# Hominy Feed Value for Bioethanol and Bakery Functional Ingredient

Mara L. Pereira[1], Manuel Malfeito-Ferreira[2], Adriana Macri[3], Nastasia Belc[3] and Isabel Sousa[1,*]

[1]*CEER–Biosystems Engineering Research Centre,* [2]*CBAA - Centre for Botany Applied to Agriculture, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa–Portugal*

[3]*National R&D Institute for Food Bioresources Baneasa, Ancuta 5, Bucharest, Romania*

**Abstract:** To increase the value of hominy feed, a by-product from corn milling, its potential for oil extraction and fermentation to produce ethanol was study. The novelty of the work was to study the use of the fermentation residue as an enhancing ingredient in bakery products. The fermentation was conducted by simultaneous saccharification and fermentation as well as saccharification followed by fermentation. After 48 h of fermentation of 30 % (w/ v) defatted hominy feed aqueous solution, ethanol concentration varied from 7.8 to 12.5 % (v/ v). The post-fermentation residue showed an increase in protein and fiber contents, from 10 % to 30 % and 14 % to 26 %, respectively, when compared with the source material. This residue presented a significant level of antioxidants, around 620 µmol/ 100g. When this residue was incorporated into bread, rolls and cookies at levels of 7%, an increment of 23% of protein content can be reached and up to 50% of total fiber as well. This fiber increment corresponded to 47% of soluble and 19% of insoluble fibers. However, a negative impact onbread volume was observed: a reduction of 1.5% of diameter, 17 – 19% of volume, 18 – 24% of height, 9 – 12% of porosity and 6–11% of elasticity. But surprisingly, the bread life time increased 100%, *i.e.*, the rolls looked as fresh as just baked, the day after.

**Keywords:** Hominy feed, Ethanol, Fermentation, Functional ingredient and Bakery products.

## 1. INTRODUCTION

Human kind faces important decisions in order to mitigate the impacts of climate change, and reduce greenhouse gas emissions (GHG's). This situation could be dramatically improved by using renewable, clean and more sustainable fuels to replace fossil fuels [1]. Coppola *et al*. [2] predict that the European market will increase the consumption of biofuels from its current 2%, up to 25% by 2030; therefore this issue was the subject of a European Directive nº 2003/30-/CE of 8th May.

Time bound targets for biofuel consumption, accelerated the interest in biofuels production [3]. However, first-generation biofuels can have a great impact on the structure and distribution of agricultural production, agricultural trade and the welfare of different households [4, 5].On average, about two-thirds of the cereals used for ethanol production are obtained from additional crop production and the remaining one-third comes from consumption changes [3].Excess demand for biofuels feed stocks increased world food prices for food and feed cereals by some 140 %, between 2002 and 2007 [3]. Nevertheless, this situation can be minimized if biofuels are produced from industrial by-products of the cereal processing manufactures.

In today's market, hominy feed (HF) is a cost-effective by-product and represents about 35% of the corn industry production volumes. It has a high percentage of starch, ranging from 37.5 up to 55.2% [6], which represents a huge potential for the conversion into ethanol, without direct competition with feed and food production.

On the other hand, functional foods that have the potential to impact positively on health are an increasing market. The increased consumption of functional ingredients in bakery products increases the quantity of fiber in the diet [7]. Cereals provide the high starch content as energy source, dietary fiber, nutritious protein and lipids rich in essential fatty acids [8]. Additionally, bread as a staple food has become a vehicle for functional ingredients [9].

These products provide new market opportunities, but drivers for acceptance of such foods are not yet well understood [10]. Consumers are rarely willing to compromise on the taste of functional foods for health benefits [11], but prefer buying enriched foods where the combination of carrier and ingredient appears natural, rather than artificial [12], preferably conveyed

*Address correspondence to this author at the CEER–Biosystems Engineering Research Centre, Instituto Superior de Agronomia. University of Lisbon, Tapada da Ajuda. 1349-017 Lisboa–Portugal, Tel: + 351 21 3653246; Fax: + 351 21 3653195; E–mail: isabelsousa@isa.utl.pt

by traditional staple foods. Some works, refer that incorporation of fiber to cookies enrichment, in levels up to 10%, still produce cookies with similar characteristics of the standard ones [13].

The aims of this study were to evaluate the potential of HF obtained from a Portuguese corn milling industry for ethanol production, determine the nutritional value of the fermentation residue, and investigate the possibility of using this residue in bakery products as a functional ingredient.

## 2. MATERIAL AND METHODS

### Hominy Feed Composition

Hominy feed (HF) was obtained from a corn milling industry located at Torres Vedras – Portugal. The samples were analyzed for moisture (method PAQF 120.2), starch (method BOE207748), oil (NP 876: 2004), fiber (MI LAQ 103.02), protein (method PAFQ 360.1) and cellulose (method EN ISO 6865: 00).

The HF composition was evaluated at two different times: before oil extraction and after fermentation.

### Oil Extraction

To increase the yield of oil extraction the HF was grounded and was sieved to obtain a particulate fraction up to 0.5 mm (Filtra Vibración–Model FTL-0200, Barcelona–Spain). The HF was defatted by Soxhlet extraction with hexane, for8 h at 80 ºC into defatted hominy feed ($HF_{DF}$).

