Vegetable protein functionality

From milk analogue to fiber

Joana Vanessa Ramos Galante

Supervisors: Prof. Marília Clemente Velez Mateus (IST)
Dr. Ir. Fred van de Velde (NIZO Food Research B. V.)
Jolan de Groot (NIZO Food Research B. V.)

October, 2013

Abstract

In this work, we present a study on the functionality of vegetable proteins, namely of *Eragrostis tef*. Protein extraction from teff flour was performed with different solvents. The best extraction involved the use of RO-water at room temperature at pH 8.0. Proteins were extracted from teff flour, with and without DTT, and were afterwards characterized by SDS-PAGE and DSC. A milk analogue from teff flour was developed and improved. Dialysis was proven to be essential in the development of a satisfactory milk drink from teff. Functional properties of water-soluble proteins from teff flour were also studied. It has been shown that teff flour proteins have high water and oil absorption capacities. This flour could therefore be useful in flavor retention, improvement of mouth feel and extension of shelf life in foods. Proteins and polysaccharides are broadly used in food production, since their interactions improve the texture of products. In the present work, the influence of various parameters in the formation of different types of fibers was studied.

Keywords: Eragrostis tef, teff, vegetable proteins, polysaccharide, functionality.

1. Introduction

Eragrostis tef, commonly known as teff, is a tropical cereal mainly cultivated and consumed in Ethiopia, where it represents 20% of cereal production [1], [2]. Teff is the staple food crop and main source of carbohydrates for the majority of the Ethiopian population [3]. Since teff contains lower amounts of prolamins, it is a gluten-free food, and it also contains more vitamin and fiber than the other commercially available gluten-free products [4]. For all these reasons, this cereal has attracted a great interest in the global market [3], [4].

Teff is a very good cereal for malting and brewing, because it has an excellent balance of amino acid content. This cereal is ground into whole-grain flour, because teff seeds are small, and that confers a high fiber and nutrient content to the obtained flour. In fact, the nutrient composition of teff indicates its good potential as ingredient in the food and beverage industry [5]. Furthermore, teff flour can be utilized to obtain leavened flatbread, which is used to produce baked goods and traditional breads, like *injera* (Ethiopian bread) [6].

Teff is a gluten-free alternative to wheat products and also a nutrient-rich ingredient, which makes it a relevant substitute for cereals in food applications [5]. For all the mentioned reasons, this project was focused on the study of teff protein functionality, along with the development of milk analogues and meat derivatives from teff.

Total extraction of proteins from cereals is challenging, due to the high complexity and heterogeneity of these proteins. In fact, these proteins interact not only with each other, but also with interfering compounds, such as polysaccharides, lipids and proteases [7]. Separation of cereal proteins can be performed by selective extraction with different solvents. Cereal proteins have been mainly separated based on Osborne's classification, according to which proteins can be divided into water-soluble proteins (albumins), salt-soluble proteins (globulins), alcohol-soluble proteins (prolamins) and acid or alkaline-soluble proteins (glutelins) [8].

The average protein content of teff seeds (9 to 11%) is comparable to that of barley, wheat and maize, and higher than the protein content of sorghum. Glutelins and

albumins represent the highest protein fractions of teff. The descending order of importance of teff proteins is glutelins (44.55%), albumins (36.6%), prolamins (11.8%) and globulins (6.7%) [5]. Prolamin bands in SDS-PAGE gels were shown to be in the range of 20 to 26 kDa [9]. Through differential scanning calorimetry (DSC), teff prolamins exhibited a single endothermic peak at 69.85°C, which indicated thermal denaturation of prolamins [6].

The study of functionality is extremely important in order to efficiently use teff flour in food products [10]. Functionality can be defined as any property of a food component, except its nutritional properties, that highly affects the utilization of that ingredient in foods [11], [12]. Functional properties of proteins in flours, protein concentrates and isolates from cereals and legumes have been investigated by several authors, in order to assess their applicability in food systems. Properties such as bulk density, gelation, emulsification and foaming properties, water and oil absorption and protein solubility are of paramount importance in the formulation and processing of food products [11], [13], [14]. In this project, in order to characterize the functionality of the water-soluble proteins present in teff flour, several functional properties were studied: bulk density (BD), water and oil absorption capacities (WAC and OAC, respectively) and protein solubility (PS).

Another functional property of vegetable proteins is the ability of forming textured structures, known as textured vegetable protein. These structures can be obtained either by fiber formation (normally using the protein isolate), and then merging the fibers in layers to attain the desirable texture (meat resemblance), or by thermoplastic extrusion (using flour, protein concentrate or isolate). Texturized protein products are used to produce meat alternatives, with reduced prices and increased product juiciness as advantages [14]. In this project, textured protein structures obtained by fiber formation (called fibers) were developed from different vegetable protein sources.

2. Materials and Methods

2.1. Materials

The materials used in this work were teff protein, polysaccharide, salt (NaCl 99.5%, Merck) sunflower oil (Albert Heijn), artificial sweetener and a food additive (Ingredient 1 – Ing.1). The origin of all of the mentioned materials whose supplier was not indicated should remain confidential.

