Protein Extractability and Thermally Induced Gelation Properties of African Locust Bean Proteins (*Parkia biglobosa* Jacq. R.Br)

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Abstract: African locust bean (AfLB) protein isolates could be an interesting alternative to the use of soy as an ingredient for the development of new protein-rich products. From AfLB seed flour, the protein extractability yields by aqueous extraction were determined as a function of pH and ionic strength. Then thermally induced gelation of various protein suspensions relating to protein concentration was studied. The most critical factors affecting extractability were the pH and the presence of fat. As a function of the extraction process, the extraction yield of protein from AfLB flour ranged from 30 to 65%. Two major fractions of proteins detected in AfLB seeds were albumins and globulins, comprising four families: legume-like protein, vicilin-like proteins, convicilin, and albumins. The globulin isolate had the lowest solubility at pH 3.5-4 and the highest at pH 8-10. The solubility of albumin isolate was lightly affected by pH and ionic strength. At pH 7, the minimum protein concentration for thermal gel formation ranged from 55 to 120 g/L as function purified state of proteins. The less purified extract with a simpler process made it possible to obtain a gel needing a lower protein concentration. This last way seems promising to the development of new foods based on African locust bean flour.

Keywords: Parkia biglobosa; African locust bean; Protein; Solubility; Thermal gel.

1. Introduction

In many rural areas in developing countries, protein malnutrition is a prime public health issue. Plant protein presents the advantage of being available in larger quantities and at a lower cost than animal protein. The African locust bean (AfLB) belongs to the family *Leguminosae*, may be a promising alternative source of plant protein. This seed is widely consumed throughout West and Central Africa, particularly in the fermented form as called "soumbala", which is a powdered condiment used as a spice. Fermented African locust beans provide energy, fibre, energy, vitamins (vitamin B, riboflavin, and vitamin A) as well as minerals. Fermented AfLB also possesses typical organoleptic characteristics, modifying smell, and taste of dishes flavoured with that [1]. Unfortunately, in the form of "soumbala", AfLB does not represent a significant source of protein. However, these seeds have a composition in proteins and lipids comparable to soybean [2]. Protein isolates of AfLB could be an attractive alternative to soya as an ingredient for the development of new protein-rich products. Indeed, in recent years, soya has come to represent two-thirds of global plant protein consumption. The widespread use of this raw material in food products has overshadowed other more local sources of plant protein from West Africa, such as African locus bean, Pigeon pea, Bambara groundnut, African yam bean [3].

Plant protein is provided by traditional foodstuffs such as legumes and cereals, but also by plant protein products. These plant protein products result from the milling of seeds, leaves, and tubers, and are used for their techno-functional and nutritional properties. Plant proteins have a wide diversity of structure and techno-functional properties according to their botanical origin, their localisation in the plant, and the technologies used to extract them [4,5]. Beyond their nutritional value, plant proteins also display specific physicochemical properties that are of interest in the context of food technology. However, as these proteins are globular and compact, it is sometimes necessary to carry out structural modifications by chemical, physical or enzymatic means to alter their properties to widen their range of applications [6]. These proteins can be used as emulsifiers, gelling agents, or foaming agents. They are also able to enhance absorption and retention of lipids and water, facilitate film formation, increase food viscosity, and control colour and expansion. These properties vary according to the source of the proteins, their concentration, the particle size, the experimental treatments, and conditions (pH, temperature, ionic strength). These functional properties determine how these proteins are used in meat-based food products, bread, pastries, diet products, and milk substitutes [7].
The major seed storage proteins of legumes are 7S and 11S globulins, with albumins constituting the remainder [8]. Various groups have done many studies on extractability conditions of these proteins, and most of them were done under alkaline conditions (pH 8-12), followed by pHi precipitation of globulin fractions [5]. Protein solubility is affected by several determining factors, including intrinsic factors such as the nature of the amino acids of the protein and their composition (hydrophilic and hydrophobic residues), and their distribution on the surface of the protein. More environmental factors such as pH or salt, play critical roles in protein solubility. Variation in pH leads to changes to the ionization of various acid-alkali groups on the protein surface, affecting the overall charge. Protein solubility can also be affected by other factors such as the preparation conditions (ratio dry matter/aqueous fraction), the extraction method used, and the drying procedure employed to obtain a purified powder from the protein solution [4].

