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HOP PELLETS TYPE 90: ESEM STUDIES OF GLANDULAR TRICHOMES MORPHOLOGICAL AND STRUCTURAL CHANGES DURING THE DIFFERENT PHASES OF HOP PROCESSING

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The results of this study show that the most usual damages of hop glandular trichomes are obtained on tunica of peltate glandular trichomes as well as cracking of bulbous glandular trichomes. In fresh hop cones any sorts of damage of glandular trichomes are obtained, on the other hand frequency of damaged hop glandular trichomes increase after drying (f_i=0.08) and after pelletization of hop cones the structure of glandular trichomes is completely destroyed. Volume of hop glandular trichomes of dried hop cones is significantly smaller in the comparison with volume of glandular trichomes of fresh hop cones ($D=0.544\times10^{-2}$ mm^3; $P<0.01$) and a phenomenon of shrinkage on tunica surface of peltate glandular trichomes of dried hop cones is also visible in comparison with the fresh one. The decrease in volume of hop glandular trichomes in dried hop cones is the result of dehydration of hop cones and consequently hops glandular trichomes during the drying process which cause shrinkage of peltate glandular trichomes. However, after pelletization the structure of hop glandular trichomes is completely destroyed.

Keywords: hop pellets type 90, hop processing, ESEM, hop glandular trichomes damage, volume of hop glandular trichomes

The glandular trichomes (peltate and bulbous) are placed on the epidermis of hop cone bracts in which the hop metabolites are accumulating (Saito et al., 1995; Hirosawa et al., 1995). Higher temperature speeds up the oxidation reactions which cause degradation of bitter and aromatic hop substances and consequently decrease the brewing value of hop pellets. The basic principle of hop chemical compounds degradation primarily means the oxidation of α-acids when they are exposed to air, particularly at high temperatures during kilning, conditioning, pressing, temporary storage, pelletization, transportation to a brewery and storage in brewery warehouse (Weber et al., 1979; Forster, 1999; 2001a; b; 2002; 2003a; b; Rossbauer & Münsterer, 2003; Virant & Majer, 2003; Srečec et al., 2009). Hop pellets type 90 are still the most frequent hop products used in brewing and their quality is diminished during harvesting and processing to hop pellets (Forster, 2001a; Srečec et al., 2009). Depending on exposure to the high temperatures during the different phases of hop processing and storage the losses of hop bitter and aromatic substances could increase (Forster, 1999; 2001a; b; 2002; 2003a; b; Rossbauer & Münsterer, 2003; Virant & Majer, 2003; Srečec et al., 2009). In order to reduce the time of hop exposure to high temperatures the integrated pelletization of hop cones into hop pellets type 90 was developed (Srečec et al., 2004b; Marić & Srečec, 2006). It means that all well-known technical solutions are linked into an

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integrated system which covers harvesting, kilning and conditioning or just cooling of dried hop cones with grounding, pelletization and packing in 3-ply Al-folium bags under inert N_2 atmosphere. Nevertheless, it is well known that even this kind of packing does not prevent degradation of α-acids when exposed to high temperatures during transportation and storage (Marić & Srečec, 2006; Srečec et al., 2009). Changes of α-acids content during the integrated procedure of hop pellets production confirm that integrated procedure considerably decrease loss of α-acids in comparison to the usual procedure (Srečec et al., 2004b; 2009). Nevertheless, time, temperature and hop damage have significant impact on losses of α-acids. However, there are no such optimal conditions, in which the losses of hop bitter compounds could be completely avoided. One of the most possible reasons is damage of lupulin glands or hop glandular trichomes during the drying and pelletizing (Forster, 2001a; b; 2002; 2003a; b; Rossbauer & Münsterer, 2003; Virant & Majer, 2003; Marić & Srečec, 2006; Srečec et al., 2009). On the basis of previous considerations the main goal of this study is to consider the morphological and structural changes or damage of hop glandular trichomes during the different phases of hop processing in order to find the causes of α-acids losses.

