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Functional properties of single cell protein produced by *kefir* microflora

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Abstract

Single cell protein (SCP) was produced by aerobic fermentation of cheese whey by *kefir* microorganisms. A feed-batch system was developed on a bioreactor of 4 1. The experiments were conducted under controlled pH (5.5) and temperature (30 °C) conditions. The biomass was analyzed for protein, lipids, carbohydrates and ash and its functional properties (emulsification, foaming, gelation) were studied. Single cell protein (53.9% protein) exhibited emulsifying properties similar to those of defatted soy flour, while its Ibaming activity and foam liquid stability were much higher. Finally, texture profile analysis of gels, formed by heating water dispersions of SCP, showed that the structures produced were stronger compared with those of gels made with soy flour.

Keywords: Single cell protein; *Kefir*; Fermentation; Cheese whey; Functional properties

1. Introduction

Increasing concern about pollution occurring from agricultural and industrial wastes has stimulated interest in converting waste materials into commercially valuable products. Additionally, there is a demand for the formulation of innovative and alternative proteinaceous food sources due to an insufficient supply from the traditional protein sources such as meat, fish or eggs.

Much interest has been focused on the potential of converting soy milk wastes, potato effluents, sugarcane bagasse, orange peels, shrimp-shell wastes, kimchi production wastes or forestry wastes (e.g. wood hydrolysates) to single cell protein (SCP) (Cheung, 1997; Choi, & Park, 1999; El-Nawwi & El-Kader, 1996; Ferrer, Paez, Marmol, Ramones, Garcia, & Forster, 1996; Parajó, Santos, Dominguez, & Vázquez, 1995; Schugerl & Rosen, 1997; Ziino, Lo Curto, Salvo, Signorino, Chiofalo, & Giuffrida, 1999). Technically, SCP is the manufacture of cell mass using microorganisms by culturing on abundantly available wastes. Algae, fungi and bacteria are the chief sources of microbial protein that can be utilized as SCP (Anupama & Ravindra, 2000). The production of the microbial biomass is done either by a submerged or solid state fermentation process. After fermentation, biomass is harvested and may be used as a protein source or be subjected to processing steps like washing, cell disruption, protein extraction and purification (Faust, 1987). In general, high production rates and protein yields as well as ease of production control makes SCP more attractive as a protein source compared with conventional plant and animal sources.

Cheese whey, a by-product of the dairy industry, is the liquid effluent remaining following the precipitation and removal of milk casein during cheese making. It represents about 85–95% of the milk volume and retains a significant amount (~55%) of milk nutrients. Among the most abundant of these nutrients is lactose (4.5–5% w/v) which is a suitable substrate for the production of value-added products using biochemical conversion processes. Whey also contains soluble proteins (0.6–0.8% w/v), lipids (0.4–0.5% w/v) and mineral salts (8–10% of dried extract), as well as appreciable

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quantities of other constituents, such as lactic and citric acids, non-protein nitrogen compounds, B group vitamins, etc. (González Siso, 1996; Marwaha & Kennedy, 1988).

Kefir, a mixed culture that ferments lactose, is known for the production of a refreshing fermented beverage popular in Eastern-European countries by inoculating milk with *kefir* grains (Farnworth, 1999; Güzel-Seydim, Seydim, Greene, & Bodine, 2000). The *kefir* grain is a symbiotic association of microorganisms belonging to a diverse spectrum of species and genera including lactic acid bacteria (*Lactobacilli, Lactococci, Leuconostoc*), yeasts (*Kluyveromyces*, *Candida*, *Saccharomyces* and *Pichia*) and sometimes acetic acid bacteria (*Acetobacter*). This microflora is embedded in a resilient polysaccharide matrix (kefiran) composed of equal amounts of glucose and galactose (Luis, Lopez, & Lemma, 1993; Rea et al., 1996).

