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Storage Life of Field-Grown Garlic Bulbs (*Allium sativum* L.) as Influenced by Nitrogen and Sulfur Fertilization

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ABSTRACT: The most important active compound in garlic is alliin. Sulfur (S) fertilization was shown to significantly increase the alliin concentration in garlic cloves, while high nitrogen (N) levels had an adverse effect. The effect of graded N and S application on the storage life of garlic has been paid little attention so far. A bifactorial field trial with 4 levels of N and S was conducted in a randomized block design. At harvest, 40 bulbs per treatment were stored under terms comparable to the storage conditions in average households (20 °C, dry, and dim) for 83 days. Every 3 weeks, samples were analyzed for their alliin and water content. The alliin concentration in peeled garlic cloves increased during storage from on average 9.2 mg g^{-1} dry weight at harvest to 21.4 mg g^{-1} dry weight after 83 days of storage. S fertilization increased the alliin concentration by a factor of 2.3 from 11.4 mg g^{-1} in the control treatment to 26.6 mg g^{-1} dry weight at the highest S level of 45 kg ha^{-1} after 83 days of storage. N fertilization decreased by a trend of the alliin content. Fertilizer rates had only a minor influence on water losses from bulbs at short-term storage. After 83 days of storage, water losses were by trend lower at higher S levels, and this relationship proved to be significant when no N was applied. Best quality in terms of high alliin contents was obtained during the entire storage time at an S level of at minimum 30 kg ha^{-1} S if no N was applied. The results show that the physiological S demand of 15 kg ha^{-1} S for optimum yield is lower than the S requirement of 30 kg ha^{-1} S for a longer storage life.

KEYWORDS: Alliin, fertilization, garlic (*Allium sativum* L.), nitrogen, storage life, sulfur

■ INTRODUCTION

Bulbs of onions and garlic are stored for varying lengths of time before being consumed. Several environmental factors such as climate, nutrient status, pests, and diseases affect the composition of *Allium* crops during growth.[1] The main factors influencing shelf life during storage are cultivar, storage duration, depth of bulb dormancy, and storage temperature.[2] The flavor compounds of onions and garlic are important quality parameters when investigating the effects of storage. Pyruvic acid is enzymatically formed during the degradation of cysteine sulfoxides. Because of its stability and ease of measurement, it is very often determined as a measure for the pungency of onions and garlics despite the fact that it is not a flavor compound in itself.[3−7] A major disadvantage of measuring the enzymatically formed pyruvic acid is the fact that it only reflects gross pyruvic acid production from all cysteine sulfoxides and not changes in individual cysteine sulfoxides such as alliin.[8] As a result, heterogeneous findings were obtained in relation to storage conditions. Increases in pungency were observed at the beginning of storage, followed by a decrease when onions were stored for longer periods.[3,5,9] Storage temperature was of prime importance, and Peterson et al.[4] demonstrated that pungency decreased if onions were stored at temperatures between 15 and 22 °C. Kopsell et al.[8] determined in a comparative study that the enzymatically formed pyruvic acid content was not correlated with the cysteine sulfoxide content in onions or with any individual cysteine sulfoxide. Therefore, the enzymatically formed pyruvic acid content seems to be no appropriate indicator for the alliin content of garlic when following up changes during storage. Yamane et al.[10] suggested the use of the formation of volatile degradation products from *S*-alk(en)ylcysteine sulfoxides during storage as a marker for the freshness of *Allium* species.

S-Alk-(en)yl-L-cysteine sulfoxides such as alliin (*S*-2-propenyl-L-cysteine sulfoxide) together with its precursors γ-glutamyl-*S*-alk(en)yl-cysteines are the primary S-containing constituents in garlic and account for about 1% of the whole biomass.[11] The characteristic flavor of garlic is released after enzymatic cleavage of alliin to allicin and its derivatives such as diallyl disulfide, dithiins, and ajoene. The enzyme alliinase is located in the vacuoles of the vascular bundle sheath cells around the phloem[12] and accounts for at least 10% of the total protein in the garlic clove.[13] Decomposition of alliin after cell disruption during harvest or processing of garlic is an extremely rapid reaction.[14] Moreover, the alliin content reacts rapidly to changing environmental factors such as water supply, temperature during growth, and mineral nutrition[15] and seems to be an appropriate indicator for the quality of garlic when following up changes during storage.