1. Materials and methods

1.1. Hop cultivar

Hop (*Humulus lupulus* L. cv. Aurora) grown in the hop garden of Hop Co-operative Gregurovec near Križevci College of Agriculture was used as experimental material. Cultivar Aurora is dual purpose hop cultivar, i.e. hop cultivar with balanced bitter and aromatic substances (Narziss, 1992; Forster & Schmidt, 1994; Srečec et al., 2001; 2004a; 2008).

1.2. Hop processing and sampling

Processing of hop cones into hop pellets was carried out according to integrated system of hop pelletization, i.e. without temporary storage of hop cones pressed into hop bales (Srečec et al., 2004b; Marić & Srečec, 2006; Srečec et al., 2009). Sampling of hop cones and hop pellets was carried out in each phase of quality chain, i.e. hop cones were sampled from the same lot after picking, drying and pelletization before packing into 3-ply Al-folium bags under inert N_2 atmosphere (Srečec et al., 2009).

1.3. ESEM studies

Environmental Scanning Electron Microscope (ESEM), working in gaseous atmosphere, represents a powerful research tool. Fresh hop cones immediately after picking could be scanned, without complex preparation (Muscariello et al., 2005). The ESEM studies of changes in morphology and structure of hop glandular trichomes during the different phases of hop processing were made with Philips XL 30 ESEM (Detector: Edax, type PV 9760/68 ME, resolution 134.30 eV. BSE detector: Philips PW 6848/00) and using the software EDAX Genesis v.5.21. Photographs were taken at an accelerating voltage of 25.0 kV under recording time of 5 s. Diameter of observed area was 10 mm. A total of 136 ESEM observations were provided through all phases of integrated system of hop pelletization, i.e. of fresh hop cones before and after picking, dried hop cones and hop pellets as well.

1.4. Calculation of glandular trichomes volume

Volume of peltate glandular trichomes was calculated by the following equation for volume calculation of spheroid bodies:

$$V = \frac{4}{3} \pi \cdot a^2 b \quad (1)$$

a: width (distance between two points on x-axis in μm);
b: height (distance between two points on y-axis in μm).

Differences between the volume of hop glandular trichomes of fresh and dried hop cones were tested by t-test for independent samples and computing the Least Significant Differences (Vasilj, 2000).

1.5. Calculation of frequency of damaged hop glandular trichomes

Calculations of frequency of damaged hop glandular trichomes were done using the following equation (Vasilj, 2000):

$$f_i = \frac{n_i}{\sum_i n_i} = \frac{n_i}{N} \quad (2)$$

where n_i: number of damaged hop glandular trichomes and $\sum_i n_i$: sum of normal and damaged glandular trichomes, i.e. total of observed glandular trichomes (N).

2. Results and discussion

In this research any damages to hop glandular trichomes were traced. Most of hop glandular trichomes are placed on the basal side of adaxial side of bracts (Fig. 1). However, the changes in volume and structure of hop glandular trichomes were obtained immediately after drying, which is the first phase of hop cones processing. Volume of hop glandular trichomes of dried hop cones is significantly smaller in comparison with volume of glandular trichomes of fresh hop cones (Table 1) and a phenomenon of shrinkage on tunica surface of peltate glandular trichomes of dried hop cones is also visible in comparison with the fresh ones (Figs 2 and 3). Moreover, frequency of damaged glandular trichomes increases following the phases of processing (Fig. 4).

It is obvious in Fig. 4 that in fresh hop cones no damage of glandular trichomes was found. On the other hand, frequency of damaged hop glandular trichomes after drying was 0.08. The most usual damages of hop glandular trichomes are obtained on tunica of peltate glandular trichomes as well as cracking of bulbous glandular trichomes (Figs 5 and 6).

