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ORIGINAL ARTICLE

Physicochemical properties of sorghum flour are selectively modified by combined germination-fermentation

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Abstract The combined effects of grain germination and of subsequent fermentation on the physicochemical properties of sorghum flour were investigated by studying the structural changes occurring in the starch and protein fractions and by assessing their effects on physical properties of the resulting materials most relevant to end use. The sequential treatments were more effective than either individual treatment in the modification of starch-related properties, whereas modification of the protein components only occurs in the fermentation step, almost regardless of a previous germination step. The resulting profile of physicochemical traits offers several hints as for the suitability of flour from treated sorghum as an ingredient for various types of gluten-free food products, and provides a basis for expanding the use of processed sorghum in applications other than traditional African foods.

Keywords Sorghum · Germination · Fermentation · Physicochemical properties · Biotechnological treatments

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Introduction

Sorghum (*Sorghum bicolor* L. *Moench*) is a staple food for millions of people in parts of Africa and Asia and constitutes a major source of proteins, calories, and minerals for those populations. Sorghum, due to its abundance in various phytochemicals (de Morais Cardoso et al. 2017), also represents an interesting ingredient in gluten-free formulations (Schober et al. 2005; Renzetti et al. 2008; Marengo et al. 2015), although the use of sorghum as a food source in developed countries has not been exploited in full.

Several traditional foods processing and preparation methods are used—at the household level—in most sorghum-producing countries to make the final product more attractive in flavour, appearance, taste, and consistency. These methods include—among others—germination and fermentation, that are commonly used to improve micronutrients bioavailability, as well as protein and starch digestibility (Dhankher and Chauhan 1987; El Tinay et al. 1979). The combined effects of germination and fermentation have a positive impact on the properties of cereal and/or legume based food formulae (Sripriya et al. 1997, Rasane et al. 2015). Germination and fermentation are simple and easily adaptable technologies for lowering bulkiness (high viscosity) and for increasing the shelf life of cereal- and legume-based food formulations (Ross et al. 2002; Gernah et al. 2011). Biotechnological pre-treatment of sorghum grains or flour have a role also in defining the overall quality of sorghum-containing Western-style foods, such as pasta (Marengo et al. 2015) or bread (Schober et al. 2007).

Knowledge about the physicochemical properties of germinated-fermented sorghum flour is of relevance to select the most adequate application for this flour in the food industry. A number of studies have focused on improving the quality of sorghum by using different traditional methods of sorghum processing, and have been devoted to the characterization of the modifications induced by fermentation or germination treatments (Elkhalifa and Bernhardt 2013; Elkhalifa et al. 2004, 2006a, b; Marengo et al. 2015, Rasane et al. 2015), but investigations on the effects induced by a combination of both treatments are very limited.

In order to expand the use of sorghum in application other than traditional African-style foods, an investigation was carried out on the combined effect of grain germination and fermentation on macromolecules in sorghum flour. This study provides an overview of various molecular and physicochemical traits that may be of interest—also from a practical standpoint—for assessing the suitability of preparations based on treated sorghum as ingredients for various types of gluten-free products.

Materials and methods

Materials

A popular and traditional Sudanese sorghum cultivar (Fetarita, from Gadarif, Sudan) was used in this study. After cleaning, grains were stored at 4 °C until used to minimize spoilage and to retard post-harvest compositional changes.

Sorghum germination

Sorghum seeds were germinated in Sudan for 72 h according to the traditional Sudanese method of germination, as described by Elkhalifa and Bernhardt (2013).

Sorghum fermentation

The roots were manually removed from germinated seeds, that were then dried at 56 °C for 16 h in a hot air oven (Heraeus UT 5042, Germany) and ground in a hammer mill to pass through a 0.4 mm screen. The resulting flour was fermented for 8 h at 37 °C as described by Elkhalifa et al. (2006b). At the end of the fermentation, samples were dried and ground into flour as described above. The same milling procedure and equipment were used to prepare control flour from untreated grains.