S fertilization was shown to increase a broad range of S-containing metabolites in plants such as cysteine, glutathione, and glucosinolates as well as cysteine sulfoxides in *Allium* species.[16−19] In a previous article, the influence of N and S fertilization on alliin, cysteine, and glutathione content in garlic during the vegetation period was shown.[20] N fertilization was of minor relevance, but S fertilization significantly increased the cysteine, glutathione, and alliin concentration in garlic bulbs. Alliin increased from 5.1 in control plots to 10.4 mg g^{-1} dry weight at the highest S rate in bulbs at harvest. In the present experiment, the bulbs from this field experiment were stored and changes in the alliin content monitored.

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Table 1. Influence of N and S Fertilization on the Alliin Concentration of Garlic Cloves in Relation to Storage Time after Harvest[a]

fertilization [kg ha^{-1}]	alliin concentration at harvest [mg g^{-1} of DW]	alliin concentration [mg g^{-1} of DW] in garlic cloves after different storage times: 6 days	21 days	42 days	63 days	83 days
N						
0	10.4	19.0	19.3	21.6	21.8	23.4
50	8.3	15.5	17.5	18.9	20.7	20.5
100	10.5	16.4	15.5	17.9	20.4	21.0
150	7.4	15.4	18.4	17.6	19.7	20.5
LSD $_{5\%}$	*3.6* ^{ns}	*2.1***	*2.1***	*2.3***	*2.5* ^{ns}	*2.9* ^{ns}
S						
0	5.1	9.3	11.1	11.7	13.5	11.4
15	10.0	17.9	17.8	19.2	21.6	21.2
30	11.2	18.1	19.4	22.7	23.3	26.2
45	10.4	21.0	22.4	22.4	24.2	26.6
LSD $_{5\%}$	*3.6***	*2.1****	*2.1****	*2.3****	*2.5****	*2.9****

[a] Two-factorial ANOVA was used to analyze the results, and the means were compared by the Tukey test at a 5% probability level; significance levels were coded as follows: ns not significant; *, significant, $p < 0.05$; **, highly significant, $p < 0.01$; ***, very highly significant, $p < 0.001$; $n = 16$.

Studies on the impact of fertilization on postharvest storage life and quality of *Allium* crops are rare. Higher N level reduced the postharvest quality of onions and shallots by increasing the rate of bulb rotting and sprouting and loss in bulb diameter and weight.[21,22] Often, such studies focus on special fertilizer practices such as the application of farmyard manures, organic fertilizers, and varying postharvest conditions on several quality parameters such as dry matter accumulation, yield, disease index, and postharvest shelf life.[23,24]

It is, however, important to know for garlic processing industries and consumers if changes in the mineral composition affect storage life and quality during postharvest storage of garlic. It was the aim of this study to address this question and to quantify the effects obtained. Alliin has been used as a marker to evaluate the effect of different fertilizer treatments and the impact of storage on the health promoting capacity of garlic.

MATERIALS AND METHODS

A bifactorial field experiment was conducted in 2002. Four S rates (0, 15, 30, and 45 kg ha^{-1} S) were applied as elemental S and 4 N rates (0, 50, 100, and 150 kg ha^{-1} N) as calcium ammonium nitrate. Cloves of garlic (*Allium sativum* L. var. *Thermidrome*) were planted on March 26th in plots of 3 × 4 m with interspaces of 10 cm and a distance between rows of 50 cm. The field trial was performed in a completely randomized block design. Each treatment had four replicates. The design of the field trial and results on the effect of N and S nutrition on the cysteine, glutathione, and alliin content of garlic are described in detail by Bloem et al.[20]

Forty bulbs per plot were stored after harvest for investigating changes in the alliin content during storage. Storage was performed at a constant temperature of 20 °C in a dry (45% relative humidity) and shaded room, which corresponds with common storage conditions in households.

