Abstract

The aim of this study was to evaluate the influence of fat substitution by fructooligosaccharide on physicochemical, technological and sensory characteristics of hamburgers. Five hamburgers formulations were prepared: F1 - standard (0% FOS) and the others added 1.25% (F2), 2.50% (F3), 3.75% (F4) and 5% (F5) of FOS. The Acceptability similar to standard sample was found for hamburgers with up to 3.75% prebiotic addition. However, all formulations had acceptability index above 70%. Cooking yield, moisture retention, shrinkage and fat retention have been increased as FOS was added. Substitution of fat by FOS increased carbohydrate and fiber content and decreased lipid and calorie hamburgers content. FOS addition did not change red and yellow values, however it increased brightness of product. FOS is an ingredient with potential for addition in beef hamburger, improving physicochemical and technological parameters and with little influence...
Effect of fat replacement by fructooligosaccharide in hamburger: 
physicochemical, technological and sensorial analysis

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E-mail: nutridai@gmail.com

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Keywords: Prebiotic; meat products; healthiness.

1. Introduction

Meat and meat products are essential diet components, providing high amounts of protein, fatty acids, vitamins and minerals [1]. Hamburgers are foods widely appreciated by different publics, due to their practicality to produce, cook and consumption. However, they are known to have low dietary fiber and high fat content, especially in saturated fatty acids [2]. Excessive consumption of this type of fat, coupled with low fiber intake, may increase the risk to developed noncommunicable diseases such as diabetes, cancer, obesity and cardiovascular disease [3,4]. In this regard, strategies are needed to add healthier ingredients in hamburgers to improve nutritional profile [5,6].

Currently, consumers are seeking a more practical and healthy diet. This fact has forced the food industries to rethink the way they produce their products, using ingredients that are more nutritious and have some health benefit. In this sense, the fat replacement in meat products by raw materials considered functional, such as dietary fibers, has gained prominence in the world scenario, beside adding economic food value to food [7,8]. The fat is an important ingredient in food as it has a positive influence on softness, juiciness and flavor, among other attributes [9]. Therefore, fat reduction may directly affect acceptability as it may interfere with technological aspects of meat product [10]. Studies have already shown the feasibility fat replacing fat in meat products with ingredients such as sugar cane and sesame oil [10], green banana flour [11], cellulose fiber [9], fructooligosaccharide (FOS), inulin [12] and pectin [8]. In these products, good sensorial acceptability and improvement in technological and nutritional characteristics were verified with incorporation of different compounds as fat substitutes.

FOS is a type of non-digestible carbohydrate knowing by body as prebiotic, as it selectively stimulates the growth and activity of particular species of bacteria in colon [13,14]. FOS is extracted from plants such as onion, artichoke, garlic, chicory root and yacon potato. It is low in calories, high in dietary fiber and not cariogenic. Furthermore, because its functional properties, it can help strengthen immune system, lower lipid, cholesterol and blood glucose levels [15]. Considering this context, the using FOS as a fat substitute in meat products becomes a viable alternative, aiming to improve nutritional profile of products and promote consumer health benefits [16]. However, research has already shown occurrences of impairments technological and sensory after prebiotics addition in different foods, such as meat products [12], pasta [17], chocolates [18] and breads [19]. Knowing this, the aim of this study was to evaluate the influence of fat substitution by fructooligosaccharide on physicochemical, technological and sensory characteristics of hamburgers.
2. Materials and methods

2.1. Beef patties processing and cooking

Five hamburgers formulations added with different FOS levels were prepared in triplicate: F1: standard (0%), F2 (1.25%), F3 (2.50%), F4 (3.75%) and F5 (5%). Percentages were defined by means of preliminary sensory tests carried out on the product. In addition to FOS percentages, following ingredients were used: beef (shoulder clod) (77.9%) ice flakes (15%), homogenized pork fat (F1: 5%; F2: 3.75%; F3: 2.50%; F4: 1.25% and F5: 0%), sodium chloride (1.5%), onion powder (0.2%), garlic powder (0.2%) and black pepper (0.2%).

To hamburgers elaboration, meat (approximately 14 kg) was ground in a meat grinder (C.A.F., Brazil) in 3 mm disk and temperature about 4 ºC. Subsequently, ground beef was homogenized in commercial blender (Super Cutter Sire, Brazil) for 1 minute. Onion and garlic powder, black pepper, sodium chloride, ice flake and pork fat were added to mixture and homogenized again for 3 minutes at temperature 7 ± 1 ºC.