Cultivation of *kefir* microorganisms on milk, as well as on various substrates (mixtures of molasses, raisin extracts and glucose-containing raw materials) has been studied (Athanasiadis, Boskou, Kanellaki, & Koutinas, 2001; Garrote, Abraham, & De Antoni, 1998; Güzel-Seydim et al., 2000). Cheese whey is a cheap and largely available raw material for microbial biomass of SCP production by *kefir* microflora.

It is well known that the proteins are one of the main constituents of foods. In addition to their nutritional function, proteins contribute significantly to the expression of sensory attributes of foods. The functional properties of proteins are important in determining their usefulness in food systems. There is limited information about single cell protein functionality (Huang & Kinsella, 1986, 1987; Otero, Wagner, Vasallo, García, & Añón, 2000; Vananuvat & Kinsella, 1975) and more knowledge is needed in order to assess their potential uses in foods.

Considering that in exploring single cell protein, isolated from cultivation of *kefir* microflora on cheese whey, as a new source of food protein, its functional properties need to be determined, this study was conducted to evaluate the emulsifying and foaming properties and the ability of the produced yeast protein to form, following heat-treatment, resilient gel structures.

2. Materials and methods

2.1. Materials

Cheese whey was obtained from MEVGAL S.A., a factory processing milk for cheese and other milk products. *Kefir* yeast commercial product, which is used in Caucasus for homemade kefir drink, was employed in this study. The oil used for preparation of emulsions was locally purchased refined corn oil, Defatted soy flour was prepared by extracting soy flour (100 g) with petroleum ether (1000 ml) under continuous stirring for 2.5 h, filtering, washing with 200 ml petroleum ether and, finally, drying at 55 °C under vacuum for 3 h. The resulting flour was then analyzed for protein (47.6%), lipids (1%), ash (6%), water (6%), and carbohydrates (31%) according to standard methods (AOAC, 1975). All the chemicals used were of analytical grade and were provided by Riedel-de-Häen.

2.2. Preparation of SCP

Kefir grains were first cultivated and proliferated in agar slants containing cheese whey (2% w/v). The cultures were successively subcultured in 5, 50, 500 ml and 2 l liquid substrate [cheese whey enriched with 2.4 g/1.5 l KH_2PO_4 and 11.3 g/1.5 l $(NH_4)_2SO_4$]. The optimal growth conditions were 30 °C and pH 5.5. The culture broth was then centrifuged at $4000\times g$ for 40 min. For SCP production in a larger scale a feed-batch system on a bioreactor of 4 l was developed. The experimental apparatus is shown in Fig. 1. The aerobic fermentation of cheese whey by *kefir* microorganisms took place at 30 °C and pH 5.5 with an air supply of 0.3 bar through a sterile filter of 0.45 μm. The nutrient medium was introduced into the reactor dropwise in about 6–9 h (the fermentation process lasts ~7 to 9 h) with the aid of a peristaltic pump The biomass was obtained by centrifugation at $4000\times g$ for 10 min, washed with distilled water and freeze dried.

2.3. Analytical characterization of yeast biomass

Single cell protein was assayed for its moisture content by drying 5 g (±0.001 g) of the sample at 98–100 °C until constant weight (AOAC, 1975). Its protein content was determined by applying the semi-micro Kjeldahl technique (N×625) (Pearson, 1976). Fat was extracted with a mixture of chloroform–methanol (1:1, v/v) according to AOAC method (1975). The fat content in the extract was estimated gravirnetrically after evaporation of the solvent at 100 °C. The phenol–sulfuric acid method was used to determine the carbohydrate content (Chaplin, 1994). The mineral content was determined by ashing 3 g at 600 °C for 15 h (AOAC, 1975). Finally, atomic absorption spectrophotometry was applied in order to determine the calcium, chromium, magnesium, iron, zinc, copper, cadmium, mercury and lead content.

