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**Exploratory study on the occurrence and dynamics of yeast-mediated nicotinamide riboside
production in craft beers**

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27 **Abstract**

28

29 Several health benefits are related to the administration of nicotinamide riboside (NR), a form of
30 Vitamin B3, and its precursors nicotinamide mononucleotide (NMN) and NAD⁺. Therefore,
31 considerable interest is currently devoted to the potential therapeutic value of their supplementation,
32 thus justifying scientific studies on the distribution of these molecules in foods and beverages. In this
33 study, the three vitamers were quantitatively analyzed in ten craft beers for the first time. All beers
34 from different commercial *S. cerevisiae* strains contained NAD⁺. NR, NMN and NAD⁺ were mostly
35 present in beers produced with *Saccharomyces cerevisiae* strain US-05. Interestingly, the three
36 vitamers were not detectable in beers produced with a commercial strain of *Saccharomyces*
37 *pastorianus*. Data from laboratory-scale beer production using *S. cerevisiae* strain US-05 showed that
38 the addition of hops during the fermentation process significantly increased NR production. The rapid
39 increase in NR formation only occurred if both hops and yeast were present, and the burst was also
40 confirmed in fermentations trials performed with *S. cerevisiae* strain CBS1171^T and by replacing
41 wort with YPD medium. The experimental model proposed in the present study can serve as baseline
42 for further research aimed at investigating the yeast-hop interaction at metabolic and molecular levels.
43 In addition to highlighting the potentialities of microorganisms to act as biological factories for
44 beneficial molecules to humans, these findings open new intriguing perspectives for the development
45 of innovative fermented foods naturally enriched in NR and its precursors.

46

47 **Keywords:** Vitamin B3, NR, NMN, NAD⁺, *Saccharomyces cerevisiae*, hop, beer, yeast and hop
48 synergy

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52

53 1. Introduction

54

55 Vitamin B3, which is also called Niacin or vitamin PP (Pellagra-Preventing), includes nicotinic acid
56 (NA), its amide nicotinamide (Nam), and nicotinamide riboside (NR), the last discovered form
57 (Bieganski & Brenner, 2004). Once taken with the diet, NA, Nam and NR are transported inside
58 the cells where they can be transformed into NAD^+ , which represents the biologically active form of
59 vitamin B3. NAD^+ itself is present in food together with the phosphorylated form of NR, i.e.,
60 nicotinamide mononucleotide (NMN). In the human gut, both dietary NAD^+ and NMN can be
61 transformed into the three forms of the vitamin by a combined action of enzymes of the intestinal
62 mucosa and microbiota (Bogan & Brenner, 2008).

63 Numerous lines of evidence indicate that administration of the vitamin NR and its precursor NMN to
64 mice at doses ranging from 100 to 500 mg/kg/day causes a significant increase in the intracellular
65 content of NAD^+ in many tissues and organs, which is reflected in improvements in energy
66 metabolism and mitochondrial function. As a result, supplementation of NR or NMN shows both
67 preventive and therapeutic properties in neurodegenerative diseases (e.g., Parkinson's and
68 Alzheimer's), metabolic syndrome (Hartnup's disease), human immunodeficiency virus (HIV),
69 autoimmune diseases, alcohol dependence, anorexia and diseases related to aging that seem to
70 reproduce the symptoms of pellagra (Chi & Sauve, 2013; Hong, Mo, Zhang, Huang, Wei, & 2020;
71 Rajman, Chwalek, Sinclair, & 2018; Ruggieri, Orsomando, Sorci, & Raffaelli 2015; Yoshino, Baur,
72 & Imai, 2018). Doses yielding health benefits in mice models are much higher than the amounts of
73 NR and NMN that have been documented to date in a common balanced diet. NMN has been detected
74 in many natural foods, such as broccoli, tomatoes, mushrooms, cabbage, shrimp, avocado and beef
75 meat, with a maximum concentration of 1.88 mg/100 g (Mills et al., 2016), whereas NR has only
76 been documented at micromolar concentrations in milk to date (Trammell, Yu, Redpath, Migaud, &
77 Brenner, 2016; Umharino et al., 2017). Considering the beneficial effects attributed to these
78 molecules, it is important to extensively investigate their distribution in food. Beside the natural