Flour characterization

The procedure described by Elkhalifa and Bernhardt (2013) was used for determination of stability and clarity of sorghum flour pastes. The freeze–thaw stability (syneresis) of samples was measured using 6% (w/w) sorghum flour pastes according to the method proposed by Zheng and Sosulski (1998). Dispersibility was measured by placing 10 g of the sample in a 100 ml stoppered measuring cylinder, adding distilled water to reach a volume of 100 ml, stirring vigorously, and allowing the suspension to settle for 3 h. The volume of settled particles was subtracted from 100 and the difference reported as percentage dispersibility. The method of Abbey and Ibeh (1998) was used to determine the swelling index, whereas the swelling power and the starch solubility were determined according to the following method. Water was added to flour slurries, that were then incubated at 60, 85, and 100 °C for 30 min with shaking every 5 min. After the samples were cooled at room temperature for 15 min, they were centrifuged at $4900\times g$ for 10 min. Supernatants were collected to measure total soluble starch, and the precipitate was used to assess the swelling power. Total soluble starch was precipitated by adding 80% ethanol (v/v) and dried at 40 °C for two days. Swelling power was calculated as the weight ratio of the wet sediment to the initial sample weight. Gel consistency and gelling temperatures were assessed as described in previous studies (Elkhalifa and Bernhardt 2013).

Starch structural indexes

Absorption spectra of iodine–starch complexes were measured according to the method of Takeda et al. (1983) with slight modifications. Sorghum flour (0.1 g) was suspended in 1 mL of ethanol. Then 9 mL of 1.0 M NaOH were added, followed by heating in a boiling water bath for 10 min with occasional shaking. The suspension was adjusted to pH 6.5 with 1 M HCl and diluted to 100 ml with distilled water. An aliquot (5 ml) of the solution was added to 1 ml of 0.2% iodine solution and made up to 100 ml with distilled water. The mixture was kept at room temperature for 15 min. The wavelength of maximal absorbance was determined by taking absorbance spectra from 450 to 800 in a JASCO V-630 spectrophotometer (Jasco Germany GmbH). The blue value of iodine–starch complexes was calculated from the absorbance at 680 nm.

Protein structural indexes

The solubility of proteins in various flour samples was determined (in triplicate independent assays) by using various buffers as described by Marengo et al. (2015). Proteins were extracted by dispersing 0.5 g of sample in 10 ml of 0.05 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl. After stirring at room temperature for 60 min and removal of insoluble materials by centrifugation ($10{,}000\times g$ for 20 min at 20 °C), the protein content in the supernatant (expressed as mg soluble proteins/g flour) was assessed by a dye-binding method (Bradford 1976). Where indicated, the buffer used for protein solubilization also contained 6 M urea or 6 M urea and 10 mM dithiothreitol (DTT). Accessible thiol groups were determined (in triplicate independent assays) as described by Marengo et al. (2015), by suspending 0.5 g of sample in 10 ml of 0.05 M sodium phosphate, 0.1 M NaCl, pH 7.0, containing 0.2 mM 5,5′-dithiobis-(2-nitrobenzoate) (DTNB, Ellman 1959), in the presence/absence of 6 M urea. After 60 min stirring at 25 °C, the suspension was centrifuged (10,000×g, 20 min, 20 °C) and the absorbance of the supernatant was read at 412 nm. Results are expressed as µmol thiols/g flour.

Pasting properties

The pasting properties of sorghum flour were measured in triplicate in a Brabender Micro-ViscoAmyloGraph (Brabender, Duisburg, Germany), using a slightly modified procedure of Marti et al. (2017). An aliquot of flour (12 g) was dispersed in 100 ml of distilled water, scaling both flour and water weight on a 14% flour moisture basis. The pasting properties were evaluated under constant conditions (speed: 250 rpm; sensitivity: 300 cmg$_f$) by using the following time–temperature profile: heating from 30 to 95 °C; holding at 95 °C for 20 min; cooling from 95 to 30 °C. The heating and cooling phases were carried out with a temperature gradient of 3 °C/min. Pasting parameters were calculated by using a specific software (Viscograph version 2.3.7).

Statistical analysis

Unless otherwise indicated, three separate batches, for a particular treatment, were taken and analyzed separately and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) and by Duncan's multiple range test with a probability $P \leq 0.05$, using SAS/STAT software, as reported by Marti et al. (2017).

