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## **Transgenic corn syrup enhances in vitro yeast ethanol production: Hidden cause of the US MASH epidemic?**

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1 **Transgenic corn syrup enhances in vitro yeast ethanol production:**  
2 **Hidden cause of the US MASH epidemic?**

3  
4 **Short title:** Transgenic corn syrup enhances in vitro yeast ethanol production

5  
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18

19

20 **Abstract**

21

22 The incidence of metabolic dysfunction–associated liver steatohepatitis (MASH) has risen over  
23 the last decade, making it a major health problem and the second leading cause of liver disease in  
24 the US. Recent studies suggest that MASH may be linked to the endogenous production of  
25 alcohol in the digestive tract after sugar ingestion, with bacteria such as *Klebsiella* and  
26 *Lactobacillus* playing a key role in this fermentation. More recently, the role of the gut  
27 mycobiota has also been suggested. In particular, MASH has been recently associated with the  
28 presence of yeast strains that produce high concentrations of alcohol in the gut microbiota. As  
29 the source of sugar varies by region - transgenic maize in the USA, sugar cane in Africa and beet  
30 in France - we hypothesised that consumption of sweetened beverages, in combination with the  
31 gut mycobiota, may be a contributing factor to MASH.

32 Analysis of American and French Coca-Cola and World-Cola from Mali revealed significant  
33 differences in carbohydrate composition, with the presence of complex oligosaccharides in  
34 American Coca-Cola. A significantly higher ethanol production was observed when a *Candida*  
35 *albicans* yeast strain was cultured in American Coca-Cola compared to the other two colas. The  
36 results suggest that consumption of transgenic maize sweeteners may increase the risk of  
37 autobrewery syndrome and underline the need for epidemiological studies to assess the  
38 association of these carbohydrates with the MASH epidemic in the US.

39

40 **Keywords:** Soda; coke; Coca-Cola, carbohydrates; transgenic maize, corn syrup, alcohol, gut  
41 mycobiota; *Candida albicans*, in vitro.

42

43

## 44 **Introduction**

45 There are several stages of liver damage in metabolic dysfunction–associated liver steatohepatitis  
46 (MASH). The first is steatosis, which in some cases can lead to fibrosis,<sup>1</sup> then to cirrhosis,<sup>2,3,4</sup>  
47 and finally to liver cancer<sup>5,6</sup>. Currently, MASH is a major liver problem, being the second most  
48 common cause of liver disease in patients awaiting liver transplantation in the United States<sup>7,8</sup>. In  
49 the last decade, the prevalence of MASH has increased by almost 100%<sup>9</sup>. Recently, several  
50 studies have shown that MASH is probably related to the endogenous production of alcohol in  
51 the digestive tract after the ingestion of sugar<sup>10,11,12</sup>. Some authors have shown that *Klebsiella*  
52 bacteria are responsible for this fermentation<sup>13</sup>; others have highlighted the role of *Lactobacillus*  
53 bacteria<sup>14,15</sup> and confirmed the presence of alcohol in the hepatic portal vein of MASH patients  
54 sampled during bariatric surgery<sup>16</sup>. Another team also showed that alcohol in stool or blood  
55 samples from MASH patients was associated with specific *Klebsiella pneumoniae* strains<sup>17</sup>.  
56 Fungal colonisation of the human gastrointestinal tract is common, particularly with *Candida*  
57 spp., and half of the human population in the US is colonised with at least one *Candida* spp<sup>18</sup>.  
58 Our team has shown that the presence of alcohol in stool samples from MASH patients is  
59 associated with *Candida* yeast colonisation<sup>18</sup>. Others have shown that alcohol in stool or blood  
60 samples from MASH patients is associated with specific *Klebsiella pneumoniae* strains<sup>19</sup> or  
61 *Saccharomyces cerevisiae* yeast<sup>20</sup>. It has long been known that MASH is associated with sugar  
62 consumption<sup>12</sup>. In addition, the use of antibiotics prior to the onset of MASH can undoubtedly  
63 explain the change in the digestive ecosystem that favours the proliferation and spread of  
64 yeasts<sup>21</sup>. Struck by the significance of the MASH epidemic in the United States, we hypothesised  
65 that it might be related to the consumption of sweetened products, since the composition of sweet  
66 soft drinks, especially colas, varies from country to country. In the USA, colas are sweetened  
67 with sugar extract from transgenic maize<sup>22,23</sup> whereas in Africa the sugar comes from sugar

68 cane,<sup>24,25</sup> and in France (Europe) from beetroot (Coca Cola European Partners).<sup>26</sup> The aim of this  
69 study was to evaluate the in vitro ethanol production of yeasts incubated in colas produced in the  
70 USA, Africa or France.