The first sampling was carried out after 1 week of storage, then after 3, 6, 9, and 12 weeks. Each time, a representative subsample of 3 bulbs (about 90 g fresh weights) per replicate (12 bulbs per treatment) was taken. Bulbs were peeled, and the cloves were shock frozen in liquid nitrogen before freeze-drying. The dry matter content of bulbs was determined after freeze-drying of the samples, and the alliin content was extracted and determined according to Hoppe et al.[25] by high-performance liquid chromatography (HPLC, Merck Hitachi, Darmstadt, Germany). Alliin was detected at 337 nm by ultraviolet (UV) detection using a LiChrospher RP-18 column (125 × 4 mm, 5 μm).

Additionally the water loss of the intact bulbs was monitored. Two-way analysis of variation (ANOVA) was used to analyze the results, and the means were compared by the Tukey test at a 5% probability level.

RESULTS AND DISCUSSION

Garlic bulbs are generally stored for a period of time: when the postharvest temperature is between 20 and 30 °C, garlic bulbs remain firm for about 60 days before they shrivel; at cool temperatures (5−18 °C), dormancy is lost after 90−120 days and sprouting occurs, while under cold conditions (−1−0 °C), bulbs remain stable for long periods.[26] Targeted S fertilization increases the content of bioactive compounds in onions and garlic.[16,20] Fertilization influenced the mineral composition too. Its significance for storage life of garlic is yet unknown and is discussed below. Experimental storage conditions were chosen equivalent to that found in average household storage rooms (20 °C, dry and dim) to uncover the effects relevant for consumers.

Changes in Alliin Content during Postharvest Storage in Relation to N and S Fertilization. It is evident that the alliin concentration in garlic cloves significantly increased in relation to S fertilization and storage time (Table 1). The statistical analysis revealed that no interactions between N and S fertilization existed. Thus, in Tables 1 and 2, only the single effects of N and S application rates are shown. N and S fertilization significantly affected the accumulation of alliin during storage, though in the opposite direction (Table 1). Garlic cloves showed a mean alliin concentration over all treatments of 9.2 mg g^{-1} dry weight at harvest, which increased up to 21.4 mg g^{-1} after 83 days of postharvest storage (Figure 1). Alliin constituted about 20% of the total S in bulbs at harvest. This value increased to approximately 50% of the total S in garlic cloves after 83 days of postharvest storage. Increasing alliin contents under different storage conditions were also reported by Ichikawa et al.[27] The reason seems to be a conversion of γ-glutamyl peptides, γ-glutamyl-*S*-allyl-L-cysteine and γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine to alliin during storage.[27]

The fertilizer effect needs to be strictly distinguished from the loss of water during storage (Figure 1 and Table 2), which

Table 2. Influence of N and S Fertilization on N and S Concentrations in Garlic Cloves at Harvest and the Water Content of Garlic Cloves after Different Storage Times[a]

		water content [%] in garlic cloves after different storage times					
fertilization [kg ha^{-1}]	nitrogen concentration [mg N g^{-1} of DW]	at harvest	6 days	21 days	42 days	63 days	83 days
N							
0	27.1	68.0	68.7	66.2	65.1	63.0	56.9
50	27.4	68.9	68.1	65.7	64.7	63.1	57.7
100	27.5	68.5	67.7	63.6	62.4	60.3	53.7
150	27.1	67.7	67.3	65.1	63.9	62.1	56.6
LSD $_{5\%}$	*1.1* ^{ns}	*1.2* ^{ns}	*1.5* ^{ns}	*1.9**	*2.0**	*2.2**	*3.6* ^{ns}

		water content [%] in garlic cloves after different storage times					
fertilization [kg ha^{-1}]	sulfur concentration [mg S g^{-1} of DW]	at harvest	6 days	21 days	42 days	63 days	83 days
S							
0	5.2	67.6	68.2	65.4	64.2	62.3	54.4
15	7.8	68.8	68.0	65.0	63.9	61.9	56.5
30	8.9	68.4	67.9	64.8	63.7	61.9	56.7
45	9.0	68.2	67.5	65.4	64.3	62.3	57.3
LSD $_{5\%}$	*0.5* ***	*1.2* ^{ns}	*1.5* ^{ns}	*1.9* ^{ns}	*2.0* ^{ns}	*2.2* ^{ns}	*3.6* ^{ns}