2.4. Preparation and study of oil in water (o/w) emulsions

One precent (w/v) dispersions of SCP or defatted soy flour in water were prepared and the pH was adjusted to

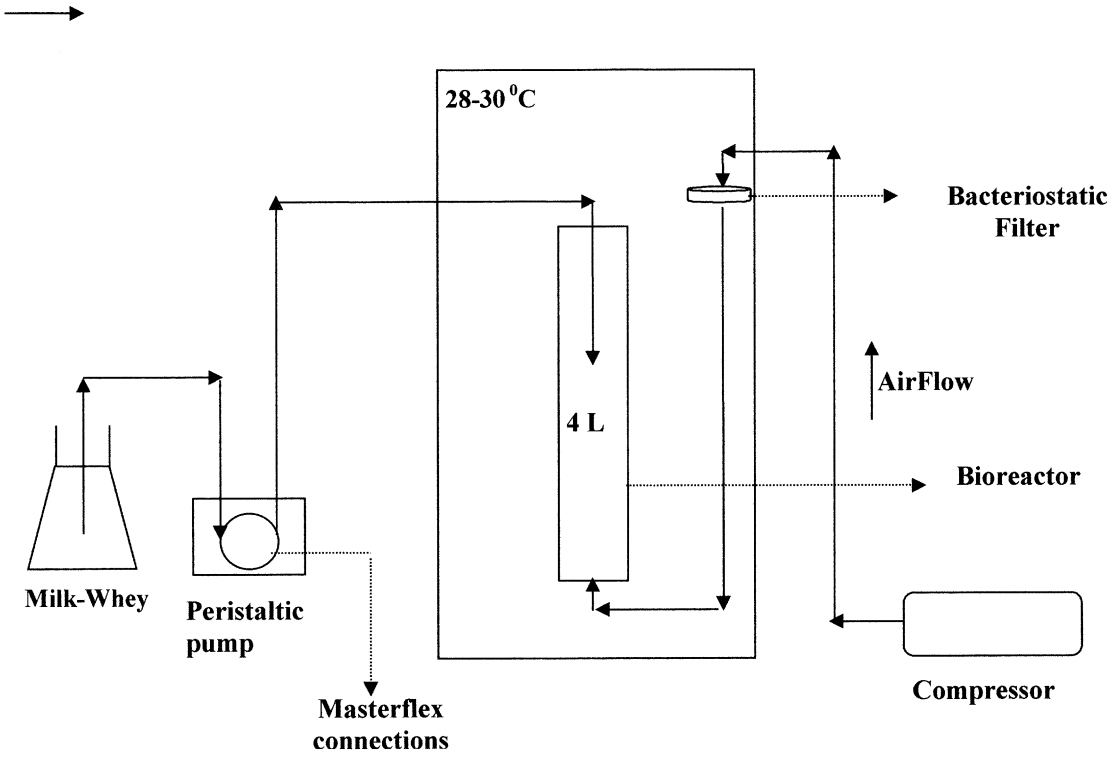

Fig. 1. Schematic layout of the experimental apparatus.

6.5. Following pretreatment (increase of pH to 10, homogenization at 24,000 rpm and readjusted of pH to 6.5) of the dispersions, if necessary, oil in water emulsions were prepared by adding dropwise, while mixing, 5 ml of corn oil into 45 ml of SCP dispersions (oil volume fraction = 0.1), with the aid of a propeller-type mechanical stirrer. The resulting crude emulsions were then homogenized for 1 min at 21,500 rpm using an Ultra-Turrax T25 homogenizer (IKA Instruments, Germany) equipped with a S25KG-25F dispersing tool. The emulsions were then stored at 5 °C and their stability against oil coalescence was determined by following the change with time of the oil droplet size distribution patterns measured by laser light diffraction using a Malvern Mastersizer 2000 (Malvern Instruments, Sweden). The refractive index of the oil was 1.4673 and the imaginary part of the refractive index (due to absorption) was fixed at 0.002. The mean surface diameter [$d_{3,2}$ (μm)] of the oil droplets were determined from the droplet size distribution. Three series of emulsions were prepared and the results are given as means of three values.

