International Journal of Scientific and Technological Research
ISSN 2422-8702 (Online)
Vol 1, No.1, 2015


# Oxidant / Antioxidant Status of Herbs *Allium Vineale* and *Chaerophyllum Macropodum* Used for Manufacture of Van Herby Cheese

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**Abstract**

The objective of this study was to determine total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), total phenolics, free sulfhydryl (SH) groups and lipid hydroperoxides (LH) contents of herbs *Allium vineale L.* (Liliaceae) and *Chaerophyllum macropodum* Boiss (Apiaceae) used for production of traditional Van herby cheese. Herbs of *A. vineale* and *C. macropodum* showed both oxidant and antioxidant properties in varying levels. TOS value of *C. macropodum* appears higher than that of *A. vineale*, while antioxidant capacities of both herbs were comparable. The lowest TAC value was found in brined *C. macropodum* (2.13 mmol Trolox equiv./g extract). TOS, OSI and LH contents of dried samples of *C. macropodum* were higher than the values of dried samples of *A. vineale* while these parameters were similar in brined samples of both herbs. The lowest SH value was determined in dried samples of *C. macropodum* (0.05 mmol/g extract) and, the highest was in brined samples of *A. vineale* (0.09 mmol/g extract). On the other hand, total phenolics values of dried samples were higher than those of brined samples. The TOS and OSI values of herbs determined in the present study can increase the TOS value during failure of antioxidant defense system in humans.

**Keywords:** Oxidant, Antioxidant, *Allium vineale, Chaerophyllum macropodum*

## 1. Introduction

For centuries, various herbs have been used as food additive due to their aromatic properties as well as for medicinal purposes due to their anti-inflammatory and antioxidative properties (Durmaz et al. 2006; Kaplan et al. 2007; Stajner and Popovic 2009). Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods to replace synthetic antioxidants (Hinneburg et al. 2006).

*Allium vineale* L. (*Liliaceae*), known as wild garlic and *Chaerophyllum macropodum* Boiss (*Apiaceae*) are traditionally added to Van herby cheese "Otlu Peynir" due to their aroma and flavor in Turkey. In addition, it is believed by local people that these plants have beneficial effects for the health. Recently, we have demonstrated that methanol, ethanol and n-hexane extracts of these plants used in cheese have

antibacterial activity in cheese (Durmaz et al. 2006).

Free radicals occurs endogenously as a result of normal oxidative metabolic reactions as well as exogenously as components of tobacco smoke, diet, drugs, and other environmental pollutants or indirectly through metabolism of certain solvents and by radiation (Lampe 1999). There is a balance between oxidants and antioxidants. When the balance shifts towards the oxidants, an oxidative stress occur, which is implicated in pathological conditions such as atherosclerosis, carcinogenesis, chronic renal failure, diabetes mellitus and aging in humans (Halliwell and Gutteridge 2000; Young and Woodside 2001). The physiological effect of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals (Young and Woodside 2001). Therapeutic effects of medicinal herbs are generally attributed to their polyphenolic contents. Polyphenolic compounds possess antioxidative activities by scavenging free radicals due to reducing properties as hydrogen or electron-donating agents (Rice-Evans et al. 1997; Young and Woodside 2001). The results of many studies (Skerget et al. 2005; Hinneburg et al. 2006; Çelik et al. 2008; Stajner and Popovic 2009) show that extracts of many plant have antioxidative activity. However, antioxidative activity is not simply related to the total phenolic components determined in extracts, as well as related to different extract constituents contributing to total extract activity (Skerget et al. 2005).

In recent years, interest in plant-derived food additives has increased. In general, plant extracts might substitute synthetic food antioxidants, which may influence public health when consumed chronically. Additionally, plant-derived food additives, especially polyphenolic compounds, have been ascribed health-promoting properties, such as to prevention of chronic cardiovascular diseases (Hinneburg et al. 2006). Stajner et al. (2008) reported that the antioxidant properties of herbs Allium species indicate their possible nutritional and medicinal value.