However, in hop pellets the hop glandular trichomes are completely destroyed (Fig. 7).

It is important to point out that the temperature during the drying of hop cones did not exceed the maximal value of 63 °C with the air flow of 0.25 m s^{-1}. However, desirable interval of hop cones drying temperatures is between 62–65 °C with the air flow of 0.25–0.30 m s^{-1} (Rossbauer & Münsterer, 2003), so that is the reason of relatively low frequency of damage of glandular trichomes after drying of hop cones (f_i=0.08). It is well known that only 10 °C increase of the drying temperature doubles the dynamics of oxidation process of α-acids

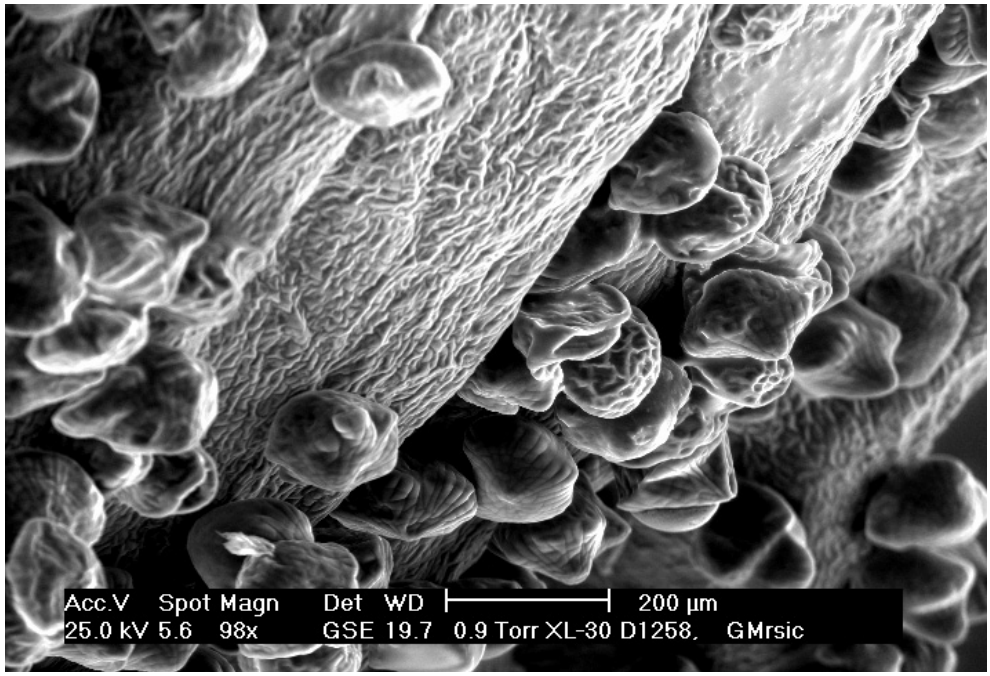

Fig. 1. Normal hop glandular trichomes in different stages of development on the base of adaxial side of hop cone bract

Table 1. Differences between the volumes of hop glandular trichomes of fresh and dried hop cones

Descriptive statistics	Volume of fresh hop cones glandular trichomes ($\times 10^{-2}$ mm^3)	Volume of dried hop cones glandular trichomes ($\times 10^{-2}$ mm^3)
Mean	1.8956	1.3516
St. Dev.	0.504	0.401
St. Error	0.126	0.092
Comparison	Volume of fresh vs. dried	–
Diff. of means	0.544**	–
St. Dev. Diff.	0.10	–
$LSD_{(P=0.05)}$	0.204	–
$LSD_{(P=0.01)}$	0.275	