FOS was incorporated into mass and homogenized for additional 3 minutes at 7 ± 1 ºC. Addition levels of ground beef and FOS varied in each formulation as described above. The resulting mass of each formulation was burger shaped (weight 100 g, 10 cm in diameter and 1 cm thick) using manual hamburger press (Picelli, HP 128, Brazil). Products were stored in plastic bags of low-density polyethylene and frozen in conventional freezer (-18 ºC) until the analysis moment.

Frozen burgers were grilled on electric plate with grill on upper and lower sides (Britânia Grill, Mega 2N, Brazil) heated to 200 ºC. Hamburger internal temperature was controlled by digital thermometer (Tp 101, Brazil) until reaching 71 ºC at its geometric center [20]. The average cooking time was 7 to 8 minutes.

2.2. Consumer study

Participated in sensory analyses 80 untrained volunteer subjects, hamburger usual consumers. Consumers had aged between 18 and 29 years and were recruited among students and staffs of Universidade Estadual do Centro-Oeste (UNICENTRO), Guarapuava, Paraná, Brazil. For conducting the sensory test, hamburgers have been cooked as previously described. All samples were evaluated by means of acceptence test using 9-point hedonic scale, with extremes ranging from: dislike extremely (1) to like extremely (9) [21]. Were evaluated attributes related to appearance, aroma, flavor, color and texture, beyond overall acceptence. For purchase intent test 5-point attitude structured scale was used, varying from: definitely would not buy it (1) to definitely would buy it (5) [21]. Sensory acceptability index (AI) was calculated by multiplying average score reported by consumers to product by 100, dividing result by the maximum score given to product within the hedonic scale for 9.0 points. Each sample was served to consumers in white plates coded with randomly selected 3-digit numbers in monadic form and using balanced design [22]. Sensory evaluations were performed by consumers under fluorescence lighting. After consuming each sample, consumer was instructed to drink water for palate cleansing. Samples were evaluated in triplicate in separate session.

2.3. Physicochemical composition

All analyzes were performed on three replicates in triplicate for cooked hamburger, FOS and pork
fat. Water activity (Aw) was determined using Aw analyzer (Novasina, Labswift model, Switzerland), at 20 °C. pH was measured using pH-meter (Tecnopon, MPA-210 model, Brazil). To establish color, five hamburgers were used per treatment, evaluated in five different hamburgers points. Color was evaluated by system of Commission Internationale de L'Eclairage (CIE), lightness ($L^*$), redness ($a^*$), yellowness ($b^*$), colorimeter reading (Konica Minolta, Chroma Meter CR 4400 model, Japan) with illuminating calibration D65 and angle of observation 10°, previously calibrated.

Moisture, ash, protein, fat and dietary fiber content were determined by AOAC methods [23]. Moisture content was determined by drying in greenhouse (105 ± 2 ºC). Fat content was determined according to Soxhlet method, using petroleum ether. Protein was analyzed according to Kjeldahl method. Factor 6.25 was used for nitrogen conversion to crude respectively. Ash was performed by muffle furnace. Total, soluble and insoluble dietary fiber was determined by enzymatic method. Carbohydrate content was evaluated by means of theoretical calculation (by difference) in triplicates results, according to the formula:

$$\% \text{ carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ lipid} + \% \text{ ash} + \% \text{ fiber dietary})$$

Total caloric value (kcal) was calculated theoretically using Atwater factors [24] for lipid (9 kcal g$^{-1}$), protein (4 kcal g$^{-1}$) and carbohydrate (4 kcal g$^{-1}$).