71

## 72 **Materials and Methods**

### 73 *Cola samples*

74 We analysed a total of three different sugared colas produced by the Coca-Cola Company in the  
75 USA and in France. In Mali, World-Cola is produced by the Castel Group, which is present in 14  
76 countries in sub-Saharan Africa. Coca-Cola original taste and World-Cola was purchased in local  
77 stores in France or Mali. Classic American Coca-Cola was purchased from the My American  
78 Market online store (21 August 2023). It is worth noting that the source of the sugar used to  
79 sweeten colas varies depending on where they are produced. Transgenic maize is used in the  
80 USA, sugar beet in France and sugar cane in Mali.

### 81 *Analysis of the carbohydrate composition of colas*

82 The carbohydrate content of colas was studied by ultra-high performance liquid  
83 chromatography-mass spectrometry (UHPLC-MS) according to an application note from Waters  
84 Corporation (Shelton, USA), with modifications.<sup>27</sup> Mono-, di- and oligosaccharides were  
85 separated by hydrophilic interaction liquid chromatography (HILIC) using a BEH amide column  
86 (2.1x50 mm, Waters) installed in an iClass UHPLC system (Waters), which in turn was  
87 connected to a Vion IMS mass spectrometer (Waters). To study the composition of free  
88 carbohydrate structures, samples were diluted in water only, whereas to study the  
89 monosaccharide composition of complex carbohydrates, samples were first evaporated and  
90 hydrolysed with trifluoroacetic acid (4 h, 100%); the acid was then evaporated and the resulting  
91 samples dissolved in water. All samples were injected directly into the amide column and the  
92 compounds were separated according to the following solvent composition gradient (A: 80:20%  
93 acetonitrile/water with 0.1% NH<sub>4</sub>OH; B: 30:70% acetonitrile/water with 0.1% NH<sub>4</sub>OH): linear  
94 increase of B from 0 to 60% between 0 and 6 min. The carbohydrates were then ionised in  
95 negative mode using an electrospray ion source and the resulting ions were monitored using an

96 accurate mass screening method including ion mobility separation and MSe data acquisition  
97 (HDMSe). Data processing was performed using UNIFI software (version 8.1.4).

#### 98 *Quantification of ethanol after inoculation with the C. albicans strain CSURL 0240*

99 Inoculum and fermentation conditions: *C. albicans* strain CSURL 0240. This yeast was  
100 inoculated in triplicate at a cell density of 3 McFarland into 1 mL of each cola sample in the  
101 flask vial tube. The inoculated flasks were incubated at 28 °C for 48 hours. Ethanol was  
102 quantified at 24, 36 and 48 hours of incubation by headspace gas chromatography-mass  
103 spectrometry (HS-GC/MS) as previously described.<sup>28</sup> Briefly, the samples and ethanol standards  
104 (20-1800 mM) were all spiked with isopropanol as an internal standard. They were then heated  
105 to 60 °C to evaporate the alcohol molecules. The pressurised headspace gases were transferred to  
106 the gas chromatography system consisting of a Turbomatrix HS110 sampler connected to a  
107 Clarus 690 chromatograph and an SQ8 T single quadrupole mass spectrometer (Perkin Elmer,  
108 Waltham, MA, USA). The analytes were separated on an Elite BAC-2 column (Perkin Elmer).  
109 Ethanol and isopropanol were selectively detected and automated quantification was performed  
110 using Turbomass 6.1 software (Perkin Elmer). The influence of both the origin of the colas and  
111 the incubation time on the ethanol concentration was tested by two-way ANOVA using the  
112 statistical software SAS Stat (ver. 9.4). A *P* value less than 0.05 was considered statistically  
113 significant.

