COMPARATIVE STUDIES ON INVERTASE ACTIVITY IN STRAINS OF GENUS SACCHAROMYCES YEAST IMMOBILIZED SODIUM ALGINATE

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Abstract

The comparative analysis of yeast strains, immobilized in sodium alginate and not immobilized (cellular density 2,74 x 10^6) shows the same pattern for all three strains . In the first 20 minutes, the invertase activity of the immobilized cells represents roughly 50% of all not immobilized cell activity, the differences are significant. For the determination after 30 minutes, the enzyme activity difference stays insignificant for Cross Evolution (-0,02850 Nm fructose), minus distinctly significant for Lallvin QA 23 (-0,2675 Nm reducing sugar) and minus very significant for Ancor (-0,3919 mM reducing sugar).

Key words: *invertase activity, yeast, , immobilized ,sodium alginate,*

INTRODUCTION

The immobilization of yeast cells requires a process of physical isolation or the localization of intact cells in a certain region of space, in the conditions of maintaining of the wanted catalytic activities (Karel et. al., 1985). Such processes take place naturally for example when the cells grow on a surface or in natural structures.

The immobilization of cells which contain specific enzymes has the advantage that it eliminates the long and expensive process of separating enzyme substrates and then purifying (Balci et al., 2002). Yeast cells are utilized on a large scale in industry because of their good invertase activity. Invertase is an enzymes which serves as a catalyst for the hydrolysis reaction of saccharide in glucose and fructose. There are several methodologies in order to increase the release of enzymes in the culture medium through the use of enzymatic inductors, like raffinose (Parascandola et. al., 1993), the application of high intensity ultrasounds in order to break the cell walls (Chisty, 2003).

The growth in interest for techniques used to immobilize cells in order to obtain fermented products is due to the numerous advantages that immobilization offers compared to traditional fermentation in which free cells are used (Korkoutas, et. al. 2004). The main advantages in the production of alcoholic beverages are (Bekatorou et al., 2010) the ones that

have to do with an increase in the production in the equipment used for fermentation (Johansen and Flink, 1986; Nigam, 2000; Rossi and Rocha, 2003; Leila et al., 2006), the elimination of unproductive phases, a high tolerance for high levels of substrate and the reduction of the risk of contamination with bacteria because of the high density of cells.

In this study we have investigated and compared the invertase activity of three different strains of commercial yeast, used in the wine making process belonging to the genus Saccharomyces immobilized in sodium alginate.

MATERIALS AND METHOD

Microorganisms

Three different strains of commercial yeast marketed under the names Lallvin QA 23, Anchor and Cross Evolution were used in the experiment. **Lallvin QA 23** is a selection of *Saccharomyces bayanus* used in the fermentation of must obtained from white grapes. The fermentation process is initialized at 13° C, the optimal temperature for fermentatoin being between 14° C and 18°C. Yeast has minimal nutritional demands and does not generate unwanted fermentation products such as SO_2, H_2S, acetaldehyde, pyruvate, ketoglutaric acid, volatile acids etc. It produces alcohol up to 14° and for every kilogram of sugar it releases 130 Kcal. **Anchor** contains hybrid yeasts between *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. It is used in the fermentation of musts which have a high sugar content. They produce a rapid fermentation with an optimal temperature situated between 14° and 16° C. They have medium requirements in comparison to nitrogen and produce small quantities of foam. They are osmotolerant yeasts (25^0 Brix ,13,9 Baume') and the alcoohol tolerance is 15 – 15.5 % alcohols in medium. **Cross Evolution** is the brand name given to a strand of yeast of *Saccharomyces cerevisiae* used to obtain white wines, roze wines and wines with certain aromas. The optimal temperature for fermentation is located between 15^0 C - 18^0 C. It has a high tolerance for alcohol (15% alcohol volume), reduced nutritional requirements in condition of a high fermentation production.

Experimental procedure

The yeasts were cultivated in a solid Sabouraud medium. The cellular suspension was attained through the stacking of cells on the medium and washing them three times in an isotonic chlorosodic solution. Out of every strain there were two suspensions in sterile water at a density of 8.9 x 10^6 and 164 x 10^6 using the densitometer DEN-1.