Results and discussion

Physicochemical properties

Sorghum contains 60–80% starch on a dry weight basis (Becker and Hanners 1991). Sorghum starch was reported to be modified by germination and fermentation (Marengo et al. 2015; Elkhalifa and Benhardt 2013; Elkhalifa et al. 2004), but the effect of a combination of the two treatments on technologically relevant properties of sorghum flour has never been assessed.

High starch flours are used to impart viscosity to food, and the clarity of the flour paste is one relevant attribute: some uses required transparent paste, (Craig et al. 1989). As shown in Fig. 1, germination and fermentation did not affect the clarity of as-prepared sorghum flour pastes, that had the same opaque character at the beginning of storage. The clarity of both treated and untreated flours increased upon storage, in particular at room temperature, and untreated flour suspensions were comparatively more transparent than germinated and fermented ones regardless of the storage temperature. These different features have been attributed to changes in the amylose/amylopectin ratio and to the different ability of starch components to interact with water (Matalanis et al. 2009).

Another relevant property for starch gels is their ability to retain water during freeze–thaw cycles (freeze–thaw stability, as reported by Aviara et al. (2010)). Such an ability relates to the potential use of starchy pastes in frozen food products (Baker and Rayas-Duarte 1998). The results shown in Fig. 2 indicate that flour derived from germinated-fermented sorghum presented higher syneresis (i.e., higher water release) than control in the first freeze–thaw cycle. However, flour derived from germinated-fermented sorghum showed lower percent syneresis than control in subsequent cycles. The higher syneresis of flour derived from germinated-fermented sorghum in the first cycle may be due to starch depolymerisation (Elkhalifa et al. 2004; Elkhalifa and Benhardt 2013). It has been reported that sorghum fermentation had no effect on freeze–thaw stability in the first cycle, but the syneresis value decreased as the number of cycles increases (Elkhalifa et al. 2006b).

Swelling power (SP) is defined as the ratio of paste weight to the dry flour weight (Loos et al. 1981). When

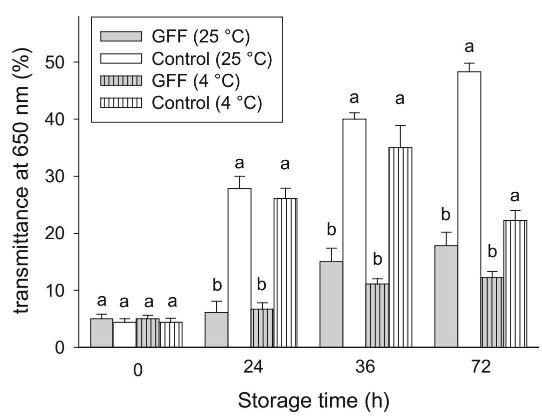

Fig. 1 Stability and clarity of germinated-fermented sorghum flour (GFF) and control pastes (1%, w\v) stored at 25 and at 4 °C, measured as transmittance (%) at 650 nm. The *error bars* represent SD from three replicates of each sample. *Bars* having different letters within the same group (GFF and control) and at the same storage temperature are significantly different ($P \leq 0.05$)

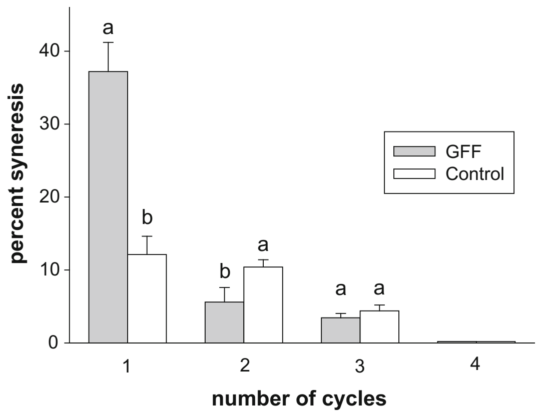

Fig. 2 Freeze-thaw stability of sorghum pastes (6%, w/w) from germinated-fermented sorghum flour (GFF) and control sorghum after four freeze-thaw cycles. Results are expressed in (%) weight of water decanted (syneresis) after thawing at 30 °C for 90 min. The *error bars* represent mean values ± SD from three replicates of each sample. *Bars* having different letters within the same group (GFF and control) are significantly different ($P \leq 0.05$)