### Fermentation Assays

Stepwise hydrolysis and fermentation [SHF] and simultaneous saccharification and fermentation assay [SSF], both combined with the application of ultrasounds [US] after enzymes addition [14, 15], with 60 ppm of $Ca^{2+}$as $CaCl_2$. $H_2O$ - Merk, Germany [Ca] [14] and with doubled amount of enzymes [DE], were tested (Figure **1**).

SHF assay was carried out in accordance to the work of Mojović [14] and Sharma [6]. $HF_{DF}$ was mixed with water to obtain 30% (w/v) in the slurry; the mixture was treated with enzymes in two steps. In the first step, liquefaction was performed at 85ºC during 1h at pH 6 [14, 16] with 0.5% v/v of α-amylase (Liquozyme Supra –Novozymes), and the second step, saccharification, was performed at 55ºC, 4h, pH 5 with 0.25% v/v glucoamylase (Optimax 4060 VHP, Genencor). This mash was subject to ethanol fermentation by $10^8$ cfu/ ml *Saccharomyces cerevisiae* ISA 1000 (PYCC 4072), at 30ºC during 48h, with stirring at a rate of 350rpm.

The SSF assay was conducted following the protocol of Sharma *et al*. [6], in conditions described previously for the SHF assay, but using both enzymes at once, with simultaneous hydrolysis, saccharification and fermentation, at 30ºC during 48h, with a stirring rate of 350rpm.

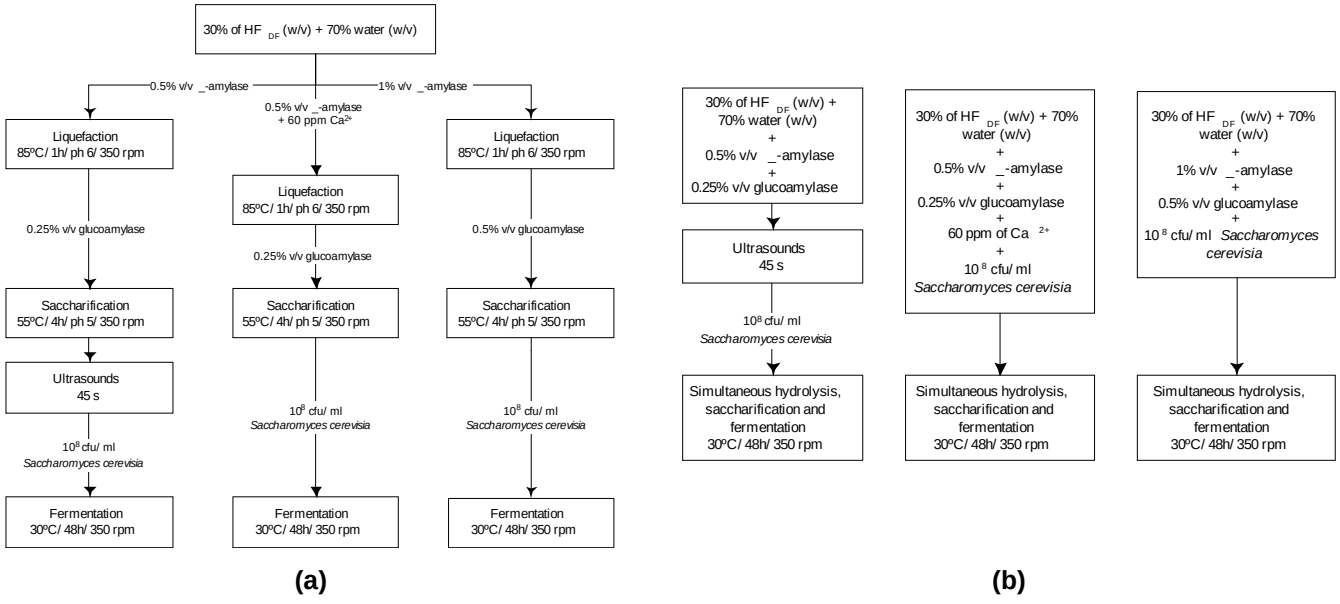



**Figure 1:** Representation of Separate Hydrolysis and Fermentation (SHF) **(a)** and Simultaneous Saccharification and Fermentation (SSF) **(b).**

### Determination of Fermentation Products

Fermentation progress was monitored by HPLC analysis using the protocol described by Sharma *et al*. [6], the mash samples were split into a two 1.5ml samples and centrifuged for 10min at 12000rpm, to obtain clear supernatant liquid. Supernatant was passed through a 0.2µm sterile syringe filter into 1ml shell vial. Filtered liquid was injected into a chromategraph column (Shodex SH 1011, Waters, USA) maintained at 50ºC. HPLC grade water containing 5mm sulphuric acid was used as the mobile phase. The column elution rate was 0.6ml/min. Separated components (glucose, glycerol, acetic acid and ethanol) were detected with a refractive index detector (Model 2410, Waters, USA). Data were processed using HPLC software (Empower 2).

### Determination of Stability of Post Fermentation Hominy Feed ($HF_{PF}$)

After fermentation, the residue was dried by lyophilization. To evaluate the stability of $HF_{PF}$ microbial analysis, micotoxin content and antioxidant capacity were measured.