2.5. Foaming studies

Foams were prepared using the method described by Poole, West, and Walters (1984). SCP was dispersed in distilled water to give a final protein concentration of 1% (w/v). The aqueous phase was submitted at various treatments concerning pH adjustment at 6.5 or 10 in combination with freezing/thawing and homogenization at 24,000 rpm for 1 min. Submission to these treatments aimed at disintegrating the SCP structure so that a greater amount of protein would become available for adsorption or interaction. The protein suspensions (100 ml) were then whipped at a speed of 1000 rpm for 5 mm in a kitchen-type Sunbeam Mixrnaster (Sunbeam Electric Co., UK). The resulting foams were immediately poured into a 1-l glass measuring cylinder and the foam expansion (FE) and foam liquid stability (FLS) were determined using the following equations (Poole et al., 1984):

$$\%\ \mathrm{FE} = \frac{\text{Foam volume (ml)}}{\text{Initial liquid volume (100 ml)}} \times 100$$

$$\%\ \mathrm{FLS} = \frac{\text{Volume of liquid (ml) retained in foam after 30 min}}{\text{Initial liquid volume (100 ml)}} \times 100$$

The volume of liquid retained in foam was calculated from the volume of liquid drained after 30 min. Additionally, % foam volume decrease was calculated from the ratio of foam volume change within 30 min divided by the initial foam volume. Three series of foams were prepared and the results are given as means of three values.

2.6. SCP solubility

SCP was dispersed (1% w/v) in distilled water. The samples (10 ml) following treatment were then centrifuged at 4500×*g* for 20 min at ambient temperature and the pH was adjusted to 6.5. The protein content in the supernatant was determined colorimetrically according to Folin-Ciolcateau method (Copeland, 1994). An index of protein solubility was calculated as % (w/v) protein in supernatant. Assays were made in duplicate.

2.7. Gels

In order to evaluate the gelling properties of SCP, gels were prepared by heating about 10 g of thin aqueous pastes containing 30 and 40% (w/v) of biomass or defatted soy flour for 30 min. The liquid samples were placed into cylindrical cells of 1 cm diameter made from aluminum foil and reinforced with several layers of plastic tape, the ends were sealed and the cells were immersed into a preheated to 90 °C water bath for 30 min. Following storage at room temperature for 24 h, the cells were cut open and the gels were cut to obtain cylindrical samples of 1 cm height.

The cylindrical gel samples were subjected to a double compression test (50% compression) using a texture analyzer (Stable Micro Systems, UK) fitted with a 2.5 cm diameter cylinder. The textural parameters (hardness, fracturability, cohesiveness and springiness) were recorded from force–time curves generated for each gel according to the texture profile analysis procedure (Bourne, 1978). All TPA data was processed with the aid of Stable Micro System Texture Expert software v. 1.00. At least six cylindrical specimens were tested for each sample.

3. Results and discussion

The composition of single cell protein obtained from the aerobic fermentation of cheese whey by *kejir* microflora is shown in Table 1. The crude protein content of the dry cells was about 54% (w/w), which was higher than the value reported for similar products (Choi & Park, 1999). The ash content was in agreement with previous results (Cheung, 1997; Konlani, Delgenes, Moletta, Traore, & Doh, 1996). The biomass is also a good source of calcium as well as a number of micronutrients such as magnesium, iron, zinc and copper.

Fig. 2 presents the influence of aging time on the mean surface diameter $d_{3,2}$ of o/w emulsions prepared with single cell protein submitted to various treatments before emulsification in comparison with emulsions prepared with defatted soy flour. Almost all the emulsions prepared with SCP, except for emulsion 3, exhibited initial droplet sizes lower than that of the soy protein stabilized one. The oil droplet size distribution for emulsions prepared with SCP (emulsion 1) and defatted soy flour (emulsion 5) is shown in Fig. 3. It appears that the SCP-stabilized emulsions exhibit broader droplet size distributions since as shown in Fig. 3, about 25–30% of the oil droplets have sizes lower than 2 μm while in the case of the soy flour-stabilized emulsions about 95% of the droplets have sizes between 2 and 12 μm. All the emulsions were very stable against oil droplet coalescence except for the one containing NaCl. This effect, of salt addition, which was consistent with results reported by Huang and Kinsella (1986, 1987) concerning salting out of phosphorylated yeast protein, was probably due to the reduced amount of protein available at the oil/water interface for emulsion stabilization.