[a] Two-factorial ANOVA was used to analyze the results, and the means were compared by the Tukey test at a 5% probability level. Significance levels were coded in the following way: ns, not significant; *, significant, $p < 0.05$; **, highly significant, $p < 0.01$; ***, very highly significant, $p < 0.001$; $n = 16$.

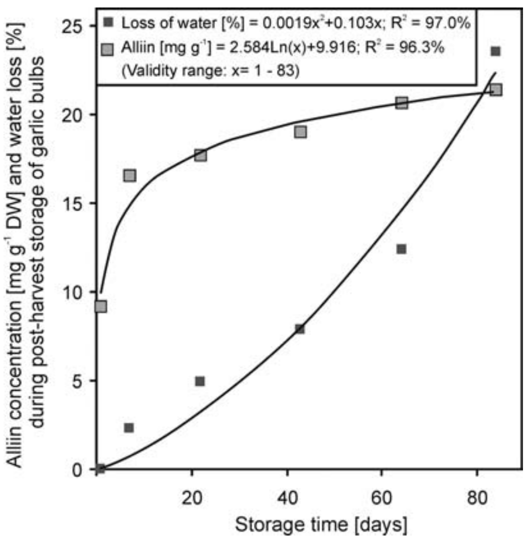

Figure 1. Change in alliin concentration of garlic cloves with storage time and relative loss of water from intact bulbs (harvest date was set to a value of 1).

resulted in higher alliin contents of fresh cloves (Table 3). In comparison, the pure N and S effect is reflected only if results are based on a dry matter basis (Table 1).

N fertilization resulted in decreased alliin contents during storage starting at the lowest level (Table 1). After six days of storage, significantly higher alliin concentrations were determined in stored bulbs, which received no N fertilization in comparison to fertilized bulbs. Such an impact lasted up to 42 days of storage and was, however, not evident at harvest time (Table 1). With longer storage times, the alliin concentration was still lower with N applications, but this effect was no longer significant because of an increasing variability between bulbs of the same treatment.

S fertilization doubled the alliin concentration in stored bulbs at all sampling dates (Table 1). At harvest, no significant differences between the different fertilizer treatments were found (Figure 2). However, after 83 days of storage, alliin was significantly increased by S application irrespective of the N rate. The control and lowest S application rate of 15 kg S ha^{-1} showed a reduced alliin concentration after 83 days of storage when compared to that of the plots which received either 30 or 45 kg ha^{-1} S. The higher S application rates resulted in similar alliin levels after 83 days of storage. These results reveal that saturation in terms of alliin has been achieved. In general, S fertilization was associated with a higher potential to produce alliin during storage. Most likely, more alliin precursors are available in bulbs that received S fertilization. These seem to be converted into alliin during storage.

In order to emphasize this assumption, the alliin content after 83 days of storage was plotted next to the factor of alliin increase from harvest to 83 days of storage in relation to N and S fertilization (Figure 3). The results show that at least an S application of 30 kg ha^{-1} S was necessary to obtain high alliin contents. The rate of increase with storage time was most pronounced in the treatment with the highest S level without N application (Figure 3).

The increase in alliin during storage is only marginally caused by the loss of water from cloves (Table 3). In Table 3, the proportion of alliin increase during storage caused by water loss was calculated on the basis of alliin content in fresh weight and the water loss in cloves of garlic during storage. Even after 83 days of storage when the water loss was highest, the increase of the alliin content due to loss of water accounted only for 6% of the total increase when compared to the alliin content at harvest.