**: 99% of significance

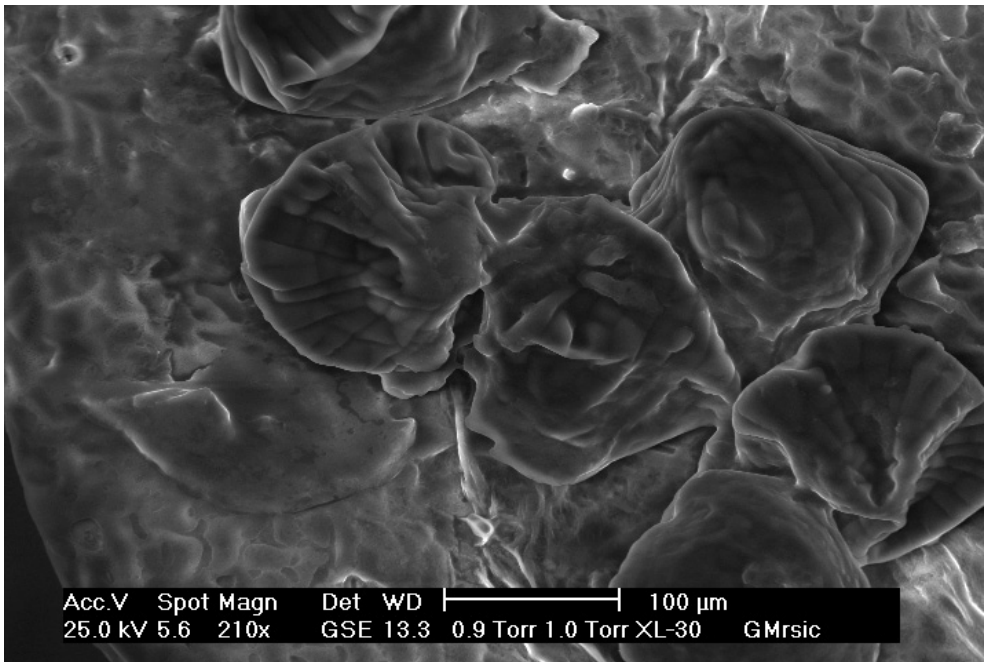

Fig. 2. Shrinkage of hop peltate glandular trichomes surface after drying of hop cones

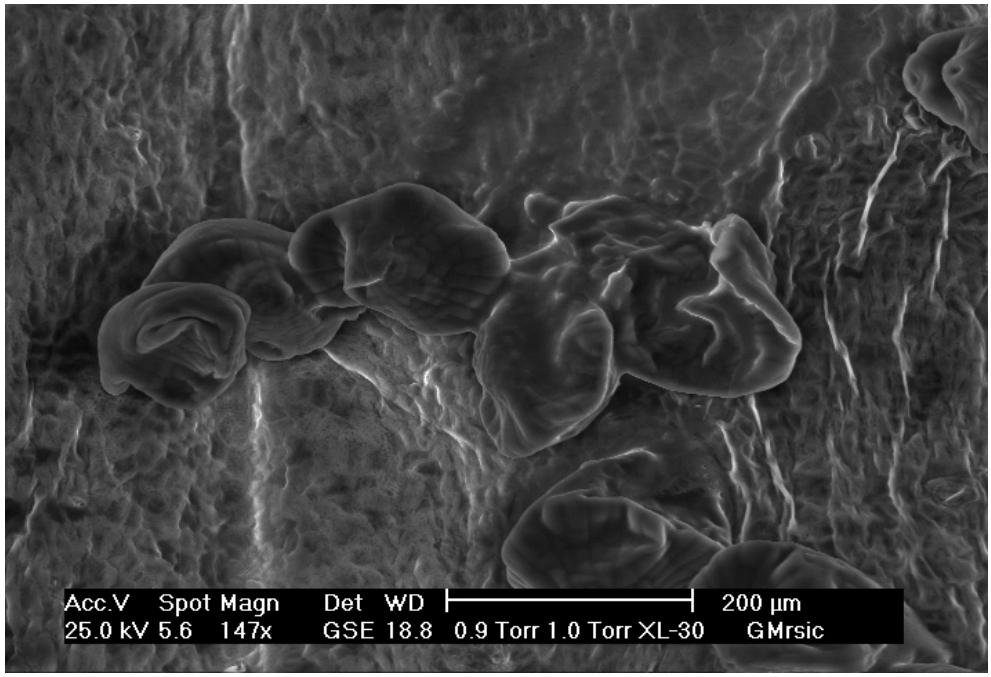

Fig. 3. Shrinkage of the top of hop peltate glandular trichomes surface after drying of hop cones