There are few studies about antioxidant activity of *A. vineale* (Stajner et al. 2006; Çelik et al. 2008; Stajner et al. 2008; Stajner and Popovic 2009) and *C. macropodum* (Stajner et al. 2006; Çoruh et al. 2007; Ebrahimabadi et al. 2010) in the literature. Although antioxidant status of *A. vineal*e and *C. macropodum* have been investigated by these authors, to the best of our knowledge, oxidant status and free sulfhydryl groups (SH) contents of these herbs has not been studied until now. In this study, total oxidant status (TOS), oxidative stress index (OSI) and lipid hydroperoxides (LH) content as well as total antioxidant capacity (TAC), SH and total phenolic contents of *A. vineal*e and *C. macropodum* were studied to determine oxidant and antioxidant status of these herbs.

## 2. Materials and Methods

### 2.1 Plant materials

*A. vineale* and *C. macropodum* were collected from Alacabuk (Pelli) mountain in Bitlis located in Eastern Anatolia in Turkey. The brined and dried samples of herbs were used in the analysis. In the brined samples, the fresh herbs were stored in 15% NaCl at 4 °C until extraction. In the dried samples, the plants were separately dried in shade, pulverized by a mechanical grinder and stored in airtight glass containers in dark until extraction.

### 2.2 Preparation of extracts

One gram of plant sample was dissolved in 50% methanol (v/v) in a final volume of 10 mL. Samples were then incubated for 2 h at 90°C in the dark, and centrifuged at 4500 rpm for 10 min. Supernatant was used for determining oxidant / antioxidant status of the samples.

### 2.3 Measurement of the TAC of the extract

The TAC of the extracts was measured by using a fully automated colorimetric measurement method developed by Erel (2004). In this method, the hydroxyl radical is produced by the Fenton reaction, and reacts with the colorless substrate *O*-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of an extract sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the extract, preventing the color change and thereby providing an effective measure of the TAC of the extract. The

assay results are expressed as mmol Trolox equivalent/g.

2.4 Measurement of TOS

TOS of the extracts was determined by using a novel automated measurement method developed by Erel (2005). Oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed as μmol $H_2O_2$ equivalent/g of extract.

2.5 Calculation of OSI

The percent ratio of the TOS to the total antioxidant status gives the OSI, an indicator of the degree of oxidative stress (Harma et al. 2003).

2.6 Measurement of total free sulfhydryl groups

Free sulfhydryl groups (SH) of the extracts were assayed according to the method of Hu et al. (1993). Briefly, 1 mL of buffer pH 8.2 containing 0.1 M Tris, 10 mM EDTA and 50 μL extract were added to cuvettes, followed by 50 μL 10 mM 5.5-dithiobis-(2-nitrobenzoic acid) (DTNB) in methanol. Blanks were run for each sample as a test, but there was no DTNB in the methanol. Following incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a spectrophotometer (Cecil 3000). Sample and reagent blanks were subtracted. The concentration of SH was calculated using reduced glutathione as free sulfhydryl group standard and the result was expressed as mmol/g of extract.

2.7 Measurement of LH

LH level of the extract were determined according to the method of Arab and Steghens (2004). LH levels were expressed as $\mu$mol $H_2O_2$ equivalent/g of extract.

2.8 Determination of total phenolics

Total phenolics in the samples were measured by using modified method of Skerget et al. (2005) based on a colorimetric oxidation/reduction reaction. Concentration of total phenolics in the samples was expressed as mg gallic acid (GA) per g of extract.

2.9 Statistical analysis

Statistical analyses of data were performed by ANOVA procedures using SAS® PROC GLM/STAT. Differences among means were identified using the Duncan multiple range test (SAS/STAT Software 1998).

**3. Results**

TAC, TOS, OSI, total phenolics, SH and LH levels of *A. vineale* and *C. macropodum* are presented in Table 1 and Fig. 1, 2, 3, 4, 5 and 6. TAC values of the herb samples were between 2.13-8.18 mmol Trolox eqv/g. The lowest TAC value was found in brined *C. macropodum*. Dried samples of *A. vineale* showed a lower oxidant status when compared to dried *C. macropodum* samples due to lower TOS, OSI and LH values ($P$<0.05); however, no significant difference was found between brined *A. vineale C. macropodum* samples for these parameters ($P$>0.05). Dried samples of both herb samples showed higher TOS levels when compared to brined samples ($P$<0.05). SH values were found lower in dried samples compared to brined samples. The lowest SH value was determined in dried samples of *C. macropodum* and, the highest SH value was in brined samples of *A. vineale*. There was not a statistically significant difference between dried samples of both herbs for total phenolics values (P>0.05). However, total phenolics values of dried samples were higher than those of brined samples (P<0.05).

