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Enhancing the digestibility of cowpea (Vigna unguiculata) by traditional processing and fermentation

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Enhancing the digestibility of cowpea (*Vigna unguiculata*) by traditional processing and fermentation

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ABSTRACT

Flatulence is an important drawback for the consumption of legumes. Therefore, the ability of traditional processing (dehulling, boiling, soaking) and fermentation (bacterial, fungal or yeast) of cowpeas to reduce flatulence was investigated. Raw and processed cowpeas were assessed for their galactose-oligosaccharide content, the amount of gas produced by *Clostridium perfringens* using *in-vitro* cowpea digests as main carbohydrate substrate (*in-vitro* fermentability index) and the alveolar hydrogen concentration of the breath of 18 healthy adults after the consumption of a cowpea porridge breakfast (*in-vivo* fermentability index). Galactose-oligosaccharides could not be detected in cowpea hulls which yielded low *in-vitro* fermentability index as compared with other treatments. Traditional processing induced a limited reduction of raffinose and verbascose content contrary to fermentations. The *in-vitro* fermentability index appeared similarly high for all processed cowpea except after *Rhizopus* and *Bacillus* fermentation. The *in-vivo* fermentability index of fermented cowpeas was significantly lower than that of traditionally processed cowpeas. Consequently, soaking and fermentation in cowpea processing deserve further investigation and promotion.

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1. Introduction

Carbohydrates are the most abundant nutrients in cowpeas (*Vigna unguiculata*). Among the carbohydrates, galactose-oligosaccharides (GOS), resistant starch (RS), and non-starch polysaccharides (NSP) withstand the digestion in the human gastrointestinal tract mainly because of the lack of secretion of some enzymes such as α-galactosidases required for their hydrolysis (Backhed, Ley, Sonnenburg, Peterson, & Gordon, 2005; Tachibe, Ohga, Nishibata, & Ebihara, 2011). Therefore, in the caecum, undigested carbohydrates can undergo fermentation by a diverse anaerobic microflora chiefly composed of *Bacteroides*, *Bifidobacterium*, *Fusobacterium*, and *Clostridium* spp. (Backhed et al., 2005; Simon & Gorbach, 1986). This anaerobic fermentation results in the formation of several gases (hydrogen, carbon dioxide, methane, etc.) and short chain fatty acids (acetate, butyrate, propionate, etc.) (Cummings, 1983; Tadesse & Eastwood, 1978). These gases represent the major fraction of the flatus generated after consumption of legumes whereas hydrogen sulphide and sulphur containing gases account for the fetid flatus (Hasler, 2006). Besides potential digestive disorders that may occur, flatulence caused by cowpea consumption is a natural and harmless process. Moreover, cowpea consumption provides substantial protein and energy to consumers. Indigestible compounds inducing flatus formation are frequently reported to protect humans from colon cancer and cardiovascular diseases (Lairon et al., 2005; McIntyre, Gibson, & Young, 1993).

Flatulence is an important limitation to cowpea' acceptance, and thus its consumption, as reported by many consumers, especially city dwellers in West Africa (Madode et al., 2011). Enhancing the consumer acceptance and thus, consumption of cowpea dishes requires the reduction of their intestinal fermentability. Several studies demonstrated the efficacy of food processing in improving the digestibility of legumes by investigating the degradation of carbohydrates (Alani, Smith, & Markakis, 1990; Egounlety & Aworh, 2003; Falkoski et al., 2006; Gote, Umalkar, Khan, & Khire, 2004; Onyenekwe, Njoku, & Ameh, 2000). In these reports, GOS were focussed on as the main cause of flatus formation. Calloway, Hickey,

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and Murphy (1971), through breath and flatus analysis, reported that a total removal of GOS from soya beans did not eliminate all flatus formation. Their results suggested that undigested carbohydrates other than GOS also play a role in the flatus formation.

Consequently, this study was undertaken to identify food processing techniques that fit with the consumers' low-flatulence quality criterion by associating three research approaches that estimate the fermentability of most indigestible carbohydrates in cowpeas. This paper discussed the potential of food processing to reduce flatulence in cowpeas and the reliability of these approaches in predicting this flatulence reduction potential.

2. Material and methods

2.1. Cowpeas

Two batches of a pigmented landrace (Adjayivi) and one batch of an unpigmented landrace (Atchawe-tola) of cowpea were purchased from the International market of Dantokpa, Cotonou, Benin.

2.2. Microbial strains

Weissella beninensis LMG25373 was cultured in a sterile de Man Rogosa Sharpe broth tube (Merck, Darmstadt, Germany) at 30 °C for 24 h to obtain 9.0 log cfu of viable cells/mL.

Bacillus subtilis M112 was cultured in a Nutrient broth (Oxoid, Basingstoke, England) at 37 °C for 24 h until a viable count of 9.0 log cfu/mL was obtained.

Rhizopus microsporus LU573 was cultured onto Malt Extract Agar (Oxoid, Basingstoke, England) slants for 6 days at 30 °C. Prior to use, spores were scraped into 9 mL of sterile peptone physiological salt solution.

A spray-dried powder of *Saccharomyces cerevisiae* (Fermipan Red, DSM, The Netherlands) was suspended into sterile peptone physiological salt solution to obtain a viable cell count of 9.0 log cfu/mL.

2.3. Cowpea processing

2.3.1. In-vitro study

Fig. 1a depicts the processing techniques implemented on cowpeas for the *in-vitro* study.

Fraction 1 of the raw beans (RB) was immersed in water at 30 °C for 15–60 min prior to a manual separation of the hulls (RH) from the cotyledons (RC). Both fractions were dried at 60 °C for 24 h.