#### *Microbial Analysis*

Aerobic mesophilic counts were made on plate count agar (PCA) (Biokar Diagnostics, Beauvais, France) and incubated at 30ºC ± 1ºC for 3days, as described by Microbiological Methods of the Member Companies of the Corn Refiners Association.

Yeasts and moulds were determined on Chloramphenicol Rose Bengal Agar Base (CRB) (Oxoid, Basingstoke, Hampshire, England), supplemented with chloramphenicol (100mg/L) and incubated during 5days at 25ºC [17].

Anaerobic and aerobic thermophilic counts were performed as described by Microbiological Methods of the Member Companies of the Corn Refiners Association. The counts were carried out on PCA (Biokar) and incubated at 30ºC ± 1ºC for 3days, after boiling at 80ºC during 10min. To anaerobic thermophilic counts the incubation occurred in anaerobic jars.

#### *Micotoxin Content*

To detect and quantify deoxynivalenol (DON) and total aflatoxins in $HF_{PF}$ the test Enzyme-linked immunosorbent assay (ELISA) was used.Imunoenzymatic kit test Ridascreen DON and Ridascreen Aflatoxin (R-Biopharm AG, Darmstadt, Germany) were used in order to detect DON and total aflatoxins, respectively. To detect DON an amount of 5g of ground sample was weighted and added to 25ml distilled water. The samples were than homogenised vigorously using a GFL 3017 shaker (GFL-Gesellschaft für Labortechnik mbH, Germany) for three minutes at 180rpm. The extract was filtered through Whatman No. 1 filter paper (What man International Ltd., UK). To detect aflatoxins,2g of the ground sample were weighted and mixed with a methanol/distilled water (70/30; v/v) solution for 10min at room temperature, using a GFL 3017 shaker (GFL-Gesellschaft für Labortechnik mbH, Germany). The extract was filtered and diluted 1:7 with distilled water. To quantify DON and aflatoxins 50µL of each filtrate were employed in the assay and absorbance of the samples was measured at 450nm using a Sunrise™ multichannel absorbance plate reader (Tecan Group Ltd., Germany).

#### *Antioxidant Capacity*

To evaluate the antioxidant capacity of $HP_{PF}$ at different conditions of drying and packaging, the residue was eitherfreeze-driedor dried in the oven at 55 ºC with ventilation. $HP_{PF}$ was then stored during 15 days both at open air and packed in polyethylene transparent, or polyethylene with metallic barrier bags.

Free radical scavenging activity assay, 2, 2-Dipheny-1-picryhydrazyl radical (DPPH), was performed in accordance to Brand-Williams, Cuvelier and Berset (1995) with various modifications. The 5mg were reacted with 4mL of DPPH solution (20% DPPH solution freshly prepared in 80% ethanol solution) for 40min. The absorbance (A) values at 517nm were read against a blank of DPPH + ethanol. The antioxidant activity was calculated using equation 1:

$$\%DPPH = \left[1 - \left(\frac{A_{Sample,\, t=40\ min}}{A_{Control,\, t=40\ min}}\right)\right] \text{x } 100 \quad (1)$$

The antioxidant activity was measured at time 0 and after 15days of storage in different conditions, for both oven and freeze-dried samples.

Statistical analysis was performed using STATISTICA version 7, using t-test for independent samples to determine if there was a significant difference ($p < 0.05$) within the results.

### Incorporation of $HF_{PF}$ in Bakery Products

To test the effect of incorporation of $HF_{PF}$ in bakery

**Table 1: Levels of Incorporation of $HF_{PF}$ and Conditions for Preparation of Bakery Products**

| | % $HF_{PF}$ | Sour Fermentation | Dough Fermentation | Proofing Dough | Baking Pieces of Dough |
|---|---|---|---|---|---|
| Cookies | 0, 5 and 7 | - | - | - | 25–30 min/ 210–220 ºC |
| Rolls | 0, 7 | - | 15–20 min/ 28–30 ºC | 35–40 min/ 28–30 ºC | 30 min/ 220–225 ºC |
| White bread | 0, 5 and 7 | - | 40 min/ 28–30 ºC | 35–40 min/ 28–30 ºC | 30 min/ 220–225 ºC |
| Rye bread | 0, 7 | 120 min/ 28 ºC | 40 min/ 28–30 ºC | 35–40 min/ 28–30 ºC | 25–30 min/ 210–220 ºC |

products, four products were formulated: cookies, rolls, wheat bread and rye bread. The bakery products were prepared according to the [18]. The levels of incorporation of $HF_{PF,}$ and conditions for the manufacture of the bakery products are shown in Table**1**.

The bakery products made with $HF_{PF}$ and the control were evaluated for different quality parameters: weight, volume, diameter, height, relation height/diameter (H/D), porosity and elasticity [19]. To evaluate the benefit of incorporation of $HF_{PF}$, these products were analyzed for moisture (gravimetric), ash [20], proteins [21], total fiber, soluble and insoluble fiber [19]. These measurements were performed three times for each bakery product and mean values are reported.

