

## **RESEARCHES CONCERNING THE CHOLESTEROL ROLE IN ALCOHOLIC FERMENTATION DYNAMIC**

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### **Abstract**

*Present research evaluates the cholesterol role upon yeast fermentative metabolism concerning two different technological processes: bread dough fermentation and grape must fermentation. The obtained results revealed that exogenous added cholesterol generate a higher yield in alcohol, a supplementary sugar transformation and a higher CO<sub>2</sub> volume. Exogenous added cholesterol produces a supplementary fermentation due to prolonged yeast cell viability.*

**Key words:** *cholesterol, yeast membranes, fermentation.*

### **Introduction**

Cholesterol, ergosterol and lanosterol are considered to be survival factors for yeasts. Sterols control cell - environment interactions being important constituents of cell membranes (Demel R., De Kruiff B., 1976). For each type of phospholipids from cytoplasmic and mitochondrial membrane structure there is a certain value of phase transition temperature; above this value, phospholipidic chains are in a rigid, pseudo-crystalline state, and beneath this phase transition temperature the phospholipidic chains are in a fluid state. Membranary phospholipids associated sterols influence the membrane to be more fluid ore more rigid, depending on the temperature, controlling the membrane state and permeability (C. Băducă, 2003).

Sterols addition to fermentation medium contributes to sterols enriching of the cell structure (Larue F., Lafon - Lafourcade, Ribereau – Gayon, 1978). A certain sterols contain is indispensable for yeast survival: beneath 0.5% sterols/yeast dry matter the multiplication is stopped, and beneath 0.2% sterols/yeast dry matter there is no fermentative activity in grape must (C. Băducă, 2003).

Sterols uptake from grape must be quantitatively significant, up to 300 mg/l – for cholesterol, but the main part is metabolized during the

yeast multiplication phase (Lafon - Lafourcade, Ribereau – Gayon, 1979).

## **Experimental**

In order to study the must alcoholic fermentation it was used an artificial must based on YPG medium for yeast; because grape must was unavailable at that time (December). The artificial must contains: glucose 150 g, peptone 10 g, yeast extract 10g, distilled water up to 1000ml. The must was autoclaved at 121°C for 20minutes and pH adjusted to 5.6.

The must was distributed in 4 fermentation containers (0.33l each) – one of them, considered blind sample (without cholesterol) and to the other three increasing cholesterol doses were added: sample 1 – 49.5mg (150 mg/g), sample 2 – 82.5mg (250 mg/g) and sample 3 – 115.5mg (350 mg/g); doses related to 1g fresh yeast (30% dry matter). Each recipient was inoculated with 1g commercial bakers yeast (RomPak S.A.) as fermentation agent. Samples were incubated at 22°C for 7 days. Then, were determined sugar and alcohol for each sample; Schoorl method was used in order to establish samples sugars contain and ebulliometric method for the resulted ethanol.

In order to study the exogenous cholesterol influence upon yeast evolution in bread dough, 5g yeast biomass were suspended in 100ml sterile water, added increasing cholesterol doses, obtaining 4 samples: a blind sample (without cholesterol), sample I with 0.75g (150 mg/g), sample II with 1.25g (250 mg/g) and sample III with 1.75g cholesterol (350 mg/g); doses related to 1g fresh yeast (30 % dry matter).

After a 24 hours period for cholesterol uptake, yeast fermentation ability in bread dough were evaluated using 985/79 STAS method (Bordei Despina, Burluc R., 2003). The dough was prepared with 100ml suspended yeast samples, 280g flour 650 type, and 60 ml 6.66% NaCl (to obtain 160 ml 2.5% NaCl as the method requires). Ingredients were incubated at 35°C; the obtained dough for each yeast sample was introduced in a tin form (also warmed) and incubated at 35°C to develop fermentation. It has measured the time between the beginning of mixing (the flour and the yeast) and the moment when fermented dough is 70mm high. This time value is correlated with yeast fermentation capacity.

## **Results and discussions**

Yeast fermentation ability in dough revealed the following results, grouped in Table 1. From this values could be observed that a 250 mg/g cholesterol dose applied to suspended yeast reduces dough's

rising time with 15 minute, which means 20%. Interesting is the fact that a 350 mg/g cholesterol dose reduces dough's rising time with only 5 minute, same as a 150 mg/g cholesterol dose. The best cholesterol dose for improving dough rising dynamic (more CO<sub>2</sub> generated in shorter time interval) is 250 mg/g.

**Table 1.** Yeast fermentation ability in dough

Yeast sample	Dough's rising time (min.)
Blind sample (no cholesterol)	75
Sample I (mg/g cholesterol)	70
Sample II (mg/g cholesterol)	60
Sample III (mg/g cholesterol)	70

Results concerning alcoholic fermentation of a simulated must with exogenous added cholesterol are showed in tables 2 and 3.

**Table 2.** Ethylic alcohol content for fermentated must samples

Must samples	Ethylic alcohol content (% volume)
Blind sample (no cholesterol)	6.3
Sample 1 (150 mg/g cholesterol)	6.3
Sample 2 (250 mg/g cholesterol)	7.8
Sample 3 (350 mg/g cholesterol)	6.3

**Table 3.** Glucose content for fermentated must samples

Must samples	Glucose content by Schoorl method (mg/l)
Blind sample (no cholesterol)	630
Sample 1 (150 mg/g cholesterol)	1260
Sample 2 (250 mg/g cholesterol)	630
Sample 3 (350 mg/g cholesterol)	940

The 250 mg/g cholesterol dose induces an alcohol overproduction of 1.5% volume compare to the blind sample. At this cholesterol dose, sugar catabolism is intensified and only 0.63 g/l glucose left unfermented. The alcohol content for sample 3 (with 350 mg/g cholesterol) wasn't superior to blind sample, but the glucose quantity left is higher (0.94 g/l). Results that sample 3 has a superior alcohol output than the blind sample, obtaining the same alcoholic grade using

a decreased glucose quantity (0.31 g/l les). Concerning sample 1, it didn't record a superior alcohol production compared to the blind sample, and a significant unfermented glucose quantity was found (1.26 g/l). Even if in this case there is the same alcohol output, it is more efficiently obtained consuming less glucose (0.32 g/l les than sample 3 and 0.63 g/l les than blind sample). It might even say that for sample 1, the alcohol output for the same glucose amount needed is higher than third sample's.

Glucose amounts left unfermented in samples 1 and 3 raise question marks concerning the sugar metabolism blocking at lower (sample 1 – 150mg/g) or higher (sample 3 – 350mg/g) cholesterol doses.

### **Conclusions**

Adding exogenous cholesterol could be improved the alcoholic fermentation dynamic both in dough and must, inducing a higher alcohol production and increasing the CO<sub>2</sub> volume, on the base of intensive sugar metabolism. This supplementary fermentation is explained by the cholesterol role in prolonging yeast viability in phase of late fermentation. Although, when exogenous cholesterol doses are to low or to high sugar metabolism is inhibited due to difficulties in yeast membranes status.

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