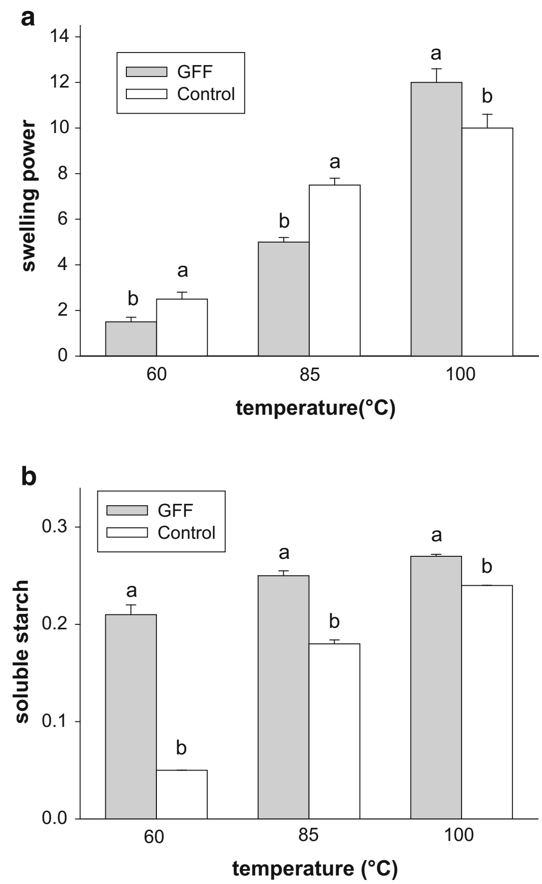

Fig. 3 Starch properties in germinated-fermented sorghum flour (GFF) and control. Swelling power (**a**) was determined after incubation of slurries at different temperatures for 30 min. Soluble starch (**b**) was assessed after precipitation with 80% ethanol and drying at 40 °C for 2 days. The *error bars* represent mean values ± SD from three replicates of each sample. *Bars* having different letters within the same group (GFF and control) and at the same temperature are significantly different ($P \leq 0.05$)

starch is pasted in an excess water system, the granules imbibe water through the amorphous regions in a reversible manner, and the amount of water imbibed increases with temperature until a critical temperature is reached (gelatinization temperature), at which the starch swells irreversibly with loss of crystalline order (Aviara et al. 2010). Results in Fig. 3a show that increasing the temperature from 60 to 100 °C increased SP. Flour obtained from germinated-fermented sorghum flour had lower SP than control at 60 and 85 °C, but not at 100 °C. Elkhalifa and Bernhardt (2013) reported that germination had little effect on the swelling power of sorghum flour, whereas structural changes due to fermentation may play a relevant role in these differences. Elkhalifa et al. (2006b) also reported that water retention of fermented sorghum flour increased as the temperature increased. The general increase in SP at high temperature is presumably due to starch gelatinization (Claver et al. 2010). The swelling power of starch depends on the capacity of starch molecules to hold water via hydrogen bonding. As the temperature is increased, intermolecular bonds are broken, allowing hydrogen-bonding sites to engage more water molecules while some short-length starch components are leached out. Further heating and further water absorption lead to the disruption and hydration of the crystalline part of the starch and—eventually—to granule rupture, resulting in an amorphous state of the starch chains (Zhu 2014). After complete gelatinization, the hydrogen bonds between starch molecules are replaced by hydrogen bonds with water (Lee and Osman 1991).

Thus, as discussed above, solubility of starch is an indicator of the degree of starch granules dispersion after cooking. SP can be positively related to the amount of soluble solids leached outside the granules. As shown in Fig. 3b, solubility increased with temperature for all the flours studied, and more soluble starch was present in germinated-fermented sorghum flour than in control regardless of temperature, in agreement with previous findings on germinated (Phattanakulkaewmorie et al. 2011) and fermented sorghum (Elkhalifa et al. 2006b).

An overview of the physicochemical starch properties considered in this study is presented in the upper part of Table 1. Flour dispersibility gives an indication of the particles suspensibility in water, which is another useful functional parameter in various food product formulations (Mora-Escobedo et al. 1991). The dispersibility of flour from germinated-fermented sorghum was significantly ($P \leq 0.05$) higher than that of control. The higher the dispersibility, the better the flour's ability to reconstitute in water to give a fine and consistent paste (Kulkarni et al.