### *Texture Measurements*

Texture of rolls, after 48h of production, were measured by puncture in a texturometer TA-XT plus (Stable Micro Systems, United Kingdom) with a cylindrical probe of 11mm diameter, at a speed of 1mm/s. The resistance to penetration was measured by the maximum force (in Newton) from the peak on the texturogram. Texture measurements were repeated five times for each sample and mean values are reported.

## 3. RESULTS AND DISCUSSION

### Hominy Feed and Post-Fermentation Hominy Feed Composition

The centesimal composition of hominy feed is shown in Table**2**. Ash and protein values were within the range reported by Raush and Belyea [22] and Sharma *et al*. [6], where values for ash varied between 0.7 and 3.2% and for protein between 9.5 and 12.3%. Our value for starch is higher than the results commonly reported in the literature, *e.g*.56.9% by Larson *et al*. [23]. When compared with these values, the concentration of fiber and fat in our hominy feed material were also slightly higher. The level of fiber reported by Larson *et al*. [23] corresponds to 6.7% compares to 7.1% of our results. The quantity of fat reported by Larson *et al*. [23], Raush and Belyea [22] and Sharma *et al*. [6] vary from 2.7 to 8.3%, in our case the level of fat in HF corresponded to 12%.

**Table 2: Centesimal Composition of Hominy Feed and Post-Fermentation Hominy Feed Residue, in Dry Basis**

| Compound | % Dry basis ± SD | |
|---|---|---|
| | HF | $HF_{PF}$ |
| Moisture | 11.9%±2.5% | 9.3 %±3.5% |
| Ash | 2.7±0.5 | 7.6±0.9 |
| Protein | 9.9±0.7 | 29.7±3.7 |
| Digestible protein | - | 23.6±2.5 |
| Fiber | 7.1±2.3 | 25.8±4.8 |
| Starch | 68.8±0.1 | 16.3±2.2 |
| Cellulose | 2.0±0.3 | 5.8±0.7 |
| Fat | 12.5±0.2 | - |

In Table **2** the composition of $HF_{PF}$ is also shown. Fermentation was effective in increasing the nutritional value of hominy feed: 20% protein, 24% digestible protein and 19% fibre. In $HF_{PF,}$ the digestible protein represents 80% of the total protein.

This $HF_{PF}$ residue has very high fibre levels which are a limiting restriction for the use in ruminant diets [22]. However, this residue can be incorporated, with benefits to health, into bakery products in human diets.

### Oil Extraction

The oil extraction from our HF samples led to 12% of fat (in dry basis). If one compares to the results for

composition on Table **2**, 12.5 % ± 0.2, it can be said that the yield for solvent oil extraction, in the described conditions, is over 95%.

### Ethanol Yield

On the SHF assay, it was possible to monitor the amount of glucose, at the end of saccharification, we could reach yields between 106 to 138g/L (Table **3**). Our values were higher than values reported by Nikolić [16] which varied from 98 to 105g/L, and within the range reported by Khanal [24], of 50 – 160g/L.

**Table 3: Glucose Concentration during Hydrolysis and Fermentation Stepwise, Combined with the Application of Ultrasounds after Enzymes Addition (SHF_US); Enrichment with 60 ppm of $Ca^{2+}$ (SHF_Ca) and Double Amount of Enzymes (SHF_DE)**

| Assay Conditions | Glucose Concentration (g/ L) | | |
|---|---|---|---|
| | Initial | End of Hydrolysis | End of Saccharification |
| SHF_US | 5.1±1.2 | 15.1±4.0 | 125.0±7.4 |
| SHF_Ca | 4.4±0.7 | 15.4±2.6 | 115.4±9.3 |
| SHF_DE | 4.6±0.3 | 14.3±3.5 | 124.7±12.9 |

During the SSF assay, the glucose content in the mash was not detected after 24h of fermentation indicating rapid sugar utilization by yeasts used for the ethanol production.

After 48h of fermentation, ethanol yields of 7.8 and 12.5% (v/v) were obtained from the hominy feed (Figure **2**). The highest ethanol levels12.1% and 12.5% (v/v), were virtually achieved in the assays were hydrolysis, saccharification and fermentation occurred simultaneously, for SSF with double amount of enzymes and with calcium ions, respectively. But statistically they do not show any significant difference ($p>0.05$). This value was similar to ethanol concentrations founded by Brethaven [25], which ranged from 11 to 12% v/v. The lowest ethanol yield was found at the control sample of SHF (7.8%).

If we convert the ethanol yield to L ethanol/ton $HF_{DF}$, the range of values goes from 260 up to 500L ethanol/ton $HF_{DF}$. The highest values achieved for the SSF assay with double amount of enzymes and SSF enriched with calcium ions samples, it will go for 444L/ton $HF_{DF}$ and 500L/ton $HF_{DF}$, respectively, which corresponded to a total increase of 11% of the scaled up final yield from the control sample. These values were higher than the values reported by Sharma [6], which reported a maximum of 380L/metric ton.