 

Table 1. Oxidant and antioxidant status in extract of *A. vineale* and *C. macropodum* samples (mean±standard deviation)

| Parameters | *A. vineale* | | *C. macropodum* | |
|---|---|---|---|---|
| | Dried samples | Brined samples | Dried samples | Brined samples |
| TAC (mmol Trolox eqv./g extract) | 7.53±0.60 | 2.73±0.03 | 8.18±0.50 | 2.13±0.17 |
| Total phenolics (mg GA /g extract) | 1.35±0.06 | 0.63±0.04 | 1.34±0.02 | 0.22±0.02 |
| SH (mmol/g extract) | 0.06±0.01 | 0.09±0.002 | 0.05±0.01 | 0.08±0.004 |
| TOS (μmol $H_2O_2$ eqv./g extract) | 14.49±3.57 | 12.14±0.49 | 83.86±35.49 | 11.44±4.45 |
| OSI | 1.94±0.56 | 4.45±0.15 | 10.28±4.43 | 5.48±2.45 |
| LH (μmol $H_2O_2$ eqv./g extract) | 10.97±2.50 | 14.55±0.39 | 59.87±23.30 | 14.21±2.13 |

## 4. Discussion and Conclusion

To our knowledge, oxidant activities of *A. vineale* and *C. macropodum*, used in herby cheese production for aroma and flavor have not been studied until now. In this study, we have analyzed oxidant and antioxidant activities in edible parts of these herbs grown in Alacabuk (Pelli) mountain in Bitlis, Turkey.

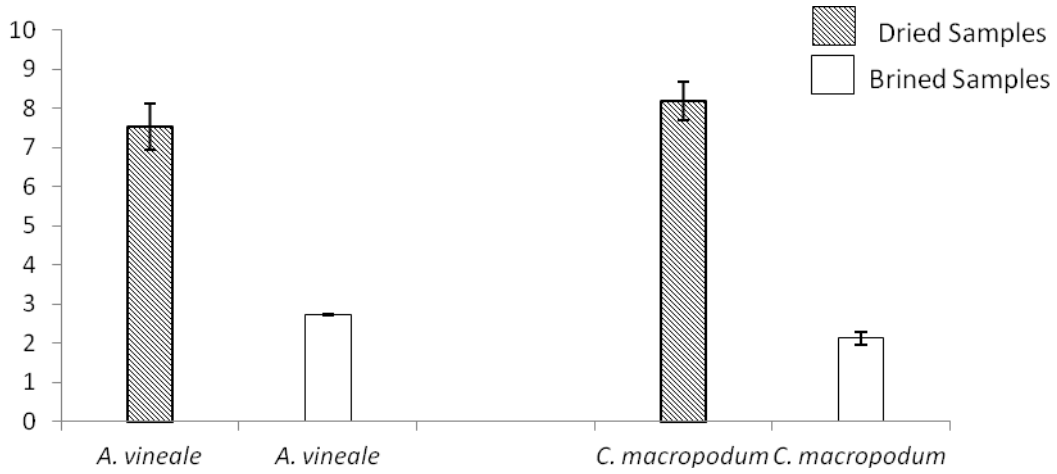


Figure 1. TAC values of *A. vineale* and *C. macropodum* samples (mmol Trolox eqv./g)

There are few studies about antioxidant activity of *A. vineale* and *C. macropodum* (Stajner et al. 2006; Stajner et al. 2008; Stajner and Popovic 2009). It has been shown that *Allium* species including *A. vineale* has effective antioxidative properties due to their high concentration of total flavonoids, high content of carotenoids and chlorophylls, and very low concentrations of toxic oxygen radicals. Our results also indicated that *A. vineale* has an antioxidant effect as reported by these authors. In the study by Çelik et al. (2008), TAC of extract for *A. vineale* samples was reported between 0.02-0.35 mmol Trolox eqv./g and TAC of extract for *C. macropodum* samples 0.03-0.35 mmol Trolox eqv./g, which were collected from different geographical locations of Van province in Turkey. These values were lower than TAC results obtained from our study (Table 1, Fig. 1). The antioxidant activities of *C. macropodum* was measured as the percent DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and percent inhibition of lipid peroxidation in study by Çoruh et al. (2007) and in DPPH test in study by Ebrahimabadi et al. (2010). Çoruh et al. (2007) studied antioxidant capacity of *C. macropodum* from Van region in Turkey and found that 50% inhibitory concentration ($IC_{50}$) values were 0.623 and 0.852 mg/mL for DPPH radical

scavenging and lipid peroxidation inhibition, respectively. Antioxidant activities of the leaves and flowers extracts of *C. macropodum* from Isfahan province in Iran were found 196.8 and 167.1 µg/mL as $IC_{50}$ values for DPPH test from Ebrahimabadi et al. (2010), respectively. The antioxidant activities of *C. macropodum* obtained from these studies cannot be compared with those obtained from our study because their values given in different units. The variability in antioxidant capacity of identical herb species may be due to the difference between methods used or geographical location and altitude.

