined when one-third of the oldest leaves had yellowed (Campbell-Nelson, 2021). For the 2018-2019 growing season, German White was harvested on June 10, 2019, while Chesnok Red, Romanian Red, and Inchelium Red were harvested on June 26, 2019. Plants from these harvests will be referred to as the "2019" plants. For the 2019-2020 growing season, all four cultivars were harvested on June 24, 2020, referred to as the "2020" plants (Fig. 1). All four cultivars were harvested on June 28, 2021, for the 2020-2021 growing season, and these plants will be referred to as the "2021"

plants. For each harvest, all plants of each cultivar were carefully removed from the ground, excess soil was removed from the bulbs, and plants were transported to the laboratory for processing. Thirty-six plants of each cultivar were randomly selected, bulbs were triple rinsed in deionized water, and roots were removed at the base of the bulb.

Each set of 36 plants were then divided into three treatment groups, with 12 plants assigned to each treatment (Fig. 2). The first treatment group, "oven-dried", had garlic bulbs cut from the leaves and placed directly into a Cascade Tek temperature controlled forced air oven (Cascade Sciences) at 55 °C. This group acted as a baseline for nutrient content for each tissue type at time of harvest (Adam et al., 2000). The second treatment group labeled "intact" left the plants intact and the leaves and bulbs remained connected. The third treatment group "separated" had the leaves cut from the bulbs, to cure separately. Both "intact" and "separated" treatments were placed in the Karls Hall greenhouse on the Missouri State University campus (Springfield, MO) to cure (Fig. 3). The average temperature of the greenhouse remained between 10-40 °C. Fresh weights of each tissue (leaves, bulbs, or leaves with bulbs) were recorded before placing them in the assigned drying environments. After approximately four weeks of drying in the greenhouse, when leaves were yellowed and dry, intact leaves were cut from bulbs of the intact treatment. All leaf and bulb samples were weighed and moved into the 55 °C Cascade Tek oven until dry weights remained constant. Bulbs were then separated into cloves and wrappers. Dry weights of cloves, wrappers, and leaves were recorded per plant. Leaf and clove samples were then ground to a fine powder using a modified coffee grinder and stored in paper envelopes in the 55 °C Cascade Tek oven.

Ground leaf and clove samples were weighed to between 0.2450 g and 0.2550 g and digested in 5 mL of trace grade nitric acid using a MARS 6 microwave accelerated reaction

system (CEM Corp.). Tissues were digested using the MARS 6 Plant Material method which included a 20-minute ramp to 200 °C, followed by a 10-minute hold in temperature, and a 20-minute cool down to 70 °C. Samples were removed from the microwave and allowed to cool to room temperature. One mL of 25 ppm yttrium, lithium, and scandium was added as internal standards. Lastly, samples were filtered using Q8 course, fast flowing, 11 cm filter paper (ThermoFisher Scientific) and diluted to 25 mL with E-Pure water (Thermo Scientific Barnstead) and stored in sterile 50 mL polypropylene centrifuge tubes (ThermoFisher Scientific). Final internal standard concentrations were 1 ppm yttrium, lithium, and scandium.

Elemental analysis of samples utilized an ICP-MS Agilent 7800 (Agilent Technologies). Fourteen elements were analyzed including aluminum, boron, calcium, copper, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, sodium, sulfur, and zinc. Argon was employed as the carrier gas for most nutrients, with helium utilized for potassium and iron to reduce interference. A set of 5 standards and a blank were analyzed and then referenced to determine sample concentrations.

The experiment was a randomized block design, analyzed as a mixed effects model. The model included cultivar and post-harvest treatment as fixed effect factors and year and block as random factors. This model was used to test the statistical significance of post-harvest treatment effects on nutrient concentrations of cultivars, as well as interactions with year using mixed-effects model Minitab 19 (Minitab, Inc.). All effects and interactions were considered significant when means differed at $P<0.05$. Due to significant interactions involving year and cultivar subsequent analyses were conducted using a one-way analysis of variance (ANOVA) with mean separation by Tukey's pairwise comparison (α=0.05).

Figure 1. Garlic plots at the Darr Center on June 24, 2020, harvest day.

Figure 2. Chesnok Red garlic at 2020 harvest, showing oven-dried (toward left), separated (middle), and intact (toward right) plants.


Figure 3. Inchelium Red garlic plants of the 2020 harvest curing in the greenhouse, with separated plants (left) and intact plants (right).

RESULTS AND DISCUSSION

Growth and Yield

In all three growing seasons in this study, most garlic plants developed successfully and resulted in bulb production. In general, garlic plants at harvest had greater clove dry weight than leaf dry weight across all cultivars and years. In 2019, leaf dry weight ranged from 3-8 g/plant with clove dry weight 6-13 g/plant (Fig. 4). Similarly in 2020, leaf dry weights were 5-16 g/plant and cloves 7-27 g/plant (Fig. 5). In 2021, leaf dry weight ranged 4-7 g/plant and cloves 9-19 g/plant (Fig. 6). Overall, Chesnok Red cultivar produced the smallest clove yield over the other three cultivars (Figs. 4, 5, and 6).

There was no effect of oven-dried, separated, and intact treatments on clove and leaf dry weight in all cultivars and years (Figs. 4, 5, and 6) with the exception of intact cloves being greater than other treatments in 2020 German White (Fig. 5).

During the 2020-2021 growing season, many of the planted cloves exhibited poor emergence rates, and the softneck cultivar Inchelium Red exhibited very poor emergence and development. Some of the few Inchelium Red plants that did emerge produced scapes. Scape production in softneck garlic is usually the result of the plants under environmental stress (Ford et al., 2014). Emergence could have been affected by below average temperatures in February 2021, reaching -26 °C for multiple days. Vegetative growth would have been occurring during this time, with leaves emerging from the ground. This extreme cold period was followed by an above average amount of perception in March of 2021 (National Weather Service, 2021) and could have contributed to the poor plant development. Due to the few numbers of produced bulbs and poor condition of the plants, Inchelium Red in 2021 were not harvested for this study. In the

remaining cultivars, a majority of the plants were harvestable, but exhibited severe rotting and degradation of leaf, neck, and clove wrappers in the field before harvest. Weather conditions prior to the harvest of 2021 could explain the sudden decrease in crop quality. Early June was dry followed by an unusual 1.4 cm of precipitation on June 21, 2021. The days following had below average temperatures near 25 °C (National Weather Service, 2021) resulting in severely diseased plants by the June 28, 2021, harvest date. Following harvest, normal curing of these plants would not have occurred with the necrotic tissues and processing of these plants would not have produced a viable product for consumption or propagation (Wright et al., 2001). As a result, Chesnok Red, Romanian Red, and German White were treated and dry weights were determined, but the plant tissues were not further processed or analyzed for nutrient content.

Nutrient Concentrations

Leaf and clove nutrient concentrations were determined for all four cultivars in the 2019 and 2020 harvests, with the exception of those below the ICP-MS limit of detection including aluminum, 2019 copper, 2020 molybdenum, and 2020 sulfur in only Romanian Red cloves. Garlic tissue contents of high mobility nutrients included the plant macronutrients of magnesium (Figs. 7 and 8), phosphorus (Figs. 9 and 10), potassium (Figs. 11 and 12), and sulfur (Figs. 13 and 14), and plant micronutrient sodium (Figs. 15 and 16). Intermediate mobility nutrients included the micronutrients boron (Figs. 17 and 18), copper in 2020 (Fig. 19), iron (Fig. 20 and 21) molybdenum in 2019 (Fig. 22), and zinc (Figs. 23 and 24). Low mobility nutrients included the macronutrient calcium (Figs. 25 and 26) and micronutrient manganese (Figs. 27 and 28).

Intact and Separated Treatments. The curing treatment of separated represents leaves being severed from the bulb at harvest and the bulb curing by itself with no further interactions

from the shoots of the plant. The curing treatment of intact represents leaves remaining connected to the bulb at harvest and through the curing process. If leaves in the separated curing treatment had higher concentration of a nutrient compared to leaves in the intact curing treatment, nutrients may be leaving the leaf tissue in the intact plants during the curing process. If cloves in the separated treatment had lower concentrations of a nutrient compared to cloves in the intact treatment, nutrients may be accumulating in the clove tissue during the curing process. Either or both differences within the leaf and clove tissues would support nutrient remobilization from leaves to cloves during curing of intact plants.

Leaf phosphorus concentrations were lower in intact compared to separated treatments in three cultivars in 2019, all but German White (Fig. 9) and three cultivars in 2020, all but Inchelium Red (Fig. 10). Similarly, leaf sulfur concentrations were lower in intact compared to separate treatments in the same three cultivars each year (Figs. 13 and 14). Although these highly mobile nutrients showed this treatment differences, other highly mobile nutrients of potassium (Figs. 11 and 12), magnesium (Figs. 7 and 8), and sodium (Figs. 15 and 16), overall did not show consistent differences between separated and intact leaves across both years.

Intermediately mobile nutrients had a few instances where intact leaves had lower nutrient content than separated. This occurred with iron and zinc in 2019 Chesnok Red (Figs. 20 and 21) and copper in 2020 Chesnok Red, German White, and Romanian Red (Fig. 19). One instance of a low mobility nutrient having lower leaf concentrations in intact compared to separated was for manganese in 2020 Chesnok Red (Fig. 28). Interestingly, low mobility nutrients in the leaves were greater in the intact than the separated treatments, including boron and calcium in 2019 Chesnok Red and Inchelium Red (Figs. 17 and 25), and calcium in 2020 Chesnok Red (Fig. 26).

In clove tissue, an increase in nutrient concentration in the intact treatment cloves compared to the separated treatment cloves would support possible remobilization from the leaves to the cloves during curing. In 2020 Inchelium Red, magnesium concentrations were higher in intact cloves compared to separated (Fig. 8). Similarly, phosphorus and potassium concentrations were higher in intact cloves in 2020 Romanian Red (Figs. 10 and 12). In 2019 Romanian Red, and 2020 Chesnok Red and Inchelium Red intact cloves also had higher potassium concentrations (Figs. 11 and 12). Additionally, highly mobile nutrient sulfur was higher in 2019 Romanian Red and Inchelium Red (Fig. 13). Intermediately mobile nutrient copper was higher in intact cloves in 2020 Romanian Red and Inchelium Red (Fig. 19). Molybdenum concentrations in 2019 Chesnok Red (Fig. 22) and zinc concentrations in 2020 Inchelium Red (Fig. 24) were also higher in intact cloves than separated cloves. However, significant differences in the low mobility nutrient calcium behaved oppositely, with intact cloves being lower in concentration than separated in 2019 Inchelium Red and 2020 Chesnok Red (Figs. 25 and 26).

Remobilization of nutrients from leaves to cloves is supported by a decrease in nutrient concentration in intact leaves compared to separated leaves coupled with an increase in nutrient concentration in intact cloves compared to separated. This pattern was significant for phosphorus in 2020 Romanian Red (Fig. 10), potassium in 2020 Inchelium Red (Fig. 12), iron in 2019 Chesnok Red (Fig. 21) and both sulfur and copper in 2019 Romanian Red (Figs. 13 and 19). Although there were limited statistically significant examples of this pattern in this study, the trends of nutrient remobilization were prevalently found across years and cultivars in phosphorus, potassium, sulfur, iron, zinc, and copper.

The low mobility categorization of calcium and boron was supported by finding greater concentrations in intact leaves than separated and with calcium found to be lower in concentration in intact cloves compared to separated. If other nutrients are being remobilized out of the leaves and into cloves, low mobility nutrients would make up a great proportion of the remaining leaf components and be diluted to lower concentrations of clove tissue as the cloves gain nutrients. It could be speculated that boron and calcium concentrations could be higher in intact leaves if other nutrients are moving out of the leaves and into the cloves, making less mobile nutrients, boron and calcium, a greater proportion of leaf composition. Similarly, intact cloves could have lower concentrations of these low mobility nutrients due to mobile nutrients moving into the cloves, diluting the concentration of nutrients. Additionally, concentration of low mobility nutrients like calcium and boron were found in much lower concentrations in the cloves compared to leaf tissue, while other nutrients tested, both of high and intermediate mobility, exhibited similar concentrations in the leaves as in the cloves. These results also support less remobilization of calcium and boron from leaves to cloves.