Effect of N and S Fertilization on the Water Content of Garlic Bulbs during Storage. The water content of garlic is an important quality parameter as only firm bulbs can be sold. It is known that high N application rates in the later stages of bulb development of onions delay maturation and produce soft bulbs with shorter postharvest shelf life.[28] Water losses from intact bulbs

Table 3. Calculation of the Proportion of Alliin Increase during Storage Caused Either by the Water Loss of Cloves or the Accumulation of Alliin

	change in alliin and water content in garlic cloves after different storage times					
		days of storage				
measured and calculated parameter	at harvest	6	21	42	63	83
alliin concentration in dry matter [mg g^{-1} of DW]	9.2	16.6	17.7	19.0	20.7	21.4
water content of cloves [%]	68.3	67.9	65.1	64.0	62.1	56.2
alliin concentration in fresh weight [mg g^{-1} of FW]	2.9	5.3	6.2	6.9	7.9	9.4
increase of alliin due to water loss [mg g^{-1} of FW]		0.0	0.1	0.1	0.2	0.4
increase of alliin due to metabolism [mg g^{-1} of FW]		2.4	3.2	3.9	4.8	6.1

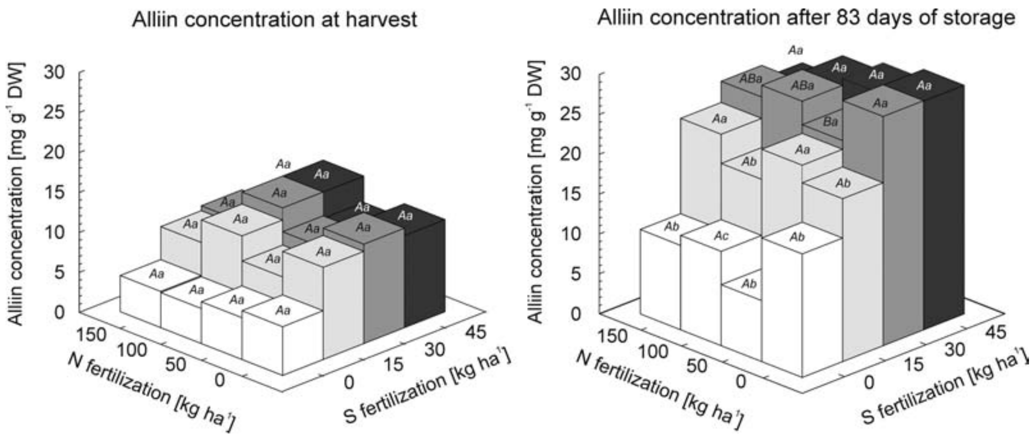

Figure 2. Alliin concentration of garlic cloves as influenced by N and S fertilization at harvest and after 83 days of postharvest storage (different letters denote significant differences between N (uppercase letters) and S (lowercase letters) at the 5% probability level by *t* test; *n* = 4).

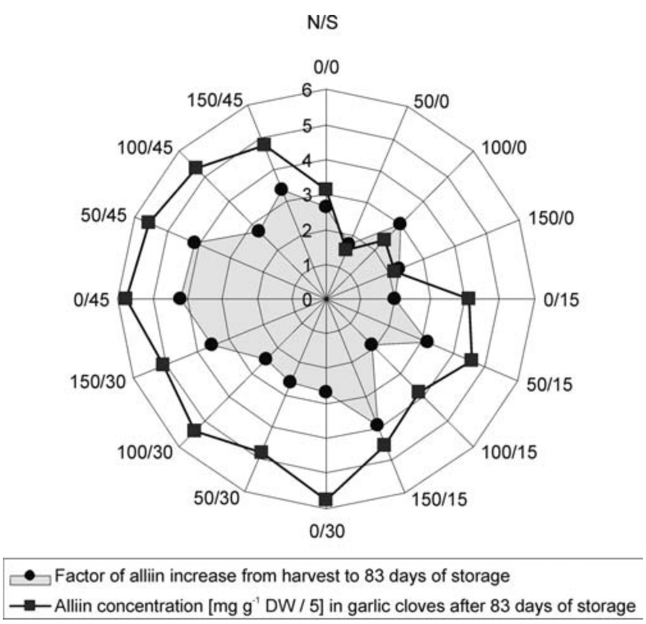

Figure 3. Alliin concentration in cloves of garlic and factor of alliin increase within 83 days of postharvest storage of intact bulbs in relation to N and S fertilizer treatments (values for alliin were divided by 5 for comparison with the accumulation rate).