Emulsions are thermodynamically unstable systems, and they immediately separate into two phases unless a surfactant is present at the interface. Even in the presence of an adsorbed surfactant at the interface, these dispersed systems are only kinetically stable; they

Table 1
Single cell protein composition

Component	% w/w
Moisture	3.0
Fat	4.0
Crude protein (N×6.25)	53.9
Carbohydrates	6.5
Ash	7.3
Ca[a]	0.012
Mg[a]	0.024
Fe[a]	0.0009
Zn[a]	0.0050
Cu[a]	0.0004

There are only traces of Cr, Cd, Hg, Pb.
[a] Estimated by use of atomic absorption.

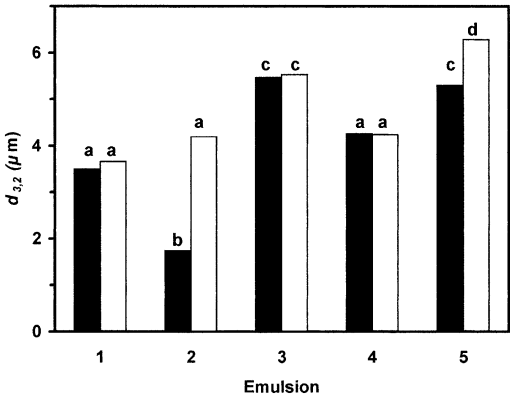

Fig. 2. Influence of aging time on the mean surface diameter $d_{3,2}$ of the prepared o/w emulsions. *Aqueous phase characteristics:* 1% w/v SCP, pH 6.5 (1); pH 6.5. NaCl 0.6 M (2); pH 4.0 (3); initial pH 10—homogenization at 24,000 rpm for 1 min—final pH 6.5 (4); 1% w/v defatted soy flour, pH 6.5 (5). Key: ■ 24 h; □ 32 days. Different letter superscripts indicate significant differences at $P<0.05$.

flocculate and coalesce and eventually separate into two phases after a period of time. The kinetic stability of an emulsion, then, depends on the physical and chemical properties of the adsorbed surfactant layer and its ability to prevent flocculation and coalescence of oil droplets (Damodaran, 1997). In general lines, SCP addition resulted in formation of emulsions with relatively low droplet sizes compared with soy flour which reflects the fact that protein molecules could form strong, rigid surface films which stabilized all droplets. Homogenization of SCP dispersion at pH 10 (emulsion 4) did not result in an improvement of the emulsifying properties in spite of the fact that an additional amount of the protein was released from the broken cells.

Foams are colloidal systems in which air bubbles are dispersed in an aqueous continuous phase. When a liquid is agitated, introduction of air takes place behind the rod, resulting in the entrapment of air bubbles by the liquid. Bubble formation and stabilization are aided by the presence of low- or high-molecular weight surface-active constituents which influence the dynamic surface properties of the foam films (Prins, 1988). The foaming properties of protein encompass two aspects: (1) the ability to produce a large interfacial area so that a large volume of gas can be incorporated into the liquid and (2) the ability to form a tenacious interfacial film that can withstand internal and external forces (Damodaran, 1997). Single cell protein is the main surface-active agent that helps in the formation and stabilization of the dispersed gas phase during foam preparation (Fig. 4). SCP seems to be a more effective foaming agent compared to soy flour, the foam of which displayed the lowest strength. The volume of foams prepared with previously homogenized or/and freezed/thawed at pH 10 biomass (dispersions 2 & 3) was the highest of all as well the most stable (Fig. 5). Treatment of the cells by freezing/thawing did not appear to influence protein solubility although its foaming properties improve considerably reflecting the fact that cell structure destruction might have contributed to this. Foam