Oven-dried and Separated Treatments. Oven-dried nutrient concentrations represent nutrient concentrations at time of harvest. If oven-dried tissue concentrations were different than the separated curing treatment, it could indicate an additional loss of nutrients from one of the drying processes. In general, leaf nutrients were similar between oven-dried and separated treatments. It was rare to find leaf nutrient concentrations to be greater in oven-dried than the separated treatments, only occurring in Inchelium Red 2020 sulfur (Fig. 14) and Chesnok Red 2019 iron (Fig. 20). More instances were found with leaf nutrients being lower in concentration in oven-dried than in the separated treatments. Highly mobile nutrients showed some instances of leaf magnesium, phosphorus, potassium, sulfur being lower in oven-dried than separated. For

example, 2019 Romanian Red leaves had lower concentrations of magnesium, phosphorus, potassium, and sulfur, in the oven-dried treatment than the separated (Figs. 7, 9, 11, and 13). Similarly, in 2020 Inchelium Red, leaf contents of potassium, sodium, and iron were lower in oven-dried tissues than separated (Figs. 12, 16, and 21). Some lower mobility nutrients were also found to be lower in oven-dried than separated, such as in 2019 Romanian Red boron and calcium (Figs. 17 and 25).

In cloves, highly mobile nutrients magnesium and sulfur were lower in oven-dried compared to separated in 2019 Inchelium Red (Figs. 7 and 13). Additionally, potassium contents in 2019 Chesnok Red and sodium contents in 2020 German White were lower in oven-dried cloves (Figs. 11 and 16). Clove copper concentrations in 2020 Chesnok Red and German White were also lower in oven-dried treatments (Fig. 19). Less mobile nutrient boron was lower in oven-dried cloves in 2019 Inchelium Red (Fig. 17). These results of oven-dried tissues being lower than separated tissues in nutrient content could indicate additional loss from tissue that was exposed to higher temperatures in the drying oven post-harvest, however these results were not consistent across years, cultivars, or nutrients. In a few instances, mobile nutrients exhibited greater concentrations in oven-dried cloves compared to the separated treatment, such as potassium and sodium in 2020 Chesnok Red cloves (Figs. 12 and 16), and iron in 2019 Chesnok Red and German White (Fig. 20). Less mobile calcium was found to be greater in oven-dried cloves in 2019 Chesnok Red and 2020 Inchelium Red (Figs. 25 and 26). Boron also exhibited greater concentrations in oven-dried in 2020 Inchelium Red (Fig. 18) and manganese in 2019 and 2020 Chesnok Red cloves (Figs. 7 and 8).

Most research on garlic and onion drying methods focuses on the impact on nutritional and flavor compounds and quality measures. Most products are processed dehydrated tissues that

are powdered, minced, granulated, chopped, and diced (Brewster and Rabinowitch, 1990). In onion, drying above 60 °C affected flavor compounds including pyruvate, sugar, L-ascorbic acid content (Adam et al., 2000). In garlic, high processing temperatures have been reported to lead to darkening of cloves and altered taste (Fenwick and Hanley, 1990b). Najman et al. (2021) also found differences in composition between freeze-drying and oven-drying garlic cloves. It is possible that differences in temperature and drying time, between the oven-dried and greenhouse curing treatments could have affected chemical compounds and nutrient content from the time of harvest. Rapid increases in temperature from the oven-dried treatment could have caused breakdown of cell walls and liquid and volatile losses from the ruptured cell walls of the tissue that the tissues in the greenhouse environment may not have experienced.

Conclusions

This study suggests that remobilization of some leaf nutrients, such as phosphorus, potassium, sulfur, iron, zinc, and copper to bulbs does occur in garlic during the curing process if leaves are left intact, with calcium and boron showing low or no mobility. However, the differences in nutrient concentrations were not found consistently in all cultivars and years of this study. Additionally, curing treatment did not impact dry weight or crop yield. Although the amount of nutrient remobilization during curing may seem negligible, continual replanting of larger higher nutrient content and quality cloves could result in larger increases over multiple generations of planting (Desta et al., 2021b; Lynch et al., 2012). Changing harvest and curing techniques to keep leaves intact could be beneficial to garlic producers, not only for the current harvest, but for future production.

Future Directions

In this study clove yield was not affected by curing treatment. However, overall plant and bulb size did differ within cultivars. In future studies, it would be beneficial to reduce variation in bulb and plant size. As planting clove size has been shown to affect final plant and bulb weight (Desta et al., 2021b), one way to achieve more consistent sizes at harvest would be to set a weight range for acceptable planting cloves. This would reduce the variation of the size of plants grown (Desta et al. 2021b) and make plant size more uniform for the curing treatments. Set parameters of acceptable bulb size could also be made at harvest when selecting plants from the field to reduce bulb size variability in the study. Another improvement on sampling would be to reduce the number of cultivars and instead focus on a larger sample set of only a few cultivars. Different cultivars of garlic have a large variation in bulbing index (Atif et al., 2020). Reducing the variation and increasing sampling numbers will increase statistical power to ascertain any small differences in dry weight and yield across curing methods.

The leaf and clove nutrient concentrations fall within reported values in garlic by (Boyhan et al., 2017; Brewster and Rabinowich, 1990). However, the reported values result in a large range of tissue nutrient concentrations (Petropoulos et al., 2018; Polyakov et al., 2020). This variability could be the result of cultivar differences (Boyhan et al. 2017; Ford et al., 2014; Gadel-Hak et al., 2011), many environmental factors, and management (Polyakov et al., 2020). Future studies of nutrient remobilization could include more controlled environments of temperature, water, and soil mineral nutrition.

Future experiments could include an oven-dried intact treatment to be able to compare both greenhouse curing treatments with an oven-dried control. Additionally, freeze-drying of tissue could more-rapidly dry tissue and prevent possible losses.

At harvest, when a plant is removed from the ground, nutrient uptake by the roots stops, reducing the movement of all nutrients in the plant (Marschner, 2012). Remobilization of nutrients from leaves to underground storage organs is likely to occur during leaf senescence. Remobilization pre-harvest as the leaves are browning could be a large contributor to bulb nutrient status and size at the time of curing. Future studies on nutrient remobilization should include a focus on harvest timing. A prolonged senescence period in the field may result in more nutrient remobilization form the leaves to the cloves prior to harvest. This could be studied by sampling at multiple stages before harvest, like Bloem et al. (2010) and Atif et al. (2020) and during curing, while analyzing both senesced (brown) and alive (green) leaf tissues. However, it would also be important to determine if delayed harvest dates to support remobilization benefits outweigh disease pressures before harvest that could yield and quality of the crop.