We must admit that most of the processes used to obtain high-purified fractions of globulin or albumin from legume seeds have low yields and use as protein ingredients in foodstuffs is limited. The dry process (air-classification) used in industry is successfully applied to soya, pea, faba bean, or lupine to obtain concentrates or isolates. Unfortunately, its application to other under-exploited legumes like the African legumes is not yet topical, cause of the costs of equipment and the variability of sourcing. The development of products from AILB grain requires a good understanding of both the factors affecting the extractability of protein from flours and the functional properties of isolated proteins and their fractions, but keeping in mind the potential use of isolates as a sustainable protein source in a low-income developing country.

The aim of this study was to determine the extractability conditions of the proteins from African locust bean flours and to investigate the solubility of the globulin and albumin fractions. Then thermally induced gelation of various protein suspensions relating protein concentrations was studied.

2. Material and methods

2.1 Plant material

Five kilograms of African locust bean (AFLB) seeds were obtained from the local market in Dalaba (Guinea). The seeds were stored in a plastic container at 4 °C in a cold room. In order to separate the AFLB seeds from their pulp, they were soaked for 30 minutes in water at 40 °C. The seeds were then washed, drained using a sieve with a 5mm mesh and hulled by hand. The seeds obtained were placed on filter paper and dried in a heat chamber at 35 °C for 12 hours. The dried seeds were then milled using an IKA Werke Model 20 mill. A sieve with a 0.71 mm mesh was then used to obtain AFLB flour that could then be defatted.

2.2 Delipidation of African locust bean flour

The AFLB flour was defatted for 3 x 2h with a ratio of 100 g per litre of petroleum ether and then 2 x 2h with ethanol (96%) with a ratio of 100 g per litre of solvent. Between each cleaning, the solvents were eliminated by filtration through Buchner filter funnel n°4. The defatted powder was then spread out on filter paper and left to dry at room temperature under a laboratory fume hood.

2.3 Chemical analyses

Dry matter of the samples was determined by drying at 105 °C overnight as per the air oven drying method [9]. Protein content was determined by the Kjeldahl method with the quantification of total nitrogen, according to AOAC method [9]. The factor N x 5.25 was applied to convert the total nitrogen to protein content. The soluble sugars, after extraction, were determined with the phenol-sulphuric acid method [10]. Total starch was quantified according to the enzymatic assay procedure (Megazyme, Co Wicklon, Ireland). Fat content was determined in the Soxhlet apparatus using petroleum ether as the solvent of extraction [9]. The ash content was determined gravimetrically [11]. Fibre amount was obtained by difference. The analyses of the proximate composition were performed in triplicates for all the analysed parameters.

2.4 Protein extractability of African locust bean flour according to pH and ionic strength

5 g of non-defatted and defatted AFLB flour were stirred in 100 ml of phosphate-HCl buffer (10 mM) of different pHs for 3 hours. Concentrations of 0.1 M and 0.6 M of NaCl were added to the different pHs of interest. The pH range studied was from 2 to 10. After the extraction time, the suspensions were centrifuged at 10,000 x g for 30 minutes at 10°C. The protein concentration in the supernatant was determined by the Kjeldahl method. The extractability was expressed in percentage (gram of extractable protein per 100g of total protein present in flour).

2.5 Preparation of protein extracts for gelation study

With the aim of developing new protein-rich foods, the aptitude for thermal gelation of three extracts of AFLB proteins were studied. Two extractions at pH 7.2 (phosphate/HCl buffer 10 mM) were conducted with a ratio of
flour /buffer: 50 g non-defatted or defatted flour / 1 L buffer for 3 hours. Suspensions were centrifuged at 10,000 x g for 30 minutes at 10°C. Supernatants were concentrated by ultrafiltration (MW cut off 10 kDa, Kvick Lab cassette, GE Healthcare, Amersham Biosciences Corp., Uppsala, Sweden) to reach a maximum protein concentration of 119 g/L. These extracts were maned PEc and DfPEc respectively to protein extract from non-defatted flour and defatted flour.

The third extraction was conducted at a higher ratio of flour /buffer: 250 g non-defatted flour/1L buffer for 3 hours. To increase the level of recovery in proteins, the suspension was filtered on nylon filter with 200-micron mesh in place of centrifugation. The protein concentration in PEf was 55 g/L (Kjeldahl method).