Fraction 2 of RB was boiled until beans could be easily squeezed between two fingers (ratio beans/cooking solution was 1:10, w/v). Three media were used for boiling: 1- water, 2- bicarbonate solution (1 g/L; pH 7.9) and kanwu solution (1 g/L; pH 9.8). Kanwu is a local rocksalt available in West-Africa, rich in bicarbonate, carbonate and minerals and used traditionally to reduce the cooking time of cowpeas. After boiling, the beans were drained to discard the boiling water and to collect the boiled beans (BBW, BBB and BBK, respectively).

Fraction 3 of RB was soaked overnight (16 h) in tap water at 4 °C to avoid acidification through uncontrolled fermentation, and subsequently dehulled by hand. The cotyledons were boiled in tap water (ratio cotyledons/water of 1:10, w/v) for 20 min, and cooled down to room temperature. Boiling water was drained and boiled cotyledons (S16BC) were separately inoculated with the different microorganisms. One portion of S16BC was inoculated with 7.0 log cfu of *B. subtilis* per gram of cotyledons, and incubated at 37 °C for 24 h (BsFC). The second portion was mixed with cooled cooking water (ratio of 1:1, w/v), inoculated with 2 × 7.0 log cfu of *W. beninensis* per gram of cotyledons, and incubated at 30 °C for 48 h (WbFC). The third portion was mixed with cooled cooking water (ratio of 1:1, w/v), inoculated with a natural enrichment of lactic acid bacteria (2 × 7.0 log cfu of total lactic acid bacteria per gram of cotyledons) and incubated at 30 °C for 48 h (NLFC). The lactic acid bacteria enrichment was obtained by a backslopping process (Nout, de Dreu, Zuurbier, & Bonants-van Laarhoven, 1987) performed on the raw beans.

Fraction 4 of RB was soaked in tap water mixed with 10% soaking water from the backslopping process at 30 °C for 24 h. After soaking and draining, cowpeas were dehulled manually and cotyledons boiled (ratio cotyledon: water of 1:10, w/v) for 20 min. Boiling water was discarded and cotyledons were cooled, superficially dried, inoculated (4.0 log spores of *R. microsporus* per g of cotyledons) and incubated at 30 °C for 48 h. This fermentation resulted in a solid cowpea-tempe cake (RmFC).

All the samples of the *in-vitro* study were produced in duplicate experiments, freeze-dried and kept at −20 °C until use.

2.3.2. In vivo study

Fig. 1b depicts the processing techniques implemented on cowpeas for the *in-vivo* study.

Fraction 1 of pigmented cowpeas was boiled in water (BBW), or kanwu solution (BBK) as described for the *in-vitro* study. Fraction 2 of pigmented cowpeas was immersed in water at 30 °C for 5 min, dried at 60 °C until a moisture content of 10% and dehulled by the Prairie Research Laboratory (PRL) dehuller (McWatters, 1990) for 4 min. We obtained the dry dehulled raw cotyledons (DRC). DRC were soaked at room temperature (28–30 °C) for 24 h allowing a natural acidification from pH 7 to pH 5 to take place. We obtained the soaked/acidified cotyledons (SAC). One share of SAC was minced in a sterile mill, inoculated (with 6.0 log cfu of *S. cerevisiae* per g) and incubated at 25 °C for 72 h. Two other shares of SAC were fermented with *R. microsporus* and *W. beninensis* as described for the *in-vitro* study.

All the processed samples prepared for the *in-vivo* study were dried at 60 °C for 24 h, and milled in a Retsch type KM 1 mill through a 0.5 mm screen to obtain a shelf-stable powder.

2.4. In-vitro digestion and fermentability

The *in-vitro* digestion (enzymatic degradation and dialysis) was performed as described by Kiers, Nout, and Rombouts (2000). The suspension obtained after enzymatic digestion was centrifuged at 3000 × *g* and 4 °C for 15 min. The pellet was washed twice and constituted the non-dissolved undigested moiety of the beans. The supernatant was dialysed in dialysis tubes against running water to collect the dissolved undigested moiety of the beans. The fermentability of these two moieties was assessed on the basis of the volume of gas released from their fermentation by *Clostridium perfringens* VWA GZ-1005 taken as a reference microorganism as defined by Nche, Nout, and Rombouts (1994). The volume of gas produced by *C. perfringens* was considered as the *in-vitro* fermentability index of the analysed product. In total, 10 cowpea products were digested and randomly fermented by *C. perfringens*. All samples were fermented and analysed in duplicate.

2.5. In-vivo digestion and breath sampling

Eighteen healthy volunteers (7 women and 11 men, aged 23–41 years with a body mass index ranging 18–25 kg/m^2) were enrolled. They were informed about the study procedures and signed a written informed consent. Three months before and during the study, none of these non-smokers took antibiotics nor reported any gastro-intestinal or lung diseases. The subjects of this study were Caucasian (4), Asian (5) and African (9).