2.2. Methods

Optimization of protein extraction from teff

Starting from a method described in the literature and available at NIZO, several trials were performed in this study, in order to optimize the protein extraction from teff flour. These trials included extractions of teff proteins from the flour using ethanol, salt and water, or extraction with water at high temperature. Only the optimized method will be described below.

The first step of the optimized method consisted in the preparation of a 20% (w/w) suspension of teff flour in reverse osmosis (RO) water, while vigorously mixing with an Ultra-Turrax (Polytron PT 3000, Kinematica AG) during 10 min at 20000 rpm, cooling with melting ice. Afterwards, the pH was adjusted to 8.0 with 4 M NaOH, followed by mixture with the Ultra-Turrax. The suspension was subsequently centrifuged for 2 min at 2000x g, at 20°C (Avanti J-26 XP Centrifuge, Beekman Coulter), in order to remove fiber and starch fractions from the alkaline dispersion. Samples were collected after the pH adjustment and after centrifugation, for Kjeldahl analysis. Samples of the end product were also collected for SDS-PAGE and DSC.

Development of a milk analogue from teff proteins

The first step of the development of a milk analogue from teff consisted in the preparation of a 20% (w/w) suspension of teff flour in RO-water and posterior pH adjustment and centrifugation, as described above. The suspension was subsequently dialyzed overnight at 4°C, using dialysis membranes of MWCO of 6000-8000 Da (Spectra/POR, Sigma Aldrich). Dialysis was done at two conditions: against RO-water or RO-water with 10 mM

NaCl. Samples were collected from the two dialyzed solutions, to perform Kjeldahl analysis.

Concentration (CF=2) with a Rotary Evaporator (Stuart) was performed, for each of the two different solutions, and samples were taken from each concentrate and submitted to total nitrogen content analysis by Kjeldahl. Then, the solutions were submitted to mixing with the Ultra-Turrax between several ingredient additions: 0.05% (w/w) salt, 0.75% (w/w) sunflower oil and 16 droplets of artificial sweetener per L of solution (0.8 g/L of artificial sweetener). The solutions were then homogenized (2 traps Homogenizer, Niro Soavi S.p.A.) at 25°C and 200/20 bar, and a sample of each homogenized solution was taken for Kjeldahl analysis. Afterwards, the solutions were heated at 74°C for 20 s. A part of each solution was not exposed to heat treatment, to be compared to the heated part in an internal and informal tasting session performed at NIZO.

Moreover, a non-dialyzed milk analogue was performed as described above, in order to assess if the elimination of the dialyzing step from the process was possible. In this trial, samples were collected for size analysis (using the Mastersizer) at different stages of the procedure (after centrifugation, homogenization and heat treatment).

Fiber formation

The formation of fibers was performed using vegetable proteins from different sources. In this article, only teff fiber formation is described. All yields of fiber formation calculated and shown in the results were associated to the wet fibers (after pressing). Fibers yield was calculated by dividing the wet mass of fibers obtained in the end by the weight of the initial fiber solution.

The first step of fiber formation consisted in the preparation of the polysaccharide solution. The polysaccharide was dissolved in RO-water, stirring during 1h at room temperature (RT) and then overnight at 4°C. Afterwards, the solution was heated for 30 min at 80°C in a waterbath, cooled down and stored until use.

Two types of protein solution were used: teff extracts at pH 6.5 and 8.0, and also the extract with ethanol; and a protein solution prepared as described in the former section, but just including the concentration step.

The two solutions (protein and polysaccharide) were mixed with an overhead stirrer. Acid was added at different speeds into the system. In the majority of the trials, the acid addition was performed through a pumping system, at a flow-rate of 0.8 mL/min until pH below the IP was reached. To pump the acid into the system, two syringes filled with acid were connected by a plastic tube to the vessel which contained the system. With lower volumes, the flow-rate was adjusted to 0.4 mL/min, allowing an easier control of the pH. When the desired pH was reached, the suspension was stirred for another 5 min. The fibers were then submitted to heat treatment in a waterbath for 30 min at 80°C, and subsequently cooled down. Then, the fibers were pressed in a sieve to remove the excess of moisture, weighed and analyzed using the CLSM.

The parameters studied to improve the quality of teff fibers were the influence of heat treatment, protein concentration in the starting solution, the addition of extra ingredients, and the effect of the presence of ethanol in the initial protein solution.

Functional properties of teff flour

In order to characterize the functionality of the water-soluble proteins present in teff flour, several functional properties were studied: bulk density (BD), protein solubility (PS), and water and oil absorption capacities (WAC and OAC). All experiments were performed in duplicate, and the respective averages and standard deviations were computed, when applied.