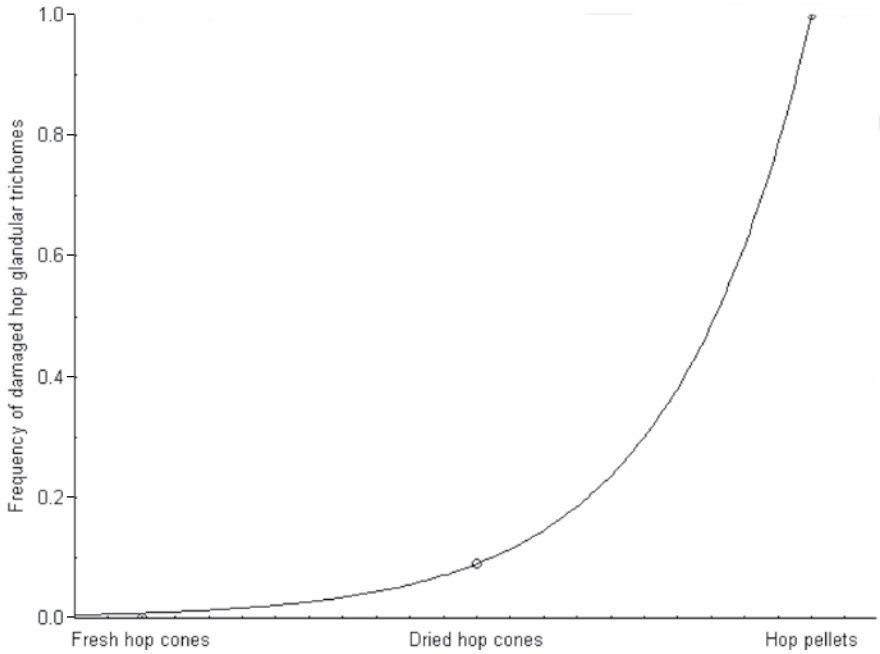

Fig. 4. Frequency of damaged hop glandular trichomes during the different phases of hop processin