The immobilization was done using a polymer matrix with the help of sodium alginate. For this purpose, 300 ml of distilled water were heated

to 60^0 C after which 2,5 g of sodium alginate was added (ApliChem Gmbh Germany) constantly stirring until the alginate was completely dissolved. The solution was left alone for 140 minutes after which 1.5 ml of the sodium alginate solution was mixed with 1.5 ml of yeast suspension. The solutions were homogenized, the mix was put through a 5 ml syringe and dripped onto $CaCl_2$ 0.1M solution. After 15 minutes, the spheres were washed four times using sterile distilled water in order to eliminate the excess Ca, they were put through a marked cylinder and brought to a volume of 30 ml with a 20% saccharose solution, through a cellular density of 2.7 x 10^6 respectively 5.48 x 10^6 cells/ml was obtained.

Enyzme assay

Invertase hydrolyses the non-reducing β-d-fructofuranoside residues of sucrose to yield invert sugar. The invert sugar released is then reacted with 3.5 dinitrosalicylic acid (DNS). The colour change (from yellow to red) produced is proportional to the amount of invert sugar released, which in turn is proportional to the invertase activity present in the sample. The absorbance is measured at 540 nm and converted into micromoles of reducing sugar produced using a calibration curve (Fig.1). Briefly, the assay consist in: at different time (presented in the experimental plan) 3ml of samples were added to 1 ml DNS reagent. The content was mixed very well using a vortex, and then the samples was immerse on the wather bath at 95^0C, for exactilly 5 minutes, and then promptly cool. The absorbance was measured at 540 nm (Schimadzu UV-Vis spectrophotometer). One unit of invertase activity represents the number of inverted sugar micromoles released by hydrolytic action of one ml invertase, during one minute.

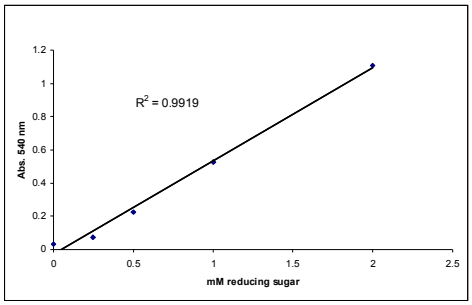

Fig.1 Calibration curve of a mixture of echimolecular glucose and fructose (reducing sugar)

RESULTS AND DISCUSSIONS

Preliminary testing shows that there are significant differences as far as the production of the invertase activity is concerned and between the

immobilized invertase and the one which has not been immobilized. One must however notice the fact that (Fig. 2) for the immobilized invertase, the hydrolysis process of saccharose starts after 10 minutes and for the immobilzed invertase it starts after 20 minutes.

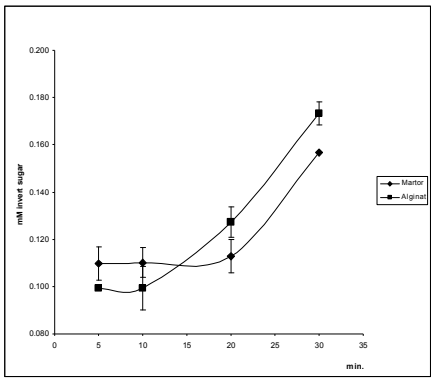

Fig.2. Invertase activity of immobilized invertase (alginat) and unimmobilized invertase (martor)

The comparative study of invertase activity for the three strands of yeast in the variant which has not been capsulated distinguishes a series of differences (Fig. 3, A). Between the Lallvin QA 23 strain and the Cross Evolution strain, the differences which have been recorded on all four time intervals are significant, the activity of the Lallvin QA 23 strain was between 1.34 and 2.4 times more intense than that of the Cross Evolution strain. Between the enzyme activity of the Cross Ecvolution and Anchor strains there were no significant differences for the determinations in the intervals between 5 and 10 minutes and distinctly significant for the following two determinations. The activity of the Cross Evolution strain was 20% and 33% more intense. Between the activity of the Lallvin QA 23 and Anchor strains there were significant differences for all determination differences.

The study of enzyme activity for cellular immobilization (Fig. 3, B) shows significant differences between the Lallvin QA 23 and Cross Evolution strains. For determinations between 10, 20 and 30 minutes the activity of the Lallvin QA 23 strain was between 31% and 58% more intense than that of the Cross Evolution strain. The Anchor strain surpasses the intensity of enzymatic activity of the Cross Evolution strain in the first 20 minutes. After this interval however, for the determination made after 30 minutes its enzymatic activity remains behind the one determined for the Cross Evolution strain, the values registered were significant (1.1632 mM reducing sugar as opposed to 0.9295 Mm reducing sugar).

