FATTY ACID PROFILE OF WET BREWER’S SPENT GRAIN
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Abstract: Wet brewer’s spent grain (WBSG) is the extracted residue remaining after the grains have been fermented during beer making process. The fatty acids composition of feed was estimated by gas chromatography. The fatty acids profile of wet brewer’s spent grain was found to contain 28.41 ± 0.30 per cent SFA and the MUFA and PUFA contents were 15.10 ± 0.25 and 55.92 ± 0.19 per cent, respectively. The PUFA: SFA ratio was found to be 1.97 ± 0.02. These unsaturated fatty acids increased the absorption of PUFA, n3, and n6 fatty acids in the intestine and accumulation in the muscles.

Keywords: WBSG and Fatty acid composition.

INTRODUCTION

Wet brewer’s spent grain (WBSG) is the extracted residue remaining after grains have been fermented during beer making process. The annual WBSG production in India is approximately 0.4 million tones. In the manufacture of beer, various residues and by-products are generated. The most common ones are spent grains, spent hops and surplus yeast, which are generated from the main raw material (Mussatto, 2009).

Spent grains are the by-products of mashing process, which is one of the initial operations in brewery in order to solubilize the malt and cereal grains to ensure adequate extraction of the wort (liquid extracted from mashing process) (Fillaudeau et al., 2006). Following different separation strategies, the amount of brewer’s spent grain (BSG) generated is 85 per cent of the total by-products (Tang et al., 2009). Thus, BSG is a readily available, high volume, low cost by-product of brewing industry and is a potentially valuable resource for industrial exploitation (Robertson et al., 2010).
Brewery spent grains have been utilized as animal feed for many years (Szponar et al., 2003). The organic matter digestibility, energy value and protein value are high. Brewer’s spent grain is used in animal feeding, primarily for beef cattle, dairy cows and also for pigs, goats, birds, fish, and other livestock. It is tasty and readily consumed by animals (Geron et al., 2010).

WBSG though have low dry matter, however, on dry matter basis, they have high content of total digestible nutrients (TDN) (Hersom, 2006) having an energy value of 71 to 75 per cent. WBSG contain 7 to 10 per cent crude fat and are a good source of protein with a crude protein content ranging from 25 to 34 per cent. Total of 26 fatty acids in the brewer’s grain, the most abundant being linoleic (C\textsubscript{18:2} n-6), palmitic (C\textsubscript{16:0}) and oleic (C\textsubscript{18:1}n-9) acids which might influence the production performance of livestock. These unsaturated fatty acids increased the absorption of PUFA, n3, and n6 fatty acids in the intestine and accumulation in the muscles. Hence, the present study had been proposed to evaluate the fatty acids composition of wet brewer’s spent grain.

**MATERIALS AND METHODS**

Fatty acids composition of feed was estimated by gas chromatography. Extraction of lipids and transmethylation were done using one-step methylation procedure described by Folch et al. (1957).

Four grams feed was finely ground and homogenized with 40 mL of Folch’s solution (containing chloroform: methanol 2:1 v/v) with high speed homogenizer for 30 sec. The homogenate was left undisturbed overnight and then filtered through Whatmann No. 42 filter paper. To the filtrate 10ml of 0.88 per cent sodium chloride solution was added. The filtrate was mixed well and left for 2 h (Folch et al., 1957).

The upper layer was siphoned off and the lower lipid layer was extracted and evaporated. Three millilitres of 10 per cent methanolic HCl was added to the brown bottles, capped tightly, vortexed and heated in a water bath for 2 h at 65°C. The samples were cooled and each of the sample, 5 ml of 6 per cent potassium carbonate solution and 2 ml of hexane were added carefully. The tubes were centrifuged for 5 min at 6,000 rpm for 10 min to separate the solvent layer. The clear upper hexane layer containing fatty acid methyl esters was transferred to 5 ml screw capped tubes and used for fatty acid analysis using gas chromatograph.
Fatty acid analysis using Gas chromatograph

The hexane layer (0.2 µl) was injected into the gas chromatograph (Chemito, India) fitted with fused silica capillary column of 30 m x 0.25 mm internal diameter, 0.25 µm film thickness of stationary phase (SGE, Australia) and connected to flame ionization detector of gas chromatograph. Ramped oven temperature condition (160 °C for 3 min and increased to 220 °C at the rate of 5 °C per 5 min and held for 5 min) was used. Temperature of injector and detector was kept at 225°C and 230°C respectively. The flow rates of nitrogen (as carrier gas), hydrogen and air were 1, 30 and 300 ml/min, respectively. The output signal from gas chromatograph was analysed based on area normalization by using Chemito software (IRIS 32 Lite). The fatty acids composition in the samples was estimated by comparing retention time of known authentic standards of methyl esters of fatty acids (Supelco, USA).

RESULTS AND DISCUSSION

The mean values of fatty acids profile of wet brewer’s spent grain analyzed in the present study is presented in Table 1.