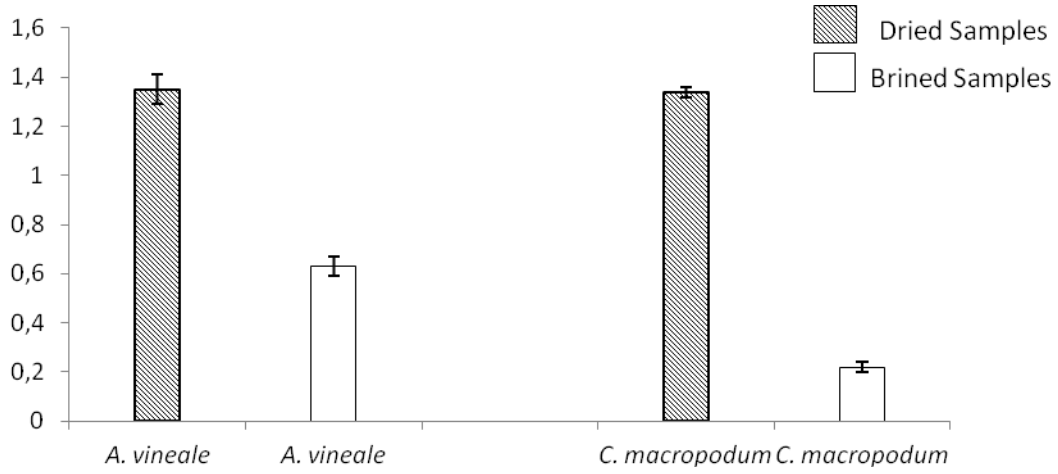


Figure 2. Total phenolics values of *A. vineale* and *C. macropodum* samples (mg gallic acid/g)

Polyphenolic compounds possess antioxidative effects by scavenging free radicals due to their reducing properties as hydrogen or electron-donating agents (Rice-Evans et al. 1997). Many herbs and spices are an excellent source of phenolic compounds, which correlate with its good antioxidant capacity (Hinneburg et al. 2006; Çoruh et al. 2007; Çelik et al. 2008). The results obtained from our study confirm these finding. However, total phenolic content was higher in brined samples of *A. vineale* when compared to *C. macropodum,* but the level of total phenolic content was comparable in both dried samples of the herbs in our study (Table 1, Fig. 2). In a study by Çoruh et al. (2007), total phenolic content of *C. macropodum* was reported to be 34 µg GA/mg of extract, which was higher than that obtained in the present study. Ebrahimabadi et al. (2010) reported that total phenolic compounds in methanol extracts of the leaves and flowers of *C. macropodum* were 29.3 µg/mg and 30.2 µg GA/mg extract, respectively. These values were higher than those from dried and brined samples of *C. macropodum* in our study, respectively.

SH proteins are mainly responsible for antioxidant response in organism and most significant correlation was found between total antioxidant activity and total SH contents of serum (Erel 2004). In our study, SH level was similar in both samples of *A. vineale* and *C. macropodum,* but it is higher in dried samples than that in brined samples of the herbs (Table 1, Fig. 3). To our knowledge, no study on SH content of *A. vineale* and *C. macropodum,* has been published until now.

The ratio of TOS to TAC was expressed as OSI, which indicates the degree of oxidative stress (Harma et al. 2003). TOS of herbs samples is measured because the measurement of oxidant molecules separately is not practical and their oxidant effects are additive (Erel 2005). In our study, mean TOS and OSI levels were higher in dried samples of *C. macropodum* than those in dried samples of *A. vineale* (Table 1, Fig. 4). The highest levels of TOS and OSI in dried samples of *C. macropodum* were determined as 83.86 µmol $H_2O_2$ eqv./g, and 10.28, respectively (Table 1, Fig. 4, 5). Harma et al. (2003) and Erel (2005) reported that TOS levels of serum or plasma in patients were significantly higher (approx. 22 µmol $H_2O_2$ equiv./L) than in those in healthy subjects (approx. 15 µmol $H_2O_2$ eqv./L) and a negative correlation was found between TOS and TAC values in patients. Therefore, the consumption of *A. vineale* and *C. macropodum* with these TOS and OSI values can increase the TOS value during failure of antioxidant defense system in humans.