2.6 Fractionation to globulin and albumin isolates

The defatted AflB flour was gradually added to the 0.1 M di-sodium hydrogen phosphate and potassium di-hydrogen phosphate buffer solution of pH 10 and stirred for 3 hours to allow solubilisation of all proteins. The solution was centrifuged at 10,000 x g for 30 minutes at 10 °C. The supernatant was filtered with Buchner filter funnel n°4, and the pH was then adjusted to 4.5 by the gradual addition of HCl at 3 % to precipitate the globulins. Then the suspension was centrifuged at 10,000 g for 30 minutes at 10 °C, to separate the soluble albumins (Alb) in the supernatant and the globulins (Glb) in the pellet. The albumin fraction was recovered and its pH was raised to 8 by the addition of 1 M of NaOH. The pellet containing the globulins was collected and suspended in a sodium-phosphate buffer (0.1 M in the presence of 5% potassium sulphate) at pH 8 with stirring overnight at 4 °C. The insoluble matter was eliminated by centrifuge (10,000 x g for 30 minutes at 10 °C). The globulin fraction and albumin fraction were concentrated by ultrafiltration and then desalted by diafiltration against 10 volumes (MW cut off 10 kDa, Kvick Lab cassette, GE Healthcare, Amersham Biosciences Corp., Uppsala, Sweden). Sodium azide at 0.02% sodium was added to the protein solution to prevent mold development, and the pH of the solutions was adjusted to 7.2. After dialysis, the solutions were freeze-dried and the powders were stored at 4°C.

2.7 Solubility of the isolates of AflB globulin and albumin

A total of 0.05 g of protein isolate (globulins or albumins) were stirred in 25 ml of a phosphate HCl/NaOH buffer for 3 hours. A concentration of 0.1 M of NaCl was added to the different pH values required. The range of pH values studies was increased to 2 to 12. The non-soluble proteins at the pH values studied were eliminated by centrifugation at 10,000 x g for 30 minutes at 10 °C. The soluble protein content was determined using the Lowry method. The reading was performed using a spectrophotometer UVISCO UV-1200, at 750nm. Solubility of protein isolate was expressed in grams of soluble protein per 100 grams of total protein.

2.8 Electrophoresis profile

The polypeptide composition of the globulin and albumin isolates was characterized by SDS-PAGE electrophoresis gel (Novex™ electrophoresis gels 8-16% Tris-Glycine). Samples were diluted at 35µg of protein per well in sample buffer: 187.5 mM Tris-HCl; pH 8.9; glycerol 10%; SDS 2%; bromophenol blue 0.05%. The SigmaMarker™ S8445, wide range, Mw 6.5-200 kDa protein markers were used. The migration was carried out at 35 mA per gel, in a migration buffer: trizma base 0.3%; glycine 1.45%; 0.1% SDS. After migration, the fixation was carried out in 3 successive distilled water baths heated for 1 min in the microwave at 550 W. The colouring was carried out with a 2.5% Coomassie blue in 25% ethanol solution with rocking stirring overnight. The gel was discoloured in several baths of distilled water, with rocking stirring until the desired result. The gels were photographed with the ChemiDoc™ XRS + System from Bio-Rad Lab.

2.9 Thermally induced gelation

The protein suspensions (PEc, DfPEc), initially at 119 g/L, were dilute in phosphate/HCl buffer pH 7, to reach protein concentration ranging between 20 to 119 g/L. The protein suspension PEf was studied at 20 and 55 g/L in proteins. Then, 5 ml of protein suspension were placed in sealed glass tube and heated from 40 to 85 °C with a heating ramp of 1°C/min, then maintained at 85 °C for 60 min. The tubes were then cooled in an ice bath for 10 min and left at 4 °C overnight. Then, the tubes were turned upside down and examined. The protein concentration for thermal gelation was considered the minimum concentration at which the gel was held to the upturned tube without falling out.