79 presence of NR in milk, it has been hypothesized that microbial metabolic activities could contribute
80 to its occurrence in fermented food and beverages (Chi & Sauve, 2013). Indeed, different authors
81 reported the ability of *Saccharomyces cerevisiae* to actively secrete this vitamin (Bogan et al., 2009;
82 Lu, Kato, & Lin, 2009). Such evidence prompted us to investigate the presence of NR and its dietary
83 precursors, NMN and NAD⁺, in craft beer. Beer is a beverage consumed worldwide that is derived
84 from a biochemical process based on the fermentation of sugary substrates present in the wort beer
85 by the action of yeast (Anderson, Santos, Hildebrand, & Schug, 2019; Nardini & Garaguso, 2020).
86 This process is an alcoholic fermentation that leads to the production of ethanol, carbon dioxide and
87 other secondary compounds, such as polyphenols particularly phenolic acids (benzoic and cinnamic
88 acid derivatives) and flavonoids, important for the characterization of the product (Nardini &
89 Garaguso, 2020). The raw materials necessary for the beer production include water, barley
90 (*Hordeum vulgare*) and other cereals eventually used, hops (*Humulus lupulus*), and yeast. Yeast
91 strains used for the brewing process belong to the genus *Saccharomyces* spp. Traditionally, these
92 yeast strains are classified as yeast for low fermentation, namely, *Saccharomyces pastorianus*
93 (operating temperature 8-15°C), and yeast for high fermentation, namely, *S. cerevisiae* (operating
94 temperature 15-23°C). The use of *S. cerevisiae* cultures (top yeast) produces a high fermentation beer
95 (top fermentation) called Ale, in which the yeasts tend to rise to the surface positioning in the foam.
96 In contrast, *S. pastorianus* (bottom yeast) produces low fermentation (bottom fermentation) in which
97 the yeast at the end of the fermentation process are found on the bottom of the beer based on their
98 ability to flocculate (Lager beer) (Iserentant, 2003; Lodolo, Kock, Axccl, & Brooks, 2008; Speers,
99 Tung, Durance, & Stewart, 1992; Verstrepen, Derdelinckx, Verachtert, & Delvaux, 2003). The
100 brewing process can be divided in four different main phases: malting (transformation of barley into
101 malt), mashing (production of wort), fermentation by yeast (transformation of sugars in ethanol,
102 carbon dioxide and secondary compounds) and downstream processes (maturing, bottling, and
103 packaging) (Anderson et al., 2019). At the end of maturation (generally 3-4 weeks), the beer must be
104 subjected to filtration processes to separate the suspended solids and to pasteurization to produce a

105 more stable final product. In a few cases, there is another step of hop addition to the beer. This step,
106 which is called dry hopping, can be performed before fermentation, at the end of fermentation, or
107 during a second fermentation in the bottle. Craft beers, unlike industrial beers, are usually subjected
108 to a second fermentation process in the bottle, by the addition of sugars and yeast. The beer produced
109 in craft breweries differs from industrial beers also because they are consumed unfiltered and
110 unpasteurized (Garofalo et al., 2015). Moreover, craft breweries produce mainly Ale beers, so they
111 utilize predominantly *S. cerevisiae* strains (Iattici, Catallo, & Solieri, 2020).
112 In this work, levels of NR and its precursors NMN and NAD⁺ have been quantified in different craft
113 beers via an enzyme-coupled assay (Ummarino et al., 2017). In addition, laboratory-scale
114 fermentations have been established using different *S. cerevisiae* strains added to wort or YPD
115 medium to shed light on the mechanism of NR production.

116

117 **2. Material and methods**

118

119 *2.1. Craft beer and wort sampling*

120

121 Ten craft beers of different brewing styles were analyzed for the presence of NR, NMN and NAD⁺.
122 In particular, two samples from different batches of each beer type were collected from two craft
123 breweries located in the Marche region (Central Italy).

124 All the worts used to produce these beers were collected at the end of boiling step and stored at 4°C.
125 Table 1 summarizes the yeast species and strains, ingredients and alcohol percentage (%) of the craft
126 beers under study, and Figure 1 shows a flow diagram of their manufacturing process.