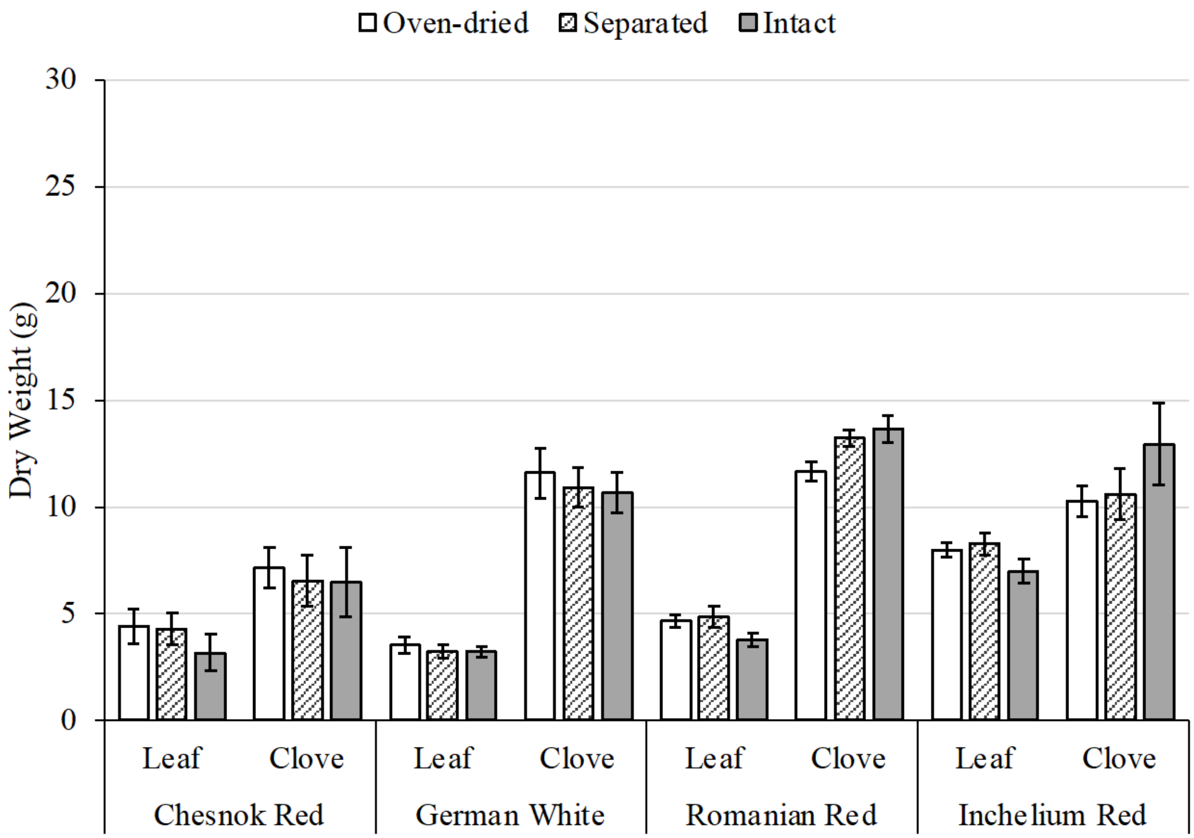

Figure 4. Dry weight of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

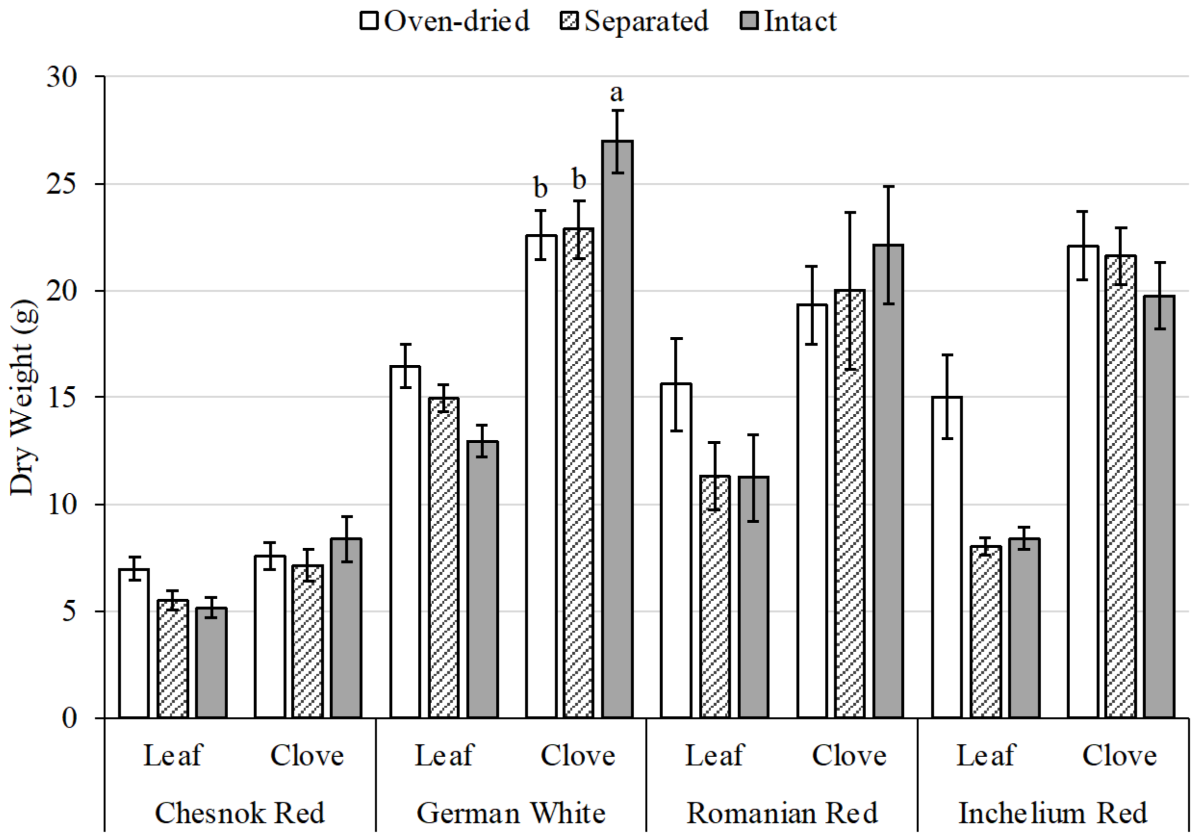

Figure 5. Dry weight of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

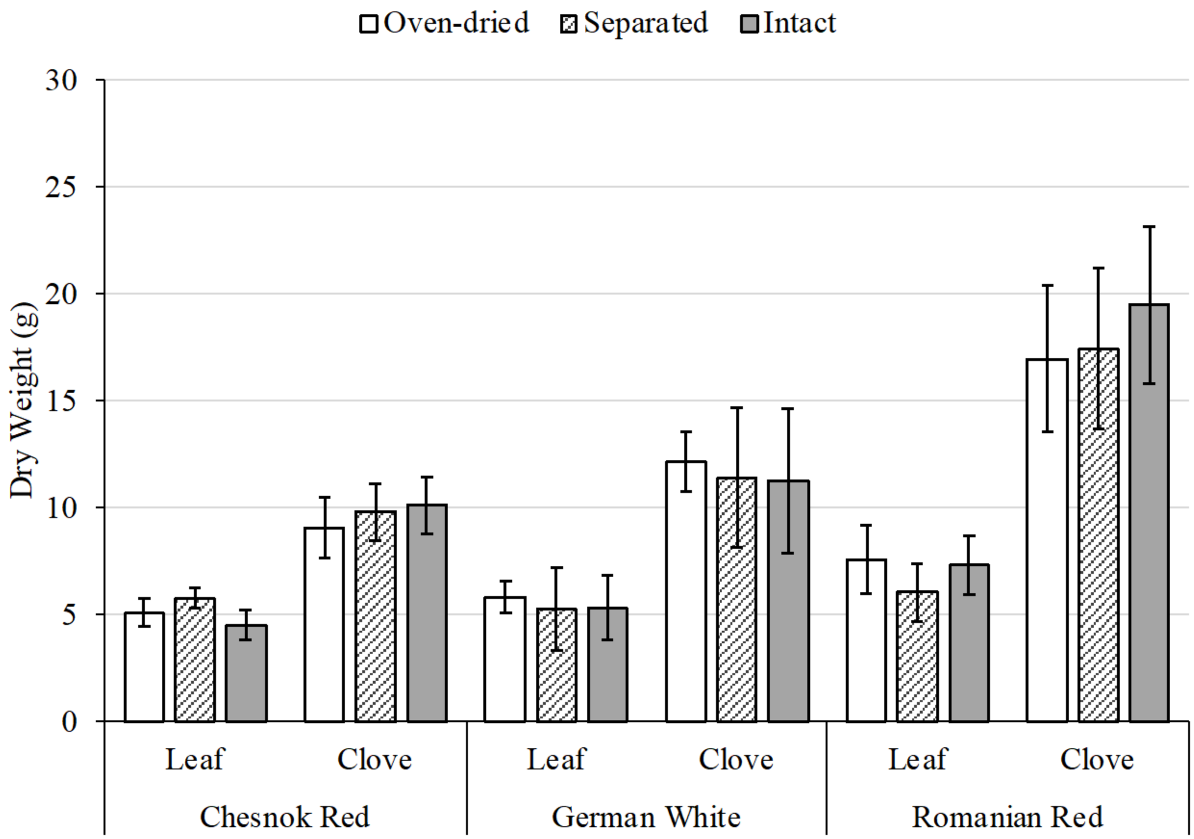

Figure 6. Dry weight of 2021 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, Chesnok Red and German White n = 12, Romanian Red n = 9. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

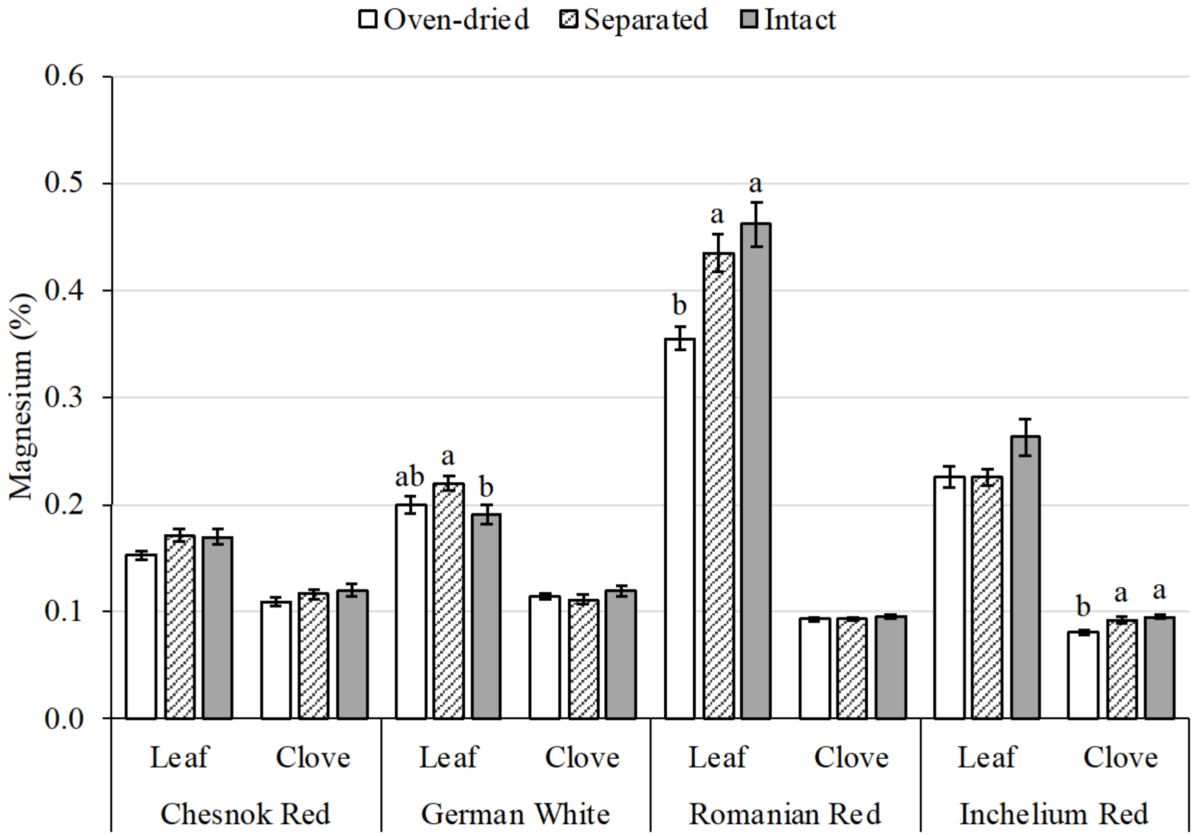

Figure 7. Magnesium content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

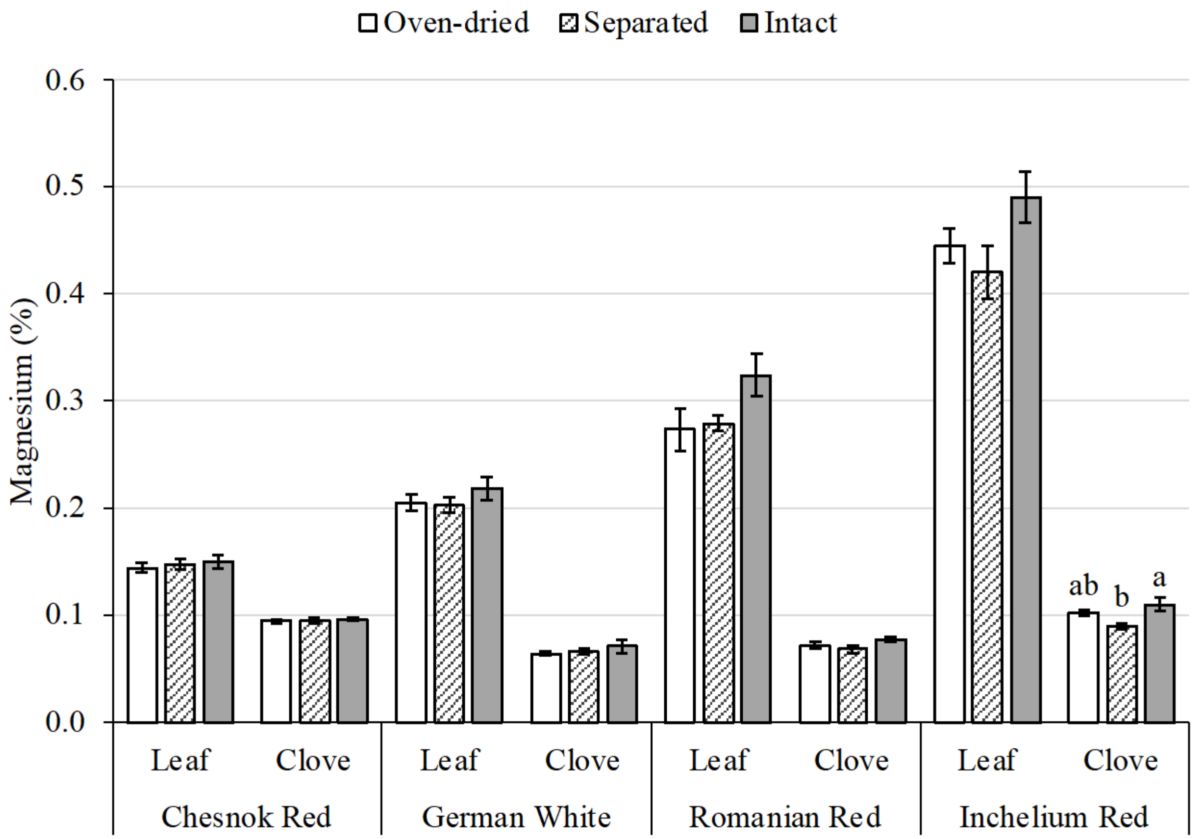

Figure 8. Magnesium content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

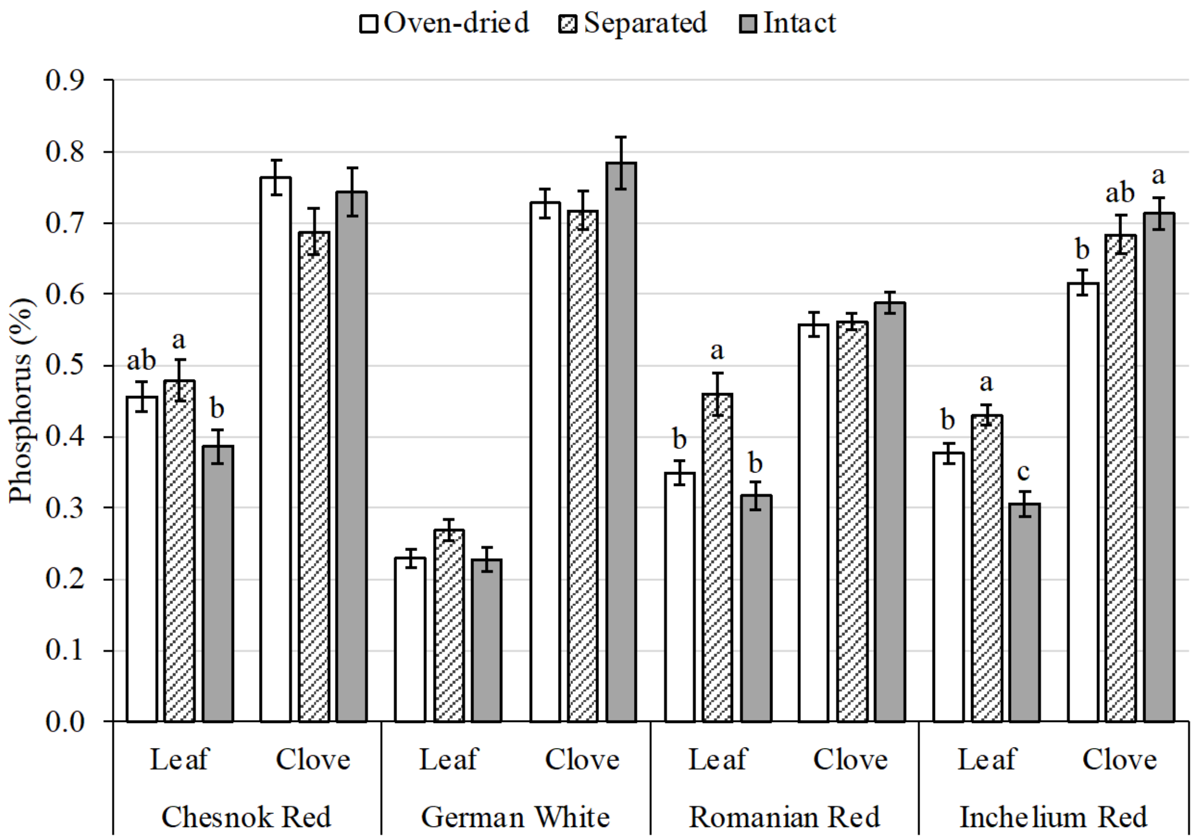

Figure 9. Phosphorus content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