Bulk density

This experiment was carried out using the procedure of Narayana and Narasinga (1984) [15]. 1.0 g of the flour sample was transferred into an already weighed measuring cylinder (W1). Packed-bulk density of the flour (PBD) was determined by gently tapping the flour sample to eliminate spaces between the flour; the level was then noted as being the volume of the sample and weighed (W2). Bulk density of the samples was calculated using Equation 1.

$$Bulk\ density\left(\frac{g}{mL}\right) = \frac{W_2 - W_1}{Volume\ of\ sample} \quad (1)$$

Protein solubility

From the water/flour mixture

This experiment was based on the procedures described on [16] and [17]. In order to assess the protein solubility of teff flour, 12 suspensions (with duplicates) with a concentration of 2% (w/v) were made from the mixture of 0.2 g of flour and 10 mL of distilled water (initial sample). Then, pH adjustments were performed to pH values ranging from 1 to 12, to investigate the influence of pH on protein solubility of the water-soluble proteins of the flour. For a better solubilization, the suspensions were stirred for 30 min at RT, using a magnetic stirrer, and the pH was adjusted to the required value with 0.5 M HCl or NaOH. The samples were then centrifuged at 4000x g for 30 min, and the total nitrogen content of the resultant supernatants was determined by Kjeldahl analysis. The solubility profile was constructed by plotting the average values of protein solubility (PS, in percentage) against each considered pH value. The percentage of soluble protein was calculated using Equation 2. The value was adjusted with the dilution resultant from the pH adjustment.

$$\text{Protein Solubility}(\%) = \frac{\text{Amount of protein in the supernatant} \times 100}{\text{Amount of protein in the initial sample}} \quad (2)$$

Water absorption capacity

Water absorption capacity (WAC) was determined by centrifugation, using the method outlined by Beuchat *et al.* [18], with modifications [19], [20]. 1.0 g of sample (teff flour) was added to pre-weighed 15-mL centrifuge tubes. Then, 10 mL distilled water was added to each sample. The suspensions were then mixed with a vortex mixer at maximum speed for 2 min. The samples were allowed to stand at RT for 30 min, and then centrifuged at 3000x g for 20 min. After centrifugation, the supernatant was discarded and the centrifugation tube containing the sediment was weighed. WAC was calculated with Equation 3, and expressed as g of absorbed water per g of sample.

$$\text{WAC} = \frac{W_2 - W_1}{W_0} \quad (3)$$

Where:

W_0 – Weight of the dry sample (g)

W_1 – Weight of the tube plus the dry sample (g)

W_2 – Weight of the tube plus the sediment (g)

Oil absorption capacity

The method previously described for WAC was applied for OAC, replacing distilled water with sunflower oil in the same concentration. Immediately after centrifugation, the supernatant was carefully poured into a 10 mL graduated cylinder, and the volume was recorded (V_2). OAC was calculated with Equation 4, and expressed as mL of oil per g of sample.

$$\text{OAC} = \frac{V_1 - V_2}{W_0} \quad (4)$$

Where:

W_0 – Weight of the dry sample (g)

V_1 – Volume of oil added to the dry sample (mL)

V_2 – Volume/amount of supernatant poured to a graduated cylinder (g or mL)

Analytical methods

SDS-PAGE

SDS-PAGE was performed using the Criterion Cell system from Bio-Rad, with 12% and 18% Criterion TGX Precast gels. Prior to electrophoresis, the protein solutions (3 to 5 mg/mL) were diluted in Laemmli buffer with DTT (54 mg/mL) in a 1:1 ratio, and were afterwards heated at 90°C for 10 min and cooled at RT. The non-homogeneous samples were centrifuged at 14000 rpm for 5 min. Then, 15 µL of each sample were loaded into the gel, which was left running for 45 min at 200 V. The protein marker used was a Protein ladder Precision Plus Protein Standards (Bio-Rad), with molecular weights ranging from 10 to 250 kDa. The staining procedure followed was the one from Instant Blue (Expedeon), based on which Coomassie Brilliant Blue was applied to the gels during one hour, followed by two changes of water.

Differential Scanning Calorimetry

Approximately 20 mg of protein dispersion (1 to 5% (w/w) protein) were sealed in aluminum hermetic pans in the DSC equipment (DSC Q1000, from TA Instruments). As a reference, a sealed empty pan was used. The samples were analyzed from 20°C to 120°C, at 5°C/min.

Confocal Laser Scanning Microscopy

CLSM (Leica TCS SP5, from Leica Microsystems Ltd.) was used with the aim of studying the microstructure of the samples. Rhodamin-B staining was used to visualize protein, using the laser DPSS 561 (with an excitation wavelength of 561 nm). Teff samples were stained with 0.2% Rhodamin-B in water (20 µL per mL of sample) and afterwards visualized with the CLSM. The 20x immersion and 63x1.2 water corrected objective lenses were utilized, and the digital images obtained had a resolution of 1024x1024. Images were acquired with zoom 1.0 and 2.0.

Mastersizer

With the aim of comparing the particle size distribution at different stages of milk development, namely after the samples have been submitted to centrifugation, homogenization or heat treatment, some droplets of protein solutions were poured into the Mastersizer device (Mastersizer 2000, from Malvern).

3. Results and Discussion

3.1. Optimization of protein extraction from teff

Overall, the best procedure was the extraction with water (Table 1, B), due to the conjugation of its better results (more protein extracted), one of the highest protein yields and its higher applicability to industry. Therefore, it was decided to perform the further extractions using this procedure.

SDS-PAGE was performed as described above. Figure 1 resumes the results obtained from all the extraction trials (identified as mentioned in Table 2). The band thought to correspond to prolamins is indicated in the figure.