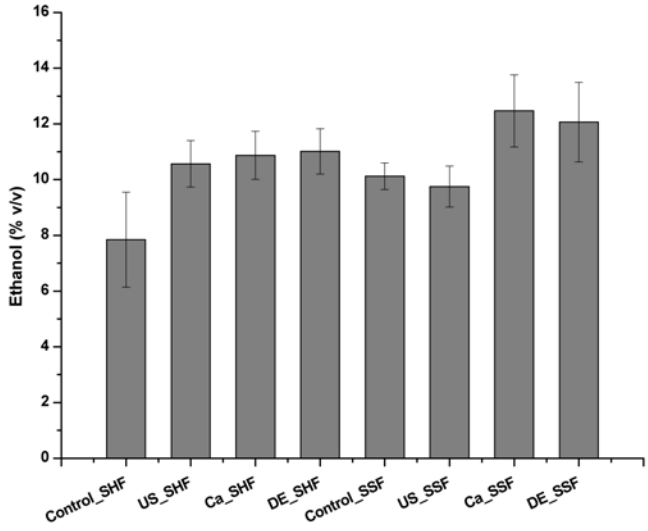



**Figure 2:** Final ethanol concentration for each tested conditions in Stepwise (SHF) and in simultaneous hydrolysis, saccharification and fermentation (SSF): control (control), application of ultrasounds after enzymes addition (US), addition of 60 ppm of $Ca^{2+}$(Ca)and application of the double amount of enzymes (DE).

The secondary products of fermentation were measured in parallel, to monitor test conditions and are given in Figure **3** for glycerol and for acetic acid. The range of glycerol was from 0.67 to 0.82% (v/v) and for the acetic acid 0.07 – 0.14% (v/v). Comparing our results with the values found by Sharma [6], the value of acetic acid was smaller (< 0.5% w/v) but the value of glycerol was somehow higher than the values found by them (< 0.4% w/v).

The highest level of glycerol occurs simultaneously with the highest concentration of ethanol, *i.e*. for SSF experiments with double amount of enzymes and with calcium ions. On the other hand, the highest level of acetic acid occurred at the lowest concentration of ethanol, in the experiments where ultrasounds were applied after enzymes addition.

### Determination of Stability of Post Fermentation Hominy Feed ($HF_{PF}$)

#### *Microbial Analysis*

The microbial counts of $HF_{DF}$ and $HF_{PF}$ are presented in Table **4**, where one can see that $HF_{DF}$ presented a considerable high microbial counting, rangingfrom 2.5 log cfu $g^{-1}$ (in moulds and yeast) to 4.9 log cfu $g^{-1}$ (in aerobic mesophilic). As it was expected, after fermentation, and subsequent enzyme inactivation by boiling for 2 h, the microbial counts in

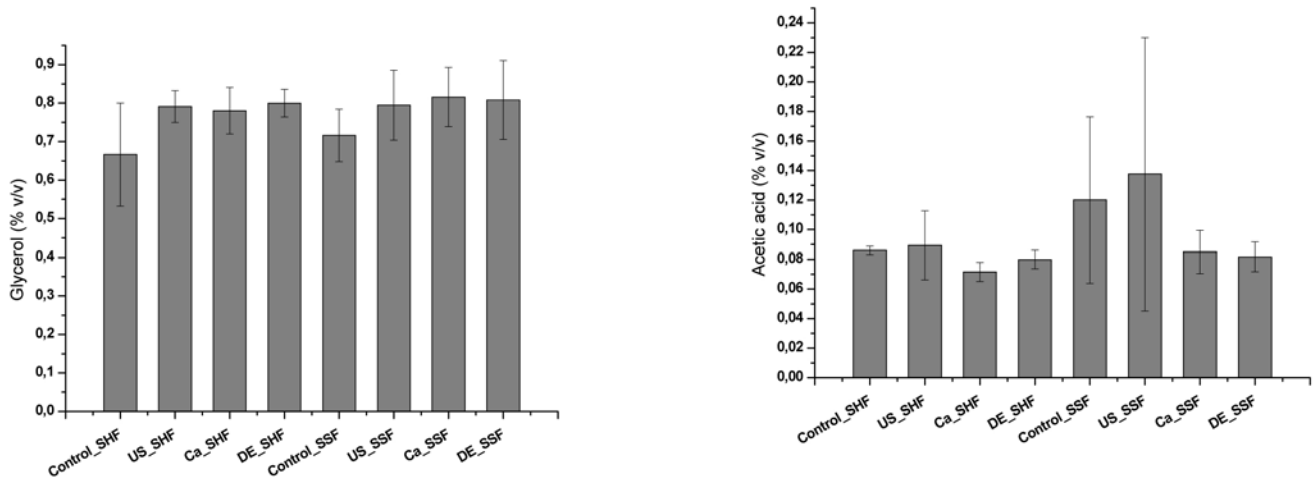



**Figure 3:** Final glycerol concentration and final acetic acid concentration for each tested conditions in separate (SHF) and in simultaneous hydrolysis, saccharification and fermentation (SSF): control (control), application of ultrasounds after enzymes addition (US), addition of 60 ppm of $Ca^{2+}$(Ca)and application of the double amount of enzymes (DE).

$HF_{PF}$ were drastically reduced down to less than 1 log cfu $g^{-1}$. These counts indicate that $HF_{PF}$ is a safe product, which can be incorporated into food products.