 

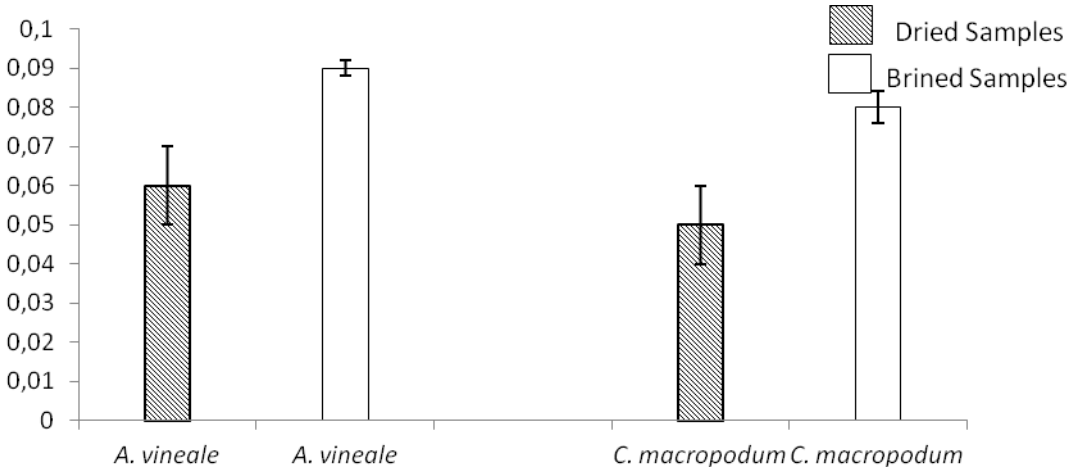


**Figure 3.** SH values of *A. vineale* and *C. macropodum* samples (mmol/g)

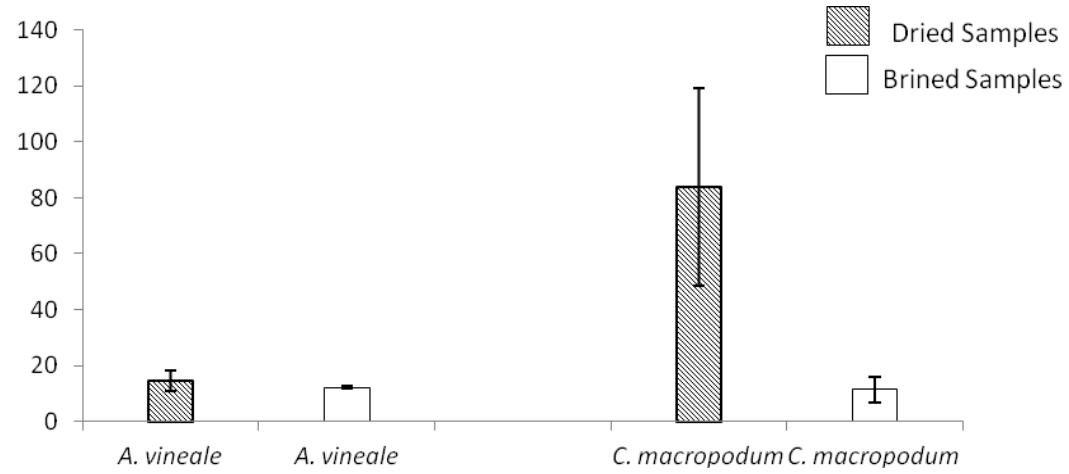


**Figure 4.** TOS values of *A. vineale* and *C. macropodum* samples (µmol $H_2O_2$ eqv./g)

One of the forms of damage resulting from oxidative stress in organism is lipoperoxidation. LHs are the first by-products of this process and therefore it is considered to be good candidates as initial biomarkers of oxidative stress (Arab and Steghens 2004). In our study, LH value was found in highest level (59.87 µmol $H_2O_2$ eqv/g) in dried samples of *C. macropodum*; however, LH level was similar in both brined samples of *A. vineale* and *C. macropodum* (Table 1, Fig. 6). Griffiths et al. (2000) reported that LH levels were 26 nmol/g in *Phaseolus hypocotyls*, 66 nmol/g and 49 nmol/g in sepals and petals of *Alstroemeria* floral tissues, respectively, 334 nmol/g in potato leaves and 568 nmol/g in broccoli florets. In our study, LH values of the samples except for dried samples of *C. macropodum*, were lower than those of Griffiths et al. (2000); however, LH value of dried samples of *C. macropodum* is lower than the values in potato