In relation to lane A, the bands aren't easily distinguished, possibly due to the presence of impurities (fat and glycoproteins). Bands of high molecular weight can be detected (at 150 kDa, for example), which might correspond to protein aggregates. Even though prolamins should not have been extracted in this trial, very thin prolamin bands are thought to be present in lane A (at about 20 and 25 kDa). The other bands present in this lane are thought to be from water-soluble proteins. These results confirm that the extraction was not very successful, probably because the extraction was performed at pH 6.5 instead of pH 8.0 (and the extraction at higher pH values is normally more effective).

Table 1 - Total amount of protein (Nx5.71 [91]), amount of water-soluble protein (WSP) extracted and visual observations relative to the supernatant of each extraction trial.

Method	Protein (%)	Protein (g)	Protein yield (%)	WSP (%)	Stability of supernatant
A	0.32	0.85	7.4	50.2	Precipitation
B	0.51	1.35	10.3	53.6	Precipitation
C	0.10	0.26	6.4	N/A	Precipitation
D	0.23	0.62	13.7	52.4	Precipitation
E	0.50	1.32	6.2	51.8	No precipitation

Table 2 - Identification of the samples present in each lane of the SDS-PAGE gel. All samples were prepared with DTT, except B2.

Lane	Sample
S	Protein standard ladder
A	Supernatant pH 6.5 at RT
B1	Supernatant pH 8.0 at RT with DTT
B2	Supernatant pH 8.0 at RT without DTT
B3	Dissolved proteins from teff flour
C	Extraction with ethanol – supernatant
D	Extraction with salt – supernatant
E	Extraction with water at high temperature – supernatant

Regarding the lanes correspondent to the samples extracted at RT and pH 8.0, with and without DTT addition (B1 and B2, respectively), the bands are also not very distinguishable, as mentioned above. However, there are pronounced similarities of these bands with the ones present in lane A, which might also be from WSP. Moreover, very thin bands at about 37 and 75 kDa are also detected in B2 (reported in the literature as 36.1 and 66.2 kDa, respectively [21]). These bands are absent when reducing conditions were used (B1), which possibly indicates that

they were polypeptides linked by disulphide bonding and might be prolamin oligomers [21].

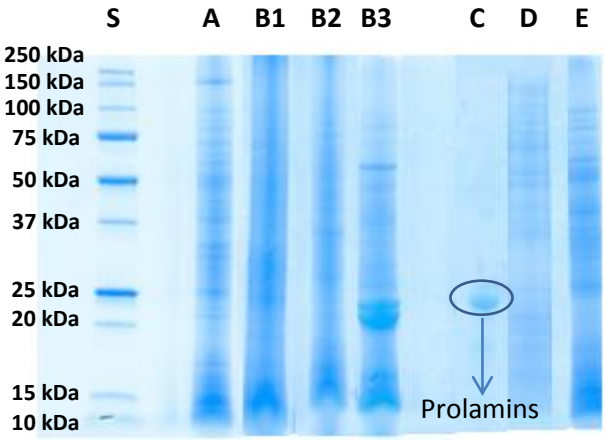

Figure 1 - Electrophoresis gel obtained from the analysis of samples from various extraction trials.

Both in reducing and non-reducing conditions (B1 and B2), the 22.5 and 25.0 kDa bands are only slightly visible, and at similar intensities. Compared to the prolamin bands in B3, there are no prolamins in the water soluble fractions (B1 and B2).

In lane B3 (dissolved proteins from teff flour – 3 mg/mL protein content), the major prolamin bands, as reported in the literature [6], are present (between 20 and 25 kDa, comparable with the reported bands at 22.5 and 25 kDa). Moreover, bands at 50 and 75 kDa can also be detected; in the literature [6], similar bands were reported (at 50.2 and 66.2 kDa, respectively), but under non-reducing conditions. The bands of low molecular weight (below 15 kDa), present both in A and all B lanes, are thought to be contaminating proteases, but no similar bands have been previously reported in the consulted literature.

In lane C, the prolamin bands between 20 and 25 kDa that have been reported [6], [21] are clearly detected. This was expected because this extraction was performed with ethanol, and prolamins are alcohol-soluble, so these proteins should be the only extracted proteins under these conditions, as it happened.

Lane D shows similar bands as lane A. However, some of these bands may correspond to globulins (salt-soluble proteins), but since these bands are not reported in the literature, no conclusions can be taken.

Finally, the extraction at higher temperature (E) shows clear bands, similar as those in lane A but slightly more concentrated.

Table 3 - Samples analyzed by DSC and respective designation (matching with that of Figure 2).

Designation	Sample
1	Supernatant 1% (w/w) protein concentration
2	Teff flour/water suspension with 5% (w/w) protein concentration
3	Previous suspension after centrifugation (precipitate and supernatant)

In this project, DSC was used to verify the denaturation temperature of teff proteins (endothermic peak in the resultant graphs), comparing it with the reference temperatures present in the literature. According to Taylor et al. [6], only one endothermic peak was expected, at 69.85°C. This peak has been reported to correspond to the denaturation of prolamins. The samples analyzed by DSC are shown in Table 3 and Figure 2.