**Table 4: Microbial Counts for HFDF and HFPF (CFU/ g)**

| | Average ± SD (log CFU/ g) | |
|---|---|---|
| | $HF_{DF}$ | $HF_{PF}$ |
| Aerobic mesophilic | 4.4±0.3 | <1 |
| Moulds and yeasts | 3.0±0.3 | <1 |
| Anaerobic thermophilic | 3.3±0.6 | <1 |
| Aerobic thermophilic | 3.6±0.6 | <1 |

### *Micotoxin Contents*

Evaluation of the micotoxin content is important because these compounds are thermo resistant and they are not destroyed by fermentation. By the results obtained (Table **5**) for DON and total aflatoxins, and compared with legal values expressed by the Commission Regulation No 1881/ 2006, one can say that $HF_{PF}$ presents a safe level of micotoxinsto be incorporated into food products.

**Table 5: Quantification of DON and Total Aflatoxins in $HF_{PF}$ (ppb)**

| | Average ± SD (ppb) | Maximum Allowed (ppb)[a] |
|---|---|---|
| DON | 258±144 | 750 |
| Total Aflatoxins | 3.0±0.5 | 4.0 |

[a]Commission Regulation (EC) No 1881/ 2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

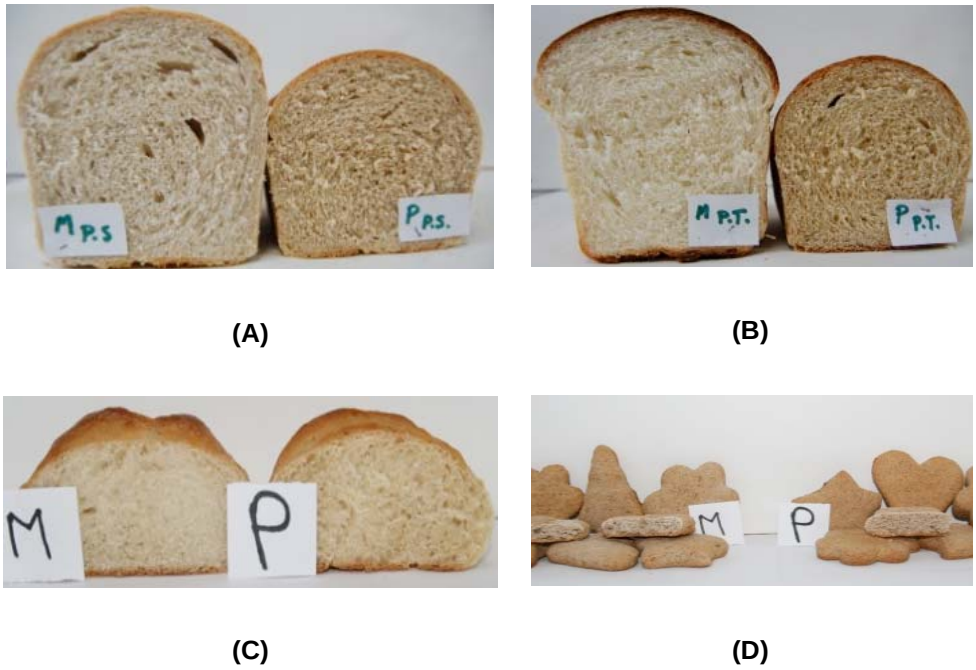
### *Antioxidant Capacity*

The antioxidant capacity for $HF_{PF}$ freeze-dried and oven-dried with ventilation, for two different kinds of packaging, is present in Table **6**, where we can observe, as expected, that $HF_{PF}$ dried at the oven with ventilation shows a lower amount of antioxidants, comparing with $HF_{PF}$ dried bylyophilization. So, for industrial purposes, it is necessary to optimize the drying process to achieve a better compromise between drying process and residual amount of antioxidants in $HF_{PF}$.

**Table 6: Quantity of Antioxidant for $HF_{PF}$ Dried by Lyophilization and in Oven with Ventilation, Packed with Different Types of Packaging Material (µmol/ 100 g)**

| | Antioxidants (µmol/ 100 g) | |
|---|---|---|
| | Start | Storage during 15 Days |
| Freeze dried $HF_{PF}$ | 688±42 | |
| Transparent packaging | | 641±94 |
| Metallic coated packaging | | 619±106 |
| Air | | 361±29 |
| $HF_{PF}$ dried in oven with ventilation | 285±28 | |
| Transparent packaging | | 231±56 |
| Metallic coated packaging | | 193±30 |
| Air | | 178±2 |

After 15 days storage, $HF_{PF}$, showed the highest values of antioxidant when packed in the polyethylene transparent packaging. The amount of antioxidants in




**(A)** **(B)**

**(C)** **(D)**

**Figure 4:** Baking products: **(A)** rye bread (control and 7% $HF_{PF}$), **(B)** wheat bread (control and 7% $HF_{PF}$), **(C)** rolls (control and 7% $HF_{PF}$) and **(D)** cookies (control and 7% $HF_{PF}$).

$HF_{PF}$ dried by lyophilization and packed with transparent polyethylene showed to be significantly higher ($P< 0.05$) than for the other tested cases (Table **6**). Therefore antioxidant capacity of $HF_{PF}$ seemed to be sensitive to the drying process but not to the type of packaging films tested since transparent bag or metallic bag showed no significant difference.

These results show that storage of $HF_{PF}$ can be made at room temperature with simple packaging of transparent polyethylene bags, which is also a barrier to water vapor.