According to the Medical and Ethical Assessment Committee of Wageningen University, this study did not fall within the scope of

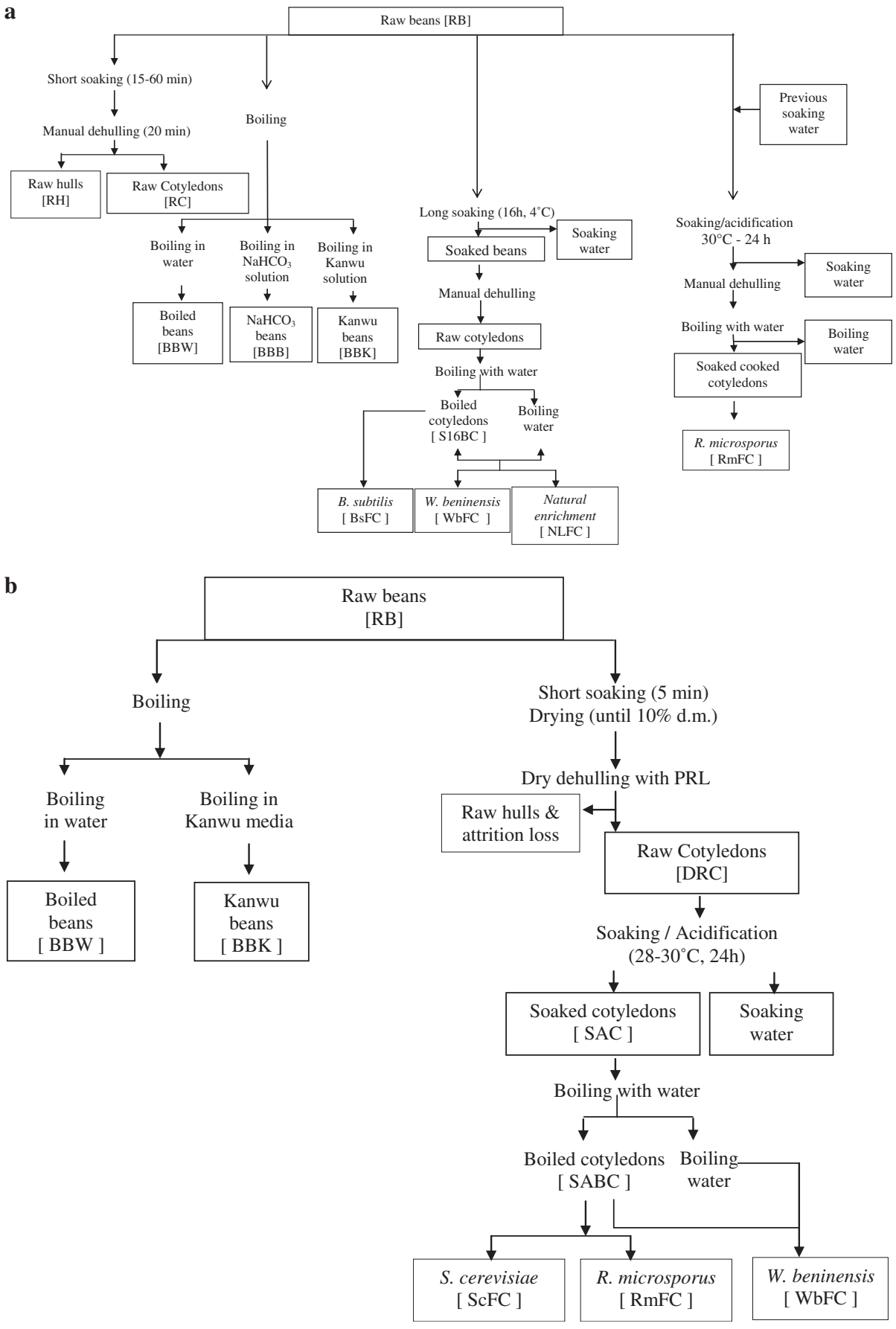

Fig. 1. Flow diagram of cowpea processing for a) the *in-vitro* and b) *in-vivo* digestion studies.

the Netherlands' law on medical scientific research on human subjects (WMO). We were advised that written informed consents from subjects would be adequate.

Raw and processed pigmented bean flours, obtained as described in section 2.3.2, were cooked with water (flour/water ratio of 1/10, w/v) for 15 min (starting from the boiling point) to obtain porridges. After cooking, water loss due to evaporation was compensated by adding water. A preliminary test was conducted during which each participant was asked to consume the porridge until he/she had enough. Three portion sizes of porridge (0.40, 0.60 and 0.75 kg) were thus identified. Each participant would consume his portion size after a fasting period of 10 h. The same portion size was maintained for each subject during the whole study. Alveolar breath was collected from the mouth with an alveo-sampler bag (QuinTron instrument company, Milwaukee, WI, USA) equipped with a 30 mL sampling syringe, on an empty stomach and hourly for 8–12 h after the consumption of the porridge. The subjects could take any beverage containing no indigestible sugars any time after the consumption of the porridge, but no solid food until 6 h after the consumption of the porridge as breakfast. The concentration of hydrogen (alveolar hydrogen) was measured in the alveolar air released by each participant using the Breath Tracker DP (QuinTron instrument company, Milwaukee, WI, USA). The alveolar hydrogen concentrations were corrected with the lowest alveolar hydrogen measured after fasting, and plotted against time (min), as shown in Fig. 2. The triangular areas under the aveolar hydrogen versus time curves were summed to obtain the area under the curve (AUC, ppm × min):

$$AUC = \Delta t \; x \sum_{1}^{n} ((C1 + C2) + (C2 + C3) + \ldots + (Cn - 1 + Cn))/2$$

where $\Delta t = 60$ min as measurements were performed hourly; C1, C2, …, Cn−1, Cn = corrected H_2 concentration (ppm) at the beginning and the end of each interval; n = number of H_2 concentration measurements.

The experimental AUCs were normalised to normal distribution by natural logarithm (ln (AUC)) and used as *in-vivo* fermentability index. The eighteen subjects received all treatments in a random order. Nine participants received two of the 9 treatments a second time, in order to investigate the within person-treatment variation. The subjects also filled a questionnaire to indicate the type of foods that they consumed before the fasting period and on the sampling day.