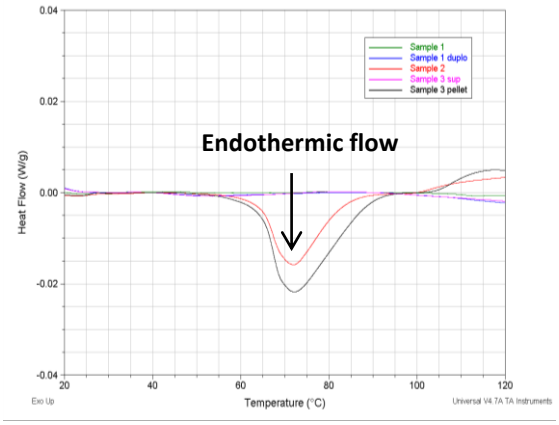

Figure 2 - DSC analysis of teff samples present in Table 3. Sample 1: 1% protein; Sample 2: 5% protein; Sample 3: 5% protein centrifuged. The temperature increments adopted were of 5°C/min.

In our studies, an endothermic peak for sample 2 was observed at 71.95°C, which is comparable to the results reported [6]. Samples 1 and 3 (supernatant) have not produced any peak. However, in the sample 3 precipitate (black curve in Figure 2), the expected behavior was observed. The fact that nothing was detected in the supernatant and that the correct behavior was observed for the precipitate may be due to the higher protein concentration in the precipitate, or even due to

the presence of different albumins in the supernatant, which make the determination of denaturation temperature difficult. From this DSC study, it can be concluded that the endothermic peak obtained was concordant with the literature [12], and may therefore be due to the prolamin, which is mainly present in the pellet (because the albumins remained soluble in the supernatant).

3.2. Milk analogue development

Firstly, it should be stated that the dialysis is a common step in milk formulation because more than proteins can be extracted: polyphenols, salt and sugars may be extracted, and dialysis is used to remove as much of these compounds as possible from the product. As for the concentration, it was an important step in this milk formulation because the protein extracted was low and the product was also diluted after dialysis. Finally, heat treatment was performed to pasteurize the milk.

Influence of method of dialysis in the final product

Protein extraction was performed as described above, and two methods of dialysis were compared: dialysis against RO-water or against RO-water with 10mM NaCl. Comparing the tasting results of the non-dialyzed product with those from the dialyzed products (see Table 4), it has been concluded that dialyzing is necessary to remove the majority of bitterness from the product. Therefore, to get acceptable milk analogues from teff, dialysis would have to be included in the processing.

The preferred product from the informal tasting sessions (see Table 4) was the product dialyzed against salt and that did not receive heat treatment. Moreover, the unheated product that was dialyzed against water was also preferred when compared to the similarly produced heated milk. Since the unheated products were better than the heated ones, further optimization of the heating step could be done, by changing the temperature and/or the time of heat treatment.

Influence of the absence of dialysis on the final product

Protein extraction was performed as described earlier, and no dialysis was done. From the informal tasting sessions performed at NIZO (see Table 4), the non-dialyzed milk was considered bitter. The product obtained after heating was even bitterer and acquired a brown coloration. When polyphenols (which were not even partly removed because dialysis was not performed in this trial) undergo heat treatment, aggregation can occur, and the formed aggregates may be bitterer than the initial compounds present in solution. Moreover, the solution may be getting browner and bitterer after heating due to a possible Maillard reaction of sugars with proteins [22].

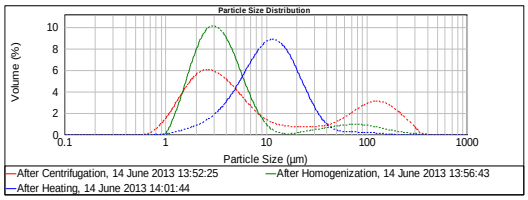

Figure 31 - Particle size distribution of teff solutions (average of two measurements). Red: teff supernatant; green: homogenate; blue: homogenate after heat treatment at 74°C for 20 s.

Table 4 - Comparison of the milk analogues produced in this project. The asterisk indicates the preferred product in the informal tasting sessions at NIZO.

Product	Dialysis method	Heat treatment	Taste
Dialyzed milk	RO-water	-	Sweet but pleasant
		Yes	Bean-like and astringent
	RO-water + 10 mM NaCl	-	Good mixture of sweet and salt*
		Yes	Astringent
Non-dialyzed milk	-	-	Bitter
		Yes	Very bitter and more aftertaste

The results of size determination by Mastersizer are presented in Figure 3. In Figure 3, it can be observed that the particles are uniform in size (narrow distribution). For the sample after homogenization, it can be seen that the mean diameter is about 2 µm, and that the system contains a small number of agglomerated particles with a diameter of about 25 µm. So, as expected, there was a higher volume of small-sized particles (less than 2 µm) after homogenization. In the heated sample, there was a shift to higher sizes when the mean diameter of the samples was

compared (approximately 10 µm), which may indicate the heat-induced formation of protein aggregates.