Table 1 Selected properties of germinated-fermented (GFF) sorghum flour

	GFF	Control
Physicochemical properties		
Swelling index	0.93 ± 0.02[a]	0.68 ± 0.00[b]
Dispersibility (%)	76.5 ± 0.50[a]	70.0 ± 0.00[b]
Gel consistency (mm)		
Acid	111.5 ± 0.00[a]	54.9 ± 7.07[b]
Water	76.0 ± 0.00[a]	34.5 ± 2.50[b]
Gelling temperature (°C)	78.5 ± 0.50[a]	73.5 ± 0.50[b]
λ_{max} (nm)	503.5 ± 1.50[a]	502.5 ± 0.50[a]
Blue value (mg/100 ml)	0.1376 ± 0.00[a]	0.1056 ± 0.01[b]
Pasting properties		
Pasting temperature (°C)	78.9 ± 0.5[b]	74.5 ± 0.4[a]
Peak viscosity (BU)	350.0 ± 48.6[b]	414.7 ± 13.6[a]
Peak temperature (°C)	92.1 ± 0.1[b]	86.6 ± 0.2[a]
Breakdown (BU)	188.3 ± 18.5[a]	179.3 ± 7.2[a]
Setback (BU)	332.3 ± 23.4[b]	612.3 ± 7.2[a]

BU brabender units

Values are mean ± SD. Values with the same superscript letter in a given row are not significantly different ($P \leq 0.05$)

1991). In the present study, the value of dispersibilty of germinated-fermented sorghum flour is relatively high, indicating that it will easily reconstitute to give fine consistency dough during mixing. Gel consistency measurements indicate that the flour from germinated-fermented sorghum produced a much thinner gel than control, independent of pH (Table 1). A similar behaviour was reported for germinated sorghum (Elkhalifa and Bernhardt 2013).

The results in Table 1 also show that flour from germinated-fermented sorghum gave higher gelling temperatures than control (78.5 vs. 73.5 °C). These differences may be attributed to differences in size, form and distribution of starch granules in the flours, and to the internal arrangement of starch fractions within the granule (Kaur and Singh 2005).

Both the blue value (BV) and the maximum absorption wavelength (λ_{max}) of starch-amylose complexes were higher in the germinated-fermented sorghum flour than in control. Yu et al. (2012) reported that the BV and λ_{max} of rice starch and flour increased with the amylose content, whereas λ_{max} has been reportedly related to the average chain length of amylose and amylopectin (Huang et al. 2008).

Pasting properties

Changes in viscosity during heating and cooling under controlled conditions—as evaluated by the MicroViscoAmylograph test (MVAG)—are reported in Fig. 4. Indices inferred from these tracings are summarized in the lower part of Table 1. The pasting temperature increased significantly ($P < 0.05$) as a consequence of treatments, indicating that starch in treated flours required higher temperatures to reach full granule swelling. Similar result was observed when either germination or fermentation was applied to sorghum as independent treatments (Marengo et al. 2015).

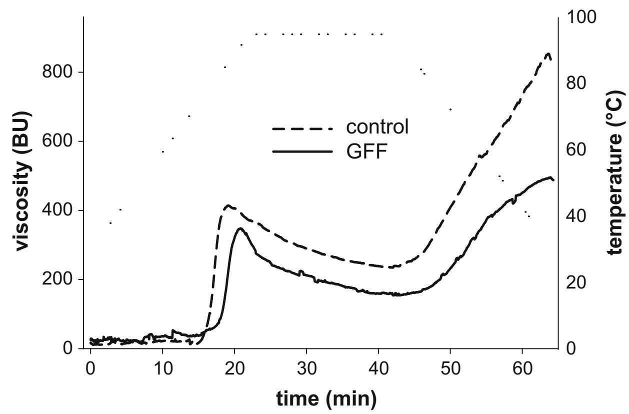

Fig. 4 Micro-visco-amylographic (MVA) tracings for germinated-fermented sorghum flour (GFF) and control

As the point of initial gelatinization and the range over which it occurs is governed by the starch structure, the observed increase in pasting temperature may relate to an increased hydrolytic starch breakdown (Elkhalifa et al. 2004). Accordingly, treatment also results in a decrease in viscosity during both heating and cooling step, resulting in a product less susceptible to retrogradation.