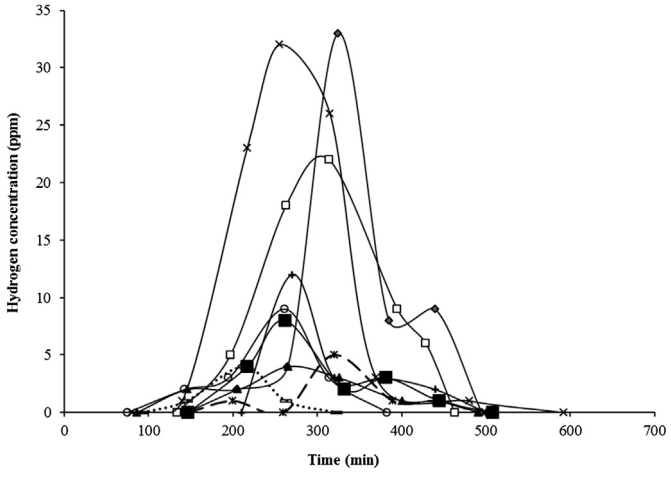

Fig. 2. Shape of H_2 concentration curves from one subject during the *in-vivo* study. RB, Raw beans; RC, Raw cotyledons; BBW, Beans boiled with water; BBK, Beans boiled with Kanwu solution; SAC, Cotyledons soaked/acidified at 28–30°C for 24 h; SABC, Boiled SAC; RmFC, *R. microsporus* fermented cotyledons; ScFC, *S. cerevisiae* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons.

2.6. Chemical analysis

An aqueous extract of sucrose and GOS in raw and processed cowpea flours was prepared by suspending 0.5 g of flour in 50 mL of demineralised water and incubating the suspension at 80 °C for 20 min (Johansen, Glitso, & Knudsen, 1996). After extraction and centrifugation at 2670 × *g* for 10 min, the supernatant was collected, mixed (ratio 1:1) with absolute ethanol, stored at −20 °C for 30 min to precipitate dissolved proteins and centrifuged at 2670 × *g* for 20 min. The supernatant was evaporated in a speedvac concentrator (ThermoElectron, Asheville, NC, USA) at 60 °C for 1–2 h. The pellet was re-suspended in water, and filtered through a 0.45 μm filter. Sucrose and GOS were separated on a Prevail carbohydrate ES 5u column (Grace, Breda, The Netherlands) (Vinjamoori, Byrum, Hayes, & Das, 2004) with an acetonitrile/water gradient elution (increased from 80:20 to 50:50) and detected by an Evaporative Light Scattering Detector (PL-ELS20100, Polymer Laboratories, Middelburg, The Netherlands). The standard raffinose and verbascose were produced by Fluka, Steinheim, Switzerland whereas standard sucrose and stachyose were made by Sigma, St. Louis, MO, USA. Extractions and measurements were performed in duplicate.

2.7. Statistical analysis

All analyses were performed in duplicate. The residual sucrose and GOS in processed cowpeas were compared using the analysis of variance followed by a Tukey posthoc test. The mean values of the *in-vivo* fermentability index (ln(AUC)) were also compared through the analysis of variance followed by a Tukey posthoc test in a block design setting, with the 18 subjects as blocks. For all these tests, a significance level was 0.05.

3. Results

3.1. In-vitro assessment of the digestibility of processed cowpea

3.1.1. Effect of traditional processing on the digestibility of carbohydrates

In this study, the pigmented and unpigmented cowpeas contained mainly stachyose (42–52 mg/g d.w.) and sucrose (18–32 mg/g d.w.), and less raffinose and verbascose (7–8 mg/g d.w. in both cases). Fig. 3a and b showed the sucrose and GOS profile of cowpeas as affected by dehulling, soaking at 4 °C for 16 h and/or boiling. The trend observed was the same for unpigmented and pigmented cowpea landraces. Raffinose and verbascose were less affected by traditional processes than sucrose and stachyose. The analysis of the hulls revealed that they did not contain significant amounts of sucrose and GOS. Taking the raw cowpeas as a reference, the use of softeners during boiling reduced the content of sucrose (60–80%) and stachyose (48–70%) significantly more than boiling in water (30–52% for sucrose and 16–33% for stachyose). Soaking at 4 °C for 16 h followed by dehulling and boiling (S16BC) greatly reduced the sucrose and stachyose content of pigmented (69%) and unpigmented (more than 75%) cowpeas.

Fig. 4a showed the *in-vitro* fermentability index of the dissolved- and the undissolved- fractions of the undigested products as metabolized by *C. perfringens*. In all cases, the undissolved moiety contained four times more fermentable substrate than the dissolved moiety, except for the hulls where these fractions were equally fermentable for pigmented and unpigmented landraces.