3. Results and discussion

3.1 Proximate analysis of African locust bean flours

The protein content of the non-defatted AflB grain flour was 24.1±2.1 g/100g (Table 1). In contrast, it was 30.7±2.3 for the defatted AflB grain flour. The amount of free sugars was 3.3%, whereas the part of carbohydrates (non-resistant starch) and crude fibres were 16.5% and 26.9%, respectively. It is important to note that defatting
results in a reduction of dry matter in defatted AfLB grain flour. This correspondingly increases the protein content, which is calculated from the dry matter. The same calculating effect is observed with the other compounds.

| Composition value          | Raw flour     | Defatted flour |
|----------------------------|---------------|---------------|
| Protein                    | 24.1±2.1      | 30.7±2.3      |
| Crude fat/oil              | 17.2±0.1      | trace         |
| Crude fibre                | 26.9±2.1      | 34.2±1.9      |
| Carbohydrates (starch)     | 16.5±1.9      | 20.8±1.2      |
| Sugars                     | 3.3±0.9       | 4.2±0.8       |
| Ash                        | 4.73±0.1      | 5.6±0.1       |
| Moisture                   | 4.5±0.4       | 7.2±0.6       |

Table 1. Proximate composition of the AfLB flour (g/100 g)

The interest of defatting is to reduce lipid-protein interactions [12], and to increase extractability. It has been found that the presence of dry matter leads to a modification of the solubility in water and increases the turbidity of protein suspensions, as in the case of soy protein solutions [13]. The protein rate in this fraction corresponded to the values cited in the literature of between 30 and 35% crude protein. This rate is higher than those of legumes traditionally consumed in Africa: beans, peanuts or Bambara peas, which contain between 16 and 29% [3].

3.2 Extractability of African locust bean flour proteins

The extractability of protein according to pH conditions and ionic strength was established for non-defatted AfLB flour, and defatted AfLB flour. Figure 1a shows that in the absence of sodium chloride, the extractability varies for about 14%, i.e. extractability at pH 10 is almost twice as extractability at pH 4. At pH 10, only 31% of available protein in the flour were solubilized. The lowest extractability obtained was at pH 4. As has already been shown in the literature, the isoelectric pH of many plant proteins, particularly globulins, is around pH 4-4.5 [14]. This explains the low extractability at this pH.

In the presence of salt, protein extractability was improved by a salting in effect which gives proteins a greater solubility by preventing them from aggregating. Thus, at pH 10 and with a NaCl content of 0.6 M, an extractability percentage of 55% was obtained.

In the absence of fats, the protein extraction percentage of defatted flour was around 57%, at pH 8 and pH 10 (Figure 1b). Defatting the flour allowed to increase extractability whatever the pH. We can notice that the effect of ionic strength is weaker on defatted flour.

This extraction yield is close to that described in the literature [15]. However, 40% of the protein content that is potentially available in flour, was unextracted. Proteins gone on trapped in the centrifuged pellet. It would be necessary to rewash the pellet several times to recover as much protein as possible. Additional washings conduct to higher volumes of supernatants with lower protein concentrations that so engage protein concentration steps to regain a suspension with an acceptable protein concentration. At this stage of our research, the potential recovery rate of the protein trapped in the pellets through rewashing has not yet been determined.

3.3 Solubility of globulin and albumin fractions

From protein suspension of defatted AfLB flour, fractions of globulin and albumin were obtained by selective precipitation at the isoelectric point of globulins. The characterization of the isolate of AfLB globulins revealed a protein content of 74.2% ± 0.2. For the albumin isolate, the protein content was 89.1% ± 0.1.
The solubility of AFLB globulin isolates presented a 'V shaped' profile characterized by a high solubility in acid pH and alkaline pH (Figure 2a). The solubility at pH 2 was of 87 and 91% without salt and with 0.1M of NaCl, respectively.

Figure 2. Study of the solubility of AFLB globulin isolate (a) and AFLB albumins isolate (b) according to pH and ionic strength (□ 0.6 M NaCl; ∗ without NaCl) in grams of soluble protein per 100g of total protein. The error bars show standard deviation.

The solubility was higher at alkaline pH. At pH 10, the solubility was of 87 and 97% without salt and with 0.1M of NaCl, respectively. The solubility was minimal, around the isoelectric point, of 41 to 68% respectively for an ionic strength of 0.1M of NaCl at pH 3 to 5. This profile is similar to globulin solubility profiles of soya and other legumes chick peas and lentils [16,17]. Generally speaking, a high solubility suggests that the extracted proteins are in a more native state [18]. The increase in the solubility can be explained by the preparation of the raw material, as prior to the freeze-drying of the protein solutions an adjustment of the pH to 8 was carried out, followed by centrifugation. Thus, when the globulins were resolubilised, a greater solubility was expected.