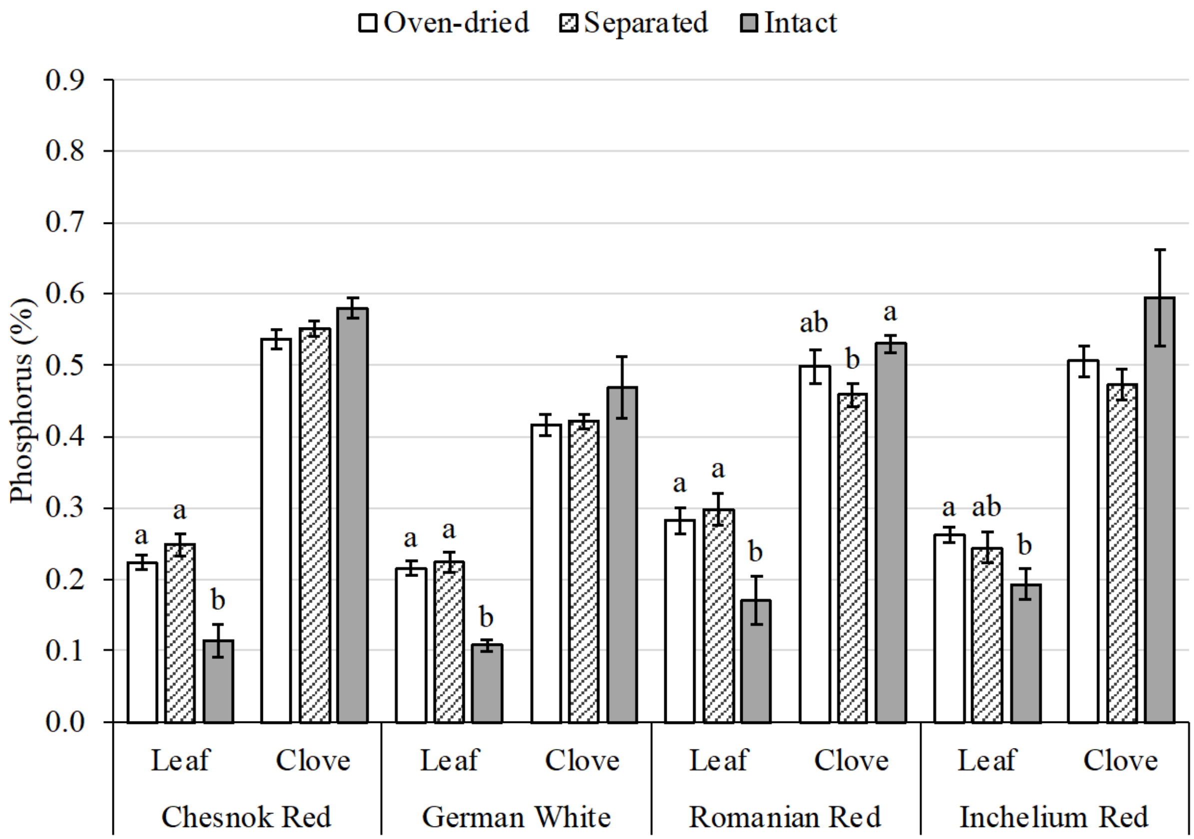

Figure 10. Phosphorus content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

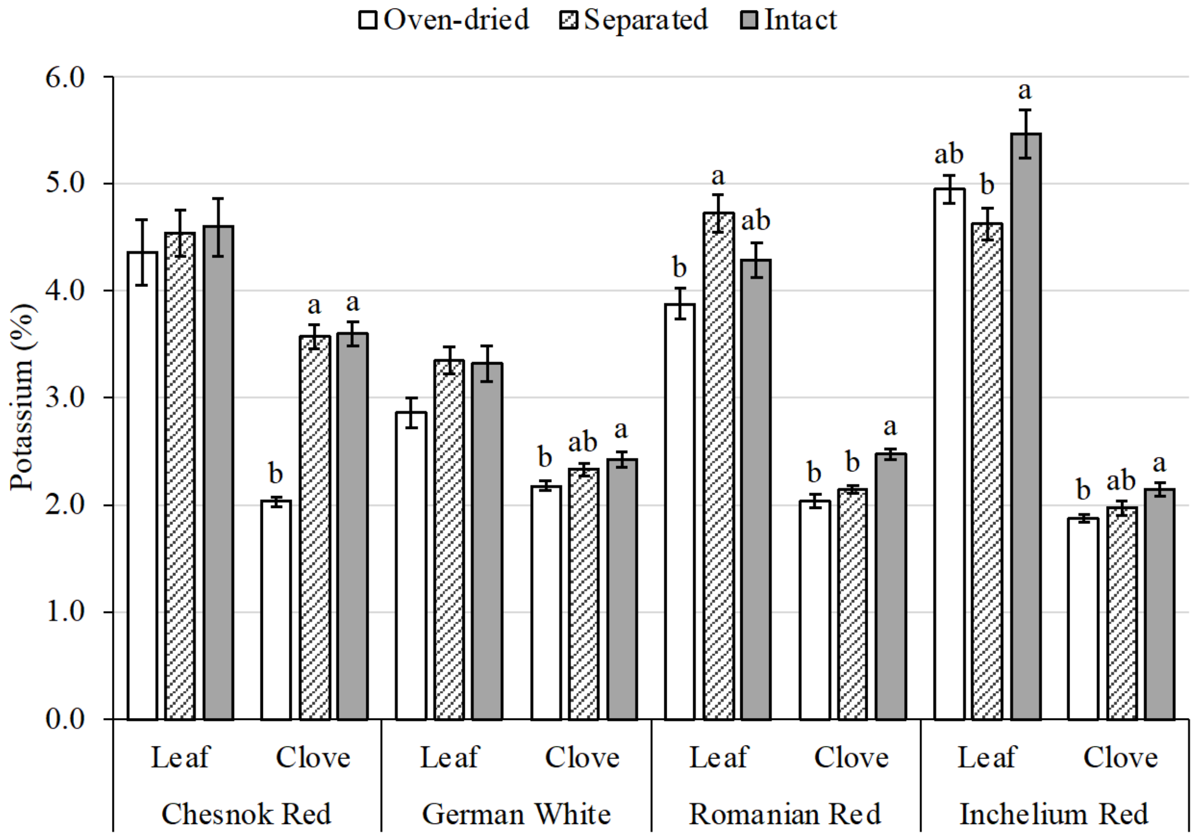

Figure 11. Potassium content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

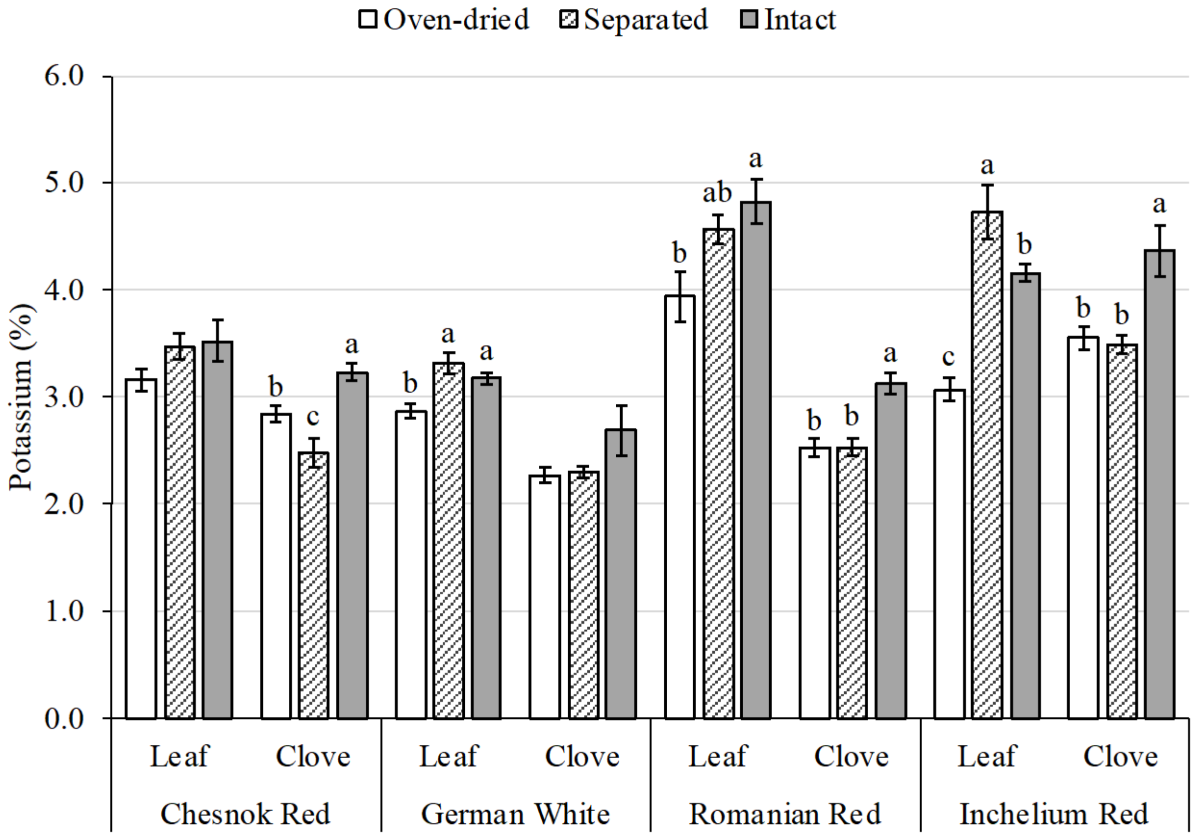

Figure 12. Potassium content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