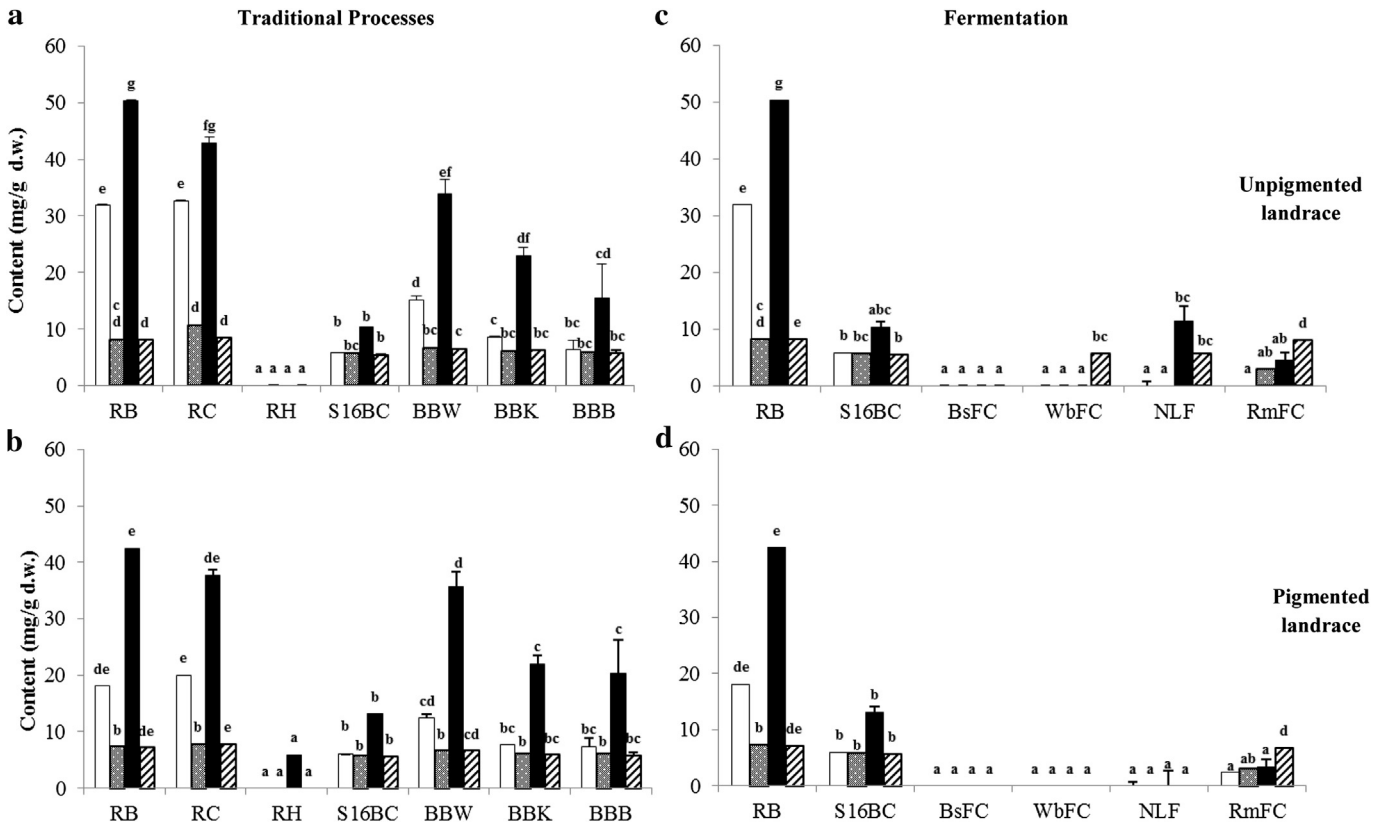

Fig. 3. Variation of the sucrose and GOS contents as a function of processing treatments and landraces (*in-vitro* study). RB, Raw beans; RC, Raw cotyledons; RH, Raw hulls; S16BC, Beans soaked (16 h at 4 °C), dehulled and boiled with water; BBW, Beans boiled with water; BBK, Beans boiled with Kanwu solution; BBB, Beans boiled with $NaHCO_3$ solution; BsFC, *B. subtilis* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons; NLFC, Cowpea cotyledons fermented with a natural lactic acid bacteria inoculum; RmFC, *R. microsporus* fermented cotyledons. □ Sucrose; ▨ Raffinose; ■ Stachyose ; ▨ Verbascose, Error bars indicate the standard deviation of the mean of sucrose and GOS contents from two trials.

Overall, the traditional processes implemented did not significantly reduce the fermentability of cowpea assessed *in-vitro*.

3.1.2. Effect of fermentation on the digestibility of carbohydrates

S16BC was the common product leading to all biological processes except the fungal fermentation. The sucrose and GOS profiles depicted in Fig. 3c and d indicated a complete removal of sucrose and all GOS of S16BC when fermented either by *B. subtilis* or *W. beninensis* except verbascose. The natural lactic acid fermentation (NFLC) showed 100% reduction of sucrose and all GOS of the pigmented landraces whereas it was less efficient to degrade stachyose and verbascose of the unpigmented landrace. The whole process of tempe production with *R. microsporus* reduced 87–100% of sucrose, 60–90% of raffinose and stachyose in raw cowpea, but hardly degraded verbascose (1–6%).

As observed previously, the undissolved moiety of the undigested residues obtained from the bioprocessing of cowpeas was significantly more fermentable than the dissolved fraction (Fig. 4b). Lactic acid fermented cowpeas produced as much or even more gas than S16BC and raw cowpea from both landraces. *B. subtilis* and *R. microsporus* considerably decreased the fermentability of RB and S16BC.

3.2. In-vivo assessment of the digestibility of cowpea

3.2.1. Effect of traditional processing on the digestibility of carbohydrates

The pigmented landrace processed for the *in-vivo* study contained 8, 5, 40 and 10 mg/g of sucrose, raffinose, stachyose and verbascose, respectively (Fig. 5). Boiling (in water or in kanwu solution) slightly decreased the sucrose and GOS contents in the pigmented landrace, whereas dehulling slightly increased the raffinose and stachyose content on dry matter basis.