**Table 1 Mean (± SE) fatty acid composition (wt %) of the WBSG**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>(%) Total Fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt; (Myristic acid)</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt; (Palmitic acid)</td>
<td>25.14 ± 0.28</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1&lt;/sub&gt; n-7 (Palmitoleic acid)</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt; (Stearic acid)</td>
<td>2.31 ± 0.06</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt; n-9 (Oleic acid)</td>
<td>14.50 ± 0.24</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:2&lt;/sub&gt; n-6 (Linoleic acid)</td>
<td>50.17 ± 0.23</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:3&lt;/sub&gt; n-3 (Linolenic acid)</td>
<td>5.09 ± 0.07</td>
</tr>
<tr>
<td>C&lt;sub&gt;20:0&lt;/sub&gt; (Arachidic acid)</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>C&lt;sub&gt;20:5&lt;/sub&gt; n-3 (Eicosapentaenoic acid)</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;22:0&lt;/sub&gt; (Behenic acid)</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>C&lt;sub&gt;22:6&lt;/sub&gt; n-3 (Docosahexaenoic acid)</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>SFA</td>
<td>28.41 ± 0.30</td>
</tr>
<tr>
<td>MUFA</td>
<td>15.10 ± 0.25</td>
</tr>
<tr>
<td>PUFA</td>
<td>55.92 ± 0.19</td>
</tr>
<tr>
<td>n6/n3</td>
<td>8.73 ± 0.15</td>
</tr>
<tr>
<td>PUFA:SFA</td>
<td>1.97 ± 0.02</td>
</tr>
</tbody>
</table>
Mean of 6 observations
Saturated fatty acid (SFA) = (C\textsubscript{14:0}, C\textsubscript{16:0}, C\textsubscript{18:0} and C\textsubscript{20:0})
Monounsaturated fatty acid (MUFA) = (C\textsubscript{16:1} and C\textsubscript{18:1})
Poly unsaturated fatty acid (PUFA) = (C\textsubscript{18:2}, C\textsubscript{18:3}, C\textsubscript{20:5} and C\textsubscript{22:6})

The wet brewer’s spent grain was found to contain C\textsubscript{14:0} (0.43±0.02), C\textsubscript{16:0} (25.14 ± 0.28), C\textsubscript{16:1} (0.60 ± 0.02), C\textsubscript{18:0} (2.31 ± 0.06), C\textsubscript{18:1} (14.50 ± 0.23), C\textsubscript{18:2} (50.17 ± 0.23), C\textsubscript{18:3} (5.09±0.07), C\textsubscript{20:0} (0.18 ± 0.02), C\textsubscript{20:5} (0.28 ± 0.0), C\textsubscript{22:0} (0.35 ± 0.02) and C\textsubscript{22:6} (0.38 ± 0.02) fatty acids. Of the total fatty acids, the SFA constituted 28.41 ± 0.30 per cent and the MUFA and PUFA contents were 15.10 ± 0.25 and 55.92 ± 0.19 per cent, respectively. The PUFA: SFA ratio was found to be 1.97 ± 0.02.

The results of present study agreed with the finding of Farcas et al. (2015) who identified a total of 26 fatty acids in the brewer’s grain, the most abundant being linoleic (C\textsubscript{18:2}n-6), palmitic (C\textsubscript{16:0}) and oleic (C\textsubscript{18:1}n-9) acids. Similarly, Niemi et al. (2012) also found that these three fatty acids as the major ones in the brewer’s spent grain. Chibisa et al. (2013) reported that brewer’s grain contained C\textsubscript{18:2}n-6 and C\textsubscript{18:3}n-3 as the major PUFA. The α-linolenic (C\textsubscript{18:3}n-3) and stearic (C\textsubscript{18:0}) acids were also present along with small levels of myristic (C\textsubscript{14:0}), vaccenic (C\textsubscript{18:1}n-7), arachidic (C\textsubscript{20:0}), 11-eicosapentaenoic (C\textsubscript{20:1}n-9), behenic (C\textsubscript{22:0}) and docosahexaenoic (C\textsubscript{22:6}) acids. Only minor changes in fatty acid composition occur during malting and mashing and therefore, the fatty acid composition of BSG was similar to that of barley (Becker, 2007).

Zinn et al. (2000) reported that unsaturated fatty acids inside the duodenum promote the absorption of PUFA by increasing the surface area of micelles. As a result, greater absorption of PUFA, n3, and n6 fatty acids occur in the intestinal lamellae which are then reflected in the muscles and finally in the meat. Further, Jenkins et al. (2002) opined that accumulation of linoleic (C\textsubscript{18:2}n-6) (the predominant fatty acid from grain) or long chain fatty acids in the rumen can stop complete biohydrogenation.

**CONCLUSION**

The fatty acids profile of wet brewer’s spent grain was found to contain 28.41 ± 0.30 per cent SFA and the MUFA and PUFA contents were 15.10 ± 0.25 and 55.92 ± 0.19 per cent, respectively. The PUFA: SFA ratio was found to be 1.97 ± 0.02.
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