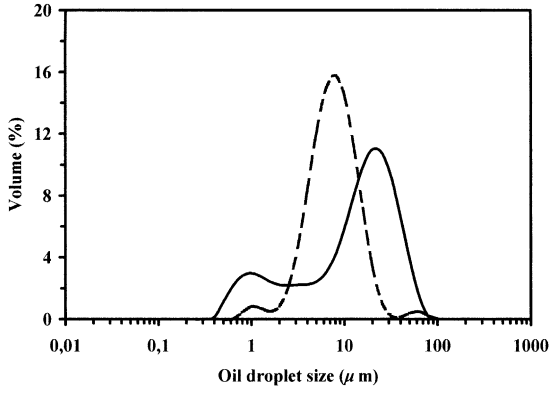

Fig. 3. Oil droplet size distribution of emulsions prepared with SCP [——] and defatted soy flour [- - - - -]. (1% w/v, pH 6.5).

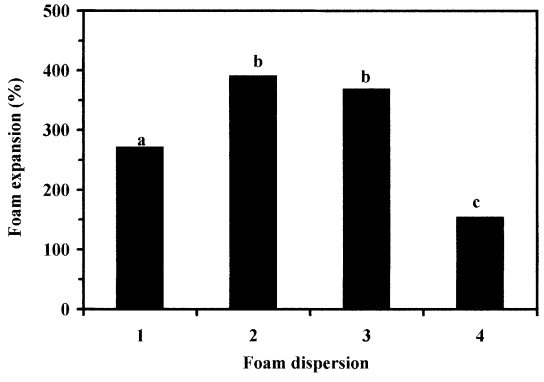

Fig. 4. Foam expansion as a function of aqueous phase treatment. *Dispersions characteristics:* 1% w/v SCP (1); Initial pH 10—homogenization at 24,000 rpm for 1 min—final pH 6.5 (2); Initial pH 6.5—freezing for 24 h—thawing (twice)—pH 10—homogenization at 24,000 rpm for 1 min—final pH 6.5 (3); 1% w/v defatted soy flour, pH 6.5 (4). Different letter superscripts indicate significant differences at $P<0.05$.

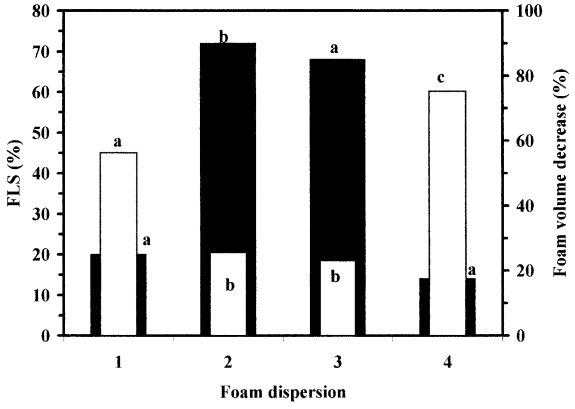

Fig. 5. Foam liquid stability (■) and foam volume decrease (□) as a function of aqueous phase treatment. *Dispersions:* as in Fig. 4. Different letter superscripts indicate significant differences at $P<0.05$.