127

128 *2.2. Fermentation trials*

129

130 The yeast strains *S. cerevisiae* US-05 (Fermentis Lessafre Italia, Parma, Italy) and *S. cerevisiae* CBS
131 1171^T (from the *Centraalbureau voor Schimmelcultures*, Filamentous fungi and Yeast Collection,
132 The Netherlands) were grown on Yeast Extract Peptone D-glucose (YPD) (yeast extract 10 g/L,
133 peptone 20 g/L, D-glucose 20 g/L) medium at 25°C for 72 hours. Yeast strains were inoculated in
134 sterile flasks containing 100 mL of wort (or 200 mL YPD) to reach a final concentration of
135 approximately 6 log₁₀ cfu/mL. In a conventional fermentation trial, after 9 days at 21°C, two different
136 hops were added in pellet form (dry hopping). These hops consisted of amarillo (alpha acid: 9.0%)
137 (4 g/L) and centennial (alpha acid: 8.5%) (4 g/L) varieties. After this addition, the maturation
138 continued at 4°C. At the 16th day of fermentation, dextrose (7 g/L) was added; it was dissolved in 600
139 µL of sterile water by heating at 100°C for 5 minutes. The brewing process continued at 4°C until the
140 45th day unless otherwise stated.

141 At different days during the fermentation, aliquots of samples were removed to analyze the following:
142 i) the yeast concentration through viable counting on YPD agar (agar 18 g/L) following decimal serial
143 dilutions on sterile peptone water (peptone 1 g/L); ii) the content of NR, NMN and NAD⁺ as described
144 in paragraph 2.3.

145

146 2.3 NR, NMN and NAD⁺ quantitation

147

148 Samples of beers and aliquots from the fermentation trials were subjected to acid-soluble nucleotides
149 extraction. To this end, 0.5 mL were centrifuged at 16000 x g for 5 minutes at room temperature
150 before adding 250 µL of 1.2 M HClO₄. After 15 minutes at 4°C, samples were centrifuged as
151 described above and 700 µL of the supernatants were added to 170 µL of 1.0 M K₂CO₃ to reach a pH
152 value of approximately 7.0. Neutralized samples were centrifuged again, and the supernatants were
153 used for the quantitation of NR, NMN and NAD⁺ through the enzyme-coupled assay described by
154 Ummarino et al. (2017). Briefly, the coupled assay consists of two consecutive reactions catalyzed
155 by recombinant bacterial NR kinase and recombinant murine NMN adenylyltransferase that

156 stoichiometrically convert NR to NMN and NMN to NAD⁺, respectively. The produced NAD⁺ is
157 then quantified by the fluorometric cycling assay described by Zamporlini et al. (2014).

158

159 2.4. Statistical Analysis

160

161 NR, NMN and NAD⁺ data are represented by boxplot that represent the interquartile range (IQR)
162 between the first and third quartiles, and the line inside the plot represents the median (2nd quartile).
163 Data were subjected to one-way ANOVA to examine the development across time. When significant
164 differences were found, Duncan's multiple range test was used. Linear regression model was used to
165 reveal the associations between NR, NMN and NAD⁺ as a function of time. A P-value of less than
166 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 21.0
167 and R software.

168

169 3. Results and Discussion

170

171 3.1 NAD⁺, NMN, NR determination in craft beers

172

173 Ten different types of craft beers were analyzed for the presence of NR, NMN and NAD⁺ as described
174 in paragraph 2.3. All the beers analyzed were prepared with *S. cerevisiae*, except beer B1, which was
175 produced by *S. pastorianus*. The results of the screening are shown in Figure 2. To the best of the
176 authors knowledge, this is the first report showing the presence of NAD⁺, NMN and NR in beer. In
177 more detail, NAD⁺ was detected in all the studied beers with the exception of B1. Its content showed
178 marked variability, even within different batches of the same beer. Levels ranged from 1.10 nmol/mL
179 to 17.80 nmol/mL and were intriguingly very similar to those determined in ovine and caprine milk
180 (Ummarino et al., 2017). The highest amount of NAD⁺ (P<0.05) was present in the two beers
181 produced using the *S. cerevisiae* strain S-04. In particular the highest value was reached in sample

182 B6 followed by sample B5 while lowest amount and minimal differences were observed among the
183 others (Figure 2). These results suggest that S-04 has the capacity among the tested strains to produce
184 and release the highest amount of NAD⁺ during the brewing process. In addition, the only beer lacking
185 NAD⁺ was the one prepared using *S. pastorianus* (sample B1), thus indicating that NAD⁺ production
186 could be species specific. Interestingly, in this latter beer, NR and NMN were also undetectable
187 (Figure 2). Notably, among the beers prepared with *S. cerevisiae*, all the beers produced with the
188 strain US-05 contained NR. Three of the beers also contained NMN (sample B7, B9 and B10),
189 although at levels generally lower than NR and without differences in the quantity among them
190 (Figure 2, P>0.05). NR concentrations ranged from 0.48 to 3.25 nmol/mL, and these values were
191 unexpectedly very similar to those determined in bovine milk (from 0.5 to 3.6 nmol/mL) (Trammell
192 et al., 2016; Ummarino et al., 2017). In particular, NR was mostly detected in samples B9 and B10.
193 However, no difference in concentration between the two was observed (Figure 2, P>0.05).
194 Furthermore, samples B7 and B8 showed comparable quantities of NR but always significantly lower
195 than B9 and B10 (Figure 2, P<0.05). In contrast to NR, NMN was not always present in both tested
196 batches of beer, and when present, its content was approximately 0.9 nmol/mL. This content closely
197 resembles that measured in ovine and donkey milk, where NMN ranges from 0 to 1.0 nmol/mL
198 (Ummarino et al., 2017). In general, NMN levels in beer and milk are lower than those measured in
199 other foods, such as tomato, avocado and beef meat (from 0.78 nmol/mg to 4.79 nmol/mg) (Mills et
200 al., 2016).