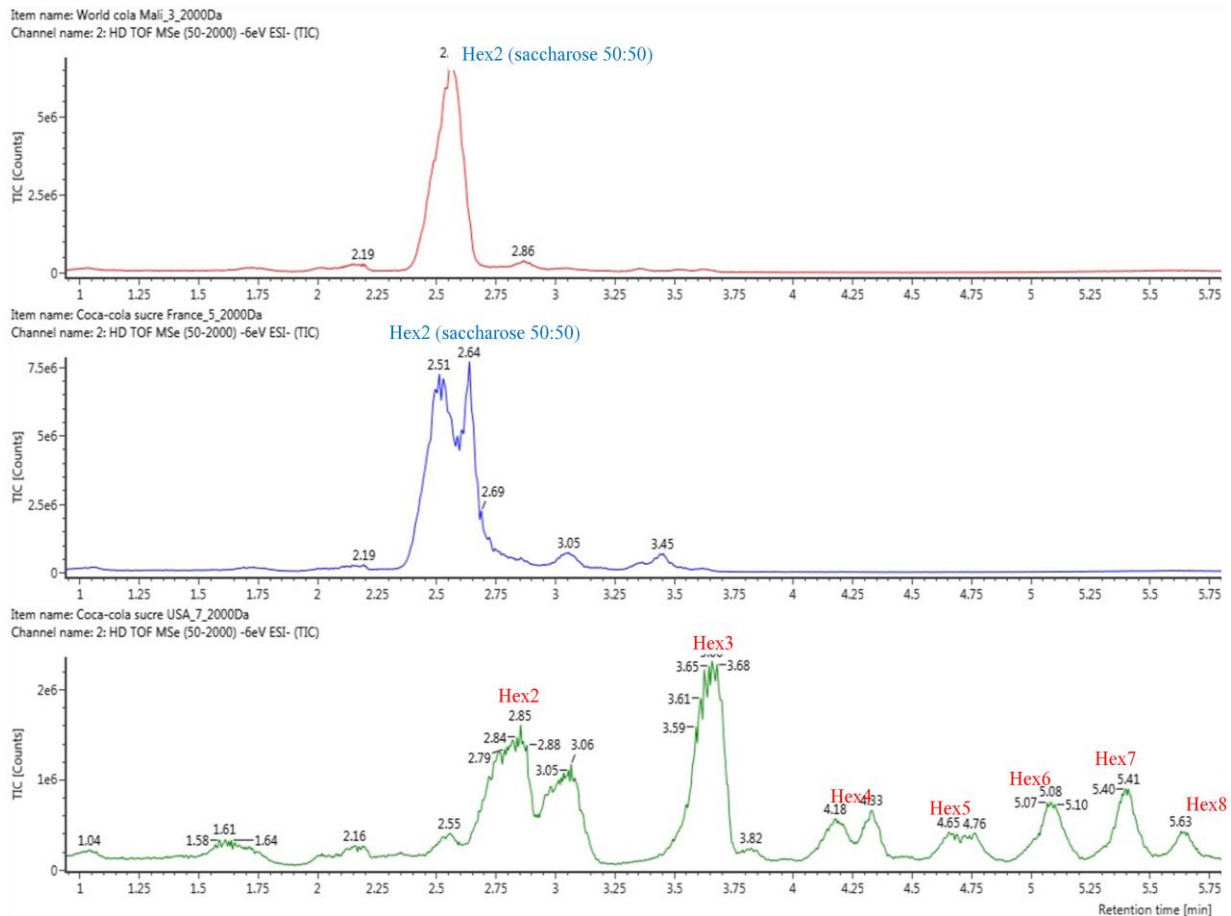
114

## 115 **Results**

### 116 *The carbohydrate composition of colas*

117 As illustrated in **Figure 1**, UHPLC-MS detected a unique disaccharide, identified as sucrose, in  
118 both Malian World-Cola and French Coca-Cola. In contrast, sugars with a high degree of  
119 polymerisation were present in American Coca-Cola. The structures of the sugars from  
120 transgenic maize are drastically different from those of sugar cane or sugar beet, as the analysis  
121 revealed several oligosaccharides with up to 8 polymerised sugar branches (hexoses), and  
122 another disaccharide of unknown composition.