So far, the results indicate that $HF_{PF}$ represents a food supplement that contains remarkable amounts of potent antioxidant activity.

### Incorporation of $HF_{PF}$ in Bakery Products

The images of each baking product are shown in Figure **4.**

After baking, the breads were cooled down to room temperature. Then the weight, volume, diameter, height, porosity and elasticity of breads were recorded (Table**7**)

This study suggests that incorporation of $HF_{PF}$ increases the weight of breads around 3%. The reduction in diameter of breads is near 1.0%, for rye bread and 1.6% for wheat bread. To resume the Table, the influence of addition of $HF_{PF}$, in both formulations, led to a decreased in volume, in height, in porosity and in elasticity of samples. The change in volume and porosity in rye bread was higher than in wheat bread, as expected since rye bread is poor in gluten proteins and has less volume from the start. These reductions correspond to 19% of volume in rye bread and to 17% for the wheat bread, and 12% and 9% of the porosity, respectively.

**Table 7: The Weight, Volume, Diameter, Height, Porosity and Elasticity for Rye and Wheat Bread, Control and with Incorporation of 7 % of $HF_{PF}$**

| | Rye Bread | | Wheat Bread | |
|---|---|---|---|---|
| | **Control** | **7 % $HF_{PF}$** | **Control** | **7 % $HF_{PF}$** |
| Weight (g) | 519 | 534±2 | 526 | 545±1 |
| Volume, $cm^3$/ 100g | 339 | 274±1.0 | 401 | 331±1.4 |
| Diameter (D) (cm) | 15.8 | 15.6±0.2 | 15.9 | 15.6±0.0 |
| Height (H) (cm) | 10.0 | 8.2±0.1 | 11.4 | 8.7±0.1 |
| H/ D | 0.6 | 0.5±0.0 | 0.7 | 0.6±0.0 |
| Porosity (%) | 85.3 | 75.2±0.1 | 86.5 | 78.5±0.7 |
| Elasticity (%) | 95.0 | 89.0±1.0 | 97.0 | 86.5±0.7 |

However, when comparing the height and elasticity of both formulations with the control bread, we observe

that changes were more evident in the wheat bread than in rye bread. The height in wheat bread decreased 24% and in rye bread 18%. With incorporation of 7% of $HF_{PF}$ the elasticity of wheat and rye bread decreased 11% and 6%, respectively.

**Table 8: Chemical Composition of Rolls Control, Rolls with 7% of $HF_{PF}$, Cookies Control and Cookies with 7% $HF_{PF}$, in Dry Basis**

| Compounds | Rolls | | Cookies | |
|---|---|---|---|---|
| (%, Dry Weight Basis) | Control | 7% $HF_{PF}$ | Control | 7% $HF_{PF}$ |
| **Moisture** | **35.40%** | **35.87%** | **17.88%** | **16.71%** |
| Total fiber | 9.4 | 11.5±0.03 | 5.8 | 8.8±0.02 |
| Proteins | 19.6 | 24.1±0.06 | 22.7 | 23.8±0.02 |
| Sucrose | - | - | 27.3 | 22.0±0.05 |
| Ash | 2.5 | 2.6±0.01 | 2.7 | 3.1±0.02 |

Simultaneously, tests were carried out to evaluate the texture of the rolls after 48h of production. The texture of rolls, expressed in terms of hardness (first peak height), of the control samples and rolls with 7% of $HF_{PF}$ was, respectively, 27.5 N ± 8.6 N and 18.0 N ± 2.4 N. These results showed a decrease on texture values for rolls with $HF_{PF}$. This softening of the rolls was persistent over 48h, and these rolls compared well with freshly baked ones. This is a practical result which leads to an increment of the shelf-life of the products with $HF_{PF}$ added.

This result is relevant, since it can have a considerable impact on the baking industry, for exporting or large distance trading of freshly baked goods.

It is important to say that in cookies no relevant impact on quality characteristics was observed when $HF_{PF}$ was added at 7% levels. These products are, by far, less sensitive to volume changes. Furthermore, the results for chemical analysis of rolls and cookies are shown in Table **8**, where it can be observed that the addition of $HF_{PF}$, in both formulations, led to an increase in total fiber content corresponding to 21.6% and 54.4%, in rolls and cookies, respectively, which is consistent with the fiber input by the $HF_{PF}$ addition. In what protein is concerned, the incorporation of 7% $HF_{PF}$ incremented differently the level of proteins in rolls and cookies, up to 22.8% (w/w) and 5.6% (w/w), respectively. This is related to the observed19% sucrose reduction on cookies with 7% $HF_{PF}$, this must be related to the higher specific surface of cookies, corresponding to a large extension of the Maillard reactions per weight unit (surface related reactions), where proteins and sucrose are involved. Furthermore, this is also consistent with the information from the ash content which showed an increment of 6.1% for rolls and 14.2% for cookies with 7% $HF_{PF}$.