3.3. Fiber formation

There was no fiber formation in the case of the unheated teff solution with ethanol. One hypothesis for this occurrence could be that the pH was too low for the fiber formation, or the pH might have been incorrectly measured because ethanol can influence the pH electrode. However, since in this trial there was more polysaccharide available (less protein concentration than in the previous trials, but the ratio between protein and polysaccharide was maintained), more fiber formation than in the previous trials was expected.

Influence of the protein concentration on the formation of fibers

The objective of the execution of this trial was to verify if there was more fiber formation when the protein content was higher (about 1% protein – concentrated trial – instead of 0.3 or 0.5% – 1st and 2nd trials). Also, some ingredients were added before the fiber formation (Ing.1 and salt).

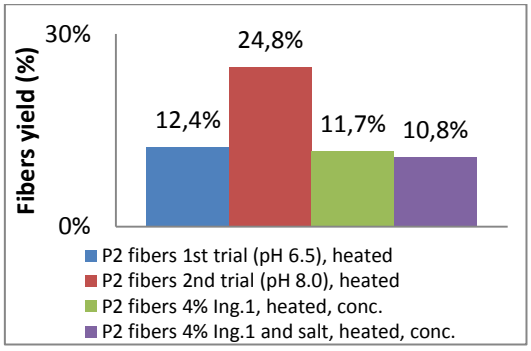

Figure 4 - Comparison between the heated P2 fibers with and without concentration, in relation to fibers yield.

The addition of ingredients was only attempted in the concentrated fibers, so there is no suitable control of concentrated fibers to compare these results with. Nevertheless, the yield reductions in this trial, shown in Figure 4, were also attributed to the addition of ingredients.

The salt addition to the fibers was 0.26% (w/w). Low salt concentrations, depending on the type of salt, usually help in the formation of complexes [23], but the contrary was verified in the obtained results. No explanation was found for that observation.

From these fiber trials, it was concluded that teff is not suitable for fiber making in the tested conditions. The exception was the simple fibers, without any additives, which although non-cohesive and watery, possessed a more neutral taste when submitted to an informal tasting.

In the CLSM images, it is clear that the fibers from the second trial (right picture of Figure 5) are longer than the ones from the first trial, and more aligned. The second extraction was more effective, like previously referred, so it is possible that the higher protein concentration in the second trial (0.5% instead of 0.3% protein content) might explain these differences in the alignment of fibers.

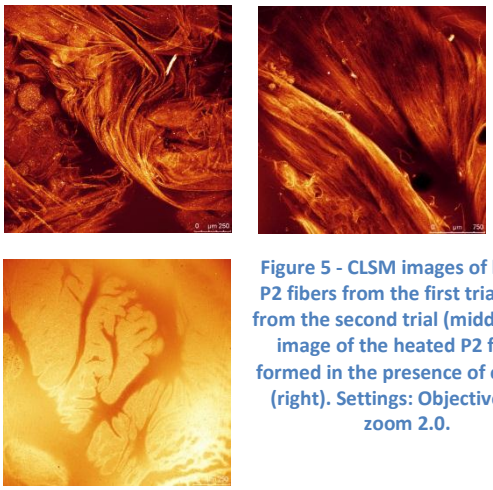

Figure 5 - CLSM images of heated P2 fibers from the first trial (left), from the second trial (middle), and image of the heated P2 fibers formed in the presence of ethanol (right). Settings: Objective 20x, zoom 2.0.

3.4. Functionality properties of teff flour

The results of water and oil absorption capacity and bulk density of teff flour are shown in Table 5.

Table 5 - Average values of functional properties of teff flour. The emulsification data represented is relative to 5% teff flour suspensions at neutral pH. All values are averages ± standard deviations of duplicate analyses.

Functional property	Teff flour
Water absorption capacity (mL/g)	3.03 (±0.05)
Oil absorption capacity (mL/g)	1.29 (±0.02)
Bulk density (g/mL)	0.40 (±0.01)

Compared to other types of sample (such as concentrates and isolates), teff flour has low functionality, as expected. Teff flour contains more carbohydrates than protein concentrates and isolates, which negatively

influences the functional properties when compared to those protein samples [24]. It has been reported that, in general, proteins have to be in solution or in fine suspension to possess desirable functional properties [25]. Compared to the literature, teff flour has a lower bulk density (see Table 5) than cowpea flour [26] and lupin flour [27].

The solubility profile for teff flour (Figure 6) is comparable to that of lima bean and chickpea flours [28], [24], although presenting much lower solubility values (less than 20% instead of 80-90%). Nevertheless, when the albumins extracted in the solubility trial were calculated, instead of the total protein extracted, the solubility results were different. As shown in Figure 7, the WSP extracted from the flour ranged from 34.7 to 73.4%. The lack of electrical charge in the IP negatively influenced the solubility of proteins (34.7%), as expected. In the optimum pH for the development of milk products (between pH 7 and 9), the albumins extraction was acceptable (more than 50%).

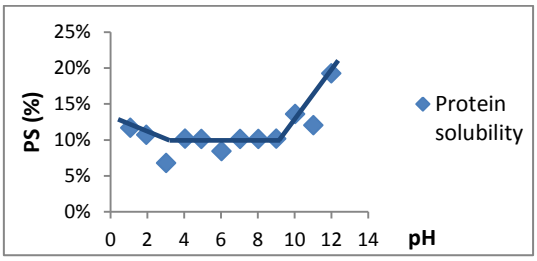

Figure 6 - Solubility profile of teff flour suspensions, obtained by plotting the averages of protein solubility (%) against the average pH, drawing a line to fit the data.