123



124

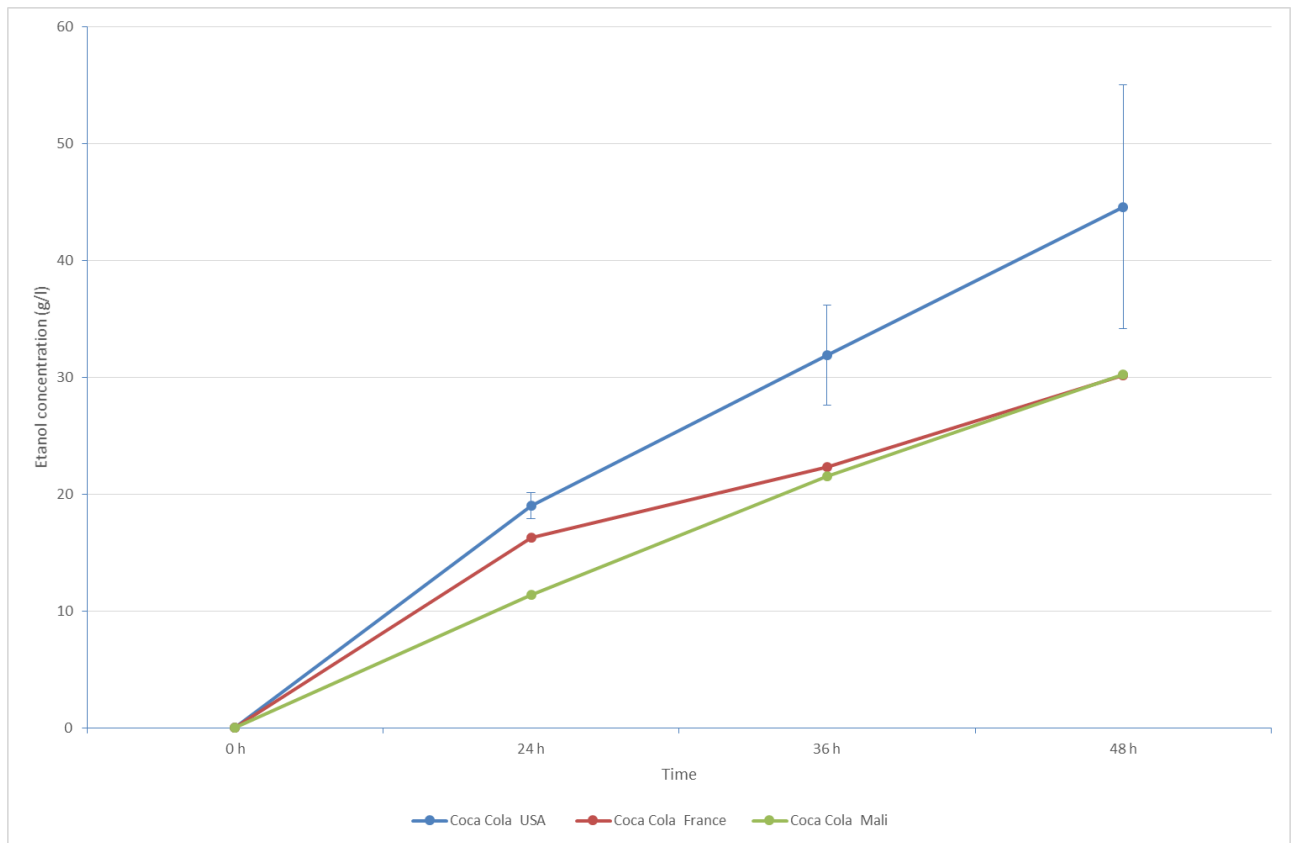
125 **Figure 1.** Ultra-high performance liquid chromatography-mass spectrometry carbohydrate  
 126 analysis showing the high degree of polymerisation of free sugars present in American Coca-  
 127 Cola, in contrast to the unique presence of saccharose in colas from France or Mali. A: Coca-  
 128 Cola from Mali; B: Coca-Cola from France; C: Coca-Cola from the USA.

129

130 *Quantification of ethanol production by C. albicans strain CSURL 0240*

131 The concentrations of ethanol produced by *C. albicans* in colas of different origins are shown in  
 132 *Figure 2*. Both incubation time and cola substrate ( $P < 10^{-4}$  each) had a statistically significant  
 133 effect on the measured ethanol concentration. As shown in **Figure 2**, yeast fermentation of  
 134 carbohydrates produced a significantly higher ethanol concentration in American Coca-Cola than  
 135 in the other colas after 36 hours of incubation. Ethanol production was similar in French and  
 136 Malian colas (**Figure 2**).

137



138

139

140 **Figure 2:** Overtime in vitro ethanol production by *Candida albicans* CSURL 0240 in colas from  
 141 the USA, France, or Mali. The yeast fermentation of carbohydrates produced a significantly  
 142 higher ethanol concentration in American Coca-Cola than in the other colas (ANOVA,  $P < 10^{-4}$ ).

143

## 144 Discussion

145 In this exploratory study, we highlighted the highly different carbohydrate composition between  
 146 the colas from the USA compared to those from France or Europe. The variety of  
 147 oligosaccharides with a degree of polymerization ranging from 3 to 8 in the American *Coca-*  
 148 *Cola* contrasted with the presence of unique shorter disaccharide structure in the other colas. In a  
 149 previous work, we have shown a very high production of ethanol by the yeasts *Pichia*  
 150 *kudriavzevii*, *C. glabrata* and *C. albicans*<sup>18</sup>. Here, we point out the association of a high degree  
 151 of polymerisation of the carbohydrates with a significantly higher yeast alcohol production.  
 152 Indeed, the ethanol production of *C. albicans* CSURL 0240 in American Coca-Cola reached up



153 to 49.7g/L at 48 hours, which is equivalent to drinking 1 litre of beer at 6% alcohol. People who  
154 have yeast in their gut microbiota and drink soda or eat any food sweetened with corn syrup  
155 derived from transgenic maize are therefore likely to be at increased risk of autobrewery  
156 syndrome. Further epidemiological studies are warranted to assess the influence of consumption  
157 of carbohydrates derived from transgenic maize on MASH epidemics in the United States. We  
158 believe that if this hypothesis is confirmed, there is likely to be a major public health problem  
159 associated with the massive consumption of glucose and fructose oligosaccharide combinations.

160

161

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