Like most proteins, solubility varies according to pH and therefore the protein's net charge. At an isoelectric pH, a protein's positive and negative charges are balanced, which facilitates interactions between proteins, to the detriment of protein-solvent interactions, which increases protein folding and consequently reduces solubility. At acid or alkaline pH values, the protein is respectively positively and negatively charged. This difference in net charge increases the forces of repulsion between particles, thereby promoting protein-solvent interactions which results in an enhanced solubility.

Figure 2b shows the solubility profile of albumin isolates according to ionic strength at different pH values. For the pH range between 2 and 8, the increase in the concentration of NaCl in the milieu led to a greater solubility of the albumins. At pH 4.5, close to the pI of the protein, a slight fall in solubility can be seen according to the ionic strength. This phenomenon may be due to a synergistic effect between the pI and the salting in effect. At very high pH values between 9 and 10, a maximum solubility higher than 90% was observed.

The albumin isolate presented a better solubility profile than the globulin isolate. Indeed, albumin remained almost completely soluble throughout the pH range studied, even at pI- 4. This high solubility is linked to the hydrophilic nature of AFLB albumins and its low hydrophobicity according to its 'water soluble' classification of Osborne. This solubility profile is similar to that observed for the albumin fraction of yellow peas [18,19] and of sunflower [20]. However, the albumins of Ginkgo biloba [16] present a minimal solubility at pH 5.

Figure 3 presents the SDS-PAGE patterns under non-reducing conditions of globulin and albumin fractions from AFLB. For the AFLB globulin isolate, the protein profile had four major polypeptides (molecular weights 35, 50, 60 and 69 kDa). This profile was greatly similar to globulins 7S and 11S in legume proteins [21–23]. We found a band at 69 kDa, which we named Convicilin in the reference to the major pea globulin. The diffuse band at 60-50 kDa was assimilated to the subunit of Legumin, and Vicilin (α, β, γ) 50kDa. The band at 35 kDa was assimilated to Vicilin (α, β) 35kDa.

The albumin isolate from AFLB also showed bands characteristic of this fraction, namely: Lipoxygenase (94 kDa), and 2S albumins at 20 kDa and 16 kDa, which are frequently described in albumin fraction from legumes [24]. Unlike the other legume albumins, we can notice the majority fraction A4 (50 kDa) and low-intensity band A3 at 45 kDa. At this step of works, we cannot state whether the bands at 50 and 45 kDa were indeed albumins or contamination by the globulin fraction. However, in legume proteins, albumin fraction was described as complex because contains various proteins as lectins, protease inhibitors and other functional proteins in addition to 2S albumins [24].
3.4 Thermal gelation

The aptitude for thermal gelation of whole AfLB proteins (globulin and albumin) was studied. With the aim of developing new protein-rich foods, it seems preferable to limit protein losses in a long process of separation/purification of protein fractions. So three kinds of AfLB protein suspensions were investigated. The composition of protein suspensions is presented in Table 2.

Table 2. Composition of AfLB protein suspensions

| Composition value g/L | PEc  | DfPEc | PEF |
|-----------------------|------|-------|-----|
| Protein               | 119.3±2.1 | 119.5±1.9 | 55.2±1.9 |
| Crude fat/oil         | 75.2±0.1 | trace     | 30.8±0.5 |
| Carbohydrates         | nd    | nd        | 26.5±1.1 |
| Dry mater % (w:v)     | 19.7±0.4 | 12.0±0.6   | 11.8±0.5 |

PEc and DfPEc suspensions, obtained after centrifugation and ultrafiltration, had comparable protein contents (119 g/L). Indeed ultrafiltration was carried out up to the maximum solute concentration before clogging of the membrane for these two suspensions. PEc being get from non-defatted flour, fat was at 75.2 g/L in the suspension. Accordingly, the dry matter of PEc suspension was 19.7% (w/v) against 12.0% for DfPEc obtained from defatted flour. The level of protein recovery was disappointing with only 35% based on initial protein content of AfLB flour. Indeed, low permeate volumes were obtained due to losses in the ultrafiltration system.