The comparative analysis of yeast strains, immobilized in sodium alginate and not immobilized (cellular density 2.74 x 10^6) shows the same pattern for all three strains (Fig. 4, A,B,C). In the first 20 minutes, the invertase activity of the immobilized cells represents roughly 50% of all not immobilized cell activity, the differences are significant. For the determination after 30 minutes, the enzyme activity difference stays insignificant for Cross Evolution (-0.02850 mM reducing sugar), minus distinctly significant for Lallvin QA 23 (-0.2675 mM reducing sugar) and minus very significant for Ancor (-0.3919 mM reducing sugar).

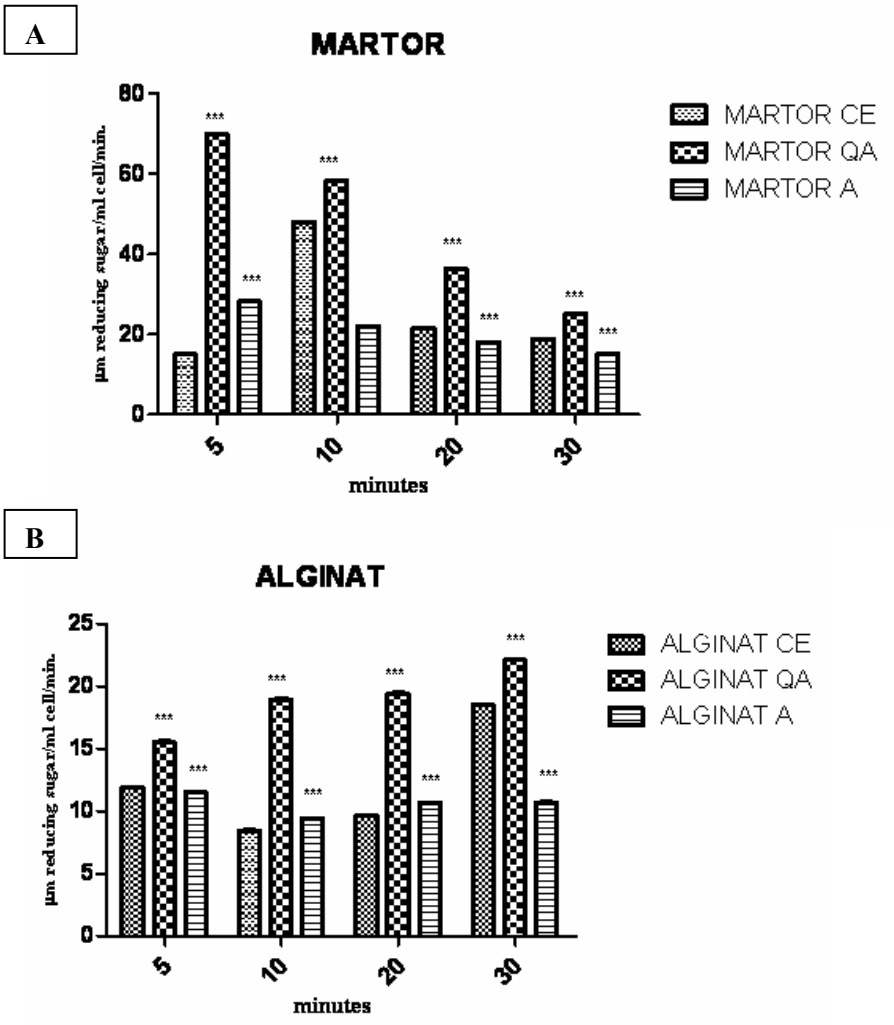

Fig.3. Invertase activity (µM inverted sugar/1ml yeast cell/min) at the first concentration (2.74 x10^6 celule/ml) from yeast uncapsulated (MARTOR) (A) and capsulated

(ALGINAT) (B). The data were expresed as means ± standard deviation (n=2) and evaluated by two-way ANOVA, Bonferroni post-test. Differences were considered to be statistically significant if $p < 0.001$ (***), martor CE vs samples and alginat CE vs samples.