201 All the worts were negative for the presence of NR and its metabolic precursors (data not shown).
202 The data obtained in the present study clearly indicate that the production of the three metabolites is
203 a typical feature of the *S. cerevisiae* species and is strain dependent.

204

205 3.2 NR, NMN and NAD⁺ determination in lab-scale produced beer

206

207 Screening of NR, NMN and NAD⁺ in the different craft beers indicated that only the beers produced
208 with *S. cerevisiae* strain US-05 contained all the three vitamers (Figure 2). Prompted by these results,
209 a replicate of the beer B9 on a laboratory scale (B9L) was established to monitor the production of
210 the molecules during the entire fermentation process. The B9 sample was chosen based on the
211 availability of the corresponding wort by the brewery. The fermentation was performed as described
212 in paragraph 2.2, and the production of the three metabolites as well as the viable yeast counts were
213 monitored during the entire process (Figure 3A).

214 On the 2nd day of fermentation, the concentration of the yeast strain significantly increased from the
215 starting inoculum ($P < 0.05$) reached the value of $7.5 \log_{10}$ cfu/mL and then remained constant until
216 the end of the process ($P < 0.05$). Different fluctuations in the levels of the metabolites were recorded.
217 After an initial lag of approximately 2 days, the amount of NR significantly increased, exhibiting a
218 burst after the addition of the hop, i.e., after the 9th day. In fact, the value of NR shifted from 1.3
219 nmol/mL on the 9th day to 3.4 nmol/mL on the 14th day, representing an approximately 3-fold
220 increase. The linear regression model showed a significant increase in the production of NR across
221 time (Adjusted R-squared: 0.9694, p-value: < 0.05). The addition of sugar after 16th days did not
222 affect the trend of NR. The trend for NAD⁺ was very different from that of NR. In more detail, in the
223 first two days of the process, this metabolite significantly increased to approximately 3 nmol/mL
224 ($P < 0.05$) and remained at this level until the addition of the hops. After the 9th day, it sharply
225 decreased to very low levels (Adjusted R-squared: 0.2701, p-value < 0.05). Regarding NMN, after a
226 sharp increase in the first two days to a value similar to that of NAD⁺, it remained constant until the
227 23rd day and then started to slowly decrease (Adjusted R-squared: 0.003437, p-value: 0.2992).

228 A comparison of the levels of the molecules of interest in the B9 beer (Figure 2) and in the lab-scale
229 beer B9L on the last day of the fermentation (Figure 3A) revealed that the final amount of NR in B9L

230 was considerably increased compared with that in B9 beer, whereas NAD⁺ was lower. On the other
231 hand, NMN levels were very similar.

232 The differences in NR and NAD⁺ content between B9 and B9L samples might be due to several
233 reasons. In fact, although we used the same ingredients at the same concentrations as in the brewery,
234 the yeast strain used by the brewery was in a lyophilized form (not grown on YPD for 72 hours), and
235 the second fermentation occurred in a closed bottle in the brewery. Furthermore, the time elapsed
236 from bottling to sampling was unknown.

237 The results in Figure 3A suggest that the addition of the hops might be responsible for boosting NR
238 and decreasing NAD⁺ during the fermentation trial. This behavior was confirmed by the linear
239 regression model where a positive relationship among NAD⁺ and NR was observed (Adjusted R-
240 squared: 0.3632, p-value < 0.05).