The *in-vivo* fermentability index of the raw cowpea porridge did not significantly differ from the index associated with the porridge made from raw cowpea cotyledons or cowpeas boiled in water. Compared with soaking of cotyledons for 24 h at 28–30 °C (SAC), boiling in water did not lower the *in-vivo* fermentability of the cowpeas (Fig. 6a).

3.2.2. Effect of fermentation on the digestibility of carbohydrates

Fig. 5 shows that SAC reduced 43% of sucrose, 61% of stachyose and 15% of verbascose content of the raw cotyledons. Compared to SAC, boiling leached out all sucrose and raffinose, 32% of stachyose and 12% of verbascose. The combined effect of dehulling, soaking/acidification and boiling was the removal of all the sucrose and the raffinose, 83% of the stachyose and 27% of the verbascose. Compared to SABC, *R. microsporus* did not reduce the sucrose and GOS contents. *S. cerevisiae* (ScFC) and *W. beninensis* (WbFC) reduced stachyose and verbascose contents, namely by 69% and 53%, respectively, for the ScFC and by 13% and 4%, respectively, for the latter treatment (WbFC).

The *in-vivo* fermentability indexes of fermented cowpeas were all low in comparison to the other treatments (Fig. 6a). Fermentation by *W. beninensis* and *S. cerevisiae* were associated with the lowest *in-vivo* fermentability index compared to raw beans, and traditionally processed beans. The stachyose content of the raw and processed cowpea samples used for in-vitro and in-vivo experiments as presented in Figs. 3 and 5 were analyzed for their correlation with the in-vitro and the in-vivo fermentability index. The

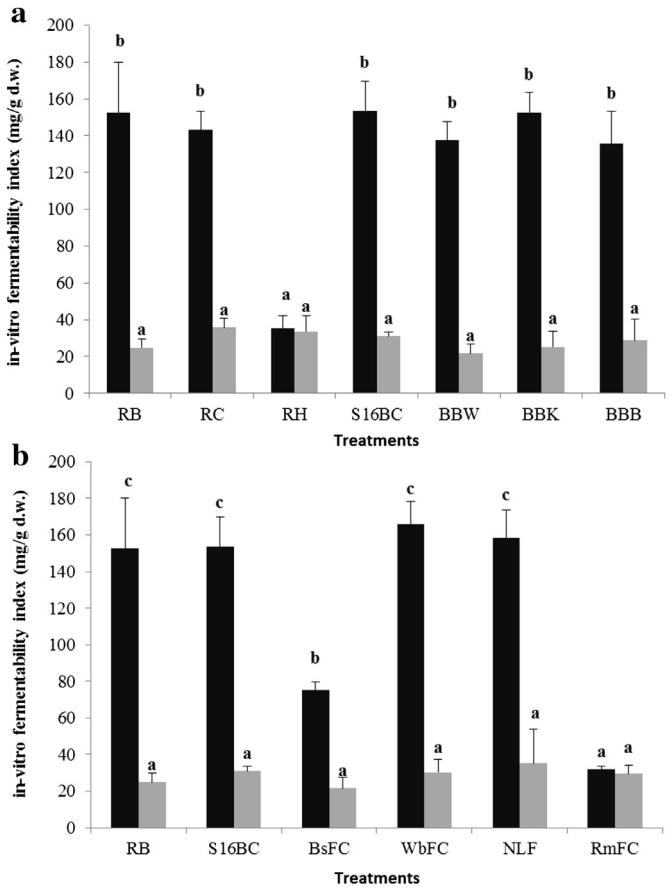

Fig. 4. *In-vitro* fermentability index as affected by processing treatments and landraces. RB, Raw beans; RC, Raw cotyledons; RH , Raw hulls; S16BC, Beans soaked (16 h at 4 °C), dehulled and boiled with water; BBW, Beans boiled with water; BBK, Beans boiled with Kanwu solution; BBB, Beans boiled with $NaHCO_3$ solution; BsFC, *B. subtilis* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons; NLFC, Cowpea cotyledons fermented with a natural lactic acid bacteria inoculum; RmFC, *R. microsporus* fermented cotyledons. ■ Retentate of dissolved fraction; ▒ Undissolved fraction, Error bars indicate the standard deviation of the *in-vitro* fermentability index from two trials.

stachyose content of cowpeas showed a high positive correlation with the *in-vivo* fermentability index ($r = 0.88$, $P = 0.004$) and no significant correlation with the *in-vitro* fermentability index ($r = 0.370$,$P = 0.099$).

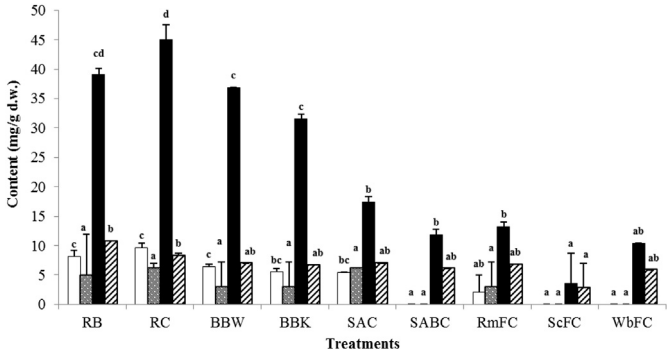

Fig. 5. Variation of the sucrose and GOS contents as a function of processing treatments (*in-vivo* study). RB, Raw beans; RC, Raw cotyledons; BBW, Beans boiled with water; BBK, Beans boiled with Kanwu solution; SAC, Cotyledons soaked /acidified at 28–30°C – 24 h; SABC, Boiled SAC; RmFC, *R. microsporus* fermented cotyledons; ScFC, *S. cerevisiae* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons. □ Sucrose; ▒ Raffinose; ■ Stachyose; ▨ Verbascose, Error bars indicate the standard deviation of the *in-vivo* fermentability index from two trials.