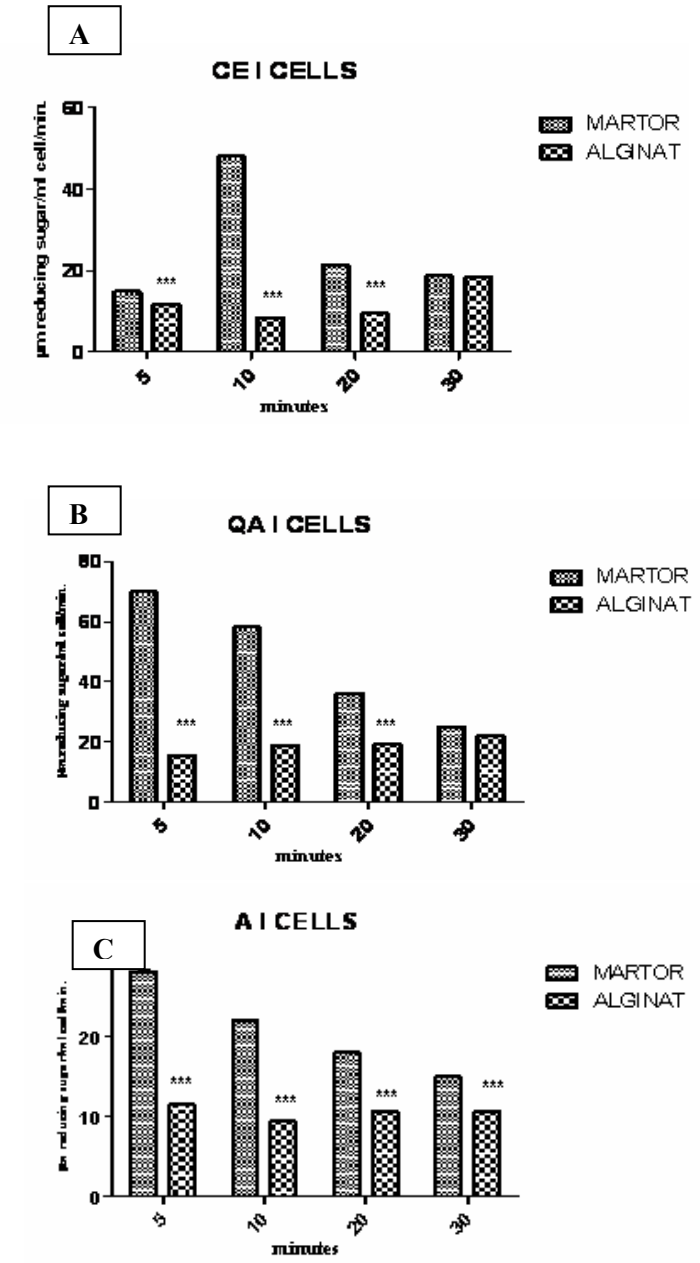

Fig.4. Invertase activity (μM inverted sugar/1ml yeast cell/min) at the first concentration (2.74 x10^6 celule/ml) of three different yeast strains (**A**:CE, **B**: QA and **C**: A). The data were expresed as means ± standard deviation (n=2) and evaluated by two-way ANOVA, Bonferroni post-test. Differences were considered to be statistically significant if $p < 0.001$ (***), martor vs samples.

The somewhat reduced production for all three strains within the first 20 minutes can be explained through the partial inactivity an invertase due to the reticular conditions of the cells within the alginate.

The results obtained through the constant maintaining of the substrate concentration (saccharose 20%) but doubling the number of cells (5.48 x 10^6 cells/ml) in the variant which was not immobilized and immobilized are presented in Fig. 5, A. As was the case with the 2.74 x 10^6 cells/ml concentration, uncapsulated, there was a more intense activity for the Lallvin QA 23 yeast at 5 and 10 minutes with positive differences which were very significant as opposed to the Cross Evolution and Anchor strains. For the determination made at 30 minutes the difference between Lallvin QA 23 and Cross Evolution was not significant which proves that the Cross Evolution yeast requires a longer period in order to get adjusted to its medium. Also, in the case of the immobilized cells in alginate there were large differences between the enzyme activity of the yeasts, differences which even though they became reduced within the last determination still remained significant between the Lallvin QA 23 and Cross Evolution strains (0.2575 mM reducing sugar) and also between Cross Evolution and Anchor (0.4080 mM reducing sugar) (Fig.6, A,B,C).