## 4. CONCLUSIONS

In this study, the potential for ethanol conversion of HF obtained from a Portuguese corn milling industry and the incorporation of the residue of fermentation in bakery products as a functional ingredient, were evaluated. Using simultaneous saccharification and fermentation, enriched with calcium ions the yield of 12.5% of ethanol was achieved, which represents 500L of ethanol/ton of hominy feed defatted. Therefore, these procedures were effective in increasing the economic value of hominy feed. Besides ethanol production, interesting hominy feed post-fermentation residue, with nutritional/functional potential, was also produced. The final composition of this material is promising for the incorporation in bakery products as a functional ingredient, with 30% of protein, being 24% of digestible protein and 26% of food fiber. The incorporation of post fermentation hominy feed residue in bakery products led to an increase of protein and fiber in the final products. This incorporation reduced the volume of breads, but increased significantly their shelf life as freshly baked products.

## REFERENCES

[1] Gnansounou E. Production and use of lignocellulose bioethanol in Europe: Current situation and perspectives. Bioresource Technology, 2010; 101, 4842–4850.

[2] Coppola, F., Bastianoni, S., Østergård, H. Sustainability of bioethanol production from wheat with recycled residues as evaluated by Energy assessment. Biomass & Bioenergy. 2009; 33,11, 1626–1642.

[3] Anonymous Biofuels and food security – implications of an accelerated biofuels production. International Institute for Applied Systems Analysis. Vienna - Austria. 2009; Pp. 9–24.

[4] Tat Tan K, Lee K T, Mohamed A R. Role of energy policy in renewable energy accomplishment: The case of second-generation bioethanol. Energy Policy, 2008; 36, 3360–3365.

[5] Qiu H, Huang J, Yang J, Rozelle S, Zhang Y, Zhang Y & Zhang Y Bioethanol development in China and the potential impacts on its agricultural economy. Applied Energy, 2010; 87, 76–83.

[6] Sharma V, Moreau R A, Singh V Increasing the value of hominy feed as a co-product by fermentation. Appl Biochem Biotechnol, 2010 149, 145 – 153.

[7] Hellyer N E, Fraser I, Haddock-Fraser J. Food choice, health information and functional ingredients: An experimental auction employing bread. Food Policy, 2012 37, 232 –245.

[8] Kadam S U & Prabhasankar P. Marine foods as functional ingredients in bakery and pasta products. Food Research

International, 2010; 43, 1975–1980.

[9] Sidhu JS, Kabir Y & Huffman F G. Functional foods from cereal grains. International Journal of Food Properties, 2007 10, 231–244.

[10] Krutulyte R, Grunert k G, Scholderer J, Lähteenmäki L, Hagemann K S, Elgaard P, Nielsen B & Graverholt J P. Perceived fit of different combination of carriers and functional ingredients and its effects on purchase intention. Food Quality and Preference, 2011 22, 11–16.

[11] Cox D N, Koster A & Russel C G. Predicting intentions to consume functional foods and supplements to offset memory loss using an adaptation of protection motivation theory. Appetite, 2004 33, 55–64.

[12] Poulsen, JB. Danish consumers' attitudes towards functional foods Nr. 62, Aarhus:MAPP, 1999 pp. 1-49.

[13] Piteira M F, Maia J M, Raymundo A & Sousa I. Extensional flow behaviour of natural fiber-filled dough and its relationship with structure and properties. Journal of Non-Newtonian Fluid Mechanics, 2006 137, 72–80

[14] Mojović L, Nikolić S, Rakin M & Vukasinović M. Production of bioethanol from corn meal hydrolyzates. Fuel, 2006 85, 1750–1755.

[15] Nitayavardhana S, Shrestha P, Rasmussen M L, Lamsal B P, van Leeuwen J H & Khanal S K. Ultrasound improved ethanol fermentation from cassava chips in cassava – based ethanol plants. Bioresource Technology, 2010 101, 2741–2747.

[16] Nikolić S, Mojović L, Rakin M, Pejin D & Pejin J. Ultrasound assisted production of bioethanol by simultaneous saccharification and fermentation of corn meal. Food Chemistry, 2010 122, 216–222.

[17] Portuguese Norm 3277-1: Food microbiology – Count of yeasts and moulds – Part I

[18] American Association of Cereal Chemists (2000) Approved methods of the AACC. The Association, St. Paul MN.

[19] SR 91. Bread and pastry products, popcorn, corn-puff snacks. 2007.

[20] ISO 2171 Cereals, pulses and by-products–determination of ash yield by incineration. (2007).

[21] ISO 20483 Cereals and pulses – Determination of the nitrogen content and calculation of the crude protein – Kjeldahl method. (2006).

[22] Raush K D & Belyea R L. The future of coproduct from corn processing. Applied Biochemistry and Biotechnology, 2006 128, 47–86.

[23] Larson E M, Stock R A, Klopfenstein T J, Sindt M H & Shain D H. Energy value of hominy feed for finishing ruminants. Journal of animal science, 1993 71, 1092–1099.

[24] Khanal, S K, Montalbo, M., van Leeuwen, J H, Srinivasan G & Grewell D. Ultrasound enhanced glucose release from corn in ethanol plants. *Biotechnology and Bioengineering*. 2007 98: 978–985

[25] Brethaven S & Wyman C E. Review continuous hydrolisys and fermentation for cellulosic ethanol production. Bioresource Technology, 2010 101, 4862–4874.

Received on 28-06-2014 Accepted on 09-07-2014 Published on 28-08-2014