### 2.4. Technological characteristics

Five hamburgers from each formulation were cooked in same procedure as mentioned previously then cooled to room temperature at 23 ºC for 2 h. Following cooking characteristics were evaluated: cooking yield and fat retention [25], shrinkage [26] and moisture retention [27]. All experiments were done in triplicate. Hamburgers were measured according to following equations:

$$\% \text{ cooking yield} = \frac{\text{weight of cooked sample}}{\text{weight of raw sample}} \times 100$$

$$\% \text{ fat retention} = \frac{(\text{weight of cooked sample}) \times (\% \text{ fat in cooked sample})}{(\text{weight of raw sample}) \times (\% \text{ fat in raw sample})} \times 100$$

$$\% \text{ shrinkage} = \frac{(\text{diameter of raw sample} - \text{diameter of cooked sample})}{\text{diameter of raw sample}} \times 100$$

$$\% \text{ moisture retention} = \frac{\% \text{ cooking yield} \times \% \text{ moisture content of cooked sample}}{100}$$

### 2.5. Statistical Analysis

Results were analyzed in randomized blocks using analysis of variance (ANOVA). The means were compared by Tukey’s test at 5% significance level ($p \leq 0.05$). The Software R was used to perform statistical calculations.
2.6. Ethical Issues

Study was approved by the Ethics in Research Committee of UNICENTRO, Brazil, under the number 2.451.570/2017.

3. Results and discussion

3.1. Consumer study

Sensory results test of cooked hamburgers added at different FOS levels are described in Table 1. Higher scores (p<0.05) for appearance, flavor, overall acceptance and purchase intention attributes were found for sample F1 compared to F5. There was no significant difference (p>0.05) between other samples. Formulations F1, F2 and F3 were better accepted than F5 for flavor. The rest samples had similar acceptance in this attribute. Hamburger texture and color were not influenced by FOS addition (p>0.05). Similar results were observed in sausage with FOS addition [28,29,30] and mortadella with inulin addition [31]. According Salazar et al. [29], FOS addition in meat products generally does not modify sensory characteristics. Thus, changes observed in hamburger can be attributed to the fat reduction, as it negatively influences some sensory aspects, reducing acceptability of the product. In the flavor case, low fat level may decrease concentration of fat-soluble compounds [32], such as aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids and esters [33], which are released during chewing [34], which reduces acceptance. Similarly, this have occurred with aroma parameter, as lipids act as solvents for aromatic compounds [35]. These results corroborate with Olivares et al. [36] who studied low-fat sausage.

Product appearance was hampered by fat reduction, although consumers did not notice significant differences in texture and color. Fat-reduced hamburgers looked drier, as also reported by Bolumar et al. [37] studying 35% fat reduced in sausages. Despite the lower grades attributed to formulation with addition of 5% FOS, all samples presented AI above 70%, classifying them with good sensory acceptance [38]. Thus, it is demonstrated the feasibility of adding FOS as a fat substitute in hamburger, which promotes healthier food intake.

Table 1 - Sensory scores (mean ± standard deviation) obtained for hamburger with different levels addition of fructooligosaccharide (FOS)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0%</th>
<th>1.25%</th>
<th>2.50%</th>
<th>3.75%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>7.70±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.23±1.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.42±1.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.19±1.27&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.06±1.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI (%)</td>
<td>85.56</td>
<td>80.33</td>
<td>82.44</td>
<td>79.89</td>
<td>78.44</td>
</tr>
<tr>
<td>Aroma</td>
<td>7.76±1.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.68±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.72±1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.36±1.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.09±1.49&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI (%)</td>
<td>86.22</td>
<td>85.33</td>
<td>86.55</td>
<td>81.78</td>
<td>78.78</td>
</tr>
<tr>
<td>Flavor</td>
<td>7.85±1.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.49±1.42&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.56±1.41&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.23±1.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.13±1.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI (%)</td>
<td>87.22</td>
<td>83.22</td>
<td>84.00</td>
<td>80.33</td>
<td>79.22</td>
</tr>
<tr>
<td>Texture</td>
<td>7.14±1.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9±1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.94±1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.91±1.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.78±1.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI (%)</td>
<td>79.33</td>
<td>76.67</td>
<td>77.11</td>
<td>76.77</td>
<td>75.33</td>
</tr>
<tr>
<td>Color</td>
<td>7.53±1.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.14±1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.20±1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.08±1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.11±1.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI (%)</td>
<td>83.67</td>
<td>79.33</td>
<td>80.00</td>
<td>78.67</td>
<td>79.00</td>
</tr>
</tbody>
</table>
3.2. Physicochemical composition

The physicochemical composition results of cooked hamburgers added at different levels of FOS are presented in Table 2. Prebiotic addition to hamburger proportionally increased moisture, carbohydrate and fiber content in product, however reduced lipid and calorie content. This is because FOS is free of lipid and contains low calorie contribute (1.46 kcal g\(^{-1}\)) [39] compared to pork fat that has 0.8 kcal g\(^{-1}\) lipid and 7.86 kcal g\(^{-1}\) [40]. There was no significant difference (p>0.05) between ash and protein content of the formulations, since both FOS [39] and pork fat [40] do not contain these nutrients in their composition. Higher moisture content in F5 is due to hygroscopic capacity of soluble fibers present in FOS (98%), which retain water within the hamburger protein matrix [41]. Similar results have been found in other surveys with the prebiotics addition in meat products [31,30,42].