PEf was obtained after a filtration step (mesh 200µm) in place of centrifugation and ultrafiltration to potentially increase the level of protein recovery. Protein and fat concentrations were 55.2 g/L and 30.8 g/L, respectively. Carbohydrates were at 26.5 g/L. So, although the protein part of PEf was lower than PEc or DfPEc, the dry matter of suspension was 11.9% (w/v) and in the same range of other suspensions. The level of protein recovery was 65% based on the initial protein content of AfLB flour. The filtration used to obtain PEf allowed higher level of protein recovery than the centrifugation-ultrafiltration process. However the three protein suspensions had different physical stability. PEc and DfPEc were stables suspensions, all insoluble matter being removed by centrifugation. In contrast, the stability of PEf was transient; sedimentation appeared after storage for 12 hours at 4°C. Indeed PEf is composed of proteins in suspension, fats, carbohydrates, and particles finer than 200 µm.

Table 3 shows the minimum protein concentration for thermal gelation for the three kinds of protein suspension. The minimum protein concentration for thermal gelation was 80g/L for the PEc (suspension of non-defatted proteins) and 119 g/L for the DfPEc (suspension of defatted proteins) respectively.
Table 3. Total protein concentration at pH 7 to obtain a thermal gel

| Protein concentration g/L | 20    | 30    | 40    | 55    | 80    | 100   | 119   |
|---------------------------|-------|-------|-------|-------|-------|-------|-------|
| PEC                       | liquid| liquid| liquid| thick | gel   | gel   | gel   |
| DPPEC                     | liquid| liquid| liquid| liquid| liquid| thick | gel   |
| PEf                       | liquid| -     | -     | gel   | -     | -     | -     |

For PEC, the dry matter of the suspension is more important due to the presence of fat. The gel was obtained with less proteins, and the presence of fat did not interfere in the building of the molecular network needed for gel formation. For DPPEC, the higher thermal gelation protein concentration may be due to a partial denaturation of the proteins because of the solvent treatment [25,26].

For PEf, the minimum protein concentration for thermal gelation was 55 g/L. Thus, the least purified protein suspension made it possible to obtain a gel with a lower protein concentration. Polysaccharides could be involved in the structure of the gel. They could increase the viscosity of water phase inside the protein network, and enhance the gel structure [27].

Our results were comparable to those of other studies on plant-protein extracts. The minimum gelling concentrations of pea globulin, and soy glycinin are 84 and 66 g/L (on a protein basis) respectively [28]. Recently, Nivala et al. (2020) [29] reported that faba bean protein isolate formed heat gel at 100 g/L concentration. Lawal et al. (2005) [30] reported a higher gelling concentration (180 g/L) for African locust bean protein fractions (prepared by isoelectric precipitation) compared to that of pea, soy and faba protein isolates. In contrast, minimum gelling concentration is 80 g/L for a whole protein extract from African locust bean flour. The least purified extract gives a gel needing a least protein concentration [30].

Thermal gelation of plant-protein isolates requires high concentrations of protein. Moreover, processes to obtain these isolates are complex and time consuming. This does not appear to be very sustainable way to produce rich-protein foodstuffs. The use of unpurified extract should be preferred. As example the production model of soy juice is interesting as it allows to obtain a thermal gel with an initial protein concentration of 30 g/L [31]. The formation of soy gel is a two-stage process requiring the denaturation of protein molecules followed by aggregation [32]. The heat treatment causes the exposure of hydrophobic groups and the exposed protein molecules are negatively charged. More, the addition of salts induces charge neutralization on these heated protein aggregates, allowing hydrophobic interactions to occur and resulting in gel formation [33,34]. This issue should be explored with AfLB proteins to improve the gelation step from low concentrate protein suspensions.

4. Conclusion

Thanks to its high protein and fat content, African locust bean is a raw material, which should find uses other than transformation into a condiment. So, our work indicates that African locust bean seeds are suitable for the development of protein-based products. The process of protein extraction from AfLB flour is a crucial stage. In alkaline conditions and the presence of salt, the extractability rate can reach 57%. From isolate of globulin and albumin, gelation was obtained with high protein concentrations. Therefore, it is reasonable to conclude that this way is not suitable for the production of protein-rich food. On the other hand, the use of a less purified extract with a simpler process made it possible to obtain a gel needing a lower protein concentration. Further investigations are necessary to improve this protein-rich food model, in particular on the mechanical resistance of gel and on the potential acceptability by consumers.

5. References

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