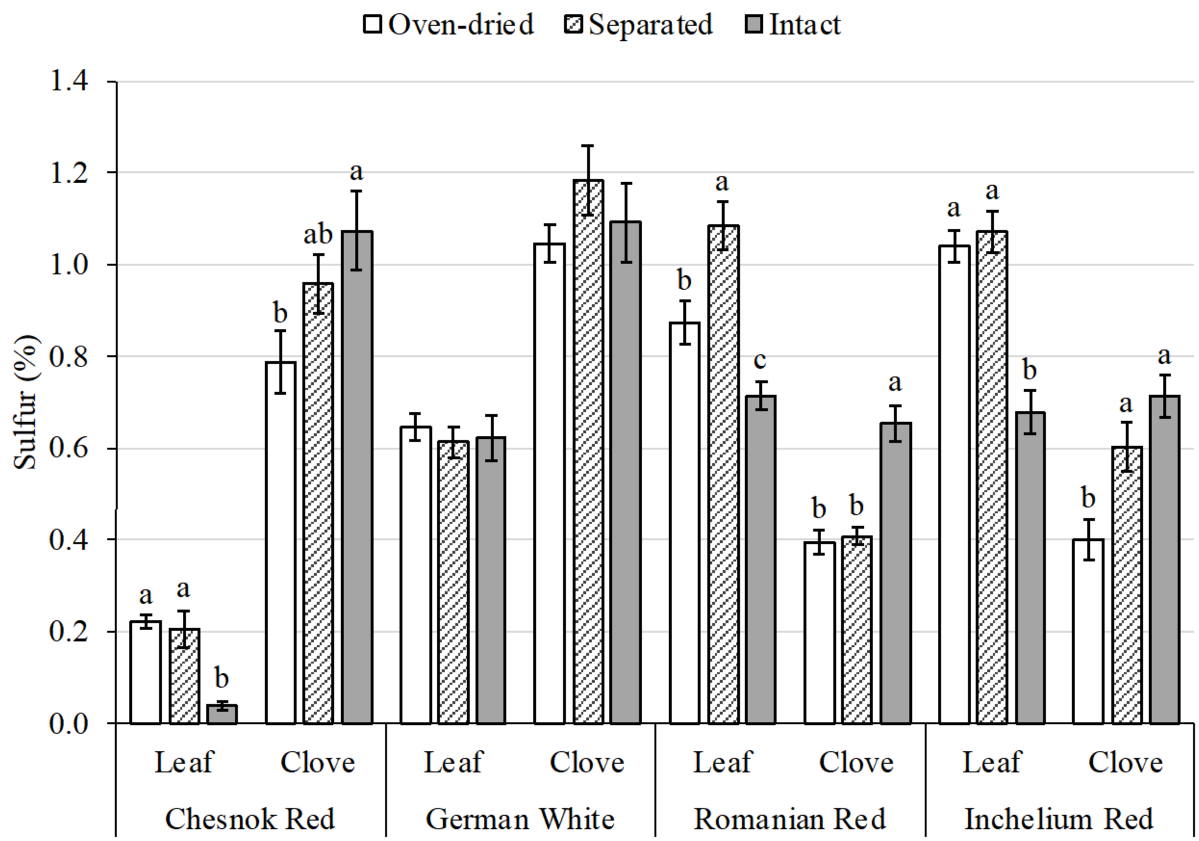

Figure 13. Sulfur content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

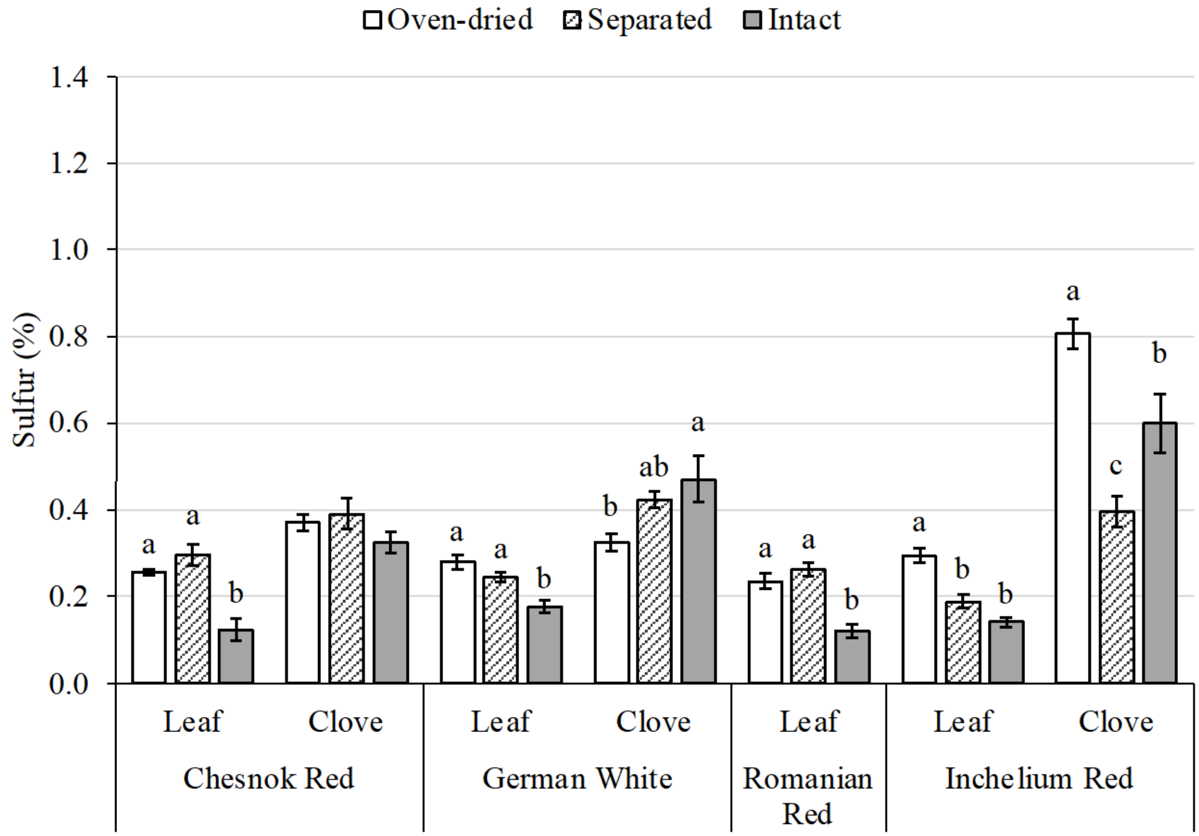

Figure 14. Sulfur content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different (p<0.05, Tukey's pairwise comparisons).