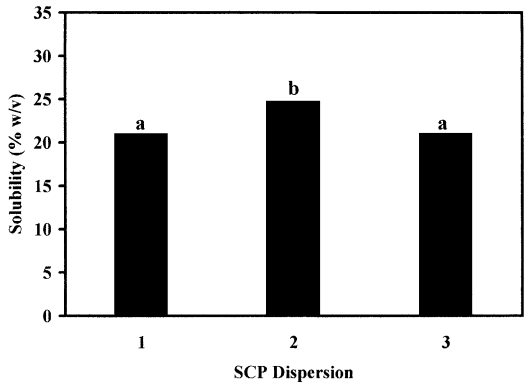

Fig. 6. Protein solubility (% w/v) of SCP dispersions submitted at various treatments. *Dispersions characteristics*: 1% w/v SCP (1); Initial pH 10—homogenization at 24,000 rpm for 1 min—final pH 6.5 (2); Initial pH 6,5— freezing for 24 h—thawing (twice)—pH 10, homogenization at 24,000 rpm for 1 min—final pH 6.5 (3). Different letter superscripts indicate significant differences at $P<0.05$.

stability refers to the ability of the protein to stabilize foam against gravitational and mechanical stresses (Damodaran, 1997). The extent and rate of liquid drainage from film lamellae are important factors determining the stability of foams, as well as protein concentration, structure and degree of denaturation, presence of lipids, etc. (Halling, 1981). The foam stability, defined as the volume of liquid remaining in the foam after 30 mm, was approximately the same for two types of SCP dispersions (2 & 3) whereas for dispersions 1 and 4 was much lower (Fig. 5). The treatment in which soy flour was submitted in order to a great amount of lipids to be removed may be responsible for the decrease in foaming ability of the defatted soy flour. When the lipid is partially or totally removed the protein structure is probably destabilized and therefore its adsorption and stabilization properties are affected. Two mechanisms, namely increased solubility and enhanced electrostatic repulsion between adjacent bubbles, may account for the increase in foamability and stability following treatment of SCP dispersions at pH 10. Generally, only soluble protein contributes to foaming (Halling, 1981). Treatment of yeast cells, such as homogenization at pH 10, weakened the cell wall and facilitated the solubilization of yeast proteins, as it is also shown in Fig. 6, thus improving foam formation and stability. The foams formed (2 & 3) appeared finer, denser and more stable. This also arises from monitoring foam volume with time (Fig. 5). The rate of foam deterioration depends mainly on the rates of drainage, coalescence and disproportionation (Prins, 1988). Other researchers also observed improved functionality of yeast protein, after cell disruption (Huang & Kinsella, 1986, 1987; Vananuvat & Kinsella, 1975).

The textural parameters of the studied gels generally exhibited higher values with the increase in total solids concentration (30, 40% w/v), but the change pattern vary with the sample (Fig. 7a & b). Gels prepared with SCP appeared to be harder, more cohesive and springier than gels prepared with defatted soy flour. This could be attributed to the higher protein concentration of SCP samples, since more protein molecules were incorporated into the gel. Additionally, delipidation of soy flour might affect protein flexibility, its ability to denature and give extended chains upon heating and hence its ability to form extensive networks by cross-linking.

Gels are a form of matter intermediate between a solid and a liquid. They are aqueous solutions or dispersions of high molecular weight carbohydrate or