The production of invertase activity of the cells which were not immoblized, compared to the immoblized ones (Fig. 5, B) is larger, the differences being between 0.3615 mM reducing sugar between the capsulated ones and the ones which were not capsulated for Cross Evolution, 0.152 mM reducing sugar and Lallvin QA 23 and 0.6455 mM reducing sugar for Anchor.

CONCLUSIONS

Between the yeasts used in the study, the best enzyme activity was recorded for the Lallvin QA 23 strain (*Saccharomyces bayanus*) and the weakest one was recorded for the Anchor strain (*Saccharomyces cerevisiae* x *Saccharomyces bayanus*).

The invertase activity of cells which were not capsulated is initially superior to that of capsulated cells but with an obvious tendency of homogenization towards the end of the observation interval.

The increase in cellular density in the condition of constantly maintaining the concentration of the substrate determines an intensification

of enzyme activity for cells which are not capsulated as well as for the ones that were with differences for the strains of yeast used. From this point of view, after 30 minutes, the Anchor strain was the one that reacted the best towards the increase in the concentration of the substrate. It is known as being osmotolerant, for both the capsulated (0.9295 mM reducing sugar at 2.74 x 10^6 cells/ml and 1.463 mM reducing sugar at 5.48 x 10^6 cells/ml) as well as the non capsulated (1.321 mM reducing sugar for 2.74 x 10^6 cells/ml and 2.108 mM reducing sugar for 5.48 X 10^6 cells/ml) versions.

A good reaction for the increase of the concentration of the substrate is also valid for the Cross Evolution strain (*Saccharomyces cerevisiae*).