leaves and broccoli florets. The LH level may be expected to vary in plants grown under conditions of abiotic and biotic stress or during the course of senescence in plant organs (Griffiths et al. 2000).

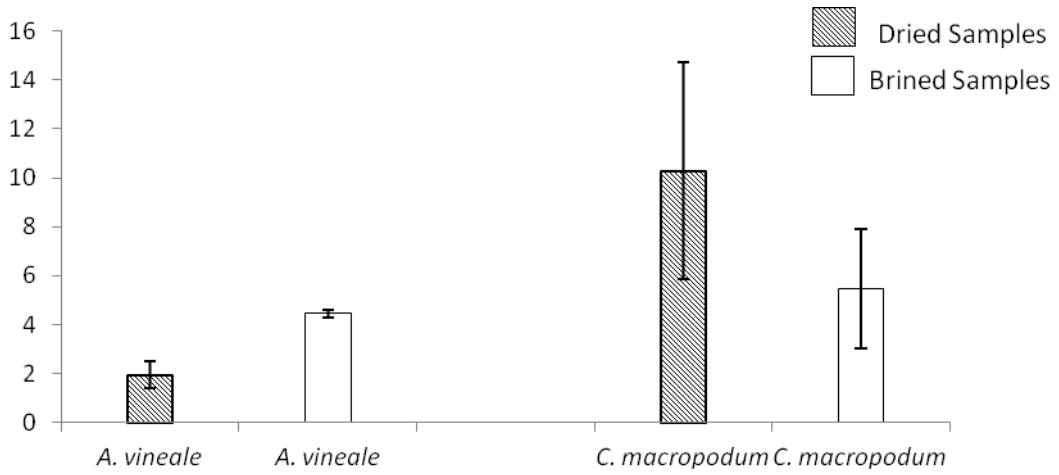


**Figure 5.** OSI values of *A. vineale* and *C. macropodum* samples

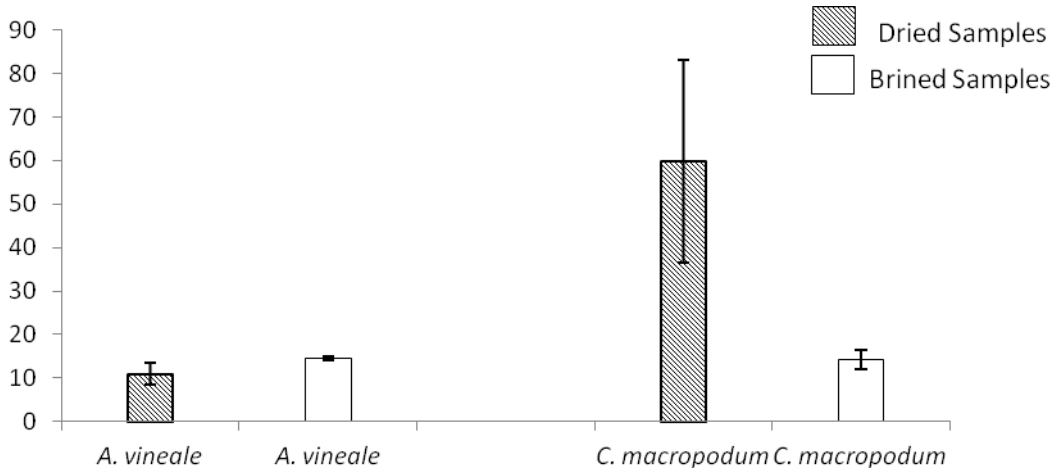


**Figure 6.** LH values of *A. vineale* and *C. macropodum* samples (µmol $H_2O_2$ eqv./g)

In conclusion, our study demonstrated that herbs of *A. vineale* and *C. macropodum* used traditionally in herby cheese production for their aroma and flavor in Turkey have both oxidant and antioxidant properties in varying levels. TOS value of *C. macropodum* appears to be higher than that of *A. vineale*, while antioxidant capacities of both herbs are comparable. The variability in antioxidant and oxidant status may be due to geographical location, altitude, growing period of herbs and analytical methods. According to the results of TOS and OSI values of herbs determined in the present study, it might be considered that the consumption of *A. vineale* and *C. macropodum* can increase the TOS value during failure of antioxidant defense system in humans.