There was no statistical difference in Aw results between samples, as already reported in literature [28,30]. FOS addition levels up to 2.5% increased pH in hamburger, while higher levels reduced this parameter. Higher FOS contents are likely to increase the lactic acid formation by the bacteria present in meat, which reduces pH in product [43,44]. Similar results were observed in sausage with addition of 0 to 30% inulin and pectin [8].

Table 2 - Physicochemical composition (mean ± standard deviation) of cooked hamburger with addition of different levels fructooligosaccharide (FOS)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0%</th>
<th>1.25%</th>
<th>2.50%</th>
<th>3.75%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (g 100 g(^{-1}))</td>
<td>52.69±0.04(^a)</td>
<td>54.95±0.05(^d)</td>
<td>56.05±0.09(^c)</td>
<td>56.55±0.06(^b)</td>
<td>62.95±0.08(^a)</td>
</tr>
<tr>
<td>Ash (g 100 g(^{-1}))(^a)</td>
<td>3.53±0.08(^a)</td>
<td>3.56±0.07(^a)</td>
<td>3.54±0.05(^a)</td>
<td>5.33±0.08(^a)</td>
<td>5.37±0.09(^a)</td>
</tr>
<tr>
<td>Protein (g 100 g(^{-1}))</td>
<td>50.55±0.10(^a)</td>
<td>50.58±0.08(^a)</td>
<td>50.66±0.11(^a)</td>
<td>50.69±0.12(^a)</td>
<td>50.57±0.10(^a)</td>
</tr>
<tr>
<td>Lipid (g 100 g(^{-1}))</td>
<td>28.79±0.08(^a)</td>
<td>27.81±0.09(^e)</td>
<td>25.90±0.10(^d)</td>
<td>24.12±0.06(^d)</td>
<td>20.38±0.08(^c)</td>
</tr>
<tr>
<td>Carbohydrate (g 100 g(^{-1}))</td>
<td>15.31±0.19(^c)</td>
<td>16.25±0.21(^d)</td>
<td>18.10±0.18(^c)</td>
<td>19.86±0.15(^b)</td>
<td>23.68±0.13(^a)</td>
</tr>
<tr>
<td>Energy value (kcal 100 g(^{-1}))</td>
<td>522.55±0.88(^a)</td>
<td>517.61±0.53(^b)</td>
<td>508.14±0.45(^c)</td>
<td>499.29±0.47(^d)</td>
<td>480.42±0.63(^e)</td>
</tr>
<tr>
<td>Soluble fiber (g 100 g(^{-1}))</td>
<td>ND</td>
<td>1.28±0.13(^d)</td>
<td>2.55±0.15(^c)</td>
<td>3.83±0.18(^b)</td>
<td>5.10±0.17(^a)</td>
</tr>
<tr>
<td>Insoluble fiber (g 100 g(^{-1}))</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total fiber (g 100 g(^{-1}))</td>
<td>ND</td>
<td>1.28±0.13(^d)</td>
<td>2.55±0.15(^a)</td>
<td>3.83±0.18(^a)</td>
<td>5.10±0.17(^a)</td>
</tr>
<tr>
<td>pH</td>
<td>5.84±0.02(^b)</td>
<td>5.98±0.03(^a)</td>
<td>6.06±0.03(^a)</td>
<td>5.55±0.08(^c)</td>
<td>5.63±0.06(^c)</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.95±0.02(^a)</td>
<td>0.95±0.03(^a)</td>
<td>0.95±0.04(^a)</td>
<td>0.95±0.07(^a)</td>
<td>0.95±0.05(^a)</td>
</tr>
</tbody>
</table>

Different letters in the same row differ significantly by the Tukey's test (p<0.05); AI: Acceptability Index.