241 To better define the role of hops in the change in metabolites levels, a control fermentation trial
242 without the addition of hops was established (Figure 3B). After the initial increase to approximately
243 3 nmol/mL, NAD⁺ continued to slightly increase throughout the fermentation process (Adjusted R-
244 squared: 0.6937, p-value < 0.05), whereas NMN remained constant (Adjusted R-squared: 0.1112, p-
245 value < 0.05). NR showed a very slight increase from the 8th day until the end of the fermentation
246 (Adjusted R-squared: 0.7835, p-value < 0.05). Altogether, these results suggest that the addition of
247 hops stimulates NR production and induces degradation of both NAD⁺ and NMN. However, the
248 relationship was verified by the linear model only by considering the behavior of NR and NAD⁺
249 (Adjusted R-squared: 0.56, p-value < 0.05).

250 *S. cerevisiae* cells constitutively produce, release and import NR (Bogan et al., 2009; Lu et al., 2009),
251 whereas no information is available on the ability of plant cells to release NR. It is therefore tempting
252 to hypothesize that hops might enhance the yeast's ability to produce and release the vitamin. In this
253 view, yeast cells would facilitate NAD⁺ supply to hop cells by providing the NR precursor. Metabolic
254 interaction between different cell-types through the exchange of extracellular metabolites is a well-
255 known mechanism, and evidence has been provided that different cell types might support each

other's NAD⁺ pools by providing NR as NAD⁺ precursor (Kulikova et al., 2015). Moreover, it is interesting to note that Steyer, Tristam, Clayeux, Heitz, & Laugel (2017) highlighted a synergy between several yeast strains and hop varieties on beer volatile compounds production, thus indicating that an interaction between hop compounds and yeast metabolism exists although it remains to be investigated. Unfortunately, the lack of information on the regulation of intracellular NR generation and release does not allow to explain the mechanism underlying the metabolic interaction between yeast and hop cells. Furthermore, to the authors' knowledge, data regarding NR, NMN and NAD⁺ dynamics during a craft beer production is lacking in the scientific literature, thereby preventing further comparison.

265

3.3 Effect of wort and *S. cerevisiae* strain on the production of NR, NMN and NAD⁺

267

The present study also investigated whether the presence of the wort was required for the NR bursting effect exerted by the hops. To this end, a fermentation trial substituting the wort with YPD medium and determining the concentrations of the molecules of interest on different days was performed. As shown in Figure 4A, the change in metabolites levels during the process closely resembled that observed in the fermentation of wort. Indeed, in YPD, the addition of hops caused a rapid increase in NR (Adjusted R-squared: 0.866, p-value < 0.05) and a decrease in NAD⁺ (Adjusted R-squared: 0.1699, p-value < 0.05) thus indicating that the presence of wort is not essential for the production of the vitamin. Even in YPD a linear trend was observed by the consumption of NAD⁺ and the production of NR (Adjusted R-squared: 0.2964, p-value < 0.05). Dextrose was not added in these fermentation trials since it was previously demonstrated that it did not influence the vitamin trends. It was therefore asked whether the presence of the yeast was required for the effect of the hops and whether the effect was strain specific. Figure 4B shows that NR and NAD⁺ are not produced in the absence of yeast. On the other hand, a slight amount of NMN was produced when hops were added to the YPD medium (Figure 4B). The amount was approximately three fold lower than that measured

282 in the presence of the *S. cerevisiae* strain US-05 and decreased to undetectable levels at the end of
283 the process.

284 Figure 4C shows the levels of the metabolites during the fermentation of YPD inoculated with *S.*
285 *cerevisiae* strain CBS 1171^T. The tendency of NR to increase following the addition of hops was
286 observed also in the presence of this strain. Strain CBS 1171^T released NR on the 4th day, whereas
287 the vitamin was detectable on the 9th day with the strain US-05 (Adjusted R-squared: 0.9138, p-value
288 < 0.05). Moreover, in the absence of hops, NR decreased in the strain US-05 under sustained
289 fermentation, whereas it continued to slowly increase when strain CBS 1171^T was present (Adjusted
290 R-squared: 0.6907, p-value < 0.05). NMN production was different in the two fermentations. The
291 strain US-05 released NMN from the beginning of the process, and the addition of hops did not change
292 its concentration up to the 15th day. Then, a progressive decrease was noted (Adjusted R-squared:
293 0.04175, p-value: 0.2346). On the other hand, strain CBS 1171^T produced NMN only after the
294 addition of hops, and the levels slowly decreased during the fermentation (Adjusted R-squared:
295 0.3007, p-value < 0.05). The trend of NAD⁺ production was similar in the two fermentations. In fact,
296 the addition of hops also caused a decrease in NAD⁺ levels with the CBS 1171^T strain. CBS 1171^T
297 released more NAD⁺ than US-05.