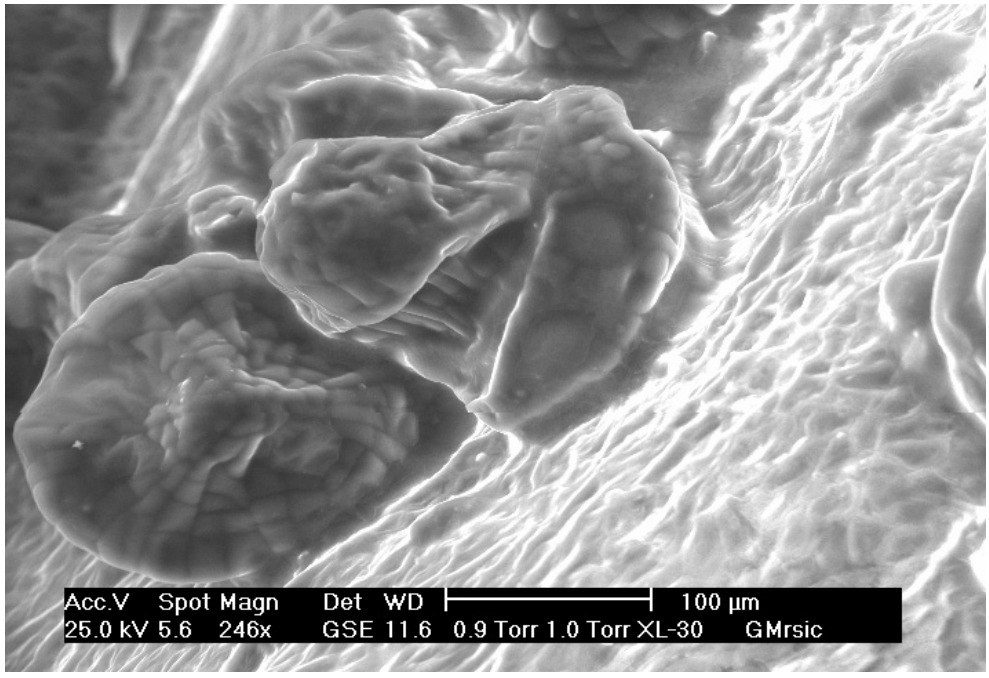

Fig. 5. Damage of hop peltate glandular trichome tunica after drying of hop cones

