SUSTAINABLE AND INTEGRAL EXPLOITATION OF AGAVE

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Index
1. Scientific trends on Agave
2. Science and technology of Agave beverages and other derivatives
3. Biological effects of Agave fructans and other by-products
4. Industrial processing of Agave wastes and subproducts

Citation example:

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Evaluation of agave fructans in starchy foods with and without thermal process

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ABSTRACT

Enzymatic/Spectrophotometric AOAC 999.03 fructan measurement method was modified with the objective to quantify agave fructans. Usefulness of the modified method was confirmed in pure agave fructans. Modified method result in about 25% of underestimation when fructans were mixed with food matrices. The effect over fructan losses resulted from thermal process was evaluated. Long chain fructans achieve better resistance in comparison with native fructans (8% of thermal loses) no matter the source of ingredient. In the case of native fructans, agave ingredients showed lower degradation in baked bread in comparison with chicory fructans.

Key words: agave fructans, soluble fibre, inulin measurement, AOAC 999.03, inulin thermal degradation.

INTRODUCTION

Fructans quantification is of a great concern because it is required for food labeling purposes to make a fiber claims. The choice of the analytical method has a significant influence on fructan quantification especially in heat-treated samples. Enzymatic-gravimetric method for total dietary fibre AOAC 985.29 it’s not a suitable method for fructan quantification because fructans do not precipitate with alcohol in a included step. There are two other official methods for fructan measurement in foods: AOAC 999.03 and 997.08. The validation of these methods has been conducted using chicory fructans and up to now there are no information about method selectivity to agave fructans. Present study shows results from agave fructans quantification in bread with and without thermic process.

METODOLOGY

Characterization of Ingredients

Chicory ingredients for evaluation: Beneo P95, Raftiline GR and Orafti HP. Agave ingredients used were Nutriagaves and AM-101. Ingredients were characterized using ultrafiltration, TLC and HPLC and classified in: a) sugars, b) fructo-oligosaccharides (FOS) and c) long chain fructans (fructans with DP>12).

Verification of agave ingredient hydrolisis

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Enzyme kinetic experiments were conducted using endo- and exo-inulinases with the object to determine the time necessary for complete hydrolysis of agave fructans. The resulting time was introduced in the method AOAC 999.03 for further evaluations.

**Bread preparation**

Different types of bread were prepared for the experiments. The general bread formulation contained: 70% flour, 15% egg, 5% fresh yeast, 5% butter, 3% sugar, 1.6% milk and 0.4% salt. For fructans incorporation in BAKING experiments, flour was partially substituted by 10-12.5% of fructans. Baking was done at 200°C for 20 min.

Types of bread:

1) White bread **without fructans** for blank and NO BAKING experiments was done using general bread formulation. After baking bread was dried and ground and mixed with pure fructans in relation 9:1.

2) Bread with **agave** fructans in relation 9:1 was performed for BAKING experiments. Fructans incorporation was done before baking. After baking bread was dried and ground.

3) Bread with **chicory** fructans in relation 9.1 was performed for BAKING experiments. Fructans incorporation was done before baking. After baking bread was dried and ground.

NO BAKING experiments were prepared adding pure fructans with the powder bread no. 1 and without thermic process. This experiment was conducted to evaluate accuracy and specificity of the modified AOAC 999.03.

BAKING experiments were conducted mixing fructans with bread formulation before baking, so this fructans had a thermal process. This experiment was performed to evaluate the fructans degradation as a result of heat-treatment.

**Evaluation of method for fructan measurement in bread**

Method AOAC 999.03 with modifications was conducted. Specificity and accuracy were evaluated through percent of fructan recovery (%R) in dry weight basis. Fructans measurement and %R were evaluated adding fructans in relation 9:1 to bread number 1) without thermic process. The effect of the thermal process in fructan content was evaluated adding fructans as mentioned above in bread no. 2 and 3. Fructans resistance was evaluated comparing percentage of fructan recovery before and after baking.

**RESULTS AND DISCUSSION**

Figure 1 shows the carbohydrate distribution of ingredients. Both native ingredients from chicory (Raftiline GR) and agave (Nutriagaves) were composed from a mixture about 40% FOS and 55% long fructans, with small quantity of sugars. The other three ingredients AM-101, Orafty HP and Beneo P95 meet their specification. AM-101 is an agave ingredient prepared in CIATEJ through ultrafiltration and contains only long fructans as is shown in Figure 1. Beneo P95 is a chicory ingredient containing more than 90% of FOS and Orafty HP from chicory contains mainly long chain fructans.