298 Altogether, these results indicated that both hops and yeast are required during the brewing process
299 to increase NR production. Moreover, the NR bursting effect seems to be *S. cerevisiae* strain and wort
300 independent. Further studies to elucidate the interaction between hops and *S. cerevisiae* cells at
301 intracellular metabolic level could shed more light on the NR bursting effect exerted by hops.

302

303 4. Conclusion

304

305 In the present work, for the first time, the presence of NR and its dietary precursors NMN and NAD⁺
306 in craft beers was quantitatively assessed, suggesting potential beneficial properties of such a low
307 alcoholic beverage. The presence of the three vitamers in the beers under study was *S. cerevisiae*

308 strain-dependent. Overall, all craft beers prepared with different *S. cerevisiae* strains contained
309 NAD⁺, further highlighting the potentialities of microorganisms to act as biological factories for
310 beneficial molecules to humans. By reproducing a lab-scale fermentation process either in wort and
311 YPD medium, a significant increase in NR levels was observed after the addition of hops, and both
312 the yeast *S. cerevisiae* and hops are required for such a burst to occur, thus indicating that a yeast and
313 hops synergy on NR production occurs. The present study represents the first attempt to provide an
314 experimental model to study the hop-yeast interaction at metabolic and molecular levels. Finally,
315 these findings open new intriguing perspectives for the development of innovative fermented foods
316 naturally enriched in NR and its precursors.

317

318 **Declaration of Competing Interest**

319

320 The authors declare that they have no known competing financial interests or personal relationships
321 that could have appeared to influence the work reported in this paper.

322

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324

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329

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410 **FIGURE CAPTIONS**

411

412 **Figure 1.** Flow diagram of manufacture of the craft beers B1-B8 (A); Flow diagram of manufacture
413 of the craft beers B9 and B10 (B).

414

415 **Figure 2.** Boxplots showing the levels NR, NMN, NAD⁺ concentrations (nmol/mL) detected in the
416 different craft beers under study.

417

418 Different letters at the base of the boxes indicate significant differences for each metabolite among
419 beers (P <0.05).

420

421 **Figure 3.** NR, NMN and NAD⁺ concentrations in B9L (A) and control (B) during the fermentation
422 process.

423

424 Measurements were performed in duplicate and the means $\pm$ standard deviation were reported.

425

426 **Figure 4.** Effect of hop addition on NR, NMN and NAD⁺ levels in YPD medium inoculated with *S.*
427 *cerevisiae* strain US-05 (A), without yeast inoculation (B), and inoculated with *S. cerevisiae* strain
428 CBS 1171^T (C).

429

430 Hop was added at the 9th day. Measurements were performed in duplicate and the means $\pm$ standard
431 deviation were reported.

432

433

Table 1. *Saccharomyces* yeast strains, ingredients and alcohol percentage (%) in craft beer samples

Sample	Yeast species	Strain	Ingredients	% alcohol
B1	<i>Saccharomyces pastorianus</i>	W34/70	H ₂ O, hop, yeast, sugar, barley and wheat malt	6.0
B2	<i>Saccharomyces cerevisiae</i>	S-33	H ₂ O, hop, yeast, sugar, barley malt	5.5
B3	<i>Saccharomyces cerevisiae</i>	WB-06	H ₂ O, hop, yeast, sugar, barley and wheat malt, wheat	6.3
B4	<i>Saccharomyces cerevisiae</i>	WB-06	H ₂ O, hop, yeast, sugar, barley and wheat malt	5.8
B5	<i>Saccharomyces cerevisiae</i>	S-04	H ₂ O, hop, yeast, sugar, barley malt	6.3
B6	<i>Saccharomyces cerevisiae</i>	S-04	H ₂ O, hop, yeast, sugar, barley and wheat malt	6.6
B7	<i>Saccharomyces cerevisiae</i>	US-05	H ₂ O, hop, yeast, sugar, barley and wheat malt	6.6
B8	<i>Saccharomyces cerevisiae</i>	US-05	H ₂ O, hop, yeast, sugar, barley malt	5.4
B9	<i>Saccharomyces cerevisiae</i>	US-05	H ₂ O, hop, yeast, sugar, barley malt, oat flakes	5.5
B10	<i>Saccharomyces cerevisiae</i>	US-05	H ₂ O, hop, yeast, sugar, barley and wheat malt, oat flakes	5.5

Fig. 1**A)** **B)**

Fig. 2.

Fig. 3

A)

B)

Fig. 4