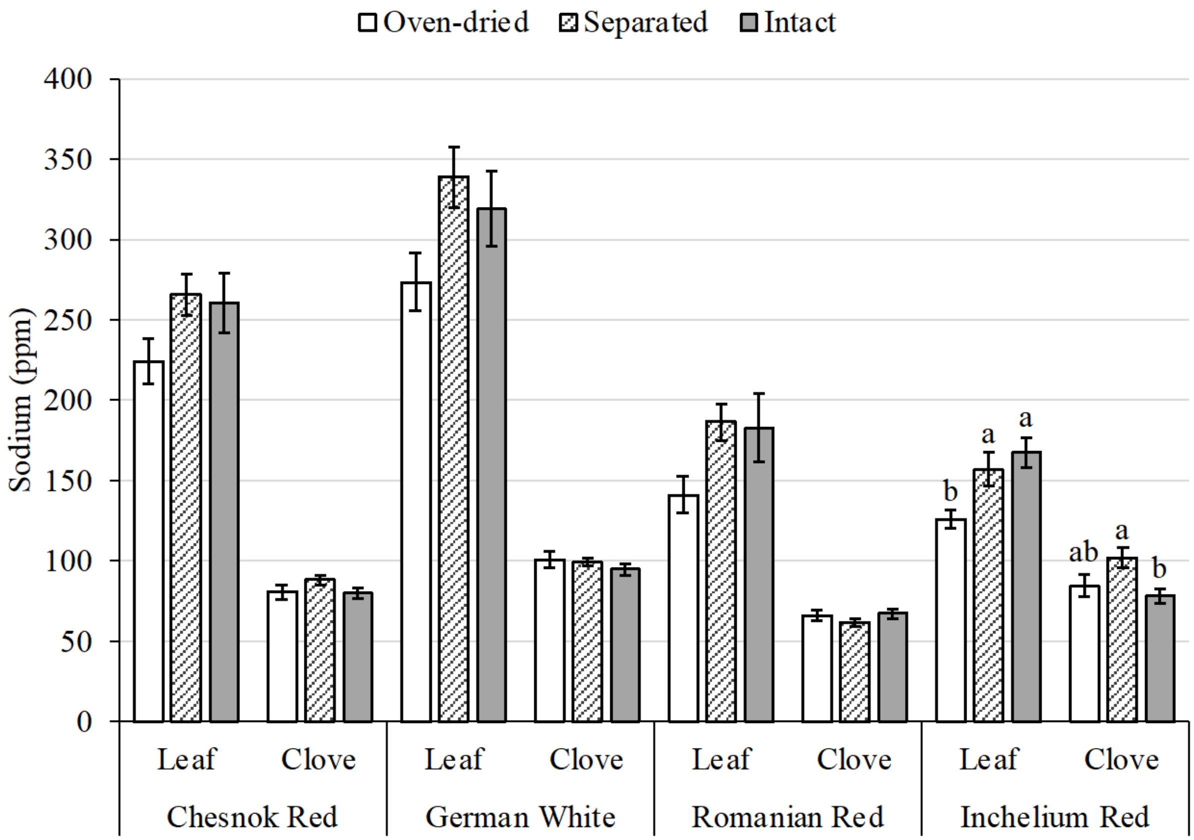

Figure 15. Sodium content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

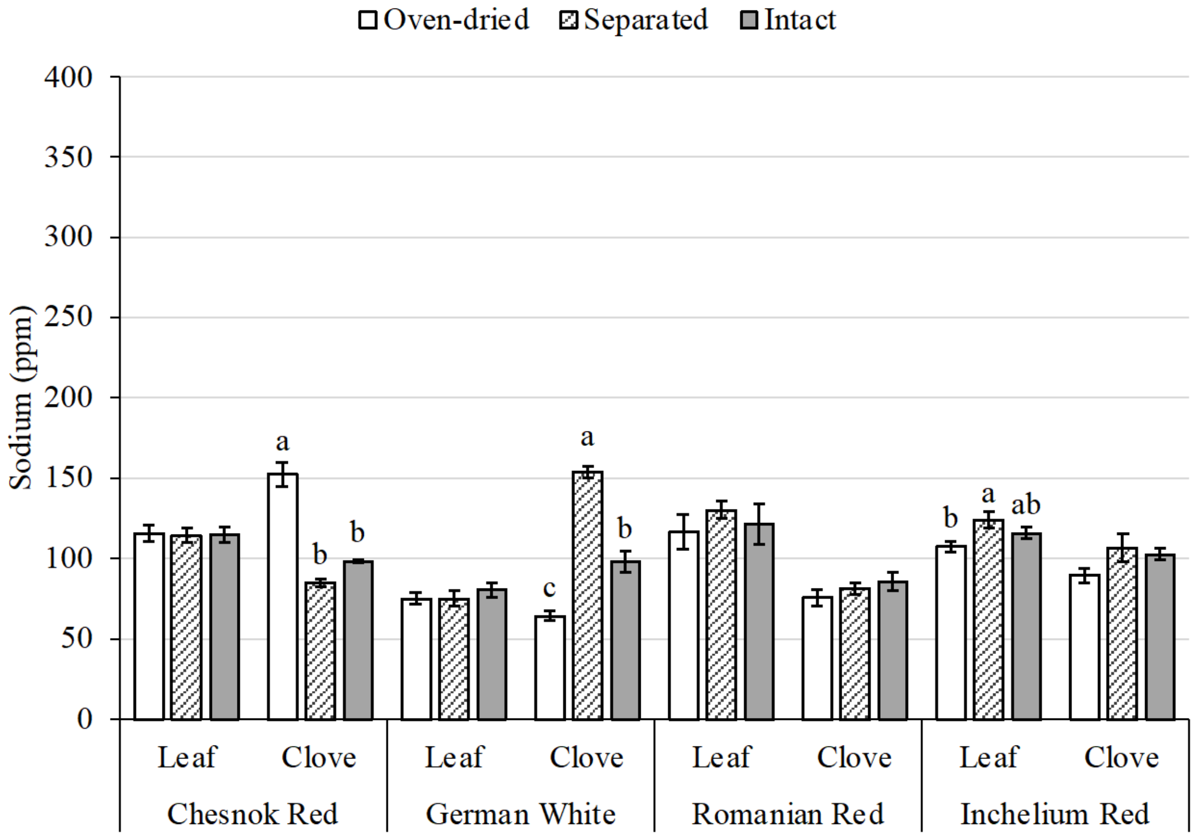

Figure 16. Sodium content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

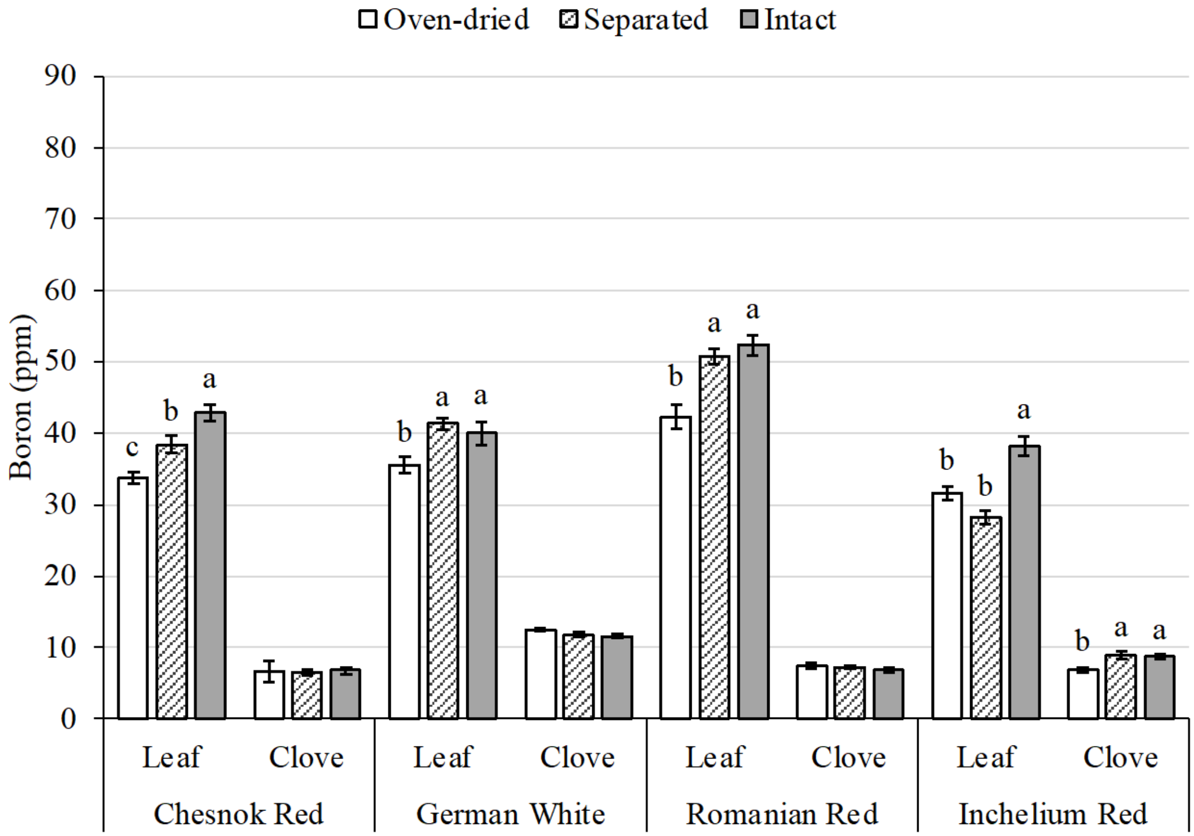

Figure 17. Boron content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

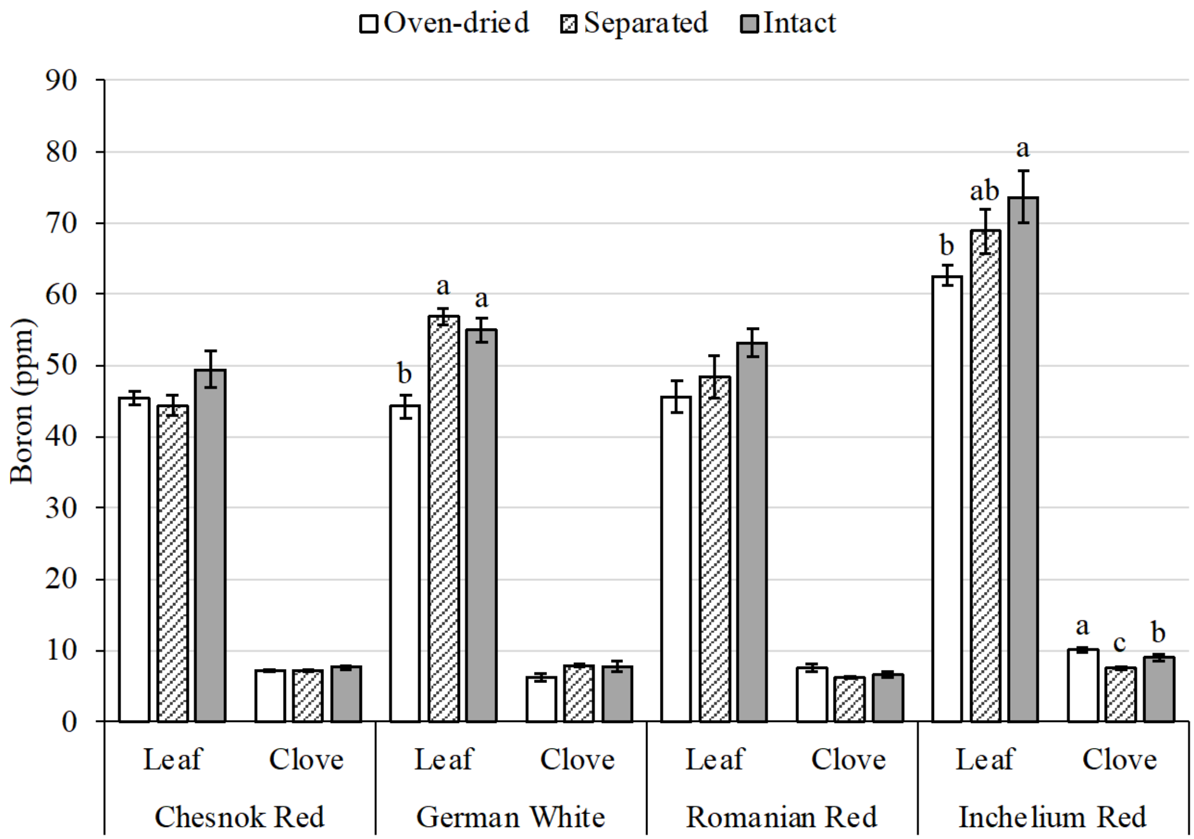

Figure 18. Boron content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

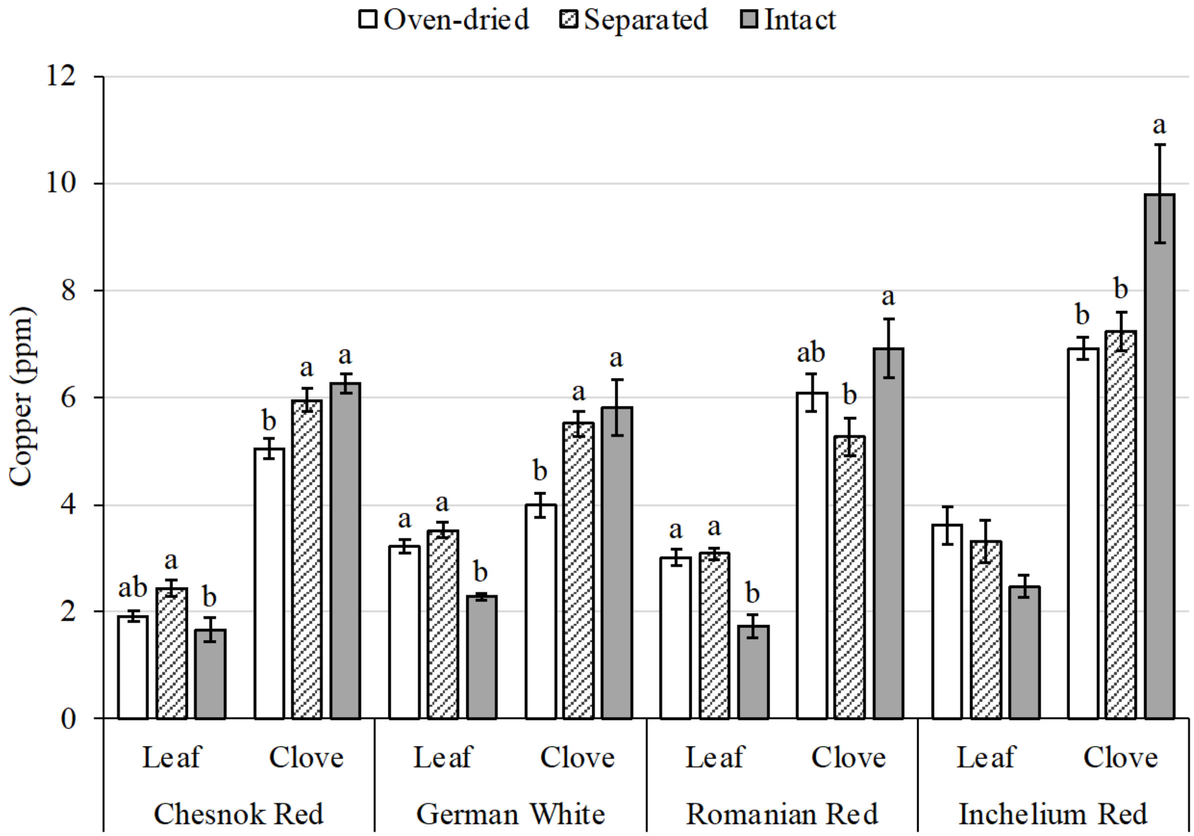

Figure 19. Copper content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

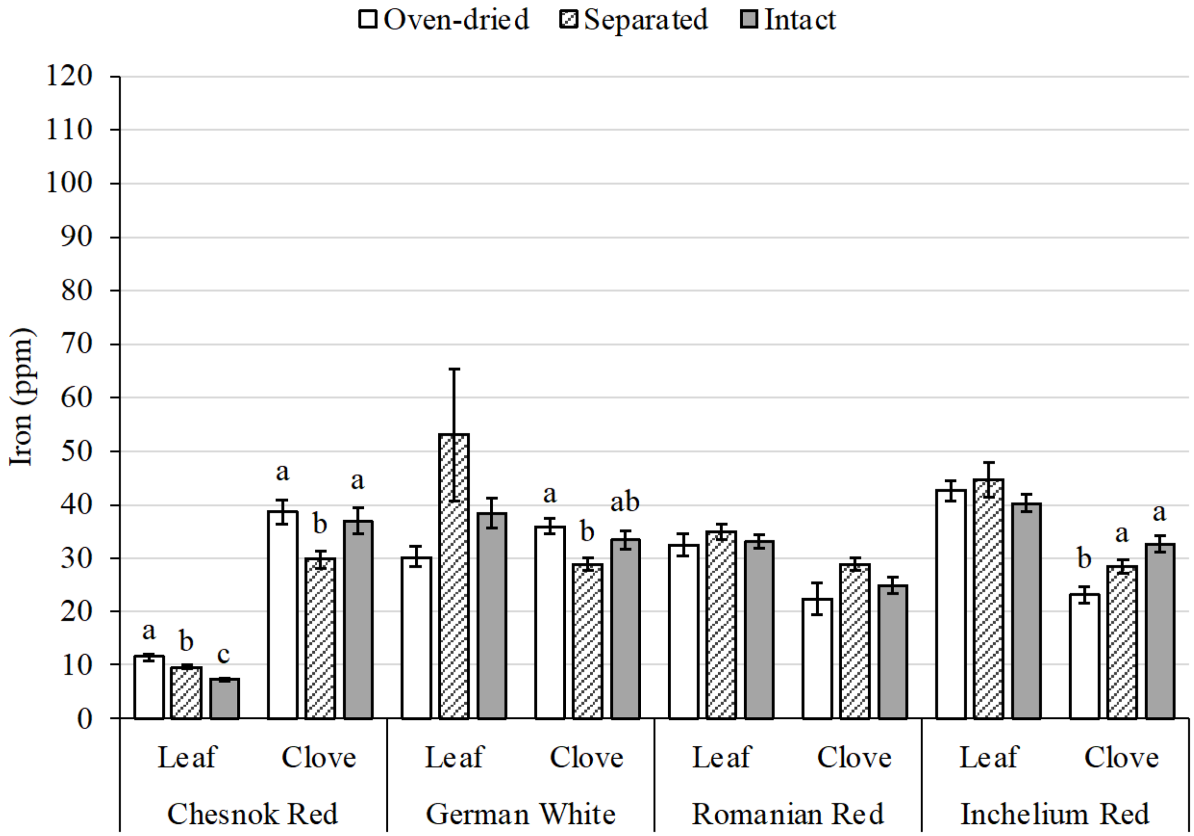

Figure 20. Iron content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different (p<0.05, Tukey's pairwise comparisons).