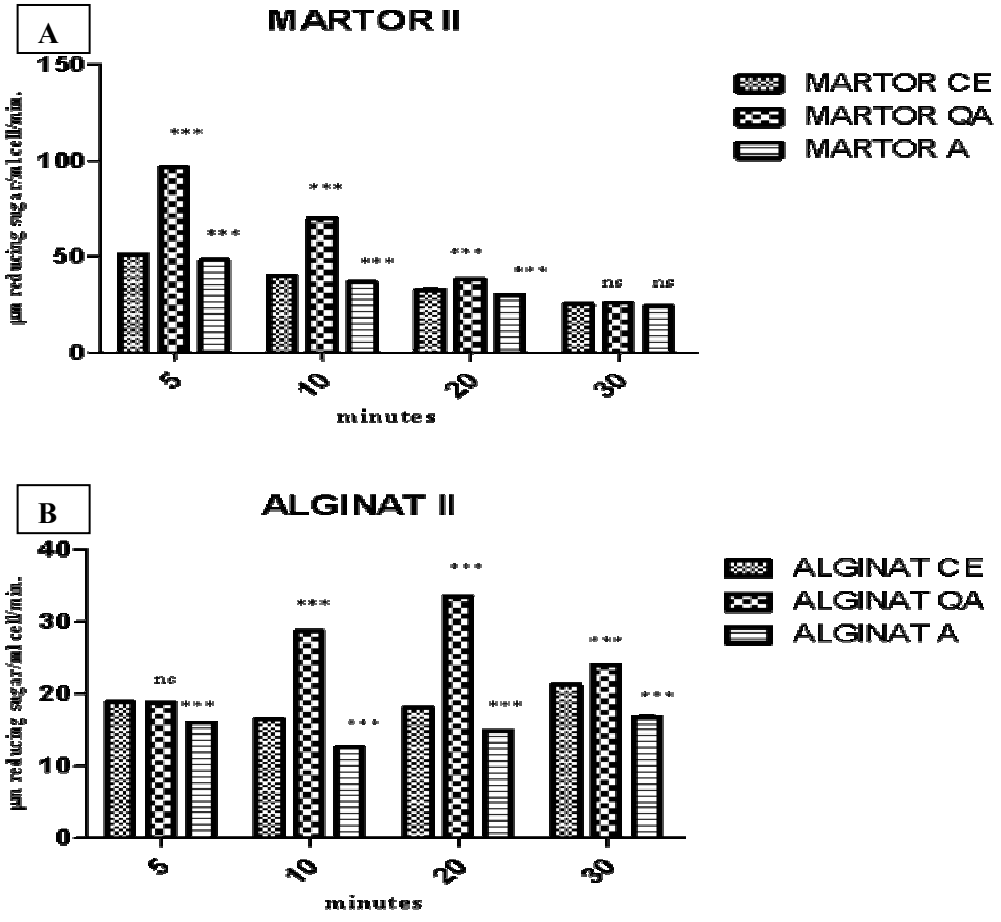

Fig.5. Invertase activity (µM inverted sugar/1ml yeast cell/min) at the first concentration (5.48 x10^6 celule/ml) from yeast uncapsulated (MARTOR) (A) and capsulated (ALGINAT) (B). (at the first concentration of yeast cells).The data were expresed as means ± standard deviation (n=2) and evaluated by two-way ANOVA, Bonferroni post-test. Differences were considered to be statistically significant if $p < 0.001$ (***), martor vs samples. ^{ns} – no significant differences

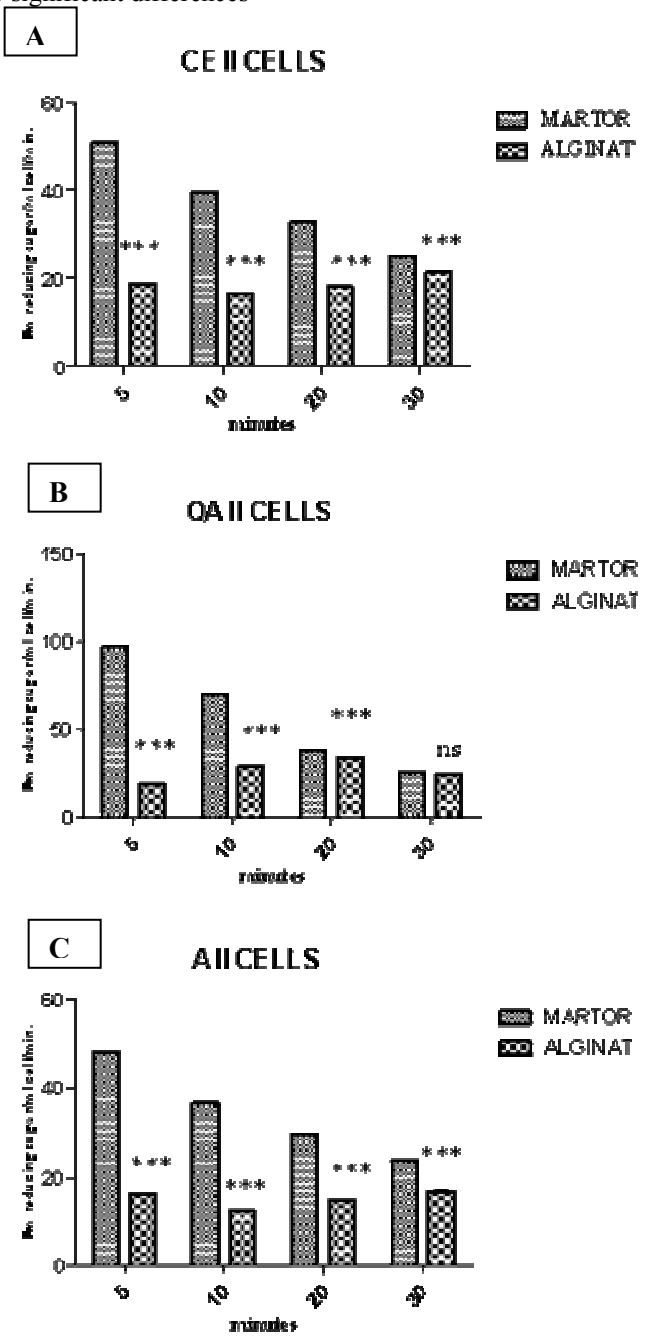

Fig.6.Invertase activity (μM inverted sugar/1ml yeast cell/min) at the first concentration (5.48 x 10^6 celule / ml) of three different yeast strains (**A**:CE, **B**: QA and **C**: A). The data were expresed as means ± standard deviation (n=2) and evaluated by two-way ANOVA, Bonferroni post-test. Differences were considered to be statistically significant if $p < 0.001$ (***), martor vs samples. . ^{ns} – no significant differences

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