Results for objective color analysis for hamburgers are presented in Table 3. The FOS addition
significantly increased the $L^*$ values as FOS have shown higher brightness (98.02 ± 0.4) than pork fat (74.43 ± 0.43). However, there was no change in $a^*$ and $b^*$ values (p>0.05). In hamburger, formation of a translucent whitish gel from the prebiotic fiber prevents changes in values of $a^*$ and $b^*$ [28], which preserves characteristics of the standard product. Similar effects were reported by Menegas et al. [45] after addition of 7% inulin in fermented chicken sausage.

Table 3 - Color parameters $L^*$, $a^*$ e $b^*$ (mean ± standard deviation) of cooked hamburgers with addition of different levels fructooligosaccharide (FOS)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Lightness ($L^*$)</th>
<th>Redness ($a^*$)</th>
<th>Yellowness ($b^*$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>45.34±1.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.36±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.34±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.25%</td>
<td>47.59±1.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.26±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.37±0.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.50%</td>
<td>47.51±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.28±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.35±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.75%</td>
<td>47.29±1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.20±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.32±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5%</td>
<td>50.38±1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.21±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.38±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Distinct letters in the same column differ significantly by the Tukey's test (p<0.05).

3.3. Technological characteristics

Hamburgers technological characteristics results with addition of different levels fructooligosaccharide (FOS) are show in Table 4. The FOS addition to hamburgers proportionally increased cooking yield, fat retention and moisture values. Only 5% increment of FOS increased (p<0.05) the product % shrinkage. The soluble short chain fibers present in FOS interact with meat proteins forming a network that prevents water migration from product to surface [46]. Thus, there was increase in moisture retention and, consequently, in yield of hamburger cooking. Nevertheless, the use of very low levels fat may negatively affect technological characteristics of this product [47,48,16]. In meat products, fat provides flavor and texture, further promotes emulsion of ingredients while maintaining structure of food. Thus, it is possible to explain the greater shrinkage found in F5, since it is fat free, which increases water loss [49]. There was greater fat retention in hamburgers added with FOS (F3, F4 and F5, p<0.05), corroborating Tornberg et al. [50], Berry [26] and Troy et al. [47]. Hamburgers with higher fat content have higher fat loss during cooking. This is due to the greater likelihood that fat droplets will meet and expand since these products naturally have a higher lipid content. In addition, protein matrix of low lipid hamburger prevents fat from escaping from the product [50].
Table 4 - Technological characteristics (mean ± standard deviation) of cooked hamburger with addition of different levels fructooligosaccharide (FOS)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Cooking Yield (%)</th>
<th>Fat Retention (%)</th>
<th>Shrinkage (%)</th>
<th>Moisture Retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>30.57±1.48</td>
<td>57.25±0.83</td>
<td>15.34±1.30</td>
<td>15.97±0.68</td>
</tr>
<tr>
<td>1.25%</td>
<td>31.36±1.76</td>
<td>57.07±0.85</td>
<td>16.70±2.80</td>
<td>17.23±0.97</td>
</tr>
<tr>
<td>2.50%</td>
<td>32.91±1.00</td>
<td>65.43±0.88</td>
<td>16.77±3.02</td>
<td>18.45±0.56</td>
</tr>
<tr>
<td>3.75%</td>
<td>33.34±1.11</td>
<td>68.83±0.99</td>
<td>16.40±1.04</td>
<td>18.85±0.63</td>
</tr>
<tr>
<td>5%</td>
<td>36.71±1.09</td>
<td>68.76±0.78</td>
<td>20.56±0.37</td>
<td>23.11±0.69</td>
</tr>
</tbody>
</table>

Distinct letters in the same column differ significantly by the Tukey's test (p<0.05).

4. Conclusions

It is concluded that FOS can be used as fat substitute in beef hamburger, withal it improves its nutritional profile, increasing carbohydrate and dietary fiber content and, reducing lipid amount and calories in product. It also has favorable influence on technological parameters of hamburger, increasing the cooking yield and moisture retention. Replacing up to 3.75% fat with hamburger FOS maintains sensory acceptability similar to standard product, with good commercialization expectations.

5. Acknowledgement

Authors thanks Araucaria Foundation of Supports Scientific and Technological Development in the Parana State - Brazil for financial support.

6. References

Trends Food Science and Technology, 44 (2) 177-188, 2015.