Fig. 6b illustrates that regardless of the processed cowpea consumed, the subjects involved in the *in-vivo* study reacted differently as they originate from Europe, Asia or Africa. Indeed, the mean *in-vivo* fermentability index associated with processed cowpeas increased significantly from Caucasians (7.1) to Asian (7.3) and African (7.5), meaning that Caucasians produced less abdominal gas than Asians who in turn produced less than Africans.

4. Discussion

Traditional processing treatments (soaking at 4 °C, dehulling, and boiling) as applied separately had a lower effect on the reduction of GOS content than fermentation (lactic, yeast, fungal). Although hulls of legume seeds are commonly considered as the main causes of the high fermentability of ingested legumes, their removal by wet-manual dehulling preceded by 1 h soaking at room temperature reduced only 17–18% of stachyose and 30–48% of raffinose (Nwinuka, Abbey, & Ayalogu, 1997; Onigbinde & Akinyele, 1983). Akinyele and Akinlosotu (1991) reported, however, a higher reduction of verbascose (76%) and raffinose (56%) and a similar stachyose reduction (17%) in cowpeas when beans were soaked for 10 min prior to wet-manual dehulling. The negligible amount of GOS that we detected in the hulls corroborated the findings of Revilleza, Mendoza, and Raymundo (1990) as only traces of GOS were found in the seed coat of *Dolichos lablab* and *Mucuna pruriens*. As far as cowpea is concerned, no previous study reported the content of GOS in hulls. Onigbinde and Akinyele (1983) analysed the raw cowpeas and their cotyledons and concluded, by deduction, that raffinose and stachyose abound in the testa without analysing the GOS content of the testa. The reduction observed subsequently to the dehulling process in the cotyledons as compared with the whole bean could be explained by the solubilisation of GOS in the water during the soaking process preceding the dehulling. In addition, the dehulling process is usually accompanied by the detachment of the embryo or germ which is rich in GOS. Kuo, Lowell, and Smith (1997) showed that during seed maturation, soya bean embryo as well as cotyledons are enriched in GOS contrary to hulls. Furthermore, the hulls may constitute a barrier for the diffusion of oligosaccharides into the soaking water. Therefore, the added-value of the dehulling process would be to favour the leaching of GOS or their breakdown products rather than the removal of the GOS from the hulls.

All boiling practices reduced the GOS content of the beans. The use of alkaline solutions (bicarbonate or kanwu) decreased the GOS content more than only water. The GOS might have been leached to the cooking solution. The alkaline conditions caused by the softeners probably tenderized the hulls and favoured a better release of GOS and/or their degradation products from the cotyledons into the boiling water. This leaching mechanism could also happen when cowpeas are soaked at 4 °C, dehulled and cooked.

The observed reduction of stachyose content when dry cotyledons were soaked at room temperature for 24 h (SAC) could be due to the leaching of GOS simultaneously with their assimilation by the naturally microbiota that acidified the soaking water. *W. beninensis* was recently identified and characterised during cassava fermentation, and its ability to metabolise GOS such as raffinose and melibiose, and produce organic acids as described by Padonou et al. (2010), was confirmed by this study. The efficiency of *W. beninensis* is comparable with the enzymatic degradation by α-galactosidase as a reduction of 93.3% of raffinose was observed by Somiari and Balogh (1992). However, the result of the fermentation by *W. beninensis* should also be considered as the sum of the effects of all the preliminary steps prior to the fermentation, namely soaking, dehulling and boiling. Although the overall effect of *R. microsporus* fermentation is high, the reduction of GOS due to the