Initial evaluation of endo- and exo-inulinases (results not shown) allow to know that agave fructans required 2 hours instead of 30 minutes indicated at original method AOAC 999.03, to complete hydrolysis the fructans, so this modification was introduced for every fructan measurement.
FIGURE 1. Carbohydrate distribution of four ingredients used in the experiments.

Table 1 shows results from NO BAKING experiments. The method underestimation was about 25%, with exception of FOS. Native ingredients had similar %R (mean 71%) no matter the source of fructans. Slightly better %R was obtained for long chain fructans (76.5-81.3%).

The experiment showed an underestimation of fructan content by method of about 27% in the case of native ingredient, 22% in long chain fructans, and 99.6% in FOS. Method produced high underestimation of FOS, which has been previously reported by McCleary & Rossiter (2004). Pure agave native ingredient was evaluated resulting in 92-104 %R, showing the suitability of the method for pure ingredient evaluation.

TABLE 1. Evaluation of fructans percentage of recovery in NO BAKING experiments.

<table>
<thead>
<tr>
<th></th>
<th>Nutriagaves</th>
<th>Raftline GR</th>
<th>AM-101</th>
<th>Orafti HP</th>
<th>Beneo P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native agave</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Native chicory</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Long chain</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>FOS</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>FRUCTAN ADITIÓN</td>
<td>7.2 ± 0.9</td>
<td>7.1 ± 0.6</td>
<td>8.1 ± 0.5</td>
<td>7.7 ± 0.8</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>g/100g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRUCTAN QUANTIFICACION</td>
<td>71.5 ± 9.2</td>
<td>70.7 ± 0.3</td>
<td>81.3 ± 5.0</td>
<td>76.5 ± 7.8</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>g/100g</td>
<td>7.2 ± 0.9</td>
<td>7.1 ± 0.6</td>
<td>8.1 ± 0.5</td>
<td>7.7 ± 0.8</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>PERCENTAGE OF</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>RECOVERY</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The possibility to get a reduction in %R because of the percentage of FOS (40% in raw ingredients) was eliminated by experiments with long chain fructans. AM-101 and Orafti HP that didn’t have FOS, resulting in 76.5-81.3% R. On the other hand, similar loses were observed when concentration ranged from 1 to 20% of long chain fructans were added to bread without thermal process (%R between 73-86% results not shown). With this evidence
we hypothesize that loses produced by the method maybe because of interaction with other ingredients of bread formulation, since experiments made with the pure ingredient didn’t show loses.

Table 2 shows the effect of fructans reduction in heat-treated samples. The difference between NO BAKING – BAKING was used for correction of the method underestimation. Long chain fructans achieve better resistance in comparison with native fructans (fructans reduction 8.2% vs. 16% respectively) no matter the source of ingredient. On the other hand, native agave fructans result in only 10.5% of loses after thermal process, against 21.5% from chicory ingredient.

TABLE 2. Comparisson of fructans percentage of recovery in bread from NO BAKING vs. BAKING experiments.

<table>
<thead>
<tr>
<th></th>
<th>AM-101 Long ch agave</th>
<th>ORAFTI HP Long ch chicory</th>
<th>NUTRIAGAVES Native agave</th>
<th>RAFTILINE GR Native chicory</th>
</tr>
</thead>
<tbody>
<tr>
<td>No baking</td>
<td>81.3%</td>
<td>76.5%</td>
<td>71.5%</td>
<td>70.7%</td>
</tr>
<tr>
<td>Baking</td>
<td>74.0%</td>
<td>67.5%</td>
<td>61.0%</td>
<td>49.2%</td>
</tr>
<tr>
<td>Difference by baking process</td>
<td>7.3%</td>
<td>9.0%</td>
<td>10.5%</td>
<td>21.5%</td>
</tr>
</tbody>
</table>

Böhm et al., (2005) observed significant loses on pure inulin by heat-treated experiments, e.g. they report degradation about 50% in 30 min., at 195° C, while we get 21.5% with similar ingredient. Differences could be explained because they used pure ingredient and we introduced the fructans into the bread formulation, then the other ingredients are maybe protecting them.

CONCLUSION
Usefulness of the modified method AOAC 999.03 for pure agave fructans quantitation was confirmed. The method result in about 25% of underestimation when fructans were mixed in food matrices with no heat process. Evaluation of the fructans resistance to heat-process showed that long chain fructans achieve better resistance (8% better) in comparison with native fructans, no matter the source of ingredient. In the case of native fructans, agave ingredients showed lower degradation in baked bread in comparison with chicory fructans.

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