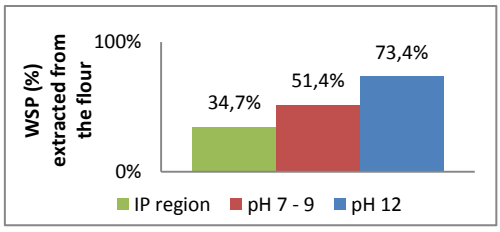

Figure 7 - Extracted water-soluble proteins (in %) as a function of pH, in the protein solubility tests. The percentages represented are referent to the estimated WSP content of the teff flour.

Compared to data from the literature, teff flour has higher water and oil absorption capacity than that of chickpea flours [11]. For that reason, teff flour may have potential as an ingredient in meat, bread, soup and cake formulations [29].

The higher oil absorption capacity compared to chickpea flours [11], [24] is probably due to the presence of more available non-polar side chains in teff proteins than in those of chickpea flours [11], [29]. These chains may bind the hydrocarbon side chains of oils among the flours, which may result in higher oil absorption [29]. It is known that teff has high carbohydrates content, so that could explain the obtained results. The higher WAC in teff flour when compared to chickpea flours [11], [24] and lupin flours [27] can be explained by the presence of more hydrophilic parts in carbohydrates, such as polar or charged side chains, which may increase water absorption [29].

4. Conclusions

The main conclusion from the extraction trials was that it is very difficult to extract protein from teff, so one idea could be to use teff flour as such in product formulation, which would imply less additional costs than performing this low yield-extraction from teff.

Regarding the milk development, a milk drink was developed from teff flour, with a fresh and nice taste. In future studies, the addition of ingredients should be adjusted, in order to obtain a desirable salt-sugar balance. Another very important conclusion from the milk development studies was that dialysis was proven to be essential in the development of a satisfactory product from teff. In fact, this step was proven crucial in the elimination of off-flavors from the teff milk obtained. Furthermore, the unheated milk products were considered better than the heated products, in terms of taste, so, the heat treatment conditions should also be optimized, either by changing the time or the temperature of the process.

Regarding fiber formation from vegetable protein extracts, it was concluded that forming fibers from teff is possible, but further improvement is required. The low amount of fibers obtained from teff is connected to the low protein concentration in teff extracts. Moreover, we can affirm that several aspects can influence fiber formation: overall protein concentration, presence of additional ingredients in the starting solution of the fibers, purity of the protein, presence of

insoluble parts and even molecular properties of each protein.

In this study, it has been shown, through detailed characterization of the functional properties, that the teff flour used possesses high water and oil absorption capacities. This flour could therefore be potentially useful in flavor retention, improvement of palatability and extension of shelf life in meat products [15].

5. References

[1] P. Belton and J. Taylor, "Millets," in in *Pseudocereals and less common cereals: grain properties and utilization potential*, Springer, 2002, pp. 177–213.

[2] J. R. N. Taylor and M. N. Emmambux, "Gluten-free foods and beverages from millets," in in *Gluten-Free Cereal Products and Beverages*, Elsevier Inc., 2008, pp. 120–148.

[3] A. Araya, L. Stroosnijder, G. Girmay, and S. D. Keesstra, "Crop coefficient, yield response to water stress and water productivity of teff (Eragrostis tef (Zucc.)," *Agricultural Water Management*, vol. 98, no. 5, pp. 775–783, Mar. 2011.

[4] E. Hopman, L. Dekking, M.-L. Blokland, M. Wuisman, W. Zuijderduin, F. Koning, and J. Schweizer, "Tef in the diet of celiac patients in The Netherlands.," *Scandinavian journal of gastroenterology*, vol. 43, no. 3, pp. 277–82, Mar. 2008.

[5] M. M. Gebremariam, M. Zarnkow, and T. Becker, "Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review," *Journal of Food Science and Technology*, Jun. 2012.

[6] A.-R. Adebowale, M. N. Emmambux, M. Beukes, and J. R. N. Taylor, "Fractionation and characterization of teff proteins," *Journal of Cereal Science*, vol. 54, no. 3, pp. 380–386, Nov. 2011.

[7] a. V. Moroni, S. Iametti, F. Bonomi, E. K. Arendt, and F. Dal Bello, "Solubility of proteins from non-gluten cereals: A comparative study on combinations of solubilising agents," *Food Chemistry*, vol. 121, no. 4, pp. 1225–1230, Aug. 2010.

[8] S. R. Bean and G. L. Lookhart, "Electrophoresis of cereal storage proteins.," *Journal of chromatography. A*, vol. 881, no. 1–2, pp. 23–36, Jul. 2000.

[9] P. Belton and J. Taylor, "The Major Seed Storage proteins of spelt wheat, sorghum, millets and pseudocereals," in in *Pseudocereals and less common cereals: grain properties and utilization potential*, Springer, 2002, pp. 9–15.