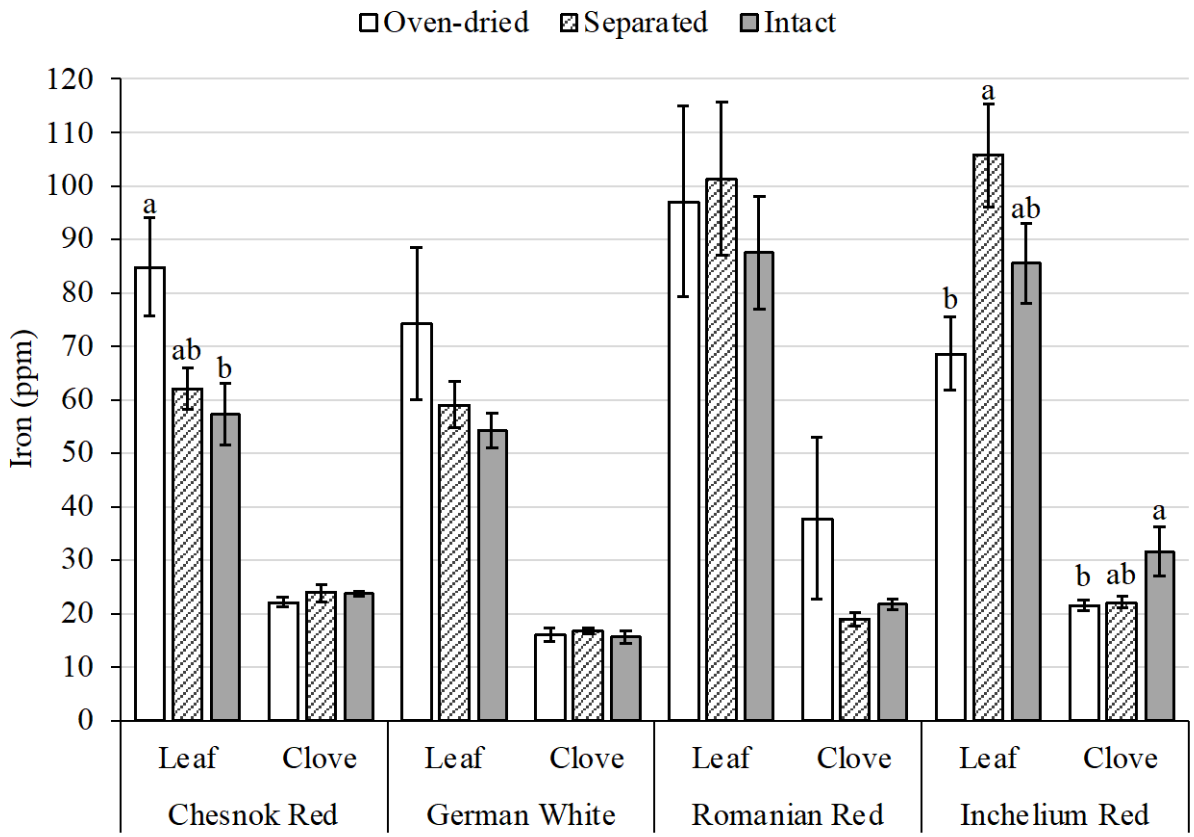

Figure 21. Iron content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

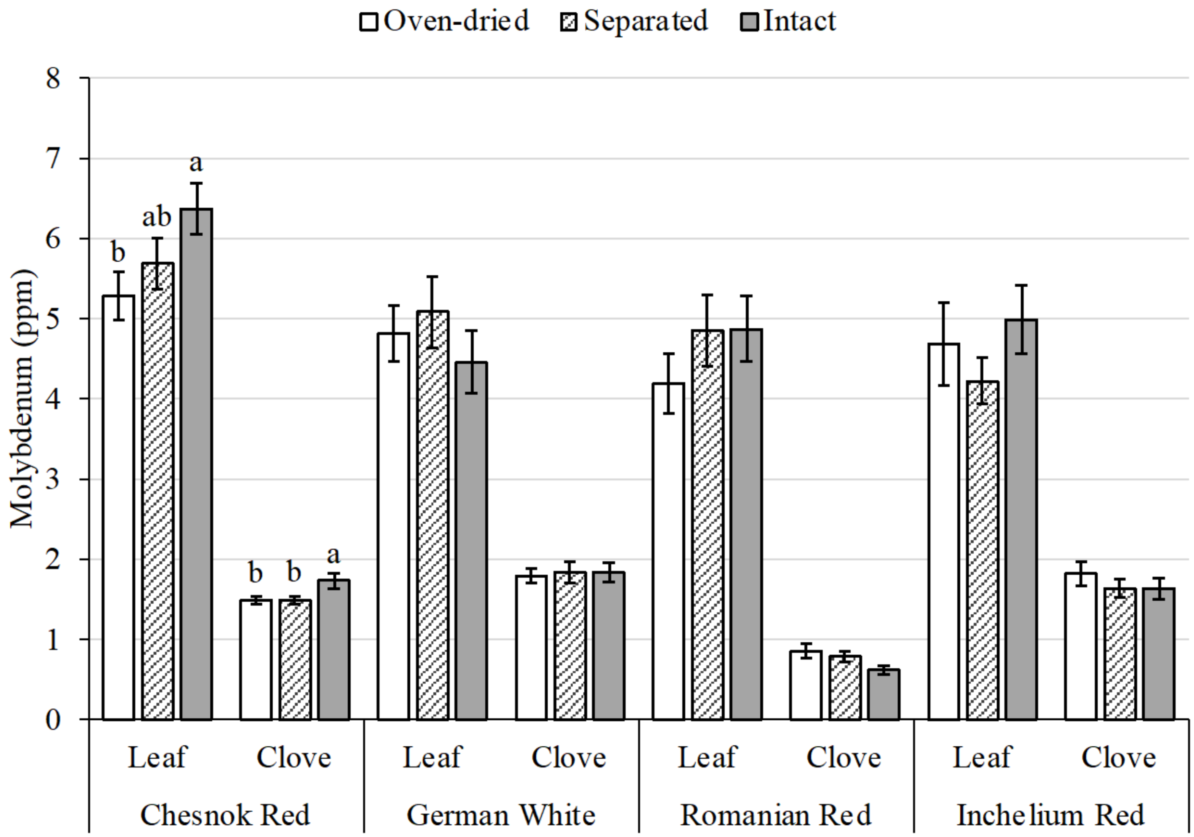

Figure 22. Molybdenum content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different (p<0.05, Tukey's pairwise comparisons).