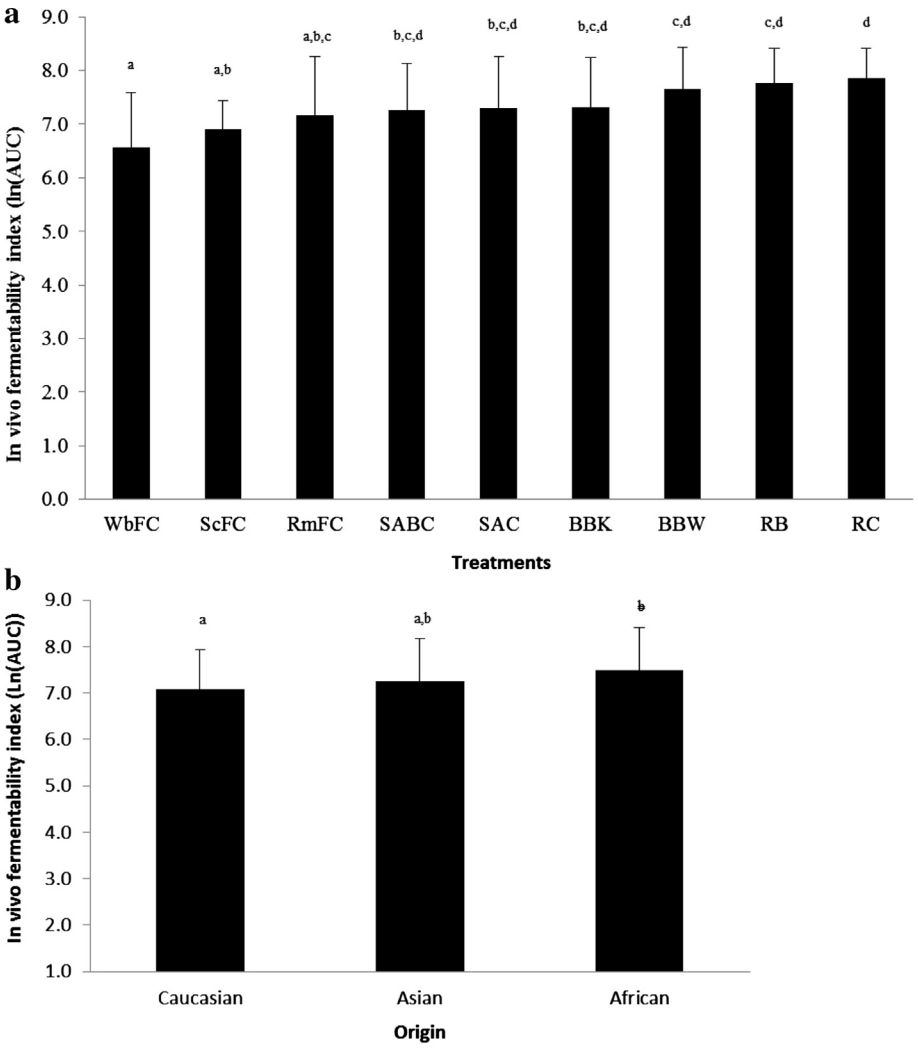

Fig. 6. *In-vivo* fermentability index as affected by a) processing treatments and b) origin of tested subjects. RB, Raw beans; RC, Raw cotyledons; BBW, Beans boiled with water; BBK, Beans boiled with kanwu solution; SAC, Cotyledons soaked /acidified at 28–30°C–24 h; SABC, Boiled SAC; RmFC, *R. microsporus* fermented cotyledons; ScFC, *S. cerevisiae* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons; AUC, Area under the curve, Bars topped by different letters relate to treatment or subject group that showed significantly different mean values of *in-vivo* fermentability index ($P < 0.05$).

activity of *R. microsporus* is rather limited. Such result suggests that the fungal strain used for tempe production is unable to produce a sufficient amount of α-galactosidase. Indeed, Rehms and Barz (1995) demonstrated that *R. microsporus* var. *chinensis*, contrary with *Rhizopus oryzae*, can produce β-fructosidase but not α-galactosidase, enabling the entire assimilation of sucrose but only a partial degradation of raffinose.

The utilization of carbohydrates, especially GOS, varies greatly depending upon the *C. perfringens* strain used. *C. perfringens* type A, which is a usual human gut inhabitant, can metabolise raffinose (Nche et al., 1994; Sacks & Olson, 1979). In our experiments, the total gas produced by *C. perfringens in-vitro* did not correlate with the amount of GOS present in the processed cowpeas. As processed cowpeas which contain a negligible amount of GOS still induce gas production, it can be suggested that *C. perfringens* can metabolize saccharides other than GOS such as resistant starch or cell wall components or their breakdown products (Calloway et al., 1971; Sacks & Olson, 1979). This hypothesis is consistent with the fact that the undissolved fraction of the analysed samples showed more than 80% of the fermentability of the undigested residues.

The difference in intestinal response of the subjects from Europe, Asia and Africa can possibly be attributed to the differences in dietary habits. These differences could highly influence the population of the gut microflora and its activity (Backhed et al., 2005). de Fillippo et al., (2010) demonstrated that the gut microbiota of infants from rural Africa was dominated by bacteria able to utilise cellulose and xylan contrary to the gut microbiota of European children. The diet of the African children was based on foods rich in starch, fibre and plant polysaccharides whereas European children were accustomed to protein and fat rich foods. Although the non-Caucasian subjects involved in our study live in Europe for a while, most of them have not shifted drastically from their original food consumption patterns. Our study recorded that the subjects still consumed many of the foods they were used to in their country of origin.

The different approaches implemented to estimate the potential of the processed cowpea to generate flatus after consumption yielded various outcomes. The *in-vivo* approach (as implemented in this research) revealed the utilisation of the undigested fraction of the ingested cowpea porridges by a large range of microorganisms (Backhed et al., 2005). The *in-vitro* approach, as implemented in this research, involved only one bacterial strain: *C. perfringens*. Therefore, the type of substrate and the metabolic ability of the microbiota present with regard to this substrate can explain the observed differences. Moreover, the sampling time scale was 8–

12 h for the *in-vivo* test and 24 h for the *in-vitro* test. It can be hypothesized that the easily fermentable compounds in the undigested bulk, namely GOS and resistant starch, induce the formation of the hydrogen measured *in-vivo* during the first 12 h after the food is consumed (Brouns, Kettlitz, & Arrigoni, 2002). Such hypothesis is in line with the correlation found between *in-vivo* fermentability index and stachyose content. The slow fermenting polymers, namely pectin, cellulose, lignin, etc. induce a significant gas release. As a matter of fact the total gas produced by *C. perfringens* from the various undigested materials is an integration of the gas generated by the fermentation of all the types of fermentable sugars. The 24 h quantification of gas production seems more appropriate to obtain a complete view of the gas production potential of processed cowpea.

5. Conclusions

The present research suggests that soaking and fermentation are the most efficient techniques to leach out and/or degrade flatus-inducing factors present in cowpea. In the context of West Africa, soaking should be promoted as a pre-treatment in any cowpea processing procedure. Moreover lactic acid fermentation, especially with *W. beninensis*, could be optimised for low flatus induction and good taste.

The various approaches used to assess flatus production appear to express different phenomena, each of which has its own merit.

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