[10] A. Chowdhury, A. Bhattacharyya, and P. Chattopadhyay, "Study on functional properties of raw and blended Jackfruit seed flour (a non-conventional source) for food application," *Indian Journal of Natural Products and Resources*, vol. 3, no. 3, pp. 347–353, 2012.

[11] E. A. A. Arab, I. M. F. Helmy, and G. F. Bareh, "Nutritional Evaluation and Functional Properties of Chickpea (Cicer arietinum L.) Flour and the Improvement of Spaghetti Produced from its," *Journal of American Science*, vol. 6, no. 10, pp. 1055–1072, 2010.

[12] T. K. Mohamed, K. Zhu, A. Issoufou, T. Fatmata, and H. Zhou, "Functionality, in Vitro Digestibility and Physicochemical Properties of Two Varieties of Defatted Foxtail Millet Protein Concentrates," *International Journal of Molecular Sciences*, vol. 10, no. 12, pp. 5224–5238, Dec. 2009.

[13] A. M. Fekria, A. M. A. Isam, O. A. Suha, and E. B. Elfadil, "Nutritional and functional characterization of defatted seed cake flour of two Sudanese groundnut (Arachis hypogaea) cultivars," *International Food Research Journal*, vol. 19, no. 2, pp. 629–637, 2012.

[14] V. A. Jideani, "Functional Properties of Soybean Food Ingredients in Food Systems," in in *Soybean - Biochemistry, Chemistry and Physiology*, T.-B. Ng, Ed. InTech, 2011, pp. 345–364.

[15] Y. A. Adebowale, I. A. Adeyemi, and A. A. Oshodi, "Functional and physicochemical properties of flours of six Mucuna species," *African Journal of Biotechnology*, vol. 4, no. 12, pp. 1461–1468, 2005.

[16] T. D. Samanta and S. Laskar, "Functional Properties of Erythrina Variegata Linn. Seed Protein Isolate," *Journal of Applied Chemical Research*, vol. 15, pp. 19–28, 2010.

[17] E. Adeyeye and F. Omolayo, "Chemical composition and functional properties of leaf protein concentrates of Amaranthus hybridus and Telfairia occidentalis," *Agriculture and Biology Journal of North America*, vol. 2, no. 3, pp. 499–511, Mar. 2011.

[18] L. R. Beuchat, "Functional and electrophoretic characteristics of succinylated peanut flour protein," *Journal of Agricultural and Food Chemistry*, vol. 25, no. 2, pp. 258–261, Mar. 1977.

[19] F. Tounkara, T. Amza, C. Lagnika, G. Le, and Y. Shi, "Extraction , characterization , nutritional and functional properties of Roselle (Hibiscus sabdariffa Linn) seed proteins," *Songklanakarin Journal of Science and Technology*, vol. 35, no. 2, pp. 159–166, 2013.

[20] T. D. Samanta and S. Laskar, "Functional Properties of Erythrina Variegata Linn. Seed Protein Isolate," *Journal of Applied Chemical Research*, vol. 15, pp. 19–28, 2010.

[21] a. S. Tatham, R. J. Fido, C. M. Moore, D. D. Kasarda, D. D. Kuzmicky, J. N. Keen, and P. R. Shewry, "Characterisation of the Major Prolamins of Tef (Eragrostis tef) and Finger Millet (Eleusine coracana)," *Journal of Cereal Science*, vol. 24, no. 1, pp. 65–71, Jul. 1996.

[22] H. Pinheiro, M. Mateus, and J. Empis, "Tecnologia Alimentar: Elementos de Apoio." Técnico, Lisboa, Lisbon, 2013.

[23] F. Weinbreck, "Whey protein/polysaccharide coacervates: structure and dynamics," Utrecht University, 1977.

[24] B. Cristina and N. Andrei, "Chemical and functional characterization of chickpea protein derivates," no. October. pp. 16–25, 2009.

[25] Y. V. Wu, "Emulsifying activity and emulsion stability of corn gluten meal," *Journal of the Science of Food and Agriculture*, vol. 81, pp. 1223–1227, 2001.

[26] K. E. Ekpo and A. M. Ugbenyen, "Comparative evaluation of certain functional properties of four different varieties of Lima Bean (Phaseolus Lunatus) flour," *Scholars Research Library*, vol. 2, no. 2, pp. 399–402, 2011.

[27] H. Tizazu and S. A. Emire, "Chemical composition, physicochemical and functional properties of lupin (Lupinus albus) seeds grown in Ethiopia," *African Journal of Food, Agriculture, Nutrition and Development*, vol. 10, no. 8, pp. 3029–3046, 2010.

[28] E. M. Ogunbusola, T. N. Fagbemi, and O. F. Osundahunsi, "Chemical and Functional Properties of Full Fat and Defatted White Melon (Cucumeropsis mannii) Seed Flours," *Journal of Food Science and Engineering*, vol. 2, pp. 691–696, 2012.

[29] M. A. A. Awad-allah, "Evaluation of Selected Nuts and Their Proteins Functional Properties," *Journal of Applied Sciences Research*, vol. 9, no. 1, pp. 885–896, 2013.