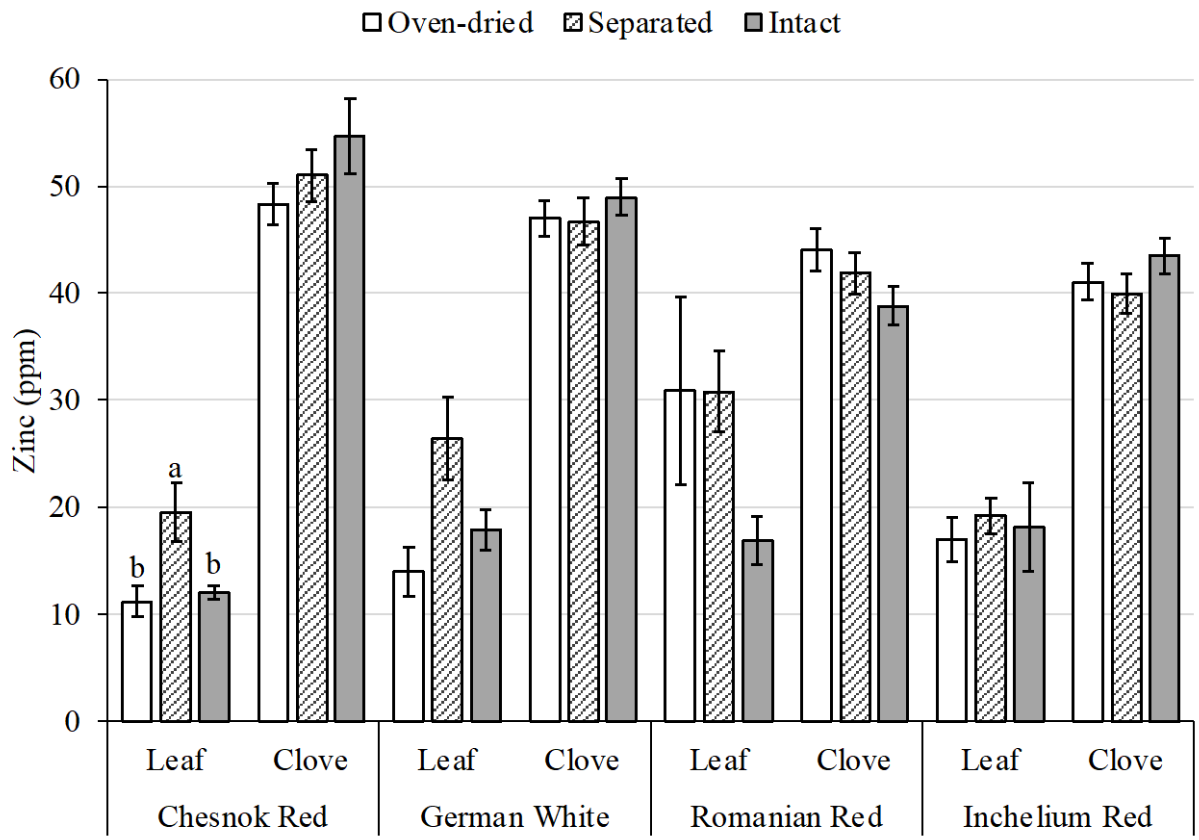

Figure 23. Zinc content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

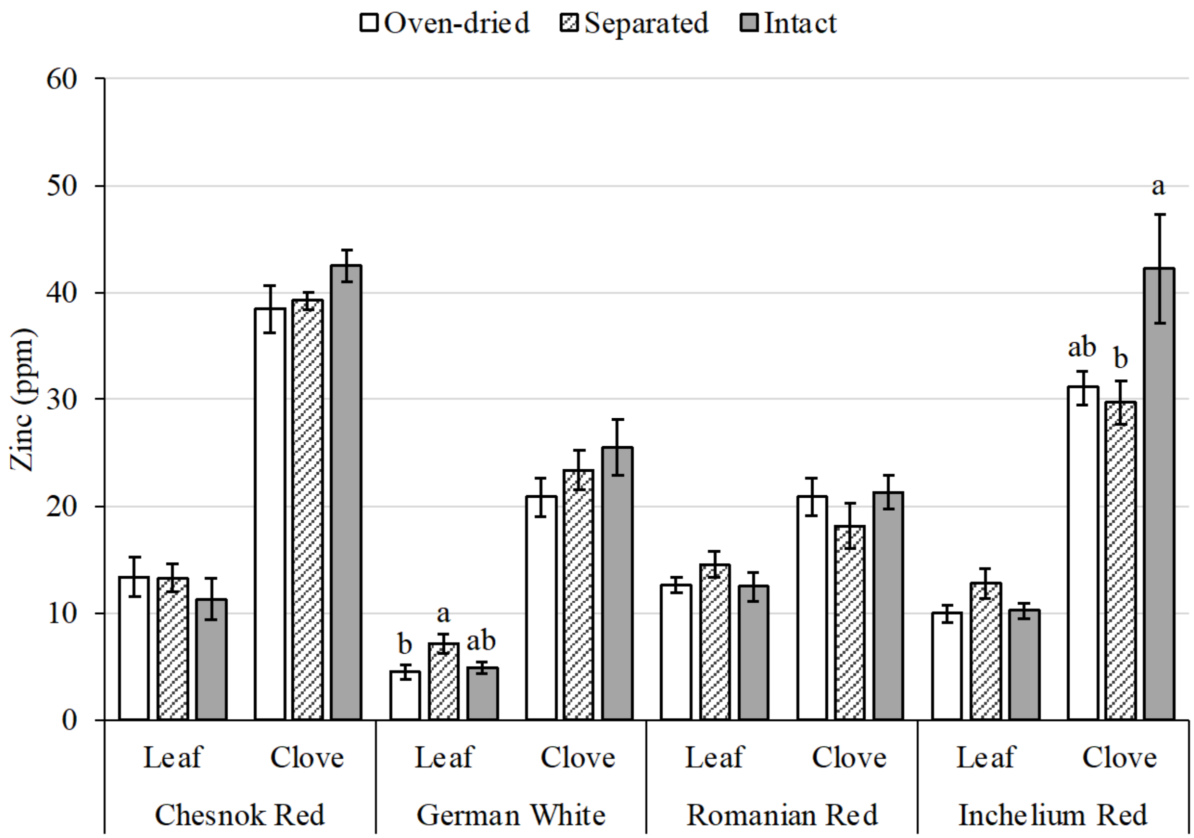

Figure 24. Zinc content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons

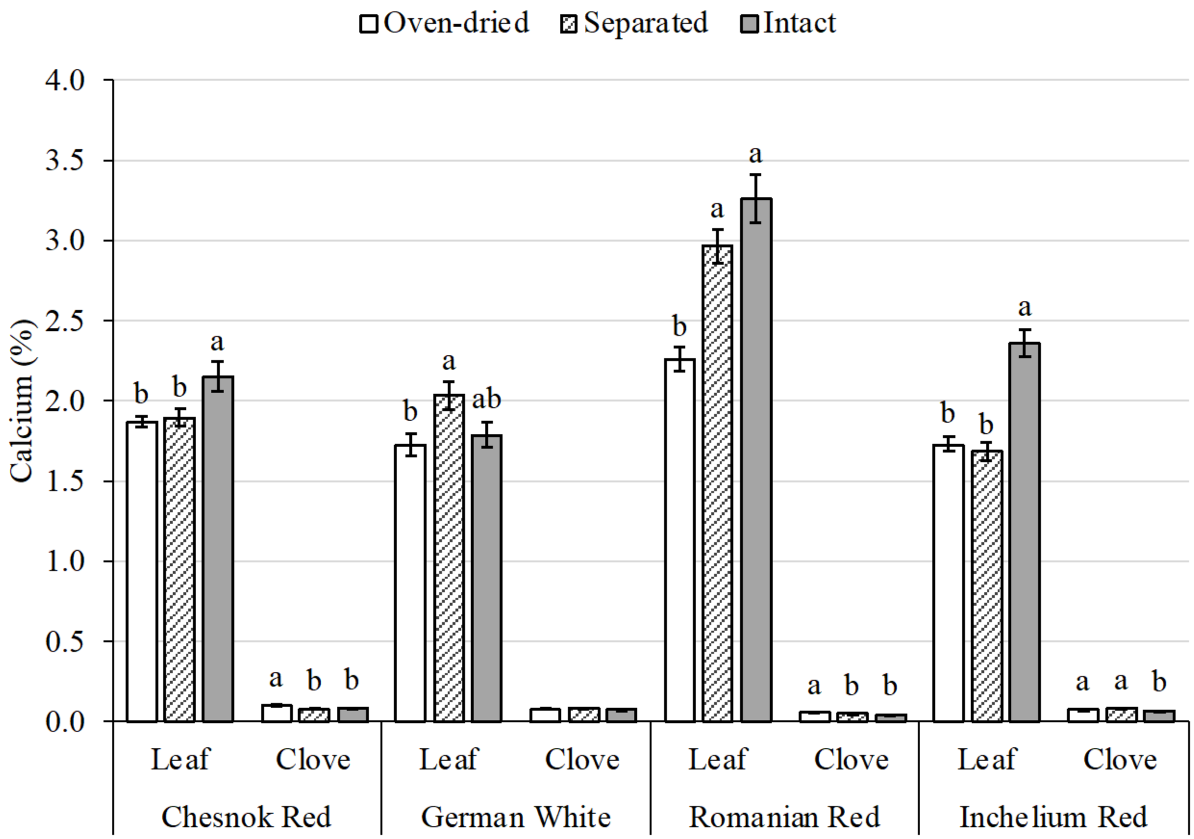

Figure 25. Calcium content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey’s pairwise comparisons).

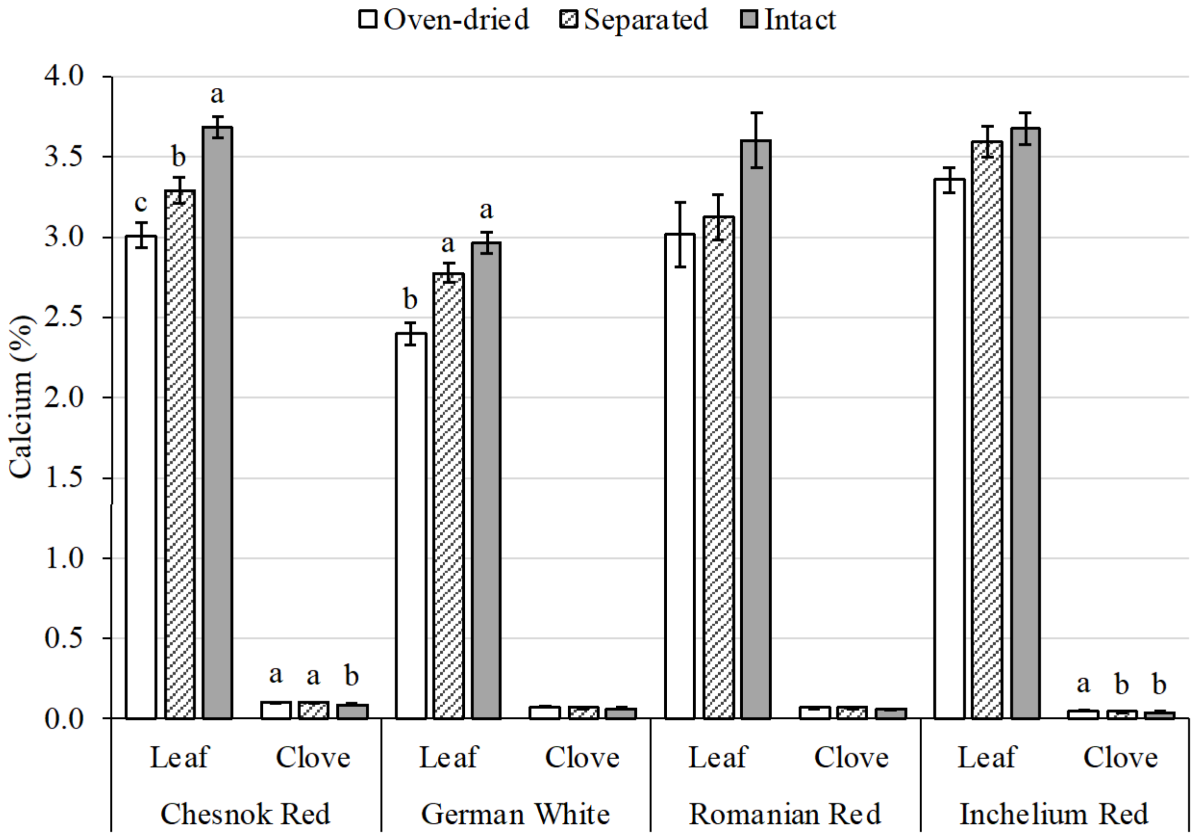

Figure 26. Calcium content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

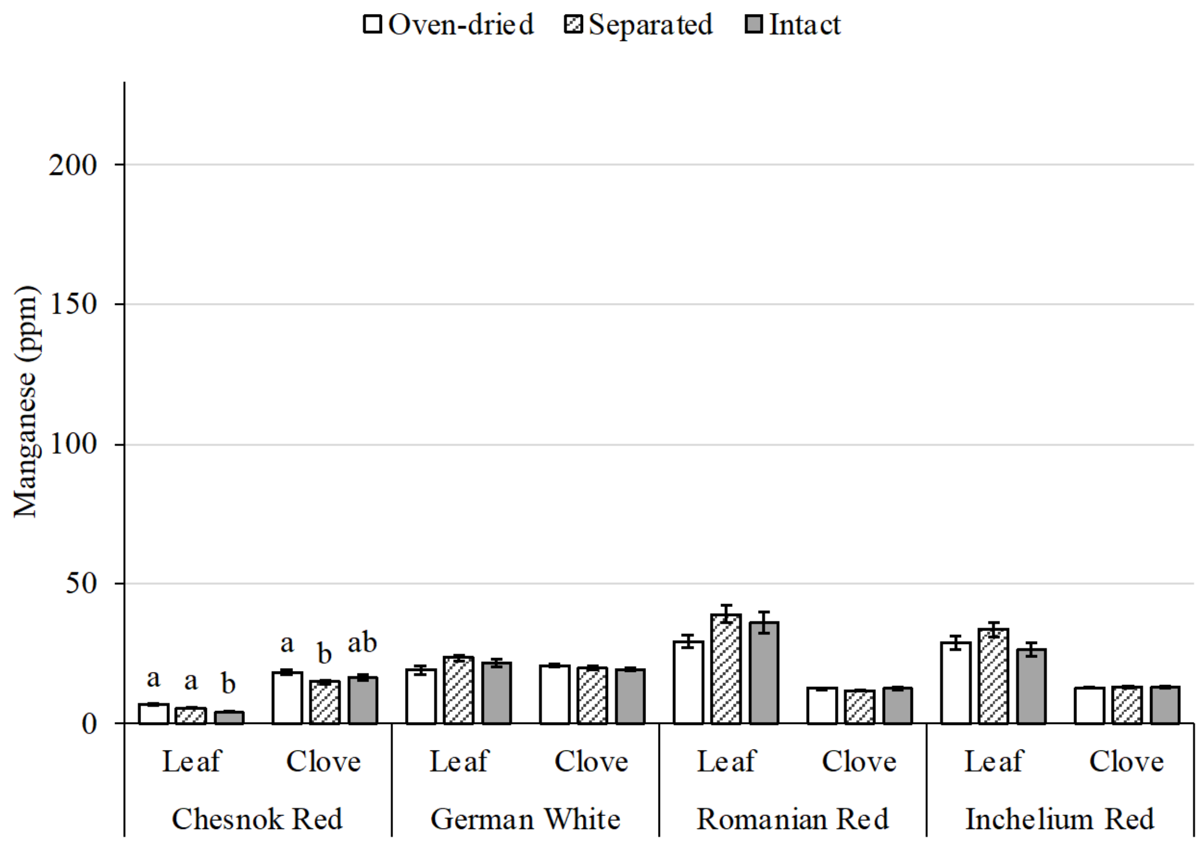

Figure 27. Manganese content of 2019 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

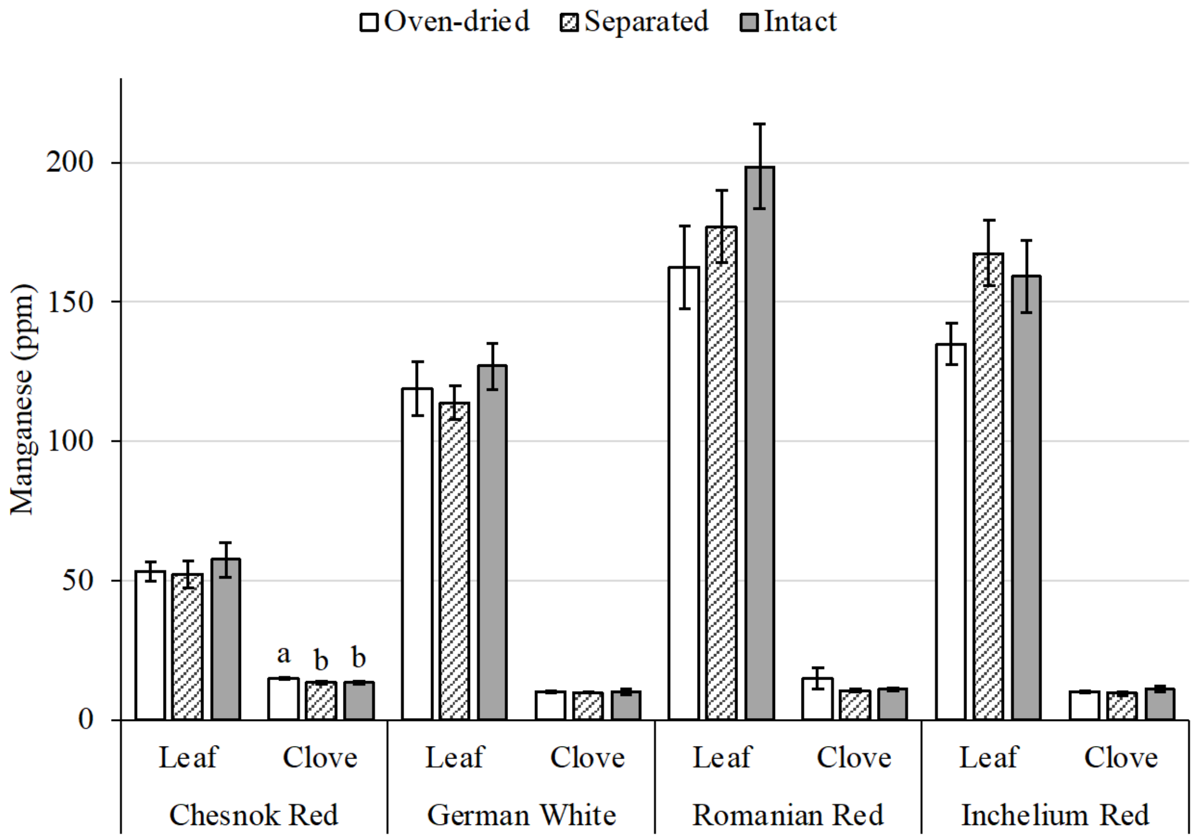

Figure 28. Manganese content of 2020 garlic leaf and clove tissue as affected by curing treatment in four cultivars. Values are means ± SE, n = 12. Within cultivar and tissue type, values not followed by the same letter are significantly different ($p<0.05$, Tukey's pairwise comparisons).

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