Characterization of the protein network

Structural features of the protein fraction and the nature and extent of inter-protein bonds were investigated by detecting the amount of protein solubilized in media of different composition (i.e., presence/absence of chaotropes and of disulfide-reducing agents). In separate measurements, the number and accessibility of protein thiols was assessed, as well as their exposure upon addition of chaotropes. These determinations represent useful indices for cereal-based matrices, as reported in a number of studies that included various sorghum-based preparations (Elkhalifa et al. 2006b; Marengo et al. 2015).

Information on the nature of bonds that stabilize the association/aggregation proteins were provided by solubility studies in media of different composition (Fig. 5a). The combined germination/fermentation treatment resulted in a decrease of all protein aggregates, consistent with previous reports on fermented sorghum flours, confirming that germination had only marginal effects on sorghum

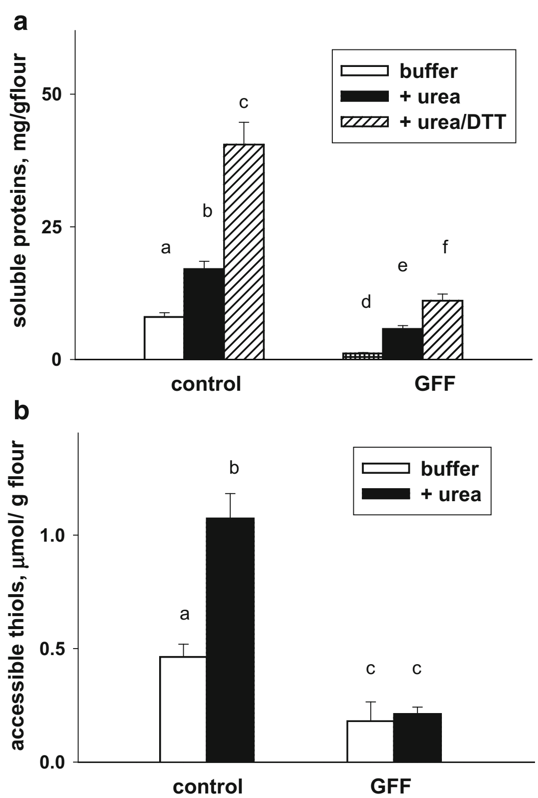

Fig. 5 Properties of proteins in germinated-fermented sorghum flour (GFF) and control. **a** Protein solubility in media of different composition. **b** Accessibility of protein cysteine thiols in the absence/presence of protein denaturing agents. The *error bars* represent mean values ± SD from three replicates of each sample. *Bars* having different letters are significantly different ($P \leq 0.05$)

proteins (Elkhalifa et al. 2006b; Marengo et al. 2015), regardless of their aggregation state.

As shown in Fig. 5b, the number of accessible thiols in the untreated flour increases by a factor of two when adding urea to the untreated samples, suggesting a high compactness of the protein network. Due to the loss of proteins (Fig. 5a) the amount of thiols in sprouted/fermented sample was much lower than in the untreated sample, again confirming previous observations on fermented sorghum (Elkhalifa et al. 2006b; Marengo et al. 2015). However, the number of accessible protein thiols in the treated material did not increase in the presence of urea, suggesting that residual proteins in the germinated/fermented flour are tightly packed.

Conclusion

The combination of germination and fermentation appear to have a positive effect on some physicochemical properties of sorghum flour. Starch-related properties are most affected by the combined treatments, whereas modifications of the protein components after the combined treatments are very similar to those induced by fermentation alone.

It seems noteworthy that the starch modifications observed after combining the two treatments have an impact much different from those observed upon individual treatments. In particular, some of the physical traits of starch in the germinated/fermented material made it fit for some specific uses, different from the ones where materials that underwent just one of the two treatments may be most appropriate. Therefore, this study suggests that appropriate selection of treatments may allow to expand use of this crop beyond the limits of traditional foods, as appropriately pre-treated sorghum flours may replace man-made and/or chemically modified natural ingredients in applications of interest for a geographically broader market, including the formulation and preparation of gluten-free foods.

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