**References**

Arab, K., & Steghens, J. P. (2004). Plasma lipid hydroperoxides measurement by an automated xylenol orange method. *Analytical Biochemistry*, 325, 158-163

Çelik, S. E., Özyürek, M., Altun, M., Bektaşoğlu, B., Güçlü, K., Berker, I., Özgökçe, F., & Apak, R. (2008). Antioxidant capacities of herbal plants used in the manufacture of Van herby cheese: 'Otlu Peynir'. *International Journal of Food Properties*, 11, 747-761

Çoruh, N., Sağdıçoğlu Celep, A. G., & Özgökçe, F. (2007). Antioxidant properties of *Prangos ferulacea* (L.) Lindl., *Chaerophyllum macropodum* Boiss. and *Heracleum persicum* Desf. from *Apiaceae* family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase. *Food Chemistry*, 100, 1237-1242

Durmaz, H., Sagun, E., Tarakci, Z., & Ozgokce, F. (2006). Antibacterial activities of *Allium vineale*, *Chaerophyllum macropodum* and *Prangos ferulacea*. *African Journal of Biotechnology*, 5, 1795-1798

Ebrahimabadi, A. H., Djafari-Bidgoli, Z., Mazoochi, A., Kashi, F. J., & Batooli, H. (2010). Essential oils composition, antioxidant and antimicrobial activity of the leaves and flowers of *Chaerophyllum macropodum* Boiss. *Food Control*, 21, 1173-1178

Erel, O. (2004). A novel automated method to measure total antioxidant response against potent free radical reactions. *Clinical Biochemistry*, 37, 112-119

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*, 38, 1103-1111

Griffiths, G., Leverentz, M., Silkowski, H., & Gill, N. (2000). Lipid hydroperoxide levels in plant tissues. *Journal of Experimental Botany*, 51, 1363-1370

Halliwell, B., & Gutteridge, J. M. C. (2000). *Free radicals in biology and medicine*. Third ed., 617-624, Oxford: Oxford Science Publications

Harma, M., Harma, M., & Erel, O. (2003). Increased oxidative stress in patients with hydatidiform mole. *Swiss Medical Weekly*, 133, 563-566

Hinneburg, I., Dorman, H. J. D., & Hiltunen, R. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry*, 97, 122-129

Hu, M. L., Louie, S., Cross, C. E., Motchnik, P., & Halliwell, B. (1993). Antioxidant protection against hypochlorous acid in human plasma. *Journal of Laboratory and Clinical Medicine*, 121, 257-262

Kaplan, M., Mutlu, E. A., Benson, M., Fields, J. Z., Banan, A., & Keshavarzian, A. (2007). Use of herbal preparations in the treatment of oxidant-mediated inflammatory disorders. *Complementary Therapies in Medicine*, 15, 207-216

Lampe, J. W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *The American Journal of Clinical Nutrition*, 70 (suppl): 475-490

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, 2, 152–159

SAS/STAT Software (1998). *Changes and enhancements through release. 6.12.* Cary NC, USA: SAS Institute Inc

Skerget, M., Kotnik, P., Hadolin, M., Hras, A. R., Simonic, M., & Knez, Z. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry*, 89, 191-198

Stajner, D., Igic, R., Popovi,c B. M., & Malencic, D. J. (2008). Comparative study of antioxidant properties of wild growing and cultivated *Allium* species. *Phytotherapy Research*, 22, 113-117

Stajner, D., Milic, N., Canadanovic-Brunet, J., Kapor, A., Stajner, M., & Popovic, B. M. (2006). Exploring *Allium* species as a source of potential medicinal agents. *Phytotherapy Research*, 20, 581-584

Stajner, D., & Popovic, B. M. (2009). Comparative study of antioxidant capacity in organs of different

*Allium* species. *Central European Journal of Biology*, 4, 224-228
Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease. *Journal of Clinical Pathology*, 54, 176-186