(Figure 1) and the water content of garlic cloves (Table 2) were determined during storage in order to evaluate if these parameters are also affected by N or S fertilization. Water losses from intact bulbs (Figure 1) are distinctly higher than water losses from cloves (Table 2). The reason is that the drying process starts from the outer hulls to the cloves.

At harvest, no differences in yield and dry matter content were determined in relation to N and S fertilization. A mean yield of 61 dt ha^{-1} was obtained.[20]

Bifactorial ANOVA revealed slightly decreasing water contents in garlic cloves during storage in relation to N fertilization, while the N concentration at harvest was not affected by N fertilization (Table 2). S fertilization had a minor effect on the water content of cloves, though S fertilization significantly increased the S concentration in cloves. The water losses were by trend higher when S levels were low after 12 weeks of storage (Table 2).

The comparison of the different fertilizer treatments shows that after 83 days of storage N fertilization had no significant effect on water losses from the plant tissue (Figure 4). In contrast, S fertilization resulted in lower water losses from cloves. This effect was significant and most pronounced when no N was applied (Figure 4).

The data further reveal that a higher S content may prevent garlic bulbs from shriveling, while a high N supply may thwart this effect. The results stress the need for a balanced nutrient supply which is implemented best by a crop-specific fertilizer design when studying the effect of storage life. It was shown for onions that bulb fresh weight and firmness of bulbs decreased linearly with increasing N concentrations in the nutrient solution,[29] while for shallots, contradictory results were reported with either no

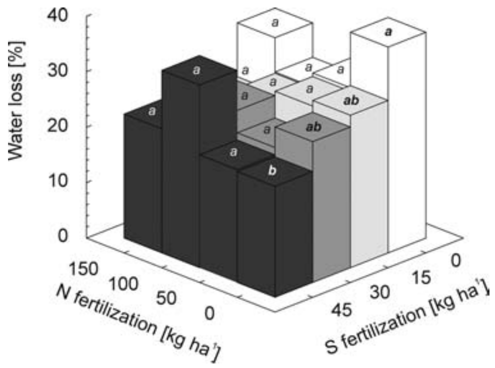

Figure 4. Water loss from garlic cloves after 83 days of postharvest storage (bold letters denote significant differences between S treatments ($n = 4$) at the 5% probability level; the effect of N fertilization was not significant).

significant effect of N fertilization on storage life of bulbs[30] or a negative effect of high N applications.[22] In contrast, Kumar et al.[31] point out that a high N fertilizer level of 200 kg ha^{-1} in combination with irrigation resulted in the best onion quality during 60 days of storage under the climatic conditions in India. Only with extended storage periods did a high fertilizer level have an adverse effect on the storability of the bulbs.

In the present article, we have shown that the duration of storage proved to be important for the content of active compounds in garlic. The alliin content increased with storage time, and this increase was most pronounced in the first days of storage. Kopsell and Randle[9] summarized the factors affecting the storage quality of onions and point out that the duration of storage and the cultivar are the most important factors determining the pungency of onions. The present study revealed that mineral fertilization also affected the quality and storage life of garlic. S fertilization increased the alliin content, and this effect was most pronounced when no N was applied. The same effect was observed for the loss of water during storage, which was reduced by S fertilization if no N was applied. In a previous study, it was shown that the best quality of garlic, in terms of its alliin content, was already achieved with an S fertilizer level of only 15 kg ha^{-1} and without additional N application.[20] The present results demonstrate that garlic bulbs fertilized with a higher level of S have a higher potential to accumulate alliin after harvest, which is important to consider as garlic bulbs are usually stored before usage. A higher S fertilization in combination with no N application resulted in high quality firm garlic bulbs, which are preferred by consumers. The presented results are an important contribution for improving the quality and storage life of fresh garlic.

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