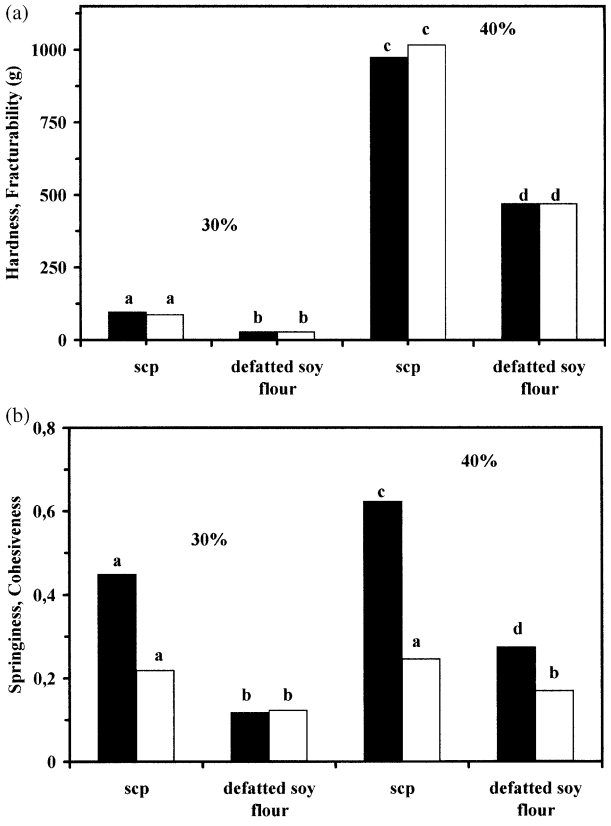

Fig. 7. Textural parameters of gels prepared with SCP or defatted soy flour as a function of solids concentration. Key: ■ hardness/springiness; □ fracturability/cohesiveness. Different letter superscripts indicate significant differences at $P < 0.05$.

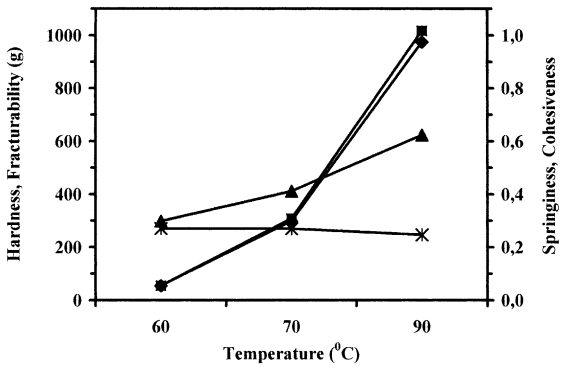

Fig. 8. Influence of heating temperature on the textural parameters of gels containing 40% w/v SCP (heating time 30 min). Key: ◆ hardness; ■ fracturability, ▲ springiness; ∗ cohesiveness.

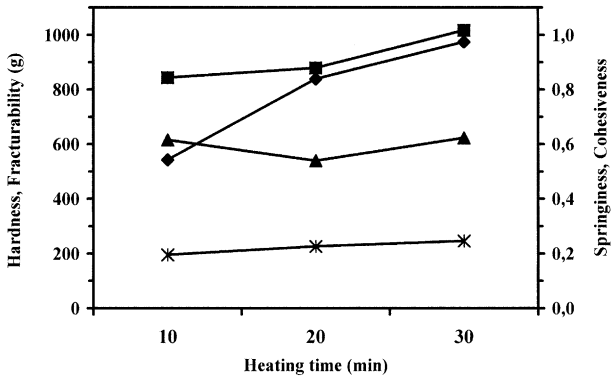

Fig. 9. Influence of heating time on the textural parameters of gels containing 40% w/v SCP (heating temperature 90 °C). Key: ◆ hardness; ■ fracturability; ▲ springiness; ∗ cohesiveness.

proteins, cross-linked so as to form an interconnected molecular network that spans the volume of the liquid medium. A gelatinous material is semi-solid; it has rigidity but readily deforms under stress. The most important characteristic of the gel state is its rheological behavior. There are two broad classes of rheological measurement: those made with ''small deformation'' and those made with ''large deformation''. TPA. which employs large stresses and strains, is useful for the characterization of ultimate mechanical gel properties relevant to eating characteristics, but it doesn't provide information on the course of structure formation and the molecular interactions underlying the final gel microstructure (Oakenfull, Pearce, & Burley, 1997).

Cohesiveness and springiness reflect the development of internal bonding in a three-dimensional gel network, and their values generally increase with increasing total solids. Cohesiveness exhibited the lowest score for gels prepared with defatted soy flour, while it was greater for gels prepared with SCP and almost the same for both concentrations studied. Differences in strength of the bonds between protein molecules may account for such behavior. An increase in heating time (10, 20, 30 min) and heating temperature (60, 70, 90 °C) resulted in harder gels, which fractured easily (Figs. 8 & 9). Cohesiveness is the only textural parameter that remained almost the same.

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