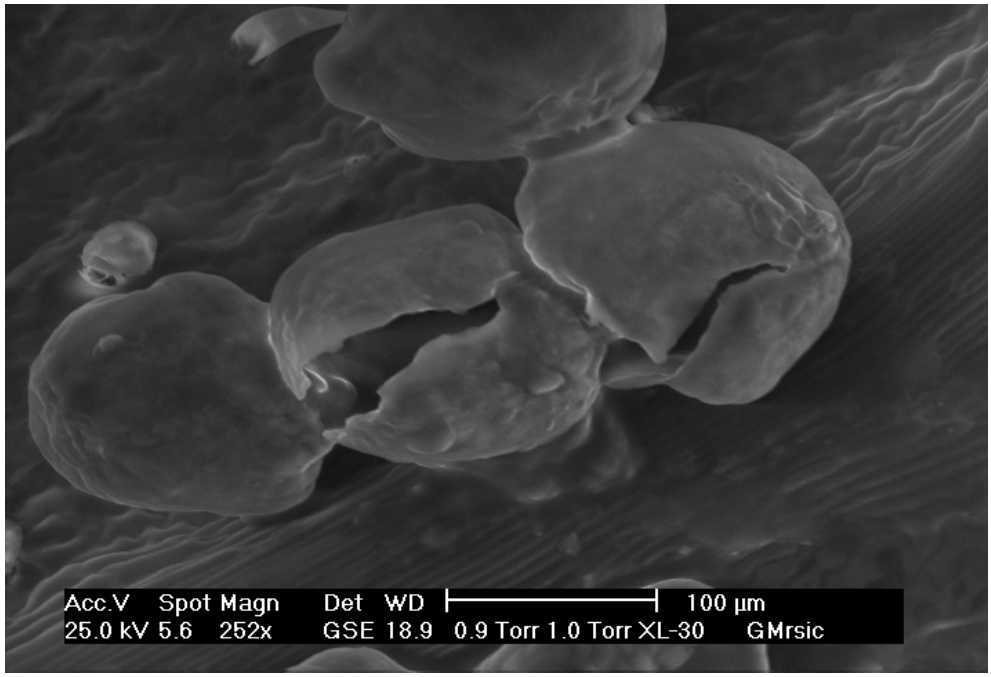

Fig. 6. Cracked hop bulbous glandular trichomes after drying of hop cones

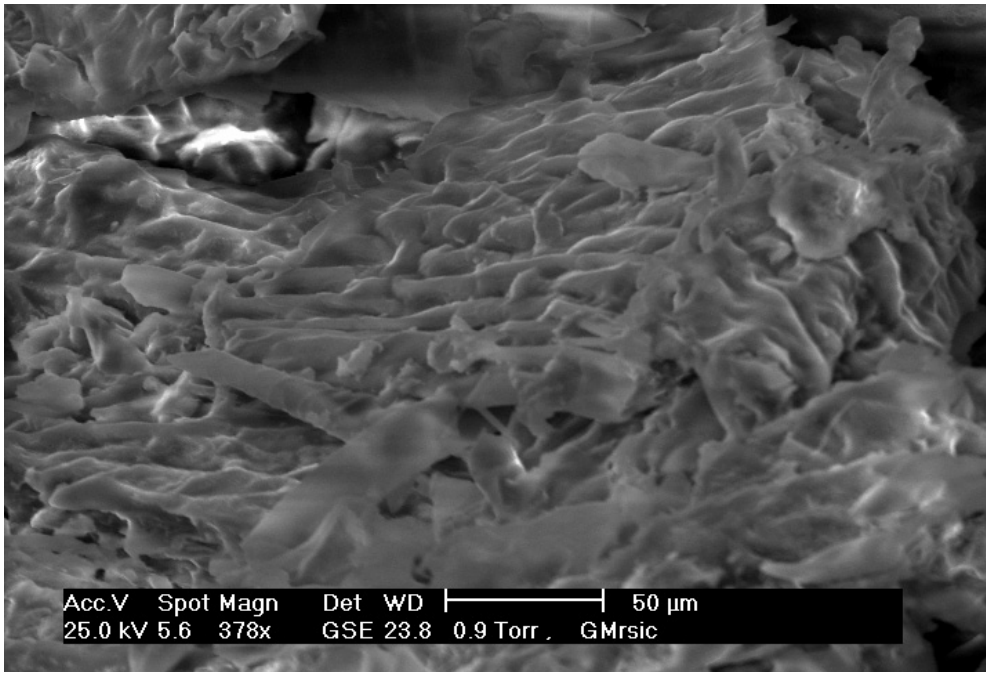

Fig. 7. Completely destroyed structure of lupulin glands in hop pellets

(ROSSBAUER & MÜNSTERER, 2003) and according to these results of ESEM studies the reason is definitely in cracking of glandular trichomes tunica. The obtained shrinkage of peltate glandular trichomes is surely a consequence of dehydration of hop cones and consequently hop glandular trichomes during the drying process. The damages of hop glandular trichomes are also the consequence of handling after the drying process and the damages of hop glandular trichomes increase during the pressing of dried and kilned hop cones into hop bales, particularly if the weight of hop cones in hop bales is higher than 150 kg m^{-3} which can also increase the oxidation processes of bitter and aromatic hop substances if the hop bales are stored under inappropriate storage conditions (FORSTER, 2001a). In this case the integrated procedure of drying and processing of hop cones into hop pellets (MARIĆ & SREČEC, 2006) was used, so the temporary storage of dried and kilned hop cones pressed in hop bales is completely abandoned and consequently the losses of hop bitter compounds, primarily α-acids before pelletization are minimalized (SREČEC et al., 2009). However, complete destroying of hop glandular trichomes structure before pelletization, during the milling of dried hop cones (FORSTER, 2001a; MARIĆ & SREČEC, 2006; SREČEC et al., 2009) speeds up oxidation of α-acids and increases HSI value of hop pellets, particularly in case of higher storage temperatures (FORSTER, 2002; VIRANT & MAJER, 2003; SREČEC et al., 2009).

3. Conclusions

The results of ESEM studies of glandular trichomes morphological and structural changes during the different phases of hop processing confirms that higher temperatures during processing of hop cones into hop pellets and during the storage and transportation of hop pellets are not the only cause of faster hop bitter substances degradation. The primary cause is mechanical damages of hop glandular trichomes during the different stages of hop harvest, drying and processing into a hop pellets. However, because of some technical limits in the construction of processing plants, sometimes it is impossible not to expose the hop cones to high temperatures, but in a short time of exposure the losses of α-acids and the increase in HSI values are not significant (SREČEC et al., 2009).

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